

# 2013 ISAPP Student and Fellows Association (SFA) Conference

Authors' Abstracts and Conference Schedule



New York, NY, USA  
June 11<sup>th</sup>-14<sup>th</sup>, 2013

## ISAPP-SFA 2012 -2013 Executive Committee

**Paul Blatchford**

President



**Ruth Toward**  
Communications  
Executive (Webmaster)



**Juhani Aakko**  
Communications  
Executive (Newsletter)



**Paul Sheridan**  
ISAPP and Industry Liaison  
Executive



**Suzanne Barnes**  
Finance Executive



**Peera Hemarajata**  
Events Executive



**The ISAPP-SFA committee would like to thank:**

- Mary Ellen Sanders, Gregor Reid, and the ISAPP Board of Directors for integrating the SFA into the ISAPP annual meeting, and for their financial support of the SFA organization.
- The New York Academy of Sciences and the Sackler Institute of Nutrition Sciences for co-hosting the conference and allowing comprehensive student participation.
- All the guest speakers for taking the time to impart their advice and insight.

## SFA Meeting Schedule

### Day 1 (Monday, June 10<sup>th</sup>)

5:00pm-7:00pm                      Registration (Chelsea Hostel Common Area)

This is a chance to get name-tags, abstract booklets and general meeting information. Directions to Tuesday's events at the Hotel Pennsylvania will be given. Arrivals to NYC may be after this time, so it is not compulsory to attend the registration.

### Day 2 (Tuesday, June 11<sup>th</sup>) - All day at Hotel Pennsylvania NYC - Madison Room 18<sup>th</sup> Floor

7:00am-9:00am                      Breakfast and walk to Hotel Pennsylvania.

9:00am-10:30am                      Welcome. SFA member introductions and presentations: (5-10 minute quick introduction and brief description of your research)

10:30am-11:00am                      Morning Tea

11:00am-1:00pm                      SFA member introductions continued....

1:00pm -2:00pm                      Lunch

2:00 –5:00pm                      Lecture Session

2:00 – 2:30pm                      **Arthur Ouwehand                      Industry and academia working together**

2:30 – 3:00pm                      **Glenn Gibson                      A case study on a spin-off company based on prebiotic research**

3:00 – 3:30pm                      **Break**

3:30 – 4:00pm                      **Gregor Reid                      Translating research into nutritional interventions**

4:00 – 4:30pm                      **Karen Scott                      Government policies directing scientific research and vice versa**

4:30 - 5:00pm                      **Tom Magaldi                      Planning for a fulfilling career**

7:30pm -                      **Dinner – SFA Only - Seven Bar and Grill**

### Day 3 (Wednesday, June 12<sup>th</sup>)

*Note: All events on this day are at 7 World Trade Center, 250 Greenwich St, 40th Fl, New York, NY. See ISAPP program for complete schedule.*

7:45 AM            **Registration, Poster Set-up, and Continental Breakfast**

8:30 AM            **Opening Remarks**

Brooke Grindlinger, PhD, The New York Academy of Sciences  
Mandana Arabi, MD, PhD, The Sackler Institute for Nutrition Science  
Patricia L. Hibberd, MD, PhD, Harvard Medical School / Massachusetts  
General Hospital for Children  
Colin Hill, MSc, PhD, DSc, MRIA, University College Cork, Ireland;  
International Scientific Association for Probiotics and Prebiotics

#### ***Session I: Putting Probiotics, Prebiotics, and the Microbiome into Translational Context***

8:45 AM            *Economic Assessment of Disease Reduction and Prevention — Challenges & Perspectives for Probiotics and Prebiotics*  
John Hutton, University of York, United Kingdom

9:15 AM            *Impact of Antibiotic Exposures on Microbiota and Implications for Public Health*  
Martin J. Blaser, MD, New York University School of Medicine

9:45 AM            **Networking Coffee Break**

#### ***Session II: Programming the Microbiome***

10:15 AM          *When and How Do Microbes and the Host First Become Aligned?*  
David A. Relman, MD, Stanford University

10:45 AM          *When the Programming Goes Awry: Diabetes, Obesity, and Beyond*  
Patrice D. Cani, PhD, Université Catholique de Louvain, Belgium

### **Session III: Future Directions for Translating Research to Transform Healthcare**

11:15 AM *Translating Research into Public Policy*  
Sir Harry Burns, DSc, MPH, Scottish Government, Scotland

11:40 AM **Open Discussion**  
*Probiotic / Prebiotic / Microbiome Research Outcomes and Policy Strategies that Have the Potential to Transform Healthcare*  
**Moderator:** Gregor Reid, PhD, MBA, Western University / Lawson Health Research Institute, Canada  
**Panelists:** Sir Harry Burns, DSc, MPH, Scottish Government, Scotland  
Martin Kullen, PhD, Pfizer Consumer Healthcare  
David A. Mills, PhD, University of California, Davis  
Bruno Pot, PhD, Institut Pasteur de Lille, France

12:30 PM **Luncheon**

### **Session IV: Who Is in Control — the Brain or the Microbes?**

1:30 PM *How Bacteria Can Influence Brain Development, Circuitry, and Behavior*  
Jane A. Foster, PhD, McMaster University, Canada

2:00 PM *Prebiotic Supplementation Alters Hypothalamic Neuronal Activity and Protects Against the Obesogenic Environment*  
Gary Frost, PhD, Imperial College London, United Kingdom

### **Session V: Hot Topics in Prebiotic and Probiotic Research and Development**

2:30 PM *Impact of a Short Chain Galactooligosaccharide on the Human Microbiome and Symptoms of Lactose-intolerant Individuals*  
Todd Klaenhammer, PhD, North Carolina State University

2:45 PM *Beneficial Effects of Prebiotics and Probiotics on the Gut-Brain Axis and Regulation of Body Weight*  
Helen E. Raybould, PhD, University of California, Davis

3:00 PM **Early Career Investigator Presentation 1**

3:15 PM **Early Career Investigator Presentation 2**

3:30 PM **Networking Coffee Break**

### **Session VI: Reaching People in Need with Innovative Probiotic Interventions**

4:00 PM *Challenges to Translating Science to the People with the Greatest Need*  
Andrew Serazin, PhD

