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June 11, 2010

Probiotics: From Bench to Market

ABSTRACTS FOR POSTER SESSION & DATA-BLITZ PRESENTATIONS

POSTER PRESENTATION

Poster set-up will be at 7:30am-8:30am.
The poster session will take place during lunch at 12:45pm – 2:00pm.

SCIENTIFIC ORGANIZING COMMITTEE

Tri Duong, PhD
Texas A&M University

Marguerite Klein, MS
Office of Dietary Supplements, National Institutes of Health

Mary Ellen Sanders, PhD
Dairy and Food Culture Technologies

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POSTER PRESENTERS – Friday, June 11

1. Felix Barron
2. Najma Bhat
3. Duane Charbonneau **
4. Kevin A Donato **
5. Eden Ephraim
6. Eden Ephraim
7. Miguel Freitas
8. Stanislaw J. Gabryszewski
9. Gianfranco Grompone
10. Rupali Gupta
11. Moti Harel
12. William Marshall
13. Linda McKibben
14. Robert Mozersky
15. Masamichi Muto
16. Marianna Naum
17. Arthur Ouwehand **
18. Erika A. Pfeiler
19. John A. Renye
20. Tamar Ringel-Kulka
21. Guy Rousseau **
22. George A. Somkuti
23. Patrick Veiga **
24. Howard A. Young **
25. Shokri Zahra

**Indicates data-blitz presentation

1. PROBIOTIC FOODS! EMERGING NEW PRODUCTS?

Felix Barron, PhD[1], Angela Fraser, PhD[1], Muthu Dharmasena[1], Megan Collins[1], Meredith Hatton[1], Lauren Peagler[1], Anna Saunders[1], Wales Watkins[1], Kenneth Herring[1]
[1]Clemson University, Clemson, SC

Probiotic organisms can be introduced into the digestive system in various forms including fermented foods, capsules or nutrition bars. In the United States, the most common products are yogurt, milk, and cheese. Because the majority of these are dairy products, there is an increasing movement for the development of non-dairy probiotic foods, such as coconut milk. The objective of this study was to determine compliance with food labels regarding the type and number of probiotic microorganisms in selected food products from a local South Carolina market. Three products containing Probiotics were selected based on the information given on the label about the number and type of Probiotics. Samples were prepared for microbiological analysis in two different growth media: MRS and RCA, incubated for 48 hours at 37C and tested for *Lactobacillus plantarum* and *Bifidobacteria*. According to the manufacturer's label of one product, the calculated average number of microorganisms for both the RCA and MRSA media combined was approximately ten times lower than the food label value. A second product contained the number of microorganisms indicated on the label while a third product showed none to minimal growth on all media samples. These preliminary results indicate that probiotic foods in the market may present a compliance problem in terms of claimed number of Probiotics, meaning consumers may not be receiving the potential health benefits they are seeking.

2. *LACTOBACILLUS RHAMNOSUS* GG INDUCES EXPRESSION AND SECRETION OF HUMAN BETA DEFENSIN 2 BY INTESTINAL EPITHELIAL CELLS

Najma Bhat[1], Jared Potts[1], Barry Goldin[2], Honorine Ward[1, 2]
Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center[1,], and Department of Public Health and Community Medicine, Tufts University School of Medicine[2], Boston, MA

The mechanisms by which probiotics such as *Lactobacillus rhamnosus* GG (LGG) exert their beneficial effects are not clear. Studies suggest that they may function by modulating innate and adaptive immune responses in the gastrointestinal tract. Recent studies have shown that some probiotics induce expression of antimicrobial peptides (AMPs) by intestinal epithelial cells. However, it is not known whether LGG has a similar effect. The goal of this study is to investigate the effects of LGG on expression and secretion of AMPs including alpha (HD5, 6) and beta (HBD-1, 2) defensins and cathelicidins (LL37) by human intestinal epithelial cell lines. Caco-2 and HCT-8 cells were grown to confluence and stimulated with increasing doses of LGG or medium alone, for 6 to 24 hrs at 37˚C. RNA was isolated and cDNA synthesized using reverse transcriptase. AMP cDNAs were amplified and quantified by real-time PCR using AMP-specific primers and expression normalized to that of beta actin. HBD-2 protein levels were measured in supernatants from stimulated Caco-2 cells by ELISA. HBD-1, HD5, 6 and LL37 were constitutively expressed and were not up-regulated in response to LGG. However, LGG induced HBD-2 expression which was maximal at a dose of10^6 pfu/ml and after 24 hours in both cell lines. LGG also induced HBD-2 protein secretion by Caco-2 cells. These results suggest that LGG may modulate innate immune responses by up regulating HBD-2 expression and secretion in intestinal epithelial cells. Current efforts are focused on determining the signaling pathways involved in up regulation of HBD-2 expression by LGG.

The present study is supported by a research grant from Dannon Company Inc.

