SESSION V: PROBIOTICS: USE AND MECHANISMS

Anaerobe 2010
The 10th Biennial Congress of the
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

PROBIOTICS: USE AND MECHANISMS

THE USE OF PROBIOTICS IN DIARRHEAL DISEASE

Gorbach, S.L.*
Tufts University, School of Medicine, Boston, MA USA

Probiotics have proven efficacious in treating, and in some studies, in preventing diarrhea. The best evidence for success in reducing the duration of diarrhea is with rotavirus diarrhea in children, where several randomized controlled trials have established a beneficial result. A few studies have shown efficacy in acute bacterial diarrhea, but the evidence is not solid. Several probiotic strains have been used with good results: Lactobacillus GG, *L. plantarum* and *L. reuteri* are the strains for which most evidence is available. Several studies in antibiotic-associated diarrhea have shown some benefit with *Saccharomyces boulardii* and Lactobacillus GG. When administered along with antibiotics for respiratory infections in children probiotics have reduce the incidence of diarrhea and abdominal cramps. However, in proven *Clostridium difficile* diarrhea the benefit has been minimal to negligible and such treatments cannot be recommended at
this time.

Anaerobe 2010
The 10th Biennial Congress of the
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

PROBIOTICS: USE AND MECHANISMS

PROBIOTICS AND NEONATAL NECROTIZING ENTEROCOLITIS

Claud, E.C.*
Departments of Pediatrics and Medicine, Sections of Neonatology and Gastroenterology,
University of Chicago, Chicago, IL USA

Neonatal Necrotizing Enterocolitis (NEC) is an inflammatory bowel disease that primarily afflicts premature infants after the initiation of enteral feeding. It is the most common gastrointestinal emergency in the neonatal intensive care unit, affecting approximately 10% of premature infants <1500gm. The primary risk factors for this disease are prematurity and bacterial colonization however the link between these risk factors and the pathogenesis of the disease has been elusive, and the pathophysiology is poorly understood. NEC is one of few diseases for which probiotics have appeared to have clear benefit in clinical trials, however safety concerns persist.

Clinical trials of probiotics have preceded our understanding of the effect of probiotics on the developing gut and microbial colonization patterns of the preterm infant. Colonization of the blank slate of the preterm intestine occurs within the confines of the neonatal intensive care unit and is influenced by iatrogenic manipulations. Resulting altered microbiota may have significant implications for the immature preterm gut and susceptibility to NEC. Data will be presented to address characteristics of the developing gut which increase susceptibility to NEC, the understanding of which is key to identifying optimal probiotic agents and treatment regimens for this unique patient population.

Anaerobe 2010
The 10th Biennial Congress of the
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

PROBIOTICS: USE AND MECHANISMS

MECHANISMS OF PROBIOTIC ACTION

Versalovic, J.*
Baylor College of Medicine, Houston, TX USA

The human gastrointestinal microbiome is composed of many bacterial species that may affect signaling pathways in intestinal epithelial cells, stem cell compartments and mucosal immune cells. Differences in microbial composition may affect the intestinal mucosa in terms of effects on cell proliferation, apoptosis, antibody and cytokine production, and cell migration. Direct effects by microbial and probiotics-derived signals on specific mammalian cell signaling pathways may explain mechanisms of mucosal immunity. The gut microbiome may affect the biology of innate and adaptive immune responses in the gut mucosa. Specific components of the microbiome may have net anti-inflammatory or pro-inflammatory effects, and the relative balance of microbes may result in different patterns of mucosal inflammation. Differences in patterns of inflammation and immune responses to the gut microbiome and probiotics may determine, in part, differences in the risk of immune-mediated disorders and infections of the gastrointestinal tract.

Anaerobe 2010

The 10th Biennial Congress of the
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

PROBIOTICS: USE AND MECHANISMS

MOLECULAR CHARACTERISATION OF ABC-TYPE MULTIDRUG EFFLUX SYSTEMS IN *BIFIDOBACTERIUM LONGUM*

Abratt, V.R.;* Moodley, C.; Reid, S.J.
Department of Molecular and Cell Biology, University of Cape Town, Cape Town, South Africa

Commercial use of probiotic bacteria is widespread, however, little is known about their potential for transferring drug resistance genes that they may harbour. This study is aimed at characterising two gene clusters in *Bifidobacterium longum* NCIMB 702259^T encoding putative membrane proteins homologous to known ABC-type multidrug efflux proteins.

