SESSION II—PROBIOTICS FOR THE PREVENTION OF ANTIBIOTIC-ASSOCIATED DIARRHEA AND CLOSTRIDIUM DIFFICILE DISEASE

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rpoE Knock-out Mutant of Lactobacillus fermentum MS79 is More Sensitive to Osmotic Stress Compared to the Wild Type Strain
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Data Mining Analysis of Relationship Between Blood Stream Infection and Clinical Background in Patients Undergoing Lactobacillus Therapy

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Functional Genomic Analyses of Reuterin Production in Probiotic Lactobacillus reuteri
PROBIOTICS: PANACEA OR POPPYCOCK?

McFarland, L.V.*
Puget Sound Veterans Administration Healthcare System, Seattle WA USA
Department of Medicinal Chemistry, U. Washington, Seattle, WA USA

Although probiotic products are used widely in Europe and Asia and are becoming popular in the U.S., a consensus on the degree of effectiveness and safety has not been reached. Probiotics are living microbes which are ingested to improve the health of the host. In the U.S., probiotics are sold as dietary supplements and less tightly regulated than prescription or over-the-counter medications. The lack of global manufacturing regulations has allowed inconsistent quality and validity of some probiotic products. In addition, as probiotics are considered 'alternative' therapies, healthcare providers often are not versed in appropriate disease indications or safety concerns. Despite these challenges, some probiotics have been well characterized using animal models, safety (Phase 1-2) studies in human volunteers and larger randomized, placebo-controlled, double-blinded clinical trials (RCT). Evidence-based efficacy for several probiotics (S. cerevisiae boulardii, Lactobacillus rhamnosus GG, and several mixes of strains) has been documented for the prevention of antibiotic-associated diarrhea. A meta-analysis of 25 RCT found probiotics were significantly protective (pooled RR=0.43, 95% C.I. 0.31, 0.58) for AAD. While only 6 RCT have been conducted with Clostridium difficile disease, the pooled RR was also significantly protective (pooled RR=0.59, 95% C.I. 0.41, 0.85). However, not every probiotic strain is effective for every disease. Which probiotic is effective for which disease is a matter of great debate. Probiotics show great promise in some diseases, but the evidence is lacking for others.
PROBIOTICS, PREBIOTICS, GNOTOBIOTICS, AND THE LIKE: WHAT IS WITH OUR FASCINATION?

Miller, M.*
McGill University, Montreal, Canada

The widespread availability of bacterial and fungal preparations for purchase and human consumption has led to a proliferation of their use by the lay public and the medical profession. Unfortunately, despite this massive uptake, very little evidence exists to support the use of these products to prevent, modify, or cure any medical condition, illness, or syndrome. Without such evidence, these products are not “probiotics,” but merely bottles and boxes of mass-produced microorganisms. Along with the exponential rise in their use, there has also been a rise in the rumours, myths, stories, and purported “evidence” as to their effectiveness.

This talk will: (1) demonstrate the poor quality control associated with many of these products, (2) review the current meta-analyses which have been performed to evaluate alleged “probiotics,” (3) look closely at the studies which have been done in the field of C. difficile-associated disease (CDAD), and (4) examine the adverse effects related to such preparations.

At the conclusion of this talk, the audience should be in a position to appreciate the paucity of evidence supporting the effectiveness of these agents, as well as the documented and potential dangers associated with their use. The subject matter presented will allow the separation of fact from fiction as they pertain to products labelled as “probiotics.”
rpoE KNOCK-OUT MUTANT OF LACTOBACILLUS FERMENTUM MS79 IS MORE SENSITIVE TO OSMOTIC STRESS COMPARED TO THE WILD TYPE STRAIN

Kim, M.H.;* Yim, M.S.; Jeon, J.M.; Lee, H.I.; Jung, M.K.; So, J.S.
Department of Marine Science and Biotechnology, Inha University, Incheon, Korea

