Probiotics, Prebiotics, and the Host Microbiome:

The Science of Translation

Poster Session

Wednesday, June 12, 2013

6:00PM – 7:30PM

SCIENTIFIC ORGANIZING COMMITTEE

Francisco Guarner, MD, PhD
University Hospital Vall d'Hebron, Spain

Patricia L. Hibberd, MD, PhD
Harvard Medical School / Massachusetts General Hospital for Children

Colin Hill, MSc, PhD, DSc, MRIA
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Mary Ellen Sanders, PhD
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Mandana Arabi, MD, PhD
The Sackler Institute for Nutrition Science
at the New York Academy of Sciences

Brooke Grindlinger, PhD
The New York Academy of Sciences

EARLY CAREER INVESTIGATOR POSTER PRIZE JUDGES

In addition to members of the scientific organizing committee, the following experts have kindly agreed to participate in the selection of awardees for the “Early Career Investigator Poster Prize”.

Samuli Rautava, MD, PhD
The Hospital for Sick Children, Toronto, Canada

Mark A Underwood, MD, MAS
University of California, Davis

Bruno Pot, PhD
Institute Pasteur de Lille, France

Jens Walter, PhD
University of Nebraska

Susan Lynch, PhD
University of California, San Francisco

POSTER PRESENTERS

1. Aakko, Juhani
2. Ambalam, Padma
3. Amoako-Tuffour, Yaw
4. Amorim, Manuela
5. Ardeshir, Amir
6. Azizpour, Khalil
7. Benezra, Amber
8. Bin Dayel, Iman
9. Bindels, Laure
10. Bisanz, Jordan
11. Blatchford, Paul
12. Cani, Patrice
13. Cardelle Cobas, Alejandra
14. Chaluvadi, Saikiran
15. Clarke, Siobhan
16. Coates, Jessica
17. Demarest, Chase
18. Doron, Shira
19. Eid, Noura
20. Eloe-Fadrosh, Emiley
21. Ettinger, Grace
22. Fricke, W. Florian
23. Gao, Chunxu
24. Hamilton, Mary Kristina
25. Hemarajata, Peera
26. Hintze, Korry
27. Jones, Mitchell L.
28. Li, Jingru
29. Livanos, Alexandra
30. Love, Bryan
31. Macklaim, Jean M. (Presenter: Enos, M)
32. Madureira, Ana Raquel
33. Mahana, Douglas
34. Manichanh, Chaysavanh
35. Mappley, Luke J.
36. Marsh, Alan
37. McMillan, Amy
38. Mogna, Luca
39. Nagpal, Ravinder
40. Nyangale, Edna P.
41. O'Shea, Eileen F.
42. Panwar, Harsh
43. Patro, Jennifer Nicole
44. Pot, Bruno
45. Quach, Darin
46. Rahat Rozenbloom, Sari
47. Renye Jr., John A.
48. Ringel-Kulka, Tamar
49. Rodes, Laetitia
50. Saha, Shyamali
51. Sela, David A.
52. Shane, Andi L.
53. Sheridan, Paul O.
54. Skuse, Peter
55. Solano-Aguilar, Gloria
56. Spinler, Jennifer K.
57. Stone, Samantha
58. Tobin, Jacinta M.
59. Tomaro-Duchesneau, Catherine
60. Tompsett, Tamara
61. Toward, Ruth
62. Urbaniak, Camilla
63. Valladares, Ricardo
64. van Hemert, Saskia
65. Veiga, Patrick
66. Vyas, Usha
67. Wang, Linda T.
68. Wirawan, Ruth

1.

EVALUATION OF STRAIN-SPECIFIC PRIMERS FOR IDENTIFICATION *OF LACTOBACILLUS RHAMNOSUS* GG

Akihito Endo, PhD, Juhani Aakko, BSc, Seppo Salminen, PhD
Functional Foods Forum, University of Turku, Turku, Finland

Lactobacillus rhamnosus strain GG (ATCC 53103) is one of the most widely studied and commercialized probiotic strains, and thus strain-specific identification for the strain is highly valuable. In this study, two published PCR-based identification methods for strain GG, a transposase gene-targeting system and a phage-related gene-targeting system, were evaluated. The former produced amplicons from eight of the 41 strains tested, and the phage-related system from five of the tested strains, including the strain GG. Fingerprinting analysis indicated that the strains LMG 18025, LMG 18030, and LMG 18038, which had an amplicon by the former system but none by the latter, were genetically distinguishable from *L. rhamnosus* GG at strain level. Strains LMG 23320, LMG 23325, LMG 23534, and LMG 25859 showed profiles very similar to that of the strain GG, suggesting that these strains might be identical to GG or derivative strains of it. The results here indicated that the phage-related gene-targeting system is a good tool for accurate identification *of L. rhamnosus* GG. This system would be able to detect both the original *L. rhamnosus* GG and its derivative strains.

2.

ANTIMICROBIAL ACTIVITY OF MULTI-STRAIN PROBIOTICS CONTAINING *LACTIC ACID BACTERIA* AND *BIFIDOBACTERIA* AGAINST *CLOSTRIDIUM DIFFICILE*

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An overuse of antibiotics may cause intestinal overgrowth of *Clostridium difficile* (CD), extended spectrum of *beta lactamase Escherichia coli* (ESBL), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococci* (VRE) and other multiple resistant bacteria. The virulence of a CD strain is strongly linked to the production of toxin A and B causing nosocomial diarrhea worldwide ranging from a mild to a life threatening disease. The epidemic CD NAP1/027 strain may produce 16 times more toxins A and B and a binary toxin than most hospital outbreak associated strains. Thus, there is an urgent need to develop alternative non-antibiotic based therapies such as probiotic and synbiotic dietary supplements, to stimulate and restore healthy indigenous gut microbes after various antimicrobial therapies. *Lactobacilli* and *bifidobacteria* produce a range of antibacterial compounds such as organic acids (e.g., lactic acid and acetic acid); oxygen catabolites such as hydrogen peroxide; peptides compounds such as bacteriocins. We now report on antimicrobial activity (AMA) of cell free supernatants (CFS) obtained from different combinations of co-cultured LAB and *bifidobacteria* against gut pathogens including *C.difficle*. Different combination of co-cultured *bifidobacteria* and *lactobacilli* in MRSC broth exhibited AMA against hypervirulent strain CD NAP1/027 and virulent strain CD 2167. Extracts obtained from ten co-cultured combinations showed AMA against CD NAP1/027 up to 1:500 dilutions, which is otherwise not observed when cells are grown as monocultures. Interestingly AMA against *C. difficile* showed strong correlation to the reduced on in toxin titers production by hypervirulent strain CD 027. These results are important for developing multi-strain probiotics and synbiotics against gut pathogens.

3.

DEVELOPMENT OF AN AUTOMATED INGESTIBLE GASTROINTESTINAL SAMPLING DEVICE

Yaw Amoako-Tuffour, BSc[1,2], Srikar Vengallatore, PhD[1], Satya Prakash, PhD[1,2]
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Existing methods of studying bacterial populations and probiotic interactions within the gastrointestinal tract are limited. They are often invasive and resource intensive, or are unable to capture high resolution temporal or spatial information. This poster presents the development of an ingestible, electromechanical capsule designed to collect physical samples from the lumen of the human gastrointestinal (GI) tract with the aims of being able to better localize gastrointestinal ailments, study the microbiome and probiotic interactions, and monitor metabolic processes. A candidate device was developed encompassing hardware, electronics, firmware, and a novel sampling mechanism leveraging the cylindrical shape of the device, enabling it to collect three discrete samples during its transit. The device may be programmed to target specific locations along the GI. The capsule was assessed for: its ability to collect samples and maintain their integrity, withstand the environmental conditions and forces associated with normal clinical use, and for its ability to transit safely through the GI tract. The device was demonstrated to be an effective and non-invasive means to study the contents of the GI tract and serve as a tool for further probiotics research and development, personalized medicine, drug delivery, and other GI interventions.

4.

PREBIOTIC PROPERTIES OF BREWER YEAST CELL WALL FRACTIONS

Manuela Amorim, BSc, Joana Pereira, BSc, Francyeli Araújo, BSc, Gullón Beatriz, PhD,
Maria Manuela Pintado, PhD
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Nowadays, it is well established that the colonic microbiota has an important influence on host health. Consequently, there is increasing interest in the development and use of prebiotics as functional food ingredients suitable for improving the composition and/or the metabolic activity of the colonic microbiota. Due to their functional properties, spent brewer´ s yeast has been proposed as an excellent candidate for a new-generation of prebiotics. Spent brewer's yeast is a natural by-product from the brewing industry. Most of this material is used in animal feeding or discarded as waste. In order to up-grade this by-product, isolation of cell wall compounds has been tentatively assessed due to its high nutritional value. These fractions are composed of mannans, beta-glucans, and glyco-proteins, which have interesting physiological and functional properties serving as sources of dietary fiber and prebiotics. Thus, the objective of this research was to evaluate the prebiotic potential of spent brewer's yeast extracts (obtained via autolysis, followed by hydrolysis and ultrafiltration) using two different protocols as carbon sources for supporting the growth of single probiotic strains *Lactobacillus acidophillus* Ki and *Bifidobacterium lactis* Bb12 and testing *in vitro* fermentation by fecal inoculum from healthy humans proving positive effects on colon conditions. The results confirmed the ability of these substrates to increase the *Bifidobacterium* population and to act as carbon sources, leading mainly to the production of acetic, propionic, and butyric acids, demonstrating their potential in the development of new functional ingredients for further application in foods and animal feeding.

5.

FORMULA-FED INFANT RHESUS MACAQUES (*MACACA MULATTA*) EXHIBIT A DISTINCT GUT MICROFLORA COMPOSITION COMPARED WITH BREAST-FED INFANTS

Amir Ardeshir, DVM, MPVM, PhD[1], Marcus Rauch, PhD[2], Nicole Narayan, BSc[1], Susan Lynch, PhD[2], Dennis Hartigan-O'Connor, MD, PhD[1,3]
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[3]Department of Medical Microbiology and Immunology, University of California, Davis, California

To assess differences in the infant rhesus macaque (*Macaca mulatta*) gut microbiome that are caused by breast vs. formula feeding, we used rRNA-targeted oligonucleotide microarrays to test the microbial composition of stool samples from animals housed in our indoor colony. Total DNA was extracted and bacterial 16S rRNA genes were amplified using a degenerate forward primer and non-degenerate reverse primer. The labeled bacterial products were fragmented and biotin labeled prior to hybridization to the PhyloChip™ Array, version G3. Cluster analysis of the bacterial population composition revealed distinct clustering of the nursery-reared infants, which were separated from the mixed breastfed subjects. Permutational multivariate analysis of variance was utilized to test the significant differences among the categorical and continuous variables. Feeding type demonstrates a statistically significant association with the bacterial community composition ($p< 0.003$). The number of relocations of the animals during the study was marginally significant ($p=0.067$). Although the bacterial richness and diversity in the stool samples were not significantly different between the two groups, the gut microbiota in breastfed infant monkeys were characterized by significantly increased relative abundance of multiple species associated with breast feeding, including members of the *Bacteroidetes,* and *Spirochaetes* phyla and *ruminococcus* family in the *Firmicutes* phylum, whereas formula-fed infants were more characterized by a relative expansion of the *Lachnospiraceae* family in *Firmicutes.* Our results suggest that breast and bottle feeding produce distinct microbiota that may influence health and disease in colony rhesus macaques.

6.

PROBIOTIC PREVENTIVE EFFECTS OF *LACTOBACILLUS DELBRUECKII* ON MCF 7 PRECIPITATED BREAST CANCER IN INBRED BALB/C MICE

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Breast cancer is a common invasive cancer that comprises 22.9% of invasive cancers in women and 16% of all female cancers. Probiotics are thought to be beneficial to the host organism. Recently, probiotics were used for improving host intestinal microbial balance, inhibiting pathogens and toxin producing bacteria, prevention and treatment of pathogen-induced diarrhea, urogenital infections, and atopic diseases. Experimental studies showed that some strains of LAB (*Lactobacillus bulgaricus*) have anti-mutagenic effects. The knowledge acquired through this investigation will pave the way to prevent or treat breast cancer by biological drugs in the future and will have an impact on medicine ultimately improving the welfare of people. In this study *Lactobacillus delbrueckii* subsp. *Bulgaricus* strain was used. MCF-7 human breast cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM). Female homozygous inbred BALB/C mice (2-3 weeks old, n=15 in each group) were obtained. Blood samples were collected by cardiac puncture at the end of experiment. Splenocyte proliferation assay (MTT assay) and Delayed Type Hypersensitivity (DTH) assay were used. Serum and supernatant cytokines were measured by ELISA kits. As a result, after 42 days, the final tumor size of mice for the probiotic group (1961.8 ± 145.32 mm3) was significantly reduced compared to the control (3811.56 ± 320.3 mm3) group ($p<0.001$, student t-test). The level IFN-γ and IL-2 were higher than control group but the level of IL-4 was lower than control group. The MTT test was showed that probiotic can enhanced the sensitivity and proliferation of cells.

7.

COMMENSALS: ANTHROPOLOGY AND THE MICROBIOME

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Commensalism literally means "to come to the table together" and has traditionally been used by anthropologists to describe activities that express solidarity and communality among human individuals, especially through practices of eating. For scientists studying the human microbiome, commensal most often refers to the trillions of microbes on and in us, constituting the majority of cells and genes in our bodies. The human gut becomes a space to creatively connect these fields and bring anthropological questions to bear on biological commensal relationships. Food, behavior, and environment are increasingly figuring into the understanding of pathogenesis, providing a unique opportunity to engage the social sciences with biological investigation. The study of metagenomics is contributing to a better scientific understanding of the invisible world of human gut microbes, and could lead to new views of our selves. This invisible world is made manifest through what and how people eat, which is situated within narratives that are both local and international. Food, therefore, provides a familiar and comprehensible way of thinking about the micro-ecology within the human body. Global public health issues such as malnutrition and obesity connect the concerns of anthropology and science. Integrating social science research into translational microbiome studies can bring to light the cultural impact and cultural challenges of public health interventions involving probiotic, prebiotic, and food therapies.

8.