3. DEVELOPMENT OF *BIFIDOBACTERIUM LONGUM INFANTIS* 35624 FOR A PROBIOTIC SUPPLEMENT

Duane Charbonneau PhD[1], Liam O'Mahony PhD[2], Fergus Shanahan MD[3], Barry Kiely PhD[4], Ray Grant PhD[1], Linda McKean BS[1], Yuli Song PhD[1]
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[4] Alimentary Health Ltd, Kinsale, County Cork, Ireland

Bifidobacterium longum subsp *infantis* 35624 (*B. infantis* 35624) was isolated from an ileocecal biopsy of a healthy individual after removal of extraneous loosely associated bacteria. This strain was selected for further study following comparative assessments to benchmark strains. The evaluation criteria were based on antimicrobial efficacy, microbial community modification, biomarker induction and efficacy in animal infection models. The impact of B. infantis 35624 on fecal floral composition and metabolism in healthy as well as Irritable Bowel Syndrome (IBS) subjects was determined. Following daily consumption, the greatest effects observed were increases in lactic acid bacteria, changes in fecal bacterial composition as well as changes in short-chain fatty acid profiles. In clinical trials, B. infantis 35624 improved certain symptoms associated with IBS, although consistent effects on symptoms were not seen in all clinical studies. Using quantitative PCR analysis of stool, it was shown that GI colonization by B. infantis 35624 was transitory in nature, and counts decreased within 2 weeks after dosing was suspended. Clinical studies that examined induction of biomarkers appeared to produce results analogous to those observed in vitro in that exposure to B. infantis 35624 increased the ratio of IL10 to IL12. These results are consistent with the conclusion that B. infantis 35624 exerts an effect both on the microbiome as well as the host.

4. *LACTOBACILLUS RHAMNOSUS* GG ATTENUATES INTERFERON-γ AND TUMOR NECROSIS FACTOR-α-INDUCED EPITHELIAL DYSFUNCTION

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Department of Laboratory Medicine and Pathobiology, University of Toronto;
Cell Biology Program, Research Institute, Hospital for Sick Children;
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The intestinal epithelial tight junctions (TJ) form a protective barrier against luminal contents and can be disrupted by infection or pro-inflammatory cytokines. Abnormalities in TJ contribute to a variety of intestinal disorders, including diarrhea and chronic inflammatory bowel diseases. Probiotics preserve intestinal TJ, but the mechanisms are not well characterized. We hypothesized that probiotics preserve barrier function by interfering with pro-inflammatory cytokine signaling. Polarized epithelial monolayers were inoculated apically with probiotic, *Lactobacillus rhamnosus* GG (LGG) 3 h prior to treatment of the basolateral medium with IFN-γ overnight. The monolayers were then placed in fresh basal medium ± TNF-α and transepithelial electrical resistance (TER) measurements were taken over the time course of TNF-α stimulation. To complement TER findings, cells were processed for zona occludens-1 (ZO-1) immunofluorescence. Basal tissue culture medium was collected after overnight TNF-α stimulation to measure secreted chemokines (interleukin-8, eotaxin). For measures of TNF-α signaling, both immunofluorescence and electromobility shift assay techniques labeling the NF-κB p65 subunit were used to detect cytoplasmic to nuclear translocation after 30 min of TNF-α stimulation. LGG ameliorated the deleterious affects of interferon-γ and tumor necrosis factor-α stimuli on tight junction architecture and barrier function. LGG dampened the NF-κB signalling response as evidenced by the reduction of NF-κB translocation and chemokine secretion. Further research will characterize the mechanism by which LGG modulates additional cell signal transduction pathways. These findings provide insight to the design of novel interventions that could be used in the prevention of both acute and chronic inflammatory bowel diseases.

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5. A PROBIOTIC STRAIN MODULATING THE IMMUNE SYSTEM OF PIGLETS DURING *SALMONELLA* INFECTION

E. Ephraim[1,3], C. Gabler[2], C. Holder[2], R. Einspanier[2], M.F.G. Schmidt[3]
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The ban of the use of antibiotics in animal production in Europe has led to increased interest in alternatives to antibiotics. In recent years, probiotic bacteria have been considered as an alternative means of reducing pathogen loads and improving the immune status of humans and animals. *Enterococcus faecium* SF68 is a probiotic that has been shown to have antibacterial effect on certain pathogens. *Salmonella* infection is a serious medical and veterinary problem world-wide and causes great concern in the food industry. Host defense against *Salmonella* is mainly based on the production of proinflammatory cytokines; such as interleukin (IL) 1, IL-6, and IL-8. A most likely mechanism of action of this probiotic against enteropathogens is thought to be a subtle modification of the immune system. In this study, piglets were divided into a control group, receiving feed with out supplement and a probiotic group, receiving 10^6 CFU *E. faecium* SF68 supplement in feed. Piglets of both groups were infected with 10^9 CFU *Salmonella* Typhimurium DT104. At different time points after infection, piglets were slaughtered and samples were collected from different organs of the piglets, from which immune cells were isolated. The FACS analyses of the relative percentages of CD8 positive cells in the intraepithelial lymphocytes of the jejunum showed a significant decrease in the probiotic group as compared to the control group. The clinical significance of this effect was not clearly seen in the animals. On the other hand, the mRNA expressions of IL-1α, IL-1β, IL-8, TLR2 and TLR9 in the PBMC of the probiotic group were significantly up-regulated at 71h after infection. The data obtained supported the hypothesis that the probiotic supplement increases the immune response of piglets during *Salmonella* infection.