- 4:20 PM *From Yoghurt to Vaccine for the Developing World*  
Gregor Reid, PhD, MBA, Western University / Lawson Health Research Institute, Canada  
Patricia L. Hibberd, MD, PhD, Harvard Medical School / Massachusetts General Hospital for Children
- 4:40 PM *Fecal Transplantation for Obesity and Type 2 Diabetes Mellitus*  
Max Nieuwdorp, MD, PhD, University of Amsterdam, The Netherlands
- 5:00 PM *Faecalibacterium prausnitzii for Inflammatory Bowel Disease*  
Joël Doré, PhD, Institut National de la Recherche Agronomique (INRA), France
- 5:20 PM *Overcoming the Regulatory Roadblocks to Non-Drug Applications of Microbiome-Based Health Interventions*  
Fred H. Degnan, JD, King & Spalding LLP
- 5:40 PM **Closing Remarks**  
Mary Ellen Sanders, PhD, International Scientific Association for Probiotics and Prebiotics
- 6:00 PM **Networking Reception and Poster Session**
- 7:30 PM **Conference Adjourns**
- 8:00PM **Dinner – SFA Only – American Flatbread Tribeca**

#### **Day 4 (Thursday, June 13th)**

Those who are invited, attend the discussion groups. Everyone else has a free day to explore NYC. Tourism options will be provided during registration but due to high costs in NYC, everything is optional.

#### **Day 5 (Friday, June 14th)**

*Note: All events on this day are at 7 World Trade Center, 250 Greenwich St, 40th Fl, New York, NY*

- |                  |   |
|------------------|---|
| 7:45am-8:40am    | Continental breakfast   |
| 8:40am-9:00am    | Report from ILSI Immune biomarkers for probiotic/prebiotic studies. Arthur Ouwehand |
| 9:00am-10:00am   | Wrap-up session lectures from discussion groups                                     |
| 10:00am-10:30am  | Morning tea   |
| 10:30am -12:00pm | Wrap-up session lectures continued  |
| 12:00pm –1:00pm  | Lunch   |

1:00pm –3:20pm

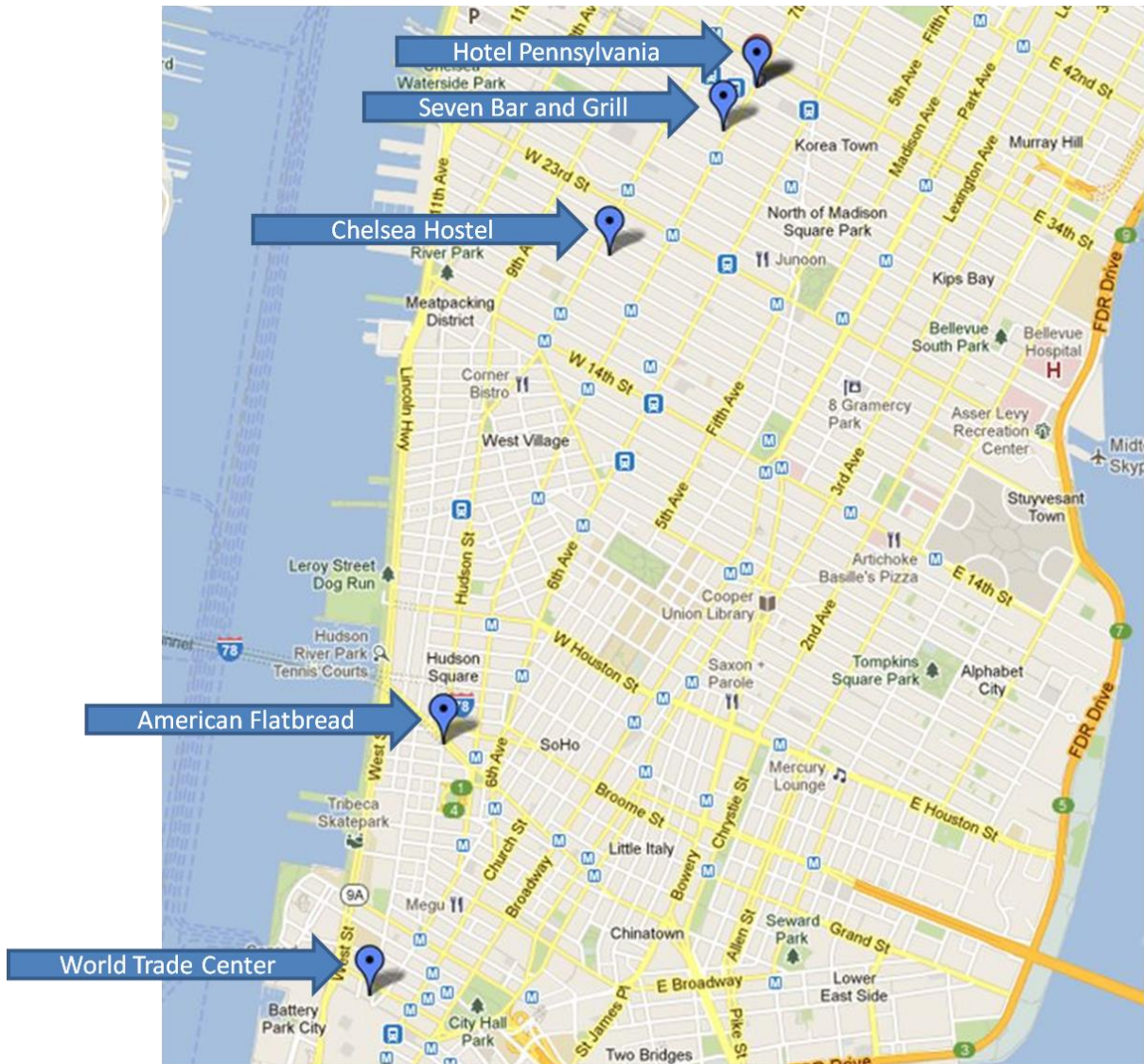
IAC Learning Forum. Effective design and reporting of clinical Trials (IAC, SFA, and ISAPP invited participants)

Discussion group topics:

- Use of evidence based nutrition concepts to develop probiotic and prebiotic dietary recommendations – scientific and regulatory perspectives. Chairs: Mary Ellen Sanders and Seppo Salminen
- Use of probiotics and/or prebiotics to program fetal and newborn health / first 1000 days of life. Chairs: Michael Cabana and David Mills
- Influence of neurochemical-producing probiotics and the microbiome on the brain and its function including behavior (appetite control, autism). Chairs: Eamonn Quigley and Ted Dinan
- Is a “normal” microbiome a “healthy” microbiome, and can a more optimal one be created through probiotic/prebiotic manipulation? Karen Scott and Todd Klaenhammer
- Personalized probiotics and prebiotics. Colin Hill and Fredrik Backhed



# SFA NYC Events Map



## Message from the President

Dear SFA members,

Welcome to the annual ISAPP SFA meeting in collaboration with the New York Academy of Sciences and the Sackler Institute of Nutrition Sciences. It has been a busy year for us on the committee, with only about eight months since our previous SFA meeting in Ireland last year. It has certainly been an eventful eight months with job positions/opportunities posted, newsletters sent out and ultimately, the preparation for this meeting in New York. It was challenging this year as we had no-one “on the ground” in New York to help coordinate events and of course, the high costs for just about everything was a constant struggle! I’d like to thank all the members of the SFA committee for their efforts in organising this conference, without which it wouldn’t have been possible.

We hope that as SFA members, in addition to getting something out of the events in this conference that you are able to give back to the running of the SFA. A new committee will need to be elected and established to continue the operation of SFA events and work towards preparing for the 2014 meeting. Please consider if you may want a position on the new committee or if you can contribute to our activities in any capacity.

I believe we are all embarking on exciting and pertinent research in an ever expanding field. Although the scope of research is vast the probiotics/prebiotics community is quite small. You will undoubtedly encounter many of the students you’ll meet at this conference again and again over the course of your career. Additionally, many of the ISAPP members both from industry and academia that you will meet may be potential collaborators, funders or employers. With that in mind, we hope you make the most of this opportunity to network and make contacts for future collaboration.

Sincerely,

A handwritten signature in black ink, appearing to read 'Paul Blatchford', with a stylized flourish at the end.

Paul Blatchford

2012-2013 SFA President

## List of Presenters

<b>Presenter</b>	<b>Country</b>	<b>Email</b>	<b>Page</b>
Alan Marsh	Ireland	alan.marsh@teagasc.ie	11
Alejandra Cardelle	Portugal	typica01@hotmail.com	12
Amandine Everard	Belgium	amandine.everard@uclouvain.be	13
Amir Ardeshir	USA	aardeshir@ucdavis.edu	14
Amy McMillan	Canada	amcmil2@uwo.ca	15
Beatriz Gullón	Portugal	bgullon@uvigo.es	16
Benjamin Offei	Belgium	benjamin.offei@mmbio.vib-kuleuven.be	17
Camilla Urbaniak	Canada	curbania@uwo.ca	18
Chunxu Gao	USA	cgao@bcm.edu	19
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Sara Quercia	Italy	saraquercia@libero.it	45
Siobhan Clarke	Ireland	siobhan.clarke@teagasc.ie	46
Tamara Tompsett	UK	tamara.tompsett@pgr.reading.ac.uk	47

## **Sequence-based analysis of Bacterial and Fungal Populations of Naturally-Fermented Beverages and Subsequent Screening for Antimicrobial Producers**

Alan Marsh,<sup>1,2</sup> P. R. Ross, Prof,<sup>1,3</sup> P. D. Cotter, PhD<sup>1,3</sup> and Prof Colin Hill, PhD<sup>1,3</sup>

<sup>1</sup>Teagasc Food Research Centre, Cork, Ireland; <sup>2</sup>University College Cork, Ireland; <sup>3</sup>Alimentary Pharmabiotic Centre, University College Cork, Ireland.

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.

## **MODIFIED CHITOLIGOSACCHARIDES 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

CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Dr. António Bernardino Almeida, 4200-072 Porto, Portugal

Chitooligosaccharides (COS) are promising substrates as source of new prebiotic ingredients. However, these COS must be modified, because the amino groups present in its 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.

Antiadhesive 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.

Acknowledgements: A. Cardelle-Cobas thanks Fundação para a Ciência e a Tecnologia (FCT) for a postdoctoral fellowship (SFRH/BDP/90069/2012). Financial support for P. Gullón and B. Gullón was provided by postdoctoral fellowships ref. SFRH/BPD/79942/2011 and SFRH/BPD/79941/2011, respectively, also issued by FCT.

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

Amandine Everard, MSc<sup>1</sup>, Florian Pierard, BS<sup>1</sup>, Lucie Geurts, MSc<sup>1</sup>, Thibaut Duparc, PhD<sup>1</sup>, Laure B. Bindels, PhD<sup>1</sup>, Giulio G. Muccioli, PhD<sup>2</sup>, Nathalie M. Delzenne, PhD<sup>1</sup>, Sylvie Robine, PhD<sup>3</sup>, Serge Luquet, PhD<sup>4</sup> & Patrice D. Cani, PhD<sup>1</sup>

<sup>1</sup>Université catholique de Louvain, Louvain Drug Research Institute, Metabolism and Nutrition research group, Brussels, Belgium; <sup>2</sup>Université catholique de Louvain, Louvain Drug Research Institute, Bioanalysis and Pharmacology of Bioactive Lipids research group, Brussels, Belgium; <sup>3</sup>Centre National de la Recherche Scientifique, Institut Curie, Paris, France; <sup>4</sup>Unité de Biologie Fonctionnelle et Adaptative, Sorbonne Paris Cité, Université Paris Diderot-Paris 7 Paris, France

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 endocannabinoid system.

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

Amir Ardeshir, DVM, MPVM, PhD<sup>1,\*</sup>, Marcus Rauch, PhD<sup>2</sup>, Nicole Narayan, BSc<sup>1</sup>, Susan Lynch, PhD<sup>2</sup> and Dennis Hartigan-O'Connor, MD, PhD<sup>1,3</sup>

<sup>1</sup>California National Primate Research Center, University of California, Davis, California; <sup>2</sup>Gastroenterology Division, Department of Medicine, University of California, San Francisco, California; <sup>3</sup>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 labelled bacterial products were fragmented and biotin labelled 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.