EFFECT OF A NOVEL SYNBIOTIC ON THE INNATE IMMUNE RESPONSE TO INFLUENZA VACCINATION IN YOUNG AND OLDER SUBJECTS

Iman Bin Dayel, MSc[1], Honglin Dong, PhD[1], Agnieszka Przemska, MSc[1], Caroline Childs, PhD[1], Ian Rowland, PhD[1], Kieran Tuohy[2], PhD, Sue Todd, PhD[1], Margot Gosney, DPhil[1], Parveen Yaqoob, DPhil[1]
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Ageing is associated with alterations in innate immunity, gut microbiota, and poor response to vaccination. The current study evaluated the influence of a novel probiotic, *Bifidobacterium longum bv. Infantis* CCUG 52486 (Silvi et al., *Journal of Food Engineering*, 2003) with a potential prebiotic (glucooligosaccharide; GlOS) (Gonzalez-Gonzalez et al., International Dairy Journal, 2011) on innate immunity and response to influenza vaccination in young (18-35y) and older (60-85y) subjects. In a randomized, double-blind, placebo-controlled, parallel study, 54 older subjects and 58 young subjects consumed either a placebo (9g/d maltodextrin) or the synbiotic (108 cfu of *Bifidobacterium longum bv. Infantis* CCUG 52486 and 8 g of GlOS/d) for a total of 8 weeks. A trivalent influenza vaccination (2010/2011 northern hemisphere season containing A/California /7/2009(H1N1), A/Perth/16/2009(H3N2), and B/Brisbane/60/2008) was administered at 4 weeks. Blood samples were collected at baseline, week 4 and after 2 and 4 weeks of vaccination. Monocyte phenotype (CD14,CD16) and expression of activation markers (CD80,CD86) were measured by flow cytometry. Serum vaccine-specific antibody titers were measured by the haemagglutination inhibition assay. Ageing was associated with a shift from classical to non-classical monocytes, but the synbiotic was associated with a shift in favor of the classical subset after one month of supplementation. High expression of CD86 pre-vaccination was associated with seroconversion to the H3N2 and Brisbane influenza strains, but there was no effect of the synbiotic on activation markers. In conclusion, supplementation with GlOS plus *Bifidobacterium longum bv. Infantis* CCUG 52486 altered monocyte phenotype, but not activation following vaccination.

9.

SYNBIOTIC APPROACH LEADS TO PROLONGED SURVIVAL OF CACHECTIC MICE WITH LEUKEMIA

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Research interest in gut microbiota-host crosstalk in pathological conditions has been growing in the past decade. Our recent results suggest that gut microbiota can control cancer cell proliferation and associated cachexia in a mouse model of acute leukemia consisting in the transplantation of Bcr-Abl expressing BaF3 cells. We have previously shown that administration of probiotics (*L. reuteri* 100-23 and *L. gasseri* 311476) lessens systemic inflammation and muscle atrophy markers, whereas administration of inulin-type fructans (ITF) as prebiotics reduces hepatic accumulation of leukemic cells in this model. Therefore, we speculated that a synbiotic approach would exert an appreciable impact on the survival of leukemic mice. First, immune-modulatory properties of *L. reuteri* 100-23 and *L. gasseri* 311476 were compared in an *in vitro* assay. *L. reuteri* 100-23 was selected based on its anti-inflammatory profile. Secondly, two set of mice were fed a synbiotic preparation (*L. reuteri* 100-23 and ITF) or vehicle, starting on day 1 after BaF3 cell transplantation. Analysis of a first set of mice at day 13 revealed that hepatic BaF3 cell accumulation and two markers of muscle atrophy (LC3 and Cathepsin L) were lessened by the administration of the synbiotic mixture. On day 14, blinded morbidity score was decreased in synbiotic-fed mice ($p=0.005$). Finally, survival was prolonged by 2 days in mice receiving the synbiotics (lifespan 18 days versus 16 days, $p=0.007$). Based on these results, we propose that synbiotic treatment might constitute a novel promising approach in the treatment of malignant diseases such as leukemia and associated cachexia.

10.

IDENTIFYING MECHANISMS THROUGH WHICH LACTIC ACID BACTERIA ACT ON ENVIRONMENTAL TOXINS AND THE POTENTIAL OF FERMENTED FOODS IN REDUCING HOST TOXIN UPTAKE

Jordan Bisanz, BMSc[1,2], Joseph, Mwanga, PhD[3], John Changalucha, PhD[3], Megan Enos, BSc[1,2], Jeremy P. Burton, PhD[1], Tamara Smokvina, PhD[4], Johan van Hylckama Vlieg, PhD[4], Greg Gloor, PhD[2], Gregor Reid, PhD, MBA[1,2]
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Toxins in the environment are ubiquitous and associated with a number of adverse health outcomes, yet exposure is unavoidable. Few have considered the use of food-grade organisms, including *Lactobacillus*, to reduce host uptake from the gut. Eighty strains of *Lactobacillus* were screened for metal binding and resistance with comparative genomics used to identify putative genes with functions in metal interactions. Through binding assays and electron microscopy, we discovered two likely mechanisms of interaction: passive sequestration and an active pathway. A cysteine biosynthetic pathway was identified as being implicated in resistance to mercury. We also aimed to explore the practical translation of this work. We carried out a randomized, controlled study in 43 school-aged children in Mwanza, Tanzania 1) The children received either milk or a *Lactobacillus rhamnosus* GR-1 supplemented yogurt over four weeks. At enrollment, these children had blood levels of lead and mercury 4.6 and 3.3-fold (respectively) higher than Canadian counterparts. At follow-up, the probiotic group had a small decrease in toxin levels. Using 16S rRNA sequencing, we have determined the intestinal microbiota of these children and will report the correlation between these abundance profiles and toxin levels. We believe that the translation of experimental microbiology and probiotic research to people who can benefit most is critically important. Particularly in developing countries; mining, manufacturing processes, and use of environmental pollutants is threatening the life and health of millions of people. It is our hope that simple foods like yogurt can at least reduce some of the burden of these contaminants.
** This clinical study was not part of the collaboration with Danone Research and TS and JvHV were not involved in the design, execution and interpretation of the results.*

11.

PREBIOTIC MODULATION OF MATERNAL DIET TO ENHANCE THE INFANT MICROBIOTA

Paul Blatchford, BSc[1,2], Kerry Bentley-Hewitt, PhD[1], Douglas Rosendale, PhD[1], Juliet Ansell, PhD[1]
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The establishment of a healthy, diverse infant microbiota is fundamental to ensuring the maturation of the naïve infant gastrointestinal tract (GIT) mucosa and immune system, which can improve long term health. It is commonly accepted that colonization of the infant GIT is through contact with vaginal bacteria and feces during birth and to a lesser extent, *in utero*. Breast milk is a source of *bifidobacteria*, *staphylococci,* and lactic acid bacteria, but has also been associated with the transfer of maternal gut bacteria. This gut-mammary lymphatic cross-talking event may transport the mother's gut microbes to the mammary glands and deliver them to the infant. By utilizing prebiotic oligosaccharides, a non-digested and non-absorbed material, we aim to improve the mother's microbial ecosystem throughout the period of microbial transfer and to observe changes in the infant microbiota. Twenty mated Sprague-Dawley rats and their offspring were used as a model to assess this hypothesis. GIT, mesenteric lymph node (MLN) and mammary tissue samples were taken from dams and GIT samples from the pups throughout the course of the experiment. Changes in the composition of microbiota at each of these points were assessed by pyrosequencing. Preliminary data shows the inulin diet initiated a positive shift in microbial composition of the dams GIT and a correlation in the profiles of the MLN and mammary associated bacteria. Although this is indicative of the transfer of bacteria to the mammary tissue, further analysis will be needed to discern whether these changes carry through to the infant microbiota.

12.

TAMOXIFEN-INDUCIBLE INTESTINAL MYD88 INVALIDATION IMPROVES DIET-INDUCED OBESITY THROUGH ENDOCANNABINOID SYSTEM

Amandine Everard, MSc[1], Florian Pierard, BS[1], Lucie Geurts, MSc[1], Thibaut Duparc, PhD[1],
Laure B. Bindels, PhD[1], Giulio G. Muccioli, PhD[2], Nathalie M. Delzenne, PhD[1], Sylvie Robine, PhD[3],
Serge Luquet, PhD[4], Patrice D. Cani, PhD[1]
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Obesity is associated with a cluster of metabolic disorders, low-grade inflammation, and gut barrier disruption. Unequivocal evidence demonstrates that gut microbiota contribute the onset of these disorders. However, mechanisms of interaction with the host remain to be elucidated. MyD88 (myeloid differentiation primary response gene 88) is a protein at the interface of interaction between microorganisms and the host. Previous studies support the notion that whole body MyD88 deletion plays a key role in energy homeostasis, however numerous discrepancies exist (i.e., protection versus worsening of diet-induced obesity). To further elucidate the exact contribution of intestinal MyD88, we have generated tamoxifen-inducible epithelium intestinal MyD88 deletion during diet induced obesity in mice. We found that intestinal epithelium MyD88-KO mice were partially protected against obesity and fat mass development (decrease of 30%). This was associated with a decrease in adipose tissue inflammation (decrease in CD11c mRNA expression). Interestingly, we found that intestinal epithelium MyD88-KO fed with a high-fat diet exhibited a modified endocannabinoid system tone. Among these endocannabinoids, we found that intestinal levels of anandamide (AEA) were decreased, whereas 2-oleoylglycerol (2-OG) and 2-arachidonoylglycerol (2-AG) were significantly increased in intestinal MyD88-KO mice. It is worth noting that AEA is known to increase gut permeability, whereas 2-OG stimulates the release of gut peptide implicated in gut barrier functions and 2-AG has anti-inflammatory properties. In conclusion, these data support that intestinal MyD88 is a target by which gut microbes interact with the host to control

obesity and associated disorders through a mechanism involving the endocannabinoïd system.

13.

MODIFIED CHITOOLIGOSACCHARIDES BY MAILLARD REACTION INHIBIT ADHESION OF ENTEROPATHOGENIC BACTERIA TO MUCIN

Alejandra Cardelle-Cobas, PhD, Maria Inês Montenegro, BSc, Beatriz Gullón, PhD, Patricia Gullón, PhD, Freni KekhasharúTavaria, PhD, Maria Manuela Estévez Pintado, PhD
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Chitooligosaccharides (COS) are promising substrates as sources of new prebiotic ingredients. However, these COS must be modified, because the amino groups present in their structure could cause an antimicrobial effect on the probiotic bacteria with possible negative health outcomes. In a previous work our research group showed, for the first time, that partial substitution of these amino groups with carbohydrates via Maillard reaction converted COS in a substrate used by probiotic bacteria indicating that these derivatives could be good candidates to be used as prebiotics. Anti-adhesive capacity is a relevant property attributed to some prebiotic oligosaccharides that may confer health benefits. Specifically, these oligosaccharides may directly inhibit infections by enteric pathogens due to their ability to act as structural mimics of the pathogen binding sites that coat the surface of gastrointestinal epithelial cells. In the present study, the objective was to evaluate, *in vitro*, the ability of the synthesized COS derivatives via Maillard reaction to inhibit the adhesion of several food pathogens to mucin. A classical mucin adhesion test was carried out using a fluorescence-based method for the detection of adhesive properties of pathogenic strains. Results showed that modified COS were capable of inhibiting the adhesion of all tested pathogens. These substrates showed a strain-dependent effect, suggesting the involvement of different carbohydrate-recognition sites. The carbohydrate used for COS modification also had a clear effect on the anti-adhesive properties of the derivative. Although more studies are necessary to further evidence of their biological effects, this work is a basis for future work showing the ability of modified COS to competitively exclude intestinal pathogens and amplify COS uses as a potential prebiotic ingredient.

14.

PROTECTION OF PROBIOTIC BACTERIA IN SYNBIOTIC MATRICES

Saikiran Chaluvadi, MSc[1,3], Arland T. Hotchkiss, PhD[2], Kit L. Yam, PhD[3]
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Probiotics, like *Lactobacillus acidophilus, Lactobacillus reuteri, Bifidobacterium breve,* and *Bifidobacterium longum,* when encapsulated with prebiotic fibers such as fructo-oligosaccharides (FOS), inulin (I) and pectic-oligosaccharides (POS), formed a synbiotic matrix system that protected the bacteria from refrigerated aerobic storage conditions. Commercial application requires at least 7 Log CFU/ml of these bacteria along with an ability to produce short chain fatty acids. Bacterial survival was determined by cell counts from different synbiotic matrices followed by analysis of fatty acids produced during anaerobic growth of the same bacteria upon revival from 28 day storage at 4 ˚C under aerobic conditions. When calcium (45 mM) was used for cross-linking the alginate-based matrices, 2-3 Log CFU/mL of *Lactobacillus acidophilus* and *L. reuteri* and 0-3 Log CFU/mL of *Bifidobacterium breve* and *B. longum* survived refrigerated aerobic storage for 28 days. Following refrigerated storage, acetic (3-9 mM), butyric (0-2 mM), propionic (5-16 mM) and lactic acids (1-48 mM) were produced during anaerobic growth of probiotics in BHI broth at 37 ˚C, suggesting their metabolic activity. When calcium cross-linking was not used in synbiotics, a much more gel-like matrix persisted and at least 7 Log CFU/mL of probiotic bacteria survived after 21 days of storage. Significantly higher levels of *Bifidobacterium breve, Lactobacillus acidophilus* and *L. reuteri* were obtained from the synbiotic matrices supplemented with FOS, POS and I compared to alginate alone. Refrigerated aerobic shelf-life of probiotic bacteria was extended to four months. These results show that synbiotics are viable delivery vehicles for health promoting probiotic bacteria.

15.

MICROBIAL COMPOSITION OF DIET INDUCED OBESE MICE WITH RESPECT TO OBESITY, ANTIMICROBIALS, AND TIME

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Obesity has become one of the most prevalent health issues of the 21st century. Recent studies linking the composition and function of the gut microbiota and obesity have led to an upsurge in interest in this area. It is still unclear if the gut microbiota represents a realistic therapeutic target. Therefore this study explores two strategies and their impact on the murine gut composition and weight gain in diet induced obese mice. More specifically, a low fat or high fat diet (DIO) was fed to C57BL/J6 mice for twelve weeks followed by an intervention period during which the high fat diet was supplemented with the glycopeptide antibiotic vancomycin, the bacteriocin producing probiotic (Bac+) *Lactobacillus salivarius* UCC118, its bacteriocin negative derivative *L. salivarius* UCC118 Bac– or was unsupplemented (9-10 mice/cohort) for eight weeks. 16S rRNA sequencing was used to analyze the impact of the interventions on the gut microbial composition of these animals. Vancomycin treatment resulted in a significant reduction in weight gain in DIO mice throughout the intervention period but the extent of this difference relative to DIO controls decreased over the eight week intervention period. A significant reduction in weight gain was also observed at week 14 in the DIO mice receiving the Bac+ probiotic compared to those in receipt of Bac-. However, this difference was not significant at subsequent time points. Here we present data relating to the gut microbial composition at week 14 and week 20 and identify populations that may contribute to the associated phenomena.