6. ANTI-VIRAL ACTIVITIES OF A PROBIOTIC STRAIN OF *ENTEROCOCCUS FAECIUM*

E. Ephraim[1, 3], M. Burwinkel[2], C. Palissa[2], Z. Wei[3], M. Sachtleben[4], J. Plendl[4] and M.F.G. Schmidt[2]
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TGEV is a highly infectious intestinal virus of pigs that causes viral enteritis and fetal diarrhea. Very few animal studies have indicated that probiotic bacteria can have a beneficial effect on virus-induced diarrhea. However, limited data are available from cell culture studies which would yield a clue on the mechanisms of the anti-viral action of probiotics. The effect of probiotics against TGEV has not been studied. Since probiotics act mainly in the intestine where TGEV-infection resides, establishing appropriate intestinal cell lines of porcine origin is crucial for *in vitro* studies with TGEV. We isolated and characterized intestinal epithelial cells from the jejunum of a piglet. TGEV grows well in the cells attaining its maximum titer at 48h after infection. Pre-treatment of these cells with the probiotic, *Enterococcus faecium* SF68, decreased virus yield and lead to an increased survival of TGEV-infected cells. The probiotic pretreatment also decreased TGEV-induced mRNA expression of IL-6, IL-8 and IFN- in the cells.

7. DEVELOPMENT OF CULTURED DAIRY PROBIOTIC FOOD PRODUCTS

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On the basis of the extensive basic and clinical research that has been conducted on dairy probiotic food products, evidence has accumulated over the recent years to support these products as an important part of the daily diet in many countries. The development of a cultured dairy probiotic food product is a complex process involving years of research. Initial steps include the collection and identification of candidate probiotic strains for a particular beneficial effect. Subsequently, several preclinical approaches, such as *in vitro* and *in vivo* models, can be used to select potential candidates. Incorporation of these candidate probiotic strains in dairy foods, including the fermentation process, is also a critical step that requires extensive trials and technical skills. Finally, the beneficial effects of a probiotic food product should be supported by reliable scientific evidence that has been validated in human trials, according to methods recognized by the scientific community. When clinical trials are performed, special attention must be placed on the intended use of the product, the design of the study, and the markers and endpoints selected. This review describes the very sophisticated chain leading to the development of a cultured dairy probiotic food, from the collection, identification, and selection process of candidate probiotic strains, to the development of the probiotic-containing food, and performing the human clinical testing of the food.

8. PROBIOTIC PRIMING OF THE RESPIRATORY MUCOSA PROTECTS AGAINST LETHAL PNEUMOVIRUS INFECTION

Stanislaw J. Gabryszewski[1], Ofir Bachar[1], Kimberly D. Dyer[1], Kristin E. Killoran[2], Joseph B. Domachowske[3], Helene F. Rosenberg[1] [1]Eosinophil Biology Section and [2]Lymphocyte Biology Unit, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; [3]Department of Pediatrics, SUNY Upstate Medical University, Syracuse, NY

Severe respiratory virus infections cause irreversible inflammatory lung injury and pose a significant societal burden, both clinically and economically. Probiotic lactobacilli have documented anti-inflammatory properties at the gut epithelium; however, oral delivery is inefficient at reducing virus-induced lung inflammation. For our study, we probed the immunotherapeutic potential of two probiotic microorganisms, *Lactobacillus plantarum* and *Lactobacillus reuteri*, when targeted to the respiratory mucosa. We observed that intranasal priming of mice with either of the *Lactobacillus* species protected against an otherwise fatal challenge with the respiratory virus pathogen, Pneumonia Virus of Mice (PVM). Survival was associated with diminished virus recovery, differential recruitment of proinflammatory leukocytes, and global suppression of a number of cytokines associated with the pathology of PVM infection (e.g. CXCL10, CCL2, CXCL1). Intriguingly, *Lactobacillus*-primed mice devoid of the Toll-like receptor (TLR) signaling adapter molecule MyD88 were protected from PVM infection, suggesting that MyD88-dependent signaling is dispensable for this response. Our findings indicate that direct contact of clinically-benign lactobacilli with the respiratory mucosa confers an effective innate immune shield against severe respiratory virus infection, providing promise for the development of prophylactic measures in the absence of specific antiviral vaccines.

9. RESHAPING IN VITRO PREDICTABILITY TO GET NEW MECHANISTIC INSIGHTS ON PROBIOTICS: HOW "BAD" ARE THE "GOOD"?

Gianfranco Grompone, PhD[1, 2], S. Legrain-Raspaud, PhD[1], R. Bourdet-Sicard, PhD[1], I. Chambaud, PhD[1], Daniel Ramón, PhD[3].
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The human immune system must deal with a huge number of microorganisms populating the gut and establish a delicate balance between destroying dangerous bacterial pathogens while preserving the beneficial gut microbiota. Getting new insights on how gut homeostasis is established, maintained or disrupted is crucial to better understand the role of the gut commensal microbiota in the health status of the host. There is increasing preclinical and clinical evidence that Probiotics play a key role as modulators of inflammatory pathways by reducing tissue inflammation. How can we improve predictability from *in vitro* tests to better select and characterize Probiotic functionality? We present here a multi-dimensional integrative approach to screen 100 LAB strains *in vitro*. We developed new host–bacteria heterotypic co-culture systems combining Lactobacilli, Bifidobacteria and Streptococci with Intestinal Epithelial Cells, Peripheral Bone Monocyte Cells and Dendritic Cells. We measured by Luminex a "customized" set of 30 pro and anti-inflammatory biomarkers from these host cell-bacteria interaction supernatants to identify new candidate strains. Moreover, we included to this approach a completely new and highly predictive anti-oxidant screening on *Caenorhabditis elegans* where worms were fed with the LAB strains. We showed correlations between anti-inflammatory *in vitro* profiles and oxidative stress resistance in *C. elegans*. Future transcriptomic analysis will dig into the identification of the signaling pathways modulated by the candidate strains in *C. elegans* which could lead us to specific mice models validation and bring new mechanistic insights on Probiotics.

