International Scientific Conference on Probiotics and Prebiotics

IPC 2016

Proceedings

June 21st – 23rd 2016, Budapest, Hungary
Organising Committee

Alojz Bomba  Chair of the Organising Committee, Slovakia
Peter Kurti  Executive Director of the Organising Committee, Denmark
Sin-Hyeog Im  President of IPC2016, South Korea
Norbert Bomba  Slovakia
Michaela Birkusová  Slovakia
Martin Haranta  Slovakia

Scientific Committee

Sin-Hyeog Im  President of IPC2016 and Chair of the SC, South Korea
Alojz Bomba  Former President of IPC, Slovakia
Ajay A. Awati  United Kingdom
Gwénaël Jan  France
Nadiya Boyko  Ukraine
Svetoslav Todorov  Brazil
Eric Guillemand  France
Wilhelm Holzapfel  South Korea
Yasuhiro Koga  Japan

Acknowledgments

The Organising Committee wish to express appreciation to the Pavol Jozef Šafárik University, Košice and particularly to the Faculty of Medicine for the scientific support of IPC2015.

Special thanks to Japanese Society for Probiotics Science and Korean Association of Lactic Acid Bacteria for their encouraging co-operation and support.

Particularly we would like to acknowledge our sponsors for their funding and support that enabled the conference.

The successful realization of the conference is especially owed to the dedication of Prof. Alojz Bomba, Prof. Sin-Hyeog Im, Prof. Gwénaël Jan and Prof. Dr. Wilhelm Holzapfel.
LIST OF CONTENTS

Conference Programme........................................................................................................... 6

Abstracts of Oral Presentations.............................................................................................. 14

Abstracts of Poster Presentations.......................................................................................... 83

Author Index.......................................................................................................................... 125

Keyword Index....................................................................................................................... 129
**PROGRAMME**

**June 20, 2016**

**Human Milk Oligosaccharides (HMO) Workshop**

**Chairman:** David A. Mills

13:00 **David A. Mills**
University of California, USA
Human Milk Oligosaccharides and Their Role in Shaping the Infant Intestinal Microbiota

14:30 **Koen Venema**
Maastricht University, The Netherlands
Fermentation of $^{13}\text{C}$-labeled 6'-sialyl Lactose by Microbiotas Originating from Babies, Adults and Elderly - Tracing the Label

15:15 **Norbert Sprenger**
Nestlé Research Center, Switzerland
Human Milk Oligosaccharides (HMOs) Biology, Lessons from Preclinical Models

16:00 **coffee break**

16:30 **Wim Soetaert**
Inbiose - Speciality Carbohydrates, Belgium
Efficient Synthesis of Specialty Carbohydrates and Human Milk Oligosaccharides Through Industrial Biotechnology

17:15 **Gert Folkerts - Liao Xing**
Utrecht University, the Netherlands
Early Supplementation of Human Milk Oligosaccharides Suppresses Spontaneous Autoimmune Diabetes in Non-obese Diabetic Mice Later in Life

**Nutrition and Health Claims - Regulatory Workshop**

13:00 **Elinor McCartney**
Pen & Tec Consulting, Spain
Regulation of Probiotics in the EU - What's New?

**Koji Design Workshop - Japanese Fermentation Food Culture**

18:00 **Hikaru Ogura**
Fermentation Designer, Japan
June 21, 2016

Session 1: Pediatric Nutrition

**Moderator:** Koen Venema  
**Chairman:** Myriam Coulet

09:00 Hania Szajewska Medical University of Warsaw, Poland  
Probiotics in Children - Do They Work?  
A critical review of the pediatric use of probiotics from a practitioner's point of view

09:50 Myriam Coulet Nestlé Research Center, Switzerland  
Establishment of the Safety in Use of Two Synthetic Human Milk, Nature-Identical, Oligosaccharides 2'-O-Fucosyllactose (2'FL) and Lacto-N-Neotetraose (LNnT) for Infant Formula

10:05 Liubov Shynkarenko Sichel Pure Research Products, USA  
Clinical Efficacy of Probiotic Lysate Del-ImmuneV® for the Treatment of Gastrointestinal Manifestations Associated With Food Allergy in Preschool Children

10:20 Erica Eliana Castro Universidad San Sebastián, Chile  
Design of Orally Administered Probiotic Formulation for the Prevention of Obesity in Children

10:35 coffee break

Session 2: Gut Barrier Function and Microbial Metabolism

**Moderator:** Koen Venema  
**Chairman:** Jerry Wells

10:55 Jerry Wells Wageningen UR, the Netherlands  
Human Intestinal Barrier Function in Health & Disease

11:20 Clara García Rodenas Nestlé Research Center, Switzerland  
Health Benefits of Probiotics by Impacting Intestinal Barrier Function

11:45 Jonathan Swann Imperial College London, UK  
Influence of gut microbial metabolism on the biochemical profile of dietary compounds

12:05 Kieran Tuohy Fondazione Edmund Mach-IASMA, Italy  
Toward Microbial Fermentation Metabolites as Markers for Health Benefits of Prebiotics

12:25 lunch break

Session 3: Prebiotics

**Moderator:** Koen Venema  
**Chairman:** Robert Rastall  
**Keynote speaker:**

13:30 Robert Rastall University of Reading, U  
Prebiotic Manipulation of the Human Gut Microbiome for Health

**Invited Speaker:**

14:10 Johan Garssen Danone/Nutricia Research, the Netherlands  
Regulatory T-Cell Depletion Abolishes the Protective Effect of Dietary Galacto-Oligosaccharides on Eosinophilic Airway Inflammation in House Dust Mite-Induced Asthma

14:25 Manuela Sailer BENEÖ-Institute, Germany  
Snapshots on Recent Prebiotic Fiber Research

14:40 Emanuela Simonetti Kamut Enterprises of Europe, Italy  
Prebiotic Potential of KAMUT® Khorasan Wheat: From in Vitro Studies to Human Clinical Trials

14:55 Monika Müller University of Vienna, Austria  
Effect of Fructans with Different Degree of Polymerization and Structure on Growth of Selected Probiotic Strains and Formation of Short Chain Fatty Acids

15:10 Quiao Shi University of Helsinki, Finland  
Synthesis of Prebiotic Isomaltooligosaccharides by Weissella Confusa Dextranucrase

15:25 Massalin Nakphaichit (YSA) Kasetsart University, Thailand  
Potential Use of *Lactococcus Lactis* KA-FF 1-4 Supplement with Non-digestible Oligosaccharide Against Vancomycin-resistant Enterococci
Session 4: Modulation of the Intestinal Micro-flora

**Moderator:** Koen Venema
**Chairman:** Gert Folkerts

Invited Speaker:

**15:35** Gert Folkerts  
Utrecht University, the Netherlands  
Microbiome Manipulation and Immune Regulation: Impact for Non Communicable Diseases?

**15:50** Hyunjoon Park (YSA)  
Handong Global University, South Korea  
Autoinducer-2 Signalling in Probiotics: A Mechanism of Gut Microbiota Modulation

**16:00** Anna Maria Szyc (YSA)  
Institute of Animal Reproduction and Food Research of PAS  
Impact of *L. bulgaricus-151* Fermented Buttermilk on Intestinal Microbiota Composition of Allergic and Non-allergic Mice Model

**16:10** Mercè Hereu (YSA)  
Institute of Advanced Chemistry of Catalonia, Spain  
Prebiotic-like Effect of D-fagomine in Gut Microbiota

**16:20** Pieter Van den Abbeele (YSA)  
ProDigest, Belgium  
Use of Microbial Networks as a Strategy to Recover From Gut Dysbiosis

16:30 *coffee break*

Session 5: Targeted Modulation of Gut Microbiota, its Transplantation and Probiotics

**Moderator:** Koen Venema
**Chairman:** Alojz Bomba

Invited Speaker:

**16:50** Alojz Bomba  
Institute of Experimental Medicine, Slovakia  
Targeted Modulation of Gut Microbiota in Chronic Disease Prevention and Therapy

**17:05** Monika Fischer  
Indiana University, USA  
Fecal Microbiota Transplantation in Gastrointestinal Diseases: 2016 Update and the Road Ahead

**17:20** Susan A. Joyce  
APC Microbiome Institute, Ireland  
Bile Alt Metabolism in the Gut : Lessons From the Microbiota

**17:35** Tarkan Karakan  
Gazi University, Turkey  
Obstacles in FMT: Methodology or donor properties?

**17:50** WORKSHOP: Simulator of the Human Intestinal Microbial Ecosystem (SHIME®): a unique technology platform to study the mechanism of action of actives in the GI tract. Examples from the lab.