16.

FIGHTING THE SUPERBUG THROUGH DIRECTED COMPETITION AND EVOLUTION: CONTROLLING MRSA INFECTIONS USING PROBIOTICS

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram positive bacterium that colonizes the anterior nares and skin. Spread through direct contact between infected individuals, MRSA can cause a range of symptoms from a common skin infection to more severe infections such pneumonia and bacteriamia. Another distinct characteristic associated with MRSA is its ability to adapt quickly to antibiotic treatments. The increased resistance to antibiotics creates a more challenging problem amongst clinical treatment therapy as many options are failing. The rapid growth of resistance and epidemiological expansion of MRSA make it a major public health concern. This study explores on alternative intervention methods that do not rely on chemotherapy but rather intervention based on the biology, fitness, and evolution of the pathogen. Through an extensive literature search we identified probiotic and bacterial candidates that could serve as competitive exclusion factors. Candidates were selected based on shared niche and resources, frequency, and shorter generation times compared to MRSA. We identify potential probiotics candidates based on competitive advantage and treatability over MRSA. We hypothesize that if such a candidate was introduced, competitive exclusion would lead to a lesser MRSA infection and could serve as an effective means for treating MRSA infections and colonization. We demonstrate this using mathematical modeling of the selected candidates.

17.

EFFECTS OF EARLY-LIFE ANTIBIOTIC EXPOSURE ON SKELETAL MUSCLE DEVELOPMENT

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The use of low-level antibiotics as growth promoters in the U.S. livestock industry is well documented, although the mechanism of this growth promotion is unclear. In the laboratory, sub-therapeutic antibiotic treatment (STAT) shifts the taxonomic community structure of the murine gut microbiome and alters expression of key metabolic genes. In the STAT mouse model, changes in body composition are evident as early as the fourth week of life, including significant developmental phenotypes in skeletal muscle, bone, and fat. Using 16S rRNA analysis, early views of the microbial populations present at four weeks show that STAT markedly perturbs community structure. Microarray analysis of STAT skeletal muscle RNA shows enrichment of genes and signaling pathways involved in growth regulation including foxO3 and the IGF-1 pathway. Additionally, STAT mice show up-regulation of gene sets involved in immune cell differentiation, including pathways involving IL-1R, IL-2, and IL-4R, as well as KEGG pathways related to Type I and Type II diabetes and energy homeostasis. Notably, in four week-old STAT mice, *leptin* gene expression is enriched, consistent with elevated serum Leptin levels and mirrored by concomitant down-regulation of Leptin Receptor expression. The increasing global burden of immune and metabolic disorders correlates with the widespread use of antibiotics. Considering their frequent use in children and the presence of residual antibiotics in food products and drinking water, future work to define a healthy human microbiome and determine the long-term effects of antibiotic-induced perturbation is essential.

18.

THE FECAL MICROBIOTA OF PATIENTS COLONIZED BY VANCOMYCIN-RESISTANT *ENTROCCOCUS* (VRE) GIVEN *LACTOBACILLUS* GG (LGG) OR PLACEBO

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VRE is a hospital-acquired organism that has become endemic in the US. It colonizes the intestine and can lead to subsequent infections. There are no proven methods or means of decreasing or eliminating the carrier state of VRE. Previously, in our laboratory, we demonstrated that LGG has the capacity to kill VRE. Two clinical studies showed reduction or elimination of VRE colonization using probiotics. We randomized seven subjects with VRE to receive two weeks of LGG or placebo in a pilot trial. Stool samples were collected at baseline, and 14, 28 and 56 days. Stool samples were cultured for the presence of VRE. These results are reported separately. DNA was extracted from stool and subjected to pyrosequencing of barcoded 16S rRNA gene amplicons. Sequences generated were processed using mothur. Community richness, evenness, and diversity were calculated for each sample. The treatment allocation remains concealed, so groups were analyzed using the designations “A” and “B”. Group A was significantly more diverse than Group B on three different measures of diversity (phylogenetic diversity, the Shannon diversity index, and the Simpson Index) and looking at post-baseline samples only using the non-parametric Kruskal-Wallis rank sums test ($p=0.0232$, $p=0.0073$, $p=0.0232$, respectively). Unweighted and weighted Unifrac measures of beta-diversity visualized using Principal Coordinate Analysis demonstrated a significant separation of Group A and Group B samples (unweighted ANOVA <0.001). Together, despite the blinded treatment allocations, there appears to be evidence of differential fecal community composition and overall diversity between the placebo and LGG groups.

19.

A RANDOMISED, PLACEBO CONTROLLED CROSSOVER STUDY TO DETERMINE THE POTENTIAL OF THE CONSUMPTION OF PALM DATE FRUITS TO BENEFICIALLY AFFECT THE COLON HEALTH

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Observational studies have shown that fruit and vegetable intake may reduce colorectal cancer risk, although the precise bioactive components remain unclear. Currently, nutritional research has been directed towards the colon, where fermentation of dietary fibers and polyphenols have been observed to modify the gut microbiota. Date fruits are rich sources of insoluble fibers and polyphenols, and therefore they may exert positive effects in gut health. A randomized, placebo controlled crossover study was conducted to determine date consumption impact on the colonic microbiota and markers of colon cancer risk. 22 healthy individuals participated in the study (consuming maltodextrin-dextrose, 50g or 7 fruits, approx.50g). Each arm was 21 days in duration, separated by a 14day washout period. Changes in the microbiota were assessed by FISH analysis. Short chain fatty acids were determined using HPLC. The biological activity of human fecal water was tested for its ability to inhibit proliferation of colon cancer cells using the Sulforhodamine B assay and in reducing colon genotoxicity using the comet assay. Date fruit consumption was observed to reduce colon genotoxicity and inhibit colon cancer proliferation following 21 days of daily consumption. FISH analysis indicated limited alterations in the growth of microbiotal groups over the period of intake. Our data indicate that although date intake may not produce selective bacterial growth changes, promising effects in reducing colon genotoxic markers appear feasible.

20.

FUNCTIONAL DYNAMICS OF THE ELDERLY GUT MICROBIOME DURING PROBIOTIC CONSUMPTION

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A mechanistic understanding of the purported health benefits conferred by consumption of probiotic bacteria has been limited by our knowledge of the resident gut microbiota and its interaction with the host. We used fecal samples from a study of twelve healthy individuals aged 65-80 to characterize the structure and functional dynamics of the gut microbiota associated with consumption of the single-organism probiotic, *Lactobacillus rhamnosus* GG ATCC 53103 (LGG). Samples were collected prior to probiotic consumption (day 0), on day 28 immediately after consuming 1010 CFU of LGG twice daily for 28 days and day 56, one month after stopping LGG consumption. Our integrative approach incorporated bacterial 16S rRNA gene sequencing, whole-community expression profiling using RNA-seq, and metagenomic sequencing. We found congruent abundances of the main bacterial taxa from the 16S, metatranscriptomic, and metagenomic datasets. The metatranscriptomic data additionally revealed highly expressed transcripts across all time points from gut archaea (on average, 12%), as well as transcripts from viral (2%) and fungal species (0.006%). While analyses using 16S rRNA sequencing did not reveal significant changes in community composition associated with probiotic intake, we identified genus-specific functional changes using a robust phylogenetically-partitioned differential expression analysis. Probiotic consumption conferred transient functional changes within the elderly gut microbiota and returned to baseline functionality 1 month after stopping LGG. We highlight the value of combinatorial 'omics methods and concomitant high-resolution informatics to probe the role that probiotics may play on the structure and function of the resident microbiota.

21.

PROBIOTIC THERAPY FOR HEART FAILURE: THE ATTENUATION OF MALADAPTIVE HYPERTROPHY AND IMPROVED CARDIAC MECHANICAL FUNCTION

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Cardiovascular disease (CVD) is a major cause of death in North America. Patients with heart failure face a 50% mortality rate within five years of first diagnosis, due to irreversible loss of working muscle and maladaptive hypertrophy of the heart. The human microbiome plays a role in cardiovascular health, as dysbiosis of the oral and gastrointestinal microbiome has been associated with an increased risk and prevalence of CVD. Probiotics are living microorganisms that when administered confer health benefits on the host. Considering the established health promoting and anti-inflammatory properties of some probiotic bacteria, we hypothesized that orally ingested *Lactobacillus rhamnosus* GR-1 (GR-1) will attenuate maladaptive hypertrophy and improve cardiac function in a coronary artery ligation rat model of myocardial infarction-induced heart failure. Rats were provided GR-1 ad libitum in their drinking water daily for 6 weeks post-infarction. Controls received water alone. Serial echocardiography revealed significant attenuation of cardiac remodeling throughout the trial ($p<0.05$), including normalization of ejection fraction and left ventricular dimensions. Haemodynamics measurements revealed a significant attenuation of left ventricular end-diastolic pressure ($p<0.05$) and improved cardiac output ($p<0.05$). Left ventricular hypertrophy was also attenuated with GR-1 treatment ($p<0.05$). Serum cytokine analysis revealed no significant changes in activity of the following cytokines/chemokines: GRO/KC, fractalkine, IFN-γ, IL-1α, IL-6, IL-10, MCP-1, MIP-1α, RANTES, and TNF-α. These results imply that the attenuation of cardiac remodeling and hypertrophy may be due to a novel mechanism of GR-1, independent of anti-inflammatory mediation, and suggest the potential for novel treatment of CVD.

22.

MICROBIOTA CHANGES AND COLONIZATION FOLLOWING FECAL MICROBIOTA TRANSPLANTATION IN *CLOSTRIDIUM DIFFICILE* INFECTION PATIENTS

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Clostridium difficile infection (CDI) is associated with an increasing clinical and economic burden. Fecal microbiota transplantation (FMT) has been used in multiple small studies to treat CDI with success rates of >95%. However, short- and long-term effects of FMT remain a clinical concern, as the gut microbiota is believed to play an important function in diseases such as obesity or inflammatory bowel disease. Furthermore, the mechanism of action for FMT remains largely unknown, hampering the development of treatments with controlled, *in vitro*-assembled mock microbiota. Using a subset of three patient/donor pairs from 25 cases of CDI patients successfully treated with FMT at Sinai Hospital (Baltimore, MD), we performed fecal microbiota analysis by 16S rRNA gene amplicon sequencing to shed light on two clinically important questions: (i) What are the characteristic microbiota changes associated with CDI and FMT treatment? (ii) Does FMT lead to stable colonization of the transplanted microbiota in the patient? We identified significant microbiota changes associated with CDI, namely a shift within the *Firmicutes* from *Streptococcaceae* or *Lactobacillaceae* (class: *Bacilli*) in CDI patients to *Lachnospiraceae* (class: *Clostridia*) in donors and post-FMT CDI patients. Our results suggest that FMT can lead to the integration of the transplanted microbiota into the recipient, as in two of the three cases individual species (i.e. operational taxonomic units; OTUs) from the donor sample were identified in patient samples up to two months post-FMT, which were not present in the CDI patient before FMT nor in any sample from the other two patient/donor pairs.

23.

ATTENUATION OF COLON INFLAMMATION BY PROBIOTIC LACTOBACILLUS REUTERI VIA HISTAMINE PRODUCTION

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Supplementation with certain probiotic *Lactobacillus reuteri* strains that naturally colonize the gut of mammals has been found effective at ameliorating intestinal inflammation in patients with IBD and in rodent colitis models, but the underlying mechanisms are unknown. Pangenomic studies revealed that among all sequenced *L. reuteri* strains, those with anti-inflammatory properties contain a complete hdc gene cluster which is responsible for synthesis and secretion of histamine, indicating a potential role for histamine in alleviation of inflammation. *L. reuteri* 6475 which contains an intact *hdc* gene cluster was found to suppress TNF production in activated THP-1 cells through the production of histamine and activation of histamine receptor 2 (H2R). Targeted mutagenesis of the *hdc* genes demonstrated diminished anti-TNF activity and loss of histamine production, indicating the anti-TNF activity of histamine in vitro. Using a trinitrobenzene sulfonic acid-induced mouse model of colitis, *L. reuteri* 6475 administration was found to protect eight-week female BALB/c mice against colitis, as indicated by significantly decreased weight loss, colonic damage graded by the Wallace score, and serum amyloid A protein concentrations compared to media control. The *hdcA* mutant of *L. reuteri* 6475 which failed to produce histamine showed diminished ability to attenuate colitis. Moreover, H2R was detected in the mouse colon by immunohistochemistry and blocking H2R with its specific antagonist ranitidine diminished the anti-inflammatory ability of *L. reuteri* 6475. These combined investigations indicate that *L. reuteri* 6475 attenuates experimental colitis via histamine production, which provides important insights into understanding the molecular mechanisms underlying probiotic immunomodulation.

24.

SYNERGY BETWEEN THE PREBIOTIC BOVINE MILK OLIGOSACCHARIDES AND THE PROBIOTIC *B. INFANTIS* IMPROVES GUT BARRIER FUNCTION IN VIVO

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Prebiotics and probiotics are used for treatment of GI disorders, such inflammatory bowel disease, irritable bowel syndrome, and recently, obesity; these disorders have altered gut microbiota, impaired intestinal barrier function, and inflammation. A synergistic relationship has been shown between prebiotic bovine milk oligosaccharides (BMO) and probiotic *Bifidobacterium longum subsp. infantis* (*B. infantis*) *in vitro* (Garrido et al, *Plos One*, 2011). The aim of the study was to assess the ability of BMO and *B. infantis* to restore intestinal permeability *in vivo.* Mice were fed a western diet (WD, 20% fat) or normal chow (NC, 10% fat) for seven weeks. For the final two weeks of the study, the diet of a subgroup of WD-fed mice was supplemented with BMO (7%). Weekly gavage of *B. infantis* was performed in all mice starting at week three. The presence of *B. infantis* was confirmed in the large intestine of all mice at the completion of the study. Intestinal tissue was mounted in Ussing chambers to evaluate intestinal permeability. Ingestion of WD compared to NC increased paracellular and transcellular flux in the large intestine; barrier function was restored by BMO supplementation. mRNA of the inflammatory marker TNFα was lower in intestine of BMO-supplemented compared to WD mice. Conditioned medium from *B. infantis*+BMO but not from *B. infantis*+lactose cultures protected against pro-inflammatory cytokine-induced alteration of barrier function in Caco-2 cells. The data support the hypothesis that *B. infantis*+BMO restores intestinal barrier function, thereby decreasing the passage of detrimental luminal contents causing inflammation.

