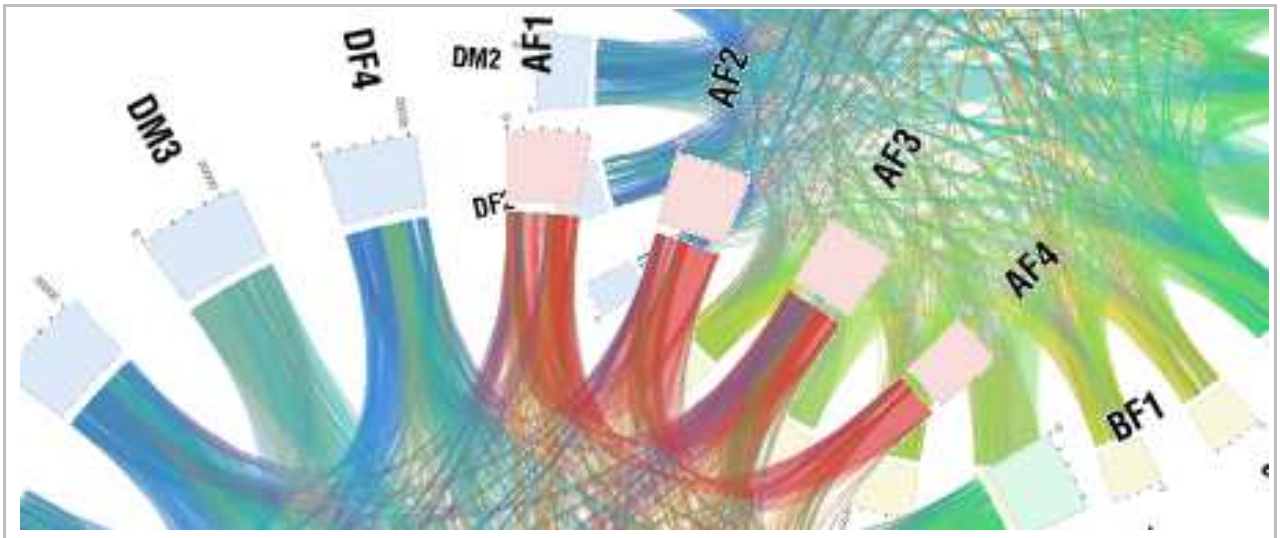

4TH THEODOR ESCHERICH SYMPOSIUM ON MEDICAL MICROBIOME RESEARCH

ABSTRACT BOOK

12.-13.10.2017 Graz, Austria

SCIENTIFIC PROGRAMME | Thursday, October 12, 2017

8.15-8.45	Registration
8.45-9.00	Welcome and introduction
9.00-9.40	Our intestinal microbiota as a source of next-generation probiotics to prevent and to treat inflammatory diseases? *Philippe Langella (MICALIS Institute, France)*
9.45-10.15	Clinical implications of respiratory tract microbiome/mycobiome data *Robert Krause (Medical University of Graz, Austria)*
10.20-10.50	COFFEE BREAK AND POSTERS
10.50-11.20	Insights into the rumen microbiome *ItzhakMizrahi (Ben-Gurion University of the Negev, Israel)*
11.25-11.35	High fat diet induces depression-like behaviour in mice associated with changes in intestinal microbiome and brain metabolome *Ahmed M. Hassan, Giulia Mancano, Karl Kashofer, Esther E. Fröhlich, Andrija Matak, Raphaela Mayerhofer, Florian Reichmann, Marta Olivares, Audrey M. Neyrinck, Nathalie M. Delzenne, Sandrine P. Claus, Peter Holzer*
11.35-11.45	Sorting and sequencing active host-compound foragers from the mouse gut microbiota *Fatima Pereira, B. Sziranyi, M. Wagner, D. Berry*
11.45-11.55	Survival of the human-associated anaerobes in the indoor environment *Manuela-Raluca Pausan, Christine Moissl-Eichinger*
11.55-12.05	Exogenous isolation of antibiotic and metal resistance plasmids from pharmaceutical wastewaters released into the environment *Juan José González Plaza, Khald Blau, Ana Šimatović, Milena Milaković, Ana Bielen, Marijan Ahel, Kornelia Smalla, Nikolina Udiković-Kolić*
12.05-12.40	LUNCH
12.40-14-10	Guided poster tour & LGC lunch talk "LGC – expertise for microbial community analysis by Next Generation Sequencing" *All poster presenters & Michel Perriere (LGC)*
14.10-14.40	Crop plants as alternative hosts for human pathogenic bacteria *Adam Schikora (Julius Kühn-Institut Braunschweig, Germany)*
14.45-15.15	Plasmid mediated adaptation and diversification of soil and plant associated bacteria *Kornelia Smalla (Julius Kühn-Institut Braunschweig, Germany)*
15.20-15.50	Endophytic colonization from roots to seeds – ecology and how plants can benefit *Angela Sessitsch (AIT, Austrian Institute of Technology, Austria)*
15.55-16.20	COFFEE BREAK

SCIENTIFIC PROGRAMME | Thursday, October 12, 2017

16.20-16.50 Human pathogenic micro-organisms in plant production systems
Leo van Overbeek (Wageningen University, The Netherlands)

16.55-17.25 Environmental and lichen-associated Pseudomonas isolates from Iceland have pathogenic potential and display behaviouralresponses to plant matter
Oddur Vilhelmsson (University of Reykjavik, Iceland)

17.30-17.40 The microbiome of alpine seeds - what is transmitted to the next generation?
Birgit Wassermann, Gabriele Berg

17.40-17.50 Pilot study of metabolisation of an Arctic root extract by gut microbiota
Ivana Turek, Kaisa Koskinen, Christine Moissl-Eichinger, Franz Bucar

17.50-18.00 The hidden power of volatiles in microbial interactions
Tomislav Cernava, Stefan Liebminger, and Gabriele Berg