10. PROBIOTIC TREATMENT OF ATOPIC DERMATITIS IN PRESCHOOL CHILDREN: A RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED CLINICAL TRIAL

Rupali Gupta, MS[1], Sergei Gerasimov, MD, PhD[2], Volodymyr Vasjuta, MD, PhD[2], Oksana Myhovych, MD[3], Lyudmyla Bondarchuk, MD[4]
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The role of probiotics in treatment of atopic dermatitis (AD) remains controversial. This was double-blind placebo-controlled prospective trial with 90 children aged 1-3 years suffering from moderate-to-severe AD and treated with a mixture of L. acidophilus DDS-1, B. lactis UABLA-12 (DDS-Junior, UASLabs) with fructooligosaccharide in a dose of 5 billion CFU twice daily for 8 weeks vs placebo. At the final visit percent decrease of SCORAD (scoring of atopic dermatitis index) was 33.7% in the probiotic comparing to 19.4% in the placebo group (p=0.001). Children receiving probiotic showed greater decrease in mean (SD) SCORAD score than did children from the placebo group at week 8 (-14.2 (9.9) vs -7.8 (7.7) respectively, p=0.001). IDQOL (infant dermatitis quality of life) and DFI (dermatitis family impact) decreased significantly from baseline by 33.0% and 34.4% in the probiotic group and by 19.0% and 23.8% in the placebo group, respectively (p=0.013, p=0.010). Use of topical corticosteroid during the 8 week trial period averaged 7.7 g less in probiotic patients (p=0.006). Percent of CD4, and CD25 decreased, and count of CD8 increased in the probiotic group at week 8 (p<0.001). The administration of probiotic mixture containing L. acidophilus DDS-1, B. lactis UABLA-12 and fructooligosaccharide was associated with significant clinical improvement in children with AD, with corresponding lymphocyte subset changes in peripheral blood. The efficacy of probiotic therapy in adults with AD requires further investigation.

11. A NOVEL PRESERVATION TECHNOLOGY FOR PROTECTING PROBIOTICS DURING LONG TERM STORAGE UNDER HUMID AND NON-REFRIGERATED CONDITIONS

Moti Harel, PhD, Jenny Tang, PhD, Krista Kaizer, Laura Marino. Advanced BioNutrition Corp., Columbia, MD

The inclusion of live probiotic bacteria in a typical non-refrigerated food product (~25°C and ~Aw>0.2) usually results in a rapid and significant loss of viability. Attempts to protect the bacteria through micro-encapsulation including spray drying, extrusion, immobilization in calcium alginate beads, emulsion, top coating and phase separation have been utilized with limited success. Here we report on a novel Targeted Delivery System developed by ABN (MicroMatrix™) aimed to stabilize and protect sensitive bioactive compounds through food manufacturing processes and long term non-refrigerated storage. The technology involves a controlled desiccation process (liquid drying) of a viscous formulation of natural polymers surrounding the probiotic bacteria or other biological active materials to be protected. As a result, the protected live probiotic bacteria remain quiescent while retaining their activity for a long period under challenging manufacturing, storage, and gastric conditions. Storage stability of preservations containing different species of *Lacto* and *Bifido* probiotic bacteria was improved dramatically to less than one log loss of viability (<1 log CFU/g) after exposure to high temperature and relative humidity (37°C and 33%RH) for over three months. This novel approach to stabilization, protection and delivery was successfully applied to various biologically active materials including probiotic bacteria, essential oils, vitamins, enzymes, and even vaccines in a variety of food or feed products. This preservation technology enables the food industry to offer a wide range of new products containing sensitive biologically active materials outside the cold chain of distribution

12. HARMLESS BACTERIA RELEASE IMMUNE-ENHANCING SMALL OLIGORIBONUCLEOTIDES

William Marshall, Ph.D., David Schaefer, MS. Immunom Technologies, Inc., Naples, FL

A growing body of knowledge is supporting a century-old notion that consuming harmless bacteria can provide a health benefit. In the1990s studies by E. Hilton at the Long Island Jewish Hospital and R. Yolken at the Johns Hopkins Univ. Hospital concluded that consuming yogurt ameliorated vaginal and intestinal infections. The research however could not reveal the molecular and structural mode of action, a requirement of the FDA to support such health claims. Our objective has been to determine this mode of action at the level of a single chemical entity and an isolated immune cell. We have found that during normal growth bacteria accumulate small ribonucleotides, which brake cell growth as populations approach stationary phase. However, when facing hostile environments like saliva, they quickly release them into the surroundings to regain active growth. Through co-evolution, imbedded sentry cells of the immune system have adapted an alert response to their sudden appearance. Feeding sterile preparations of these released oligos totally protected mice from the lethality of LPS-induced septic shock and reduced by 45% the lethality of a white spot virus challenge to shrimp. Exposing neutrophils to a 9-membered oligoribonucleotide, presumed to occur in the preparation, triggered a significant oxidative burst without apparent toxicity. In all procedures, efficacy was related to dose and strain; no toxicity was observed. The fastest growing strains appear to accumulate and release the most protective oligos.
Our studies indicate that commercial production of probiotic bacteria does not allow retention of the immune-enhancing oligos.