RT-PCR experiments conducted indicate that these gene clusters are arranged in 2 operons, 1 comprising two genes, excluding a putative MarR-type regulator upstream (BL0162/3), and the other, 3 genes inclusive of another putative regulator (BL1765/6/7). BL0162/3 was cloned and expressed in *Lactococcus lactis* using the Nisin Controlled Expression System and an increase in resistance was observed for both erythromycin (4-fold) and tetracycline (2-fold). Antibiotic induction experiments in *B. longum* revealed a significant increase in erythromycin resistance (>512-fold) after pre-exposure to sub-lethal doses of the antibiotic. RNA hybridization analysis showed a notable increase in transcription levels of all the genes under investigation under these inducing conditions, and this has been quantified using qRT-PCR. Putative MarR-type regulators found upstream of both operons may function as negative regulators of the operons in question. The genes encoding these proteins have been cloned and the proteins purified, and they have been used in gel retardation experiments to demonstrate their binding to DNA promoter regions.

This data indicates the ubiquity of MDR genes in *B. longum.* It also highlights their role in conferring antibiotic resistance, especially when pre-exposed to antimicrobial substances. The presence of regulators in these operons may indicate MDR regulation at a global level.

Anaerobe 2010

The 10th Biennial Congress of the
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

PROBIOTICS: USE AND MECHANISMS

TWO PROBIOTICS SUCCESSFULLY INTRODUCED IN VETERINARY PRACTICE: MAIN PRINCIPLE OF CREATION AND MECHANISMS OF ACTION

Boyko, N.V.;*[1] Karganyan, A.K.[2]
[1]Department of Medicine, Uzhhorod National University, Uzhhorod, Transcarpathian Region, Ukraine
[2]Biofactory "Niva LTD", Crimea, Ukraine

Development of and search for the new domains of use of probiotics is an extremely topical task.

Based on typical biotechnological approaches the two bacterial preparations Monosporyn and Bacell has been recently developed, stately registered, and applied in veterinary practice. Monosporyn is based on strain 090 of *Bacillus subtilis* and Bacell is composed from representatives of *Bacilli*, *Lactobacilli* and *Ruminococci* genera. Three medical forms were used in industrial long term testing for the prophylaxis and treatment of infections caused by opportunistic pathogens in chicken, calves and piglets. Animal weight gains and increased productivities in comparison to the usage of traditional antibiotics have been observed. Monosporyn leads to the mobilization of thyroid gland function detected by triiodothyronine, thyroxin, thyrotropic hormones levels and serum content of total and protein-bind iodine. Prophylactic and therapeutic usage of the Monosporyn significantly reduces the animal mortality caused by introduction of salmonella and staphylococcus toxin. Both *B. subtilis* strains from Monosporyn and Bacell are characterized by a high level of amylolytic, pectolytic, lipolytic, proteolytic, lysozyme activity and are able to produce defined bioactive substances. Most probable mechanisms of anti-bacterial efficiency of Monosporyn and Bacell are competition for nutrients, moderate and strong adhesion properties, and stimulation of the humoral and cellular systemic and local host immune response.

Bacell as a complex microbial-enzymatic probiotic is able naturally decompose of indigestible amylaceous polysaccharides: celluloses, hemicelluloses, pentazanes, β-glucans, pectins. Due to the use of Bacell the fodder consumption has been reduced up to 15% with the cost per product unit going down by 20%; the reproduction of animal is improved, the livestock's survivability is increased and the breeding cost is decrease respectively. Cellulosolytic properties of *R. albus* and ability of *B. subtilis* strain 8130 to produce endoglucanase lead to de-polymerizing of cellulose at primary stages of its digestion and isolate high-energy carbohydrates, proteins and fats. *L. casei* strain regulates normal microbiocenoses formation. Bacell added to mixed fodder substantially decreases the concentration of type-A trichothecene mycotoxins (T-2 and HT-2) by its biotransformation into harmless metabolites.

Both preparations passed the national state registration procedure. The each package of Technical Documentation consists among of other required regime documents the specification of manufacture and table-sheet of newly proposed and original scheme of its application based on synergy to others preparation (antiviral vaccines, etc.).