## The vaginal metabolome in health and dysbiosis

Amy McMillan, BMSc<sup>1,2</sup>, Jean Macklaim, BSc<sup>1,3</sup>, Mark Sumarah, PhD<sup>4,5</sup>, Jonathan Swann, PhD<sup>6</sup>, Jeremy Burton, PhD<sup>1,7</sup> and Gregor Reid, PhD<sup>1,2,7</sup>.

<sup>1</sup>Human Microbiology and Probiotics, Lawson Health Research Institute, Western University, London, Ontario, Canada; <sup>2</sup>Department of Microbiology and Immunology, Western University, London, Ontario, Canada; <sup>3</sup>Department of Biochemistry, Western University, London, Ontario, Canada; <sup>4</sup>Agriculture and Agri-food Canada, London, Ontario, Canada; <sup>6</sup>Department of Food and Nutritional Sciences, University of Reading, Reading, United Kingdom; <sup>7</sup>Department of Surgery, Western University, London, Ontario, Canada.

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 Canada at any given time. Current diagnostic techniques are unreliable and treatment with antimicrobials has poor efficacy and a high recurrence rate (58%). High throughput sequencing studies by our group have uncovered over 250 bacterial species in the vagina, and an increased diversity in BV. The final piece in understanding the vaginal environment is to characterize the metabolome (the complete set of small molecules in a given environment). We hypothesize that small molecules produced by the microorganisms that thrive in vaginal infections such as BV play a role in the etiology of the condition, and therefore the metabolome of women with infection will be distinct from healthy women. Using untargeted gas chromatography mass spectrometry (GC-MS) techniques we have identified over 100 compounds in vaginal samples. Using principle component analysis we have demonstrated that the vaginal metabolome of women with BV is distinct from a healthy profile (n = 39). Compounds responsible for these differences include sugars, short chain fatty acids, and the amines tyramine and cadaverine, which are known to cause malodor in women with BV. Future work will combine the metabolome, bacterial transcriptome and microbiome to attempt to map metabolites back to their species of origin. This work will improve our understanding of the metabolic interactions between the bacteria in BV so that better treatments can eventually be developed.



## Antiadhesive properties assessment of prebiotic oligosaccharides derived from agroindustrial hemicellulosic wastes

Beatriz Gullón<sup>1\*</sup>, Patricia Gullón<sup>1</sup>, Alejandra Cardelle-Cobas<sup>1</sup>, M. I. Montenegro<sup>1</sup>, Freni Tavaría<sup>1</sup>, Ana María Gomes<sup>1</sup>, Maria Manuela Pintado<sup>1</sup>

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Prebiotic oligosaccharides are defined as nondigestible food ingredients that provide beneficial effects to the host by stimulating the growth of selected microbial members of the gastrointestinal tract among other relevant effects.

Besides their direct activity on microbiota composition, another mechanism by which prebiotic oligosaccharides may confer health benefits is via inhibition of intestinal infection through their antiadhesive activity. It is known that certain exogenous oligosaccharides structurally resemble the receptor sites coating the intestinal epithelial cell to which intestinal pathogens recognize and adhere. Accordingly, these oligosaccharides may act as molecular receptor decoys or antiadhesives that can competitively inhibit pathogen adherence. The search of oligosaccharides that combine direct effect on microbiota with antiadhesive activity is a relevant issue in the last decade. Therefore, this bioactivity could be an encouraging factor in the manufacture of future prebiotic oligosaccharides. The prebiotic properties of oligosaccharides derived from agro industrial hemicellulosic wastes were extensively studied (1); however, other activities such as antiadhesive properties have not yet been investigated. The aim of this work was to evaluate the ability of several oligosaccharides derived from different agroindustrial wastes (*Eucalyptus globulus*, rice husks, barley wastes, wheat bran) to inhibit the adhesion of *Bacillus cereus* to mucin. So, the results of the present study indicated that oligosaccharides from agroindustrial wastes can act as efficient molecules against *Bacillus cereus* adherence to mucin, opening new opportunities for application of these functional ingredients.

(1) Gullón, P., González-Muñoz, M. J. and Parajó, J. C (2011). Manufacture and prebiotic potential of oligosaccharides derived from industrial solid wastes. *Bioresources Technology*, 102 (10), 6112- 6119.

Acknowledgements: B. Gullón thanks Fundação para a Ciência e a Tecnologia (FCT) for a postdoctoral fellowship SFRH/BPD/79941/2011. Financial support for A. Cardelle-Cobas and P. Gullón was provided by postdoctoral fellowships ref. SFRH/BDP/90069/2012 and SFRH/BPD/79942/2011, respectively, also issued by FCT.

## **IN VITRO ASSESSMENT OF GASTRO-INTESTINAL SURVIVAL SUPERIORITY BETWEEN SACCHAROMYCES CEREVISIAE VAR. BOULARDII AND VARIOUS SACCHAROMYCES CEREVISIAE STRAINS.**

Benjamin Offei, MSc<sup>1,2</sup>, Maria Foulquié-Moreno, PhD<sup>1,2</sup>, Françoise Dumortier, PhD<sup>1,2</sup>, Johan Thevelein, PhD<sup>1,2</sup>

Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, KU Leuven, Heverlee, Flanders, Belgium<sup>1</sup>; Department of Molecular Microbiology, Flanders Institute for Biotechnology, Heverlee, Flanders, Belgium<sup>2</sup>.

