Poster Session I — 4:30 – 5:30

PS1-1: Molecular Mechanisms of *Clostridioides difficile* Biofilm Formation

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Despite the increasing burden of *Clostridioides difficile* infections in healthcare settings, significant gaps remain in our understanding of the genetic and molecular basis for *C. difficile* colonization. To elucidate these fundamental processes, our group has identified Type IV Pili (T4P) as a colonization factor. T4P are extracellular helical fibers composed of diverse protein subunits (pilins). These appendages have diverse functions, including twitching motility, cellular adhesion, horizontal gene transfer, and biofilm formation in bacteria. Previous investigations by our group and others have revealed the importance of T4P in biofilm formation and demonstrated a role in colonization using animal models by *C. difficile*. To identify the mechanism(s) by which *C. difficile* T4P promotes biofilm formation, we investigated biomolecular interactions known to occur in bacterial biofilms, including interactions between proteins and extracellular DNA (eDNA), which are known to stabilize biofilm in other bacterial species. Here we report the ability of two *C. difficile* T4P subunits, PilJ and PilW, to directly bind recombinant plasmid DNA via electrophoretic mobility shift assay, suggesting a potential role in biofilm formation via eDNA binding. PilJ initiates binding of plasmid DNA non-specifically at 10μM, whereas the initial binding by PilW is between 40μM to 250μM between different plasmids. Ongoing work aims to determine the DNA-binding interfaces of PilJ and PilW using our previously determined high-resolution structure of PilJ and a recently determined x-ray crystal structure of PilW, as well as the use of gene-interruption mutants of *pilJ* and *pilW* to investigate their role in *C. difficile* biofilm formation.

PS1-2: A Microbiome Restoration Strategy Modulates the Gut Microbiome and Metabolic Markers in Healthy Adults

Fuyong Li (1), Anissa M. Armet (1), Junhong Liu (1), Rodrigo Margain Quevedo (1), Francesco Asnicar (2), Tianna B. S. Rusnak (1), Janis Cole (1), Zhihong Zhang (3), Adele Gagnon (1), Dale Archer (4), Andrea M. Haqq (1,5), Laurie Mereu (6), Nicola Segata (2), Catherine J. Field (1), Liang Li (7), Carla M. Prado (1), Jens Walter (1, 4, 8)
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Increased chronic disease prevalence in industrialized societies might be partially driven by disrupted gut microbiome composition and function. We tested the effects of a microbiome restoration strategy on the gut microbiome and host health, which included a non-industrialized-type (Non-Ind) diet and a probiotic *Limosilactobacillus reuteri* (species rarely found in industrialized microbiomes). Using a randomized pilot study, 30 participants consumed the Non-Ind diet or their usual diet in a crossover fashion for three weeks each. Participants also consumed a single dose of either one of two *Lm. reuteri* strains or placebo in each diet period. The Non-Ind diet enhanced the temporal persistence of one *Lm. reuteri* strain, but the administered bacteria had no measurable effects on the microbiome or host. The diet significantly shifted overall fecal microbiome composition (R2=0.015, p=0.001; ADONIS), and altered 56% of Amplicon Sequence Variants and 21% of metabolic pathways (FDR<0.05). Fiber fermentation was enhanced, exhibited by increased total fecal short-chain fatty acid concentrations (+10.7%, p=0.03) and reduced fecal pH (-3.8%, p=0.002). Six metabolic markers were reduced (all p<0.01): total cholesterol (-14.1%), low-density lipoprotein cholesterol (-16.8%), high-density lipoprotein (HDL) cholesterol (-11.3%), non-HDL cholesterol (-15.2%), glucose (-6.3%), and C-reactive protein (-14.2%). Furthermore, the diet reduced fecal calprotectin (-21.0%, p=0.002) and zonulin levels (-14.9%, p=0.025), markers of gut inflammation and barrier function, respectively. Our study demonstrates pronounced beneficial effects of a Non-Ind diet on metabolic markers, informing future strategies to improve health in modern societies. Ongoing analyses are exploring mechanistic links between the diet-induced gut microbiome and host physiological changes.

PS1-3: Genome mining for strain level variations among Roseburia spp in carbohydrate utilization

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The health promoting *Roseburia spp.* are significantly abundant in the human gut microbiome. It is known that *Roseburia spp.* play an essential role in butyrate production via complex carbohydrate utilization, but their carbohydrate degradation capabilities may vary across different species and strains. There are no genomic studies of *Roseburia* carbohydrate utilization differences on the strain level, largely due to the lack of sufficient isolate Roseburia genomes in the database. Here we took advantage of the availability of thousands of high-quality *Roseburia* metagenome assembled genomes (MAGs) from diverse habitats and hosts including human gut, cow rumen and pig gut for a comparative genomics analysis, with a goal to reveal the genomic variations in carbohydrate utilization loci in *Roseburia*. We hypothesized that the differences of carbohydrate-active enzymes (CAZymes) in numbers, types, and the changes of genes within CAZyme gene clusters (CGCs) across *Roseburia* genomes would indicate the efficiency and specificity of utilizing carbohydrates between *Roseburia* species and strains. As a work in progress, we have identified at least four *Roseburia* species-level clades by constructing phylogenetic tree and found the *R. faecis* clade was specifically different from the others. Using pan-genome analysis, we studied conserved and unique CGCs with gene synteny analyses to investigate gene order changes of specific CGCs. Altogether, through strain-level differences between *Roseburia* MAGs, our study can provide further understanding of the role of *Roseburia* spp on carbohydrate utilization in the human gut microbiome.

PS1-4: Molecular mechanisms underlying bacterial colonization of intestinal mucus layer

Benjamin S. Sidner (1), Kurt H. Piepenbrink (1,2,3)
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Many members of the intestinal microbiota reside within the mucus layer that overlays the epithelial cells of the gut. The mucus layer is composed of mucins, highly glycosylated proteins secreted by goblet cells, which can provide growth substrates and also act as ligands for bacterial adhesins. It is therefore likely that adhesion to mucins plays a key role in intestinal colonization and persistence of both commensal and pathogenic microbes. Using the nosocomial gut pathogen *Clostridioides difficile* (*C. difficile*) as a model organism, our investigation seeks to evaluate the potential role of two classes of bacterial extracellular appendages, type IV pili (T4P) and flagella, in mediating this host-microbe interaction. Both pili and flagella are generally thought to be adhesive appendages and have been implicated in glycan-binding (including mucin-binding) in several other bacterial species. The significance of these extracellular proteinaceous fibers will be evaluated through *in vitro* adherence assays utilizing immobilized human cell-derived mucins and gene interruption mutants of the primary T4P and flagellar subunits. Understanding the genetic and molecular basis for mucosal adherence may provide context for mechanisms underlying colonization and persistence of pathogenic and commensal microbes; particularly in terms of the inter-microbial competition which helps to determine the composition of the human gut microbiome.