18:20 *networking bar, poster visit and welcome drink*
June 22, 2016

Session 6: Human Milk Oligosaccharides

Moderator: Koen Venema
Chairman: David A. Mills
Plenary Speaker:
09:00 David A. Mills University of California, USA
Establishment of a Milk-oriented Microbiota in Infants: New Insight into Probiotics and Prebiotics
Keynote Speaker:
09:50 Norbert Sprenger Nestlé Research Center, Switzerland
Human Milk Oligosaccharides (HMOs): From Observation to Clinical Intervention
10:30 Wim Soetaert Inbiose - Speciality Carbohydrates, Belgium
Efficient Synthesis of Specialty Carbohydrates and Human Milk Oligosaccharides Through Industrial Biotechnology
10:45 coffee break

Session 7: Clinical Trials and Health Claim Substantiation

Moderator: Koen Venema
Chairman: Yasuhiro Koga
Invited Speaker:
11:05 Yasuhiro Koga Tokai University School of Medicine, Japan
Functional Dyspepsia: A Novel Field for the Introduction of Gastric Probiotics
11:20 Markus Lehtinen DuPont Nutrition and Health, Finland
Bifidobacterium Animalis Ssp. Lactis 420 With or Without Litesse®Ultra Controls Body Fat Mass and Waist Circumference in Overweight and Obesity- Randomized, Double-Blind, Multicenter Clinical Study
11:35 Junichi Minami Morinaga Milk Industry, Japan
Effects of Bifidobacterium Supplementation on Mild Anemia Women in a Randomized Controlled Trial
11:50 Christiane Laue Clinical Research Center, Germany
Immunomodulatory effects of non-digestible polysaccharides - a double-blind, randomized, controlled clinical trial

Session 8: Microbiome of the GIT and URT

Moderator: Koen Venema
Chairman: Yasuhiro Koga
Keynote Speaker:
12:05 Kathleen McCoy University of Bern
The Role of Gut Microbiota in Immune Development and Homeostasis
12:45 Sara Ramos-Romero Institute of Advanced Chemistry of Catalonia, Spain
Changes in Gut Microbiota Subgroups Differently Relate to Progression of Obesity, Hypertension and Insulin Resistance in Rats
13:00 lunch break
14:00 Eric Guillemard Danone Nutricia Research, France
Probiotics and Respiratory Tract Infections: What's New?
14:15 Linda Mulder Winclove Probiotics, the Netherlands
Microbiota Management for the Prevention of Recurrent Ear and Throat Infections

Session 9: Models to Study Intestinal Interactions

Moderator: Koen Venema
Chairman: Gwénal Jan
Keynote Speaker:
14:30 Michiel Kleerebezem Wageningen UR, the Netherlands
Small Intestine Microbiology and Probiotic Modes of Action
Invited Speaker:
15:10 **Gwénaël Jan** INRA, France
The Probiotic Propionibacterium Freudenreichii as a New Adjuvant for TRAIL-Based Therapy in Colorectal Cancer

Invited Speaker:
15:25 **Wilhelm H. Holzapfel** Handong Global University, South Korea
Using a Diet-induced Obesity Murine Model for Studying Active and Whole Microbial Communities and Their Probiotic Modulation

15:40 **Sae Hun Kim** Korea University, South Korea
Probiotics Modulate the Gut-bone-microbiota Axis in Ovariectomized Murine Model

15:55 **Poonam Singh** (YSA) Universidade de Coimbra, Portugal
Characterization of Cellulose Based Hydrogels as Probiotics Delivery Vehicles

16:05 **Jos van der Vossen** TNO, the Netherlands
Intestinal Microbiota Screening Platform (I-Screen) Provides Insights Into the Effects of Food Ingredients and Medication on Gut Microbiota Composition and Activity

16:20 **coffee break**

**Session 10: Delivery Vehicles**

Moderator: **Koen Venema**
Chairman: **Bruno Pot**

16:40 **Fernanda B. Haffner** CNRS-Université de Lorraine, France
Encapsulation of *L. rhamnosus* GG (LGG) in Alginate-Silicate Hybrid Beads

16:55 **Katja Krizman** (YSA) National University of Singapore, Singapore
Growth and Fermentative Activities of Probiotic Starter Culture Treated With High Intensity Ultrasound in Mono- and Co-Culture

17:05 **Song Huang** (YSA) STLO, Agrocampus Ouest, INRA, France
Major Breakthrough in Probiotic Production. The Two-in-One Use of Sweet Whey Affords Yet Unknown Probiotic Viability and Stability

What's in name? – Expert panel discussion on the terms and meanings around probiotics

17:15 **Bruno Pot** - Chairman Institut Pasteur de Lille, France
**Sin-Hyeog Im** Institute for Basic Science (IBS) and POSTECH, South Korea
**Carine Lambert** International Probiotics Association – IPA Europe
**Magali Cordaillat-Simmons** Pharmabiotic Research Institute, France
**David A. Mills** University of California, USA
**Linda Mulder** Winclove Probiotics, The Netherlands
**Elinor McCartney** European Union Food Chain Legislation Expert, Switzerland

20:00 **conference dinner**
June 22, 2016

Paralel Sessions
Bacteriocins and Antimicrobial Peptides

**Chairman:** Svetoslav Todorov

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution/Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:00</td>
<td>Svetoslav Todorov</td>
<td>Universidade Federal de Viçosa, Brazil</td>
</tr>
<tr>
<td>10:40</td>
<td>Itaru Dekio</td>
<td>Tokyo Women's Medical University, Japan</td>
</tr>
<tr>
<td></td>
<td><strong>Bacteriocins - Answer to Increasing Problem of Antibiotic Resistance?</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Clinical Effect of Novel Probiotic Product for the Skin Containing Staphylococcus Epidermidis Isolated From Customers</strong></td>
<td></td>
</tr>
<tr>
<td>10:55</td>
<td><strong>coffee break</strong></td>
<td></td>
</tr>
<tr>
<td>11:15</td>
<td>Dzung Diep</td>
<td>Universiteit for Miljoog Bovitenskap, Norway</td>
</tr>
<tr>
<td></td>
<td><strong>A Breakthrough in Identification of Bacteriocin Targets</strong></td>
<td></td>
</tr>
<tr>
<td>11:40</td>
<td>Corli Witthuhn</td>
<td>University of the Free State, Republic of South Africa</td>
</tr>
<tr>
<td></td>
<td><strong>Control of Tuberculosis Using Bacteriocinogenic LAB</strong></td>
<td></td>
</tr>
<tr>
<td>12:05</td>
<td>Baljeet Singh Saharan</td>
<td>Kurukshetra University, India</td>
</tr>
<tr>
<td></td>
<td><strong>Bacteriocins From LAB</strong></td>
<td></td>
</tr>
<tr>
<td>12:20</td>
<td>Yujin Kim</td>
<td>Konkuk University, South Korea</td>
</tr>
<tr>
<td></td>
<td><strong>Evaluation of the Intranasal Administration of Live Lactobacillus Sakei Protection Against Different Subtype Influenza Virus Infection in Mice</strong></td>
<td></td>
</tr>
<tr>
<td>12:35</td>
<td>Yeonhee Lee</td>
<td>Seoul Womens University, South Korea</td>
</tr>
<tr>
<td></td>
<td><strong>Antimicrobial Susceptibility of Lactobacillus and Bifidobacterium Isolated From Healthy Elderly People Living in the Korean Longevity Village</strong></td>
<td></td>
</tr>
<tr>
<td>12:50</td>
<td>Shakhl Mirdjamalovna Miralimova</td>
<td>Institute of microbiology of AS of Uzbekistan</td>
</tr>
<tr>
<td></td>
<td><strong>Bacteriocinogenic Lactic Acid Bacteria Isolated From Different Local Sources In Uzbekistan And Their Antimicrobial Activity</strong></td>
<td></td>
</tr>
<tr>
<td>13:05</td>
<td>Aleksandra Tymoszewska</td>
<td>Institute of Biochemistry and Biophysics, Poland</td>
</tr>
<tr>
<td></td>
<td><strong>Garvieacin Q - the Wide-Spectrum Class IId Bacteriocin That Targets Man-PTS</strong></td>
<td></td>
</tr>
<tr>
<td>13:20</td>
<td>lunch break</td>
<td></td>
</tr>
</tbody>
</table>