13. CAN FDA COORDINATE THE REGULATION OF DIETARY SUPPLEMENTS AND INVESTIGATIONAL, NEW DRUGS TO IMPROVE CLINICAL SAFETY OF PROBIOTICS?

Linda McKibben MD DrPH, Robert Mozersky DO, Sixun Yang, MD PhD
Food and Drug Administration, Silver Spring, MD

The Food and Drug Administration (FDA), an agency of the Department of Health and Human Services, is responsible for protecting the public health by assuring the safety of America's foods; the safety, efficacy, and security of human and veterinary drugs, biological products and medical devices; and the safety and security of cosmetics and products that emit radiation. FDA is also responsible for advancing public health by helping to speed innovations that make medicines safer and more effective, as well as by providing the public with accurate, science-based information about medicines and foods to improve their health. A recent systematic review of probiotics used as nutritional supplements in hospital patients (Whelan, Myers 2010) suggests additional research is needed in the safety of these products when used in this particular population. To further evaluate the safety of probiotic products, a pilot project is underway to assess the value of sharing probiotic product safety information between FDA's (1) Center for Food Safety and Applied Nutrition (CFSAN), which has regulatory authority over probiotics in dietary supplements, foods, drinks, and cosmetics; and (2) Center for Biologics Evaluation and Research (CBER), which regulates probiotics as investigational new drugs when the intent is to treat, prevent or cure human diseases before these may be lawfully marketed as live, bio-therapeutic products (LBPs); currently, there are no licensed LBPs. While FDA cannot disclose specific information about investigational products or studies unless these are publicly disclosed by the sponsor, we will summarize our experience reviewing proposed clinical trials and published reports of serious adverse events (SAEs) related to use of probiotic products. We will also describe the results of a review of the CFSAN Adverse Events Reporting System SAE data related to probiotics in dietary supplements.

14. CHARACTERIZATION OF SERIOUS ADVERSE EVENT DATA ON DIETARY SUPPLEMENTS FORMULATED WITH LIVE MICROBIAL ORGANISMS FROM FDA'S CENTER FOR FOOD SAFETY AND APPLIED NUTRITION (CFSAN) ADVERSE EVENTS REPORTING SYSTEM (CAERS)

Robert Mozersky, D.O.
Food and Drug Administration, Silver Spring, MD

The mission of the FDA's CFSAN is to ensure that the US food supply is safe, sanitary, wholesome, and honestly labeled, and that cosmetic products are safe and properly labeled. To help monitor safety, the CFSAN Adverse Events Reporting System (CAERS) collects voluntary adverse event (AE) reports from the MedWatch program, emails, telephone calls, faxes, letters, and the FDA's Office of Regulatory Affairs District Offices' Field Accomplishments and Compliance Tracking System. CAERS also collects mandated industry reports of serious AEs (SAEs) defined as deaths, life-threatening experiences, inpatient hospitalizations, persistent or significant disabilities or incapacities, congenital anomalies or birth defects, and outcomes requiring medical intervention to prevent permanent impairments. Physicians review SAE reports using the Council for International Organizations of Medical Sciences classification algorithm to determine plausibility of association between events and reported product(s)/ingredient(s).

CAERS is a passive surveillance system that captures voluntary AE reports from all sources and mandatory SAE reports from industry. It lacks a population denominator for calculating rates. CAERS also lacks co-morbidity, co-consumption, and other individual data on factors that could contribute to SAEs. Low numbers of voluntary versus mandatory reports may create bias in the overall results, as may differences in individuals who do versus do not report SAEs. Here, we characterize CAERS SAE reports on dietary supplements formulated with live microbial ingredients

15. SAFETY EVALUATION OF COMMERCIAL BIFIDOBACTERIA STRAINS BY MUCIN DEGRADATION AND TRANSLOCATION ABLITY

Masamichi Muto[1], Fumiaki Abe, PhD[1], Tomoko Yaeshima, PhD[1] and Keiji Iwatsuki, PhD[1]
[1] Food & Technology Institute, Morinaga Milk Industry Co., LTD, Kanagawa, Japan

Beneficial effects of probiotics on human health have been widely known and probiotics have been applied to various dairy foods. On the other hand, severe diseases like sepsis caused by probiotics, especially in immunocompromised persons have been reported.
To evaluate commercial probiotic strains*, Bifidobacterium longum* subsp. *longum* BB536 (BB536), *B. breve* M-16V (M-16V) and *B. longum* subsp. *infantis* M-63 (M-63), for safety, the abilities of mucin degradation and translocation were observed. Addition of mucin into culture medium as the only carbon source did not stimulate the growth of the tested strains. And mucin degradation in each cultured medium was not observed when SDS-PAGE analyses stained by coomassie blue and PAS were performed, indicating all tested strains can not degrade mucin unusually.
For translocation tests, both conventional and immunocompromised mice were used. BB536 did not occur the translocation in various organs, such as blood, liver, spleen, kidney and mesenteric lymph nodes, when the high number cells were administered into conventional mice for one week. Also, no disturbance of epithelial cells and mucosal layer in the ileum, cecum and colon was detected in the same experiment.
Even when immunocompromised mice occurred by administration of cycrophophamide were used, no translocation of BB536, M-16V, and M-63 was observed in various organs, although these mice were caused sepsis by administration of pathogenic bacteria. These results strongly suggest that BB536, M-16V and M-63 are safe from the points of view of mucin degradation and translocation ability.