PS1-5: The structural basis for DNA-uptake by *Acinetobacter*

Yafan Yu (1), Kurt Piepenbrink (1,2)
(1) Department of Food Science; (2) Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, Nebraska USA

Naturally competent bacteria take up DNA from the environment through horizontal gene transfer, leading to increased genetic diversity, including the spread and development of antibiotic resistance. Environmental, commensal, and opportunistic pathogens from the genus *Acinetobacter* are universally naturally competent, contributing to antibiotic-resistant infections. Multidrug-resistant strains now account for more than half of *Acinetobacter* infections. Natural competence in *Acinetobacter* is dependent upon DNA uptake mediated by type IV pili (T4P). T4P are extracellular appendages composed of protein subunits (pilins) polymerized into helical fibers. Although it has been determined that deletions of pilA (the primary T4P subunit) and pilT (a cytosolic enzyme necessary for T4P retraction) abrogate natural competence, the mechanism remains unclear as the DNA-receptor has not been identified. Previously our lab has determined strain-specific functions of pilA in T4P, i.e., biofilm, twitching, and natural competence. To identify the structural mechanism T4P in *Acinetobacter* uses for horizontal gene transfer, we propose identifying DNA-receptors incorporated into T4P by directly measuring the affinity of recombinantly-expressed pilins to double-stranded DNA. Additional studies using a combination of structural biology and biochemistry will be used to elucidate the structural basis and specificity of these DNA binding interactions. We have purified *Acinetobacter* pilins individually and in multimeric complexes and assessed their ability to bind plasmid DNA using EMSA (electrophoretic mobility shift assays). Preliminary data indicates that two minor pilins, PilE1 and PilE2, can bind plasmid DNA, and we have identified reproducible crystallization conditions for chimeric fusions of these proteins to Maltose-binding Protein (MBP).

PS1-6: Differences in Host Responses to C. difficile Infection Modulated by Microbial and Host Factors
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Affiliations: (1) Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, Nebraska USA

Clostridioides difficile is recognized as one of the most important pathogens in hospital and community healthcare settings. The clinical outcome of infection of toxigenic *C. difficile* infection (CDI) ranges from asymptomatic colonization to fulminant pseudomembranous colitis and death. In recent studies, it has been suggested that a high proportion of nosocomial CDI cases are transmitted from asymptomatic carriers which might be acting as infection reservoirs. Understanding what causes the different responses to infection could lead to the development of novel prevention and treatment strategies. Although several explanations have been proposed to explain variations in susceptibility, understanding of the exact mechanisms that underlie the spectrum of variation in CDI disease severity remains limited and further research is needed to determine what factors are responsible for these variations. In this work, we establish different human microbiota-associated (HMA) mouse models. By analyzing innate immune responses to CDI, we demonstrate that these models reproduce differences in disease severity during infection based upon differences in mouse strain background (C3H/HeN and C57BL/6J) and microbiome composition that are independent from *C. difficile* burden or toxin activity. Altogether, our HMAmouse models demonstrated the potential to study interactions between microbiome, pathogen, and host inflammatory responses in the context of CDI.

PS1-7: The Gut Microbiota Modulates the Severity of Experimental Autoimmune Myocarditis
Paul Velander (1), **Xu Shi (1)**, Robert Schmaltz (1), Jeff Price (1), Amanda Ramer-Tait (1)
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Myocarditis is an inflammatory disease of the heart muscle caused by infectious agents or other triggers that induce autoimmune responses toward heart-specific antigens. For some patients, myocarditis can progress to dilated cardiomyopathy and even heart failure. Although the gut microbiota has been linked to cardiovascular diseases, a role for the microbiome in the pathogenesis of inflammatory myocarditis has not yet been established. We therefore investigated whether the gut microbiota modulates disease severity in a mouse model of Experimental Autoimmune Myocarditis (EAM). Germ-free male C3H/HeN mice were conventionalized with one of three distinct mouse micorbiomes (M31, MC608 and W116) prior to the induction of EAM via injection of adjuvanted myosin. After 21 days, heart tissues were evaluated for microscopic inflammatory lesions and cardiomyopathy, and autoimmune responses were assessed by qualifying myosin-specific IFN-gamma and serum IgG levels. Compared to mice carrying the M31 microbiome, mice harboring the MC608 or W116 microbiomes exhibited significantly higher inflammatory heart scores characterized by moderate to marked chronic diffuse myocarditis. T cells isolated from mice colonized with MC608 or W116 also produced significantly greater levels of myosin-specific IFN-gamma versus those from mice harboring M31. The presence of the MC608 microbiome induced a stronger anti-myosin autoantibody response compared to the W116 or M31 microbiomes. Together, these results demonstrate that the gut microbiota influences EAM severity and autoreactive immune responses. The mechanisms of this regulation are being further investigated using 16S rRNA gene sequencing to determine if specific gut microbiota members are linked to disease severity and autoimmune responses.

PS1-8: dbCAN-profiler: automated carbohydrate-active enzyme annotation using raw sequence reads
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Since 2012, dbCAN has become the most popular bioinformatics tool for automated **C**arbohydrate-active enzyme **An**notation in microbiome research. Currently, dbCAN only allows assembled genomes as the input for CAZyme annotation. With cheaper DNA sequencing, it is now easy to obtain a massive amount of sequence reads from a large number of metagenomic DNA/RNA samples. There is an urgent need from microbiome researchers to predict CAZymes using raw reads without assembling them into contigs, which is very time confusing and error prone. This demands an assembly-free method to annotate CAZymes, which can also allow quantification of the CAZyme abundance across multiple samples. As a work in progress, we are developing dbCAN-profiler, a new software to allow users to input raw reads from multiple microbiome samples. The output will be the occurrence and abundance of CAZymes in different samples. In addition to the assembly-free module, dbCAN-profiler also implements a module to calculate the CAZyme abundance for the assembly-based approach by mapping reads back to annotated CAZyme contigs. Using 22 complete bacterial genomes recommended by the international CAMI (Critical Assessment of Metagenome Interpretation) community, we have simulated raw sequence reads to compare the performance of dbCAN-profiler's two modules (assembly-based and assembly-free) against the ground truth (CAZymes predicted from 22 complete genomes). Our preliminary results indicate that (i) the assembly-free method is much faster in runtime, (ii) has a better accuracy for CAZyme occurrence prediction when the sequencing coverage is low, but (iii) is generally less accurate in CAZyme abundance prediction than assembly-based method.

