Global Engage’s

Microbiome R&D and Business Collaboration Forum: USA

Collocated with the Probiotics Congress: USA

Poster Presentation Abstracts

San Diego, USA
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² Department of Veterinary Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark  
³ Instituto de Investigación Biomédica de Málaga (IBIMA)  
⁴ Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición, CIBERobn  
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²Department of Medicine, University of Otago, Christchurch, New Zealand  
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²THE NINTH HOSPITAL SHANGHAI SECOND MEDICAL UNIVERSITY, Shanghai, China  
³BAO GANG HOSPITAL OF SHANGHAI SECOND MEDICAL UNIVERSITY, Shanghai, China  
⁴SHANGHAI HUADONG HOSPITAL, Shanghai, China  
⁵LONGHUA HOSPITAL OF SHANGHAI UNIVERSITY, OF T.C.M, Shanghai, China  
⁶RAILWAY HOSPITAL OF TONGJI MEDICAL COLLEGE, Shanghai  
⁷Shangdong Longlive Bio-Technology Co., Ltd, Shanghai, China  
⁸AIDP, Inc., CA, USA. |
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<td>Dept. Biol. Env. Sc. College of Arts and Sc. Qatar University</td>
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**Poster Title**

Use of quantitative PCR (qPCR) and flow cytometry to enumerate Viable but Not Culturable (VBNC) bacterial cells in Direct-Fed Microbial products

**Abstract**

In response to environmental stressors, many bacteria are known to enter a state of low metabolic activity known as the Viable but Nonculturable (VBNC) state. Although these bacteria are alive and capable of resuming metabolic activity under more favorable conditions, they are incapable of forming colonies on agar plates. Many regulatory agencies rely upon aerobic plate counts or similar plating assays to evaluate the microbial activity of probiotic products and ensure the accuracy of label claims. However, not only do these assays have a well-known propensity for the underestimation of microbial titer, but they are not sufficient to enumerate VBNC bacteria. In the present study, we monitored the stability of several direct-fed microbial (DFM) products using both benchtop and molecular methodologies. Benchtop assays included plating on selective and nonselective agars as well as Most Probably Number (MPN) assays, all of which work under the assumption that live cells are capable of division. Molecular assays included quantitative Polymerase Chain Reaction (qPCR) with taxon-specific primers and probes, as well as flow cytometry using dyes which indicate membrane permeability. Results indicate that in many instances, cells which are not detectable using benchtop assays such as plating and MPN are detectable using molecular methods. Furthermore, the results of field trials demonstrate a continuity of product efficacy (improvements to ADG, FCR and biomass accumulation) despite a loss of activity in plating assays. This suggests that the presence of colony forming units (CFUs) may not be an absolute indication of product efficacy, as CFU-based assays are unable to detect VBNC cells. It may be advantageous for regulatory agencies to consider molecular approaches when evaluating the viability of bacterial cells in probiotic formulations, to ensure that effective products of the appropriate titer are not erroneously denied entry into the market.
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<td><strong>Abstract</strong></td>
<td>Recent advances in sequencing technologies and metagenomics have revealed statistical associations between the abundance of taxonomic groups (or their genetic repertoire) and a number of pathologies. Such descriptive, profiling investigations offer important insights into taxonomic and functional variations relevant to host health and disease; yet, a mechanistic and comprehensive understanding of those observed results remains elusive. If available, a comprehensive map of molecular interactions between microbial species could be used to integrate the vast collection of previous findings into a global network context. To this end, we present a global interspecies metabolic interaction network of the human gut microbiota. The information upon which the network architecture stands is primarily from literature-based annotations of ~570 microbial species and 3 human cell types metabolically interacting through &gt;4,400 small-molecule transport and macromolecule degradation events. To demonstrate the utility of our network, we developed a mathematical framework for analyzing gut microbial communities in a given population, such as a cohort of type 2 diabetes (T2D) patients. In a patient population with a specific set of socio-demographic characteristics, the microbial entities abundant or scarce in T2D, and the metabolic influence connections surrounding each microbial entity, were shown in a community-scale, metabolic influence network. The influence network suggests the presence of microbial entities that impose a relatively high degree of metabolic influence to other entities. Our network presents a foundation towards integrative investigations of community-scale microbial activities within the human gut.</td>
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**Keywords:** Microbiome, Ecological Systems Biology, Bioinformatics, Multi-cellular Systems
### Contributing Author(s)
Inga L. Bazukyan

### Organisation
Yerevan State University

### Email Address
bazukyan@ysu.am

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<th>The effect of different types of irradiation on the lactic acid bacteria isolated from Armenian dairy products</th>
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| Abstract     | Ionizing radiation affects the chemical components of cell and changes their properties. Currently, the ionizing radiation is widely used in medicine, industry, agriculture and other areas. Clinical studies have shown that 100 Grey X-ray irradiation leads to the development of the radiation syndrome, which is followed by disbalance in normobiota. Lactic acid bacteria (LAB) are the main component of the human microbiota and play an important role in digestion, immunity and resistance to pathogenic bacteria.  
   The aim of this study is the investigation of the radioresistance of *Lactobacillus rhamnosus* R-2002 (KY054594) and the effect of radiation on the antibacterial activity and antibiotic resistance of strain. UV irradiation was emitted by quartz lamp 50-60 Hertz during 45 min, the dose of γ- irradiation was 30-35 μSv/h and the dose of X-rays was 2-1000 Sv. The antibacterial activity of LAB was investigated by well diffusion method against 8 different Gram positive and negative bacteria. The antibiotic resistance to 22 different antibiotics was carried out by disc-diffusion method.  
   It is the first time when the radio sensitivity of LAB isolated from Armenian dairy products was investigated. The most resistant strain was found to be *L. rhamnosus* R-2002. The exposition by both UV and γ- radiation did not affect the growth. The survival rate of LAB after irradiation by 1000 Sv X-ray was 10⁸ CFU from 10⁹. The UV and γ- radiations have not influenced the antibacterial activity of *L. rhamnosus*, while 500 Sv of X-ray led to 50% inhibition of antibacterial activity. The antibiotic resistance of LAB was not affected by any type of radiation.  
   Thus, because of the high radioresistance *L. rhamnosus* R-2002 could be interesting as a probiotic for the mitigation of the gastrointestinal disorder associated with radiation syndrome.  
   The work was supported by grant ANSEF biotech 4431. |
### Poster Title

**Inferring Microbial Interactions in the Gut of the Hong Kong Whipping Frog (*Polypedates megacephalus*) and a Validation Using Probiotics**