16. TAXONOMIC DATABASE ASSEMBLY OF BACTERIAL STRAINS USED AS FOOD INGREDIENTS

Marianna Naum, PhD, Socrates Trujillo, PhD, Rohini Dave, William Sacks, Cathryn Bolger, and Dan Levy, PhD
Food and Drug Administration, College Park, MD

Bacteria, which have long been used in food production, are found commonly as viable organisms in a wide range of foods. In addition to use in traditional food fermentations, strains thought to have nutritive value as "probiotics" are also added to food. However, bacteria with traits inappropriate for use in human food have been inadvertently added into products. Therefore, it is important to properly characterize bacteria used as food ingredients For example, some bacteria used in fermentation are not easily distinguished from closely-related pathogens. Furthermore, use of non-standard genus and species names for bacterial ingredients can be confusing and even misleading as to the nature of the

ingredient. To address this, we have constructed a taxonomic database of representative *Bacillus* species encompassing both pathogens and organisms used in traditional food fermentations. 16S rDNA gene sequences were used to determine the evolutionary relationships of 135 Bacilli, followed by sequencing of the gyrA gene of the Bacillus subtilis clade to further discriminate among closely-related microorganisms frequently used as food ingredients. In addition, we mapped the antibiotic resistance profiles of these Bacilli on the resulting phylogenies to assess the prevalence and possible transfer of antibiotic resistance. Preliminary results from Lactobacillus and other genera have also been collected. This novel genetic database will be published and can serve as a reference resource for identifying bacteria used in food such as those described in new dietary ingredient notifications to the FDA's Center for Food Safety and Applied Nutrition

17. ADMINISTRATION OF PROBIOTIC *BIFIDOBACTERIUM LACTIS* 420 REVERSES DIABETIC STATUS IN MICE UNDER HIGH-FAT DIET

Arthur Ouwehand[1], A. Waget[2], P. Klopp[2], Kaisa Olli[1], Sampo Lahtinen[1], Didier Carcano[3], Nina Rautonen[1], Remi Burcelin[2]

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[2] Institut National de la Santé et de la Recherche Médicale (INSERM), U858, Toulouse, France
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Recent findings indicate that a high-fat diet (HFD) induces metabolic endotoxemia (elevated plasma lipopolysaccharides; LPS) leading to inflammation, and is an early triggering factor of metabolic syndrome and type II diabetes. We hypothesized that administration of a probiotic *Bifidobacterium* may reduce endotoxemia and subsequently inhibit inflammation, improve glucose tolerance and contribute to weight maintenance. C57Bl6 mice and CD14 knock-out mice were fed with normal or high-fat diet to induce diabetic state. *Bifidobacterium lactis* 420 was administered daily for four weeks. Diabetic status was measured by IPGTT and hyperinsulinemic euglycemic clamp. RNA was extracted from tissues for the determination of inflammatory cytokines. Body composition was measured by ECO-MRI and bacteriological analyses were done by qPCR. LPS was determined from blood. Epithelial integrity was measured with cell culture. B. lactis 420 reduced fasting glycemia, improved glucose tolerance and reduced insulin resistance and tissue inflammation. Weight gain, total fat mass, mesenteric adipose tissue weight and plasma LPS were lowered by the treatment. Probiotic effect was blunted in knock-out mice lacking CD14 (LPS receptor), suggesting a central role of LPS. Additional mechanistic studies showed that *B. lactis* 420 improved epithelial barrier function and reduced bacterial translocation from gut into mouse tissues. Administration of *B. lactis* 420 may offer a new strategy for treatment of metabolic syndrome, type II diabetes, and co-morbidities including obesity and chronic inflammation. The mechanism associated with the positive effect of *B. lactis* 420 involves reduction of translocation of gut-derived LPS into the host, thus reducing inflammation.

18. REGULATORY REVIEW OF LIVE MICROBIAL INGREDIENTS FOR DIETARY SUPPLEMENTS

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The Dietary Supplement Health and Education Act (DSHEA) of 1994 defines a dietary supplement as "a product intended to supplement the diet that bears or contains one or more dietary ingredients." Firms wishing to manufacture or distribute a dietary supplements containing new dietary ingredients (NDIs, those not marketed in the U.S. before 1994) may be required to notify the Food and Drug Administration and provide history of use or other evidence supporting a reasonable expectation of safety before marketing the product in the United States. The statute does not authorize FDA to evaluate efficacy (e.g. whether a microbe has probiotic properties) during premarket safety reviews.

New dietary ingredient notifications describing 21 live microbial ingredients have been submitted to the FDA as of December 2009. These have included notifications for species of *Lactobacillus*, *Bifidobacterium*, *Bacillus, Enterococcus*, *Saccharomyces*, *Streptococcus*, and *Clostridium*. Six of these NDIs were acknowledged by the FDA. The agency objected to the marketing of the remaining products. A number of safety concerns were raised in the responses to these notifications including questions of taxonomy, fermentation conditions, quantity and viability of the microorganisms, and the presence of toxin and antibiotic resistance genes. These concerns will be listed as illustrations of how these products lacked sufficient evidence to demonstrate a reasonable expectation of safety.