Animal Health Session

**Chairman:** Ajay Awati

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution/Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:20</td>
<td>Ajay Awati</td>
<td>DuPont Industrial Biosciences, UK</td>
</tr>
<tr>
<td></td>
<td><strong>Feed Enzymes, Prebiotics, and Gut Health: a Vital Part in Feed Additive Strategy in Post-anti Microbial Growth Promoter Era</strong></td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td>Gholamreza Salehi Jouzani</td>
<td>Agricultural Biotechnology Research Institute of Iran</td>
</tr>
<tr>
<td></td>
<td><strong>Designing Specific and Efficient Probiotics for Broiler Chickens Based on Native Lactobacillus Strains</strong></td>
<td></td>
</tr>
<tr>
<td>15:15</td>
<td>Karoline Sidelmann Brinch</td>
<td>Novozymes, Denmark</td>
</tr>
<tr>
<td></td>
<td><strong>Novel Bacillus Subtilis Strain Brings Health Benefits and Improvement of Performance in Broilers</strong></td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td>Ramona Cernat</td>
<td>Chr Hansen A/S, Denmark</td>
</tr>
<tr>
<td></td>
<td><strong>New Probiotic Bacillus spp. Improves Gut Health in Piglets</strong></td>
<td></td>
</tr>
<tr>
<td>15:45</td>
<td>Brian B. Oakley</td>
<td>Western University of Health Sciences, USA</td>
</tr>
<tr>
<td></td>
<td><strong>Research and Development for an Effective Avian Probiotic</strong></td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td><strong>coffee break</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Chairman:** Ajay Awati

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution/Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:20</td>
<td>Jeihyun Jeong</td>
<td>Konkuk University, South Korea</td>
</tr>
<tr>
<td></td>
<td><strong>Adjuvant Potential of Lactic Acid Bacteria With Inactivated Oil-Emulsion H9N2 Vaccine in Chicken</strong></td>
<td></td>
</tr>
<tr>
<td>16:35</td>
<td>Zdenka Hertelyová</td>
<td>Institute of Experimental Medicine, Slovakia</td>
</tr>
<tr>
<td></td>
<td><strong>Effect of Potentiated Probiotics on Fatty Acid Composition in Weaning Piglets</strong></td>
<td></td>
</tr>
<tr>
<td>16:50</td>
<td>Guanhong Li</td>
<td>Jiangxi Agricultural University, China</td>
</tr>
<tr>
<td></td>
<td><strong>Peptidoglycan from Lactobacillus rhamnosus Promotes Avian Beta-Defensin 9 Gene Expression in Immune Cells and Intestine of Chicken</strong></td>
<td></td>
</tr>
<tr>
<td>17:05</td>
<td>Wootack Hong</td>
<td>Konkuk University, South Korea</td>
</tr>
<tr>
<td></td>
<td><strong>Oral Administration of Lactobacillus Confers Beneficial Effects Against Salmonella Infection in Chickens</strong></td>
<td></td>
</tr>
<tr>
<td>20:00</td>
<td>conference dinner</td>
<td></td>
</tr>
</tbody>
</table>
June 23, 2016

Session 11: Mechanisms of Action

Moderator: Koen Venema
Chairman: Sin-Hyeog Im

09:00 **Sin-Hyeog Im**
Institute for Basic Science (IBS) and POSTECH, South Korea
Probiotics for Hyper-immune Disorders: Selection and Mode-of-action

09:15 **Nicolas Szita**
University College London, UK
Real-Time Monitoring of Specific Oxygen Uptake Rates of Adherent Cells in a Microfluidic Device

09:30 **Tsuguhito Ota**
Kanazawa University, Japan
*Lactobacillus Pentosus* Strain S-PT84 Attenuates Lipotoxicity-Induced Hepatic Insulin Resistance and Steatohepatitis by Maintaining Gut Permeability and Inducing Macrophage Alternative Activation in Mice

09:45 **Soyoung Park** (YSA)
Handong Global University, South Korea
Study on the Functionality of Two Different *Lactobacillus Rhamnosus* Strains in a Murine Model

10:00 **Lyudmila Lazarenko**
Zabolotny Institute of Microbiology and Virology, Ukraine
Probiotic Strains Alter the Cytokines Production at Monosodium Glutamate-Induced Obesity in Rats

10:20 **Soyoung Park** (YSA)
Handong Global University, South Korea
An ATP-Binding Cassette (ABC) Uptake System Governs the Preference of Probiotic *Bifidobacterium Animalis Subsp. Lactis* for β-Galactosides

10:40 **Kohei Kamikado** (YSA)
Ezaki Glico Co., Japan
Effect of *Bifidobacterium Animalis Ssp. Lactis* GCL2505, a Probiotic Strain That Proliferates in the Gut, on Visceral Fat Accumulation: Evidence From Human and Mice Studies

10:50 **Cristian Botta** (YSA)
University of Turin, Italy
Comparative Genomics and Functional Analysis of *Lactobacillus Plantarum* Probiotic Candidates Highlighted a Strain-Dependent Capability to Produce Butyric Acid by Fatty Acid Biosynthesis Pathways

11:15 **Priti Mudgil** (YSA)
United Arab Emirates University, UAE
Pancreatic Lipase Inhibitory Activities of Lactic Acid Bacteria Isolated From Raw Camel Milk

11:25 **Sejong Oh**
Chonnam National University, South Korea
The Doubts and Truths of Plant-Origin Probiotics: Is It Really More Beneficial to Human Health Than Human (Animal)-Origin?

11:40 **Elinor McCartney**
European Union Food Chain Legislation Expert, Switzerland
Regulation of Probiotics in the EU - What's New?

12:20 **Henk van Loveren**
European Food Safety Authority - EFSA
EFSA update on the scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms

13:00 **coffee break**

Session 12: Probiocuticals

Moderator: Koen Venema
Chairman: Elinor McCartney

11:15 **Priti Mudgil** (YSA)
United Arab Emirates University, UAE
Pancreatic Lipase Inhibitory Activities of Lactic Acid Bacteria Isolated From Raw Camel Milk

11:25 **Sejong Oh**
Chonnam National University, South Korea
The Doubts and Truths of Plant-Origin Probiotics: Is It Really More Beneficial to Human Health Than Human (Animal)-Origin?

Session 13: Regulatory Issues and Barriers

Moderator: Koen Venema
Chairman: Elinor McCartney

11:40 **Elinor McCartney**
European Union Food Chain Legislation Expert, Switzerland
Regulation of Probiotics in the EU - What's New?

Keynote speaker:

12:20 **Henk van Loveren**
European Food Safety Authority - EFSA
EFSA update on the scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms

13:00 **lunch break**
Session 14: Novel Strains and Approaches

Moderator: Koen Venema
Chairman: Sin-Hyeog Im
Invited Speaker:
14:00 Sarah Lebeer       Uantwerpen, Belgium
Molecular Approaches to Explore Potential of Probiotics for Non-intestinal Applications
14:15 Ales Berlec       Jozef Stefan Institute, Slovenia
Engineering Lactic Acid Bacteria With Cytokine/Chemokine Binding Ability and Infrared Fluorescence for Imaging-Guided Treatment of Inflammatory Bowel Disease
14:30 Walter Chingwaru   Bindura University of Science Education, Zimbabwe
Two Novel Lactobacillus Plantarum Strains (CLP1 and CLP4) With Great Potential for Use as Prophylactic / Therapeutic Probiotics Against Diarrhoea Caused by Escherichia Cali in Infants

Session 15: Future of Probiotics and Prebiotics - Visions and Opportunities

Moderator: Koen Venema
Chairman: Sin-Hyeog Im
Keynote Speaker:
14:45 Tadao Saito       Graduate School of Agricultural Sciences, Japan
Development of New Functional Yogurts Using Probiotics Lactic Acid Bacteria (LAB) and/or Bifidobacteria and the Future Strategy in Japan
15:25 Sam Possemiers    ProDigest, Belgium
Use of Microorganisms for Carotenoids Delivery: Next Generation of Probiotics for Cardiovascular Disease
15:40 coffee break
16:00 Nadia Recine      Sapienza University of Rome, Italy
Long-Term Probiotic Implementation to Re-Create a Balanced Vaginal Ecosystem: A Promising Boost Against HPV-Infection
16:15 Harsh Panwar      Guru Angad Dev Veterinary and Animal Science University, India
Probiotic Stimulation of Incretin Hormone Secretion as a Potential Therapeutic Strategy for Type 2 Diabetes Mellitus: Examining the Mechanism of Action
16:30 Cristina Barrera  Universitat Politècnica de València, Spain
Improving the Probiotic Effect of Lactobacillus Salivarius spp. Salivarius in Mandarin Juice by Adding Trehalose and/or Applying High Pressure Homogenization
16:45 Natarajan Ranganathan
Pro/prebiotic formulation towards modulation of Gut Microbiome towards Chronic Kidney Disease application
17:00 Young Scientist Awards and Best Poster Awards ceremony
17:15 farewell drink

End of the Conference
**Abstracts of Oral Presentations**

**FEED ENZYMES, PREBIOSIS, AND GUT HEALTH: A VITAL PART IN FEED ADDITIVE STRATEGY IN POST-ANTI MICROBIAL GROWTH PROMOTER ERA.**