### Abstract

The concerted activity of intestinal microbes is crucial to the health and development of their host organisms. Investigation of microbial interactions in the gut should deepen our understanding of how these micro-ecosystems function. Due to advances in Next Generation Sequencing (NGS) technologies, various bioinformatic strategies have been proposed to investigate these microbial interactions. However, due to the complexity of the intestinal microbial community and difficulties in monitoring their interactions, at present there is a gap between the theory and biological application. In order to construct and validate microbial relationships, we first induce a community shift from simple to complex by manipulating artificial hibernation (AH) in the treefrog *Polypedates megacephalus*. To monitor community growth and microbial interactions, we further performed a time-course screen using a 16S rRNA amplicon approach and a Lotka-Volterra model. Lotka-Volterra models, also known as predator–prey equations, predict the dynamics of microbial communities and how communities are structured and sustained. An interaction network of gut microbiota at the genus level in the treefrog was constructed using Metagenomic Microbial Interaction Simulator (MetaMIS) package. The interaction network obtained had 1,568 commensal, 1,737 amensal, 3,777 mutual, and 3,232 competitive relationships, e.g., *Lactococcus garvieae* has a commensal relationship with *Corynebacterium variabile*. To validate the interacting relationships, the gut microbe composition was analyzed after probiotic trials using single strain (*L. garvieae*, *C. variabile*, and *Bacillus coagulans*, respectively) and a combination of *L. garvieae*, *C. variabile*, and *B. coagulans*, because of the cooperative relationship among their respective genera identified in the interaction network. By taking advantage of microbial community shift from simple to complex, we thus constructed a reliable microbial interaction network, and validated it using probiotic strains as a test system.
Contributing Author(s)  Heidi L. Rowles
Organisation   University of Cincinnati Clermont College
Email Address  rowles.heidi@gmail.com

Poster Title  Probiotics: Growth patterns of Lactobacilli and their effects on antibiotics, pathogenic bacteria, and other Lactobacilli strains

Abstract  Statement of the Problem: Incidences of bacterial infections continue to increase and alternative treatments need to be developed. People are seeking more natural and holistic medical care which includes probiotics. The microbiome has become a focus of interest in medicine and study of the gut bacteria is needed to determine the health impact of probiotics. Exploring the interaction of antibiotics and probiotics is an important area of research as antibiotics cause wide-spread devastation throughout the microbiome. Developing a probiotic that can combat the effect of an antibiotic on the microbiome is essential to eliminating the decimation caused by antibiotic therapy. Studying the growth of individual probiotic strains and combinations thereof is an important foundation in probiotic research and necessary in developing effective probiotic supplements. Methodology & Theoretical Orientation: Standard bacterial growth curves were established for several commercial probiotics and compared to pathogenic bacteria. Probiotics and pathogenic bacteria were combined to establish growth curves for these amalgamations. Growth curves were established for individual strains of Lactobacilli and some initial combinations of these strains. Ampicillin and penicillin susceptibility discs were used to treat plates of varying concentrations of probiotic bacteria to determine the effect by measuring the zones of inhibition. Findings: Ampicillin and penicillin are less effective against a combination of Lactobacillus and Bifidobacterium than on the individual genera. A probiotic containing Lactobacillus fermentum exhibited a growth rate similar to the pathogenic bacteria. Conclusion & Significance: The interactions of indigenous gut and probiotic bacteria are very complex and research into these interactions is needed to develop a deeper understanding of the role the microbiome plays in overall health. Research of probiotic bacteria is needed to develop effective probiotics. Emphasis should be placed on the study of Lactobacillus fermentum in the microbiome based on the results of this research.
Abstract

**Background:** More extensive use of metagenomic shotgun sequencing in microbiome research relies on the development of high-throughput, cost-effective sequencing. Here we present a comprehensive evaluation of the performance of the new high-throughput sequencing platform BGISEQ-500 for metagenomic shotgun sequencing and compare its performance with that of the Illumina platform.

**Findings:** We evaluated intra-platform variations for metagenomics sequencing on BGISEQ-500 and cross-platform variation against Illumina HiSeq 2000. A dataset from 20 healthy individuals was generated, including 8 library replicates and 8 sequencing replicates on BGISEQ-500, and 20 pairwise cross-platform replicates on BGISEQ-500 and Illumina HiSeq 2000.

By a newly developed overall accuracy quality control method, an average of 82.45 million high quality reads (96.06% of raw reads) per sample with 90.56% of bases scoring Q30 and above was obtained using BGISEQ-500. After discarding human reads, 77.77% of the remaining reads could be mapped to the integrated gene catalog. Quantitative analyses revealed extremely high reproducibility between BGISEQ-500 intra-platform replicates. Cross-platform replicates showed a slightly greater difference than intra-platform replicates but still a high consistency was observed. In total, the abundance of 11,350 (3.25%) genes showed significant differences between platforms, with a bias towards genes with higher GC content being enriched on the HiSeq 2000 platform.

**Conclusion:** Our study provides the first set of performance metrics for human gut metagenomic sequencing data using BGISEQ-500. The high accuracy and technical reproducibility confirm the applicability of the new platform for metagenomic studies, though caution is still warranted when combining metagenomic data from different platforms.
BioCyc.org is an extensive web portal for microbial genomics and metabolic pathways. BioCyc contains 11,000 microbial genomes including 929 organisms from the Human Microbiome Project. Two BioCyc databases are noteworthy as bacterial references: the EcoCyc database for Escherichia coli has been curated from 33,000 publications, and the BsubCyc database for Bacillus subtilis has been curated from 4,000 publications. In addition, the MetaCyc database is a multi-organism metabolic pathway database describing 2,400 experimentally elucidated pathways. BioCyc contains an extensive set of bioinformatics tools. Genome-related tools include a genome browser, sequence searching and alignment, and extraction of sequence regions. Pathway-related tools include a tool for navigating zoomable organism-specific metabolic map diagrams that can be painted with metabolomics and gene-expression data, and a new Omics Dashboard for interactive exploration of omics datasets.

The new multi-organism metabolic route search tool enables the user to explore biochemical conversions among metabolites that are accomplished by multiple microbiome organisms. To specify a route search problem the user provides a starting and an ending metabolite of interest, and a set of organisms. The organism set could span just a handful of named BioCyc organisms, or it could include hundreds of organisms, such as from the pre-defined gut microbiome set within BioCyc. The route-search tool then computes a set of optimal routes connecting the starting and ending compounds. Each route is a linear sequence of reactions that converts the starting compound to the ending compound. An optimal route minimizes the number of reactions used while maximizing the number of atoms from the starting compound that are incorporated into the ending compound. The set of reactions from which the routes are computed is the union of all reactions in the metabolic networks of the selected organisms -- all reactions in those organisms are assumed to be accessible, independent of transport considerations.
Contributing Author(s) | Trevor Kirby¹, Sara L. Colpitts², Eli Kasper², Abigail Keever¹, Caleb Liljenberg¹, Krisztian Magori¹, Lloyd H. Kasper² and Javier Ochoa-Repáraz¹

Organisation | ¹Department of Biology, Eastern Washington University, Cheney, WA 99004, USA ²Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth College, Hanover, NH 03755 USA.