Poster Session II 8:00 – 9:00

P2-1: Gut microbes participate in food preference alterations during obesity

Alice de Wouters d'Oplinter (1), Marialetizia Rastelli (1), Matthias Van Hul (1), Nathalie M. Delzenne (2), Patrice D. Cani (1) and Amandine Everard (1)
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Obesity is mainly associated with unappropriated food intake. The dopaminergic mesocorticolimbic system, responsible for the hedonic responses to food intake is altered during obesity. Gut microbes are others key players involved in obesity. Therefore, we investigated whether the gut microbiota plays a causal role in reward alterations contributing to obesity. We transferred fecal material from lean or obese mice into recipient mice and evaluated the hedonic food intake using a food preference test comparing the intake of control and palatable diets (HFHS, High-Fat High-Sucrose) in donor and recipient mice. Obese mice ate significantly less HFHS than the lean donors, suggesting a dysregulation of the hedonic food intake during obesity. Strikingly, the reduction of the pleasure induced by eating during obesity was transferable through fecal transplantation since obese recipient mice exhibited similar reduction in HFHS intake during the food preference test ($p<0.01$). We also pinpointed a highly positive correlation between HFHS intake and *Parabacteroides*, which represents a potential actor involved in hedonic feeding through the gut-brain axis. We further demonstrated key roles played by gut microbes in hedonic food intake since depletion of gut microbiota using broad-spectrum antibiotics also altered HFHS intake during food preference test in lean mice. In conclusion, we discovered that gut microbes regulate hedonic aspects of food intake. Our data demonstrate that gut microbiota modifications associated with obesity participate to dysregulations of the hedonic components of food intake. They provide evidence that gut microbes could represent interesting targets to tackle hedonic disorders linked with obesity.

P2-2: Bacterial-Derived Outer Membrane Vesicles as a Novel Oral Gene Delivery System

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Affiliations: (1) Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, Nebraska USA; (2) Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, Nebraska USA; (3) Department of Surgery and Mary and Dick Holland Regenerative Medicine Program and Center for Drug Delivery and Nanomedicine, University of Nebraska Medical Center, Omaha, Nebraska USA

Non-viral gene delivery via the oral route is a desirable delivery method due to high rates of patient compliance, ease of administration and large cellular surface area present within the gastrointestinal tract for transfection. Applications for non-viral oral gene delivery include nucleic acid-based vaccination and gene therapy for intestinal diseases. However, the success of oral gene delivery is limited due to harsh conditions within the gastrointestinal tract. To overcome challenges associated with oral gene delivery, we are developing a novel delivery system by loading outer membrane vesicles (OMVs) derived from commensal gut bacteria with plasmid DNA via electroporation to create DNA-loaded OMV nanocarriers (DNA-OMV NCs). OMVs are produced by blebbing of the outer membrane of gram-negative bacteria, including strains present in the gut microbiome. Orally administered OMVs have been shown to cross the intestinal mucosal barrier and facilitate immune modulation. Optimization of the electroporation loading process showed voltage but not DNA: OMV ratio influenced DNA loading efficiency into DNA-OMV NCs. The highest DNA loading at 31.8% ± 10.9% was achieved using a 1:2 DNA: OMV ratio, and DNA-OMV NC were able to mediate transfection of HEK 293T cells. DNA-OMV NCs showed low cytotoxicity when applied to Caco2 cells and effectively protected loaded DNA from simulated gastric fluid mediated degradation. DiO-labeled OMVs were able to survive gastric transit and associate with intestinal epithelial cells in vivo 24 hours after administration. Together, these data suggest that DNA-OMV NCs derived from gut bacteria are a promising new delivery platform for oral gene therapy.

P2-3: Genetic analysis of human gut microbiome-active traits in common bean

Mallory J. Van Haute (1), Qinnan Yang (1), Nate Korth (1), Mary M. Happ (1), Car Reen Kok (1), Jennifer L. Clarke (1), Kelsey Karnik (1), Kent M. Eskridge (1), Carlos A. Urrea (1), David L. Hyten (1), James C. Schnable (1), Devin J. Rose (1), and Andrew K. Benson (1)
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Common bean (*Phaseolus vulgaris L.*) is an important food legume with tremendous genetic and phenotypic diversity. Common beans are widely recognized for a diverse array of bioactive molecules, but little is known about how variation in bioactive compounds relates to genetic variation and population structure of the species. To our knowledge nothing has been reported about the influence of molecular diversity or genetic variation on the human gut microbiome. Using a powerful new Automated *in vitro* Microbiome Screen (AiMS), we measured common bean phenotypes based on *in vitro* fermentation patterns of human gut microbiomes on individual cultivars of common bean. Pilot studies using AiMS-based phenotypes from 12 human microbiomes across a representative subset of 24 lines from the Middle American Diversity Panel (MDP) showed a significant association of multiple microbiome phenotypes with population structure. Three diverse microbiomes were subsequently phenotyped by AiMS across the entire 300-line MDP. GWAS results of the phenotypes identified seven major effect loci (MEL) on six chromosomes where genetic variation in the beans has significant effects on multiple microbial taxa from two or three of the human microbiomes. Linkage disequilibrium analysis of the MEL on *Pv*05 localized the peak to a 900 kb region, and genomic analyses of this region identified variation in genes that may be causal for the *Pv*05 MEL. We conclude that genetic analysis of AiMS-based phenotypes provides a powerful approach for identifying new types of traits (microbiome-active traits) in food crops that may have significant impacts on human health.

P2-4: The role of *Bifidobacterium pseudocatenulatum* in the utilization of xylose-based glycans

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Xylans, a family of xylose-based polysaccharides, are resistant to digestion and reach the large intestine intact, where they are utilized by members of the gut microbiome. They are initially broken down by primary degraders from the genera *Bacteroides*, *Prevotella*, *Eubacterium* and *Roseburia* that secrete extracellular xylanases to cleave xylan into smaller oligomers. These xylooligosaccharides (XOS), depending on degree of polymerization or linkages, can then either be further hydrolyzed by primary degraders or cross-fed by secondary consumers, like *Bifidobacterium*. While several *Bifidobacterium* species have metabolic systems for XOS, most grow poorly on longer XOS and xylan substrates. However, we observed some bifidobacteria display growth on long chain XOS, suggesting the XOS phenotype is strain specific. In this study, we explored the ability of five *Bifidobacterium pseudocatenulatum* strains to grow on XOS and xylan. Two distinct phenotypes were observed, with some strains capable of growth on xylan in both pure and fecal culture. In-silico detection of an extracellular GH10 endo-1,4-beta-xylanase in xylan+ strains, indicated potential extracellular xylan degradation. To test this hypothesis, xylan and XOS were incubated with *B. pseudocatenulatum* supernatants, and hydrolysis products visualized via thin-layer chromatography. Results indicated only supernatants from xylan+ strains hydrolyze xylan into products xylobiose and xylotriose. Furthermore, xylan- strains saw improved growth on xylan when combined with supernatant from xylan+ strains, indicating crossfeeding had occurred. This research suggests that some strains of *B. pseudocatenulatum* serve as primary degraders of xylan through extracellular enzymatic degradation. Collectively, this research also provides new insight into xylan utilization by bifidobacteria.