*Saccharomyces cerevisiae var. boulardii* (*S. boulardii*) is a thermotolerant yeast strain that has been employed for several decades as a probiotic. Probiotics are live microorganisms that confer beneficial effects on their hosts when administered in adequate amounts. Common probiotics are bacteria (*Lactobacillus* and *Bifidobacteria*), therefore *S. boulardii* appears unique as the only commercially available yeast for the treatment of gastrointestinal disorders in humans. *S. boulardii* has been demonstrated as being closely related to *Saccharomyces cerevisiae* (*S. cerevisiae*) genetically. However these yeast strains exhibit dissimilar attributes physiologically and metabolically. These differences are thought to confer on *S. boulardii*, the ability to act as a probiotic. The phenotypic differences of probiotic relevance between these two strains, if clearly defined, may be exploited to determine the polygenic basis behind the probiotic properties in *S. boulardii* or other *S. cerevisiae* strains. The objective of this work was to screen different *S. cerevisiae* strains vis-à-vis *S. boulardii* from various sources for differential low pH and bile tolerance. By means of colony counting and a dye exclusion method based on flow cytometry, we have observed superior tolerance by *S. boulardii* strains towards a low pH environment akin to the gastric milieu. We have additionally observed that *S. boulardii* strains from various sources exhibit different levels of tolerance on exposure to low pH environments. On the other hand, spot assays on rich medium after yeast exposure to oxgall revealed no significant differences in tolerance to bile between *S. boulardii* and *S. cerevisiae*

## CHANGES IN THE MICROBIOTA OF BREAST MILK AFTER CHEMOTHERAPY

Camilla Urbaniak, MSc<sup>1,2</sup>, Michelle Angelini, RN<sup>3</sup>, Jeremy Burton, PhD<sup>1,2</sup>, Gregor Reid, PhD<sup>1,2</sup>

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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 9 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 4 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.

## 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.

## 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.

## Isolation and Characterization of Biosurfactants Synthesized by Probiotic Lactic Acid Bacteria

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Biosurfactants are amphiphilic compounds produced by microorganisms with pronounced surface and emulsification activities. Some lactobacilli play a protective role by producing compounds such as hydrogen peroxide, lactic acid, bacteriocins and biosurfactants, which restrain the growth of potential pathogens. Besides, lactobacilli interfere with pathogens by competitive exclusion from receptors present on the surface of the epithelial cells and by co-aggregation with them, which contributes to create a barrier that prevents colonization by pathogens. Various probiotic lactic acid bacteria have been isolated from different fermented and tribal foods across the country.

All the strains were screened for the production of biosurfactant using various methods such as surface tension measurement, emulsification index and micro titer plate assay. Further, all the strains were identified using their carbohydrate metabolization, 16S rRNA amplification and Sanger dideoxy sequencing. Various strains of *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus helveticus*, *Lactococcus lactis*, *Enterococcus faecium* and *Lactobacillus fermentum* were found as putative biosurfactant producer. Further, structural composition was determined using TLC, FTIR, NMR and GCMS. Biosurfactant was tested for their antimicrobial and antiadhesive properties against food borne pathogens at different concentrations. The result obtained suggests the possible use of this biosurfactant as an alternative antimicrobial to conventional antibiotics. Biosurfactant produced by different lactobacilli and their composition has not been extensively studied and only a few have been partially characterized.

## **Extracellular enzymes of *Pediococcus acidilactici*: A Lactic Acid Bacteria with Probiotic Potential**

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Natural Health Products (NHP) regulations define probiotic as a monoculture or mixed culture of live microorganisms that is beneficial to the microbiota indigenous to humans. Though, safety of traditional LAB is not in question, but according to NHPr, products containing some strains/species will be rejected without further consideration. *In vitro* testing critical for assessing probiotic potential of *Pediococcus acidilactici* has given encouraging results (unpublished results). Though there is insufficient research on how probiotic microbes benefit the host. Probably they benefit by producing some antimicrobial compounds, lowering the blood ammonia and also by producing digestive enzymes. Understanding of enzymes secreted by probiotic bacteria will help us to understand the underlying mechanism. We have already explored some exopeptidases with synthetic substrates. Isolation, purification and characterization of endopeptidase(s)/ lipase(s), esterase(s) and peptidoglycan hydrolase are under study. Our studies indicate that *Pediococcus acidilactici* under study has endopeptidase(s)/ lipase(s), esterase(s) and peptidoglycan hydrolase activities in extracellular medium. 36 h old extracellular fluid of *Pediococcus acidilactici* was concentrated (0-80%  $(\text{NH}_4)_2\text{SO}_4$  precipitation) and dialysed sample was subjected to successive chromatographies. The enzyme(s) were screened using different substrates and also characterized by zymography. A casein hydrolyzing protein that also hydrolyses p-nitrophenyl phosphate at alkaline pH has been purified and characterized. Its MALDI-TOF analysis has revealed a peptide fragment whose sequence shows 100% matching with human phosphate binding protein and one cysteine is at conserved position of that of DING proteins. Further studies on the characterization, functional and application aspects of these enzymes are in progress in our laboratory.

## **AN *IN VITRO* INVESTIGATION INTO THE EFFECT OF A 28 DAY PROBIOTIC SUPPLEMENT GANEDENBC<sup>30™</sup> ON THE RESPONSE OF HUMAN FECAL MICROBIOTA OF OLDER PERSONS: THE POTENTIAL ROLE OF SYMBIOSIS**

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Composition of the gut microbiota that during the birth process and the first two years of life 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. have been seen to decline. Several studies have investigated the role of probiotic supplements on modulation of the microbiota in older persons and the favourable 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 faecal 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 faecal microbiota of those on different treatments. This could suggest a link between probiotic supplementation and beneficial effects of prebiotics in a symbiotic relationship



## **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.<sup>[1]</sup> 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* 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.

[1] Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C & Gahan CG (2007) Bacteriocin production as a mechanism for the anti-infective activity of *Lactobacillus salivarius* UCC118. *Proc Natl Acad Sci U S A* 104:7617-21.

## 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 remodelling 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- $\gamma$ , IL-1 $\alpha$ , IL-6, IL-10, MCP-1, MIP-1 $\alpha$ , RANTES, and TNF- $\alpha$ , however there was a significant down-regulation of leptin and up-regulation of adiponectin with GR-1 treatment ( $p < 0.05$ ). These results imply that the attenuation of cardiac remodelling and hypertrophy may be due to a novel mechanism of GR-1, independent gut microbial composition or anti-inflammatory mechanisms, and suggest the potential for a novel treatment of heart failure.

## 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.

## ***In vitro* evaluation into the prebiotic activity of a novel trans-galactooligosaccharide to potentially reduce the risk of contracting travellers' diarrhoea using faecal samples from athletes in batch culture systems**

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**Background:** Traveller's diarrhoea (TD) affects over 20 million travellers worldwide every year, the most common cause of which are bacterial enteropathogens. At the Beijing Olympics 2008 and the commonwealth games in New Delhi, India 2010, there were many athletes marred by gastrointestinal complaints. Prolonged endurance exercise has been linked with immune impairment and reduced gastrointestinal blood flow, both of which are associated with increased risk of infections.