In a previous study, we have isolated Lactobacillus sp. MS79, showing high resistance to various environmental stresses. MS79 was identified as L. fermentum using PCR-RFLP and 16S rDNA sequencing. In order to determine the mechanism of stress resistance at the molecular level, we attempted to clone rpoE gene from MS79. In various other bacteria, rpoE is known to play a role in adaptation to environmental stress and the cellular morphology during growth. Initially, we cloned partial rpoE gene using degenerate primers. The cloned rpoE gene of MS79 is similar to that of the DNA-directed RNA polymerase delta subunit of Lactobacillus reuteri F275, displaying sequence identity and similarity of 86% and 95%, respectively. Using Northern blot analysis, two transcripts were observed from the salt stressed cells having similar levels of intensity. This result suggests that the extreme osmotic stress response of MS79 is under transcriptional control of rpoE and possibly cotranscription of two promoters like in other bacteria. To confirm the involvement of rpoE in osmotic stress response, rpoE knock-out mutant was constructed and compared with the wild type in terms of their response to salt stress. Briefly, rpoE fragment of MS79 was cloned into non-replicative delivery vector pRV300, and the recombinant plasmid was successfully inserted into MS79 chromosome by single-crossover integration. MS79 survived approximately 6-fold higher than the rpoE knock-out mutant, after 40 min of osmotic stress (2 M NaCl). It has been shown that rpoE is induced and required during survival at extreme osmotic stress. Currently, we are comparing the stress responses between rpoE knock-out mutant and MS79 to determine the direct involvement of rpoE in other stress responses.
DATA MINING ANALYSIS OF RELATIONSHIP BETWEEN BLOOD STREAM INFECTION AND CLINICAL BACKGROUND IN PATIENTS UNDERGOING LACTOBACILLUS THERAPY

1Osaka General Medical Center, Osaka, Japan, 2Koden Industry, Tokyo, Japan
3Sakai Municipal Hospital, Sakai, Japan, 4Gifu University, Gifu, Japan
5Shimane University, Izumo, Japan

The aim of this study is to analyze the effects of lactobacillus therapy and the background risk factors on blood stream infection in patients from our hospital clinical microbiology database by data mining.

We analyzed our data on positive blood cultures to assess the incidence of blood stream infection and the effect of this infection patient’s care at Osaka General Medical Center.

Usually, there are various bacteria in human body, such as, intestinal tract, oral cavity, and skin etc. And those bacteria form normal bacterial flora. While human maintains good health, the bacteria flora will keep balance and will act as a barrier against infection. However, by external factor, such as medication, stress, diarrhea, etc, the normal bacteria flora gets off balance and loses a barrier against infection. Consequently, bacteria may invade into blood stream of human body. This bacteria invasion into blood stream is called bacterial translocation, which may cause blood stream infection.

In our medical center, lactobacillus therapy (probiotic product) has been used for patients’ recovery from surgery and prevention of postoperative infection, since early 1990s. Currently, lactobacillus preparation is used in the most departments of our center.

As the analytical methods, we used decision tree, if-then rule, chi-square test, odds ratio, logistic regression and adjusted residue. Data mining software (“ICONS Miner”, Koden Industry Co., Ltd.) was used in our study.

The subjects were divided into two groups by the absence or presence of lactobacillus therapy. Lactobacillus group patients were administrated lactobacillus preparation or yoghurt within 5 days from microbial detection in blood cultures, and control group patients never took those preparations.

Result-1: From odds ratio of lactobacillus absence to lactobacillus presence, bacteria detection risk of lactobacillus absence was 2.17 (95%CI: 1.57-2.99). The p-value of chi-square test was 0.000000159 < 0.01. Thus, lactobacillus therapy might be significantly effective to prevent the bacteria detection on blood sample.

Result-2: Decision tree was obtained as the relationship between the bacteria detection and the various factors, such as diarrhea, lactobacillus therapy, anti-biotics, surgery, tracheotomy, CVP/IVH catheter, urethral catheter, drainage, other catheter, etc. The first node of the decision tree is lactobacillus therapy. Therefore, lactobacillus therapy might be the most significant factor for prevention of blood stream infection.

Result-3: The significant “If-then rules” were extracted from the decision tree between bacteria detection on blood samples and patients’ treatments, such as lactobacillus therapy, anti-biotics, various catheters, etc. By If-then rule, lactobacillus therapy presence might prevent bacterial translocation when patient has no diarrhea and has central venous pressure (CVP) catheter and intravenous hyper-alimentation (IVH) catheter after the surgery.

Result-4: From logistic regression equation, lactobacillus therapy has the biggest absolute value among the standard partial regression coefficients. Therefore, lactobacillus therapy might be the most significant factor for prevention of blood stream infection.

Result-5: By the result of adjusted residue, lactobacillus therapy might be especially effective for the reduction of blood stream infection by Anaerobes, Fungi, and Resistant microbes.