19. EXPRESSION OF PEDIOCIN IN DAIRY LACTIC ACID BACTERIA TO ENHANCE THEIR POTENTIAL AS PROBIOTICS

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Pediococcus acidilactici is considered a food-grade bacterium which produces the antilisterial peptide pediocin. The production of this bacteriocin contributes to the potential for using *P. acidilactici* as a probiotic to prevent Listeria contamination of foods. The use of pediococci in dairy foods is limited due to their inability to metabolize lactose; thus effort has been made to express pediocin in dairy cultures to enhance their value as probiobics. The pediocin operon (*papA-D*) was cloned downstream of the nisin-inducible promoter in pMSP3535. The vector, pRSNPed, was introduced into *Streptococcus thermophilus, Lactococcus lactis* ssp*. lactis*, and *Lactobacillus casei*. Following nisin induction all strains expressed pediocin and prevented the growth of *Listeria monocytogenes*. However, only the *L. casei* strain inhibited *L. monocytogenes* NR30, a nisin resistant mutant. Pediocin production in *L. casei* was determined to be 3,200 arbitrary units (AU) ml^{-1}, whereas expression in *S. thermophilus* and *L. lactis* ssp. *lactis* resulted in 400 and 1600 AU ml^{-1} respectively. To increase production in *S. thermophilus* and *L. lactis* ssp. *lactis* the pediocin operon was cloned into a modified NICE vector, pMSP3535H3, which leads to increased nisin-induced expression due to presence of the nisin immunity gene. Proper construction of the new vector, pRSNPedH3, was confirmed by PCR, showing a 2.2 kb product corresponding to *papC*, and a 0.8 kb product containing the *nisA* promoter and *papA*. The newly constructed vector was introduced into all three dairy strains, with work continuing to increase pediocin output.

20. PREVALENCE AND CHARACTERISTICS OF PROBIOTIC USE IN SUBJECTS WITH FUNCTIONAL GI SYMPTOMS

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Consumer-targeted advertising has led to an increased use of probiotics for a range of gastrointestinal (GI) symptoms. Inexpensive and readily available, these products are attractive to consumers, yet clinical trials show that the benefit from their use may be product, bacterial species and condition/symptom specific.
Aim: To determine the prevalence, characterize, reasons and motivations for probiotic use in subjects with functional GI (FGI) symptoms.
Methods: Subjects completed questionnaires capturing information on type and severity of GI symptoms, time and duration of probiotic use, source of recommendation, improvement of symptoms and satisfaction with use and quality of life.
Results: Out of the 131 subjects enrolled 35.1% had intentionally consumed probiotics. 40% of these did so following the recommendation of a friend or family member, 38% did so on their own, and only 22% did so at the recommendation of a health care provider. No statistical differences were found between the two groups regarding subjects' perceived improvement in symptoms and satisfaction with treatment. Subjects consuming probiotics were not statistically different from subjects not consuming probiotics in frequency and severity of their GI symptoms and quality of life. Baseline demographics were similar among the two groups.
Conclusions: The majority of the population consuming probiotics are doing so based on anecdotal information and at their own behest. The lack of increased satisfaction indicates that self-treatment did not meet consumers' expectations. Better education, preferably by health-care providers, regarding appropriate and effective use of probiotics may improve beneficial effects and satisfaction with probiotics use.

21. PROBIOTICS INHIBIT BEHAVIORAL SIGNS OF DEPRESSION AFTER A MYOCARDIAL INFARCTION IN A RAT MODEL

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We have previously demonstrated that pre-treatment with probiotics reduces apoptosis observed in the limbic system after myocardial infarction (MI). This study tested whether probiotics could also attenuate post-MI depressive behavior. **Methods:** MI was induced in anesthetized rats via a 40-minute transient occlusion of the left anterior coronary artery. Sham rats underwent the same surgical procedure without actual coronary occlusion. For the 7 days before MI and between the 7th and 14th day post MI, half the MI and sham rats were given > 1 billion live bacterial cells of *Lactobacillus helveticus R0052* and *Bifidobacterium longum R0175* per day dissolved in water while the rest of the rats received only the vehicle. Depressive behavior was evaluated 14 days post MI using Social Interaction, Forced Swimming and Passive Avoidance tests. **Results:** Vehicle-treated MI rats showed the expected behavioral syndrome of depression. Probiotics-treated MI rats displayed more Social Interactions and a better performance in the Forced Swimming and the Passive Avoidance tests compared to vehicle-treated MI rats ($p < 0.05$). Probiotics had no impact on behavioral performance in sham rats. **Conclusion:** Probiotics can interfere with the development of post-MI depressive behaviour. These results further suggest a role of the gastro-intestinal tract in mediating this response.