P2-5: Experimental evaluation of ecological principles to understand and modulate the outcome of bacterial strain competition in gut microbiomes

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It is unclear if coexistence theory can be applied to gut microbiomes to understand their characteristics and modulate their composition. Through experiments in gnotobiotic mice with complex microbiomes, we demonstrated that strains of *Akkermansia muciniphila* and *Bacteroides vulgatus* could only be established if microbiomes were devoid of exactly these species. Strains of *A. muciniphila* showed strict competitive exclusion, while *B. vulgatus* strains coexisted but populations were still influenced by competitive interactions. These differences in competitive behaviour were reflective of genomic variation within the two species, indicating considerable niche-overlap for *A. muciniphila* strains and a broader niche space for *B. vulgatus* strains. Priority effects were detected for both species as strains' competitive fitness increased when colonizing first, which resulted in stable persistence of the *A. muciniphila* strain colonizing first and competitive exclusion of the strain arriving second. Based on these observations, we devised a subtractive strategy for *A. muciniphila* using antibiotics and demonstrated that a strain from an assembled community can be stably replaced by another strain. Altogether, these results indicate that competition outcomes in gut ecosystems depend on niche characteristics and are historically contingent. These findings provide explanations for ecological characteristics of gut microbiomes and a basis for their modulation.

P2-6: Effect of resistant starch on gut microbial communities of mice with human microbiomes

Anthony F. Juritsch (1), Qinnan Yang (1), Sukaina al-Hamedi (1), Kristin Beede (1), Jing Shao (1), Robert Schmaltz (1), Jeff Price (1), Devin Rose (1), Scott Sattler (1,2), Andrew Benson (1), Amanda Ramer-Tait (1)
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Resistant starch (RS) interventions targeting the gut microbiome may improve gastrointestinal health and limit chronic disease progression by preventing the loss of beneficial gut bacteria and/or the expansion of disease-associated taxa. Previous studies from our group demonstrated that in vitro fermentation of RS from sorghum flour (*Sorghum bicolor* L.) with human fecal microbiomes promoted potentially favorable changes in the gut microbiota, including an increased abundance of *Roseburia* species and decreased levels of *Escherichia coli*, when compared to RS from other cereal grains. This project sought to determine whether dietary sorghum RS could reproduce these gut microbial community changes in mice colonized with the human fecal microbiotas used in the *in vitro* assays. Germ-free C57BL/6 mice were colonized with one of four human fecal microbiotas and fed a diet containing either 20% whole grain, Wheatland sorghum flour or sorghum flour from an isogenic, waxy mutant, with negligible RS content. Consuming sorghum RS was sufficient to induce global changes in the fecal microbiota structure of all recipient mice, but specific taxonomic changes were donor-dependent. In particular, RS feeding enriched for *Roseburia hominis* and *R. inulinivorans* in mice harboring one of the donor microbiomes but decreased levels of these species in mice colonized with a different donor microbiome. RS consumption reduced *E. coli* abundance in one of four donor microbiomes. Together, these results demonstrate individualized gut microbiota responses to sorghum RS *in vivo*. Future experiments will determine if the microbiome-targeted effects of sorghum RS are protective against intestinal inflammatory insults in mice.

P2-7: Assessing the beneficial claims of mass-marketed probiotic strains.

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Probiotic supplements or formulae espousing a suite of health advantages are retailed the world over. Consuming living microorganisms solely based on label promises is not worth the risk since numerous examinations have revealed a product deficiency problem. The study evaluates the commercialized products for their core microbial components in terms of quantity and quality. Only a third of the tested products were found to comply with their label claims, with lower counts per dose and unwanted microbial flora being the most typical problems. Sequence types based on multi-locus sequence typing and ribotypes showed different genome sequences of *L. acidophilus* LA-5, L. rhamnosus LGG, *B. animalis* BB12, and *C. butyricum* being used both within different batches of the same product as well as in different brands. *L. reuteri* DSM17938 was detected to have geno-and phenotypic resistance to more than three antibiotic classes. A fourth of the product range examined showed microbial viability in the gastric and intestinal phases when subjected to a static in-vitro cost INFOGEST digestion model. Weak antagonistic activity against potential human pathogens, poor functional attributes, and poor survival in GIT compromise their usefulness. Current regulatory procedures fail to address these industry-related inconsistencies.

Conference Day 2

Tuesday, October 12
8:00 – 4:00 pm US Central Time

Poster Session III 3:00 – 4:00

P3-1: Major changes in seed protein composition of parental and quality protein popcorn derivatives influence growth of beneficial, health-promoting bacteria from the human gut microbiome

Nate Korth (1,2), Leandra Parsons (3,4), Mallory Van Haute (1,2), Qinnan Yang (1,2), J. Preston Hurst (3,4), James C. Schnable (2,3,4), David R. Holding (3,4), Andrew K. Benson (1,2)
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Americans consume an average of 40 liters of popcorn a year; improvement of popcorn's protein quality could prompt human nutrition outcomes without depending on consumers changing their diets. Over half the protein content in popcorn seed is comprised of zein storage proteins, which are deficient in the essential amino acids: tryptophan and lysine. Quality protein popcorn lines, developed at the University of Nebraska utilizing a natural mutation in maize, have substantially altered protein composition, including elevated levels of lysine and tryptophan. We employed *in vitro* fermentation to study the effect of the quality protein popcorn on the human gut microbiomes of four human subjects. Comparisons of the effect of multiple pairs of popcorn hybrids with and without the quality protein phenotype on *in vitro* human gut microbiomes identified a significantly higher microbial diversity in microbiomes treated with quality protein popcorn relative to wildtype controls. The abundance of multiple genera of butyrate-producing gut bacteria including *Dorea*, *Coprococcus*, and *Butyricicoccus* increased in microbiomes fed quality protein popcorn relative to wildtype controls. Direct measurement of butyrate produced results consistent with the predicted increase in butyrate production among samples fed quality protein popcorn in two of the subject's microbiomes. Collectively these data suggest the opportunity to employ maize genetics as a tool to achieve targeted changes in the human gut microbiome, both to study interactions between the composition of human gut microbiome and health and, perhaps, to ultimately achieve desirable health outcomes.