**Objective:** To assess the influence of a commercial GOS (B-GOS), on the gut bacteriology of athletes to potentially reduce the risk of Travellers' diarrhoea.

**Design:** A set of batch cultures were used under anaerobic, pH controlled conditions set at 37°C. Faecal specimens were taken from athletes that had consumed either 4 weeks of B-GOS or placebo (maltodextrin) and were then stimulated with pathogenic bacteria (*Salmonella enterica serovar Typhimurium*, entero-toxicogenic *Escherichia coli* or entero-aggregative *Escherichia coli*). Samples were taken at 0, 5, 10, 24 and 48 hours and analysed for major bacterial groups using fluorescent *in situ* hybridisation (FISH), and production of short-chain fatty acids (SCFAs) using gas chromatography (GC).

**Results:** There were found to be no significant changes in any of the bacterial groups measured in the B-GOS group compared with the placebo group over time. There were no significant differences in pathogen levels between the two groups, and no significant changes in SCFA profiles.

**Conclusions:** Pre-existing high levels of bifidobacteria (the target bacteria for B-GOS), and low levels of less beneficial bacteria such as clostridia were found in the original faecal samples collected. This could potentially explain why no effect has been seen with B-GOS supplementation.

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

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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<sup>1</sup>). 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.

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

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*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.

## DIRECTED GENERATION OF PREBIOTICS USING REVERSE ENZYME SYNTHESIS IN LACTOBACILLI

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Coronary heart disease (CHD) is a major cause of death in the UK costing the NHS around £19 million a year. It is caused by the generation of atherosclerotic lesions in the arterial linings; the primary cause of these lesions is due to cholesterol accumulation. Anti thrombotic and lipid lowering drugs exist to help combat CHD however these can often lead to side effects and are expensive to produce. A new potential therapy includes the use of pro and prebiotics. Both of these therapies target the gut microbiota to enhance population levels of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*. Current research has shown certain lactobacilli species have potential in cholesterol assimilation such as *Lactobacillus fermentum* and *Lactobacillus acidophilus*. This is partly due to their bile salt hydrolase (BSH) activity, allowing them to deconjugate bile salts enhancing their faecal excretion. Lactobacilli also harbor  $\beta$  galactosidase activity; this enzyme allows the bacterium to synthesize a self specific galacto-oligosaccharide (GOS). The aim of this project is using reverse enzyme synthesis to develop a novel GOS that targets lactobacilli. Initial screening is underway to assess abilities of different lactobacilli to undergo cholesterol assimilation, to possess  $\beta$  galactosidase activities in a range of different media and BSH activity. A targeted GOS generation should therefore give rise to targeted prebiotic functions.

## 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, immunomodulatory 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.



## LACTOBACILLI ANTAGONISE *BRACHYSPIRA PILOSICOLI* AND REDUCE IT'S 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 colonisation of the caeca and colon 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 antagonise 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 caecal *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 microscopical 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.

## PREBIOTIC PROPERTIES OF BREWER YEAST CELL WALL FRACTIONS

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Nowadays, it is well established that the colonic microbiota has an important influence on the 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, brewer's yeast spent has been proposed as excellent candidates for a new-generation of prebiotics. Brewer's yeast spent 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 byproduct, isolation of cell wall compounds has been tentatively assessed due to its high nutritional value. These fractions are composed by mannans, beta-glucans and glycoproteins 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 brewer yeast spent extracts (obtained via autolysis, followed by hydrolysis and ultrafiltration) using two different protocols: as carbon sources for supporting the growth of single probiotic strains viz *Lactobacillus acidophilus* Ki and *Bifidobacterium lactis* Bb12 and testing *in vitro* fermentation by fecal inoculum from healthy human proving positive effects on colon conditions. The results confirmed the ability of these substrates for increasing the *Bifidobacterium* population and for acting 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.

## THE EFFECT OF NUTRITION ON THE MICROBIOME IN PREGNANT WOMEN AND THE USE OF MICRONUTRIENT SUPPLEMENTED PROBIOTIC YOGURT TO IMPROVE OUTCOMES

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Relatively little is known about the gut, oral and vaginal microbiome of pregnant women, especially those who are under-nourished. In Africa, maternal and infant morbidity and mortality are major problems with an aberrant vaginal microbiota and poor nutrition being contributing factors. We hypothesize that by using next generation sequencing we will observe a difference between the microbiotas of under-nourished, healthy and obese women. Thus, in order to target these populations, we chose to perform our study in Mwanza, Tanzania. We also hypothesized that daily supplementation of the diet with probiotic yogurt containing *Lactobacillus rhamnosus* GR-1 and nutrient-rich ground Moringa leaf extracts, will 'normalize' the microbiota and improve pregnancy outcomes. Many challenges arose, from language/cultural barriers, weather extremes, lack of resources, and the need to supervise yogurt production and quality. Nevertheless, we recruited 67 subjects (18 under-nourished, 18 nourished, 14 undernourished and 11 nourished randomized to receive probiotic yogurt and 6 obese). Anthropometric measurements and 48 hr dietary recall interviews, 16s Ion Torrent sequencing and metabolomic data from 700 vaginal, oral and fecal samples, along with maternal and newborn health status has revealed how complex and interlinked these parameters are. Of interest, the incidence of bacterial vaginosis, a risk factor for preterm labour, was 32% which is similar to Canadian women, and three babies died before term. We hope that this work will lead to novel and practical ways of improving the health of mothers, fetuses and infants, and stimulate microbiome and probiotic research in Tanzania and other developing countries.

## **A randomised, placebo controlled cross-over 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 fibres (1) and polyphenols (2) have been observed to modify the gut microbiota. Date fruits are rich sources of insoluble fibres (3) and polyphenols (4), and therefore they may exert positive effects in gut health. A randomised, placebo controlled cross-over 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 14 day washout period. Changes in the microbiota were assessed by FISH analysis. Short chain fatty acids were determined using HPLC. The biological activity of human faecal 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 microbial 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.

