

## Proceedings of the Inaugural International Conference on Probiotics Health and Nutraceuticals (IPN-2016)

### Keynote Presentations

#### **Safety Aspects of Application of Lactic Acid Bacteria and Their Bacteriocins**

**Svetoslav Dimitrov Todorov**

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#### **Abstract**

Due to their lack of pathogenicity, most LAB species have received the GRAS (Generally Recognized as Safe) status by the U.S. Food and Drug Administration. In addition to their important technological properties in food production, various species of LAB have been shown to possess therapeutic properties since they are able to prevent the development of some diseases as shown mostly using animal models and have the capacity to promote beneficial effects in human and animal health. In recent years, the number of functional food products enriched with live probiotic microorganisms, has increased exponentially since it is known that these can confer health benefits on the host. Besides all beneficial properties studied for various LAB, a special attention need to be pay on the possible presence of virulence factors, production of biogenic amines and antibiotic resistance. This virulence determinant has been well detected and studied in *Enterococci* and *Streptococci*, however, in last few years' report on presence of virulence factors in otherwise GRAS *Lactobacilli* have been showing the potential upcoming problems. Horizontal gene transfers of virulence factors between pathogenic and LAB, including probiotics is a highly possible scenario in case of uncontrolled application of probiotics. In addition, some of the antimicrobial peptides expressed by LAB may be associated with high cytotoxic properties. A special attention need to be pay on the possible cytotoxicity levels of the expressed bacteriocins in order to drown conclusion for the safe application of the producer or antimicrobial peptides in the bio-preservation and as probiotics.

#### **Manipulation of the Gut Microbiome to Improve Health Outcomes. Which is Best?**

**Gerard E. Mullin**

*John Hopkins Hospital, Baltimore, MD, USA*

#### **Abstract**

The gut microbiome is a highly organized ecosystem that has a diverse array of functions that impacts the body's physiology and health. There is emerging evidence that manipulation of the gut microbiome can alter health outcomes-both positively and negatively. The proposed session aims to succinctly review how diet and lifestyle factors influence the gut microbiota and how best to manipulate it's the composition and function to provide positive health outcomes.

## Probiotics in the Treatment of (*Vulvovaginal candidiasis*) VVC and (*Bacterial vaginosis*) BV

Franco Vicariotto

University of Milan, Italy

### Abstract

The human vaginal microbiota plays an important role in the maintenance of a woman's health, as well as of her partner and newborns. When this predominantly *Lactobacillus* community is disrupted or decreased in abundance, Vaginitis may occur. Of the millions of cases of vaginitis each year, most are caused by *Bacterial vaginosis* (BV), followed by *Vulvovaginal candidiasis* (VVC). The dominance of *Lactobacilli* in healthy vaginal microbiota and its depletion in BV and VVC has given rise to the concept of oral or vaginal use of probiotic *Lactobacillus* strains for treatment and prevention of vaginitis.

Probiotics, defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host, are considered a valid and novel alternative for the prevention and treatment of female urogenital tract infections. Probiotics are well known for their ability to lower intravaginal pH, thus establishing a barrier effect against many pathogens. Some strains are also able to create additional and more focused antagonistic activities mediated by specific molecules such as hydrogen peroxide and bacteriocins. In any case, despite some undeniable positive evidence, other intervention studies have at least partially failed to highlight a statistically significant alleviation of BV and VVC symptoms. This is most likely attributable to the lack of a specific inhibitory activity of the strains used towards the bacteria commonly causing BV, such as *G. vaginalis* and *E. coli*, and VVC such as *C. albicans*.

We present clinical data to assess the effectiveness of specific probiotic strains taken orally or topics directly into the vagina, for the treatment of BV and VVC, and the prevention of recurrences.

## Development of Clinical Stage Oral $\beta$ -Lactamase Therapies Designed for the Prevention of Antibiotic-Induced Disruption of the Gut Microbiome

John F. Kokai-Kun\*, Olivia Coughlin, Tracey Roberts, Heidi Whalen, Klaus Gottlieb and Joseph Sliman

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### Abstract

The gut microbiome is a complex and delicate balance of microorganisms which is essential to human health. Disruption of this balance can lead to various maladies from opportunistic infections like *Clostridium difficile* to various chronic disorders. The balance of the gut microbiome can be disrupted by various factors including antibiotic administration.  $\beta$ -lactam-containing antibiotics like penicillin's and cephalosporin's are commonly used and some of the most effective antibacterial but can also be highly disruptive to the gut microbiome. Even when administered intravenously (IV),  $\beta$ -lactam antibiotics like piperacillin and ceftriaxone are excreted into the gut through the bile where they remain active and may disrupt the balance of the gut microbiome. SYN-004 is a novel, orally-administered recombinant  $\beta$ -lactamase enzyme designed to be given during treatment with certain IV  $\beta$ -lactam antibiotics for prevention of the adverse effects caused by antibiotics in the intestine. SYN-004 is released in the small intestine when the pH > 5.5 to degrade  $\beta$ -lactam antibiotics excreted into the intestine. This degradation is expected to protect the gut microbiome from disruption by these residual antibiotics, thus preventing opportunistic infections like *C. difficile*. SYN-004 degrades most penicillin's and cephalosporin's, and has been shown to be well tolerated in phase 1 clinical trials in normal healthy volunteers. Phase 2a clinical trials have now demonstrated that orally administered SYN-004 catabolizes IV-administered ceftriaxone excreted into the intestine of volunteers with functioning ileostomies which allows sampling of their intestinal chyme. Importantly for SYN-004 to be an effective drug, it did not affect the systemic concentrations of ceftriaxone. SYN-004 has now progressed into a phase 2b clinical trial in patients receiving ceftriaxone as treatment for lower respiratory tract infections. The endpoint for this study is prevention of *C. difficile*-associated disease and antibiotic-associated diarrhea.

## Featured Presentations

### Phytochemicals and Aging: Current Insights

Hongwei Si

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#### Abstract

Food-derived small molecules, also called phytochemicals, have been extensively investigated the potential anti-aging effects in cells, animals and humans. In this presentation, the effects of the major anti-aging phytochemicals including resveratrol, epicatechin, quercetin and curcumin based on our and others' studies will be summarized. The cellular and molecular mechanisms such as ROS, AMPK, IGF-1 and inflammation underlying the anti-aging actions by these molecules will be further highlighted.

### Probiotics, Functional Foods and Adverse Food Reactions: Is there a Link?

Alessandro Di Cerbo<sup>1</sup>, Giuseppe Terrazzano<sup>2</sup>, Gianandrea Guidetti<sup>3</sup> and Sergio Canello<sup>3</sup>

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<sup>2</sup>University of Basilicata, Italy

<sup>3</sup>Forza10 USA Corp., Orlando, FL, USA

#### Abstract

Gut microbiome is not a silent ecosystem but exerts several physiological and immunological functions, as well as favor the homeostasis recover in tissues and organs other than gastro-intestinal tract. It is of note that *Lactobacilli* and functional foods (even herbal extracts) have been used as an effective therapy for treatment of several inflammatory conditions displaying positive effects. In addition, gut microbiome also has a role in contrasting adverse reactions to peculiar food and to food contaminants. In this regard we recently demonstrated that adverse food reactions in pets, derived from the massive presence of food contaminants (such as the antibiotic oxytetracycline, largely used in livestock), exhibited an inflammatory background mainly characterized by the pro-inflammatory lymphocyte cytokine release, as represented by INF- $\gamma$ . Based on literature reports that pointed out the efficacy of some *Lactobacilli* in reducing the secretion of INF- $\gamma$  during autoimmune disease simulation, we developed some functional foods able to relieve the inflammatory conditions in dogs and, as a concrete perspective, in humans.