Email Address | jochoareparaz@ewu.edu

Poster Title | Exploring the bidirectional link between central nervous system inflammatory demyelinating disease and the intestinal microbiota

Abstract | The gut microbiome plays a critical role in the regulation of central nervous system (CNS) demyelination in experimental models. Gut microbes influence the response of regulatory immune cell populations and disease progression in acute and chronic models of experimental autoimmune encephalomyelitis (EAE). Recent studies suggest that there is a bidirectional communication between the host and the microbiota. We hypothesized that the gut microbiota differs between the acute inflammatory and chronic progressive stages in a bi-phasic murine model of multiple sclerosis (MS) induced in non-obese diabetic (NOD) mice. Approximately 70% of NOD mice develop a severe form of EAE while the remaining develop no or mild symptoms of disease. We compared the gut microbiota of NOD mice with either mild or severe disease states to healthy control mice. Moreover, we evaluated whether or not the outcome of progressive stages of EAE in the NOD model was modified by the administration of a broad-spectrum antibiotic cocktail. We observed significant changes in the microbiota of NOD mice that developed a severe secondary form of EAE when compared with healthy control mice. Our results indicated reduced mortality and clinical disease severity in mice treated with antibiotics compared to untreated mice. To assess the functionality of the differing microbiomes between disease severity states, we generated putative annotations on bacterial operational taxonomic units (OTUs) using PICRUSt. To determine whether different autoimmune diseases affect the microbiome similarly we extended the comparisons to severe EAE versus diabetic mice, and evaluated the effects of transplantations stool samples obtained from EAE, diabetes and control mice on diabetes progression. Our findings support the hypothesis that there are reciprocal effects between experimental autoimmune diseases and modification of the microbiome; early therapeutic intervention that target the gut microbiome could potentially limit disease progression.
**Contributing Author(s)**
Nesreen ALJahdali¹, Pascale Gadonna-Widehem ², Pauline M Anton PhD² and Franck Carbonero PhD¹,³*

**Organisation**
¹Cell and Molecular Biology Program; and ³Department of Food Science; University of Arkansas, Fayetteville, AR, USA 
²Expression des Gènes et régulation Epigénétique par l’Aliment UP 2012.10.101.; Institut Polytechnique UniLaSalle, Beauvais, France

**Email Address**

nhaljahd@uark.edu; fgcarbon@uark.edu; pascale.gadonna@unilasalle.fr; pauline.anton@unilasalle.fr

**Poster Title**
Determination of the Effects of Different Maillard Reaction Products on the Murine Gut Microbiota

**Abstract**

**Background:** The Maillard Reaction (MR) is a non-enzymatic chemical reaction which results in the linkage between the amino group of amino acids and the carbonyl group of reduced sugars. This reaction generates Maillard reaction products (MRPs) are not naturally in foods and are responsible for a range of colors, odors, flavors, and palatability. Conflicting reports of MRPs impacts on human health are probably due to the fact that bioconversion of these digestible molecules by the gut microbiota has been marginally taken into account. Here, we assessed the impact of MRPs on the murine gut microbiota through two studies.

**Methods:** Study 1 focused on the impact of model bread crust melanoidins (and model bread crumbs) on healthy and experimentally-induced inflammation mice. Study 2 focused on the impact of consumption of increased concentration of melanoidin-rich malts on short and long-time.

**Results:** Melanoidin-rich bread crust had a significant impact on the gut microbiota, with significant decrease of Enterobacteriaceae and increase of Ruminococcaceae, Oscillibacter and Lachnospiraceae, while surprisingly bread crumbs stimulated Lactobacillus. Experimental colitis appeared to buffer the intensity of gut microbiota responses. High amounts of melanoidins rich malt rapidly and persistently led to significantly different gut microbiota profiles. There was a trend for decrease of Lactobacillus and Ruminococcus and increase of Akkermansia and Bifidobacterium with higher amounts of dietary melanoidins.

**Conclusion:** Overall, our findings suggest that melanoids appeared to have a prebiotic-like impact, with inhibition of potentially pathogenic Proteobacteria, and stimulation of fermentative Firmicutes for bread crust, or probiotic genera for the malts. Melanoids structure are extremely variable from one food to another, therefore additional studies will be needed to better assess their potential beneficial properties on gut health.
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<td>Abstract</td>
<td>The number of microbes within the human body outnumbers our own cells by an order of magnitude with gene content that outnumbers ours by at least 100:1. Most of these microbes reside within the GI tract and their role in health and disease has been the subject of many studies. The impact of the microbiome on the immune system has also been documented. Oh et al, (2014) demonstrated that microbiome activation of TLR5 enhanced the immune response to a trivalent inactivated influenza vaccine and to polio vaccines. These studies highlight the underappreciated role of the microbiome on the immune response to a vaccine and potential impact on efficacy. Probiotics, defined as ingested bacteria with beneficial health effects, have been found to influence the microbiome composition and the immune response in the gut and the genitourinary system. Probiotics, even as transient inhabitants of the gut, act to shift the resident microbiome and can help exclude harmful bacteria by enriching those commensal residents. While it is clear that the microbiome greatly influences the state of mucosal health, how it influences, and is influenced by, the mucosal immune response to vaccines is poorly understood. We have developed an orally-delivered, mucosal vaccine platform that employs <em>Lactobacillus acidophilus</em>. This platform offers several important advantages as it is inexpensive to produce, does not require cold-chain, and is needleless. We have inserted a linear epitope from the membrane proximal external region of the HIV-1 envelop protein into the highly expressed bacterial surface layer protein. Using this vaccine-probiotic (vaccine vector) goal of this study was to evaluate microbiome-immune system-vaccine-vaccine vector interactions. Our results show a profound shift in the intestinal microbiome affected by the presence of the vaccine and the vaccine vector and that is potentially driven by the mucosal immune response.</td>
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### Contributing Author(s)

Joshua Wollam¹, Yong-Jiang Xu¹, Andrew M. F. Johnson¹, Jachelle M. Ofrecio¹, Wei Ying¹, Mathew Riopel¹, Dalila El Ouarrat¹, Luisa S. Chan², Andrew W. Han², Nadir A. Mahmood², Caitlin N. Ryan², Yun Sok Lee¹, Jeramie D. Watrous¹, Mahendra D. Chordia³, Dongfeng Pan³, Mohit Jain¹, Jerrold M. Olefsky¹

### Organisation

¹University of California, San Diego; ²Second Genome, Inc.; ³University of Virginia