**Awati A.**
DuPont Industrial Biosciences, UK

**Introduction:**
An increasing number of trials demonstrate the impact nutrition has on the animal’s gut health and performance. Enzymes are a key nutritional consideration and player in these effects. Exogenous enzymes are categorised according to the substrates they target, exogenous xylanases target the soluble and insoluble arabinoxylans in cell walls. Use of xylanase has multiple benefits, from releasing encapsulated nutrients such as starch and protein from the cells to reducing the viscosity of the digesta, both leading to improvements in digestibility (Choct, 2006,). Non starch polysaccharide (NSP) content in animal feed is one variable needing to be managed as it affects satiety, gut motility, nutrient digestion and absorption, as well as changes in gut microbiota. The breakdown of NSPs by xylanase can create a positive environment for beneficial bacteria to grow by reducing viscosity and producing small oligomers. Recent scientific studies shed light on enzymes effectiveness in maintaining a stable gut environment by favouring host and beneficial microflora and creating specific conditions detrimental to the growth of non-beneficial bacteria. The mechanism of action behind the effectiveness of enzymes differ in the upper and lower gastrointestinal tract (GIT). In the upper GIT, exogenous enzymes increase the digestibility of nutrients, leading to a reduction in the availability of indigestible substrate for microbial growth. Furthermore, viscosity of the chyme is also reduced when feeding viscous grains such as wheat or barley, increasing the passage rate of digesta. These conditions lead to a reduced microbial population in the upper GIT, consequently reducing the threat of proliferation of non-beneficial bacteria. While degrading viscous β-glucans and arabinoxylans from wheat and barley, small oligomers and free sugars are produced and some of these are poorly absorbed in the upper intestinal tract. These oligomers and sugars are utilised by certain beneficial bacteria in the hind gut leading to increased volatile fatty acids (VFA) production and containment of proliferation of non-beneficial bacteria. Choct et al (1999) found that VFA production was lower in an enzyme treated group in the ileum, while in contrast in the caecum VFA production of the enzyme treated group was higher than the control. These results underline the earlier mentioned degradation of fiber fractions into smaller oligomers and sugars which are fermented further down the tract in caecum. This shift can benefit intestinal health and microbial balance in the lower GIT. These effects were evident in several published studies, showing higher performance of the animals along with reduction of non-beneficial bacteria in the GIT. Amerah et al (2012), showed a significant reduction in Salmonella prevalence in broilers, when xylanase was added to the diet. Some studies reported positive effects of xylanase inclusion on gut barrier function when birds were challenged by C. perfringens (Liu et al. 2012). These positive effects of enzymes, in particular xylanases, on gut environment, have been shown also to help boost effects of other feed additives such as probiotics (Romero et al 2013; Mathijs et al 2013)

Enzymes are known for their effects on the anti-nutritional factors in the feed, however, their impact on the gut environment, and consequently on gut health is receiving growing attention. The ability of enzymes to boost animal performance, reduce feed cost and positively affect gut environment and gut health, support their use as an important and essential feed additive in the post-AGP era.

**References:**


Choct, M., Hughes, R.J., Bedford, M.J. (1999): Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat, British Poultry Science, 40:3, 419-422
Liu, D., Guo, S., Guo, Y. 2012. Xylanase supplementation to a wheat-based diet alleviated the intestinal mucosal barrier impairment of broiler chickens challenged by Clostridium perfringens, Avian Pathology, 41:3, 291-298


**Keywords:** Animal’s gut health, Exogenous enzymes, Xylanase, Non starch polysaccharide

### ENGINEERING LACTIC ACID BACTERIA WITH CYTOKINE/CHEMOKINE BINDING ABILITY AND INFRARED FLUORESCENCE FOR IMAGING-GUIDED TREATMENT OF INFLAMMATORY BOWEL DISEASE

Berlec A.; Zadravec P.; Škrlec K.; Kosler S.; Turk B.; Štrukelj B.
Jozef Stefan Institute

**Introduction:**

Inflammatory bowel diseases (IBD) are idiopathic chronic intestinal inflammations that include ulcerative colitis and Crohn’s disease. The disease represents a significant health burden and its treatment costs exceed 1.7 billion dollars annually in the US. The exact etiology of IBD is unknown, but it is hypothesized to be a combination of genetic factors and alterations of the microbiota which together cause a dysregulation in the immune response and intestinal inflammation. Altered pattern of cytokine production offers an opportunity for therapeutic intervention by neutralization of pro-inflammatory cytokines, as already successfully demonstrated by monoclonal antibodies against TNFα. Lactic acid bacteria (LAB) are natural members of intestinal microbiota and are used for the treatment of IBD. Their probiotic activity can be upgraded by introducing cytokine/chemokine binding ability with genetic engineering or heterologous protein coating.

**Methods:**

Small protein cytokine/chemokine binders (TNFα-binding affibody, IL-17-binding fynomer, IL-23-binding adnectin and chemokine-binding evasin-1, evasin-3 and evasin-4 with the ability to bind CXCL-2, CXCL-3, CXCL-8, CCL-3, CCL-4 and CCL-5) were fused to secretion signal and cell wall anchor domain and inducibly expressed in model LAB Lactococcus lactis. Fusion proteins were secreted to the growth medium and enabled display of binding proteins on recombinant Lactococcus lactis and, upon producer cell removal, coating of non-recombinant species of LAB, thus providing a non-genetically-modified organism alternative. Surface display was confirmed with flow cytometry and fluorescent microscopy, while the amount of cytokine/chemokine binding was assessed with whole-cell ELISA and Luminex. Infrared fluorescent protein (IRFP) was expressed in L. lactis and Lb. plantarum. Infrared bacteria were tracked in vivo in mice using Ivis spectrum imager.

**Results:**

Several non-recombinant species of Lactobacillus were coated with small model protein binder. Coating was particularly effective on Lactobacillus salivarius ATCC 11741, which has been suggested as an optimal non-recombinant host for surface display. Lb. salivarius coated with individual binders removed up to 78.3% of IL-17, 79.0% of TNFα and 26.7% of IL-23. Heterologous display of multiple binders on Lactobacillus salivarius enabled the construction of bacteria with concomitant IL-17-, IL-23- and TNFα-binding ability. Evasin-displaying bacteria bound from 37.5% to 67.0% of chemokines in vitro and neutralized CXCL-8 in colon epithelial cell model. IRFP-expressing L. lactis and Lb. plantarum were administered to healthy mice and imaged in vivo to demonstrate the ability of quantifying the bacteria and determining their transit time. IRFP-expressing bacteria that were coated with cytokine binder will enable concomitant in vivo imaging and therapeutic cytokine binding.
Discussion:
We have shown that engineered probiotics can bind various cytokines/chemokines, including their mixtures, and can interfere with cytokine signaling. We have also established a platform for in vivo fluorescent imaging of IRFP-expressing LAB. These two modalities were combined to engineer LAB that will concomitantly enable amelioration of excessive immune response in inflammatory bowel disease, as well as in vivo monitoring of bacterial fate.

Keywords: Inflammatory bowel disease, Chemokine binding, Cytokine binding, Fluorescence animal imaging, Lactococcus lactis, Lactobacillus salivarius

TARGETED MODULATION OF GUT MICROBIOTA IN CHRONIC DISEASE PREVENTION AND THERAPY

Bomba A.; Strojný L.; Kuzma J.
Institute of Experimental Medicine, Faculty of Medicine, Pavol Jozef Šafárik University in Košice

Results:
Gut microbiota play a very important role in health maintenance and disease prevention in humans. Therefore, dysbiosis of gut microbiota which leads to the inflammation and metabolic disorders, plays an important role in the pathogenesis of chronic diseases. Better understanding of the bacterial communities inhabiting human gut and the role of dysbiosis in various disease states will certainly result in new therapeutics in the coming decade and a number of diseases both within and outside the gastrointestinal tract may soon be treated with microbiota (Kelly et al., 2015). Current knowledge shows that sophisticated modulation of gut microbiota using functional foods, probiotics, prebiotics and natural bioactive substances could effectively decrease the measure of health risks. A new and underexplored method to alter the gastrointestinal microbiota involves fecal microbiota transplantation (FMT) (Smits et al. 2013). Fecal microbiota transplantation involves administration of fecal material containing distal gut microbiota from a healthy person (donor) to a patient with a disease or condition related to dysbiosis or an alteration in their normal gut microbiota. The goal of FMT is to treat disease by restoring phyllogenetic diversity and microbiota more typical of a healthy person. FMT is the first and crudest way to alter the intestinal microbiome, and both patients and providers have become more aware of this highly effective option for Clostridium difficile infection (CDI) (Kelly et al., 2015).