1. Connolly, M. L.; Lovegrove, J. A.; Tuohy, K. M., In vitro evaluation of the microbiota modulation abilities of different sized whole oat grain flakes. *Anaerobe* **2010**, *16*, 483-488.
2. Tzounis, X.; Rodriguez-Mateos, A.; Vulevic, J.; Gibson, G. R.; Kwik-Urbe, C.; Spencer, J. P., Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. *The American Journal of Clinical Nutrition* **2011**, *93*, 62-72.
3. Baliga, M. S.; Baliga, B. R. V.; Kandathil, S. M.; Bhat, H. P.; Vayalil, P. K., A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). *Food Res Int.* **2010**, *In Press, Corrected Proof*.
4. Eid, N. M. S.; Al-Awadi, B.; Vauzour, D.; Oruna-Concha, M. J.; Spencer, J. P. E., Effect of Cultivar Type and Ripening on the Polyphenol Content of Date Palm Fruit. *Journal of Agricultural and Food Chemistry* **2013**, *61*, 2453-2460.

## Protective activity assessment of hemicellulosic oligosaccharides in lactobacilli survival under simulated gastrointestinal conditions

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The interest towards prebiotic compounds has increased in the last decade, and they were used as functional compounds in synbiotic products, since they enhance and extend colonization by the probiotic bacteria. Therefore, the study of the protective function of prebiotics upon probiotic bacteria in stress environments, such as the gastrointestinal conditions, seems appropriate and interesting. To the best of our knowledge the resistance to gastrointestinal conditions of *Lactobacillus* strains grown in prebiotic carbohydrates has rarely been considered. Growth of some *Lactobacillus* strains on different prebiotics could help increase their resistance to gastrointestinal conditions, thus, enhancing their survival through the gastrointestinal tract. With this idea in mind, in this work we proposed to test the effect of four oligosaccharide mixtures from several hemicellulosic agroindustrial wastes (with different structure, including chain length, branching, linkage types and the presence of mixtures of different molecules) and fructooligosaccharides (a known commercial prebiotic) on survival of lactobacilli to different gastrointestinal conditions (lysozyme, low pH, bile extract and pancreatin). The obtained results showed that the growth of *Lactobacillus* strains on tested oligosaccharides could help increase their resistance to gastrointestinal conditions, thus, enhancing their survival through the gastrointestinal tract. These findings may help to expand the applications of hemicellulosic oligosaccharides in synbiotic products with important applications in the design of new functional food ingredients with added functionality.

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## PREBIOTIC MODULATION OF MATERNAL DIET TO ENHANCE THE INFANT MICROBIOTA

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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 faeces 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.

## 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 characterisation 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.

## 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 to 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.



## **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.

## **Studies of $\beta$ -galactosidase and Proline iminopeptidase from Lactic Acid Bacteria of Probiotic Significance**

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Microbial enzymes account for about 60% of total worldwide sale of enzymes. There is increasing demand of industrial enzymes with novel characteristics for different applications. Among microorganisms lactic acid bacteria have gained great attention because of their GRAS (generally regarded as safe) status.  $\beta$ -galactosidase is an enzyme of nutritional, environmental, therapeutic and industrial significance and mainly used for combating the problem of lactose intolerance, synthesis of galacto-oligosaccharides and whey disposal. *Pediococcus acidilactici* is a potential probiotic bacterium and further studies are required to confer probiotic status to this. *In vitro* studies of *Pediococcus acidilactici* for  $\beta$ -galactosidase have shown  $65 \pm 0.9$  Miller Units in uninduced state which is much higher as compared to the normally observed values of this enzyme in uninduced state (1.5-3 Miller Units). The enzyme has been partially purified by ammonium sulfate fractionation (30-55%) and successive chromatographies on ion exchangers and gel filtration. Proline iminopeptidase is another important enzyme of food industry used for reducing bitterness in enzyme-modified cheese. It hydrolyses Pro containing peptides thus minimizes bitterness in dairy and food products. Because of GRAS status, different LABs were screened for this enzyme. *Lactobacillus plantarum* had the highest activity in membrane bound form. The enzyme was solubilized in presence of 1.5% Triton X-100 and further purified using successive chromatographies. Further studies on functional characterization of both  $\beta$ -galactosidase and proline iminopeptidase are in progress in our laboratory.

## **INFLUENCE OF POLYPHENOL RICH HERBS IN GUT REPRESENTATIVE 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 Savoury. 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 °C, samples were taken to enumerate viable cells in specific media. At same time, centrifugation of the samples was performed and the supernatants were analysed 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.

## 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 randomised, 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, faecal and urine samples were collected and stored for analysis of; faecal 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-Clostridium 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.

## **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 7 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.

## Microencapsulation of *Kluyveromyces marxianus* B0399: development of novel strategies for improving the probiotic potential

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Probiotics market, which is estimated to grow further in the next years, is mainly constituted by fermented dairy product containing probiotics bacteria belonging to the health-promoting genera *Bifidobacterium* and *Lactobacillus*. Conversely, the utilization of probiotic yeast is limited to date. Recently, Maccaferri *et al.* (2011) described a new microorganism with probiotics effect, *Kluyveromyces marxianus* B0399. *K. marxianus* B0399 has been demonstrated to positively modulate the gut microbiota composition, increasing the total amount of bacteria belonging to the genus *Bifidobacterium* and enhancing the production of acetic and propionic acids. In order to improve the survival of *K. marxianus* B0399 in the human intestine and to enhance the stability and the shelf-life of the probiotic yeast in the food vehicle (fermented dairy products or non-dairy beverages), we aim at assessing the impact of new strategies for the microencapsulation of *K. marxianus* B0399. A mixture of *K. marxianus* and partially hydrogenated oil was used to create the microcapsule core and then it was sprayed at very low temperature, allowing the creation of droplets of 100µm diameter. After creating an array (10-40%) of microcapsule with different percentage of inclusion of yeast cells, 25% inclusion rate has been selected on the basis of the most effective ration between cost and release rate. Afterwards, the survival to pH 2 and physiological concentration of bile acids, the stability to accelerated shelf-life conditions and the maintenance of all the probiotic skills of the yeast after treatment will be evaluated using the same experimental approaches used by Maccaferri *et al.* (2011), in order to evaluate the characteristics of this new product and to guarantee high microorganism viability.