18.00-18.05 Information for the next day

SCIENTIFIC PROGRAMME | Friday, October 13, 2017

8.55-9.00	Welcome and Introduction
9.00-9.40	Mechanistic studies of the impact of the gut microbiome on obesity and type 2 diabetes *Fredrik Bäckhed (University of Gothenburg, Sweden)*
9.45-10.15	The microbiome in intestinal inflammation and tumorigenesis *Alexander Moschen (Medical University of Innsbruck, Austria)*
10.20-10.40	COFFEE BREAK AND POSTERS
10.40-11.10	Impact of plant species and performance on the establishment of E. coli O157:H7gfp+ and the microbial phyllosphere community structure on leafy green vegetables *Beatrix Alsanius (Malmö University, Sweden)*
11.15-11.45	Computational metaproteomics of plant and human interactions with bacteria *Judith Klein-Seetharaman (University of Warwick, UK)*
11.50-12.00	From community organisation to complex behaviour: Competition and keystones in the lung microbiome of persons with cystic fibrosis *Stefanie Widder*
12.00-12.10	TraG acts as a lytic transglycosylase and scaffolding protein in the pIP501 encoded type IV secretions system of *Enterococus faecalis* *Andreas Aufschnaiter, Verena Kohler, Sabrina Büttner, Ines Probst, Elisabeth Grohmann and Walter Keller*
12.10-12.20	Effect of a high fat diet on the bile acid metabolism and resistance of bacteria in the mouse gut *Nicole Simone Treichel, Blaž Stres, Michael Schloter, Anne Schöler*
12.20-12.30	Closing remarks and prizes

SHORT TALK 1

High fat diet induces depression-like behaviour in mice associated with changes in intestinal microbiome and brain metabolome

Ahmed M. Hassan, Giulia Mancano, Karl Kashofer, Esther E. Fröhlich, Andrija Matak, Raphaela Mayerhofer, Florian Reichmann, Marta Olivares, Audrey M. Neyrinck, Nathalie M. Delzenne, Sandrine P. Claus, Peter Holzer

Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Graz, Austria

The biological mechanisms linking obesity and depression remain unclear. In this work, we explored the effects of obesity induced by high fat diet (HFD) on emotional-affective behaviour of male C57Bl/6J mice. Potentially involved biological factors including the intestinal microbiome, brain metabolome, and neuropeptide Y (NPY) were examined in parallel. HFD for 8 weeks led to the development of a depression-like phenotype as revealed by reduced sociability and sucrose preference. 16S rDNA sequencing of caecal contents disclosed significant changes in response to HFD, including a diminished relative abundance of the phylum Bacteroidetes, and an increased relative abundance of the phyla Firmicutes and Cyanobacteria. In the brain, analysis of the metabolome by 1H nuclear magnetic resonance in the prefrontal cortex and striatum revealed HFD-induced alterations in the concentrations of molecules involved in energy metabolism, such as lactate, and in molecules relevant to neuronal signalling, such as GABA. Moreover, HFD caused a region-specific reduction of NPY expression, whereas the plasma levels of both NPY and its catabolizing enzyme activity, dipeptidyl peptidase-4 (DPP-4), were increased. The HFD-induced anhedonia was not affected by a 4-week treatment with imipramine (7 mg/kg/day) or sitagliptin (50 mg/kg/day). The current study shows that obesity induced by HFD is associated with imipramine-resistant-depression-like behaviour in mice along with distinct alterations in the intestinal microbial community, distinct brain metabolites, the NPY system, and DPP-4 activity. The microbial and molecular changes associated with HFD-induced depression-like behaviour provide new insights into the pathophysiological links between obesity and depression.

SHORT TALK 2

Sorting and sequencing active host-compound foragers from the mouse gut microbiota

Fatima Pereira, B. Sziranyi, M. Wagner, D. Berry

Department Microbial Ecology, University of Vienna, Vienna, Austria

The secreted mucus layer that separates the mammalian intestinal epithelium from the lumen provides a habitat and serves as a nutrient source for a subset of gut bacteria. Due to the complexity of mucin and the diversity of the O-glycan carbohydrate chains that it consists of, our knowledge about the molecular strategies employed by gut bacteria to utilize mucin glycans still remains incomplete. To study the capacity of the mouse colon community to forage on mucin and to metabolize monosaccharides originating from O-glycans, we used a stable isotope probing approach that employs heavy water (D2O)-based activity labelling and Raman microspectroscopy. With this approach we could observe that a significant percentage of the microbial community was stimulated by the addition of each of the O-glycan monosaccharide constituents (i.e., sialic acid, fucose, N-acetylglucosamine, N-acetylgalactosamine and galactose), or by mucin itself. By Raman-based cell sorting of active (D2O-labeled) cells with optical tweezers and subsequent whole genome amplification and sequencing, near-complete genomes of mouse gut microbes that can forage on N-acetylglucosamine were recovered. Genes predicted to encode the necessary enzymes for complete or nearly complete N-acetylglucosamine catabolism were identified in all of the cell-sorted genomes or on the genomes of the next closest relatives, including N-acetylglucosaminidases that allow these organisms to scavenge the N-acetylglucosamine monosaccharide from the mucin O-glycans. This approach successfully enabled the establishment of a link between an organism's function in the context of a complex microbial community and its genomic content, and will further help in dissecting the identities and functions of key-players in the mucin-associated niche.

SHORT TALK 3

Survival of the human-associated anaerobes in the indoor environment

Manuela-Raluca Pausan, Christine Moissl-Eichinger

Department of Internal Medicine, Medical University of Graz, Graz, Austria

The indoor microbiome has been extensively studied in the last couple of years. Since we spend most of our time indoors, the microorganisms present in our houses highly influence our lives and health. The environment is also considered an important source of microorganisms for infants during their first years of life as their gut microbiota undergoes rapid changes towards an adult like microbiota. 90% of all microorganisms in the human gut are (strictly) anaerobic microorganisms, and thus are affected by oxygen. Many of them are most-likely acquired by direct transfer from mother to child during birth; however, C-section born infants acquire most of their microorganisms from other sources including the environment, i.e. their family and from the house environment. Most of the studies focus on the acquisition of bacteria, while the human-associated archaea are overlooked. The goal of this study is to determine whether the house environment can harbour anaerobic microorganisms, especially human-associated archaea, serving as a source of anaerobic microorganisms. A NGS approach was used to explore the anaerobic microbial community present in the house environment, followed by a qPCR approach to determine the ratio between bacteria and archaea. The microbial community was also visualized using fluorescent in situ hybridization. The results show that the bacterial community present on the sampled surfaces in the house is dominated by microorganisms from Firmicutes, Actinobacteria and Proteobacteria phyla. Archaeal signals from Thaumarchaeota and Euryarchaeota have also been identified. The ratio between bacteria and archaea has been determined to be 100:1.