Another way of gut microbiota modulation is administration of defined microbiota. It was shown that the administration of a Microbial Ecosystem Therapeutic (MET-1) consisting of 33 bacterial strains, isolated from human stool and previously used to cure patients with recurrent CDI, may be protective against enteric infections besides C. difficile infection such as S. typhimurium disease (Martz et al., 2015).

A new method involves taking a patient’s stool sample before antibiotic treatment. If the patient develops C. difficile infection then the stool samples are processed into capsule form and consumed by the patient, helping to restore the microbiota balance prior to antibiotic treatment.

We are developing original solution of gut microbiota modulation, which could be helpful in the prevention and treatment of diseases and could possibly meet criteria of the personalized medicine approaches. Our approach has a unique advantage because it eliminates the risks connected with the transplantation of the gut microbiota from donor and it allows the targeted modulation according to specific needs of the patient. Further research will be needed to clarify which way of gut microbiota modulation is more effective to restore and maintain the physiological status of the microbiota in patients with chronic diseases.

References:
Martz S.-L. E et al., Scientific Reports, 2015, 5:16094 | DOI: 10.1038/srep16094

Acknowledgements:
This work was supported by the project VEGA 1/0309/16.

Keywords: Transplantation, Disease prevention and treatment, Modulation , Gut , Microbiota
IMPROVING THE PROBIOTIC EFFECT OF LACTOBACILLUS SALIVARIUS SPP. SALIVARIUS IN MANDARIN JUICE BY ADDING TREHALOSE AND/OR APPLYING HIGH PRESSURE HOMOGENIZATION

Burca C.; Barrera Puigdollers C.; Betoret Valls N.; García Hernández J.; Hernández Pérez M.; Seguí Gil L.
Instituto Universitario de Ingeniería de Alimentos para el Desarrollo. Universitat Politècnica de València

Introduction:
Given the high prevalence of infection caused by Helicobacter pylori (Go, 2002) and the side effects associated to its treatment with antibiotics (Graham y Yamaoka, 2000), it becomes interesting to develop natural alternatives to decrease this pathogen colonization. There are clinical studies and in vitro models confirming that Lactobacillus bacteria (particularly L. salivarius spp. salivarius) compete with and inhibit the colonization by Helicobacter pylori (Hamilton-Miller, 2003). However, there is little information about how the antibacterial activity or the survival of this probiotic to the digestion process could be affected by the presence of other compounds such as vitamin C and trehalose, or by the application of food processing unit operations, such as the homogenization, what is the object of this study.

Methods:
Probiotic beverages were prepared by inoculating commercial mandarin juice with 4 mL/L of MRS Broth containing 109 CFU of Lactobacillus salivarius spp. salivarius per mL. Yeast extract (5 g/L) and sodium bicarbonate (9.8 g/L), apart from trehalose of food grade (0, 0.1 and 0.2 g/g), were added to the juice formulation to improve the microbial growing. Once inoculated, the liquids were incubated for 24 hours at 37 ºC and submitted to pressure homogenization (from 0 to 150 MPa) before physicochemical and microbial characterization.

Results:
Among all the physicochemical properties tested, only the particle size was significantly affected by the homogenization pressure. As expected, both the distribution average diameter (D[4,3]) and the average size of 90% of the particles present in the samples decreased significantly with the homogenization pressure. Regarding the trehalose content, increasing its concentration from 0 to 0.2 g/g resulted in a significant decrease in water activity values and a considerable increase in both pH and Brix values. Since Lactobacillus salivarius spp. salivarius are lactic acid bacteria that convert carbohydrates into acids, beverages with no trehalose seemed to be the best for the microorganism growing. However, as the homogenization pressure effect on the number of viable cells was dependent on the trehalose concentration, beverages containing 0.1 and 0.2 g of trehalose per gram and submitted to homogenization pressures below 50 MPa had similar or even greater counts than for the correspondent juice without trehalose. About the other two microbial properties, the antimicrobial activity was evidenced but not improved by the presence of trehalose and/or the pressure homogenization while the probiotic resistance to gastric and intestinal conditions was significantly improved by both adding 0.1 g of trehalose per gram of commercial juice and applying 50 MPa to the fermented juice. The latter could be promoted by the osmotic stress undergone by the probiotic in the presence of trehalose and its increase in hydrophobicity by the homogenization at moderate pressure.

Discussion:
Mandarin juice containing 10% (w/w) of trehalose and homogenized at 50 MPa 24 hours after the inoculation with Lactobacillus salivarius spp. salivarius showed the best microbial properties and changes in its physicochemical properties did not adversely affect its quality.

Keywords: Lactobacillus salivarius spp. salivarius, Homogenization, Trehalose, Mandarin juice, Gastrointestinal simulation, Helicobacter pylori

DESIGN OF ORALLY ADMINISTERED PROBIOTIC FORMULATION FOR THE PREVENTION OF OBESITY IN CHILDREN

Castro E.; Asenjo S.; Bustos A.; Bórquez R.; González M.; Durán D.
Departamento de Bioquímica Clínica e Inmunología, Facultad de Farmacia, Universidad de Concepcion, Chile.

Introduction:
Obesity corresponds to an intake/output energy imbalance, but also is influenced by genetic, physiological,
metabolic, social and cultural factors. It was recently reported that the microbiota that colonizes the human gut may play an important role in the development of obesity. This study summarizes research in the development of a new oral anti-obesity formulation based on a probiotic strain isolated from breast milk.

Methods:
Was evaluated the effect of diet supplementation with L. salivarius LPLM-01 strain in a murine diet-induced model of obesity and in a clinical study with schoolchild.

Murine model: Mice were randomized. One group feeding with D12450H diet (Low fat diet; LFD) and the second group feeding an isocaloric diet D12451 (High fat diet: HFD). After three months was observed a significant weight increase in the HFD-fed mice versus LFD-fed mice (>30% weight). Then, each group was newly randomized. One subgroup started a diet supplementation with placebo (fruit jelly; LFD-placebo and HFD-placebo). The other subgroup started a diet supplementation with fruit jelly containing 1x109 CFU/g Lactobacillus salivarius LPLM-O1 (LFD-Lac and HFD-Lac). Animal weight was assessed weekly. At the end of the study, the animals were sacrificed and plasma triglycerides (TG), total cholesterol (TC), plasma alanine aminotransferase, aspartate aminotransferase, blood glucose, insulin, leptin and Interleukin 6 were measured.

Clinical study: A randomized, placebo controlled study was conducted, in which patients with higher BMI (obese or overweight) were compared to patients with normal BMI. Patients of both weight conditions receive fruit jelly containing 1x109 CFU/g Lactobacillus salivarius LPLM-O1 strain or placebo for a period of four weeks. Assessment of body composition parameters, biochemical and immunological measures and fecal bacteria in stool was performed at baseline and at week 8.

Results:
Supplementing the diet with the strain LPLM-O1 does not significantly modify glucose tolerance or insulin response, but it did reduce body weight gain in both diet group when compared to the placebo (p<0.05). Weight loss was related to a reduction of BMI, fasting glucose, insulinemia, and subcutaneous and periovarian adipose tissues, without being statistically significant. However, weight loss was also related to a significant reduction of leptinemia in the HFD+LPLM-O1 group, in comparison with the placebo group (p<0.05). Clinical study showed that the application of the probiotic strain is safety in children, no significant differences in body composition and metabolic markers were observed. But important tendencies were shown.

Discussion:
Discussion. LPLM-O1 diet supplementation reduces adipose tissue mass associated with decreased adipocyte size in obese mice. Altogether, diet supplementation with probiotic strain improves obesity markers in a murine diet-induced obesity model. Even a discrete effect on glucose tolerance and insulin sensitivity, diet supplementation with LPLM-O1 strain reduces total weight in obese mice associated with an improvement in inflammatory markers and reduced circulating basal glucose and insulin levels compared to lean-mice. Clinically, no significant differences were observed, however the probiotic diet could have impact decreasing BMI, glycemia and High Sensitive C reactive protein (PCRhs). The probiotic oral formulation is now in patenting process.

Keywords: Probiotic, Obesity, Lactobacillus, children, Prevention

NEW PROBIOTIC BACILLUS SPP. IMPROVES GUT HEALTH IN PIGLETS

Cernat R.; Nielsen B.
Chr Hansen A/S

Introduction:
The scheduled phase-out of specific antibiotic growth promoters in the EU requires management changes in the pig industry and a need for cost-effective feed additives with high efficacy and thus the need for new probiotics. Bacillus spp.-based feed additives are known for their positive effects on health and production in pigs and are highly relevant for the feed industry since spores are heat stable and can withstand the pelleting process when temperatures reach up to 90-95 °C.