P3-2: Developing a human microbiome associated mouse model of peanut allergy to study immunomodulation by live microbial products

Morgan Cade (1,3), Tasneem Ali (1,3), Anthony Juritsch (2,3), Kristin Beede (2,3), Robert Schmaltz (2,3), Bethany Henrick (4), Amanda Ramer-Tait (2,3)
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Over 1.2 million children in the United States currently live with peanut allergy. Results from recent studies suggest a link between food allergies and the gut microbiota, thus prompting the hypothesis that interactions between gut microbes and early immune programming influence the development of food allergies. In particular, previous studies have shown that administration of select live microbial products, including *Bifidobacterium*, induce expansion of regulatory T cells, which maintain oral tolerance and prevent immune responses against ingested food antigens. To further investigate how specific members of the gut microbiota influence the onset of food allergies in children, we developed a human microbiota-associated mouse model of peanut allergy. Germ-free C3H/HeN mice were colonized with an infant stool microbiome. Sensitization material, dose, route, and frequency as well as adjuvant dose were all optimized to yield the most robust combination of anaphylaxis, antigen-specific IgE responses, and serum mast cell protease (mMCP) levels following a peanut challenge. Weekly oral sensitization with 5 mg defatted peanut flour adjuvanted with 10 µg cholera toxin induced clinical signs of anaphylaxis as well as stronger peanut-specific IgE and mMCP responses upon challenge compared to lower peanut doses. Weekly oral gavages generated lower variability in immune responses compared to intraperitoneal injections and/or bi-weekly oral sensitizations. Future studies will use this optimized mouse model to determine if the development of peanut allergy is attenuated following administration of species of gut bacteria previously shown to regulate the abundance and/or function of regulatory T cells.

P3-3: The Gut Microbiota Regulates the Metabolic Benefits Mediated by Red Raspberry Polyphenols
Ashley Mulcahy Toney (1,2), Yibo Xian (1), Jing Shao (1), Car Reen Kok (1), Duncan Works (1), Mahaa Albusharif (1), Kristin Beede (1), Jeff Price (1), Robert Schmaltz (1), Virginia Chaidez (1), Soonkyu Chung (3), Amanda E. Ramer-Tait (1)
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Red raspberry (RR) polyphenols, including ellagic acid and their gut-derived metabolites, the urolithins, reduce inflammation and improve insulin sensitivity. However, it is unknown whether the gut microbiota is directly responsible for these benefits. To determine the role of the microbiome in mediating the health effects of RR polyphenols, C3H/HeN mice with or without a microbiota were fed either a low fat (LF), high fat (HF), or HF diet with RR polyphenols. Feeding a HF diet increased body weight and fat mass for both germ-free and conventionalized mice compared to feeding a LF diet. Feeding a HF diet with RR polyphenols significantly decreased visceral adipose tissue and liver triglycerides compared to a HF control diet in conventionalized but not germ-free mice. We next tested whether a specific gut microbe (*Gordonibacter urolithinfaciens*) previously shown in vitro to metabolize ellagic acid into urolithins, could enhance metabolic benefits when administered with RR polyphenols. *G. urolithinfacien*s administration to mice receiving a HF diet and fructose water significantly reduced body weight, body fat percentage, fasting blood glucose, and subcutaneous adipose tissue mass compared to controls. Notably, mice supplemented with both *G. urolithinfaciens* and RR polyphenols experienced no metabolic improvements compared to controls. Our results demonstrate that the microbiome is required for mediating RR polyphenol metabolic benefits. Supplementation of *G. urolithinfaciens* improved metabolic health; however, those beneficial effects were abrogated in the presence of RR polyphenols. Together, these results highlight the importance of considering specific diet-microbiota interactions when developing foods for preventing and treating obesity-related diseases.

P3-4: Isolation and characterization of new wild type bacteria originating from Greek fermented foods
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Fermented foods represent an important category of functional foods, due in part, to the presence of live lactic acid bacteria (LAB) that may contribute to gut health. Greece has a large variety of unique fermented foods with many of them containing lactic acid bacteria. IFUNFoods is an ambitious 3-year European research project that initially aims to isolate "wild" lactic acid strains from traditional Greek fermented foods. Then, cultures immobilized on natural prebiotic substrates (nuts, fruits, cereals) will be used to produce novel foods. Collectively, this project focuses on developing innovative technological processes for the production of health-promoting ingredients, evaluating their effectiveness using experimental models, and ultimately confirming their beneficial properties by conducting clinical trials. During this part of the project, sixty-eight wild-type bacterial strains derived from Greek fermented foods (table olives, dried raisin, dried figs) and human stool samples were isolated and evaluated for probiotic properties. Identification of bacterial strains was conducted by 16s rRNA sequencing. Twenty-two representative samples from the different food/stool sources were selected for whole genome sequencing (WGS). Assembly, annotation, and comparative analysis were performed by applying a combination of different pipelines and wrappers (SPAdes, PROKKA, ANVIO, DbCAN etc.) at the in-house HPC facility. Results & Conclusions: The comparative genome analysis of the isolated bacteria led to identification of unique gene clusters and enzymes relevant to their physiological role in fermented foods, as well as traits that may contribute to health benefits. The information gained through this project could lead to development of new functional foods and also to new probiotic strains that could provide health benefits to people with particular diseases.

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P3-5: Gastrointestinal microbiota modulation of egg white anti-hypertensive effect
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Hypertension affects approximately one billion people worldwide, with less than 1 in 5 people having this condition under control. Present pharmacological interventions require long-term adherence to the therapy and are often associated with undesirable side effects. However, food-derived bioactive peptides have exhibited anti-hypertensive properties and could be used as a natural alternative for preventing and managing hypertension. Our central hypothesis states that egg white hydrolysate (EWH) anti-hypertensive activity is dependent on several signaling pathways and the gut microbiota plays a critical role in modulating blood pressure development through gastrointestinal-renal axis. Preliminary studies showed that EWH, but not egg white (EW), peptides after gastrointestinal (GI) digestion, exert anti-hypertensive activity through the Renin-Angiotensin System (RAS) in Spontaneously Hypertensive Rats (SHRs), an animal model of essential hypertension. In addition, Angiotensin-II Receptor Type 1 expression, responsible for initiating vasoconstriction, was reduced by EWH compared to the untreated SHR group. Furthermore, *in-vitro* microbial fermentation under anaerobic conditions using fecal microbiomes of healthy laboratory mice showed that the digestion-resistant fraction after simulated GI digestion of EWH (EWH-GI-U) increased Lachnospiraceae and Ruminococcaceae, both butyrate producers, when compared to EW-GI-U. Furthermore, metabolites of the EWH-GI-U treated microbiome increased the expression of the peptide transporter PepT1 in gastrointestinal epithelial cells, potentially increasing EWH anti-hypertensive peptides bioavailability. Thus, our study aspires to understand food protein and peptide's bioactivity, its interaction with the gut microbiota, and its impact on cardiometabolic health.