25.

IDENTIFICATION OF A PROTON-CHLORIDE ANTIPORTER (ERIC) BY HIMAR1 TRANSPOSON MUTAGENESIS IN *LACTOBACILLUS REUTERI* AND ITS ROLE IN HISTAMINE PRODUCTION

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The gut microbiome may modulate intestinal immunity by luminal conversion of dietary amino acids to biologically active signals. The human microbiome-derived probiotic organism *Lactobacillus reuteri* ATCC PTA 6475 converts the amino acid L-histidine into the biogenic amine histamine. Histamine suppresses TNF production by human myeloid cells and is a product of L-Histidine decarboxylation, which is a proton-facilitated reaction. A transposon mutagenesis strategy was developed based on a single-plasmid nisin-inducible Himar1 transposase/transposon delivery system for *L. reuteri*. A highly conserved proton-chloride antiporter gene (eriC), a gene widely present in the gut microbiome was discovered by Himar1 transposon (Tn)-mutagenesis presented in this study. Genetic inactivation of eriC resulted in reduced ability of *L. reuteri* to inhibit TNF production by Toll-like receptor (TLR) 2-activated human myeloid cells and diminished immunomodulatory histamine production by *L. reuteri*. We also observed downregulated expression *of histidine decarboxylase (hdc)* cluster genes, which are required for the conversion of histidine to histamine by the bacteria, compared to those of wild-type *L. reuteri* 6475. EriC belongs to a large family of ion transporters that includes chloride channels and proton-chloride antiporters. This antiporter relieves the accumulated inside-negative transmembrane potential generated during amino acid decarboxylation and facilitates the availability of protons for the amino acid decarboxylation reaction, resulting in histamine production by *L. reuteri*. This report highlights the widely conserved nature of ion transporters in the intestinal microbiome and the coupling of ion transporters with amino acid decarboxylation and immunomodulation by gut microbes and probiotics.

26.

BROAD SCOPE METHOD FOR CREATING HUMANIZED ANIMAL MODELS FOR ANIMAL HEALTH AND DISEASE RESEARCH THROUGH ANTIBIOTIC TREATMENT AND HUMAN FECAL TRANSFER

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We have developed a method to humanize non germ-free mice with human intestinal microflora. The method involves depleting intestinal microflora with broad-spectrum antibiotics, introducing human microflora from frozen fecal samples by weekly gavage and maintaining mice in HEPA-filtered microisolator cages. Pyrosequencing of 16S rRNA genes from cecal microflora demonstrated that recipient mice adopt a humanized microbiota profile analogous to their human donors, yet distinct from mice treated with only antibiotics (no fecal transfer) or untreated control mice. In the humanized mice, 75% of the sequence mass was observed in their respective human donor and 68% of the donor sequence mass was recovered in the recipient mice. Principal component analysis of GC- and HPLC-separated cecal metabolites from mice humanized by different donors clustered near each other, yet were sufficiently distinct that separate clusters were apparent for each donor. Metabolite profiles for mice treated with only antibiotics (no fecal transfer) and control mice were very dissimilar from each other and the humanized mice. These data demonstrate that our protocol can be used to humanize non germ-free mice and is sufficiently robust to generate phenotypic differences between mice humanized from different human donors. Our method overcomes several limitations of humanized mouse models. Most inbred or transgenic mouse strains are not commercially available as germ-free, limiting researchers who aim to examine the impact of human microflora populations in animal models of human disease, but lack relevant strains of germ-free animals. Also of note, our approach could be extended to other highly used model species.

27.

MODULATION OF THE MICROBIOME WITH *L. REUTERI* NCIMB 30242 REDUCES CHOLESTEROL, INFLAMMATORY MARKERS AND INCREASES 25(OH) VITAMIN D

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Cardiovascular disease (CVD) is the leading cause of global mortality and morbidity, is responsible for 16.7M deaths worldwide, and carries direct and indirect costs of $403.1B in the US alone. Epidemiological and clinical evidence have established a clear link between elevated serum cholesterol and CVD. Recently, it has been proposed that changes to the microbiome may be associated with elevated cholesterol and CVD. We have conducted two clinical studies evaluating bile-salt hydrolase active *L. reuteri* NCIMB 30242 in hypercholesterolemic adults and found significant reductions in lipid markers in separate interventional, multicenter RCTs of six (ITT N=114) and nine (ITT N=127) weeks. We have found significant reductions in LDL-C (11.6%; P=0.001), TC (9.1%; P<0.001), non-HDL (11.3%; P<0.001), apoB-100 (8.4%; P=0.002), saturated cholesterol esters (8.8%; P=0.002), as well as in CVD risk factors hs-CRP (1.05 mg/l; P=0.005) and fibrinogen (14.3%; P=0.004). We found that baseline LDL-C levels were associated with reduced deconjugated bile acids in subjects with hypercholesterolemia, and that individual changes were associated with changes in LDL-C in treated subjects. We found that changes in hs-CRP were positively correlated to changes in Il-6 and TNF-α, and that surrogate markers for cholesterol absorption were significantly decreased suggesting inhibition of intestinal cholesterol absorption as a mechanism. Finally, serum 25(OH)D, associated with CVD risk at low levels, was increased by 22.4% (P=0.011) compared to placebo, and changes were negatively correlated with hs-CRP. These results indicate the potential of *L. reuteri* NCIMB 30242 as a novel adjunctive therapy for the treatment of hypercholesterolemia acting through the gut microbiome.

28.

VK2 CO-INFECTION MODEL WITH *LACTOBACILLUS RHAMNOSUS* AND *GARDNERELLA VAGINALIS*

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Lactobacillus is the predominant bacterial species in the healthy balanced vaginal microbiome, and plays an important role in the maintenance of vaginal health by preventing colonization of pathogenic bacteria and inducing host defense responses. This study was designed to determine the protective effect that a probiotic *Lactobacillus rhamnosus* provides for vaginal epithelial cells against a pathogenic bacterium *Gardnerella vaginalis*, which is associated with bacterial vaginosis. A co-infection assay showed that pretreatment *of L. rhamnosus* was able to reduce viable *G. vaginalis* and decrease vaginal epithelial cell death. In addition, menopause is commonly associated with a decrease of *lactobacilli* in vaginal microbiome and an increase in the risk of vaginal infections, which is often attributed to the loss of estrogen. Research has shown that estrogen can stimulate glycogen deposition in the vaginal epithelial tissues, which promotes *lactobacilli* growth and in turn controls vaginal pathogens. This study also evaluated how administration of estrogen affects adhesion capability of *L. rhamnosus* and *G. vaginalis* on the vaginal epithelium in the co-infection model.

29.

EFFECT OF ANTIBIOTIC-INDUCED GUT MICROBIOTA PERTURBATION ON THE DEVELOPMENT OF TYPE 1 DIABETES IN NON-OBESE DIABETIC MICE

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Since 1950, type 1 diabetes (T1D) incidence has greatly increased in developed countries, most rapidly in young children. This increase cannot be explained by altered genetic susceptibility, but suggests that changing environmental factors, such as a perturbed gut microbiome could modulate disease risk. In the non-obese diabetic (NOD) mouse model of T1D, disease incidence is higher in SPF than conventional facilities, reinforcing the importance of commensal microbes. We hypothesize that early-life antibiotic use alters gut microbiota essential for immune development, accelerating T1D onset. We compared male and female control NOD mice (n=37) to mice exposed to either subtherapeutic antibiotic treatment (STAT, n=41) or pulsed antibiotic treatment (PAT, n=32). In STAT, low-dose penicillin was administered late in pregnancy until 12w; in PAT, tylosin (macrolide) was given as 3 therapeutic pulses early in life, mimicking repeated childhood antibiotic exposures. PAT male mice had increased T1D (53%) compared to control males (26%) by 31w ($p=0.04$). At 6-15w, female mice had significantly higher insulin autoantibody (IAA) levels than males, but there were no significant differences by treatment. Flow cytometry of small intestinal lamina propria (SI-LP) cells showed that in pre-diabetic control mice, males had higher percentages of CD4+RORγT+(Th17)($p=0.01$) and CD4+FOXP3+(Treg)($p=0.008$) cells than females. In males, PAT reduced the percent of SI-LP Th17 ($p<0.01$) and Treg cells ($p<0.01$); however, with no similar effect in females. Percent of Th17 cells was lower in males than in females in STAT ($p=0.03$) and in PAT ($p<0.001$). These findings provide evidence that early-life antibiotic exposures both alter SI-LP T-cell populations, and accelerate T1D onset.

30.

ANTIBIOTIC EXPOSURE AND THE RISK OF FOOD ALLERGY IN YOUNG CHILDREN

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A diverse microbiome is essential for normal gut immune response which leads to appropriate development of food tolerance. We hypothesize that altering the normal gut flora in infants with early use of antibiotics might contribute to the increasing prevalence of childhood food allergy (FA). South Carolina Medicaid billing data for children born between 2007 and 2009 were obtained. Children with a diagnosis of FA before 12/31/2010 were identified as cases and matched by birth year, sex, and race to controls without FA. Both cases and controls were limited to children with uninterrupted Medicaid coverage during the first year of life. Logistic regression was used to model the odds of any diagnosis of FA. A total of 1,105 cases and 6,433 controls were identified. The mean number of antibiotic courses was 2.65 for cases and 1.84 for controls ($p<0.001$). Among those receiving antibiotics, the mean time (days) to first antibiotic course was 181.5 for cases and 190.1 for controls ($p=0.009$). Additionally, fewer controls received an antibiotic (67% vs. 76%; $p<0.001$). The overall risk of FA was higher with antibiotic exposure (OR 1.71; 95% CI 1.43-2.05). “Late” antibiotic exposure (between days 183-365) conferred a greater risk (OR 1.98; 95% CI 1.61-2.42). Three or more courses increased the odds of FA, but >/=5 courses had the highest odds (OR 2.15; 95% CI 1.74-2.66). Antibiotic exposure in the first year of life is associated with an increased risk of FA. Multiple courses and “late” exposure confer a greater risk.

31.

USING META-RNASEQ TO UNCOVER THE FUNCTIONAL CONTRIBUTION OF THE VAGINAL MICROBIOME

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High-throughput 16s rRNA sequencing of the vaginal bacterial microbiome has uncovered distinct profiles in healthy conditions and dysbiotic states like bacterial vaginosis (BV). Though the biota structures have been well characterized, we don't yet understand how the organisms function and contribute to the community. We therefore used meta-RNAseq to uncover genes and pathways that potentially differentiate dysbiotic states, such as BV, from healthy communities dominated by *Lactobacillus iners* and *L. crispatus.* Comparative transcriptomics of *L. iners* and *L. crispatus* show differing gene expression patterns that may explain their differing ability to persist. Unlike *L. crispatus, L. iners* is often present in BV-like conditions and drastically modulates its gene expression in response to this environment. Most notably: *L. iners* increased expression of a cholesterol-dependent cytolysin, mucin and glycerol transport and related metabolic enzymes, and genes belonging to a CRISPR system - suggesting that bacteriophage influence the community. Although diverse in biota structure, we show that BV communities share similar functions including preference for glycogen and glycerol as carbon sources under BV conditions. The predicted end-products of metabolism under BV conditions include an abundance of succinate and other short-chain fatty acids. We further show that the different biota profiles can be clustered by similarity in transcriptional function and the clusters possibly represent different risks or outcomes for the host. Our study underscores the importance of understanding the functional activity of the bacterial community in addition to characterizing the population structure when investigating the human Microbiome.

32.

INFLUENCE OF POLYPHENOL RICH HERBS IN REPRESENTATIVE GUT BACTERIAL SPECIES

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The importance of polyphenols in human health, as well their natural sources such as medicinal herbal extracts, is well discussed and supported by scientific studies. Sage and Savory are popular medicinal herbs rich in rosmarinic acid and quercetin. Following the ingestion of such polyphenols or their extracts, if they are not adsorbed in small intestine, they reach the colon where they are transformed by the colonic microflora. Thus, a preliminary study was made on the interactions between bacterial strains representative of the stimulant colonic microflora (*Lactobacillus spp.* and *Bifidobacterium spp.*) and polyphenolic compounds of medicinal herbal extracts such as Sage and Savory. In a 96-well microplate, MRS broth medium with and without glucose and supplemented with the herbal extracts were inoculated with 1% (v/v) inoculum of bacterial strains. Simultaneously, a negative control without herbal extracts were used using the same conditions. At time 0 and 24 hours of incubation at 37 ºC, samples were taken to enumerate viable cells in specific media. At same time, centrifugation of the samples was performed and the supernatants were analyzed to quantitatively determine the polyphenols and metabolites generated by using Foulin method and by High Performance Liquid Chromatography (HPLC). Differences between the growth of the bacterial strains in the presence of the herbal extracts were evident. In general the polyphenol compounds present in the extracts were hydrolyzed by the bacterial strains and different metabolic compounds were also generated.

33.

A MURINE MODEL OF ANTIBIOTIC-INDUCED OBESITY AND ALTERED METABOLIC STATUS

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In America, about one-third of adults and 20% of children are clinically obese, contributing a major risk factor for type 2 diabetes and other complicating diseases. While genetics and lifestyle play roles, the human microbiome may be an important and modifiable factor. We hypothesize that sub-therapeutic antibiotic treatment (STAT) predisposes hosts to obesity by altering their metabolic status. Mice received benzylpenicillin (17 µg/ mouse/ day) in drinking water beginning prior to day 0 of life and at week 13 were changed from normal chow (10% kcal fat) to high-fat chow (45% kcal). Fasting blood was collected at weeks 20 and 32 for analysis by MILLIPLEX® MAP magnetic bead panel, including tests for C-peptide, ghrelin, insulin, and leptin. Body weight and composition were tracked, glucose and insulin tolerance assayed, and livers examined for steatosis. STAT mice were significantly heavier than controls, with fat comprising most of the difference. At week 20, weight and fat mass diverged, but not serum tests. By week 32, STAT mice showed glucose intolerance and insulin resistance, particularly in females. STAT mice scored higher (4.8±1.7 vs. 2.5±1.0; $p<0.0001$) on the Non-Alcoholic Steatosis (NAS) scoring system and were more likely to develop NASH (72% vs. 0%) compared to controls. STAT had elevated C-peptide ($p=0.018$, males), insulin ($p=0.004$, males), and leptin ($p=0.018$, males and females). Control mice had significantly higher levels of ghrelin ($p=0.026$). Determination of microbial taxa is in progress. These findings provide evidence that low dose antibiotics can promote adiposity, development of type 2 diabetes, and steatohepatitis.