SHORT TALK 4

Exogenous isolation of antibiotic and metal resistance plasmids from pharmaceutical wastewaters released into the environment

Juan José González Plaza, Khald Blau, Ana Šimatović, Milena Milaković, Ana Bielen, Marijan Ahel, Kornelia Smalla, Nikolina Udiković-Kolić

Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia

Antibiotic resistance (AR) is currently one of the most significant epidemiological risks. It is linked to anthropogenic activities, as release of pharmaceutical industry wastewaters, which often contain high concentrations of antibiotics and metals. Release into aquatic environment is assumed to promote dissemination of antibiotic resistance genes (ARGs) among environmental bacteria, and potentially to human pathogens. We need more information to understand the mobilization of ARGs in the environment influenced by antibiotic pollution to control the emergence of resistant pathogens. Studies of sediments at discharge points of two pharmaceutical factories in Croatia showed a high proportion of antibiotic resistant bacteria (ARB), which may lead to increased AR in human pathogens by horizontal gene transfer (HGT). Conjugation is one of most important HGT mechanisms for the spread of ARGs. Once established on plasmids, resistance genes may spread across different strains, species, or genera. Moreover, multiple ARGs are often co-localized on the same plasmid (co-resistance), allowing for the spread of multidrug resistance. We have assayed whether plasmids containing ARGs genes from pharmaceutical discharge sites are transferable to other bacteria through exogenous plasmid transfer. Wastewater or sediments influenced by antibiotic pollution, as well as sediments from upstream and downstream sites, were mixed with model recipient E. coli. Transconjugants were selected based on AR markers and green fluorescent signal emission. Frequency of transconjugation was calculated for each site. Besides, we have characterized the presence/absence of ARGs as well as other metal/biocide resistance genes through qPCR and PCR, and antibiogram profiles for unique isolates were determined.

SHORT TALK 5

The microbiome of alpine seeds - what is transmitted to the next generation?

Birgit Wassermann, Gabriele Berg

Graz Technical University, Institute of Environmental Biotechnology, Austria

While the microbiome of various crop plants have been extensively studied within the past decades, the seed microbiome and the vertical transmission of the microbiota was comparatively little investigated. We selected eight plant species from the East Alpine region with different diaspore types (capsules and achaenes) and subjected their seeds into a multifactorial analysis. Scanning electron microscopy was used to visualize native micro-niches and colonization patterns of seed-adherent microbes. Sequencing of the ITS region and 16S rRNA gene revealed a plant genotype-depending structure and diversity of the micro- and mycobiome, while the effect of the diaspore type was insignificant. Focusing on plant life cycle, annuals and perennials showed distinct differences towards their microbiome composition. The bacterial families of the putative plant beneficials, Pseudomonadaceae and Enterobacteriaceae dominated the core microbiome which was shared across all alpine seeds, whereas the mycobiome harbored frequently described fungal plant pathogens including Alternaria, Phoma, Boeremia and Botrytis. Alpine plants represent, not only due to the low anthropogenic influence, valuable research objects and our results can contribute as important supplementary information for international seed banks. Following analyses will investigate the metagenome of Gentiana asclepiadea (seeds and adult plant) that is able to grow at a wide range of altitudes and further define a microbiome that serves as a bedrock for tolerance and adaptation.

SHORT TALK 6

Pilot study of metabolisation of an Arctic root extract by gut microbiota

Ivana Turek[1], Kaisa Koskinen[2], Christine Moissl-Eichinger[2], Franz Bucar[1]

[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Graz, Austria, [2]Department of Internal Medicine, Medical University Graz, Graz, Austria

Arctic root, the roots and rhizomes of Sedum *roseum* (L.) Scop. (syn. *Rhodiola rosea* L., Crassulaceae), have been traditionally used for the relief of mental and physical symptoms of stress. Currently salidroside as well as rosavins are regarded as the quality determining compounds1. As these glycosides most likely act as prodrugs, a metabolic study of a root extract was performed. The extract was incubated with freshly harvested human gut microbiota taking samples after 0, 4 and 24 hours. The samples were analyzed using UHPLC-PDA-HRMS analysis. The obtained raw data was processed with Proteowizard2 and Python 2.7 using the Pyteomics3 v. 3.4.1 module. The preliminary results showed that whilst some of the main compounds like lotaustralin and rosavin were present at 0 hrs, they were not present at 4 and 24 hrs, respectively. Interestingly, cinnamyl alcohol was increased within 4 hours, however was present at lower amounts after 24 hours indicating metabolisation. The microbial community was analyzed by 16S rRNA gene sequencing. After 24 hrs the changes in community composition affected mainly Bacteroidales (S24-7-296340; *Bacteroides uniformis*), Ruminococcaceae (359950, *Ruminococcus*), and Enterococcaceae (*Enterococcus*). These results have provided insights into the interplay of a plant extract and the colon microbial community.

SHORT TALK 7

The hidden power of volatiles in microbial interactions

Tomislav Cernava, Stefan Liebminger, and Gabriele Berg

The indigenous microbiota of plant hosts fulfills a central role in terms of protection against biotic stresses. A variety of microorganisms produce highly efficient metabolites to block pathogen attacks and thus maintain plant health under unfavorable conditions. The utilization of volatile organic compounds (VOCs) is a powerful strategy to overcome distance boundaries. Bioactive VOCs are employed by beneficial microorganisms to establish and maintain a 'protective shield' around the host. It was demonstrated that distinct plant-associated bacteria, e.g. the genera of Bacillus, Pseudomonas, Stenotrophomonas, and Paenibacillus, are major contributors to the recruitment of volatile and highly active antimicrobial substances. Identified diazines from the Bacillus and Paenibacillus clusters drastically reduced the viability of the pathogenic fungi Botrytis cinerea, Verticillium dahliae, and Candida albicans. Moreover, analogous diazine derivatives were demonstrated to inhibit the growth of various human pathogenic bacteria including Listeria monocytogenes, Salmonella typhimurium, and Staphylococcus aureus when applied in low concentrations. We also demonstrated that various volatiles can be employed as long-distance messengers and play a central role in inter-species interactions. In the environmental context we found that VOCs-driven effects highly depend on i) the availability of nutrients, ii) the development stage of the microorganism, and iii) strain-specific interactions, e.g. synergistic effects of compatible microorganisms. The overall findings provide strong evidence for the importance of microbial VOCs in the maintenance of host wellbeing and additionally increase the repertoire for upcoming biotechnological applications.