### Functional Constituents of Yacon (*Smallanthus sonchifolius*)

Maria Lucia Masson<sup>1</sup> and Eriel Forville de Andrade<sup>2</sup>

<sup>1</sup>Universidade Federal do Parana, Brazil

<sup>2</sup>Fundacao da Universidade Federal do Parana, Brazil

#### Abstract

*Smallanthus sonchifolius* (Poepp & Endl) H. Robinson (yacon) presents in its composition bioactive materials in all botanical structures: root, flower, leaves. The root of the most studied and best-known, whose composition is high in fructose polymers - fructooligosaccharides and inulin compounds having prebiotic properties. The leaves and flowers are plant components not or less known. We used three methods of extraction to obtain extracts from leaves and flowers of yacon, using methanol and water (infusion and decoction with water) as solvents. Compositions of the extracts from leaves and flowers of yacon were quite different, extracts with methanol showed the absence of some bioactive compounds usually found when the solvent employed is water. Although the extraction with methanol, as solvent, yield superior total solids content than water solvent, aqueous extracts were more efficient to obtaining phenolic compounds. Similarly, the content of flavonoids and therefore antioxidant activity of extracts were significantly higher in the extracts of leaves and flowers in water. Among the flavonoids and phenolic, gallic acid was the most abundant in the leaves aqueous extracts. Extracts of flowers, however not showed phenolic acids concentrations in the same order of magnitude of the leaves extracts. Were also identified the presence of rutin, quercetin, kaempferol, and myricetin in extracts, with the emphasized rutin content in the composition of the extracts of leaves by water and methanol and myricetin in aqueous extracts of flowers. Both the test of DPPH as the ABTS, aqueous extracts showed antioxidant activity superior to methanol extracts.

## Fermented Milk with Probiotic *Lactobacillus rhamnosus* S1K3 (MTCC5957) Protects Mice from *Salmonella* by Enhancing Immune and Non-Immune Protection Mechanisms at Mucosal Level after Prolonged Consumption

Tanedjeu S. Kemgang<sup>1,2\*</sup>, Suman Kapila<sup>1</sup>, Venkatesa P. Shanmugam<sup>1</sup>, Srinu Reddi<sup>1</sup> and Rajeev Kapila<sup>1</sup>

<sup>1</sup>National Dairy Research Institute, India

<sup>2</sup>University of Ngaoundere, Cameroon

### Abstract

*Salmonella enterica* is a Gram-negative bacterium that causes gastroenteritis, bacteremia and typhoid fever in several animal species including humans. With an increase in the epidemiology worldwide, added to the occurrence and spread of resistance among serovars which shrink considerably the efficacy of chemical antibiotic, alternative methods (preventive) are needed to combat *Salmonella* spp. With this respect, probiotic culture with anti-*Salmonella* and immunomodulatory potentials offer a suitable solution. Here, we explored *in vitro* and *in vivo*, the mechanism by which an indigenous probiotic culture, *Lactobacillus rhamnosus* S1K3, could overcome the pathogenic strain *Salmonella enterica* using a mice model with an emphasis on the response at the intestinal mucosal level after long term consumption. Results showed that S1K3 was able to produce antimicrobial compounds against the pathogens. The probiotic adhered strongly to intestinal epithelium and maintained its integrity in presence of *Salmonella* through stimulation of tight junction and antimicrobial peptide genes *in vitro*. Mice pre-fed for 30 days with S1K3-fermented milk exhibited low incidence of pathogenic *Salmonella* compared to control skimmed milk at mucosal and systemic levels. The probiotic induced TLRs expression at the Peyer's patches, followed by an increase in the S-IgA in intestinal fluid, the IgA-secreting and Goblet cells in lamina propria of small intestine and the IgA level in serum. Moreover, S1K3 maintained the level of IL-12 (Th1 cytokine), increased the IL-4 (Th-2 cytokine) and surprisingly reduced the level of regulatory cytokine (TGF- $\beta$ ) in intestinal fluid/serum at the later stage of infection. All these protective actions concurred to lower the invasion of *Salmonella* in feces, spleen, liver and intestine tissues and improved the health status of probiotic fed group. In view of this performance, *L. rhamnosus* S1K3 appear to be a suitable candidate for the development of nutraceutical food against one of the most devastating pathogen of today.

## Immunomodulatory Effect of Low Molecular Weight Fractions of G<sup>+</sup> and G<sup>-</sup> Bacteria

Eva Kmoníková<sup>1\*</sup>, Zuzana Zákostelská<sup>2</sup>, Miloslav Kverka<sup>2</sup>, Helena Tlaskalová-Hogenová<sup>2</sup> and Zdeněk Zidek<sup>1</sup>

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<sup>2</sup>Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic

### Abstract

Major medical indications of probiotic bacteria are conditions associated with the gastrointestinal tract. The aim of the study was to investigate the effect of lysates of probiotic bacteria on severity of intestinal inflammation and to analyze immunobiological properties of bacterial lysates and their fractions. *Lactobacillus casei* (Lc) probiotic strain DN-114 001 and *Escherichia coli* Nissle 1917 were employed. Lysates were prepared by passing bacteria through a French press. The fractions were prepared by the microfiltration of the crude lysate using the 3-, 10-, 30-, 50-, and 100- kDa cutoff filters (Amicon® Ultra 0.5 ml, Millipore Corp.). Acute colitis was induced by 3% dextran sodium sulfate. Effects of microfiltrates on the *in vitro* production of nitric oxide (NO), cytokines, and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) were investigated in macrophages. We found that oral administration of Lc lysate attenuated the acute colitis in BALB/c mice and decreased the production of cytokines IL-6, IFN- $\gamma$  and IL-10. Lc lysate prevented LPS-induced TNF- $\alpha$  expression in RAW 246.7 cells. The microfiltration procedure of bacterial lysates completely removed macromolecules such as peptidoglycan, lipoteichoic acid, lipopolysaccharide (LPS). Original lysates and molecular weight cutoff fractions (MWCF) of both G<sup>+</sup> and G<sup>-</sup> bacteria showed NO- and cytokine-stimulatory (IL-1 $\beta$ , TNF- $\alpha$ , IL-6, IL-10) activities. The original crude lysate also activated the biosynthesis of PGE<sub>2</sub>. The molecules with the molecular mass  $\leq$ 3 kDa were responsible for approximately 45% and 83% of the NO- and PGE<sub>2</sub>-enhancing activities of the crude lysate, respectively. The probiotic strains contain low molecular mass ( $\leq$ 3 kDa) molecules possessing immunostimulatory properties.

## Isolation of Probiotic and Lactic Acid Bacteria: Development of an Original, Sustainable and Robust Starter by Spray-Drying/Freeze-Drying

Antonio F. Carvalho<sup>1</sup>, Andreza Ferreira<sup>1</sup>, Evandro Marins<sup>1</sup>, Rosangela de Freitas<sup>1</sup>, Italo Perrone<sup>1</sup>, Pierre Schuck<sup>2</sup> and Gwénaél JAN<sup>2</sup>

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### Abstract

The isolation of probiotic and LAB strains from artisanal cheeses and the processing environment has made it possible to build up culture banks with a wide variety of microorganisms. These banks have allowed it to both preserve and valorize the microbiota from socially, economically and historically important regional cheeses. In the specific case of our researches, culture banks were formed with isolates from traditional cheeses of Marajo (LAB) and Campos das Vertentes (*Propionibacterium*) regions. Further investigations made it possible to select isolates with characteristics of industrial interest, including bacteria with probiotic relevance. The identification of probiotic strains is a promising approach because it allows the formulation of novel products with healthy characteristics. However, health benefits may depend on the viability of probiotic cells and their maintenance in commercial cultures. Bacterial strains from natural resources can show higher resistance to congelation or drying, which can be considered advantageous during the production of cultures for industrial applications. Probiotic cultures are largely commercialized in lyophilized or frozen form. However, it is necessary to maintain the cold chain throughout all steps of production and distribution and the cold chain is directly influencing the final price of cultures. To ensure the viability of long-term cultures at low cost, the preservation by alternative methods, such as spray drying, has been proposed. The cost of spray-drying is more than 10 times lower than freeze-drying, making it possible to elaborate cheaper probiotic starters. In addition, dehydrated cells have sustained high viability over time even when exposed to room temperatures.