34.

A ROBUST MICROBIOTA AGAINST FUNCTIONAL BOWEL DISORDERS

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Background: Functional bowel disorders (FBD) represent a clinical challenge due to their heterogeneous symptoms. There is increasing evidence that the gut microbiota influences FBD; characterization of dysbiosis and potential biomarkers are thus critical for developing diagnostic and therapeutic strategies. Methods: Here, we used quantitative PCR and high-throughput sequencing of 16S rRNA genes to analyze the gut microbiota of FBD patients enrolled with a common symptom of excessive gas production. Results: We found that, relative to healthy controls, FBD patients presented significant differences in the abundance of several taxa and a larger microbial phylogenetic core. When challenged with a flatulogenic diet, the patients' microbiota developed a large instability, exhibiting variations in the main phyla, a reduction of microbial diversity and a loss of taxa from their phylogenetic core. Several taxa from the *Bacteroides fragilis* group and *Bilophila wadsworthia* were positively correlated with the number of expulsions and the volume of gas evacuated respectively. Conclusion: Therefore, inability to stabilize more heterogeneous microbial populations, together with the prevalence of particular bacterial species, may be key determinants for this common form of FBD.

35.

LACTOBACILLI ANTAGONIZE *BRACHYSPIRA PILOSICOLI* AND REDUCE ITS CLINICAL PATHOLOGY *IN VIVO*: A POTENTIAL FOR PROBIOTICS AS AN INTERVENTION AGAINST AVIAN INTESTINAL SPIROCAETOSIS

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Avian intestinal spirochaetosis (AIS) results from the colonization of the caeca and colorectum of poultry by pathogenic *Brachyspira*. The number of cases of AIS has increased since the 2006 EU ban on the use of antimicrobial growth promoters which, together with emerging antimicrobial resistance in *Brachyspira*, has driven renewed interest in alternative intervention strategies. Probiotics have been reported as protective against infection with common enteropathogens in livestock. Here, we investigate their potential to antagonize aspects of the pathobiology of avian *Brachyspira* and their potential to protect against experimentally-induced AIS *in vivo*. The cell-free supernatant of two *lactobacilli* of poultry origin, *L. reuteri* LM1 and *L. salivarius* LM2, suppressed the growth of *B. pilosicoli* B2904 in a pH-dependent manner. In *in vitro* association assays using HT29-16E 3D cells and a novel avian cecal *in vitro* organ culture model, the adherence and invasion of *B. pilosicoli* to gut epithelial cells was reduced significantly by the presence of viable *lactobacilli* ($p<0.001$). *Lactobacilli* inhibited the motility of *B. pilosicoli*, regardless of whether they were live or heat-inactivated. Electron microscopic observations indicated that contact between the *lactobacilli* and *Brachyspira* was crucial in inhibiting both adherence and motility. A novel model for AIS was developed using laying chickens experimentally challenged with *B. pilosicoli* B2904. In this model, all aspects of the clinical pathology were reduced significantly when *L. reuteri* LM1 was delivered in the drinking water. These data suggest the use of probiotics in chickens intervenes against AIS and encourages further investigation of their use to treat other *Brachyspira*-associated diseases.

36.

SEQUENCE-BASED ANALYSIS OF BACTERIAL AND FUNGAL POPULATIONS OF NATURALLY FERMNETED BEVERAGES AND SUBSEQUENT SCREENING FOR ANTIMICROBIAL PRODUCERS

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Several natural, fermented beverages produced via a symbiosis of bacteria and yeast are popular due to their purported nutritional and therapeutic benefits. Indeed, consumption of such foods is on the increase, primarily due to consumers growing desire for functional foods. Examples of natural, fermented beverages include Kefir, a lactic-fermented milk, and Kombucha, an acetic-fermented sweetened tea. Though these beverages have been produced for >2000 years, relatively little is known about the composition and complexity of the associated microbial populations which are key to their production. Here, we apply culture-independent, high throughput DNA sequencing to assess, in detail, the microbiota of Kefir, Water Kefir, and Kombucha sourced from several countries in order to identify core populations and to examine the environmental and regional influence on the microbial diversity of these products. Additionally, high-throughput robotic screening of these fermented cultures was performed to reveal antimicrobial-producing isolates with interesting spectra of activity. Peptide analysis is currently ongoing.

37.

THE VAGINAL METABOLOME IN HEALTH AND DYSBIOSIS

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A healthy vaginal microbiota is dominated by *Lactobacillus* species, but it can rapidly shift to a diverse biota and a condition termed bacterial vaginosis (BV) that afflicts 30% of women in North America at any given time. Current diagnostic techniques are unreliable and treatment with antimicrobials has poor efficacy and an unacceptable recurrence rate (58%). High throughput sequencing studies have uncovered over 250 bacterial species in the vagina, and an increased diversity in BV. In order to better understand what is happening at the molecular level, we decided to undertake studies of the vaginal metabolome (the complete set of small molecules in a given environment). We hypothesized that the small molecules produced by microorganisms that thrive in BV will be distinct from healthy women. Using untargeted gas chromatography mass spectrometry (GC-MS) we have identified over 100 compounds in vaginal samples. Using principle component analysis we demonstrated that the vaginal metabolome of BV is distinct from a healthy profile (n = 39). Compounds responsible for these differences include glucose, maltose, succinate, butyrate, as well as the amines tyramine and cadaverine, which are known to cause malodor women with BV. Transcriptomic studies will correlate bacterial species with metabolic products. In addition we have acquired 140 samples from women in Kigali, Rwanda to determine if different profiles occur with race, diet, and pregnancy. We have also identified potential amine producing and degrading bacteria. We hope this work will improve our understanding of the metabolic interactions of bacteria in the vagina.

38.

MICROENCAPSULATION AS THE NEW HORIZON FOR PROBIOTIC PRODUCTS: THE INCREASED EFFICACY OF GASTRO-PROTECTED PROBIOTICS

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In order to be effective and confer health benefits to the host, probiotics must be able to survive passage through the stomach and upper intestine and be present in sufficient amount to impact the colon microenvironment. This feature is strongly strain-dependent, even if it could be stated that only an average of 10 to 25% of the ingested probiotic cells is able to survive and reach the gut. In any case, some strategies were employed to significantly improve the effectiveness of probiotics. Microencapsulation of bacteria with a gastro-protected material was successfully applied to anticipate and amplify the onset of the beneficial effects. Probiotical SpA has developed an exclusive, internationally patented lipidic microencapsulation technology able to coat the bacterial cells with a matrix of food grade vegetable fatty acids. The mortality of cells during the process is close to zero, thus optimally preserving the probiotic potential of beneficial strains. In order to reliably predict the number of probiotic bacteria able to reach the gut after the oral intake, *in vitro* studies were performed with real human gastric juice and bile or with specific simulations. This study highlighted the significantly improved survival of microencapsulated *Lactobacillus rhamnosus* GG (LGG®, ATCC 53103) and *Bifidobacterium lactis* BS01 (LMG P-21384) to the contact with gastric juice, bile salts, and pancreatic

secretion, as well as their better stability in prototype formulations of two widely used dosage forms of finished products, especially monodose vials containing a plug cap for powder.

39.

PROTECTIVE EFFECT OF A PROBIOTIC FERMENTED MILK AGAINST DIET-INDUCED HYPERCHOLESTEROLEMIA AND DMH-INDUCED COLORECTAL CARCINOGENESIS

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A probiotic fermented milk product, prepared by co-culturing *L. acidophilus* and *L. plantarum*, was fed separately to diet-induced hypercholesterolemic rats and rats with DMH-induced colorectal tumors. The probiotic milk attenuated diet-induced hypercholesterolemia and deposition of cholesterol and triacylglycerols in aortic and liver tissue. Plasma total cholesterol and HDL-cholesterol increased in fermented product without probiotics; while on probiotic milk, these decreased significantly. The VLDL + LDL-cholesterol level and plasma TAGs were also significantly lowered than in control group. Atherogenic index decreased significantly on probiotic group as compared to control group without probiotics. The probiotic product also attenuated DMH-induced gastrointestinal carcinogenesis. It decreased the progression of preneoplastic lesions such as aberrant crypt foci and mucin depleted foci, and prevented the rise in proliferating cell nuclear antigen labeling index, a marker for progression of carcinogenesis in GI tract. The probiotic milk augmented the glutathione-S-transferase activity in liver and colorectal tissues. The lipid peroxidation (thiobarbituric acid reactive substances in liver and colorectal tissue, and b-glucuronidase activity in feces) were also lowered in DMH-induced rats, which further correlated with decreased tumor incidence, tumor multiplicity and tumor volume in GI tract. Feeding rats with this probiotic fermented milk product increased expression of pro-apoptotic gene Bax and decreased expression of anti-apoptotic gene Bcl2, proto-oncogene c-myc and cell cycle check point gene cyclin D1, which correlated with decreased initiation and progression of carcinogenesis in GIT. The studies evidently advocate a potential protective and preventive effect of probiotics against lifestyle disorders.

40.

AN *IN VITRO* INVESTIGATION INTO THE EFFECT OF A 28 DAY PROBIOTIC SUPPLEMENT GANEDENBC30™ ON THE RESPONSE OF HUMAN FECAL MICROBIOTA OF OLDER PERSONS: THE POTENTION ROLE OF SYMBIOSIS

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During the birth process and the first two years of life, composition of the gut microbiota is influenced by the delivery process and dietary choices thereafter, but generally remains stable during adult life. However, in advancing age most notably the population of *Bifidobacterium spp.* has been seen to decline. Several studies have investigated the role of probiotic supplements on modulation of the microbiota in older persons and the favorable effects associated in terms of gut and immune function. Several live cultures of *Lactobacillus spp* and *Bifidobacterium spp*, have been investigated with positive results. This *in vitro* investigation aims to study the possible synergistic effect of probiotics and prebiotics via the use of a single stage batch culture model studying the response of fecal microbiota obtained from volunteers after a 28 day treatment of either the GanedenBC30™ or a placebo, and the response to prebiotic supplements fructooligosaccharide (FOS) and galactooligosaccharides (GOS). Bacterial enumeration will be carried out using fluorescent in situ hybridization and short chain fatty acids by gas chromatography. Both prebiotics increased populations of *Bifidobacterium spp, Lactobacillus spp., Eubacterium rectale* and *Faecalibacterium prausnitzii.* GOS specifically increased populations of *Clostridium lituseburense* and *Bacteroides spp*. Samples from volunteers on treatment-B increased populations of both *Clostridium lituseburense* and *Faecalibacterium prausnitzii* more than those on treatment-A. This data shows a different response to prebiotic supplements by the fecal microbiota of those on different treatments. This could suggest a link between probiotic supplementation and beneficial effects of prebiotics in a symbiotic relationship.

41.

BACTOFENCIN, A NOVEL BACTERIOCIN PRODUCED BY PORCINE INTESTINAL ISOLATES OF *LACTOBACILLUS SALIVARIUS*

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Bacteriocin production can be considered an important probiotic trait of intestinal Lactobacillus salivarius isolates in that it may assist colonization of the producing strains and has been shown to provide in vivo protection against gastrointestinal pathogens (Corr et al., *PNAS*, 2007). In this study, a novel bacteriocin, bactofencin, produced by the porcine-derived intestinal isolate *L. salivarius* DPC6502 has been identified and purified, and its potency against a variety of pathogenic species including *Staphylococcus aureus* and *Listeria monocytogenes,* was demonstrated. The genome of the bactofencin producing strain was sequenced with a view to establishing the identity of the corresponding genetic determinants. The mature 22 amino acid bactofencin peptide corresponds to a molecular mass of 2,782 Da, is highly basic (pI = 10) and is encoded on a chromosomally-located gene cluster. Bactofencin contains two cysteine residues which form an intramolecular disulfide bond. The bacteriocin locus also encodes an ABC transporter and a transport accessory protein, and unusually, a DltB homologue. The dlt operon is responsible for D-alanylation of teichoic acids in the cell wall of many Gram-positive bacteria and has previously been associated with bacterial resistance to cationic antimicrobial peptides. Heterologous expression of the dltB homologue in various host strains resulted in enhanced resistance to the bactofencin peptide indicating the corresponding protein is involved in bactofencin immunity. Following the identification of the relevant gene cluster, the distribution of the corresponding bacteriocin structural gene, bfnA, was assessed. Its presence in five additional isolates derived from porcine origin, which also produce the class IIb bacteriocin salivaricin P, was revealed.

42.

NF-κB AS A POTENTIAL BIO-THERAPEUTIC TARGET FOR MANAGEMENT OF DIABETES BY PROBIOTIC INTERVENTION

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Nuclear factor kappa B (NF-κB) has been recognized as a major biomolecule responsible for β cell apoptosis in pancreatic cells. Hence, an attempt was made to target the anti-inflammatory potential of probiotic *lactobacilli* for their NF-κB modulation capabilities in human pancreatic (Panc-1) cells. Probiotics are known to play an important role in modulation of immune responses in epithelial cells and may have equivalent effects on pancreatic cells. To validate this possibility, ten of the proven probiotic strains characterized as *L. plantarum* Lp9, Lp65, Lp75, Lp78, Lp91, LpS7, *L. fermentum*21, 100, Lf1 and *L. bulgaricus*CH4, were screened for their capacity to modulate NF-κB expression in Panc-1 cells. Panc-1/NF-κB/Renilla luc reporter cells were used to measure effects of different preparations of *Lactobacillus* strains on cellular expression of a reporter system for NF-κB under TNF-α stimulated conditions, which could bring about an upregulation of around 2.5 fold relative to control. Panc-1 reporter cells were treated with live probiotic *lactobacilli* and culture supernatant of THP-1 macrophages treated with live, heat killed and cell free conditioned medium of *Lactobacillus* strains in co and post-treatment conditions with TNF-α. Co and post-challenge with live probiotic *lactobacilli* significantly downregulated NF-κB expression (27 to 59% and 28 to 43%) with most significant down regulation by *L. plantarum* S7 (59%) and *L. fermentum* 100 respectively. Culture supernatant of live and heat killed *lactobacilli* treated THP-1 macrophages also showed equal efficacy in NF-κB modulation (25 to 54%). However, conditioned medium of majority of strains was not found to be much effective.