SHORT TALK 8

From community organisation to complex behaviour: Competition and keystones in the lung microbiome of persons with cystic fibrosis

Stefanie Widder

Center for Molecular Medicine, Medical University of Vienna, Vienna, Austria

Microbes are everywhere and make up most of the biomass on earth. Frequently, they form microbial communities (MCs) and conduct complex, collective functions that are of highest importance for biogeochemical cycles on earth and human well-being alike. For example, the human gut microbiome can actively promote human health or be etiologic for chronic diseases or cancer. These emergent community functions are driven by microbial interactions. To build predictive understanding and manage microbial functions for the human context, research needs to address all scales involved from metabolic interactions up to ecological roles and community dynamics. In my talk I will present our modelling approach that allows detection of keystone species from NGS data. Such keystones are not only relevant for community persistence, but are also prime targets for improving human health. I will show how networks and graph theory are applicable for pinpointing the dynamics of the human microbiome in airways of cystic fibrosis (CF) patients and how our generic framework enables prediction of drug targets in metabolic networks of the CF microbiome. Moreover, the presented concepts are directly transferable to other lung disorders with poly-microbial implication, such as COPD or asthma.

SHORT TALK 9

TraG acts as a lytic transglycosylase and scaffolding protein in the pIP501 encoded type IV secretions system of *Enterococus faecalis*

Andreas Aufschnaiter, Verena Kohler, Sabrina Büttner, Ines Probst, Elisabeth Grohmann and Walter Keller

University of Graz, Austria

Conjugative transfer is an important molecular mechanism responsible for the spreading of antibiotic resistances and thus represents a major health issue. It describes the transmission of a plasmid from a donor to a recipient cell, equipping these bacteria not only with antibiotic resistance genes but also with the whole machinery needed to further spread these plasmids. The aim of our project is to study the molecular details of the conjugative model plasmid pIP501 with the broadest transfer host-range known so far, frequently found in E*nterococcus faecalis* and *Enterococcus faecium* clinical isolates. Here, we describe for the first time a physical interaction of the peptidoglycan metabolizing enzyme TraG and the membrane translocation channel protein TraM, two of the 15 proteins of the pIP501 type IV secretion system. We observed a spot-like localization of both proteins at the cell membrane of E. faecalis and revealed that correct membrane localization of TraM requires the transmembrane helix of TraG, but not its enzymatic active domains. Deletion of TraG resulted in complete abrogation of conjugative transfer, which could be complemented by providing full length TraG in trans. We propose that the enzymatic activity of TraG is required for peptidoglycan digestion in order to insert the type IV secretion system in the cell membrane and that TraG acts as a scaffolding protein to maintain correct localization of the pore complex during conjugative transfer. Thus, inhibition of TraG might represent an attractive new target to prevent spreading of antibiotic resistances via conjugative transfer.

SHORT TALK 10

Effect of a high fat diet on the bile acid metabolism and resistance of bacteria in the mouse gut

Nicole Simone Treichel, Blaž Stres, Michael Schloter, Anne Schöler

COMI, Helmholtz-Zentrum München, Germany

High fat diet was shown to alter the gut microbiome of mice by several studies, but the reasons for this are not fully understood. As a high fat diet increases bile acid concentrations in the gut, it is argued that this shift is a major contributor to the alterations of the gut microbiome. Bile acids are amphipathic molecules responsible for emulsifying fat, enabling lipid digestion and uptake. They are toxic to bacteria primarily by causing membrane damage. However bacteria can also modify bile acids. If this is for detoxifying or for obtaining energy and nutrients is still under investigation. The products of these modifications are called secondary bile acids and in humans can only be produced by bacteria which harbor bile salt hydrolases. Genes encoding bile salt hydrolases have been found in C. perfringens, Lactobacillus plantarum, La. johnsonii, Bi. longum, *Bi. bifidum*, *Bi. adolescentis*, and *Listeria monocytogenes*. Secondary bile acids may contribute to the pathogenesis of colon cancer, gallstones, and other gastrointestinal diseases. Therefore, we investigated how high fat diet is influencing the secondary bile acid forming bacterial community and their metabolism. We used a metagenomics approach in combination with a targeted metabolomics to assess bile acid composition and to connect bacterial gene abundances to changes in the bile acid composition.

POSTER 1

Knockout of peptide YY induces critical changes in gut microbiota composition specifically in response to high fat diet

Aitak Farzi, Karl Kashofer, Felicia Reed, Lei Zhang, Peter Holzer, Herbert Herzog

Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Graz, Austria

The gut hormone peptide YY (PYY) is a member of the neuropeptide Y (NPY) family and is being expressed by endocrine L cells of the lower gastrointestinal tract. PYY levels increase upon ingestion of a meal, slow the transit of food through the gastrointestinal tract and inhibit food intake. As the effects of gut-derived PYY on gut microbiota composition and its metabolic consequences have not been investigated, this work aimed at exploring the differences of intestinal microbiota composition of wild-type (WT) and PYY-knockout (KO) mice under basal conditions, as well as in response to dietary interventions. Therefore, the artificial sweetener sucralose was added to the drinking water of male WT and PYY-KO mice at a concentration of 1% for a treatment period of 1 week, while subsequently high fat diet (HFD) was given for a period of 3 weeks. Faecal samples were collected at baseline, as well as after sucralose and HFD treatment. PYY-KO lead to an increased abundance of the phylum Bacteroidetes, while both sucralose and HFD decreased the abundance of the phylum Bacteroidetes. In addition, LEfSe analysis revealed distinct genotype differences in gut microbiota composition, specifically in response to HFD. Thus taxa belonging to the phylum Firmicutes were enriched in WT mice, while taxa belonging to the phylum Bacteroidetes were enriched in PYY-KO mice in response to HFD. Together these results highlight a critical role of gut-derived PYY in the control of gut microbiota composition, specifically in response to HFD.

POSTER 2

Changes of the microbiome in critically ill patients

Lena Horvath, Gregor Gorkiewicz, Bettina Halwachs, Kaisa Koskinen, Christine Moissl-Eichinger, Maximilian Mora, Robert Krause, Christoph Högenauer.