## The Impact of an Antibiotic and a Probiotic on Antibiotic-Associated Diarrhea, the Microbiota and the Resistome: a Randomized, Double-Blind, Placebo-Controlled Study

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### Abstract

One-hundred sixty healthy participants were randomized into two groups (80/arm). Both groups received 875 mg amoxicillin with 125 mg clavulanic acid per day for 7 days and either placebo or Lacidofil® Strong. They were monitored for 10 weeks for antibiotic-associated diarrhea (AAD). The occurrence rate of AAD of the entire study population was approximately 11%. Thus the probiotic did not prevent the AAD, however post-hoc analysis demonstrated that the duration of the AAD was significantly shorter by one full day (probiotic,  $2.70 \pm 0.36$  days; placebo,  $3.71 \pm 0.36$  days;  $p = 0.037$ ; effect size = 0.52). Real-time PCR of the fecal samples demonstrated excellent retention of the two bacterial strains that compose Lacidofil® Strong (*Lactobacillus rhamnosus* R0011 and *Lactobacillus helveticus* R0052). Microbiome analysis using 16s rRNA gene sequencing indicated that there were significant changes to the microbiota in both groups at the end of antibiotic intake but it quickly reverted within one week. In particular, during antibiotic use there was a significant increase in Enterobacteriaceae. Investigation of the resistome, which is the complete set of microbial genes associated with antibiotic resistance (ABR), was achieved using a custom-designed microarray capable of detecting 354 known ABR genes. We observed significant changes to the resistome during antibiotic intake and this quickly reverted one week after completion of the antibiotic. The main differences were due to increases in the abundance of ABR genes for aminoglycosides, beta-lactams and tetracyclines. Changes in the microbiome and resistome are complementary molecular biology tools which could be used to further decipher the underlying mechanism of action of probiotics on the clinical improvement of AAD.

## Anaerobically Cultivated Human Intestinal Microbiota - an Effective and Safe Alternative to the Use of Fresh Feces in Fecal Microbiota Transplantation (FMT)

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### Abstract

For close to 20 years we have been working with a human intestinal microbiota cultured under strict anaerobic conditions as an *in vitro* batch culture. This microbial ecosystem has been re-inoculated every week, and the microbiota has retained a microbial diversity comparable to that of the feces from the healthy donor, containing the major bacterial phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*, as confirmed by gene analyses. The batch-cultured microbial ecosystem has been tested for safety and clinical efficacy for many years in more than 400 patients, most of them suffering from *Clostridium difficile* infection. No adverse events and a cure-rate close to 90% have been reported. Results will be presented from a randomized clinical study in which the efficacy of the culture is being compared to conventional antibiotic (vancomycin) treatment of *C. difficile*-infections. Clinical results with patients suffering from other diseases associated with gut dysbiosis will as well be reported briefly.

## The Relationship of Human Gut Microbiota with 25(OH)D and HbA1c Could Identify Species with Probiotic Properties

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### Abstract

The majority of known probiotics are species of human gut microbiota (GMB) and have functions similar to symbiotic microbiota. This study investigated the relationship between microbiota and metabolic biomarkers in African American men (AAM) with pre-diabetes. The study was ancillary to a randomized controlled trial of vitamin D supplementation (weekly ergocalciferol 50,000 IU vs. placebo) conducted in AAM over 12 months (Barengolts et al. 2015). GMB was analyzed in stool collected at the exit (n = 115). Glycemic groups (Gr) were characterized based on changes in oral glucose tolerance between baseline and exit. Subjects with stable normal glucose tolerance were assigned to Gr-1 and those with stable pre-diabetes (impaired glucose tolerance and impaired fasting glucose) to Gr-2. Comparisons were done between Gr-1 and Gr-2 and between the lowest and the highest quartiles of dietary intake of energy and fat, HbA1c, serum 25(OH)D and bone mineral density (BMD). Differences between Gr-1 and Gr-2 included the Bacteroidetes/Firmicutes and Bacteroidales/Clostridia ratios and differences in genera such as *Ruminococcus* and *Dialister*. Specific taxa and biomarkers of inflammation and "leaky gut" (zonulin, sCD14) were associated with the lowest and highest 25(OH)D quartiles. These taxa (*Ruminococcus*, *Roseburia*, *Blautia*, *Dorea*) were distinct from those differentiated by quartiles of dietary intake (Bacteroides, Bacteroides/Prevotella ratio) or HbA1c (*Faecalibacterium*, *Catenibacterium*, *Streptococcus*). There were no differences between BMD quartiles. In conclusion, despite of high inter-individual GMB variability, specific taxa were associated with specific metabolic profiles. Understanding relationship of GMB with 25(OH)D and HbA1c could identify GMB species with probiotic properties.

## Dietary Interventional Approaches to Mitigate Air Pollution Cardiovascular Effects

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### Abstract

Air pollution is known to cause adverse health effects. Fine particle pollution, or PM<sub>2.5</sub>, is of particular concern. This pollutant is emitted from motor vehicles, power plants, industry, and forest fires, and unlike summertime ozone, can occur year-round. Particle pollution is a contributor to heart attacks and sudden death or morbidity in people with cardiovascular disease or risk factors for the disease such as high blood pressure or high cholesterol. Everyone is exposed to some level of air pollution, the attributable health burden can be high, particularly for susceptible populations. Interventions can be effective actions to

reduce the health impacts of air pollution. Dietary fatty acid supplementation has been shown to decrease cardiovascular risk through multiple mechanisms. We conducted studies evaluated the efficacy of supplementation with marine fish oil (FO) or olive oil (OO) in protecting against cardiovascular effects induced by controlled exposure of middle-aged healthy volunteers to concentrated ambient air pollution particles. Subjects (ages 50 to 72 years), were randomly assigned to receive 3 g/d of fish oil (FO, 1.2 g EPA and 0.82 g DHA), or olive oil (OO, 3g/d) for 28 days. Supplementation resulted in statistically significant increases in plasma EPA levels in the FO group and oleic acid levels in OO group. Subjects were then exposed to concentrated ambient air pollution particles (CAP, mean mass concentration  $253 \pm 16 \mu\text{g}/\text{m}^3$ ) or filtered air for 2 hours on sequential days. Heart rate variability (HRV), plasma lipids, coagulation markers, and endothelial function measured by flow-mediated dilation of the brachial artery (FMD) were assessed pre-, immediately post-, and 20 hours' post-exposure. Short-term exposure to CAP resulted in autonomic nervous system imbalance and impaired vascular endothelial function. FO supplementation attenuated CAP-induced reductions in HF/LF ratio, elevations in nLF domain of HRV, and increases in plasma triglycerides and VLDL. OO supplementation ameliorated CAP-induced reduction of FMD and changes in blood markers associated with vasoconstriction and fibrinolysis. The study of healthy middle-aged adults found that short-term exposure to ambient air pollution particles results in acute cardiovascular effects in healthy middle-aged adults, and suggest that supplementation with FO is protective against changes in autonomic balance and plasma lipids, while OO supplementation blunts adverse vascular responses of air pollution inhalation. This study could have impact on public health: people at risk of heart disease can change their behavior during bad air quality days to limit their exposure. The study also suggests that fish oil may provide protection from harmful cardiac effects and olive oil may provide protection from the harmful vascular effects of air pollution exposure.

**This abstract of a proposed presentation does not necessarily reflect EPA policy.**

## Nutrient and Food Groups Intakes Following Laparoscopic Roux-en-Y Gastric Bypass (RYGB)

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### Abstract

**Overview:** Serial changes in dietary intake, including specific food groups and nutrients during the first year following RYGB are of interest due to surgically-induced alterations in meal size, food intolerances present after surgery, and potential nutrient deficiencies. It was of interest to examine changes in macro- and micronutrients, food groups, and plasma vitamin and mineral status in post RYGB individuals.

**Materials and Methods:** RYGB patients (n = 17) completed four-day food records to assess intake of macro- and micronutrients and food groups at baseline (prior to surgery) and then at 3-wks, 3-mos, 6-mos, and 12-mos after surgery. Blood was obtained and analyzed for vitamin and mineral concentrations.