43.

UTILITY OF THE FDA "GUTPROBE" METAGENOMIC MICROARRY FOR IDENTIFICATION, GENOTYPING, AND COMPARATIVE COMMUNITY ANALYSIS OF COMMERCIALLY AVAILABLE PROBIOTICS

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Lactic acid bacteria are important microbes added to many food products and dietary supplements for their purported health benefits. Proper identification and strain specific subtyping is important for assessing safety as well as proper labeling and post-market product surveillance. We have developed the FDA "GutProbe" (gut-probiotic) array to quickly identify and genotype these strains. The array contains genes from 92 genomes and 229 plasmids of the most commonly used species found in probiotics and food ingredients that are also represented in the gut commensal environment, namely: *Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus, Bacillus, Clostridum, Leuconostoc, Pediococcus,* and *Bacteriodes*. Validation studies were completed using pure cultures of our in-house type strain collection containing 34 strains of *Lactobacillus* representing 20 different species. Applied utility of the array was conducted with commercially available probiotics to determine whether it could be utilized to quickly identify species of bacteria present for surveillance and product labeling concerns. In this regard, use of GutProbe revealed some differences that may result from improper strain designations, particularly with *L. acidophilus*, and therefore may be useful for routine monitoring of batch variation as part of a "Good Manufacturing Practices" (GMP) process. The FDA GutProbe is an efficient platform to identify important live microbial ingredients and probiotics on the market. It has effectively distinguished between strains of various Lactobacillus. The GutProbe will have the capability to identify DNA from pure culture isolates with potential applicability towards direct community or food matrix sampling.

44.

EFFECT OF PROBIOTIC CONSUMPTION ON OBESITY AND RELATED INFLAMMATION IN MICE

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The gut microflora plays an important role in maintaining human health. More recently, undesired alterations in the microbiota composition (known as dysbiosis) were suggested to play a role in the development of obesity. Obesity is associated with a state of low-grade, chronic inflammation influencing the development of obesity-related insulin resistance. Selected probiotics have been successfully used to prevent and treat inflammatory bowel diseases and, as such, could represent interesting alternatives to counteract the low-grade inflammation associated with obesity. We thus evaluated whether the oral consumption of probiotic strains or mixtures - selected for their potent anti-inflammatory capacities - could help improve metabolism and inflammation in diet-induced obesity in mice. We observed a strain-specific effect; some strains having no beneficial impact while other strains led to significant limitation of body weight gain, fat mass development, as well as to an improvement of metabolic and immune parameters, including insulin resistance. The anti-obesity effect was associated with, on the one hand, a strong decrease in the visceral adipose tissue of pro-inflammatory genes characteristic for the pro-inflammatory infiltrated macrophages, and, on the other hand, caused an increase of the expression of PPARg and FoxP3 (both markers corresponding to regulatory T-cells), also contributing to the observed protective effect. We therefore postulate that supplementation with selected probiotics can counteract diet-induced obesity by reducing the pro-inflammatory environment, including the reduced recruitment of pro-inflammatory macrophages.

45.

PREVENTION OF MENOPAUSE RELATED OSTEOPOROSIS IN MICE BY TREATMENT WITH PROBIOTIC LACTOBACILLUS REUTERI

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Osteoporosis and osteopenia are estimated to affect over 200 million people worldwide. While men, women, and children can be afflicted with various maladies that contribute to bone loss, the largest population affected by osteoporosis is post-menopausal women. Current therapies that prevent bone loss have undesirable side effects, thus the development of novel treatments is critical. Increased levels of pro-inflammatory cytokines contribute to the development of osteoporosis mediated during estrogen deficiency by disrupting the balance between bone resorption and bone formation. Moreover, inflammatory cytokines have been shown to favor bone resorption. The increase in osteoclast formation, the cell type responsible for bone resorption, is integral to the pathophysiology of postmenopausal osteoporosis. We investigated whether the anti-inflammatory probiotic strain *Lactobacillus reuteri* (*L. reuteri*) ATCC PTA 6475 is capable of preventing osteoporosis in an ovariectomized (OVX) mouse model. Our studies demonstrate that *L. reuteri* 6475 is effective in preventing osteoporosis in OVX mice, causing a complete suppression of bone loss after 4 weeks of treatment. This effect appears to be specific to *L. reuteri* because several other probiotic strains tested in the OVX model do not offer complete protection from bone loss. Using an *in vitro* cell culture assay, our data suggests that *L. reuteri* suppresses osteoclast formation by ~70%. We are currently focused on identifying both the host mechanisms impacted by *L. reuteri* 6475 as well as the bacterial factors mediating protection from bone loss.

46.

EVIDENCE THAT COLONIC SCFA PRODUCTION IS GREATER IN OVERWEIGHT THAN LEAN SUBJECTS

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Background: Animal studies suggest that the obese colonic microbiome, characterized by a low *Bacteriodetes:Firmicutes* ratio (B:F), promotes obesity because of excess short-chain fatty-acid (SCFA) production and increased colonic energy availability. A low B:F has been found in obese humans, but it is unknown if obese humans have increased SCFA production. Objectives: to compare fSCFA concentration, rectal SCFA absorption, diet and microbial profiles in lean (BMI≤25) and overweight/obese (OW)(BMI>25) subjects. Methods: 22 subjects (11 lean and 11 OW) participated in the study. Fecal samples were assessed for fSCFA concentration and microbial composition. A three day diet record was used to determine nutrients intake. SCFA absorption was assessed by disappearance of SCFA from a dialysis bag that was inserted into the rectum for 30min. Results: fSCFA concentrations were higher in OW subjects compared to lean subjects (121.1±16.4 vs.97.7±9.4mmol/kg, respectively p=0.023). Both SCFA absorption and dietary intakes were not significantly different. OW subjects had lower Bacteroidetes compositions than lean subjects (0.06±0.04 vs. 0.19±0.06, respectively p=0.044) and higher Firmicutes compositions than lean subjects (0.83±0.04 vs. 0.70±0.05, respectively p=0.03). Conclusions: Our findings suggest that the higher fSCFA concentration in OW subjects may be explained by a greater colonic SCFA production of the lower B:F, and not by different dietary intake or SCFA absorption. Further studies are needed in order to determine the effects of the increased fSCFA concentrations in OW individuals on obesity.

47.

EXPRESSION OF BLP SUBUNITS IN *STREPTOCOCCUS THERMOPHILUS*

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Bacteriocin production has been suggested as a probiotic trait since these antimicrobial peptides can prevent the growth and colonization of potential pathogens. In *Streptococcus thermophiles,* a gene cluster has been identified for the production of a bacteriocin-like peptide (BLP). In the sequenced strain LMD-9, this gene cluster is inactive due to insufficient production of the quorum sensing induction peptide (QSIP) BlpC. However, bacteriocin production was reported if exogenous QSIP was provided. In this study, a screen using synthetic QSIP identified two strains, ST106 and ST118, capable of producing an active bacteriocin. Real time PCR analysis showed that the addition of synthetic QSIP increased blpC expression by 92 and 95-fold for ST106 and ST118 respectively, and resulted in distinct zones of inhibition, using ST113 as the target bacterium. The same QSIP preparation increased blpC expression by only 35-fold in LMD-9 and zones of inhibition were faint or nonexistent. In ST110, which naturally produces bacteriocin, blpC expression was approximately 11 and 53-fold higher than that observed for induced ST106 and LMD-9 cultures respectively. Additionally, expression of blpD and blpU were analyzed by RT-PCR. Results showed blpD expression increased 91 and 67-fold in strains ST106 and LMD-9; but only 19-fold in ST118; and blpU expression increased 9-fold for ST118, and only 2 and 3-fold for LMD-9 and ST106 respectively. This study demonstrates that the components required for optimal bacteriocin activity may differ between strains, and work continues to define the essential components in ST106 and ST118.

48.

THE INTESTINAL MICROBIOTA IN HEALTHY CHILDREN IS NOT ESTABLISHED UNTIL AFTER TODDLERHOOD - AN OPPORTUNITY FOR PROBIOTICS/PREBIOTICS INTERVENTION

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It is generally believed that the infant's microbiota is established during the first 1-2 years of life. However, there is scarce data on its characterization and comparison to the adult microbiota in consecutive years. Aim: To characterize and compare the intestinal microbiota in healthy young children and adults from the USA using high-throughput bacterial phylogenetic microarray analysis. Methods: Characterization and comparison of the intestinal microbiota of healthy children (1-4 years; n=28) and healthy adults (21-60 years; n=23) was carried out using the Human Intestinal Tract Chip (HITChip) phylogenetic microarray targeting the V1 and V6 regions of 16S rRNA and quantitative PCR. Results: The HITChip data indicate that *Actinobacteria*, *Bacilli*, *Clostridium cluster IV* and *Bacteroidetes* are the predominant phylum-like groups that exhibit differences between children and adults. The phylum-like group, *Clostridium* cluster XIVa was equally predominant in young children and adults and is thus considered to be established at an early age. The genus-like level show significant 3-6 fold (higher or lower) differences in the abundance of 26 genera between children and adults. Young USA children have a significantly 3.5-fold higher abundance of *Bifidobacterium* species than adults from the same location, however, their microbiota is less diverse than that of adults. Conclusions: We show that the establishment of an adult-like intestinal microbiota continues through a later age than previously reported. Characterizing the microbiota and its development in the early years of life may help identify 'windows of opportunity' for interventional strategies such as probiotics/prebiotics to promote health and prevent disease processes.

49.

ANTI-INFLAMMATORY PROPERTIES OF PROBIOTIC *LACTOBACILLUS* AND *BIFIDOBACTERIUM*: AN *IN VITRO* STUDY USING A HUMAN COLONIC MICROBIOTA MODEL AND RAW 264.7 MACROPHAGE CELLS

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Current therapies have been developed to target the human gut microbiota in order to reduce endotoxaemia-induced inflammation. Probiotics are biotherapeutics used to shift the gut microbiota towards health-promoting bacterial populations. This study investigates the properties of probiotic candidates to modulate colonic lipopolysaccharide concentrations and inflammatory cytokines production. *Lactobacillus reuteri* NCIMB 701359, *L. rhamnosus* ATCC 53103, *L. plantarum* ATCC 14917, *Bifidobacterium animalis* ATCC 25527, *B. bifidum* ATCC 29521, *B. longum* ATCC 15707, and *B. longum subsp. infantis* ATCC 15697 were administered daily for 14 days to a well-established in vitro human colonic microbiota model. RAW 264.7 macrophage cells were stimulated with bacterial supernatant from the colonic microbiota model. Concentrations of colonic lipopolysaccharides, colonic Gram-positive and -negative bacteria, and TNF-α, IL-1β, and IL-4 cytokines were measured. Results demonstrated that lipopolysaccharide concentrations were significantly reduced following supplementation with *B. bifidum* (-46.45±5.65%; P<0.05), *L. rhamnosus* (-30.40±5.08%; P<0.05), *B. longum* (-42.50±1.28%; P<0.05), and *B. infantis* (-68.85±5.32 %; P<0.05). In addition, there was a probiotic strain-specific effect on the immunomodulatory responses of RAW 264.7 macrophage cells. Specifically, *B. infantis* decreased pro-inflammatory TNF-α cytokines secreted (-69.41±2.78%; P<0.05) and increased anti-inflammatory IL-4 cytokines (+16.50±0.59 %; P<0.05) the most. Interestingly, lipopolysaccharide concentrations were significantly correlated with TNF-α (r=0.447, P=0.002) and IL-1β (r=0.504, P=0.002) levels. As well, lipopolysaccharide concentrations were negatively correlated with IL-4 concentrations but the effect was not significant (r=-0.160, P=0.170). These findings suggest that specific probiotics, such as *B. infantis*, may decrease colonic lipopolysaccharide concentrations, thereby improving the inflammatory status.

50.

PROBIOTIC BIO-THERAPEUTIC FOR THE PREVENTION AND TREATMENT OF DENTAL CARIES

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Dental caries (DC) affects almost 100% of the world's population. The primary causative organism of DC is *Streptococcus mutans*. Current DC prevention/treatment methods have important limitations. Research has turned to probiotics as biotherapeutics for treating/preventing DC. This work investigates *L. reuteri* NCIMB 701359, *L. reuteri* NCIMB 701089, *L. reuteri* NCIMB 11951, *L. reuteri* NCIMB 702656, *L. reuteri* NCIMB 702655, *L. fermentum* NCIMB 5221, *L. fermentum* NCIMB 2797, *L. fermentum* NCIMB 8829, *L. acidophilus* ATCC 314, *L. plantarum* ATCC 14917 and *L. rhamnosus* ATCC 5310 for their ability to inhibit *S. mutans*, using semi-quantitative and quantitative evaluations. For semi-quantitative evaluation, 0.5% (v/v) of a *S. mutans* overnight culture was incorporated into molten MRS-agar and live probiotic cultures/cell-free extracts were incorporated (4 concentrations) in preformed wells. Following incubation, a clearance zone around the wells indicated probiotic inhibition of *S. mutans. L. fermentum* NCIMB 5221 (p=0.008) and *L. reuteri* NCIMB 701359 (p=0.047) demonstrated the largest clearance zones. For quantitative evaluation, all the *L. fermentum* and *L. reuteri*, selected from the semi-quantitative assay, were grown in simulated saliva with *S. mutans* for 24 h at 37ºC and 75 rpm. Selective agar was used for determining the viability of *S. mutans* and the probiotic using standard colony forming units. *L. reuteri* NCIMB 702656, *L. reuteri* NCIMB 11951, *L. reuteri* NCIMB 701359, and *L. reuteri* NCIMB 701089 demonstrated the strongest inhibition (p=0.00001) with no detectable *S. mutans*

remaining following 24 h of co-culture. This research demonstrates the potential of probiotic biotherapeutics for the prevention/treatment of DC.

51.