Methods:
This work aimed at screening a pool of 260 new spore formers previously isolated from fermented food, healthy pig feces, soil and different culture collections, and selecting the two best candidates for subsequent in vivo trials. One of the two candidates is presented herein. The strain was identified as Bacillus subtilis subsp.
subtilis based on 16S rDNA, gyrB and rpoB gene sequencing. Its antibiotic susceptibility was established by minimal inhibitory concentration (MIC) which was found below the accepted breakpoint values. Other analyses included bile and acid tolerance, growth in different media, sporulation and antimicrobial activity against Clostridium perfringens Type A and Type C, Salmonella typhimurium, Staphylococcus aureus and porcine pathogenic E. coli strains belonging to serotypes O147:K89:F4, O149:K91:F4 and O101:K-:F5. In vitro adhesion to porcine jejunal IPEC-J2 cell line, human Caco-2 and mucus-secreting HT-29 MTX cell lines was also investigated. The IPEC-J2 cell line and the porcine pathogenic E. coli strains were further used as in vitro challenged models to assess the probiotic protective effect on small intestinal epithelium. For the in vivo trial, 216 four weeks old newly weaned piglets were randomly allocated to control or Bacillus subtilis (DSM25841) treatment group, respectively, balanced for sex and liveweight. Piglets were fed equal standard diets based on corn, soybean and barley strain or no Bacillus spp.

**Results:**
In vitro adhesion of Bacillus spp. vegetative cells to porcine jejunal IPEC-J2 cells was 12.2 ± 1.93 and comparable to the adhesion to human mucus-secreting HT-29 MTX cells (12.7 ± 0.9) but higher than the adhesion to Caco-2 cells (10.7 ± 1.5). Our data are in agreement with the few similar in vitro studies conducted so far on other probiotic Bacillus spp. and emphasize the ability of our selected candidate to adhere in vitro, and the differences in characteristics and functions exhibited by the three cell lines.

In vitro challenge data showed the protective effect of Bacillus spp. candidate on the porcine IPEC-J2 intestinal cells against the attachment of the porcine pathogenic E.coli. The protective effect proved to be probiotic- and pathogen-specific. Most notably, the in vitro adherence of E.coli O101:K-:F5 decreased significantly (P < 0.05) from 14.1 ± 1.1 to 0.4 ± 0.8 when co-cultivated with Bacillus spp. candidate. Results showed that feed supplementation with the selected Bacillus spp. DSM 25841 strain had numeric or significant effect on daily gain (235 g/day vs. 218 g/day) and feed conversion (1.15 kg/kg vs. 1.21 kg/kg; P<0.05) as well as improved fecal scoring (P < 0.01) compared to the control group.

**Keywords:** Swine, Piglets, Gut health, Probiotics, Bacillus spp.

---

**OMEGA 3, L. REUTERI AND VITAMIN D COLLABORATE IN DIMINISHING GUT INFLAMMATION.**

*Cesi V., Costanzo M., Palone F., Vitali R., Pierdomenico M., Colantoni E., Negroni A., Stronati L. Cucchiara S.*

Department of Radiobiology and Human Health, ENEA, Rome, Italy; Department of Pediatrics and Infantile Neuropsychiatry, Pediatric Gastroenterology and Liver Unit, Sapienza University of Rome, Rome, Italy

**Introduction:**
krill oil (KO), an extract prepared from a species of antartic krill, Euphasia superba, containing omega-3 fatty acids, phospholipids and the natural pigment, astaxantin, has emerged for its health benefits in treatment of inflammatory and metabolic disorders. Recently our group published a work to demonstrate its efficacy in counteracting intestinal inflammation, induced either by pro-inflammatory cytokines or by adherent-invasive Escherichia coli (AIEC) administration *in vitro.*

Lactibacillus Reuteri is a well-documented probiotic, whose efficacy in lessening intestinal inflammation has been proven in different systems.

Our aim was demonstrating that co-administration of KO, L.Reuteri and vitamin D is effective in decreasing inflammatory cytokines increase, stress fiber formation and in diminishing adhesion and invasion on AIEC, in a model of *in vitro* intestinal inflammation.

**Methods:**
Immunofluorescence

Cells exposed to the cytomix or co-exposed to cytomix and KO, L. Reuteri and vitamin D were grown at confluence and analyzed with standard fluorescence microscopy techniques for the expression of F-Actin, as markers of tissue stress.

Bacterial adhesion and invasion assays

Adhesion assay: cells were grown to confluence and infected with LF82. Adherent bacteria were recovered and plated on LB agar plates and then the colonies were counted for statistical analysis.

Invasion assay: To assess the invasiveness of LF82, CACO2 and HT29 were infected and incubated, as
above. After incubation cells were incubated in gentamicin to kill extracellular bacteria. Lysis, incubation and counts were performed as in the adhesion assay.

Quantitative PCR
Expression of TNFα and IL-8 was detected by quantitative PCR. RNA integrity was checked by agarose-formaldehyde analysis.

To ensure maximum reproducibility, accuracy and statistical significance, all the experiments were carried out in triplicates.

Results
While in inflamed cells F-actin polymerization increased stress fibers, a combination of KO, L. Reuteri and vitamin D restored initial conditions. Such combination markedly reduced AIEC adhesion/invasion in epithelial cells: from 100±4.5% to 60±2.9% in CACO2 and from 100±3.8% to 39±5.2% in HT29. Finally, the same combination reduced LF82-induced mRNA expression of pro-inflammatory cytokines: TNF-alpha from 100±5.4% to 56.12 ±5.8% in CACO2 and from 100±5.8% to 51.16 ±6.2% in HT29. IL-8 from 100±5.4% to 49.5 ±5.8% in CACO2 and from 100±4.8% to 82.31 ±3.3% in HT29.

Discussion
Omega-3 and probiotics are both beneficial to the intestinal health: the advantage of using a combination of Omega-3, phospholipids and astaxanthin (KO), a probiotic (Lactobacillus Reuteri) and vitamin D is that each experimental relevant endpoint is mainly achieved by one or more of the components, so that the overall results are generally more pronounced that by using the single components separately. Our group is currently working on more endpoints to broaden the knowledge and in highlighting the overlapping and different molecular mechanisms of action.

Keywords: Omega 3, Lactobacillus Reuteri, Inflammation, Krill oil, adhesion & invasion assay

TWO NOVEL LACTOBACILLUS PLANTARUM STRAINS (CLP1 AND CLP4) WITH GREAT POTENTIAL FOR USE AS PROPHYLACTIC / THERAPEUTIC PROBIOTICS AGAINST DIARRHOEA CAUSED BY ESCHERICHIA COLI IN INFANTS.

Chingwaru W.
Bindura University of Science Education

Introduction:
Background: We sought to evaluate a selection of our putative probiotic strains of Lactobacillus plantarum as prophylactic or therapeutic candidates against diarrhoea in infants. A number of probiotic products or strains are widely claimed to be highly efficacious against diarrhoeal pathogens including strains of pathogenic Escherichia coli.

Methods:
Methods: We tested the inhibition of 6 clinical isolates or a standard strain of E. coli (ATCC) by 4 strains of L. plantarum obtained from Balkan cheeses or 3 commercial paediatric antidiarrhoeal probiotic products using the well diffusion assay.

Results:
Results: CLP1, CLP2 or CLP3 had significantly greater inhibition against clinical strains of E. coli, namely 6479, 6525, 6488 and ATCC (11105) than probiotic products PJ or PL (p < 0.05), and 6597 than all probiotic products tested (p < 0.0001). L. rhamnosus LGG exhibited significantly greater inhibition against the following strains of E. coli: 6479, 6497, 6961 and 6488 compared to PJ or PL, and 6525 against PJ only (p < 0.05). BG showed significantly greater inhibition against 6479 and 6497 than probiotic products PL and PJ, and 6961 and 6488 than PJ only (p < 0.05). Exposure to the probiotic product PL or LGG had no inhibitory effect against E. coli strain 6597 (clinical). PL had significant inhibitory effect against E. coli 6479 (p < 0.0001).

Discussion:
We therefore present 2 new strains of L. plantarum bacteria with great potential for use against diarrhoea caused by E. coli in infants. Studies to further characterise the new strains are necessary.

Keywords: Probiotic, Inhibition, Escherichia coli, Diarrhoea, Strains, Products
Establishment of the Safety in Use of Two Synthetic Human Milk, Nature-Identical, Oligosaccharides 2′-O-Fucosyllactose (2′FL) and Lacto-N-Neotetraose (LNnT) for Infant Formula

Coulet M.; Constable A.; Röhrig C.; Penard L.; Aujoulat M.; Schilter B.
Nestec SA

Introduction:
In order to match the composition of human breast milk more closely, it is now possible to supplement commercial infant formula with synthesised oligosaccharides that are chemically identical to human milk oligosaccharides. There is currently no official guideline available specifically addressing the data required for safety assessment of such materials. The safety approach developed for two new human-identical milk oligosaccharides (HiMOs), 2′-O-Fucosyllactose (2′FL) and Lacto-N-neotetraose (LNnT) is presented here.