**Results:** A dramatic decrease in mean ( $\pm$  SEM) daily energy intake occurred:  $2,150 \pm 165$  kcals at baseline vs.  $649 \pm 40$  kcals at 3-wks; energy intake continually increased to a high of  $1307 \pm 129$  kcals by 12-mos. More than 50% of patients had low intake of vitamins D, E, C, folate, and calcium, magnesium, and potassium at 12-months. Servings from vegetables, grains, fats, and sweetened beverages were lower, whereas, meats, dairy, fruits, and sweets showed only small, transient changes following surgery. Suboptimal micronutrients status was common in these patients at 12-months post-surgery.

**Conclusions:** The reduction in energy intake following RYGB is from selected food groups and not solely a reduction in portion sizes across the diet. The lower intake and serum levels of micronutrients indicate potential risk for deficiencies and supports use of supplements. These findings can help in the clinical management of surgical patients to improve nutritional health.

## My New Gut: Insulin Resistance - Linking modulation of the Gut Microbiome to Dietary Recommendations and Health Claims

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## Abstract

Obesity is one of the greatest public health challenges of the 21st century. Its prevalence has tripled in many countries of the World Health Organization (WHO) European Region since the 1980s, and the numbers of those affected continue to rise at an alarming rate. In addition to causing various physical disabilities and psychological problems, excess weight drastically increases a person's risk of developing a number of non-communicable diseases, including cardiovascular disease, cancer and diabetes. Worldwide, current dietary recommendations don't refer to the role of the gut microbiome in health. Nevertheless, from a public health perspective, establishment of a physiologically relevant, beneficial health effect following modification of the microbiome on obesity should best be established by the favorable modification of one common denominator that is directly linked to a physiological beneficial effect. As common denominator of studies into the dietary modulation of the microbiome by probiotic and prebiotic foods, 'insulin resistance' has been proposed. Not only is insulin resistance a hallmark in the development of obesity and type 2 diabetes, but it is also implicated in a plethora of disease states and diseases associated with metabolic syndrome, like excessive weight, hypertension, atherogenic dyslipidemia and chronic low-grade inflammation.

This notion implies that insulin resistance should be one of the important, if not the most important primary outcome measure in clinical studies, applying pre- and probiotic interventions and performed in the context of applied microbiome research.

Taken together, in order to make the shift from microbiome research towards its application in dietary strategies and recommendations, as well as into health claims made on newly developed microbiome-modifying foods, we hypothesize that microbiome research should, at least in part, be targeted at deciphering the impact of (un)favorable modulation of the microbiome on insulin resistance.

## Synbiotics: Traditional Approach, Present Status and Future Outlook

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### Abstract

Synbiotics have been defined as the foods having characteristics of both probiotics and prebiotics. It has more promising ability to impart health benefits over the probiotics owing its prebiotic components like xylobiose, inulin, lactulose, resistant starch etc in promoting the growth of probiotic microorganism inside the colon. Although the term synbiotic is new in use but many traditional foods like kanji, lugri, sur, pozol, sufu, bushera etc. have been consumed since time immemorial. Recent investigations revealed their synbiotic characteristics. Presently, studies have been directed towards the development of synbiotics rather than probiotics due to their ability to support the gut microflora. Many researches has been going on for the development of new foods using cereals, fruits and other factors which may add prebiotics and can improve the overall nutrition of the food. The future food needs to be safer, tastier, easy to consume, energy dense, low GI, nutritionally sound and healthy. In this regard, synbiotics can provide a perfect option as the incorporation of wide variety of components in a single food can improve the overall nutritional quality. So, we can say that synbiotics are the next generation foods and there is an ample need to develop such foods using indigenous knowledge and modern scientific approaches.

## Structural Insights into Pilus Formation in Probiotic *Lactobacillus rhamnosus* GG

Priyanka Chaurasia<sup>1,2</sup>, Shivendra Pratap<sup>1</sup>, Ingemar von Ossowski<sup>3</sup>, Airi Palva<sup>3</sup> and Vengadesan Krishnan<sup>1\*</sup>

<sup>1</sup>*NCR Biotech Science Cluster, India*

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### Abstract

Sortase-dependent pili are lengthy projections on the cell surface of certain Gram-positive bacteria. Functionally, these structures are implicated with cellular adhesion, colonization, immunomodulation, and biofilm-forming processes. Each pilus is built up of different types of proteins subunits called pilins, where multiple numbers of the "major" pilin form the backbone structure, which itself is adorned by various types of "ancillary" pilins that are for adhesion and other functions. Sortase enzymes are a key component of pilus assembly and covalently connect together the pilin-proteins. As these pili were first characterized in harmful pathogens, they are seen as important virulence factors given their role in promoting host-cell adhesion and pathogenesis. This particular view of sortase-dependent pili has recently changed following their detection in beneficial

commensal bacteria, after which they have come to be regarded as niche-adaptation factors. We have initiated a structural examination of the pilins constituents from the SpaCBA sortase-dependent pili of gut-adapted *Lactobacillus rhamnosus* GG. Our overall aim is to achieve a structure-function understanding of the pilin-proteins in commensal bacteria. As a first, we solved the X-ray crystal structure of the backbone pilin GG-SpaA. GG-SpaA consists of two tandem CnaB-type domains, each possessing an isopeptide bond and E-box motif. Although the isopeptide bond in the N-terminal domain forms between lysine and asparagine residues, in the C-terminal domain, it atypically involves an aspartate instead. The effect of these bonds on thermal, proteolytic, and structural stabilities, along with pilus polymerization, was investigated. This presentation covers our recent and ongoing structural investigations.

## Arming our Primitive Bowels to Stand Against Modern Elements

**Kris A. Fuehr**

*Wholesome Yogurt, Inc., USA*

### Abstract

Recent Western culture has become reactionary. One where we look to eliminate what we are sensitive to or pop a pill to stop our symptoms. Instead of avoidance and cover-ups, what if we were able to proactively channel and nurture our own personal resistance and set ourselves up to be more hardy, more resilient– or PROSILIENT with functional foods like yogurt and kimchee. This session is an exploration into how we've applied the new science of probiotics into the food we eat every day and how researchers and food manufacturers can work together to come to the aid of our culture's health.

## Cranberries and Urinary Tract Health: How Can the Same Evidence Lead to Conflicting Advice?

**DeAnn Liska**

*Biofortis Research, a Merieux NutriSciences Company, USA*

### Abstract

Cranberry has been used traditionally to prevent urinary tract infections (UTIs). Although a number of published clinical studies in healthy women prone to recurrent UTIs have supported this benefit, meta-analyses on cranberry and UTI have reported conflicting conclusions. This talk will review the literature on cranberries and UTI and the reasons for discrepancies when the totality of the evidence is reviewed. Specifically, this discussion will highlight the differences in populations used across studies, as well as the various ways of measuring the outcomes. Considerations for designing and assessing research on UTI, as well as understanding the implications of the differing approaches to defining the outcome, will be presented. Understanding these factors is essential in translating the science into clinical practice.

## The Potential of Probiotics: Their Application in the Health and Food Areas

**Ozlem Osmanagaoglu**

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### Abstract

Probiotic is a relatively new word meaning 'for life', which is used to name live good microorganisms that are associated with the beneficial effects for humans and animals, when administered in adequate amounts, according to the World Health Organization. Their benefits to both human and animal health have been proven in hundreds of scientific research. The reported strain-specific beneficial effects of probiotic consumption include improvement of intestinal health, protection against diarrhea (antibiotic-associated and traveller's diarrhea), alleviation of constipation, enhancement of the immune system, amelioration of symptoms of lactose intolerance, prevention/treatment of allergy, reduction of serum cholesterol, increased resistance to malignancy and infectious illness, reduction of the risk of various diseases such as urogenital diseases, vaginal infections (e.g., candida or thrush), Irritable bowel disease (IBD) (e.g., ulcerated colitis and Crohn's disease), auto immune diseases (e.g., rheumatoid arthritis). This list continues to increase day by day as the pace of the researches in recent years have accelerated.