Department of Internal Medicine/Division of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria

The human microbiome of multiple body sites is affected by critical illness and clinical interventions. When and how microbes change in intensive care unit (ICU) patients is not fully understood. This study investigated the short and long-term changes of microbiota composition in pharynx, feces, tracheobronchial and gastric secretion of 6 ICU patients. The association of clinical factors (medication, infections, mechanical ventilation) was studied for each case. The microbiota of clinical samples was phylogenetically characterized by 16S rRNA sequencing. All samples were divided into 3 groups (early, mid, late), depending on sampling day. Overall, the microbiome of both respiratory and gastrointestinal tract showed a clear loss of species-richness over the course of hospitalisation (seen by decreasing Chao1 indices). Low-diversity communities (one genus comprising over 75%) were detected in all patients, especially in pharyngeal, tracheobronchial and gastric samples, the majority after longer ICU stays. Microbial composition showed great interpatient differences, but Staphylococcus and Enterococcus were pathogens frequently observed in several patients. We found that patients with an infection showed the infecting pathogen in at least one sample before (3 of 5 patients) or after (all patients) the clinical diagnosis of the infection. In most of these cases, the same pathogen was detected simultaneously in multiple sample areas, suggesting colonization of different body habitats. Other clinical factors were not directly related to microbial changes in this study. Assessing the clinical influence of microbiota diversity-loss in relation to the abundance of nosocomial pathogens in critically ill patients is of great interest for the future.

POSTER 3

Understanding the Mechanisms of Receding Intestinal Inflammation after Fecal Microbiota Transplantation

Barbara Jelusic, Phillip Wurm, Patrizia Kump, Christoph Högenauer, Paul Kohl, Stefan Schild, Gregor Gorkiewicz

Institute of Pathology, Medical University of Graz, Graz, Austria

The success of fecal microbiota transplantation (FMT) as therapy for gastrointestinal disorders varies greatly across different diseases. Our research focuses on predicting the outcome of FMT and revealing the elements that dampen intestinal inflammation. For people suffering from chronic ulcerative colitis non-responsive to conventional therapy, FMT is a promising option. In an open controlled trial of repeated FMT after antibiotic pre-treatment, we found 10 out of 17 patients with clinical response. Different donors participated in the study. With 16S rRNA gene-based microbiota analysis and real-time PCR, we investigated taxonomic characteristics of donors´ microbiota. A prominent finding is a high abundance of Akkermansia muciniphila associated with remission. A. muciniphila is a common mucin-degrading bacterium that resides in a close proximity to the colon epithelium. One of the communication pathways between microbiota and the host are bacterial outer membrane vesicles (OMVs). They carry bacterial effectors to the epithelium and lamina propria, with the potential to modulate the immune response. We isolated OMVs from A. muciniphila, as well as Fusobacterium nucleatum, a pathogen associated with inflammatory bowel diseases and colorectal cancer. Our work in progress comprises analyzing the effects of OMVs on myeloid cells like macrophages and dendritic cell lines. We hypothesize that OMVs from A. muciniphila, in comparison to F. nucleatum, lead to a different and possibly unique cytokine profile. Using immunohistochemistry, we will analyze the changes in macrophage phenotype after FMT associated with disease remission in patient samples.

POSTER 4

The human gastric microbiome is predicted upon infection with *Helicobacter pylori*

Ingeborg Klymiuk, Ceren Bilgilier, Alexander Stadlmann, Jakob Thannesberger, Marie-Theres Kastner, Christoph Högenauer, Andreas Püspök, Susanne Biowski-Frotz, Christiane Schrutka-Kölbl, Gerhard G. Thallinger, Christoph Steininger

ZMF/Medical University of Graz, Graz, Austria

The human gastric lumen is one of the most hostile environments of the human body and was suspected to be sterile until the discovery of Helicobacter pylori (H.p.). State of the art next generation sequencing technologies multiply the knowledge on H.p. functional genomics as well as on the colonization of supposed sterile human environments like the gastric habitat. Here we studied in a prospective, clinical trial the 16s rRNA gene amplicon based bacterial microbiome in a total of 30 gastric biopsy samples. The evaluation of the samples for H.p. infection status was done by histopathology and a quantitative real time PCR assay. CagA status was determined by a CagA-specific PCR assay. Patients were grouped accordingly as H.p.-negative, H.p.-positive but CagA-negative and H.p.-positive and CagA-positive (n=10, respectively). Here we show that H.p. infection of the gastric habitat dominates the gastric microbiota in most patients and is associated with a significant decrease of the microbial alpha diversity from H.p. negative to H.p. positive with CagA as a considerable factor. The genera Actinomyces, Granulicatella, Veillonella, Fusobacterium, Neisseria, Helicobacter, Streptococcus and Prevotella are significantly different between the H.p.-positive and H.p.-negative sample groups. In H.p. negative samples, microbiota were similar to those found in the oropharynx. Differences in microbiota found between CagA-positive and CagA-negative patients were not statistically significant and need to be re-evaluated in larger sample cohorts. In conclusion, H.p. infection dominates the gastric microbiome.

POSTER 5

TraN: a novel repressor of an *Enterococcus* conjugative type IV secretion system

Verena Kohler, Andreas Aufschnaiter, Sabrina Büttner, Ines Probst, Elisabeth Grohmann and Walter Keller

Institute of Molecular Biosciences, University of Graz, Graz, Austria

The perpetual increase of antibiotic resistant strains among bacterial pathogens represents one of the most immanent challenges to human health care in the 21st century, annually accounting for estimated 700,000 deaths worldwide. Plasmid-born conjugative transfer is the most prevalent mechanism, which requires a type IV secretion system, a multi-protein machinery, responsible for the transfer of plasmid DNA to recipient cells. The production of these multi-component complexes bears tremendous energetic effort and thus must be tightly controlled. Although pharmacological manipulation of these regulatory mechanisms might be a promising therapeutic target against the dissemination of antibiotic resistances, insights into these processes remained elusive. In this study, we identified the small cytosolic transfer protein TraN as a novel repressor of the Gram-positive conjugative plasmid pIP501. TraN has an essential role in the regulation of the conjugative transfer, as a deletion variant exhibited up-regulated gene expression, leading to increased protein load and elevated transfer efficiency. Since the pIP501 conjugative plasmid shows an exceptionally broad host-range and has been frequently identified in diverse clinical isolates, an inhibition of its transfer would be crucial to block further dissemination of resistance factors and thus the generation of multi-resistant bacteria. To this end, TraN presents itself as a promising pharmacological target.