### Email Address

jwollam@ucsd.edu

<table>
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<tr>
<th>Poster Title</th>
<th>The microbiota-produced N-formyl peptide fMLF promotes obesity-induced glucose intolerance</th>
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<tr>
<td>Abstract</td>
<td>The commensal microbiota of the gastrointestinal (GI) tract serve as a key link between diet and metabolism, producing numerous metabolites that influence host physiology. The composition of GI microbiota and associated metabolites changes dramatically with diet and the development of obesity. Although many correlations and associations have been described, specific mechanistic links between these changes and dysregulated glucose homeostasis remain to be defined. Here we show that blood and intestinal levels of the microbiota-produced N-formyl peptide, formyl-methionyl-leucyl-phenylalanine (fMLF), are elevated in high fat diet (HFD)-induced obese mice. Genetic or pharmacological inhibition of the high affinity N-formyl peptide receptor Fpr1 leads to increased insulin levels and improved glucose tolerance, dependent upon the enteroendocrine-produced hormone glucagon-like peptide-1 (GLP-1). Compared to obese wild-type mice, obese Fpr1 knockout mice display an altered microbiome composition, suggesting feedback mechanisms exist between microbiota and Fpr1 signaling. Overall, we describe a specific new mechanism by which gut microbiota interact with the host via the fMLF / Fpr1 system to modulate glucose metabolism, providing a potential new approach for therapeutic treatment of metabolic disease.</td>
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Ovarian cancer (OC) is the most lethal gynecological malignancy and the fifth leading cause of cancer death in the United States. Metformin, an anti-diabetic drug, has been shown to decrease the risk of mortality in cancer patients, particularly those with OC. Though the drug acts through inhibition of the PI3K/mTOR/AKT pathway, which is commonly altered in OC, knockout of key proteins in the pathway does not attenuate metformin's anti-diabetic effects. Further, intravenous metformin administration is less effective than oral, implying that some crucial interaction takes place in the gut. The gut is home to some $10^{13}$ bacterial cells, collectively known as the microbiota. These bacteria play important roles in fermentation of fiber, producing short-chain fatty acids (SCFA) that have positive health benefits, including prevention of metabolic syndrome, bowel disorders, and certain cancers. Mechanistically, SCFAs inhibit the PI3K/mTOR/AKT pathway, similar to metformin. Conditions such as obesity, metabolic syndrome and diabetes significantly alter gut microbiota composition, possibly leading to a reduction in beneficial SCFAs. However, in type II diabetes, treatment with metformin leads to an increase in SCFA-producing bacteria, suggesting that metformin may positively influence the microbiota to improve patient metabolic profiles. In OC, metformin may play a similar role in promoting the presence of SCFA-producing bacteria. We hypothesized that metformin increases the abundance of SCFA-producing bacteria in the gut, with which it acts synergistically to decrease growth of OC cells in combination with OC chemotherapy. To investigate our hypothesis, we employed the OC patient-derived xenograft (PDX) mouse model established at Mayo Clinic. Immunocompromised SCID mice received OC cells from Mayo Clinic patients and the tumors were allowed to grow to an appropriate size before treatment was started. Cage mates were split into one of four treatment arms: a control (no treatment) group, metformin only, carboplatin/paclitaxel (CP) chemotherapy only, or CP with metformin. Mice received 2mg/ml metformin in their water continuously and/or 51mg/kg carboplatin and 15mg/kg paclitaxel intravenously weekly. Stool was collected weekly prior to tumor injection, during tumor growth, and through the four weeks of treatment. DNA was extracted from stool and bacterial 16S rRNA amplicon sequencing was used to determine microbiota profiles. Mice with chemosensitive tumors treated with only CP chemotherapy had significant reductions in tumor growth along with significantly less microbial gut diversity than pre-tumor and pre-treatment mice. Chemosensitive mice treated with metformin alone did not lose significant microbial diversity but did not have significant reductions in tumor growth as compared to the control mice. CP in combination with metformin both reduced tumor growth and protected against loss of microbiota diversity. Further, mice treated with only metformin had significant increases in abundance in the Firmicutes genera Ruminococcus and Oscillospira, SCFA-producers that are associated with leanness and health. Mice treated with both metformin and CP showed inhibition of tumor growth with metformin in combination with CP, implying that metformin-associated changes in the gut microbiota may increase sensitivity to CP. These results suggest that metformin protects against detrimental loss of gut microbiota diversity induced by chemotherapy treatment, which may preserve beneficial SCFA producers. This work implies a role for the gut microbiota in OC treatment and provides support for metformin’s potential use as a beneficial addition to chemotherapy treatment.
Contributing Author(s) | George W. Agak, Stephanie Kao, Kelsey Ouyang, Min Qin, Ahsan Butt, David Moon, & Jenny Kim
---|---
Organisation | David Geffen School of Medicine, UCLA
Email Address | gagak@mednet.ucla.edu

**Poster Title**
Antimicrobial activity of Th17 cells induced by *Propionibacterium acnes* strains associated with healthy and acne skin

**Abstract**
Studies of human skin microbiome suggest that *Propionibacterium acnes* strains may contribute differently to skin health and disease. However, the immune phenotype and functions of Th17 cells induced by healthy (*P*<sub>H</sub>) vs. acne (*P*<sub>A</sub>) skin-associated *P. acnes* strains are currently unknown. We observed that *P*<sub>A</sub> strains induced higher IL-17 levels than *P*<sub>H</sub> strains in PBMCs from healthy human donors. We next generated *P*<sub>H</sub> and *P*<sub>A</sub>-specific Th17 clones, which we categorize as protective (prTh17) or pathogenic (paTh17). We show that *P*<sub>H</sub> strains induced prTh17's in contrast to *P*<sub>A</sub> strains, which induced the paTh17 phenotype. Furthermore, supernatants derived from activated prTh17, but not paTh17, demonstrate antimicrobial activity against *P. acnes*. Electron microscopic studies revealed that supernatants derived from activated prTh17 clones exhibited robust bactericidal activity against *P. acnes*, and complete breaches in the bacterial cell envelope were observed. This antimicrobial activity was independent of IL-26, as both natural IL-26 released by Th17 clones and rhIL-26 lacked antimicrobial potency against *P. acnes*. Overall, our data suggest that *P. acnes* strains may differentially modulate the CD4<sup>+</sup> T cell responses, leading to the generation of a heterogeneous population of Th17 cells that may contribute to either homeostasis or pathogenesis in acne vulgaris.
INTRODUCTION: Despite recent advances in the diagnosis of periprosthetic joint infection (PJI), identifying the infecting organism continues to be a challenge, with up to a third of PJIs reported to have negative cultures [1]. Current molecular techniques have thus far been unable to replace culture as the gold standard for isolation of the infecting pathogen. Next-generation sequencing (NGS) is a well-established technique for comprehensively sequencing the entire pathogen DNA in a given sample and has recently gained much attention in many fields of medicine [2,3]. Our aim was to evaluate the ability of NGS in identifying the causative organism(s) in patients with PJI.