## Controlled, Randomized Double-Blind Study on the Effect of *Bifidobacterium animalis* ssp. Lactis Growth Activator (NCP<sup>®</sup>) on Irritable Bowel Syndrome and Intestinal Disorders

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### Abstract

This study includes 96 volunteers with moderate irritable bowel syndrome (IBS). Control group consumes a standard yoghurt (2x125 g/day for 33 days). Verum group (NCP-Bif) consumes the same yoghurt, enriched by a growth factor (NCP<sup>®</sup>) from a specific process of lacto-fermentation of barley (1.5 g/yoghurt) associated with *Bifidobacterium* ssp. (>1.00E+07 cfu/g product). We compared frequency related to consistency of stools (Bristol Stool Scale), Transit Score TS (frequency x consistency) and colonic transit time CTT using radio-opaque markers. Pain, quality of life and intestinal discomfort was assessed by questionnaires IBQS-QOL & WBFQ.

### Results

- Frequency of stools further increases in NCP-Bif (+1.7; p = 0.0045) vs +0.8 (p = 0.022)
- Consistency improves in both NCP-Bif (+0.7; p = 0.0045) vs control (+0.8; p = 0.022)
- TS increases in NCP-Bif (+11.9; p = 0.0003) vs control (+11.6; p = 0.012)
- CTT decreases for NCP-Bif (-33 h; p = 0.022) and not for control (+0.5 h.; p = 0.96).

The comparative number of days with pain significantly decreases in NCP-Bif (p = 0.05), respectively 4.6 to 3.7; vs 4.0 to 5.2; All scores of the IBS-QOL improved significantly for both groups, i.e. p = 0.0001 for the global score, so as for the intestinal discomfort and transit scores of the WBFQ.

**Conclusion:** The NCP-Bif association reinforces the effectiveness of the yoghurt by significantly improving consistency, frequency of stool and transit time. The product tends to reduce the number of days with pain. In accordance with the literature, these effects are probably in relation with an improvement and the quality of the intestinal microbiota.

## Cowpea Peptides Could be used to Alleviate Insulin Resistance

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### Abstract

Plant biomolecules such as cowpea peptides could hold the keys to preventing the development of diabetes and the medical complications associated with it. Phytochemicals from cowpea are beneficial in the reduction of hyperglycemia and strengthens the antioxidant status of the diabetic. L6 rat skeletal muscles were exposed to various doses of cowpea extract (0.1, 1, 10, 100 ng) for 20 hours or insulin (100 nM) for 30 minutes. Proteins isolated from treated cells were analyzed by Western blot to express the phosphorylation of Akt. Findings from this study demonstrate that cowpea extract can increase the phosphorylation of Akt in cell culture. This indicates that administering cowpea peptides to skeletal muscles activate the insulin signaling cascade. It may be deduced that cowpea peptides can mimic the actions of insulin by activating the same signaling cascade.

## Functional and Molecular Characterization of Genetically Manipulated Probiotic *B. coagulans* and Process Optimization for its High Cell Density Fermentation

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### Abstract

A leading Indian pharmaceutical company was facing the problem of repeated bacteriophage attacks. The problem was

tackled by strain improvement to develop phage resistance by genetic manipulation of *B. coagulans*.

In addition to parameters enlisted by FAO/WHO, probiotic strains were evaluated for PUFA production, scavenging activity and siderophoregenic properties. Phylogenetic relationship of strains was studied. RAPD and 2D gel electrophoresis were used to study the genome and membrane protein profiles of phage resistant and sensitive types. Statistical techniques were used to optimize conditions for maximum biomass production. The process was scaled up to achieve high cell density fermentation (HCDF).

The improved strains displayed several beneficial alterations in their functional attributes, some also produced PUFAs like EPA and DHA. Phylogenetic tree analysis revealed that phage sensitive strain was an out group in the cluster. RAPD profile exhibited several polymorphisms. Proteins like polysaccharide biosynthesis protein, YhgE/PiP N-terminal domain protein *etc.* led to development of phage resistance. Use of statistical tools enhanced the productivity by 52%. For the first time, lab scale fermentation process for HCDF of *B. coagulans* was developed yielding approximately 500% of shake flask productivity.

This was the first endeavor to develop a HCDF process for *B. coagulans*. The work reported here may prove useful in development of efficient formulations for enhanced health applications.

## The Inflammasome: Critical Regulator of Intestinal Microbiota and Inflammatory Disorders

Hasan Zaki

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### Abstract

The intestinal mucosal immune system of mammals evolved to adapt with densely populated microorganisms that reside in the intestinal lumen. The central physiological process for homeostatic immune response in the gut is the recognition of pathogen-associated molecular patterns (PAMPs) by host's pattern recognition receptors (PRRs). Perturbation of host-pathogen interaction leads to uncontrolled immune responses resulting in intestinal inflammatory disorders such as inflammatory bowel disease and colorectal cancer. Therefore, PRRs play a central role in the maintenance of intestinal homeostasis. Upon activation by specific PAMPs, several cytosolic PRRs trigger inflammatory responses by forming a multi-protein complex called the inflammasomes. The inflammasome functions as a molecular machinery for the activation of caspase-1, which subsequently processes proinflammatory cytokines IL-1 $\beta$  and IL-18 into their active forms. Our studies demonstrated that mice defective in the inflammasome pathways are susceptible to experimental colitis and colorectal tumorigenesis. Increased inflammatory responses in inflammasome-deficient mice during experimental colitis are associated with altered gut microbial ecology, particularly an abundance of *Escherichia coli*. Further investigation revealed that the inflammasome controls *E. coli* growth via the production of antimicrobial peptides. This presentation will discuss recent advances on the role of the inflammasome in the regulation of intestinal microbiota and inflammatory disorders.

## The Fiber-Microbiota-Butyrate Axis in Tumor Suppression

Scott Bultman

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### Abstract

It is controversial whether dietary fiber protects against colorectal cancer because of conflicting results from human epidemiologic studies. However, these studies and mouse models of colorectal cancer have not controlled the composition of gut microbiota, which ferment fiber into short-chain fatty acids such as butyrate. Butyrate is noteworthy because it has energetic and epigenetic functions in colonocytes and tumor-suppressive properties in colorectal-cancer cell lines. We utilized gnotobiotic mouse models colonized with wild-type or mutant strains of a butyrate-producing bacterium to demonstrate that fiber does have a potent tumor-suppressive effect but in a microbiota- and butyrate-dependent manner. Furthermore, due to the Warburg effect, butyrate was metabolized less in tumors where it accumulated and functioned as an HDAC inhibitor to stimulate histone acetylation and affect apoptosis and cell proliferation. To support the relevance of this mechanism in human cancer, we demonstrate that butyrate and histone-acetylation levels are elevated in colorectal adenocarcinomas compared to normal colonic tissues.

**Significance:** These results, which link diet and microbiota to a tumor-suppressive metabolite, provide insight into

conflicting epidemiologic findings and suggest that probiotic/prebiotic strategies can modulate an endogenous HDAC inhibitor for anticancer chemoprevention without the adverse effects associated with synthetic HDAC inhibitors used in chemotherapy.

## Intramammary Infusion of a Live Culture of *Lactococcus lactis* in Lactating Ewes with Subclinical and Clinical Mastitis: A Promising New Alternative for the Treatment of Mastitis?

Cinzia Marianelli<sup>1\*</sup>, Sebastian Mignacca<sup>2</sup>, Simone Dore<sup>3</sup>, Liliana Spuria<sup>4</sup>, Pietro Zanghi<sup>2</sup>, Benedetta Amato<sup>2</sup>, Ilaria Duprè<sup>3</sup>, Federica Armas<sup>1,5</sup>, Elena Biasibetti<sup>4</sup>, Cristina Camperio<sup>1,4</sup>, Stefano Lollai<sup>3</sup>, Maria Teresa Capucchio<sup>4</sup>, Agnese Eugenia Cannas<sup>3</sup> and Vincenzo Di Marco Lo Presti<sup>2</sup>

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### Abstract

Alternatives to antibiotic therapy for mastitis in ruminants are needed. We present an evaluation, in two trials, of the efficacy of an intramammary infusion of a live culture of *Lactococcus lactis* for the treatment of subclinical and clinical mastitis in lactating ewes. Sixty-seven animals were enrolled: 19 lactating ewes (Study 1), including healthy (N = 6) and CNS-infected ewes (N = 13); and 48 lactating ewes (Study 2) with either CNS mastitis (N = 32), or *S. aureus* mastitis (N = 16), for a total of 123 mammary glands. Intramammary infusions were performed with either *L. Lactis* or PBS for three (Study 1) or seven (Study 2) consecutive days. Antibiotic-treated and untreated control glands were included. Milk samples for microbiology, somatic cell analysis and milk production were collected before and after treatment. *L. lactis* rapidly activated the mammary glands' innate immune response and initiated an inflammatory response as evidenced by the recruitment of PMNs and increased somatic cell counts. But while leading to a transient clearance of CNS in the gland, this response caused mild to moderate clinical cases of mastitis characterized by abnormal milk secretions and udder inflammation. Moreover, *S. aureus* infections did not improve, and CNS infections tended to relapse. Further studies are needed to elucidate the efficacy and potential risks involved in the use of bacterial cultures as alternatives for the prevention and treatment of mastitis in ruminants. This work was supported by the Italian Ministry of Health, grant number RF-2010-2313040.