INFANT-ASSOCIATED BIFIDOBACTERIUM LONGUM SUBSP. INFANTIS AND *BIFIDOBACTERIUM BIFIDUM* UTILIZE HUMAN MILK OLIGOSACCHARIDES BY DIVERGENT MECHANISMS

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Species belonging to the genus *Bifidobacterium* often constitute a large fraction of the neonate's colonic microbiota. Likewise, most *Bifidobacterium* species form commensal partnerships with their animal hosts and are regarded as beneficial in several capacities. It is believed that specific *bifidobacteria* are nourished, and thus enriched, by soluble oligosaccharides transferred in mother's milk. The prominent infant-associated commensal *B. longum subsp. infantis (B. infantis)* deploys an array of solute binding proteins to facilitate import of human milk oligosaccharides (HMO) prior intracellular glycolytic digestion and downstream metabolism in the bifidus shunt. In contrast*, B. bifidum* extracellularly degrades HMO to subsequently import liberated carbohydrate products, most notably lacto-N-biose. Comparative and functional genomics have been employed to characterize and verify these disparate physiological operations. In part, RNA-seq has elucidated regulatory networks and expression profiles while subsisting on a range of HMOs. Indeed the temporal transcriptomes of *B. infantis* and *B. bifidum* exhibit divergent means by which they sense, respond, and metabolize HMO. Furthermore, previously characterized HMO-active gene suites are differentially regulated and expressed dependent on the specific HMO substrate. In sum, these data buttress the model that infant-associated *bifidobacteria* have evolved divergent physiological solutions to exploit the relative abundance of HMO localized to the nursing infant's colon.

52.

LACTOBACILLUS REUTERI ATCC 55730 SUPPLEMENTATION ENHANCES INFANT ROTAVIRUS VACCINE RESPONSE

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Improving the immunogenicity of rotavirus (RV) vaccines with probiotic supplementation was evaluated in a prospective randomized double-blind, placebo-controlled trial. Eight healthy infants received either the probiotic *L reuteri* ATCC 55730 (Lr) 108 colony forming units per day, or placebo from 10 days before receipt of their first RV immunization (enrollment) until 10 days following their last RV immunization (conclusion). Stool and serum samples were collected from 8 infants; 4 received Lr and 4 placebo. All had similar gestational ages, birth weights, and diets at enrollment. Four infants received 3-dose RV5 vaccine and 4 infants received 2-dose RV1 vaccine. Three of 4 subjects (Lr) and 1 of 4 (placebo) mounted a > 2 fold increase in serum RV immunoglobulin (Ig)G titers at conclusion compared to enrollment. Three of 4 subjects who received Lr and 2 of 4 who received placebo had serum RV IgA titers >500 at study conclusion. Two of 4 who received Lr and 1 of 4 who received placebo had a > 2 fold increase in stool IgA at conclusion compared with enrollment. C-reactive protein values were within normal range in 6 subjects at enrollment and in 3 of 3 who received Lr and 2 of 3 who received placebo at conclusion. Adverse events were rare and comparable between infants who received Lr and placebo. Among subjects, there was a trend toward increased RV IgG and IgA serum titers and fecal RV IgA in the Lr compared with placebo recipients, suggesting that Lr supplementation may boost RV vaccine immunogenicity.

53.

TECHNIQUES FOR GENETIC ANALYSIS OF SELECTED BUTYRATE-PRODUCING BACTERIA OF THE GUT MICROBIOTA

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The human gastrointestinal tract (GIT) provides a habitat for a complex microbial community, collectively known as the gut microbiota. This community has been the focus of much research in recent years in light of increasing evidence for its importance in maintaining health. The majority of the microbial community in the colon consists of strictly anaerobic bacteria belonging to two phyla: the low-G+C content Gram-positive *Firmicutes* phylum, mainly members of the *Lachnospiraceae* and *Ruminococcaceae* families; and the Gram-negative *Bacteroidetes* phylum. The butyrate-producing bacterial members of the *Firmicutes* phylum are of particular interest, as butyrate has been implicated in promoting health in the human intestine. Genome sequencing of several *Firmicute* species has recently been completed, including some of the highly oxygen-sensitive butyrate-producing bacteria, belonging to the *Lachnospiraceae* and *Ruminococcaceae* families, which have been isolated in our lab. However, detailed knowledge of the biochemistry and physiology of these bacteria has been limited by a lack of genetic manipulation techniques. Therefore, the aim of this work is the establishment of genetic manipulation techniques for a selected group of these bacteria, specifically *Faecalibacterium prausnitzii* and members of the *Roseburia/Eubacterium* rectale group. Preliminary work involved the characterization of the restriction activity of selected bacterial species and the identification of genes potentially involved in host interaction from genomic sequences. This was followed by the design of suitable methods to allow native genes to be selectively inactivated, and the establishment of methods to allow heterologous genes to be expressed in these bacteria. These data will be presented.

54.

THE EFFECT OF DAIRY WHEY PROTEIN ISOLATE ON ADIPOSITY AND THE GUT MICROBIOTA IN MICE

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Obesity has more than doubled worldwide since 1980. As obesity is linked with a wide range of diseases, developing novel therapies which would have a beneficial effect on weight gain have become extremely desirable. The gut microbiota has been recently associated with obesity and thus targeting its composition with prebiotics, probiotics or in this instance food components/bioactives is one mechanism by which a desirable effect on weight management may be achieved. Here we used 50 C57BL mice to examine the effect of supplementing a high fat diet with increasing percentages (20%, 30% and 40% respectively) of whey protein isolate (WPI) in terms of adiposity and total body weight gain. To identify the changes in the gut microbiota that result from the ingestion of WPI, total genomic DNA was extracted from fecal pellets at the trial end point and processed to facilitate culture independent analysis by high throughput sequencing. Our findings revealed that the supplementation of a high fat diet with WPI reduced the total body weight gain in the mice with the effect becoming more significant as the percentages of whey protein consumed increased, where, on average, the 20% WPI supplemented diet animals were 6.7%, the 30% WPI supplemented diet animals were 8.6% and the 40% WPI supplemented diet animals were 26.4% lighter than their high fat diet counterparts. Analysis of high throughput sequence data highlighted a number of changes in the gut microbiota that corresponded with the inclusion of WPI into the murine diet.

55.

TRANSCRIPTOMIC PROFILE OF WHOLE BLOOD CELLS FROM ELDELRLY SUBJECTS FED PROBIOTIC BACTERIA *LACTOBACILLUS RHAMNOSUS* GG ATCC 53103 (LGG)

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Probiotics are purported to confer health benefits, but little is known about their mechanism of action. Given interest in using probiotics to promote health in the elderly, we analyzed the effect of daily intake of 2 x 1010 cfu of *Lactobacillus rhamnosus* GG ATCC 53103 (LGG) for 28 days on 11 healthy elderly subjects in an open label study. We used RNA sequencing (RNA-seq) to identify differentially expressed genes (DEGs) in whole blood cells prior to consuming LGG (day 0), at the end of LGG consumption (day 28), and one month after feeding of LGG stopped. A list of DEGs after pair-wise analysis were generated using the edge-R statistical package with an FDR adjusted p-value at p=0.1. Analysis revealed a total of 44 DEGs (39 down-regulated, 5 up-regulated) with an absolute fold change (FC) between1.3-2.0 in whole blood cells on day 28. Changes resolved one month after LGG was stopped. To investigate the functional relationships between genes which are differentially expressed at day 28 and predict possible outcomes, we used Ingenuity Pathway Analysis (IPA) package to create networks in which the DEGs can be related to molecular and cellular functions based on cited scientific literature. Examination of the top gene networks activated by feeding LGG were linked to expression of eight genes related to changes in cell to cell signaling, hematological system development and function, and immune cell trafficking with an increased proliferation of lymphocytes. Overall, LGG consumption transiently modified whole blood cell transcriptomic expression in healthy elderly subjects.

56.

SUPPLEMENTATION WITH LACTOBACILLUS IS A VIABLE OPTION FOR PREVENTATIVE TREATMENT OF RECURRENT CLOSTRIDIUM DIFFICILE-ASSOCIATED DIARRHEA

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Vertical transmission of *Lactobacillus spp*. from mother to child at birth is important in establishing a healthy microbiome during the first years of life. Nourishment and secondary inoculation of the infant GI microbiota from breast milk provides another maternal source of microbes rich in lactic acid bacteria. Metagenomic studies have indicated >10-fold difference in the abundance of *Lactobacillus* in the GI tract of healthy preadolescent children compared to healthy adults. Incidence of *C. difficile* infection (CDI) increases with age and is rare in children even in the presence of this pathogen. We hypothesize that decreased abundance and diversity of *Lactobacillus* may contribute to increased susceptibility to CDI. Metagenomic data analysis of stool specimens from adults with recurrent CDI showed the relative abundance of *Lactobacillus* is significantly decreased. *Lactobacillus spp.* isolated from infant feces screened for activity against *C. difficile*-induced IL-8 production by colonocytes identified two vancomycin-resistant isolates that secreted a large (>100kDa), heat stable protective factor. Protective effects of these isolates were confirmed in a mouse model of recurrent CDI. We propose that *lactobacilli* isolated from the infant GI tract represent candidate probiotic strains that naturally interfere with *C. difficile* cytotoxicity, and repopulation of known lacto-deficient GI microbiomes with such strains may serve as an efficient means to protect against recurrent CDI.

57.

TO DIGEST OR NOT TO DIGEST — THE STORY OF BACTERIAL MUCIN METABOLISM

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The characterization of oral bacteria has been well elucidated. Numerous studies have looked into the colonization and the role this microbiome plays in health and disease. Depending on the oral health status, the mouth can be colonized by either commensal or pathogenic bacteria. This project hypothesized that oral colonization can be manipulated to give rise to beneficial health advantages to the host. It is proposed that salivary mucins can be used as a natural prebiotic for commensal bacteria. Mucins play a large role in helping commensal bacteria to colonize by acting as nutrients and a site for physiochemical protection and adherence. Mucin metabolism by commensal bacteria was tested by an oral microbial enrichment experiment. Bacteria was obtained from seven sites (front of mouth teeth-inside and outside, back of mouth teeth-inside and outside, top of tongue, inside of cheek, and between bottom front teeth) using a sterile dental brush. These bacteria were then inoculated into a basal nutrient media containing 1.25% porcine gastric mucin. After inoculation these samples were subjected to 24 and 48 turnovers in aerobic as well as anaerobic conditions. Changes in the microflora will be examined by DGGE-PCR and 454 pyrosequencing techniques. It is hypothesized that a change in the number and amounts of bacteria will be observed as the turnovers progress enabling some of the key players in mucin degradation to be identified. From this, more about the potential role mucin can play in beneficially altering the microbiota will be elucidated.

58.

PROPREMS; A RANDOMISED CONTROLLED TRIAL INVESTIGATING PROBIOTICS BB-02;BB12;TH-04 IN CULTURE-PROVEN LATE ONSET SEPSIS, IN VERY PRETERM INFANTS

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Specific probiotic strains may reduce mortality and necrotising enterocolitis (NEC) in preterm infants, with unclear effect on culture proven late onset sepsis (CLOS).This study assessed effects of a probiotic combination, on the incidence of CLOS in preterm infants< 32 weeks gestation and <1500g. A multi-centered, double-blinded, placebo-controlled, randomized trial compared daily supplementation with a probiotic combination (*Bifidobacterium infantis* BB–02 300 x 10 6, *Bifidobacterium lactis* BB-12 350 x 10 6, *Streptococcus thermophilus* TH–4 350 x 10 6 containing 1 x 10 9 total organisms,Solgar®) with placebo(maltodextrin), on the incidence of CLOS in infants. Between October 2007 and November 2011, 1099 preterm infants from ten perinatal centers in Australasia were randomized. There was no significant effect of probiotics on CLOS incidence overall but in the pre-determined stratified subgroup of infants ≥28 weeks, sepsis was reduced (10.8% versus 5.5%; Relative Risk 0.51, Confidence Intervals 0.29, 0.88, P=0.01). NEC of modified Bell stage 2 or more was also reduced (2.0% versus 4.4%; RR 0.46, 95% 0.23,0.93, P=0.03; number needed to treat 43, 95% CI 23,333). There was no effect on mortality which was low overall (5.1%). This is the largest international study of its kind worldwide with the in-hospital phase completed now and with further follow-up continuing. As most infants born prematurely on a world-wide basis are >28 weeks the sepsis reduction in this group may prove valuable with potentially greatest impact globally when translated as an adjunct to best practice in settings with highest rates of NEC, mortality and CLOS.

59.

ORAL ADMINISTRATION OF PROBIOTIC *L. FERMENTUM* NCIMB 5221 IN ZUCKER DIABETIC FATTY RATS ALLEVIATES MARKERS OF METABOLIC SYNDROME

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Metabolic syndrome (MetS) is a growing health concern of industrialized countries. This disorder encompasses type 2 diabetes mellitus, obesity, dyslipidemia, chronic low-grade inflammation, and cardiovascular disease. Ferulic acid (FA) is a phenolic acid naturally found in foods consumed by humans (ex: wheat bran, beet, coffee) that has demonstrated antioxidant activity, cholesterol-lowering capabilities, and anti-tumorigenic properties. Selected probiotic bacteria, including *Lactobacillus fermentum* NCIMB 5221, produce FA due to intrinsic ferulic acid esterase (FAE) activity. The goal of this research was to investigate a FA-producing probiotic's potential as a biotherapeutic for the treatment and prevention of MetS. *L. fermentum* NCIMB 5221 (1 x 1010 cells/dose) was orally administered to Zucker Diabetic Fatty (ZDF) rats, a model of hyperlipidemia and hyperglycemia, daily for eight weeks (n = 8). Following eight weeks of administration, the probiotic formulation reduced fasted serum insulin levels and insulin resistance, significantly reduced serum triglycerides ($p = 0.016$), significantly lowered serum low-density lipoprotein cholesterol levels ($p = 0.008$) and significantly reduced the atherogenic ($p = 0.016$) and atherosclerosis ($p = 0.012$) index, as compared to untreated animals. In addition, the probiotic formulation significantly increased high-density lipoprotein cholesterol levels ($p = 0.041$), as compared to the control animals. These results indicate that FA-producing *L. fermentum* NCIMB 5221, when administered orally, can reduce insulin resistance, hyperinsulinemia, hypercholesterolemia, and other markers involved in the pathogenesis of MetS. Further studies are required to investigate the exact mechanism(s) of action and the dose required for better modulation of the markers and pathogenesis of MetS.

60.