METHODS: After obtaining Institutional Review Board approval and informed consent for all study participants, samples were prospectively collected from 148 revision total joint arthroplasty procedures (83 knees, 65 hips). Synovial fluid, deep tissue and swabs were obtained at the time of surgery and shipped to the laboratory for NGS analysis (MicroGenDx). Deep tissue specimens were also sent to the institutional laboratory (Rothman Institute) for culture. PJI was diagnosed using the Musculoskeletal Infection Society (MSIS) definition of PJI [4]. Statistical analysis was performed using SPSS software.

RESULTS: 55 revisions were considered infected; culture was positive in 40 of these (40/55, 72.7%), while NGS was positive in 47 (47/55, 85.5%). Among the positive cultures, complete concordance between NGS and culture was observed in 33 cases (33/40, 82.5%). One case was partially discordant between NGS and culture, with culture detecting three organisms as opposed to one organism on NGS. One case showed complete discordance with NGS and culture detecting different organisms. Three patients with negative NGS results had positive cultures. In another two cases culture simply reported the gross morphology of the organism but the phenotype was not identified, while NGS was able to definitively identify an organism. Among the 15 cases of culture-negative PJI, NGS was able to identify an organism in 10 cases (10/15, 66.7%).

93 revisions were considered to be aseptic; NGS exclusively identified microbes in 15 of 93 “aseptic” revisions (16.1%) and culture exclusively isolated an organism in 4 of 93 cases (5.3%). One case was positive on both NGS and culture, however the results were discordant from each other. The remaining cases (73/93, 78.5%) were both NGS and culture negative. NGS detected several organisms in most positive samples, with a greater number of organisms detected in aseptic compared to septic cases (6.8 vs. 4.0, respectively).

DISCUSSION: NGS was able to detect a pathogen in two-thirds of culture-negative cases and demonstrated a high rate of concordance with culture in culture-positive cases. The rate of false positives was low compared to earlier studies using molecular techniques. Our findings also suggest that some cases of PJI may be polymicrobial and escape detection using conventional culture. NGS may be a useful tool for identifying the causative organism in PJI, particularly in the setting of negative cultures. Further study is required to determine the significance of isolated organisms in samples from patients who are not thought to be infected.

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<th>Poster Title</th>
<th>e[automateddatascientist] - A Questions-based System for Scientific Data Discovery and Exploration</th>
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| Abstract          | The productivity of research groups within the life sciences, personal care, and nutrition/food industries today is increasingly dependent on their ability to process and extract value from a range of diverse and complex datasets (internal and external). Scientific datasets are managed for their primary use only, as industry lacks the technologies to efficiently promote data re-use and cross-functional integration. Furthermore, traditional tools for storing and accessing information have significant limitations in enabling scientists to understand and exploit information in the required manner, e.g. due to information silos and a lack of essential metadata. Current information management technologies require a vast amount of time and dedicated resources on analyzing data to find, access, understand, curate, and integrate the required input data assets. e[automateddatascientist] employs probabilistic machine-learned models to address these problems, providing harmonization, curation, evaluation and exploitation of federated datasets by scientists in pursuance of their research goals. The system is questions-based, allowing the researcher to rapidly discover and explore relevant data from internal and external data sources, saving significant time and money, while promoting innovation/discovery and collaboration. Example applications of e[automateddatascientist] include:  
- Comparison of different samples i.e. different body sites, different treatments resulting in assessment of product effectiveness and better product claims.  
- Relating phenotype (e.g. disease) with microbiome composition, resulting in the discovery of new targets.  
- Integration of microbiome data with host data measurements (e.g. RNAseq), resulting in new biological insights.  
- Identification of properties of microbiome that promote product discovery e.g. identification of anti-oxidant properties of bacteria to help anti-aging.  
- Biomarker discovery and identification, assisting in patient stratification and the discovery of new therapies.  
- Identification of drugs that have potential therapeutic effects for target diseases. |
Contributing Author(s) | Randi Lundberg¹,², Isabel Moreno-Indias³,⁴, Lukasz Krych⁵, Philip E. Dubé¹, Stine Broeng Metzdorff², Witold Kot⁶, Dennis Sandris Nielsen⁵, Camilla Hartmann Friis Hansen², Axel Kornerup Hansen²

Organisation | ¹ Taconic Biosciences  
² Department of Veterinary Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark  
³ Instituto de Investigación Biomédica de Málaga (IBIMA)  
⁴ Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición, CIBERobn, Spain  
⁵ Food Microbiology, Faculty of Science, University of Copenhagen, Denmark  
⁶ Department of Environmental Science, Faculty of Science and Technology, Aarhus University, Denmark

Email Address | randi.lundberg@taconic.com  
philip.dube@taconic.com

Poster Title | Diet Alters Fecal Microbiota Transplantation Efficiency in Germ-Free Mice

Abstract | INTRODUCTION: Fecal microbiota transplantation (FMT) in germ-free mice is a common approach in microbiome research and drug testing. However, FMT with complex, human microbiotas in mice does not fully capture the microbial community of the donor, nor does it stimulate the murine immune system in the same way as a murine microbiota. It was hypothesized that diet affects the efficiency of a human microbiota to establish in recipient germ-free mice and subsequent immune responses. Therefore, it was tested whether custom diets with altered fat content or fat/protein sources (soybean oil vs. milk fat or soy protein vs. casein), compared to a standard rodent chow, would improve human microbiota establishment and immune system characteristics. METHODS: Germ Free C57BL/6NTac mice were colonized with human or mouse microbiota and fed different test diets (n=8-10): (1) animal-sourced (with casein and 4.3% milk fat), (2) human profile (grain-based with 10.5% soybean oil), or (3) control rodent chow. Fecal microbiota were characterized by 16S rRNA amplicon sequencing of the V3 region. Lymphocyte and dendritic cell populations were measured in mesenteric lymph nodes and Peyer’s patches by flow cytometry. Gene markers for lymphocytes, dendritic cells, and pro- and anti-inflammatory cytokines were measured in ileum and colon by qPCR. RESULTS: It was found that the animal sourced diet increased the colonization efficiency of mouse microbiota-colonized mice significantly and differentially altered dendritic cell populations. In contrast, the human profile diet improved the colonization efficiency of human microbiota-colonized mice, although only slightly. The immunological phenotype of the human microbiota-colonized mice was characterized by an increase in regulatory T cells in mesenteric lymph nodes and inflammatory cytokines in colon, and this effect was greater in recipient mice on the animal-sourced diet. CONCLUSIONS: Altering the diet of mice transplanted with complex human fecal microbiotas is a promising approach for optimizing FMT efficiency in mice and for modulating subsequent immune system function. Further studies to optimize fat content and diet constituents are warranted in order to successfully model the human microbiota in mice and its effects on the immune system.
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<th>Contributing Author(s)</th>
<th>Emily A. Stout, Rosemary Sanozky-Dawes, Yong Jun Goh, Alexandra B. Crawley, Todd R. Klaenhammer, and Rodolphe Barrangou*</th>
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<td>Organisation</td>
<td>North Carolina State University- Food Science Dept</td>
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<tr>
<td>Email Address</td>
<td><a href="mailto:rsdawes@ncsu.edu">rsdawes@ncsu.edu</a></td>
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<th>Poster Title</th>
<th>Deletion-based escape of Type II CRISPR-Cas Targeting in <em>Lactobacillus gasseri</em></th>
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<tr>
<td>Abstract</td>
<td>CRISPR arrays, together with the CRISPR-associated proteins (Cas) form adaptive immune systems in bacteria and archaea that protect against invasive mobile genetic elements (MGE). Surviving MGEs often escape cleavage via CRISPR-Cas due to mutations that occur in sequences involved in the CRISPR interference process. In this study, a plasmid interference model was used to screen for CRISPR escapees in <em>Lactobacillus gasseri</em>. Plasmids containing a protospacer, a validated protospacer adjacent motif (PAM), and an antibiotic resistance gene were transformed into strains JV-V03 and NCK1342 where they were targeted by the native CRISPR-Cas system. Mutants able to escape plasmid cleavage via CRISPR targeting were recovered using selective media. As CRISPR escape is an elusive phenomenon, the mechanism of survival in these escapees was then investigated to better understand this biological process. No mutations were observed in the endonuclease domains of Cas9 or in the protospacer-PAM region of the plasmid, but mutations in the CRISPR array were the dominant means of escape in both strains, appearing in every independent experiment. Analysis of sequence mutations revealed polarized excisions from the leader end that always included the targeting spacer. Apparently, some cells adapt to evade CRISPR targeting, while maintaining both the protospacer-PAM target sequence and the functionality of the CRISPR-Cas system (i.e. ability to take up potentially beneficial plasmids). Further experiments performed on NCK 1342 escapees that had lost spacers revealed a number of them possessed phenotypes (and some genotypes extrinsic to CRISPR loci) distinct from that of the wild type strain, demonstrating that mutations and deletions can be detected throughout the CRISPR arrays and beyond.</td>
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**Contributing Author(s)**
Y. Jun Goh, Todd R. Klaenhammer, Rodolphe Barrangou