POSTER 6

Microbial dynamics of a full duration flight to planet Mars simulation

Kaisa Koskinen, Petra Schwendner, Alexander Mahnert, Christine Moissl-Eichinger, Simon Barczyk, Reinhard Wirth, Gabriele Berg, Petra Rettberg

Department of Internal Medicine, Medical University of Graz, Graz, Austria

A manned flight to planet Mars will confront its crew with an extremely isolated built environment. Despite rigid confinement the crew and their spacecraft have to share their habitat with microorganisms. The MICHA ("MIcrobial ecology of Confined Habitats and humAn health") experiment monitored microbial dynamics inside a confined sealed spacecraft mock-up for more than 500 days in the frame of the so-called Mars500 project. Over the full duration of this project 360 samples from the air and different surfaces were collected and analyzed by 16S rRNA gene amplicon sequencing, supported by PhyloChip G3 analysis and extensive cultivation. The microbiome was dominated by human-associated bacteria assigned to Corynebacterium, Ralstonia and Staphylococcus. According to next generation sequencing the microbial diversity decreased significantly over time. However, the proportion of opportunistic pathogens, stress-tolerant or potentially mobile element bearing microorganisms increased. Temporary fluctuations of microbial diversity and composition could be linked to procedures of microbial maintenance conducted by the crew. The microbial communities did not only show a time-dependent, but also a location-dependent composition: microbial communities in wet rooms, on table surfaces or in greenhouse could be further linked to different surface materials and surface orientation, but only slightly to climatic variables. This study shows that despite confined conditions, human associated microbiota is still subject to fluctuations. A detailed monitoring of microbial abundance and diversity will be essential to counteract threatful developments, such as an increase of highly resistant microorganisms or labile and low microbial diversity, to guarantee a safe journey to planet Mars.

POSTER 7

Characterization of TraF, a member of a Gram-positive Type IV Secretion System

Anna Lammer, V Kohler, I Probst, E Grohmann, W Keller

Institute of Molecular Biosciences/University of Graz, Graz, Austria

The frequent occurrence of antibiotic resistances of pathogenic bacteria, especially of those which cause nosocomial infections, is a big problem for the health system nowadays. Bacterial conjugation, the transfer of plasmid DNA, carrying resistance genes, from a donor to a recipient cell, is the major cause for the spread of antibiotic resistance. The transfer is preceded by the formation of a plasmid-encoded multiprotein complex, called type IV secretion system (T4SS). Although conjugation is rather well characterized in Gram-negative bacteria, there is hardly any information how the transfer in Gram-positive organisms works. Our group is working on the biochemical and functional characterization of the transfer-proteins from the conjugative model plasmid pIP501, to find out how the conjugative transfer in Gram-positive bacteria works. pIP501 was originally isolated from Gram-positive Streptococcus agalactiae and shows the broadest host range in Gram-positive bacteria known to date. At the moment, we focus on the essential transfer factor TraF. TraF is likely to participate in the formation of the T4SS pore complex, which facilitates the actual transfer of the DNA. Utilizing quantitative immunoblotting, qPCR, in vivo- and in vitro-crosslinking approaches and interaction studies provide us with more details about the protein. Furthermore, with the results we can reinforce or rather confirm the assumption, that TraF is a pore complex member.

POSTER 8

Microbiota profiling of cutaneous tumors identifies a putative driver of squamous cell carcinoma development

Nandhitha Madhusudhan[1], Manuela-Raluca Pausan[1], Christine Moissl-Eichinger[1], Jürgen C Becker[2], Gregor Gorkiewicz[1]

[1]Medical University of Graz, Graz, Austria; [2]DKTK, Essen, Germany

Malignant epithelial skin tumors (e.g. cutaneous squamous cell carcinoma, cSCC) arises from specific precursors (e.g. actinic keratosis, AK). During tumor development the host-microbiota equilibrium is altered and a selected pro-inflammatory cutaneous microbiota might drive cancer progression. Thus to assess the impact of microbiota in progression of AK to cSCC, we used comparative 16S rDNA based microbial community profiling, expression analysis and immunophenotyping of human skin cancer samples. These analyses showed that AK and SCC have a higher microbial richness but lower evenness and diversity compared to basal-cell carcinoma (BCC) which served as a control. Each tumor entity harbored a distinct microbial community type signified by an overabundance of Staphylococcus in AK and SCC and Streptococcus in BCC. qPCR confirmed these findings and indicated the presence of Staphylococcus aureus in AK & SCC. S. aureus was predominantly found in the hyperkeratotic region of AK & SCC as assessed by FISH. Interestingly, AK and SCC showed overexpression of anti-microbial peptides (AMPs) hBD-2 and 3, known to cause hyperproliferation of squamous epithelia. Also, hBD-2 expression was significantly upregulated in cSCC cells when challenged with live S. aureus. Furthermore, the severity of inflammation significantly correlated with increased species richness and Staphylococcus abundance in AK. We, therefore, hypothesize that during neoplasia development, change of the microbial habitat, e.g. by hyperkeratosis, leads to overgrowth of S. aureus, which contributes to a proinflammatory and hyperproliferative state, both features which are known to be associated with the development & progression of cSCCs.

POSTER 9

Life from space: Microbes from indoor surfaces of the International Space Station

Maximilian Mora, Lisa Wink, Ines Kögler, Christine Moissl-Eichinger

Medical University of Graz, Graz, Austria

In Space Sciences, microorganisms onboard spacecraft pose a potential hazard for astronauts and spacecraft material but they also provide possible means for an autonomous maintenance of such isolated environments, e.g. by improving indoor air quality, waste recycling or food production. Understanding the microbial population able to thrive and evolve in this special type of environment is critical for planning future long term space missions. The best model system to date to investigate the microbiome of a confined space environment with constant human occupation is the International Space Station (ISS), which is constantly inhabited by humans since November 2000. In the scope of the ARBEX project (ARchaeal and Bacterial EXtremophiles onboard the International Space Station ISS), designed to elucidate the population of hardy, extremophilic bacteria and archaea onboard the ISS, fresh wipe-samples were taken from inside surfaces of the ISS and are currently investigated at the Medical University of Graz. Together with other ongoing studies supported by ESA, NASA and Roscosmos, the data obtained here will provide insight into the complete microbial inventory of the ISS and lay the base for future space travel. Here we will describe the sampling procedure onboard the space station in more detail and show recently obtained data of microbial isolates from the ISS environment.