Anaerobe 2010

The 10th Biennial Congress of the
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

PROBIOTICS: USE AND MECHANISMS

BIO-K+CL-1285® DECREASING THE INCIDENCE OF ANTIBIOTIC-ASSOCIATED DIARRHEA (AAD) AND *CLOSTRIDIUM DIFFICILE* INFECTION IN A DOSE RESPONSE BASED STUDY

Gao, X.W.;*[2] Mubasher, M.;[1] Fang, C.Y.;[2] Reifer, C.[1]
[1]Sprim Advanced Life Sciences, Plano TX USA
[2]Xinhua/Yuyao Hospital, Shanghai Jiao Tong University, PR China

Study purposes: To evaluate the effects of 1 or 2 capsule(s) of BIO-K+CL-1285® vs. placebo, on a) (primary) incidence of AAD and b) (secondary) i) severity of AAD and ii) incidence of *Clostridium difficile*.
Design/Methods: A double blind 3-arm randomized study, conducted at Xinhua/Yuyao Hospital, Shanghai Jiao Tong University, China between Oct 2008-Mar 2009. 255 eligible men and women; 50-70 yrs, initially hospitalized, were recruited and randomized to three study arms; group A: 2 capsules of placebo (n=84); group B: one capsule of placebo and one capsule of Bio-K+CL-1285® (n=85) or group C: 2 capsules of Bio-K+CL-1285® (n=86). Participants were administered their assigned study products while being on their prescribed antibiotics (3-14 days), for 5 more days thereafter and then followed for 21 more days. Incidence and severity of AAD data were collected using questionnaire and diaries given to participants upon discharge. Stool samples were regularly collected by study nurses. The primary outcome measure was incidence of AAD and secondary measures included (a) severity of AAD (total days of diarrhea (≥ 3 episodes of liquid to soft stool/day)) and (b) incidence of *C. diff*. Data were analysed according to ITT principles.
Results: Demographics characteristics were balanced among the three groups. **Incidence of AAD:** Overall: 74 cases (29%); group A: 37 (44.1%); group B: 24 (28.2%) and group C: 13 (15.5%). Controlled group comparisons were statistically significant indicating a dose-response pattern.
Severity of AAD (mean ± sd of days with AAD): group A: 6.4 ± 1.8; group B: 4.1 ± 1.5 and group C: 2.8 ± 0.8. Group comparisons were statistically significant indicating a dose-response effect.
Incidence of *C. diff.:* group A: (23.8%); group B: (9.4%) and group C: (1.2%). Group comparisons were statistically significant also indicating a dose-response effect.
Conclusions: One or two capsules of Bio-K+CL-1285® significantly reduced the incidence and severity of AAD and also the incidence of CDAD. Effects indicated a statistically significant dose-response effect.

Anaerobe 2010
The 10th Biennial Congress of the
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

PROBIOTICS: USE AND MECHANISMS

IN VITRO SCREENING FOR PROBIOTICS, PREBIOTICS AND SYNBIOTICS ANTI-INFLAMMATORY AND ANTI-PROLIFERATIVE EFFECTS IN HUMAN GUT

Grimoud, J.;*[1,2] De Souza, S.;[1,2] Durand, H.;[2] Theodorou, V.;[3] Monsan, P.;[4] Ouarné, F.;[5] Roques, C.[1]
[1]Laboratoire de Microbiologie Industrielle, Université Paul Sabatier, Toulouse, France
[2]Département Nutrition Humaine, Lallemand, Blagnac, France
[3]Unité de Neurogastroentérologie et Nutrition, INRA ESAP UPS, Toulouse, France,
[4]Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, INSA INRA CNRS, Toulouse, France,
[5]CRITT Bio Industries, INSA, Toulouse, France

Colorectal cancer and inflammatory bowel diseases are among the most prevalent intestinal pathologies in occidental populations. Even if the pathophysiology of these diseases is not clearly understood, some data suggest that gut flora abnormalities could play a key role. Indeed, correcting flora imbalance by probiotics could lead to protective effects against such diseases[1,2].

The purpose of our study was to screen some probiotic lactic acid bacteria and some prebiotic glucooligosaccharides for *in vitro* anti-inflammatory and anti-proliferative effects. Each tested strain was preselected on basis of probiotic properties such as survival to gastrointestinal conditions, epithelial cells adhesion and pathogens inhibition and for their ability to use glucooligosaccharides as carbon sources. HT-29 cells monolayers were inflammatory activated by IFN-γ+LPS and co-cultured with the selected strains. Then, activated NF-κB and secreted IL-8 were assayed by ELISA tests. Some *Bifidobacterium*, *Lactobacillus* and *Lactococcus* strains exerted significant inhibitions. Some of these results were consistent with those obtained in a second assay using Caco-2 cell lines transfected with a reporter gene under control of NF-κB inducible promoter. Furthermore, three of the potent anti-inflammatory strains showed anti-proliferative effects on cancerous HT-29 cells when used in combination with prebiotic glucooligosaccharides. Furthermore, the observed effects were dose-dependant for both probiotic and prebiotic. Inhibition of proliferation could be explained by an induction of apoptosis pathway or to a differentiated phenotype of the cancer cells[3]. Thus we assayed caspase-3 by ELISA and intestinal alkaline phosphatase (IAP) gene expression by RT-PCR to assess respectively for the involvement of apoptosis or differentiation in HT-29 cells challenged by anti-proliferative compounds. We showed that IAP expression was increased in treated cells whereas capsases-3 was not activated suggesting that the observed proliferation inhibitions were due to an induction of the HT-29 cells differentiation.