**Organisation**
North Carolina State University

**Email Address**
yjgoh@ncsu.edu

**Poster Title**
*In vivo* transcriptome of probiotic *Lactobacillus acidophilus* NCFM during murine gut colonization.

**Abstract**
*Lactobacillus acidophilus* is the most ubiquitously formulated probiotic culture in the dairy and dietary supplement industries. Over the past decade, our genetic and *in vitro* functional studies have begun to elucidate the mechanisms through which *L. acidophilus* exerts its probiotic attributes. To establish key genotypes contributing to its gut fitness and host interaction *in vivo*, we employed RNA-seq transcriptome analysis to identify genes implicated in adaptation to the gut environment using germ-free murine model. The highest number of differentially expressed genes were observed in NCFM population residing in the cecum (49% of the ORFeome), followed by ileum and colon. Significant induction was observed for genes encoding carbohydrate metabolism (e.g. prebiotic sugar transporters, glycogen metabolism), amino acid and nucleotide metabolism, cell division, DNA repair, stress/bile defence, mucus-binding/cell surface adhesins, and numerous unknown proteins. A set of 139 *in vivo* core genes were consistently over-expressed throughout gut transit. These core genes are predominantly involved in carbohydrate metabolism, host adhesion/immunomodulation and stress signalling pathways, indicating their important roles in nutrient acquisition and gut colonization. The core genes appeared to concentrate at specific chromosomal regions, suggesting the presence of gut-adaptive genomic islands that likely confer competitive advantage and reflect the specialized adaptation of *L. acidophilus* to the mammalian gut. Analysis of host expression in response to NCFM colonization revealed significant down-regulation of IL-12b and tight junction proteins OCLN and TJJP1 solely in the small intestine. The lower turnover rates of tight junction proteins is likely attributed by a more stabilized intestinal barrier integrity promoted by the presence of NCFM. The down-regulation of IL-12b signalled an anti-inflammatory response conferred by interaction of NCFM with the host epithelium. These results further indicate the small intestinal region as a primary probiotic effector site where *L. acidophilus* modulates the host immune and intestinal barrier functions.

(297 words)
Contributing Author(s)  
Paul Blatchford 1, Halina Stoklosinski 1, Sarah Eady 1, Alison Wallace 1, Christine Butts 1, Jennifer Gu 4, Richard Gearry 2, Glenn Gibson 3, Juliet Ansell 1.

Organisation  
1 The New Zealand Institute for Plant & Food Research Limited, New Zealand  
2 Department of Medicine, University of Otago, Christchurch, New Zealand  
3 Department of Food and Nutritional Sciences, University of Reading, United Kingdom  
4 AIDP, Inc. City of Industry, CA 91748

Email Address  
jennifer@aidp.com

Poster Title  
Consumption of kiwifruit capsules increase *Faecalibacterium prausnitzii* abundance in functionally constipated individuals: a randomised controlled human trial

Abstract  
This study investigated the impact of ACTAZIN™ green (2400 mg and 600 mg) and Livaux™ (2400 mg) gold kiwifruit supplements on faecal microbial composition and metabolites in healthy and functionally constipated (FC) participants. The participants were recruited into the healthy group (n = 20; one of whom did not complete the study) and the FC group (n = 9), each of whom consumed all the treatments and a placebo (isomalt) for 4 weeks in a randomised cross-over design interspersed with 2 week washout periods. Modification of faecal microbiota composition was determined by 16S rRNA gene sequencing, organic acid concentrations were measured by gas chromatography and colonic pH was calculated using SmartPill® wireless motility capsules. In the functionally constipated group, *Faecalibacterium prausnitzii* abundance significantly increased (P = 0.024) from 3.4% to 7.0% following Livaux™ supplementation. Lower proportions of *F. prausnitzii* are often associated with gastrointestinal disorders; especially those with an inflammatory pathology. The discovery that Livaux™ supplementation increased *F. prausnitzii* abundance offers a potential strategy for improving gut microbiota composition, as *F. prausnitzii* is a butyrate producer and has also been shown to exert anti-inflammatory effects in many studies.
Application of Xylooligosaccharides (XOS) in Improving Intestinal Dysfunction in Chinese Constipated IBS Patients

This study was designed to test the effect of XOS in 100 patients with IBS. 106 patients with IBS were recruited from 6 hospitals in Shanghai, China. Six cases had to follow-up, 100 cases complete the study including 56 males and 44 females, Aged between 17 and 61 years old, average 38+2.1 yrs. Clinical symptoms were graded as Mild, moderate and severe degree and scored 1, 2, 3 respectively, according to the following criteria:

Mild (1): bowel movement every 1-3 days, the quality of faeces was dry at first and soft at the end, and each bowel movement lasted 10-20 minutes
Moderate(2): 1 bowel movement every 4 days, the quality of faeces was dry, and each bowel movement lasted 21-30 minutes
Severe(3): 1 bowel movement every 5 or more than 5 days, the faeces was dry, ball-like or with blood, and each bowel movement lasted more than 30 minutes;

Xylooligosaccharides capsules were given at 1.4g daily to IBS patients with constipation for 2 weeks. Results: Total effective rate of constipation in IBS-c group was 88%. The effective rate is higher in mild and moderate IBS-c groups as compared to the sever IBS-c group. No side effects were found during the administration. So, XOS is an ideal prebiotic fiber for constipation relief in IBS patients.
**Contributing Author(s)**  
Hunt von Herbing, I.