POSTER 10

Soil-borne fungal pathogens: Biocontrol of sclerotia – the imperishable survival structures

Pascal Mülner, Philipp Wagner, Alessandro Bergna, Barbara Gstöttenmayr, Kristin Dietel, Tomislav Cernava and Gabriele Berg

ABiTEP GmbH, Berlin, Deutschland

Fungal pathogens such as Sclerotinia sclerotiorum and Rhizoctonia solani are the cause of extensive crop damage across the globe. Sclerotia, consisting of an outer black layer containing cells with high concentration of melanin and medullary hyphae with extended vacuoles, are formed by both fungi to persist severe environmental conditions. These survival sctructures preserve their viability and ability to infect potential hosts up to eight years. Microbial communities associated with both fungal pathogens were explored to identify potential biocontrol agents. Cultivation-dependent and –independent methods were combined to obtain deepening insights into the microbiome of sclerotia. Data from the 16S rRNA amplicon study showed that distinctive bacterial communities were associated with healthy and sclerotia-affected potato peel. Flavobacteriaceae and Caulobacteraceae were primarily found in unaffected areas. Bacterial cultures were harvested from both model organisms and tested for their antagonistic potential against fungal pathogens. In addition to a wide range of Bacillus species, isolates assigned to the Enterobacter, Pseudomonas and Buttiauxella genus exhibited auspicious antagonistic acitivities. In a subsequent approach the most promising bacteria were screened for bioactive volatiles. Among other substances, a wide range of alkylated pyrazines were detected in the volatilome of antagonists belonging to various species of Bacillus. Previous studies displayed the powerful antimicrobial effect of these diazine derivatives. Moreover, distinct combinations of preselected antagonists led to a significant increase in the pathogen inhibition. The so far conducted experiments set the basis for further molecular studies and will facilitate the identification of novel biocontrol agents.

POSTER 11

Gut microbiota diversity is not related to intestinal permeability measured by serum zonulin in women

Sabrina Mörkl, Sonja Lackner, Andreas Meinitzer, Gregor Gorkiewicz, Karl Kashofer, Andreas Oberascher, Sandra Holasek

Medical University of Graz, Graz, Austria

Objective: Increased gut permeability causes the trespass of antigens into the blood stream which subsequently leads to neuroinflammation. It is unknown whether gut microbial diversity contributes to gut permeability in women. In this cross-sectional study, we aimed to investigate serum zonulin, a marker of intestinal permeability, along with the gut microbiota composition of a large female cohort of different BMI groups and activity levels (represented by athletes). Methods: 102 female participants were included (BMI range 13.24-46.89): AN patients (n=17), athletes (n=20), normal weight (n=25), overweight (n=21) and obese women (n=19). DNA was extracted from stool samples and subjected to 16S rRNA gene analysis. Quantitative Insights Into Microbial Ecology (QIIME) was used to analyze microbiome data. Serum zonulin was measured with ELISA. Additionally, nutrient intake was assessed by analyzing repeated 24-hour dietary recalls. Results: We used the median serum zonulin concentration (53.64 ng/ml) to divide our sample into a "high zonulin" (>53.64ng/ml) and "low zonulin" (<53.64ng/ml) group. Alpha diversity (Shannon-Index, Simpson-Index, Equitability) was not significantly different between the high and low zonulin group. Zonulin concentration showed positive correlations with total calorie intake (r=0197, p=0.047) and Vitamin B12 intake (r=0.201, p=0.043). Lefse analysis identified Ruminococcaceae as significantly more abundant in the low Zonulin group. Discussion: The composition of the gut microbiota in woman seems not to be associated with serum zonulin concentration. Ruminococcaceae have been shown to induce anti-inflammatory effects and could be a regulating factor of gut permeability. Further studies are needed to investigate serum zonulin as a biomarker for increased gut permeability.

POSTER 12

Studying *Helicobacter pylori* as a manipulator of the human immune system

Margit Neger, Gregor Gorkiewicz

Medical University of Graz, Graz, Austria

The human immune system is trained and shaped by the symbiotic microbiota to maintain a balance between protection against pathogens and tolerance towards innocuous antigens. Disruption and reduction of the microbiota through antibiotics and increased hygiene standards are both proposed to account for the rise in autoimmune and inflammatory disorders in high-income countries. An interesting bacterium in this context is Helicobacter pylori. It colonizes 50% of the world's population during early childhood and usually persists for a lifetime. 15% of the carriers develop gastric pathologies ranging from chronic gastritis and peptic ulcers to different forms of gastric cancer. However, the other 85% of the carriers remain asymptomatic. In recent years mounting clinical and epidemiological evidence suggests that H. pylori can protect from the development of various diseases including early-onset asthma and allergies. This two-sided effect may be explained by H. pylori's impact on the immune system: Infection induces a mixed pro-and anti-inflammatory reaction that recruits high numbers of immune cells but down-regulates immune activity to allow stable bacterial colonization. An immune factor that might play a role in this context is the immune receptor NKG2D. This molecule is expressed on NK- and T cells and helps them to detect and destroy cells that are stressed, infected or neoplastic. We have observed a reduction of the NKG2D receptor in the stomach mucosa of H. pylori carriers. Now we aim to study the underlying molecular mechanisms and effects of NKG2D reduction during H. pylori colonization in regard to immune regulation and tumorigenesis.