Methods:
This consisted first of chemical characterization to establish the structural equivalence with the natural endogenous molecule and to get insight on potential impurities resulting from processing. Pre-clinical in vitro and in vivo investigations were then conducted to establish the safety boundaries of the materials and to identify potential unexpected concerns. In vitro genotoxicity testing (bacterial reverse mutation, mouse lymphoma and micronucleus tests) was first conducted. Then, to establish the toxicological profile of the materials, 2′FL and LNnT were administered each separately via gavage in a model representative of the intended target population (a juvenile adapted sub-chronic 90 day rat study). Data from juvenile adapted 14 and 28 day rat studies were also available for LNnT. Fructooligosaccharide (FOS) currently approved for use in infant formulae was used as a reference control.

Results:
2′FL and LNnT were non-mutagenic in in vitro assays. Oral administration up to 5000 mg/kg bw/day of either 2′FL or LNnT to rats (starting to post-natal day 7) over 90 days was not associated with any adverse effects, based on clinical observations, body weight, feed consumption, clinical pathology, organ weights and histopathology findings. The 90-day study together with genotoxicity data was considered most relevant to identify potential toxicological effects resulting from the presence of process-related, uncharacterized impurities, whilst the shorter studies better addressed potential sub-acute gastrointestinal tolerance aspects. These later effects may be missed in longer-term studies because of reversibility resulting from lower susceptibility in adulthood. In addition, pre-clinical data provided insight into the upper bound gastrointestinal tolerance of the ingredient in order to establish a margin of exposure compared to the anticipated human intake. These findings allowed further investigation in clinical trials.

Discussion:
These findings in the juvenile rat have supported the safety of LNnT and 2′FL for use in infant foods and have supported authority decisions for such applications and target populations. Both 2′FL and LNnT have been generally recognized as safe (GRAS) by the US FDA. In Europe, the NDA Panel at EFSA concluded that LNnT is safe for infants (≤ one year of age) when added to infant and follow-on formulae, and for young children (> one year old) when added to follow-on and young-child formulae in combination with 2′-FL, at concentrations up to 0.6 g/L of LNnT and up to 1.2 g/L of 2′-FL, at a ratio of 1:2 in the reconstituted formulae.

Keywords: Safety, Human Milk Oligosaccharides, Lacto-N-Neotetraose, 2′-O-Fucosyllactose, Preclinical studies

Clinical Effect of Novel Probiotic Product for the Skin Containing Staphylococcus Epidermidis Isolated from Customers

Dekio I.
Tokyo Women’s Medical University

Introduction:
Although many pre/probiotic products to promote healthier skin have come to the market worldwide, there has been no attempt to develop probiotic skincare product containing species that reside on the human skin.
As lactic acid bacteria species are usually not detected on the skin, it seems even more reasonable to develop products containing such natural inhabitants. We focused on Staphylococcus epidermidis as the candidate microorganism. S. epidermidis is an abundant Gram-positive bacteria on the facial skin, with up to 2 x 10^5 cells/cm^2 density or 2 x 10^8 cells/face. Its symbiosis with humans is unique, producing not only glycerin and related substances that moisturize the skin surface, but also an active antimicrobial peptide against harmful Staphylococcus aureus attaching from the outer environment to induce dermatitis. Moreover, the species rarely causes infectious disease in clinical settings although it is widely and densely populated. Therefore we assumed that this bacteria can be a candidate for such skincare products.

**Methods:**
We developed the powdery product containing 1.2 x 10^9 freeze-dried cells of S. epidermidis per vial with dried soy broth to be suspended in liquid or cream before application. Our preliminary experiment showed >90% of the bacteria quickly revive when added to vehicle liquid/cream widely distributed in Japan for skincare purpose. We recruited 21 healthy females (aged 22–57 years) who wanted to improve the skin condition enrolled the study. The comparison experiment was designed as two-group crossover and subjects were randomized in a double-blind manner. Group I applied the powder containing S. epidermidis with vehicle liquid, twice a week for eight weeks, followed by placebo soy broth powder with the same liquid. On the other hand, Group II applied the placebo first, then the bacteria. The skin water content, water evaporation, pH, redness, and melanin were measured by using Cutometer MPA580 (Courage+Khazaka, Germany).

**Results:**
At the end of the first 8-week term, Group I showed significant increase (+39%) in water content and decrease (-24%) in water evaporation compared with Group II, together with 14-times increase in S. epidermidis cell number. No significant change was observed in pH, redness, and melanin. During the second term, the water content gradually increased and the water evaporation gradually decreased significantly in Group II, as in the first term of Group I. On the other hand, the second term in Group II showed the former decreased and the latter increased. No adverse effect is observed in both groups.

**Discussion:**
Our study showed the strong skincare effect of S. epidermidis-containing probiotic skin product for the first time. After this study, the product passed the Japanese regulation and brought into the market in 2013. However, there are still significant regulation hurdles to distribute the product worldwide.

**Keywords:** Skin, Staphylococcus epidermidis, Skincare product, Clinical trial, Powder

---

**NOVEL BACILLUS SUBTILIS STRAIN BRINGS HEALTH BENEFITS AND IMPROVEMENT OF PERFORMANCE IN BROILERS**

**Devillard E.; Nelson A.; Brinch K.; Rayat L.; Nielsen P.**
Novozymes

**Introduction:**
Gut health is a major factor to be taken into consideration for the optimum performance of birds. In all animal species, it is becoming increasingly clear that intestinal microbiota mediates key physiological processes thereby influencing the host. Probiotics can positively impact these processes, however their effects are strain dependent. In the present study, we describe a novel strain of Bacillus subtilis that was phylogenetically different from commonly applied Bacillus strain. It was found to have specific impact on animal performance, gut microbiota and host intestinal functions.

**Methods:**
A novel strain of Bacillus subtilis was identified by comparisons of whole genome sequences. To study the phylogeny of the selected strain (B. subtilis DSM29784), a comparative analysis of the gyrB gene sequences was performed using about 1200 nt partial gyrB gene sequences.

Animal trials were conducted in three different trial facilities to assess the impact of strain DSMZ29784 on broiler performance. Broilers were fed 1.0E8CFU/kg feed and Feed conversion was measured at day 28 and 42. Furthermore, samples from a 42-day in vivo performance study in broilers were used for assessment of effects on epithelial cells and gut response. Samples from the small intestine were examined by electron
microscopy to measure gut morphology and microvillus length comparing untreated controls to animals receiving DSM29784. The changes in microbiota in cecum were assessed by Illumina MiSeq.

**Results:**
The gyrB gene analysis suggests that DSM29784 is a novel subspecies of B. subtilis.
In vivo results showed a significant improvement in animal performance in all three animal studies when compared to negative controls with an average FCR improvement of 3.8% at day 35 (p<0.05) when compared to untreated controls.
In the 42 day study FCR improved by 5.7%, (p<0.05) when compared to untreated controls of broilers. Samples from the small intestine showed significantly longer microvilli (p<0.0001) in treated (0.8 um) compared to untreated (0.68 um) animals. Treatment with DSM29784 also resulted in changes in the cecal microbiota with increase in butyrate producing species such as Ruminococcus (increased from 1.6 to 5.1%).

**Discussion:**
Bacillus subtilis is a bacterial species that covers quite extensive diversity. As a consequence the species have currently been divided into three subspecies B. subtilis subsp. subtilis, spizizenii and inaquosorum. The phylogenetic studies suggest that the Bacillus subtilis DSM29784 belongs to a new fourth subspecies.
This novel strain was tested in animal studies. The results of these in vivo tests showed significant improvement in animal performance which was found to be linked to the strain’s impact on the host microbiota and host epithelial wall. These data support that the effects of probiotic bacteria are strain specific and that a probiotic strain with certain specific properties can benefit the health and growth of commercial broilers.

**Keywords:** Probiotics, Microbiota, Animal performance, Novel Bacillus subtilis, Host response

---

**A BREAKTHROUGH IN IDENTIFICATION OF BACTERIOCIN TARGETS**

*Diep D.*
Universiteit for Miljoog Biovitenskap, Norway

**Introduction:**
Bacteriocins have been extensively studied during the last 50 years for their potential in antimicrobial applications. Still they are in general very much under-exploited for commercial use in food safety and in medicine, and they are at best in infancy with regard to detailed knowledge of their mode of action. The latter is crucial in order to develop bacteriocins into effective and safe antimicrobial interventions. The nature of the receptor is the holy grail in bacteriocins' mode of action research. It reveals the Achilles’ heel where the bacteriocin attacks and the answers how cells trigger adaptive response or resistance mechanisms. This set of information is invaluable to help design strategies optimized for the individual bacteriocins and the type of applications.