**Organisation**  
University of North Texas

**Email Address**  
vonherbing@unt.edu

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<tr>
<th>Poster Title</th>
<th>Healing Our Inner Oceans with Probiotics: How knowledge of the origins of our gut microbiome can improve human and animal health under chronic stress.</th>
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| Abstract     | Human and animal health is reliant on natural gut microbial populations. Until recently we knew little about the origins of the gut microbiome. Recent results from a three-year research cruise, which sampled plankton throughout our oceans revealed: >73% of the diversity of the ocean’s microbes is shared with the human gut microbiome. In the human as well as the fish gut, microbes maintain intestinal cell tight-junction integrity, produce vitamins and fight pathogens, among other functions. But “chronic stress” can change gut microflora, resulting in homeostatic imbalance, disease susceptibility, and even death. In agriculture and more recently in aquaculture, antibiotics used to treat fish disease brought on by stress from overcrowding and handling, disrupted gut microflora and development, resulting in less resilient adult livestock. In our studies on fish early life history stages, we tested a broad-spectrum antibiotic (e.g. ampicillin) and several strains of probiotics (live microorganisms beneficial to host health) as alternative biological control agents to antibiotics. Results showed that antibiotics retarded zebrafish (*Danio rerio*) intestinal development (e.g. serotonin and enteric innervation). But a single-strain probiotic (*Lactobacillus rhamnosus* 501 IMC) enhanced growth of juvenile Mozambique Tilapia (*Oreochromis mossambicus*), and a multiple-strain probiotic PrimaLac® (*Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidium, and Enterococcus faecium*) improved condition (immune and endocrine health) in juvenile red drum (*Sciaenops ocellatus*). Further, a genetically modified probiotic (*Lactobacillus reuteri*) served as a vehicle for enzyme delivery to treat the human disease Phenylketonuria (PKU)* in the PAHenu2 homozygous (PKU) mouse. Thus, despite the physicochemical differences among these ecosystems (ocean, fish & mammal) microbes in the form of probiotics may improve gut microbial diversity and confer adaptive advantages for better health.  
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<tr>
<th>Contributing Author(s)</th>
<th>E. Bruning, Y. Chen, K. A. McCue, J R. Rubino, J. E. Wilkinson, A.D.G. Brown</th>
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**Poster Title**  
28-Day Clinical Study Assessing the Impact of Age and Product Use on the Vulvar Microbiome

**Abstract**  
Although there is an increase in feminine topical vulvar wash products on the market, a lack of clinical studies exist in the medical literature assessing their impact on vulvar skin tolerance and its resident microbial communities. In a similar vein, there is an abundance of information on the vaginal microbiota, whereas the vulvar microbiome composition and how it may be impacted by age differences is not well documented. A feminine gel wash with antimicrobial activity containing 2% lactic acid intended for external use was tested over a 4-week period to assess its tolerance and impact on vulvar skin pH, and the vulvar skin microbiome at Days 0, 14 and 28. Following a 1 week wash out period, 36 healthy female subjects were enrolled in 3 balanced age groups of 18-29, 30-44, and 45-55 and instructed to use the topical wash to cleanse their external genital area at least once per day for 28 days. Tolerance criteria were assessed by a gynaecologist and effects of the vulvar skin microbiota were evaluated via bacterial 16S rRNA and fungal ITS microbial diversity analysis. Results showed that the gel wash was well tolerated with no signs of increased dryness, redness, oedema, itching, stinging or burning. There was no detectable statistically significant (p<0.05) impact on the natural skin pH as well as the bacterial and fungal microbiome species richness or diversity of the vulvar skin (alpha diversity analysis). In contrast, an impact was observed in the microbiome communities between the age groups of women across time points overall but not at the baseline time point (beta diversity analysis). Topical washes can play an important role in intimate health and women are encouraged to carefully select clinically tested products for tolerance that provide targeted antimicrobial benefits without disrupting the natural pH and healthy balance of commensal microbiota.
The genus *Lactobacillus* encompasses a diversity of species that occur widely in nature and encode a plethora of metabolic pathways reflecting their adaptation to various ecological niches, including human, animals, plants, and food products. Accordingly, their functional attributes have been exploited industrially and several strains are commonly formulated as probiotics or starter cultures in the food industry. Although divergent evolutionary processes have yielded the acquisition and evolution of specialized functionalities, all *Lactobacillus* species share a small set of core metabolic properties, including the glycolysis pathway. Thus, the sequences of glycolytic enzymes afford a means to establish phylogenetic groups with the potential to discern species that are too closely related from a 16S rRNA standpoint. Here, we identified and extracted glycolysis enzyme sequences from 52 representative species and carried out individual and concatenated phylogenetic analyses. We show that a glycolysis-based phylogenetic tree can robustly segregate lactobacilli into distinct clusters and discern very closely related species. We also compare and contrast evolutionary patterns with genome-wide features and transcriptomic patterns, reflecting genomic drift trends. Overall, results suggest that glycolytic enzymes provide valuable phylogenetic insights and may constitute practical targets for evolutionary studies.
Abstract

Microbiome changes were characterized in response to dietary interventions that were devised to determine interacting effects of protein source and saturated fat level on blood lipoproteins and risk for cardiovascular disease. One hundred and ten healthy normolipidemic men and women aged 21-65, with BMI of 20-35 were randomized, after a 2 wk baseline diet, to high (15% E) or low (7% E) saturated fat groups and randomly received diets (4 wks) in which the major protein source (25% E) was red meat (beef, pork), white meat (chicken, turkey), and non-meat sources (nuts, beans, soy). Taxonomic characterization using 16S ribosomal RNA was performed on fecal samples collected at each diet completion. The overall effect of these diets did not significantly alter the microbiome community in PCoA ordination space with unweighted UniFrac distance, and there were minimal differentially abundant OTUs between the protein sources. However, there were 175 significantly different OTUs between high and low saturated fat. Interpersonal differences were greater than dietary influence, and most samples clustered by patient. The significant factors influencing community composition were age, triglycerides, body fat (%), systolic blood pressure, height, diet order, sex, and alpha diversity. In addition, alpha diversity was positively correlated with age and HDL cholesterol and negatively correlated with triglycerides and systolic blood pressure. Samples were clustered into two groups (ie. enterotypes) using dirichlet multinomial mixtures, and these groups happened to differ in alpha diversity. When samples were separated into low and high alpha diversity groups, the high diversity group had more significant differences in the change of clinical traits from the baseline. These results suggest that inter-individual differences like sex and age outweighed the influence of these specific dietary changes on the microbiome, but that microbiome diversity mediated the response of clinical traits to the diets.
### Poster Title

The Gut Microbiome: An Advanced Understanding of Microbial Health Across Countries and Cultures

### Abstract

**Background:** Recent research has shown that the microbiome of the human gut plays a significant role in an individual's health. Gut microflora composition can be correlated with health or disease, such as Crohn's disease and colon polyps. The gut microbiome is known to vary among individuals and across geo-locations. Therefore, measuring the extent of such variation is critical in determining the baseline microbiome.