## Gut Microbiome Modulators to Regulate Energy Balance

Hariom Yadav

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### Abstract

Obesity and type 2 diabetes are highly epidemic around the world. Microbiome contributes significantly in pathophysiology of obesity and diabetes. Role of probiotics in obesity is questionable due to the fact that probiotics have been used as growth promoter in farming industry. Our initial studies show that probiotics supplemented fermented milk products (Indian yogurt/dahi) exhibit beneficial effects against diet induced insulin resistance and hyperglycemia. Recently, we demonstrated that selected probiotics are not detrimental for induction of high fat diet induced obesity (DIO) in mice. Interestingly, we have observed that one of probiotics (VSL#3) was potent to suppress and reverse diet induced obesity and insulin resistance. Beneficial effects of VSL#3 feeding were due to decreased food intake via increased hypothalamic satiety signals and decrease hunger signals. We found that VSL#3 treatment significantly changed gut microbiome that resulted to produce differential metabolites i.e. short chain fatty acids. Specially, VSL#3 feeding increased butyrate production that resulted to increased production of gut hormones such as glucagon like protein-1 (GLP-1). Here we described mechanism(s) of action of probiotics (i.e. VSL#3), suggesting that VSL#3 modulated gut microbiome derived short chain fatty acids (butyrate) and gut hormone axis to modulate energy homeostasis. Our further studies are aimed to describe detailed role of short chain fatty acids, and molecular mechanisms to regulate energy metabolism against obesity and diabetes.

## Soy Milk: Another Potential Source of Probiotic Bacteria

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### Abstract

Lactic acid bacteria (LAB) have been utilized in the food industry for several decades. They are referred to as probiotics because they confer beneficial health effects to their host. Such effects include balancing the microbial flora, boosting the immune response and lowering cholesterol. Probiotic bacteria from different sources have been isolated and are commercially available. However, due to their extensive use, LAB are ought to adapt to different environmental conditions which might reduce their robustness. In this study, 28 bacterial strains isolated from spontaneous fermentation of soymilk were assessed for probiotic characteristics such as low pH resistance, bile salts resistance, antibacterial activity and hemolysis test. From the 28 organisms, 9 were selected as probiotics because they showed resistance to pH 2.5, bile salt (0.4%) and none of them had hemolytic activity. They also showed resistance to a spectrum of antibiotics and were able to inhibit the growth of pathogenic Gram positive and Gram negative indicator organisms. The amplification and sequencing of the 16S rRNA gene revealed the isolates to be *Leuconostoc pseudomesenteroides* (6), *Enterococcus mundtii* (2) and *Lactobacillus plantarum* (1). In addition, 6 of these strains were able to degrade both raffinose and stachyose which are regarded as non-digestible oligosaccharides by humans. In conclusion, lactic acid bacteria of great interest can be isolated from their natural habitat and therefore serve as starter cultures in the food industry to improve organoleptic quality of soy derived products.

### Poster Presentations

## *Lactobacillus rhamnosus* GG conditioned Media Regulates Free Radicals Homeostasis in J774 Murine Macrophages

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### Abstract

Altered reactive oxygen species (ROS) and nitric oxide (NO) homeostasis is associated with many inflammatory and neurodegenerative disorders. However, the use of ROS and NO as physiological molecules by macrophage during phagocytosis suggests that macrophages were able to achieve a high degree of control over ROS and NO toxicity. Formation of free radicals in macrophages may be regulated by probiotic bacteria. However, their exact mechanism of this regulation is not clear. Thus, studies were carried out to determine the role of probiotic in the acute regulation of both ROS and NO production in the murine J774 macrophages, using a cell free *Lactobacillus rhamnosus* GG culture medium (LGG-CM). J774 macrophages were loaded with either H2-DCFDA for monitoring reactive oxygen species or with DAFFM-DA for nitric oxide. Acute free radicals' production was measured using a fluorescence microplate reader and changes were analyzed by cumulative sum (CuSuM) calculations. LGG-CM significantly enhanced ROS generation ( $p \leq .01$ ) but also significantly reduced NO ( $p \leq .05$ ) level in both ingestion and digestion phase of phagocytosis. ROS production in the ingestion phase was significantly higher than that of the digestion phase ( $p \leq .001$ ). These effects of LGG-CM were not altered in the presence or absence of *E. coli*. Pattern of ROS production was found to be oscillatory. In this study, a pulse of excessive ROS production to LGG-CM seems to be targeted for rapid digestion of *E. coli* without causing any tissue injury. Therefore, the ability of probiotic to balance NO and ROS generation may be of clinical significance in improving intestinal homeostasis.

## Viability of Probiotic Cultures in Greek Yogurt during Refrigerated Storage

Rabin Gyawali<sup>\*</sup>, Kayla Harris, Nadia Idris, Reza Tahergorabi, Valerie L. Giddings and Salam A. Ibrahim

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## Abstract

Greek yogurt has recently gained much attention as a popular, healthy snack option. This increased popularity is most likely due to the high protein content in Greek yogurt. This high protein content could also promote the viability of probiotic cultures in Greek yogurt compared to regular yogurt. Therefore, the objective of this study was to determine the viability of probiotic cultures in Greek yogurt during refrigerated storage. A total of 16 yogurt samples (8 Greek and 8 regular) of the same brand were obtained from a local store in Greensboro, North Carolina. The yogurt samples were kept at 4 °C for 4 weeks and analyzed weekly for probiotics viability. Ten grams of each yogurt were mixed with 90 ml of sterile peptone water. Appropriate dilutions were plated on MRS agar, M-17 agar and modified BIM-25 for the enumeration of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacteria* respectively. Our results showed that the initial populations of yogurt and probiotic cultures were approximately 6.5 log CFU/g in both Greek and regular yogurt samples. During the refrigeration period, the yogurt culture population remained within the same range in all tested products. However, the population of *Bifidobacteria* were maintained in Greek yogurt, whereas in regular yogurt, the *Bifidobacteria* population decreased significantly ( $P < 0.05$ ) by 2 log CFU/g during refrigerated storage. Thus, our study revealed that Greek yogurt provides higher *Bifidobacteria* compared to regular yogurt and should be recommended as a healthy probiotic option.

## The Long Term Exposure Effects of Aspirin on Growth, Functionality and Protein Profile of *Lactobacillus rhamnosus* (ATCC 53103)

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## Abstract

The present study was conducted to determine the long term exposure effects of aspirin on the growth and functionality of *Lactobacillus rhamnosus*. One isolated colony of *L. rhamnosus* was propagated in deMan, Rogosa and Sharpe (MRS) broth and incubated at 37 °C. An active strain was then harvested and washed with 0.1% peptone water. Cells were transferred into 9 mL MRS broth containing approximately 6 mg/mL aspirin, vigorously mixed and incubated for 4 h. The cells were further harvested, transferred into MRS broth and incubated at 37 °C. The exposure protocol was repeated for five sequential transfers within a week and the exposed strain cell was then surface plated onto MRS agar containing equal amount of aspirin. One isolated colony of aspirin exposed *L. rhamnosus* from aspirin was further activated in MRS broth. This procedure was repeated sequentially for 12 consecutive weeks. Bacterial populations,  $\beta$ -galactosidase activity, and protein expression were determined. A strain without aspirin exposure was used as the control. Our results showed that *L. rhamnosus* could survive after a long term exposure to a sub inhibitory concentration of aspirin. The average  $\beta$ -gal activity of *L. rhamnosus* in unexposed cells was  $153 \pm 2.5$  Gal U.; however, the production of  $\beta$ -gal activity was completely inhibited in exposed cells throughout the exposure period. There was approximately 54% more protein concentration in the long term aspirin exposed strain compared to the unexposed strain as determined by BCA assay. In addition, the SDS-page gel indicated synthesis of more protein in the exposed type *L. rhamnosus*. Regular intake of medical drugs such as aspirin could thus affect beneficial gut microflora. Therefore, foods containing probiotics and other functional foods are important for maintenance of optimal gastrointestinal health.