P3-6: Influence of Substrate Diversity on Interspecies Interactions between Human Fecal Bacteria
Hugh C. McCullough (1,2), Hyun Seob Song (1,2,3), and Jennifer M. Auchtung (1,2),
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The human gut microbiome, the collection of microorganisms in the gastrointestinal tract, is a rich collection of life, and like any other ecosystems, their composition and functions are susceptible to biotic and abiotic factors. Understanding what ecological interactions are present within the gut microbiome can aid in understanding rules dictating resilience and resistance to perturbations. Despite rapid advancements in the field, we still lack fundamental principles that dictate the adjustment in microbial populations and interactions in response to environmental variation. In this work, we investigate how microbial populations and interactions in human fecal bacterial communities shift across growth cultures with varying substrate diversity. We cultured fecal microbiota from different healthy humans in media of varying carbohydrate composition in anaerobic continuous-flow bioreactors and analyzed changes in community composition through 16S rRNA gene amplicon sequencing. Based on the resulting time-series population data, we inferred media-specific microbial interaction networks using a regression-based approach termed LIMITS. From our networks we were able to find a correlation between species richness and substrate evenness, division of conserved interactions by predicted CAZyme function, and the role of interspecies interactions on the theoretical stability of these cultured fecal communities.

P3-7: Characterizing prebiotic responders using metagenomics and machine learning approaches
Car Reen Kok (1), Robert Hutkins (1)
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In prebiotic studies, responders and non-responders are often observed. These phenotypes are thought to occur due to the highly individualized nature of the microbiome, emphasizing the need for a personalized approach towards prebiotic supplementation. However, it remains a challenge to identify, *a priori*, personalized responses to specific prebiotics. Based on ecological theory, we hypothesize that the fecal microbiome of prebiotic responders share carbohydrate degradative systems required to compete for, degrade, and utilize a prebiotic, producing measurable responses such as Short Chain Fatty Acids (SCFA). Accordingly, we developed an *in vitro* phenotyping method across 3 different prebiotics; xylooligosaccharides (XOS), fructooligosaccharides (FOS) and inulin using acetate and butyrate as our major response markers. Through differential analyses of metagenomic sequences, we discovered prebiotic-specific biomarkers in the form of carbohydrate gene clusters and incorporated a random forest analysis to select for top ranking features. From this, we identified 24 XOS, 12 FOS and 8 inulin prebiotic-associated genes and have designed primers to target and quantify these genes in baseline microbiomes. We also confirmed the expression of these genes in the presence of the prebiotic substrate. Subsequently, we built support vector machine models for each prebiotic that can accurately (AUC > 0.9) discriminate between responders and non-responders using a defined set of genetic features. Moreover, we verified the prevalence of our biomarkers across over 7000 global metagenomes. In conclusion, using a rational approach, we successfully characterized prebiotic-specific biomarkers and implemented them in models that are able to predict response status by genotyping baseline microbiomes.

Conference Day 2

Tuesday, October 12
8:00 – 4:00 pm US Central Time

P3-8: Quinoa, pulses, and other grains nutritional improvement from Soil to Society
Franck Carbonero (1), Robert S Brueggeman (1), Kimberly G Campbell (1), Jessica Fanzo (2), Michael Frazier (3), Girish M Ganjyal (1), Deidre Griffin-LaHue (1), Stephen S Jones (1), Gabriel T LaHue (1), Vicki A McCracken (1), Rebecca McGee (1), Pablo Monsivais (1), Clark B Neely (1), Martine M Perrigue (1), Katalin Szlavecz (2), Patrick M Solverson (1), Juming Tang (1), Andrew L Thorne-Lyman (2), Anna Warner (3) and Kevin M Murphy (1)
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Quinoa and under-utilized grain crops such as pulses and pseudo/ancient cereals are already demonstrated to be part of a healthy diet. However, the limited agronomy and plant breeding efforts have so far been focused on environmental and stress adaptation. Our preliminary data has shown that their nutritional profile is highly variable and has the potential to be optimized. In this context, we are aiming at combining traditional agronomy-oriented crop breeding programs with human nutritional characterization through a Soil to Society pipeline. Starting with the soil component, the potential for innovative and sustainable agricultural practices will be tested both in terms of yield and grain nutrient profile. Traditional and marker-assisted plant breeding will be performed in concert to further optimize these two major outcomes. Resulting lines/varieties will be tested for their nutritional potential through in vitro gut microbiome and host biomarkers assays, culminating with human dietary interventions. In parallel, innovative food products will be developed based on grains physiochemical properties with the aim of obtaining flavorful, attractive, and accessible food products. All these objectives will be guided by epidemiological studies covering a range of crucial population nationwide and internationally. Our transdisciplinary team will work as a cohesive unit to achieve these ambitious yet attainable goals, which directly address the USDA Science Blueprint's long-term goal of Food and Nutrition Translation, with the long-term aim that our research will catalyze changes that "affect food quality, bioavailable nutrients, and access to food, impacting health, community prosperity, and overall quality of life."

Conference Day 3

Wednesday, October 13
8:00 – 3:30 pm US Central Time

Poster Session IV 8:00 – 9:00

P4-1: Genetic analysis of microbiome active traits in Sorghum identifies tannin biosynthesis loci
Qinnan Yang (1), Mallory J. Van Haute (1), Nate Korth (1), Scott Sattler (1,2), John Toy (1,2), James C. Schnable (1), Devin J. Rose (1), and Andrew K. Benson (1)
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Genetic analyses in food crops holds promise for identifying components that affect human gut microbiome-activity but is complicated by cost and technical difficulty of using human feeding studies to phenotype effects on the microbiome. Here we describe a new, automated *in vitro* microbiome screening (AiMS) strategy for phenotyping human gut microbiome and use AiMS-based phenotypes for genetic analysis of human gut Microbiome-Active Traits (MATs) in the BTx623 X IS3620C Recombinant Inbred Line (RIL) population of *Sorghum bicolor*. Quantitative Trait Locus (QTL) mapping of AiMS-based abundances of individual microbial genera across 298 of the BTx623 X IS3620C RILs identified significant QTLs for 13 different microbiome genera that are dispersed across loci on seven of the ten S. bicolor chromosomes. Overlapping QTL peaks for MATs, seed color and tannin contents were detected on Chr2 and Chr4 and are proximal to the *Tan2* and *Tan1* loci known to control tannin synthesis. Segregation analysis of seed color, tannin contents, and microbiome phenotypes matches the pattern of duplicate recessive epistasis that is expected by inheritance from the BTx623 (*Tan2/Tan1-b*) and IS3620C (*Tan1/Tan2-c*) parents. Effects of tannin on microbiome were confirmed using Near-Isogenic Lines (NILs) differing in tannin production. Our study illuminates a new way to exploit existing genetic resource populations of crop plants for discovery of novel traits that can influence human health. Furthermore, our results extend the known pleiotropic effects of tannin production in sorghum from agronomically-important traits to effects on the gut microbiome and potential human health traits.