THE PAMS STUDY: A PREBIOTIC APPROACH TO METABOLIC SYNDROME

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Systemic and adipose tissue inflammation are associated with obesity, insulin resistance and the onset of type 2 diabetes (TD2).The gut microbiota confers a pool of potentially inflammatory mediators such as lipopolysaccharide (LPS). Levels of systemic LPS have been observed to generate a low grade chronic inflammation, termed metabolic endotoxaemia, leading to the onset of insulin resistance, a situation reversed by prebiotic use in animal experiments. Plasma levels of LPS have been found to be elevated within individuals with metabolic syndrome (MetS) and in patients with T2D. MetS is a constellation of heterogenous factors: raised blood pressure, dyslipidemia, central obesity, and insulin resistance, which increase risk of T2D and cardiovascular disease. The PAMS study has recruited 59 individuals at risk of developing MetS. The study is a randomized double blind placebo controlled cross over design using a placebo or a prebiotic, galactooligosaccharide mixture (2.75 g incorporated into 2 slices of bread and 250 ml of orange juice). The volunteers have consumed either bread or orange juice throughout the intervention. Samples have been collected of feces, blood, and 24h urine to assess changes in fecal microbiota, lipid profiles, and inflammatory markers. The intervention is complete and initial blinded analysis of the bacterial content of fecal samples show significant increases in *bifidobacteria* were produced by one of the orange juices furthermore this product has reduced elevated levels of serum triglycerides within the volunteers. Initial data looks encouraging for using a product targeting the gut microbiota to reduce some of the risk factors associated with MetS.

61.

THE EFFECT OF PREBIOTIC B-GOS ON IMMUNITY, GUT MICROBIOTA, AND METABOLISM IN THE ELDERLY

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The colonic microbiota undergo certain age related changes that may affect health. To date, research into the immunostimulating effect of prebiotics on the elderly is limited. A recent study has shown promising results with a novel prebiotic mixture, significantly enhancing the gut microbiota and immune response in the elderly. In the current study, the aim is to determine if, through consumption of a trans-galactooligosaccharide mixture (B-GOS), modification of the gut microbiota can impact on immunity and metabolomic biomarkers for immunosenescence. 40 volunteers aged 65 – 80 yrs have completed this randomized, double-blind, placebo controlled, cross-over study. 2.75g of B-GOS was consumed daily for 10 weeks, followed by a 4 week washout period and then 10 weeks of placebo (or vice versa). Treatments were coded L and T for blinding purposes. Blood, fecal, and urine samples were collected and stored for analysis of fecal bacterial populations, cytokines, inflammatory biomarkers, oxidative stress, metabolic biomarkers for immunosenescence, and bacterial endotoxins. Volunteers kept food, mood, and stool diaries throughout the study. Initial findings show *Bifidobacterium spp., Bacteroides spp.,* and *Eubacterium rectale-Clostridum coccoides* numbers significantly increased following treatment L compared to treatment T. NK cell activity significantly increased and IL-6 cytokine levels significantly decreased following treatment L compared to treatment T. Initial results seem promising, however to date the authors are still blinded. This study will lead to further knowledge of the impact of prebiotics on immune changes that occur with age.

62.

CHANGES IN THE MICROBIOTA OF BREAST MILK AFTER CHEMOTHERAPY

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It has long been known that a variety of bacteria exist in human milk, but less is known about what factors change the composition of this microbial community. Here we report a case study of a 25-year-old breast-feeding mother undergoing 4 months of chemotherapy for non-Hodgkin's lymphoma. Breast milk was collected at baseline and then before and after each of her nine chemotherapy sessions. Bacterial analysis was performed using culture, denaturing gradient gel electrophoresis, and Ion Torrent 16s rRNA sequencing. Changes in metabolite composition were assessed using gas chromatography- mass spectrometry (GC-MS). Surprisingly, culture analysis revealed a drastic drop in bacterial numbers as soon as two hours after chemotherapy. While bacterial numbers did increase during the time in between therapy, the numbers were still lower than that observed at baseline. As well, bacterial profiles changed over time and by the end of her four month treatment, we observed the presence of potentially pathogenic organisms. While metabolite data is still pending, we believe that differences will exist over the course of her treatment. Since both bacterial and non- bacterial components in breast milk from "healthy" mothers have been shown to promote gastrointestinal, immunological, and neurological development in newborns; this case study raises important questions as to the effects these observed changes would have on the breast fed infant. It also raises the question as to whether probiotics or prebiotics should be administered to breast fed infants of mothers undergoing chemotherapy in order to counteract the changes resulting from therapy.

63.

ORAL *LACTOBACILLUS* SUPPLEMENTATION ALTERS TRYPTOPHAN METABOLITE LEVELS IN BIOBREEDING RATS: THE ROLE OF HYDROGEN PEROXIDE AND INDOLEAMINE 2,3-DIOXYGENASE

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Members of the gut microbiota influence the immunological and metabolic status of their mammalian hosts through a variety of mechanisms. Our previous work showed that feeding the intestinal commensal *Lactobacillus johnsonii* to diabetes-prone rats decreased the incidence of disease development. This decrease correlated with lower levels of ileal indoleamine 2,3-dioxygenase (IDO), a central immunoregulatory enzyme and the rate-limiting enzyme in tryptophan catabolism. A follow-up study was performed to characterize the effect of *L. johnsonii* feeding on IDO activity in juvenile pre-diabetic rats. *L. johnsonii* feeding resulted in a significant reduction of serum kynurenine when compared to vehicle-fed controls, correlating with a 1.4-fold elevation in 5-hydroxytryptamine levels. *In vitro* analysis using affinity-purified IDO found that H_2O_2 production by *L. johnsonii* abolished the activity of this redox sensitive enzyme. Interestingly, *L. johnsonii* feeding resulted in a 3.9-fold increase in ileum lumen H_2O_2. A 47% reduction in IDO activity in IFNγ stimulated HT-29 intestinal epithelial cells when compared to vehicle treated controls, an effect eliminated by catalase treatment. Immunofluorescence microscopy analysis of total kynurenine-induced protein modifications and H_2O_2 levels in intestinal epithelial cells treated with *L. johnsonii* and *L. johnsonii* cell-free supernatant support the role of both bacterially-derived and host-derived H_2O_2 in altering IDO activity. This work highlights H_2O_2 as a significant signaling molecule in commensal-host interactions and IDO as a target of these interactions.

64.

THE EFFECT OF DAILY ORAL INTAKE OF PROBIOTICS ON THE FREQUENCY AND INTENSITY OF MIGRAINE ATTACKS – A PILOT STUDY

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Migraine prevalence is increased in disorders associated with a leaky gut, possibly because undigested food particles and bacterial components leak from the intestine into the circulation inducing pro-inflammatory immune responses associated with migraine. Probiotics may decrease the intestinal permeability, and therefore may help to reduce the frequency and/or intensity of migraine attacks. To assess clinical effect, feasibility, and adverse reactions of probiotic treatment in migraine, 29 migraine patients took 2 grams of a probiotic food supplement (Ecologic®Barrier, 2.5x109 cfu/gram) per day during 12 weeks. Participants recorded frequency and intensity of migraine (10-points Likert scale) in a daily headache diary and completed the Migraine Disability Assessment Scale (MIDAS) and Henry Ford Hospital Headache Disability Inventory (HDI) at baseline and after 12 weeks of treatment. 27/29 (93%) completed the study. Compliance with the ingestion of the probiotics was 95%. Four patients reported obstipation during the first two weeks of treatment, which diminished in the third week. The mean number±SD of migraine days per month decreased significantly from 6.7±2.4 at baseline to 5.1± 2.2 [p=0.008] in week 5-8 and to 5.2 (2.4) in week 9-12 [p=0.001] of treatment. The mean intensity of migraine (SD) decreased significantly from 6.3± 1.5 at baseline to 5.5±1.9 after treatment [p=0.031]. The MIDAS(SD) score improved from 24.8± 25.5 to 16.6±13.5 [p=0.031]. However, the mean HDI did not change significantly. Probiotics may decrease migraine attack frequency. Feasibility and lack of adverse reactions warrant further placebo-controlled studies.

65.

SPECIES-LEVEL METAGENOMIC ANALYSIS REVEALS SUBTLE CHANGES OF THE GUT MICROBIOME FOLLOWING CONSUMPTION OF A FERMENTED MILK PRODUCT CONTAINING *BIFIDOBACTERIUM LACTIS* CNCM I-2494

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The gut microbiota is an open ecosystem composed of resident commensals exposed to transient bacteria conveyed by the diet. Fermented Milk Products (FMPs) contain <109 bacteria/g. When FMPs-derived bacteria reach the colon, their relative abundance is estimated to be < 1% of the colonic microbiota. Consequently, only subtle changes to the resident microbiota are expected. Such changes, that may have important consequences for gut ecosystem functioning and host health, can only be observed by using a high-resolution technology that allows species and genes identification. Recently, the MetaHIT consortium developed a bioinformatic pipeline that enables i) a species-level resolution and ii) the assignment of microbial genes to unknown species based on the principle that genes of the same species must co-vary in abundance due to their linkage on bacterial chromosomes (Nielsen et al., submitted). Hence, clusters of genes that co-vary across a large sample set are likely to belong to the same species. We applied this approach to understand the impact of the daily consumption of a FMP on the human gut microbiome in comparison to an acidified milk product (MP) in patients with Irritable Bowel Syndrome (IBS) with predominant constipation (n=28). Fecal samples originated from a 4-week intervention study (Agrawal et al., *Alimentary Pharmacology & Therapeutics*, 2009.) We observed a modulation of identified and unknown species in subjects consuming the FMP versus MP. Metabolic reconstruction of the known and unknown species suggests that metabolic cross-feeding potentially occurs between the administered bacteria and commensals which may be related to the improvement observed in IBS patients consuming FMP.

66.

DOSE ESCALATION, SAFETY, AND IMPACT OF RENADYL™, A PROPRIETARY PROBIOTIC DIETARY SUPPLEMENT ON CKD III AND IV PATIENTS- PRELIMINARY OUTCOMES

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In continuation with the previous multisite trial an open label, dose escalation study was carried out at Thomas Jefferson University. The safety and impact of an orally administered dietary supplement Renadyl™ (*S. thermophilus* KB19, *L. acidophilus* KB27 and *B. longum* KB31-proprietary formulation containing 30B CFU per capsule) in CKD stage 3 and 4 patients with compromised renal function was studied. Following baseline data collection, 31 patients were started with an initial dose of 90B CFU per day at month 1 followed by 180B CFU per day (Month 2) and 270B CFU per day (month 3 and 4). This was followed by a two month washout period. Statistical analysis was done using SAS software and mixed model methodology for repeated measurements. Out of 31 participants, 28 (90.3%) completed the study. None showed any adverse effects. The dose of 270B CFU/day was well tolerated by all. Uremic markers such as BUN declined (baseline vs month 4, -3.55 mg/dL, p=0.089), (month 1 vs month 4, -3.8mg/dL, p=0.069), creatinine declined (month 2 vs. 6, -0.23mg/dL, p=0.0126, baseline vs month 6 p=0.089) and potassium declined (month 1 vs 6 -0.213mEq/L, p=0.059). Statistically significant improvement was seen in hemoglobin (p=0.0003), hematocrit (p=0.005) and RBC (p <0.0001). Middle molecules such as β2 microglobulin and protein bound solutes like indoxyl sulfate, serum

pentosidine and p-cresyl sulphate varied widely between patients and thus failed to show any statistical significance. QOL showed an improvement in emotional wellbeing (p=0.10) and energy/fatigue (p=0.12), with no change in physical, mental, pain, and social well-being.

67.

SAFETY OF LGG PROBIOTIC IN HEALTHY, ELDERLY VOLUNTEERS

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Lactobacillus rhamnosus GG (ATCC 53103), or LGG, is a probiotic consumed to confer "health benefits" by 2-5 million people daily since the mid 1990s. Since the FDA had concerns about its safe use, particularly as a potential vaccine adjuvant, we conducted three studies all under investigational new drug applications (IND) (one with only LGG, no vaccine administration, followed by two randomized trials with LGG or placebo and influenza vaccine administration.) All studies evaluated the safety and tolerability of oral LGG (1010 CFU twice daily for 28 days) in healthy, elderly volunteers aged 65-80. Safety was evaluated from medical histories eliciting information about side effects, vital signs, physical examinations, and laboratory tests before, during, and one month after the consumption of LGG or placebo. We recorded side effects reported daily over the same period. In the 15 open label study subjects, there were 47 non-serious AEs, 35 (74% judged to be unrelated to LGG.) In the two randomized trials where 58 subjects received the influenza vaccine and either LGG or placebo, there were 477 adverse events, 464 (97%) judged unrelated to LGG or placebo. The blind has not been broken, but rates of adverse events are similar in the two groups. The majority of side effects in all three studies were mild gastrointestinal complaints of short duration that resolved without therapy and did not require discontinuation of study drug. Our study found LGG had a comparable safety profile to placebo and is safe and well tolerated by healthy, elderly subjects.

68.

β-CAROTENE-PRODUCING GUT-COLONIZERS TO ERADICATE VITAMIN A DEFICIENCY: PROOF OF CONCEPT

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Vitamin A Deficiency (VAD) is a public health problem with detrimental effects on the growth and development of millions of children worldwide. Current strategies to alleviate VAD include food fortification, supplements, and introducing horticulture education, which rely on continuous supplementation. We explored the possibility of creating "friendly" gut-colonizing probiotics that synthesize β-carotene, the most abundant and well-characterized vitamin A precursor, thus providing the body with vitamin A regardless of food intake. As proof of concept, *Escherichia coli* MG1655*, a strain with high efficiency of intestinal colonization, was transformed with the pAC-BETA plasmid carrying the genes crtB, crtI, crtY and crtE that encode enzymes required for β-carotene synthesis from isopentenyl pyrophosphate. This transformant strain was administered to 5-week old mice lacking β-carotene-15,15'-oxygenase (CMOI), which cleaves β-carotene to generate vitamin A in mammalian tissues. Due to their inability to cleave β-carotene in any tissue, these mice represent a suitable model to demonstrate that β-carotene produced in the intestine by *E. coli* MG1655* (pAC-BETA) can indeed be absorbed and distributed to various sites in the body. Our data support this hypothesis. Enumeration of *E. coli* in feces showed consistent levels (107–108 cfu/g) suggesting colonization. *E. coli* (105–108 cfu/g) were also recovered from the mucosa of the intestine and colon of the colonized mice. Furthermore, we detected β-carotene not only in the feces, but also in the serum and liver, by HPLC analysis. Further studies are being carried out to introduce the carotenoid genes into a vector for use in probiotic bacteria.