## Intestinal Epithelial Cell Adhesion of Potential Probiotic Strains: *In vitro* Study

Fadime Kiran<sup>1\*</sup>, Tugce Onbas<sup>1</sup>, Yavuz Emre Arslan<sup>2</sup>, Tugba Sezgin Arslan<sup>2</sup> and Ozlem Osmanagaoglu<sup>1</sup>

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## Abstract

Bacterial adhesion to intestinal epithelial cells is considered to be one of the most important characteristic of probiotic strains which protect the host organism from pathogen invasion and adhesion. Probiotic strains with their high adherence capacity effectively provide protection against disease such as pathogenic infections, diarrhea and modulate inflammatory responses. The aim of the presented study was to investigate the adherence capacity of potential probiotic strains isolated from various human sources (infant feces and breast milk) and traditional food products (yogurt, pickle juice and salgam juice) to human colon cancer cell line (Caco-2, ATCC-HTB-37), as an intestinal model. Cytotoxic effect of the strains on Caco-2 cells was also determined.

Adhesion of probiotic strains was examined from early stationary growth phase and determined by microbiological counting

assay. Adhesion was also visualized by light, fluorescent and Scanning Electron Microscope. The effect of probiotic strains on the proliferation of Caco-2 cells was assessed by tetrazolium salt XTT assay.

The results indicate that probiotic strains were characterized as highly adhesive and anti-proliferative. The strains were found to inhibit the growth of Caco-2 cells in a dose-dependent manner as detected by the XTT assay. Between the strains *Lactobacillus plantarum* PTR-1 was selected as exhibiting the best results. Our findings are the important step for the selection and *in vivo* assessment of target and function specific probiotics. In conclusion, with the aim to meet the increasing demand of individuals and markets, new functional strains might have used as probiotics in functional food or for colon cancer.

## Anti-obesity Effects of Potential Probiotic Strains on 3T3-L1 Adipocytes

G. Eda Yildirim<sup>1</sup>, Fadime Kiran<sup>1</sup>, Yavuz Emre Arslan<sup>2</sup>, Tugba Sezgin Arslan<sup>2</sup> and Ozlem Osmanagaoglu<sup>1</sup>

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### Abstract

The significant increases in the worldwide numbers of obese people are mostly due to diet and lifestyle changes. Among the various beneficial health effects of probiotics, their biological impact on obesity by modulating the lipid metabolism, has generated considerable interest. The aim of the presented study was to investigate the anti-adipogenic effect of potential probiotic strains isolated from various human sources (infant feces and breast milk) and traditional food products (yogurt, pickle juice and salgam juice) in 3T3-L1 (ATCC CL-173™) preadipocytes, as an *in vitro* model of adipogenesis.

3T3-L1 cells were cultured with bacterial cell extracts and filtered supernatant from overnight bacterial cultures and cells were allowed to differentiate in the presence of different dose levels of appropriate treatments for 10 days. The XTT assay was used to determine the cellular growth. After the induction, the cellular lipid content was assessed by Oil Red-O staining and the quantification of lipid content was determined by the AdipoRed assay.

Our results indicate that treatment with probiotic strains inhibited the proliferation and differentiation of adipocyte cells, as assessed by morphological analysis and Oil Red O staining of fat. Oil-red-O staining demonstrated that *Lactobacillus plantarum* strains reduced the number of lipid-containing rounded cells and reduced lipid accumulation in fully differentiated 3T3-L1 adipocytes. Our findings suggested that potential strains might be used as an important strategy for the potential treatment of obesity and further studies are needed to elucidate the mode of actions in efficacy tests of probiotic strains *in vivo*.

## Viability of Encapsulated *Pediococcus pentosaceus* OZF in Yogurt

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### Abstract

Encapsulation technologies are used to keep probiotic cell viable during storage, commercialization and use in food products. This viability is important while cells are passing through the gastrointestinal tract. In this research, we investigated the effect of encapsulation techniques on *Pediococcus pentosaceus* OZF strain that was isolated from breast milk in our previous studies. The strain that was shown to have some probiotic properties was identified by molecular techniques. OZF, encapsulated using sodium-alginate and whey protein was used for double coating by extrusion technique. Nonencapsulated OZF, alginate capsules and whey capsules were incubated in simulated gastrointestinal conditions for 3-4 hours. The morphology and size of the microcapsules were visualized by SEM. The survival of OZF in yogurt (nonencapsulated OZF, alginate capsule and whey capsule 10<sup>7</sup> cfu/gr) was monitored at +4 °C for 30 days. When compared with control, the results obtained indicated that the survival of microencapsulated probiotic increased significantly in simulated gastrointestinal condition (P<0.05) and there was no significant difference between the capsules coated with alginate and whey in gastrointestinal condition. SEM micrographs have shown the presence of rough surface on capsules and random presence of OZF strain both in and on the surface of capsules. However, when images of capsules coated with alginate and double coated with whey proteins under SEM are considered, a much more protective effect of double capsulation has noticed. Yogurt experiment results showed that alginate capsulation and whey coating protected the OZF approximately 92% and 95% respectively, when compared with the control.

## Common Probiotics Preparations and Use within the Acute-Care Setting

Brittney Patterson<sup>\*</sup>, Jessica Keating, Roberta Spier, Caroline Wiswell, Luz Gonzalez, Hema Kundargi, Tugba Bayrak and Colette LaSalle

San Jose State University, San Jose, CA, USA

### Abstract

**Background:** Over the past few years there has been a surge in research and interest in using probiotics within the acute-care setting. Recent ASPEN guidelines suggest using probiotics in select medical and surgical patient populations where research shows benefit.

**Purpose:** To determine the current landscape of probiotics on the market and analyze which probiotics strains and doses have an effects on many medical conditions seen in the acute-care setting.

**Methods:** Online search via Google Scholar, PubMed, and ScienceDirect for systematic reviews, meta-analysis, and individual studies using probiotics in the acute-care setting.

**Results:** A total of 27 common probiotic strains on the market place where found. The following disease and medical states were reviewed with number of article found: antibiotic associated diarrhea (n = 3), *Clostridium difficile* (n = 5), vancomycin-resistant *Enterococci* (n = 3), ventilator-associated pneumonia (n = 3), major abdominal surgery (n = 4), and trauma (n = 2). Further, probiotic strains and dosing were compared among the studies reviewed.

**Discussion:** Each study and review used different strains, dosing, and timing, making it difficult to ascertain if probiotic strains continually have an effect. Overall, it appears that probiotics can be helpful in certain medical/disease states but caution should be applied when prescribing. Specifically, those who are immune compromised or in the ICU should not routinely be given probiotics.

**Conclusion:** Further research needs to decrease confounding variable and consistently research specific strains and dosing to determine if probiotics are indeed effective.

## Benefit of Probiotics and Prebiotics in Elderly Population

Hema Kundargi<sup>\*</sup>, Jessica Keating, Roberta Spier, Brittney Patterson, Caroline Wiswell, Luz Gonzalez, Tugba Bayrak and Colette LaSalle

San Jose State University, San Jose, CA, USA

### Abstract

**Background:** Studies have shown a decline in microbiota composition and activity in gut bacteria in elderly population, due to physiology of aging, prevalence of disease(s), and other factors like malnutrition, polypharmacy and environment. Dietary modulation with prebiotic and probiotic foods may be an alternative to improve elderly microbiota.

**Purpose:** To review the literature of addition of probiotics and prebiotics in the gut modulation of elderly population.