22. PROPERTIES OF A NEW PLASMID-ENCODED ANTILISTERIAL DURACIN

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The beneficial traits of probiotic bacteria may include bacteriocin production with potential in developing strategies for food protection. The pathogen *Listeria monocytogenes* causes outbreaks of listeriosis, with ca. 25% mortality rate. Since this pathogen can grow in harsh environments (low pH and temperature), contaminated food is often the source of listeriosis outbreaks. The widespread presence of *Listeria* requires the utilization of every available means, including bacteriocins, to control its growth in foods. In this study, we characterized the anti-listerial bacteriocin of a lactic fermentation bacterium recovered from cheese samples (designated as Enterococcus 41D) and evaluated the genetic elements responsible for bacteriocin production. Enterococcus 41D was identified by 16S rRNA analysis as *Enterococcus durans*, which may be a probiotic member of the the Enterococcus group. The bacteriocin was stable between pH 2 to 7 and could be stored for at least 4 days at room temperature, and 14 days under refrigeration conditions, without the loss of activity. Treatment with proteases or heat (121°C, 15min) inactivated the bacteriocin. PCR analysis revealed that primer pairs for all known enterococcal bacteriocin (enterocin, duracin) genes failed to amplify DNA from strain 41D. Plasmid curing experiments suggested the association of the bacteriocin gene with a plasmid. Research is in progress to complete the characterization of resident plasmids in Enterococcus 41D and the genes responsible for bacteriocin production. The apparently new new, plasmid-encoded antilisterial bacteriocin from the putative probiotic strain *E. durans* 41D may be useful to protect food products under low temperature storage conditions.

23. STUDY OF THE INTERPLAY BETWEEN GUT MICROBIOTA AND INGESTED BENEFICIAL BACTERIA IN IRRITABLE BOWEL SYNDROME SUBJECTS WITH PREDOMINANT CONSTIPATION

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Previously, we showed in a randomized, double-blind, controlled, parallel group study that a 4-weeks consumption of a fermented milk containing *Bifidobacterium lactis* DN-173010 and strains of *Lactococcus lactis*, *Steptococcus thermophilus*, *Lactobacillus bulgaricus* led to an improvement of IBS symptoms in women with predominant-constipation (IBS-C) (Agrawal *et al.*, 2008). Fecal samples (n=34) from this study were analyzed with the aim to identify potential changes in the gut microbiota (GM) induced by product consumption. Samples acquired before and after the consumption of test product or a control product (a non-fermented acidified dairy product) were analysed using phylogenetic microarrays (HitChips) and qPCR. Intragroup HitChips analysis (confirmed by qPCR) showed that the consumption of the test product did not affect the global structure of the GM whereas some specific phylogenetic groups were rearranged as a result of i) the recovery of the ingested bacteria and ii) the rearrangement of the resident GM. In addition, fecal abundance of ingested beneficial bacteria was shown to correlate with the abundance of specific gut microbial components before the intervention. In conclusion, we showed that the improvement of symptoms in the studied IBS-C population correlates with GM rearrangement and that the resident bacteria can predict the susceptibility of ingested beneficial bacteria to transiently integrate the gut microbial community. Future studies will be needed to establish whether the titers of live bacteria in the gut microbial community are a key factor underlying the variability in the efficacy of beneficial ingested bacteria

Référence: Agrawal A. et al.,2008, PubMed PMID: 18801055.

24. EXACERBATION OF DSS-INDUCED COLITIS BY LOCALIZED DELIVERY OF IFN-BETA SECRETED BY *LACTOBACILLUS ACIDOPHILUS*

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There have been conflicting reports of the role of Type I interferons in gut inflammation and inflammatory bowel diseases. While in some patients with active ulcerative colitis (UC), treatment with Type I interferons induced remission of symptoms, other reports have documented the spontaneous occurrence of UC in multiple sclerosis and chronic hepatitis C patients receiving IFN-beta therapy. In order to evaluate the potential for IFN-beta as a therapeutic for IBD, we developed a delivery system involving probiotic bacteria. Treatment of healthy mice with *Lactobacilli* secreting IFN-beta results in increased IFN-gamma positive cells within the gut as well as a reduction in the percentage of T-regulatory cells. When mice were pretreated with *Lactobacilli* secreting IFN-beta prior to induction of acute DSS colitis, we observed more weight loss and a worsening of intestinal thickening/shortening. In addition, there was an increase in the percentage of Th17 cells, as well as an increase in the production of TNF-alpha by colonic tissue. This study demonstrates that localized delivery of IFN-beta by probiotic bacteria results in an exacerbation of DSS induced colitis in a preventative model.

25. SPRAY DRYING OF *BIFIDOBACTERIUM BIFIDUM*

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Spray drying is a suitable method for production of probiotic powder enriched with high numbers of viable bacteria. Using High temperatures in spray drying process cause low moisture content and high stability during storage of powder, while low probiotic survival can often occure. The purpose of this research was to investigate the effect of spray drying conditions on production of *Bifidobacterium bifidum* powder and also we were going to determine the optimum conditions for spray drying of *B.bifidum* suspension. The main process variables were inlet air temperature, air pressure and malto dextrin concentration. The survival and moisture content of *B.bifidum* following spray drying were determined as two responses for optimization. *B.bifidum* PTCC 1644 was inoculated in a sterilized medium containing of permeate powder, saccharose, csl, and malto dextrin. After 48hours incubation at 37°C, the suspension was dried in Buchi B-191 mini spray-dryer (Buchi, Flawil, Switzerland) with predetermined spray drying conditions in each run. Response Surface Methodology (RSM) was used for analysis of experiments, process modeling and optimization. From the statistical point of view, in order to maximize survival and minimize moisture of spray dried powder, the optimum conditions are inlet air temperature of 103.15°C , air pressure of 4.0 bar and malto dextrin concentration of 0.15$\frac{gr}{ml}$. Under these conditions, the survival of *B.bifidum* was 32.77% while the powder has the moisture of 2.99% .

The predicted models indicate that the moisture of *B.bifidum* powder depends on the malto dextrin concentration rather than air pressure whereas the viability of *B.bifidum* is more affected by air pressure.

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