Poster Session IV 8:00 – 9:00

P4-2: Determining the Requirement of the Gut Microbiota in Developing Diet-induced Obesity in Female Mice
Jing Shao (a), Yibo Xian (a), Ashley Mulcahy Toney (b), Kristin Beede (a), Robert Schmaltz (a), Alison Ermisch (c), Jennifer Wood (c), and Amanda E. Ramer-Tait (a)
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Interactions between high-fat, high-sugar Western diet (WD) consumption and the gut microbiota promote the development of obesity in male C57BL/6 (B6) but not C3H/HeN (C3H) mice. However, experiments investigating the effects of a WD diet on female germ-free (GF) mice versus conventionalized (CVZ) mice harboring a gut microbiota are lacking. This gap in knowledge may be due in part to previous studies reporting that female mice experience only modest changes in weight and percent body fat in response to a WD compared to males. In this study, we tested whether a WD could induce obesity and metabolic disease in female GF and CVZ B6 and C3H mice compared to a low-fat (LF) diet and evaluated microbiome changes in CVZ animals. Compared to a LF diet, feeding a WD increased weight gain, white adipose tissue accretion, and blood glucose levels in both GF and CVZ female C3H mice. However, B6 females gained weight on a WD when CVZ but not while GF. Although WD feeding induced global alterations to gut microbial community structure in both CVZ B6 and C3H females, the changes were more notable in B6 versus C3H mice. Altogether, our results demonstrate a requirement for the gut microbiota for weight gain in female B6 but not C3H mice, which is consistent with previous studies using male mice. These findings provide new insights into the role of the microbiota in female animal models of diet-induced obesity and may help further development of microbiota-based treatment strategies for obesity based on gender.

P4-3: Do Lactic Acid Bacteria in Fermented Foods Persist in the Gastrointestinal Tract: an *in vitro* Investigation
Chloe M. Christensen (1), Car Reen Kok (1), Robert W. Hutkins (1)
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It is well established that the gastrointestinal (GI) microbiota plays a major role in human health. Research in our lab focuses on understanding how specific dietary compounds can modulate microbial GI communities and ultimately improve human health. One such approach is through consuming diets rich in fermented foods, especially those that contain live microbes. However, persistence of these exogenous or allochthonous microorganisms within the GI tract is limited by colonization resistance and other host factors, resulting in their transient presence. In this research, we examined whether fermented food-derived microbes, using *Lactobacillaceae* as a proxy group of organisms, can persist within a simulated *in vitro* GI environment, and if this persistence could be altered by the addition of prebiotics. Daily stepwise fecal fermentations were performed with three live microbe fermented foods – kefir, sausage, and sauerkraut. Real Time qPCR, with *Lactobacillus* group specific primers was used to quantify lactobacilli, and 16S rRNA sequencing was used to assess other microbial changes. We demonstrated that lactobacilli derived from these foods were unable to persist in the *in vitro* environments, reaching undetectable levels within 48 hours of the stepwise fermentations. Although prebiotics enhanced persistence of lactobacilli under some conditions, 16S rRNA gene sequencing showed the main effect of the prebiotics was to increase bifidobacteria abundance. Collectively, these results support previous *in vivo* studies that demonstrate the transient nature of fermentation-derived bacteria in the GI tract.

P4-4: Impacts of dietary copper on macronutrient metabolism predicted by modeling
Naeun Lee (1), Joseph Brake (2), Heejeong Kim (2), Jaekwon Lee (2) and Hyun-Seob Song (1,3)
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Minerals, including copper (Cu), are essential nutrients for mammals and most, if not all, species in the gut microbiome. Conversely, minerals are toxic by excess accumulation or by catalyzing uncontrolled chemical reactions. Therefore, appropriate amounts of minerals in food are vital for staying healthy. In particular, Cu deficiency or excess causes the onset or the progression of human diseases, such as metabolic-associated liver disease, cardiovascular disease, and cancer. The liver plays a key role in Cu homeostasis by storing and excreting excess Cu; in turn, the functions of the liver are dependent on Cu-requiring enzymes. Therefore, it is critical to understand the Cu dysregulation-associated metabolic consequences and the underlying mechanisms. To predict Cu deficiency-induced metabolic changes in hepatocytes, we performed flux balance analyses of a genome-scale human cell network under the two conditions with vs. without constraints on a few key reactions that are known to be directly affected by Cu limitation. As major findings, our simulation showed that Cu deficiency alters fatty acid metabolism and increases mitochondrial net activity. Predicted flux distributions are being validated by comparison with transcriptomic data collected from the control and Cu-limited liver of mice where the CTR1 gene encoding a Cu importer is deficient. The extension of the current study to integrate gut microbiome models is an important next step. The outcomes are expected to lead to a more holistic understanding of the impacts of Cu on the nutrient metabolism and therapeutic intervention of metabolic diseases.

P4-5: Breast milk and its role in the neonatal gut resistome
Silvia Saturio (1), Marta Suárez (2), Laura Mantecón (2), Nuria Fernández (2), Gonzalo Solís (2), Silvia Arboleya (1), Miguel Gueimonde (1)
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The gut microbiota is a reservoir of antibiotic resistance genes (ARGs) known as the intestinal resistome which is established from the beginning of life. However, this process, affected by many factors, remains mostly unknown. The aim of this work was to analyse the burden of ARGs in the microbiota of neonates according to the type of feeding (breast milk or mixed feeding). A cohort of neonates recruited at the Hospital Universitario Central de Asturias (HUCA), consisting of 47 born at term (≥37 weeks of gestation) by vaginal delivery, was studied. 32 were exclusively breast-fed and 15 were mixed fed. Fecal samples were collected at 2, 10, 30 and 90 days of life. After DNA extraction, the concentration of the most common ARGs at the neonatal resistome was determined by qPCR: *bla*SHV, *bla*TEM, *mec*A, *tet*O, *tet*M, *cml*A1 and *aac(6')-Ie-aph(2")*. We observed that infants exclusively breastfed generally carried lower levels of ARGs. Out of the seven genes studied, four showed significant differences according to the type of feeding at different time points. Our results show that the type of feeding during the first months of life has an important effect on the levels of ARGs in the infant gut microbiota. Breastmilk may be playing a protective role. However, further studies are needed to assess the impact of perinatal factors on the intestinal resistome. The ultimate goal is the development of nutritional intervention strategies for the reduction of this ARGs burden.