These results suggest that probiotic strains and prebiotic oligosaccharides can be clearly differentiated by their *in vitro* immunomodulatory properties. Further investigations on animal models are needed to evaluate the correlation between current data and actual probiotic, prebiotic or synbiotic activity of these strains.

[1]Rafter J, Benett M, Caderni G, *et al* (2007). *Am J Clin Nutr* 85, 838-849.
[2]Geier MS, Butler RN, Howarth G (2006). *Int J Food Microbiol*. 1115, 1-111.
[3]Liong MT (2008). *Int J Mol Sci*, 9, 854-863

Anaerobe 2010
The 10th Biennial Congress of the
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

PROBIOTICS: USE AND MECHANISMS

SCREENING OF GLUCOOLIGOSACCHARIDES FIBRES AS A SPECIFIC CARBON SOURCE FOR PROBIOTIC LACTIC ACID BACTERIA

Grimoud, J.;*[1,2] Courtin, C.;[1,2] Durand, H.;[2] Ouarné, F.;[3] Monsan, P.;[4] Theodorou, V.;[5] Roques, C.[1]
[1]Laboratoire de Microbiologie Industrielle, Université Paul Sabatier, Toulouse, France
[2]Département Nutrition Humaine, Lallemand, Blagnac, France
[3]CRITT Bio Industries, INSA, Toulouse, France,
[4]Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, INSA INRA CNRS, Toulouse, France
[5]Unité de Neurogastroentérologie et Nutrition, INRA ESAP UPS, Toulouse, France

Gut microbiota plays a key role in the host overall health[1] through its metabolic activities and physiological regulations. Alteration of microbiota may cause some diverse digestive pathologies. This led to the development of strategies aiming to restore or to maintain intestinal ecosystem through probiotics, prebiotics and more recently synbiotic, which were shown to be more effective than probiotics or prebiotics alone in microbiota positive regulation[2].

In an attempt to develop synbiotics efficient in the prevention of intestinal disorders, the purpose of our study was to screen some lactic acid bacteria for their ability to use as a specific carbon source some potential prebiotic glucooligosaccharide (GOS). The GOS used in this study are a mixture of different degree of polymerisation (DP) of short polymers of glucose units linked by specific bonds or organized in crystalline structure (GOS α-1,3/α-1,6, GOS α-1,6, GOS α-1,6/α-1,2 and resistant starch). These characteristics confer them some resistance properties against digestion in the gastrointestinal tract by host enzymes and most of the gut microbiota. Lactic acid bacteria belonging to the genus *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Streptococcus*, preselected on the basis of probiotic characteristics (gastrointestinal survival rates, pathogen inhibition and adhesion to *in vitro* cells cultures) were used to evaluate the prebiotic properties of these GOS. The test was based on the ability of the GOS to promote growth of the probiotic strains but not of gastro-intestinal pathogenic bacteria.

GOS consumption by probiotic was kinetically assayed in microplates filled with reconstituted MRS medium without carbohydrates complemented by GOS, and incubated in an automated reader. Growth parameters (turbidity, pH and generation time) showed that only GOS α-1,6 and less efficiently GOS α-1,3/α-1,6 were used mainly by the six strains of bifidobacteria. Only two strains of lactobacilli and *Lactococcus lactis* were able to metabolize GOS α-1,6. HPLC analyses were conducted on supernatant obtained after culturing bacteria with GOS to check the consumption of each DP. GOS α-1,6 of DP3 to DP6 were significantly consumed whereas only GOS α-1,3/α-1,6 DP3 was consumed. Intestinal pathogenic microorganisms *Candida albicans*, *Clostridium difficile*, *Campylobacter jejuni*, *Enterococcus faecalis*, *Escherichia coli* serotype O157:H7, *Listeria monocytogenes* and *Salmonella enterica ssp. enterica* serotype Typhimurium were unable to grow in presence of GOS as sole carbon source.

The selected bifidobacteria and lactobacilli, associated with GOS α-1,6 and/or GOS α-1,3/α-1,6 enriched by preferentially consumed DP are good candidates for incorporation in a formula to be further evaluated for beneficial effects in prevention of gastrointestinal pathologies.