**Methods:** Electronic databases PubMed, Google Scholar, and ScienceDirect were searched for clinical trials and systematic reviews Key word search: prebiotics, probiotics and elderly.

**Results:** Prebiotics and Probiotics have shown significant changes are elderly with low microbiota diversity with an increase of fecal *Bifidobacteria*, total numbers of *Lactobacilli*, and numbers of *B. bifidum*. It plays a role to reduce malnutrition, stimulate the immune system, and ease constipation problems in the elderly subjects. Prebiotics and probiotics consumption increased the size and diversity of protective fecal *Bifidobacterial* populations, which are often reduced in older people.

**Discussion:** While various studies support the use of probiotics and prebiotics among elderly population there is a lack in findings according to bacteria strains, dose, treatment duration, study protocol, and prebiotic type used. General dosage of pre-and-probiotic supplements for elderly population cannot be reached at this time and caution should be applied when prescribing.

**Conclusion:** The results are encouraging and further large-scale studies are warranted to establish the effectiveness of prebiotic and probiotic foods on elderly population

## The Benefits of Prebiotics and Probiotic Usage among Chronic Kidney Disease Patients

Roberta Speir<sup>\*</sup>, Luz Aceves Gonzalez, Brittney Patterson, Jessica Keating, Hema Kundargi, Caroline Wiswell, Tugba Bayrak and Colette LaSalle

San Jose State University, San Jose, CA, USA

### Abstract

**Background:** It is known that Chronic Kidney Disease (CKD) patients present an increase in uremic toxins from loss of kidney function and intestinal dysbiosis from various factors. The combination of dietetic probiotic and prebiotics may be an alternative to manage CKD symptoms.

**Purpose:** Review the literature on the benefits and sources of dietetic probiotics and prebiotics could be use in the management of CKD symptoms and improvement in quality of life.

**Methods:** Electronic databases PubMed, Google Scholar, and ScienceDirect were searched for clinical trials and systematic reviews using probiotic and prebiotic` foods and supplements in adult CKD patients.

**Results:** Probiotic and prebiotic shown to moderately reduce levels of uric acid, indoxyl-and p-cresyl sulfate, and BUN, and improve bowel movements and microbiome. The most beneficial strains were *L. acidophilus* and *casei*, *B. longum* and *breve*, and *S. thermophilus*, easily found in fermented foods. Better outcomes were found associating the usage of probiotics with prebiotic supplements, such as fructo-oligossacharides, galacto-oligossacharides, and inulin. Prebiotic foods were not used as intervention due to difficulty to include them in the patient`s diets mainly restricted in protein and phosphorous rich foods.

**Discussion:** Studies differed in findings levels on the effects of probiotic and prebiotic among CKD patients according to bacteria strains, dose, treatment duration, study protocol, and prebiotic type used. General dosage of pre-and-probiotic supplements for CKD symptoms and quality of life improvement cannot be reached at this time.

**Conclusion:** In order to determine if probiotic and prebiotic supplements can effectively manage CKD symptoms and improve quality of life of these patients, further research is needed.

## Probiotics and Behavioral Modulation

Jessica Keating<sup>\*</sup>, Brittney Patterson, Roberta Speir, Caroline Wiswell, Luz Aceves Gonzalez, Hema Kundargi, Tugba Bayrak and Colette LaSalle

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### Abstract

**Background:** The connection between “dysbiosis” and behavioral abnormalities mediated by the central nervous system has led many researchers to test the effectiveness of administering probiotics as a method of modulating behavior. The mechanistic factors behind this connection is thought to be in relation to the ability for specific probiotic strains to improve epithelial barrier integrity which reduces the likelihood of bacterial translocation and aids in restoration of serum metabolite levels.

**Purpose:** To review current literature and determine, which, if any, probiotics (genus/species) show consistent efficacy within the ability to influence behavior.

**Methods:** Online search via Google Scholar and ScienceDirect for systematic reviews, meta-analysis, and individual studies assessing use of probiotics as a means for influencing behavior in humans.

**Results:** The genus types that consistently induced behavior affect were *Lactobacillus*, *Bifidobacterium*, and *Bacteroides fragilis*. The specific genus species used varied between studies and demonstrated that different species of the same genus induce behavioral affect in different ways.

**Discussion:** The differences pertaining to genus species, probiotic dosage amounts, and experimental methodology demonstrates the need for further research to better understand the varying ways in which each species is inducing affect. This knowledge will potentially allow researchers to make smart “pairings” with specific species types in order to induce specific behavioral changes.

**Conclusion:** More research with standardized protocol pertaining to genus species, dosage amounts, and experimental methodology is needed to understand the varying ways in which specific probiotic strains are inducing behavioral affect. Until said research is completed, clinical application is not advisable.

## The Effects of Probiotics on IBS Symptoms

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### Abstract

**Background:** Given the increasing prevalence of IBS and non-existent cure, probiotics via supplements and functional foods may be viable therapeutic treatments to ameliorate this condition's symptoms while improving the quality of life among affected adults.

**Purpose:** Review the literature on the efficacy of supplements and functional foods containing probiotics for the management of IBS symptoms and quality of life.

**Methods:** Google Scholar, PubMed, EBSCOhost, and ScienceDirect electronic databases were searched for systematic reviews and individual studies using probiotic supplements and functional foods with IBS adult patients according to Rome II and III diagnostic criteria to improve their symptoms and quality of life.

**Results:** Reviews and studies on supplements and fermented milk products consisting of single and multiple probiotic strains were found. The degree of adequate relief of symptoms (abdominal pain, bloating, discomfort, and stool consistency) and quality of life among participants using probiotic supplements varied depending on IBS subtype, treatment duration, dose, and strain(s). This was also demonstrated in participants exposed to fermented milk products containing different probiotic strains.

**Discussion:** Each study and review showed differing findings on the effects of probiotic use among IBS participants according to subtype, Rome criteria, treatment duration, study protocol, dose, and strains presented. General recommendations on supplements and functional foods with probiotics for IBS symptoms and improving quality of life cannot be reached at this time.

**Conclusion:** In order to determine if probiotic supplements and functional foods can effectively manage IBS symptoms and improve quality of life of those affected, further research is needed.

## Does Gut Microbiome Link to Obesity?

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### Abstract

The fact that one third of Americans is obese or overweight displays the necessity of taking action in the treatment of obesity. When obesity related to chronic diseases is considered, understanding of obesity gains more importance. Therapeutic studies explore new approaches in the treatment of obesity. Novel approaches seek the relationship between obesity and gut microbiome. The purpose of this article is to search the link between gut microbiota and obesity, and to inquire whether targeting gut microbiome may become an approach in treating obesity. In this study, a total of 20 meta-analysis, individual studies and systemic reviews were included after online search via PubMed, ScienceDirect and CINAHL. Evidence acclaims that certain types of microbiome-firmicutes and bacteroidetes-in human gut may be related to obesity in certain mechanisms-weight loss/gain, nutrient absorption/utilization and energy production. Nevertheless, researchers are still uncertain about the mechanisms and/or clinical importance of targeting gut microbiota in the treatment of obesity.

## Protective Effects of a Yeast Fermentate in Heat Stress Conditions

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## Abstract

Exposure to high temperature can lead to chronic, acute and even lethal illnesses in human and animals. Injury and death from heat stress arise from the loss of the gut barrier integrity, resulted in the increased translocation of lipopolysaccharides (LPS) from the gut. This situation triggers a systemic inflammatory response that then leads to disseminated intravascular coagulation, necrosis of organ tissues, and multi-organ failure. New approaches for mitigation of heat stress adverse effects are of great importance for protecting the health of humans working in extreme conditions and for animal health. We studied the efficacy of a yeast fermentate EpiCor in the prevention of complications related to heat stress in rats. Oral treatment of animals with EpiCor before exposure to heat stress conditions prevented traumatic changes in gut morphology, elevation of LPS level in serum, increased vesiculation of erythrocytes, and increase in number of white blood cells. Thus, we demonstrated a protective effect of EpiCor in heat stress conditions. Future directions of this study will help to understand the feasibility of our approach not only in environmental heat stress but also in heat stress, related to physical activity.

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