**Methods:**

CosmosID has developed an innovative analysis platform to detect, identify, and characterize microorganisms in a metagenomic sample, using its ultrafast bioinformatics tool and curated databases containing more than 65,000 genomes. Over 500 whole genome shotgun (WGS) metagenomic sequenced samples of human stool of healthy and diseased subjects were downloaded from public databases, including the Human Microbiome Project (HMP). Samples from geographic locations, including Spain, Denmark, Tanzania, India, Japan, and the United States, comprised the dataset. The samples were analyzed using CosmosID's automated cloud-based metagenomics software to investigate correlation of geographical location with the microbiome and to analyze differences between healthy and diseased microflora.

**Results:** Diverse gut microflora, including bacteria, viruses, fungi, and protists, were identified in samples collected from different geographic regions. Samples from the same geographic locations, such as Denmark and Spain, showed differences in microbiome composition, but more limited compared to samples from Tanzania. Additionally, the gut microbiome of healthy individuals from all locations revealed the presence of pathogens with genomic potential for causing disease. These results will be used to assess differences in gut microbial composition of individuals from different geographic locations and to characterize baseline compositions for future analyses.

**Conclusion:** Subspecies and strain level data describing the microbiome of healthy and diseased individuals provide valuable in-depth information for the clinician, including identifying the community resistome and virulence.
### Poster Title
Flow cytometry as a superior method for the enumeration of specific microbial product formulations

### Abstract
Probiotics are typically enumerated using a culture-based colony formation unit (CFU) assay. The assay is highly variable and cannot measure viable but not culturable (VBNC) bacterial cells. VBNCs can occur due to the stress of the manufacturing process on bacteria. These cells typically cannot grow in traditional CFU assay medium. However, many of these cells can become active upon reaching the more optimal environment of the lower gut. By not measuring these cells, only a small portion of the product is enumerated.

Flow cytometry is a laser-based method to detect individual cells. It has the ability to measure multiple parameters on individual cells at the same time. This method can enumerate live, injured, and dead bacteria based on DNA-binding or membrane potential fluorescent dyes irrespective of the ability to culture them. Additionally, flow cytometry may differentiate populations of cells based on antibody markers. Due to limited differences in strain protein profiles, developing antibodies specific to strains has been difficult. Here we show the use of a highly specific antibody to the strain L. Rhamnosus GG. Such antibodies may be used to enumerate specific strains within a mixed population.
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<th>Poster Title</th>
<th>Effects of <em>Bacillus coagulans</em> V5-1 Probiotic Gummy on Functional Bloating &amp; Occasional Diarrhea: A Randomized, Double-Blind, Placebo-Controlled, Parallel Study</th>
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| Abstract     | Up to 45 million Americans suffer from irritable bowel syndrome (IBS); most are women. IBS is characterized by bloating, abdominal pain and discomfort, gas, nausea, and incontinence, often reducing quality of life. Bloating is reported in approximately 76% of those with IBS.\(^1\)\(^2\) IBS etiology is poorly understood and interventions are often ineffective and costly, with undesirable side effects.\(^3\) Probiotic supplementation has been investigated as a new approach to the management of IBS. However due to the paucity of methodologically sound and rigorous studies to date, there is limited application of probiotics in the management of functional bowel issues.  

*Bacillus coagulans* is one of the most studied probiotics among spore-forming bacteria. It forms a durable and protective endospore coating that renders it exceedingly stable and enables it to survive transit through the stomach into the intestines, and withstand extreme heat and moisture, thereby increasing the appeal for various manufacturing processes. Inside the colon, *B. coagulans* germinates and colonizes, crowding out more harmful bacteria as it competes for the finite number of available nutrients. This process positively supports digestive and immune health.\(^4\)\(^5\) The V5-1 strain of *Bacillus coagulans* used in this study was specifically chosen due to previously published research and its superior stability. |
A probiotic product was developed to improve broiler chicken health, increase productivity, and outcompete antibiotic resistant bacterial pathogens. This study was designed to determine if this probiotic product, containing four bacterial strains (*Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactobacillus plantarum*, and *Bacillus subtilis*) could colonize the gut compartments of broiler chickens, would be found in the feces, and would improve animal growth. Ninety six broiler chickens were equally divided into experimental and control pens, and experimental birds were given the probiotics in their drinking water at 10⁴ CFU/ml for 22 days. Feces were collected daily, and 6 birds from each group were sacrificed for gut sampling on day 22. Lumen and mucosa samples were collected from each bird’s duodenum, jejunum, ileum, and ceca. The probiotic bacteria was detected by PCR using primers designed to be strain-specific. *B. subtilis* was detected in feces within one hour of probiotic administration and beyond, predominantly in experimental birds. This strain was only rarely detected in gut samples suggesting quick passage through the intestines. *P. pentosaceus* was observed predominantly in experimental gut lumen samples, but rarely in mucosa samples suggesting a lack of colonization. The *P. acidilactici* and *L. plantarum* primer sets were found to be non-specific; they amplified products from chicken feed, and from fecal and gut DNA in both control and experimental birds. Consistent detection of target in the cecal mucosa suggested that *P. acidilactici* and *L. plantarum* colonize this compartment, but it was not possible to distinguish between endogenous and probiotic colonizers. Although the four bacterial strains were detected in the gut and feces, probiotic supplementation did not result in significant differences in body weight, rate of weight gain, feed intake, or feed conversion ratio. However, birds growing in a more crowded, stressful environment may have exhibited more probiotic-related effects.
### Abstract

Qatari strains of *B. thuringiensis* strains were particularly selected for the forms of their parasporal crystals. The morphology of the crystals produced by the strains during sporulation was investigated using SEM microscopy showing Spherical-shaped crystals produced by Bti-like and non Bti-like strains and cuboidal and bipyramidal crystals produced by Btk strains. QBT strains show different large plasmids and protein patterns. QBT strains harbours genes *cry4B, cry1I, cyt1a, cry2, vip3A* and *cry1Ia* depending on their crystal forms. Many gene sequences show polymorphism compared to reference strains and others have cytotoxicity against cancer cells.