In our study, we could extract the hypothesis that lactobacillus therapy might be the significant factor for prevention of blood stream infection and the detection of resistant strains by a comprehensive analysis (If-then rules, chi-square test, odds ratio and logistic regression). We consider it necessary to take the more specific measures for the prevention of hospital infections based on these analytical results.
MOLECULAR CLONING OF cspA GENE IN BIFIDOBACTERIUM BREVE KB 69; REGULATION OF ITS EXPRESSION BY VARIOUS STRESSES

Department of Marine Science and Biotechnology, Inha University, Incheon, Korea

Bifidobacteria are Gram-positive, anaerobic branched or pleomorphic rods, and one of the normal flora in the large intestine of humans and animals. They play an important role in maintaining health by producing antimicrobial substances to inhibit undesirable pathogens. In the food industry, bifidobacteria are widely used as food additives. During production of viable cells, bifidobacteria can be exposed to various stresses such as low temperature, osmotic, and oxidative stress. Therefore studies on the response of Bifidobacterium spp. to various stresses including cold shock are required. In this study, cspA gene was isolated from B. breve KB 69 by PCR using degenerate primers designed based on the two conserved regions of cold shock domain among previously known cspA genes of other bacterial species. Subsequently, we performed the whole cspA gene sequencing by single primer PCR technique. The cspA gene encodes 240 bp, which displays 99% identity with cspA gene of B. breve UCC 2003. B. breve KB 69 cspA have two RNA-binding motifs that have been suggested that they are highly conserved amino acids of other CspB, CspA and Y-box factors. Moreover, an inverted repeat (IR) of eight nucleotides was present in 5'-UTR which might function as a transcription terminator. The regulation of cspA gene was investigated under various stresses (cold shock, osmotic, oxidative, and ethanol) at transcriptional level. The amount of cspA mRNA was measured by using reverse transcriptase polymerase chain reaction (RT-PCR). cspA transcript in B. breve KB 69 was maximal during early exponential phase but was hardly detectable during stationary phase, indicating cspA expression might be dependent on growth phase. Moreover, the level of cspA mRNA was increased by cold shock, and the expression was transiently triggered under other stresses. Further studies are underway to find cis-acting elements and corresponding trans-acting factors responsible for the cspA regulation.
FUNCTIONAL GENOMIC ANALYSES OF REUTER IN PRODUCTION IN PROBIOTIC LACTOBACILLUS REUTERI

Departments of Molecular Virology & Microbiology and Pathology, Baylor College of Medicine, Houston, TX USA
Department of Pathology, Texas Children's Hospital, Houston, TX USA
Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI USA

Probiotic Lactobacillus reuteri is an indigenous anaerobe in the human gastrointestinal tract that produces a potent broad-spectrum antimicrobial agent, reuterin. In L. reuteri, glycerol dehydratase (gdh) converts glycerol to reuterin, and some reuterin is converted to 1,3-propanediol by 1,3-propanediol oxidoreductase (1,3-pdo). While the synthesis and antimicrobial effects of reuterin are well established, the regulatory mechanisms of reuterin production are unknown. Four L. reuteri strains (ATCC 55730, ATCC PTA 6475, ATCC PTA 4659 and ATCC PTA 5289) were evaluated for reuterin production from late-stationary phase cultures. L. reuteri strains 6475, 4659 and 5289 produced similar amounts of reuterin, while 55730 produced 3-fold more reuterin. L. reuteri strains 55730 and 6475 were analyzed further to assess reuterin production during different growth phases. L. reuteri 55730 increased reuterin production to ~150 mM during late-log and early-stationary phases, while L. reuteri 6475 consistently produced ~50 mM reuterin overtime. We compared gene expression of gdh and 1,3-pdo at late-log and early-stationary phase to early-log-phase in 55730 using two-color microarray. These genes were 2-fold up-regulated in 55730 correlating with the pattern of reuterin production. The relative expression of these genes in 6475 is unknown. Microarray analyses also showed increased expression of a putative transcriptional regulator and a glycerol uptake facilitator indicating these genes might participate in reuterin production. Sequence analyses of these genes suggest structural differences exist within functional regions of the predicted proteins that could account for the strain-dependent variation in reuterin production. Targeted insertional mutagenesis has been used to generate L. reuteri mutants inactivated in one of four genes: 1) gdh, 2) 1,3-pdo, 3) putative transcriptional regulator and 4) glycerol uptake facilitator. While gdh and 1,3-pdo are well characterized, preliminary data indicate the putative transcriptional regulator, but not the glycerol uptake facilitator, is essential for reuterin production. Characterization of these mutants is ongoing and may enhance our understanding of the regulation of reuterin production. Ultimately these studies may enhance the understanding of antimicrobial or microbial community-remodeling functions of probiotics.