POSTER 13

Bioprospecting a Moss Metagenome for Biotechnological Treasures

Melanie M. Obermeier, S.N. Hollauf, C.A. Müller, G. Berg

Environmental Biotechnology TU Graz, Graz, Austria

Natural product discovery from microorganisms is still essential for the identification of industrially valuable compounds. Yet, it is impeded by the inability to cultivate up to 98% of the bacteria. Exploring microbial community DNA, metagenomes, directly allows to omit culturing and grants access to untapped sources and their hidden treasures. One such unexplored treasure chest is the bacterial community associated to the moss Sphagnum magellanicum. Adapted to harsh abiotic conditions and known to have a rich secondary metabolism as well as a high share of antimicrobial properties, the moss microbiome represents a rich source for enzymes and metabolites of biotechnological interest.1 We investigate the antibiotic resistome, in vitro and in silico, and source the metagenome for novel antimicrobials targeting several plant and human pathogens. Thereby, we are especially interested in bioactive volatile organic compounds (VOCs). Additional focus lies on the identification of novel nonribosomal peptide synthetases (NRPS) as well as polyesterases. Sequence based screenings have led to the identification of one novel NRPS potentially involved in the synthesis of homopoly(amino acid)s. The isolated NRPS could be relevant for the production of natural food preservatives. Further, by functional metagenomics six novel polyesterases were identified which break down synthetic polyesters and possess industrially valuable properties making them promising candidates for plastic recycling2. Antimicrobial VOCs can be used in bio-fumigation processes to develop crop well fare and control microbial hazards in human environments. Finally, evaluating the resistome will promote our understanding of the potential of this bacterial community towards the identification of novel antimicrobials.

POSTER 14

Isomerization of urocanic acid by ultraviolet radiation and its role in modulation of skin microbiome, antimicrobial peptides, and immune function

VijayKumar Patra, Mina Bashir, Meghana Somlapura, Harald C. Köfeler, Ingeborg Klymiuk, Thomas Pieber, Peter Wolf

Department of Dermatology, Medical University of Graz, Graz, Austria

Trans-urocanic acid (UCA) is a natural photoreceptor present in the stratum corneum of the skin. Upon exposure to ultraviolet-radiation (UV-R) trans-UCA is isomerized to cis-UCA. Several studies indicate that cis-UCA induces local and systemic immune suppression via various underlying mechanisms. However, microbes are established all over the surface of the skin and the interplay between cis-UCA and the skin microbiome is not completely understood. In this study, we investigated the effects of cis-UCA on the skin microbiome and antimicrobial peptides (AMPs) expression using mouse models. We employed HPLC to determine quantitative isomerization of trans-UCA to cis-UCA by UV-A, UV-B, and Psoralen+UV-A (PUVA). We further made use of the model of contact allergy to assess the percentage of immune suppression by UV-A, UV-B, PUVA and topical application of cis-UCA to the contact allergen DNFB. Next, we treated mice with UV-A, UV-B, PUVA and cis-UCA and collected skin swabs for 16S rRNA gene sequencing and AMP gene expression, at 8h and 24h after treatment. We noted that UV-B ($p=0.002$) and PUVA ($p=0.023$) significantly increased the formation of cis-UCA, whereas UV-A exposure alone showed no significant formation of cis-UCA in the skin. Utilizing the contact allergy model, we observed a dose-dependent increase in immune suppression (by up to 100%) against the contact allergen DNFB, when mice were pretreated with cis-UCA. Furthermore, application of cis-UCA on the skin altered the microbial landscape of the skin both at 8h and 24h, correlating with a change in expression of various AMPs. Collectively our results suggest that, cis-UCA alters the skin microbial landscape and AMP expression. This imbalance in the skin microbial landscape and altered AMP expression may be crucial in immune suppression upon UV-R exposure mediated through cis-UCA.

POSTER 15

Effects of antibiotic feed administration on the conjugal plasmid transfer in piglet faeces

Manuela Perebner, Viviana Klose, Gerd Schatzmayr

BIOMIN Research Center, Tulln, Austria

The spread of antimicrobial resistance genes on mobile genetic elements, such as plasmids, poses a serious threat to the successful treatment of bacterial infections. To study the effect of tetracycline on the conjugal plasmid transfer in pig production, a feeding trial with 16 piglets housed individually in metabolic cages under controlled environmental conditions was conducted. Using a randomized block design (blocking factors: sex, mother sow, room), the piglets were allocated to two different groups (n = 8): negative control (basal diet) and antibiotic group (basal diet + in-feed antibiotic). The in-feed antibiotic was provided for seven consecutive days at a dose of 40 mg oxytetracycline-hydrochloride/kg body weight per day. Faeces was sampled on day 0 (prior to antibiotic supplementation), day 8 (last antibiotic day) and day 21. Tetracycline resistance (TetR) carrying plasmids were exogenously isolated from fresh piglet faeces using tetracycline for transconjugant selection and a gfp-tagged E. coli recipient. Transfer frequencies per recipient were significantly higher for captured plasmids from antibiotic-fed piglets than for those of the control, at day 8 and day 21. Phenotypic assessment of a selection of transconjugants (n=57) revealed various antibiotic resistance patterns (many of them carrying resistances against tetracycline, streptomycin, ampicillin and trimethoprim). PCR-based replicon typing using a commercial kit (DIATHEVA, Italy) showed a variety of broad- and narrow-host range plasmids belonging to incompatibility groups IncF, IncI1, IncB/O and IncP.

POSTER 16

Vesiculation impacts the pathophysiology of the gastrointestinal pathogen Vibrio cholera

Franz G. Zingl, Paul Kohl, Fatih Cakar, Debora Leitner, Sandro Roier, Katherine E. Bonnington, Meta J. Kuehn, Joachim Reidl, Stefan Schild

ZMBE/ Institiute of Molecular Biosciences, Graz, Austria

Gram-negative bacteria produce outer membrane vesicles (OMVs) which are spherical bilayered facsimiles of the bacterial surface. The vesicle amount can be modified via a retrograde phospholipid (PL) transporter, which is highly conserved in Gram-negative bacteria. Recent data indicates that vesiculation is enhanced by the host environment i.e. envelope stress and iron limitation which can be shown to downregulate the PL transporter. Thus the gastrointestinal tract is constantly bombarded with OMVs at doses even higher that previously reported.

We used the facultative pathogen V. cholerae as a model organism as it can be triggered to activate the virulence cascade in vitro, mimicking their natural transition from the aquatic environment into the human host. Therefore V. cholerae is the ideal model organism to study the impact of vesiculation within this transition. Our data demonstrate that a hypervesiculating variant of V. cholerae has a significant advantage in colonizing the murine model. This can be linked to faster adaption to polymyxin B and bile via a rapid exchange of surface components, which is crucial to counteract these host derived antimicrobial substances. Concordantly, vesiculation plays a major role in the rapid surface exchange and is therefore a key feature for adaptation facilitating infection, survival and colonization fitness.

This study provides insights into an adaption mechanism, based on vesiculation allowing an effective surface exchange during transition events. Thus, it could be a global mechanism for bacteria to adapt to different conditions.