P4-6: Behavioral changes in common marmosets after antibiotic administration
Shivdeep S. Hayer (1), Mackenzie Conrin (1), William Gasper (1), Shayda Azadmanesh (1), Missy Briardy (1), Skyler Gebers (1), Aliyah Jabenis (1), Jeffrey French (1,2), Jonathan B. Clayton (1,2)
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Depression is a complex multifactorial disease and affects 4% of the global human population. Recent epidemiological studies indicate that antibiotic treatment predisposes humans to the development of neuropsychiatric disorders. It has been postulated that gut microbiota-brain communication plays a vital role in the etiology of antibiotic-mediated depression. We tested whether antibiotic administration leads to development of depressive-like behaviors in common marmosets (*Callithrix jacchus*). Antibiotics (enrofloxacin, neomycin, vancomycin) were administered orally to 8 adult marmoset monkeys for 28 days. A control group of 8 marmosets were administered vehicle. Based on fecal 16S rRNA gene analysis, there was a statistically significant decrease in Shannon diversity ($p<0.05$) in the gut microbiome of antibiotic-treated group as compared to control group. Relative abundances of *Fusobacterium* spp. increased and *Bifidobacterium* spp. decreased significantly ($p<0.05$) in antibiotic-treated marmosets as compared to vehicle-treated marmosets. Daily behavioral observations revealed a statistically significant ($p<0.05$) increase in interaction and proximity (time spent near cage mate) during last 2 weeks and after end of antibiotic treatment, respectively. Increase in social interactions between marmosets might be the result of mate-seeking behavior which characteristically acts as a social buffer to minimize stress in these monkeys. Considering the drastic changes in gut microbiome and observed altered behaviors in antibiotic-treated marmosets, it can be hypothesized that there might be causal relation between gut microbiome altered by antibiotics and depression-related behaviors in marmosets. We are currently investigating changes in gut and plasma metabolites and circulating inflammatory cytokines to further strengthen the aforementioned hypothesis.

Accepted Abstracts Not Presented

RG-I modulates gut microbiota and stimulates innate immune responses in humans

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Dietary polysaccharides are known to influence host-gut microbiota symbiosis and some pectic polysaccharides from traditional medicinal extracts, like ginseng, show beneficial immunostimulatory effects against respiratory (viral) infections. We aimed to identify active polysaccharide constituents from affordable and renewable crops (bell pepper and carrot), evaluate the *in vitro* gut microbiota- and immune-modulating effects of an enriched extract, and assess innate immune and gut microbiota responses in healthy adults. We used activity-guided fractionation to characterize the nutricophore, colonic batch incubations to determine gut bacterial metabolic activity and community composition, *in vitro* immune function assays to evaluate cytokine secretion profiles and phagocytic activity, and a randomized, double-blind, placebo-controlled study to assess microbiota and immune responsiveness. Rhamnogalacturonan-I (RG-I) was identified as the nutricophore responsible for the immunostimulatory and gut microbiota modulatory effects, with both *in vitro* and human intervention study data supporting the biological effects. Extracts enriched with RG-I displayed a dual mode of action by exerting 1) an immunomodulatory effect on phagocytosis, and 2) a gut microbiota modulating effect, with concomitant enhanced production of short chain fatty acids. In healthy humans, the RG-I-enriched extract was well tolerated, and it modulated gut microbiota and stimulated a dose dependent innate immune response. RG-I-enriched extracts from bell pepper and carrot showed similar gut microbiota and immune modulatory activities. Both appear to be efficacious solutions, aimed to support protective innate immune responses and increase resistance to (respiratory) infections, that are safe, sustainable, affordable and can easily be integrated into food products or dietary supplements.

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Feeding with sustainable sourdough bread has the potential to promote healthy microbiota metabolism at the colon level

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The contribution of sustainable food processing to healthy intestinal microbial functions is of current research interest. The sourdough fermentation fits with the most sustainable bread making. We manufactured baker`s yeast (BYB) and sourdough (t-SB30) breads, which first underwent an in-depth characterization. According to nutritional questionnaires, we selected 40 volunteers adhering to the Mediterranean diet. Data on their fecal microbiota and metabolome allowed the selection of two highly representative fecal donors to separately run the Twin Mucosal-SHIME under two-week feeding with BYB and t-SB30. Bread feeding did not affect the phylum and family composition of both donors, in all Twin M-SHIME colon tracts, and lumen and mucosal compartments. The genus core microbiota showed only few significant fluctuations, which regarded the relative abundances of *Lactobacillus* and *Leuconostoc* according to feeding with BYB and t-SB30, respectively. Compared to BYB, the content of all SCFA and isovaleric and 2-methylbutyric acids significantly increased through feeding with t-SB30. This was evident for all Twin M-SHIME colon tracts and both donors. The same was true for the content of Asp, Thr, Glu, GABA and Orn. The bread characterization made it possible to identify the main features responsible for these rseults. Compared to BYB, t-SB30 had much higher contents of resistant starch, peptides and free amino acids, and inhomogeneous microstructure. We used the most efficient approach to investigate a staple food component, excluding interferences from other dietary factors and attenuating human physiology overlaps. The daily consumption of sourdough bread may promote the healthy microbiota metabolism at colon level.

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In vitro impact of agavins on the gut microbiota and the levels of antibiotic resistance genes

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Agavins (agave fructans) have been proposed as new prebiotics. Some beneficial effects of these compounds have recently been shown in the obesity context. However, scientific knowledge of its impact on the microbiota is still limited. Furthermore, there is no information about their effect on gut antibiotic resistance genes (ARGs) levels. Therefore, this work aimed to evaluate the in vitro effect of agavins on gut microbiota composition and ARGs reduction. *In vitro* fecal cultures were performed from normal-weight and obese adults for 48 h at 37 oC under anaerobic conditions. Each fecal sample was added with agavins, inulin, or glucose at a final concentration of 0.3% (v/v). A bottle with no carbon source added was used as a control. Also, pH and gas production in fecal cultures were monitored. The production of short-chain fatty acids was analyzed by gas chromatography and the microbial composition of the samples by 16S rRNA gene sequencing. The absolute levels of the ARGs blaTEM, blaSHV, *cml*A1, *mec*A, *tet*O, *tet*M, and *aac (6 ") - leaph (2")* was quantified by qPCR. The results showed a modulating effect of agavins on the composition and activity of the human gut microbiota, inducing a decrement in pH and an increment of gas production throughout the fermentation. In addition, changes in the relative abundances of different microbial groups and a reduction in the levels of some ARGs were observed. These results suggest that agavins have a modulating effect on the human gut microbiota and reducing ARGs.