## 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 21<sup>st</sup> 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 12 weeks followed by an intervention period during which the high fat diet was supplemented with the glycopeptide antibiotic vancomycin, the bacteriocin producing probiotic (Bac<sup>+</sup>) *Lactobacillus salivarius* UCC118, its bacteriocin negative derivative *L. salivarius* UCC118 Bac<sup>-</sup> or was unsupplemented (9-10 mice/cohort) for 8 weeks. 16S rRNA sequencing was used to analyse 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 8 week intervention period. A significant reduction in weight gain was also observed at week 14 in the DIO mice receiving the Bac<sup>+</sup> probiotic compared to those in receipt of Bac<sup>-</sup>. 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.

## THE PAMS STUDY: A PREBIOTIC APPROACH TO METABOLIC SYNDROME

Tamara Tompsett, BSc, Adele Costabile, PhD, Gemma E. Walton, PhD, Julie Lovegrove, PhD, Glenn R. Gibson, PhD.

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Systemic and adipose tissue inflammation are associated with obesity, insulin resistance and the onset of T2D. 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 heterogeneous factors: raised blood pressure, dyslipidemia, central obesity and insulin resistance which increase risk of T2D and CVD. The PAMS study has recruited 59 individuals at risk of developing MetS. The study is a randomised 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 trial. Samples have been collected of faeces, blood and 24h urine to assess changes in the faecal microbiota, and to monitor indices of MetS including lipid profiles and inflammatory markers. The intervention is complete and initial blinded analysis of the bacterial content of faecal 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.



## Guest Speakers



***Dr. Arthur Ouwehand, Research Manager, Danisco***

Dr. Arthur Ouwehand has a research background in both academia and industry. His main interest is in functional foods, in particular probiotics and prebiotics and their influence on the intestinal microbiota. He is an active member of the International Life Science Institute Europe, the International Dairy Federation and the International Scientific Association for Probiotics and Prebiotics. Dr. Ouwehand received his MSc degree (1992) in cell biology from Wageningen University (the Netherlands) and his PhD degree from Göteborg University (Sweden). In 1999 he was appointed adjunct professor at the University of Turku (Finland). He is the author of more than 150 journal articles and book chapters on pre- and probiotics.



***Dr. Glenn Gibson, Professor of Food Microbial Sciences; Head of FMSU Research Group, University of Reading***

Professor Gibson heads the Food Microbial Sciences Research Unit. His main research interest is human gut bacteriology. PhD research (Dundee) was on the microbiology of sea loch sediments in Scotland. 8 books, 241 research papers, 144 review articles, >300 published abstracts, 10 patents, >13,000 career citations (h factor = 58), over 60 PhD students, more than 300 conference lectures in the last 5 years (>70 plenary), principal Investigator on over 100 research grants, H factor = 58, 6 advisory boards. Past-President of the International Scientific Association for Probiotics and Prebiotics. He currently researches acute and chronic gut disease, autism, obesity, novel prebiotics, and human metabonomics. Specific projects on pro/prebiotics, the molecular genotyping of gut bacteria, H<sub>2</sub>S production, gastroenteritis in sportspersons, metabolic syndrome, IBS, IBD, gut flora development with age and colonic homeostasis are being carried out.



**Dr. Gregor Reid**, Chair, Human Microbiology and Probiotics, and Assistant Director (International), Lawson Health Research Institute; Director, Canadian Research and Development Centre for Probiotics; Professor, Microbiology & Immunology, and Surgery, University of Western Ontario

Gregor Reid developed an interest in Microbiology during his BSc Honours in Glasgow University. Through an International Rotary Scholarship, he did a PhD at Massey University on *E. coli* uropathogens. In 1982, he joined Bill Costerton's team in Canada and set up a collaboration in Toronto with Dr. Andrew Bruce. Together, they studied the vaginal microbiota and the ability of lactobacilli to restore and maintain health, publishing a number of 'firsts', and developing a probiotic that has subsequently been used by millions of women worldwide. Dr. Reid moved to the University of Western Ontario in 1990 as Director of Research Services, then to Lawson Health Research Institute in 1996. He received an MBA from Monash University in 1998, an Honorary Doctorate in Biology from Orebro University in 2008. He has published over 400 papers, given over 500 talks in 50 countries and has a lab currently comprising 16 students.



**Dr Karen Scott**: Senior Research Fellow, Microbial Ecology Group, Rowett Institute of Nutrition and Health, University of Aberdeen, Greenburn Road, Bucksburn, Aberdeen, Scotland, UK. AB21 9SB.

Karen Scott's main research involves investigating the molecular microbiology of gastrointestinal tract bacteria, focussing on the changes that occur in the microbial diversity due to dietary changes, and on the utilisation of prebiotic, and other dietary, substrates by human commensal gut bacteria. Microarray technology has been used to investigate differential gene expression in an obligate gut anaerobe, leading to the identification of a novel metabolic pathway enabling the bacterial strain to use host derived epithelial glycoconjugates as energy sources during times of dietary starvation. We have shown that many of the numerically and metabolically important gut bacteria are able to use specific dietary substrates, including resistant starch and prebiotics, for growth. This is indicative of alternative mechanisms for the efficacy of prebiotics in improving gut health. She has published more than 50 journal articles and book chapters. Karen is Vice President of International Scientific Association for Probiotics and Prebiotics.



***Dr Thomas Magaldi***, *Director of Science Alliance at the New York Academy of Sciences*

With over 6 years of experience working as a molecular biologist studying viral oncogenes and infectious entry of DNA tumor viruses, Dr. Magaldi received his PhD in genetics from Yale University in 2012. He then continued his postdoctoral research at the National Cancer Institute, discovering a new family of viruses not previously known to infect vertebrates. Dr. Thomas Magaldi has a long history working in science education. He is interested in enhancing and transforming the training and education of students at all levels of learning; in particular, undergraduate students, graduate students and postdocs within the sciences. In addition to his scientific research and his interest in the improvement of education, Dr. Thomas Magaldi has a passion for science policy. He has worked as an intern for the US Department of State, on a research project looking into identifying ways in which the United States can assist scientific research and education improvement abroad. He has also been an advocate for science funding, as part of the American Society of Biochemistry and Molecular Biology's 100 Meeting Challenge.