**Methods:**
We have developed a simple but powerful strategy to identify receptor genes. Briefly, this approach is composed of three steps: step 1, generation of resistant mutants by random mutations; step 2, the mutations are revealed by whole genome sequencing and SNP identification; and step 3, receptor candidate genes are confirmed by complementation or by heterologous expression that renders a resistant host sensitive to the bacteriocin.

**Results:**
By this approach many receptor genes are now identified, including a maltose-transporter for the circular bacteriocin garvicin ML; UppP, a protein involved in lipid II synthesis-for the two-peptide bacteriocin lactococcin G; RseP, a Zn-dependent protease-for the LsbB-like bacteriocins; APC, an amino acid transporter-for another two-peptide bacteriocin plantaricin S; and PspC, a stress responsive protein-for two unrelated bacteriocins.

**Discussion:**
In general we have observed that related bacteriocins employ the same receptors on target cells. This is true for the pediocin-like bacteriocins which employ the sugar permease man-PTS as receptor on target cells, and the LsbB-like bacteriocins which employ RseP, a membrane-bound Zn-dependent protease. But there are also cases where bacteriocins belonging to different subclasses attacking the same receptors. We see a great diversity in the nature of the receptors, implying that bacteriocins have different modes of action and that there are likely different...
mechanisms to develop resistance. All the receptors identified have one feature in common, in that they all are membrane-bound proteins, supporting the general notion that bacteriocins are membrane-active peptides.

Remarks:
Two interrelated aspects connected to the mode of action have been the major bottlenecks in the field for decades: (i) the nature of the receptor on which bacteriocins bind specifically to target cells, and (ii) the molecular nature of the damage triggered by the interaction between a bacteriocin and its receptor. Now we have now cracked the code of the first aspect. The second aspect is more challenging but necessary to gain a full insight as to how a bacteriocin kills a target cell, i.e., whether it forms pores causing leakage of cellular solutes that disrupts the membrane integrity and proton motive force, or it destroys an enzyme or a metabolic pathway important for cell survival, etc. This second aspect directly links to the mechanisms by which bacteria develop resistance.

Keywords: Bacteriocin, Resistance development, Bacteriocin receptor, Antimicrobial development, Antibiotics.

FECAL MICROBIOTA TRANSPLANTATION IN GASTROINTESTINAL DISEASES: 2016 UPDATE AND THE ROAD AHEAD

Fischer M.
Indiana University

Discussion:
Fecal microbiota transplantation (FMT) has been rapidly adopted as a major therapeutic modality for the treatment of recurrent Clostridium difficile infection (CDI) given its consistent and extraordinary clinical cure rates > 85 percent. FMT shows promise in reducing colectomy and mortality rates associated with severe and complicated CDI. With the emergence of FMT in clinical practice, the vital role of gut microbiota has become more apparent in health and disease. A myriad of ailments, such as chronic inflammatory diseases, irritable bowel syndrome, obesity, diabetes, autism, NASH, hepatic encephalopathy among others, are linked to abnormal gut microbiota composition and function. In the treatment of recurrent CDI, FMT was observed to reverse CDI related dysbiosis and to sustainably restore healthy gut microbial communities. Current and future trials focus on harnessing the gut microbiota to preserve health and cure disease. Despite FMT’s widespread use and success, numerous questions and concerns surrounding its short-term and long-term safety remain. As therapeutic product, FMT has been evolving from blended fresh stool to readily available frozen-and-thawed full spectrum microbiota based preparations and future “synthetic” products with well-defined microbial composition in liquid or encapsulated formulations.

Keywords: Fecal microbiota transplantation, C. difficile, Gut microbiota, Inflammatory bowel disease, Irritable bowel syndrome

EARLY SUPPLEMENTATION OF HUMAN MILK OLIGOSACCHARIDES SUPPRESSES SPONTANEOUS AUTOIMMUNE DIABETES IN NON-OBESE DIABETIC MICE LATER IN LIFE

Folkerts G.; Xiao L.; Vos A.; Nato A.; Bastiaans J.; Leusink-Muis A.
Division of Pharmacology, Department of Pharmaceutical Sciences, Utrecht University, The Netherlands

Introduction:
Early life nutrition such as breast milk plays a crucial role in the development of type 1 diabetes. Human milk oligosaccharides (HMOS) are important bioactive components of human milk. We evaluated the effect of early supplementation with HMOS on the autoimmune diabetes incidence in Non-obese diabetic (NOD/ShiLtJ) mice, and correlated the protective effect with regulation of immune system and modulation of gut microbiota. Methods: NOD mice were fed with HMOS containing diet from week 4 to 10 or normal diet. Diabetes incidence was determined by urine glucose tests. Pancreatic insulitis was characterized histologically. Naturally occurring regulatory T cell and T helper cell frequencies in the spleen were analyzed by flow cytometry. Cytokines profile in serum were evaluated by Luminex assays. Intestinal microbiome composition was analyzed by 16S rRNA amplicons derived from fecal sample. Short chain fatty acids...
Acids (SCFAs) were measured in cecal and fecal samples by HPLC. Results: Early supplementation with HMOS significantly reduced the incidence of diabetes up to the age of 30 weeks (p=0.03). Suppressive effects were corroborated by lower pancreatic insulitis and decreased T-cell activation markers (CD25 and CD69) expression in the spleen, although the ratio of Th1/Th2 as well as Th17 cells remained unchanged. Spleen regulatory T cells (CD4+CD25+FOXP3+) were reduced in the HMOS group as well. Reduction in serum concentrations of IL-17 was observed, no significant differences were observed in other cytokines. Total cecal SCFAs were relatively elevated by HMOS diet. Moreover, Furthermore, bacteriological examination of the gut microbiota composition revealed that HMOS diet significantly changed the gut microbiota composition compared with control diet. Particularly, a decreased ratio of Bacteroides/Firmicutes was observed in HMOS group.

Results:
Temporary dietary exposure of NOD mice to HMOS in early life reduced the incidence of autoimmune diabetes beyond the intervention period. This immunomodulatory effect is assisted via directly interacting on immune system, and dependent on the positive alterations of gut microbiota, which may in turn influence mucosal immune system.

Keywords: Early life nutrition, Human milk oligosaccharides, autoimmune diabetes, Non-Obese Diabetic Mice

MICROBIOME MANIPULATION AND IMMUNE REGULATION: IMPACT FOR NCD'S?

Folkerts G.1, Garssen J.2
1 Utrecht Institute for Pharmaceutical Sciences, Utrecht University
2 Nutricia Research, Utrecht, The Netherlands

Introduction:
Our body is attacked continuously by many different danger signals. An effective immune system is essential in order to protect. Unbalanced immune reactivity seems to play a key role in non-communicable diseases (NCDs) such as for example chronic pulmonary disease (COPD), allergies, asthma, diabetes, cancer and even cardiovascular diseases and obesity. According to the WHO NCDs are not only relevant for high income countries but even for middle and low income areas. It is essential to develop new avenues to prevent the enormous increase in incidence and severity of NCDs. Early life programming including proper education of our immune system (a.o. inflammation management) has been recognized as one of the most promising approaches.

The awareness of the importance of a diverse microbiome in immune-regulation/inflammation management and as a consequence impact on NCDs is growing exponentially. After birth, the development of a “healthy” gut microbiome is considered to play an important role in the susceptibility for a.o. immune related diseases including NCDs. Non-digestible prebiotic oligosaccharides and even some unique microbes are transferred by the mother through the breastmilk to the child. Some of these affect the composition and/or activity of the gastrointestinal microbiome leading to health benefits (prebiotic function). As an example it has been shown that specific non-digestible oligosaccharides (GOS/FOS) induce a gut microbiome comparable at least in part to breastfed infants. In addition to indirect effects on the immune system via microbiome changes prebiotic oligosaccharides can affect immune cells in a direct fashion as well.

Recent clinical trials indicated that unique prebiotic fibers can impair the incidence and severity of allergic disorders. The majority of the studies so far indicated immune effects in individuals still having an immature immune system (infants and toddlers). However, very recent data indicated significant impact on other immune related diseases in other life stages as well such as HIV infections, copd, cancer and asthma.

Results:
Both pharma as well as specialized–nutrition companies do see the highly relevance of microbiome manipulation for both prevention as well as treatment of some immune related disorders linked to NCDs. However more research, multicenter trials, and long-term follow-up studies are needed in order to validate the uniqueness of a.o. non-digestible oligosaccharides and microbiome management in both classical pharma approaches as well as specialised and medical nutrition aimed at NCD management.
HEALTH BENEFITS OF PROBIOTICS BY IMPACTING INTESTINAL BARRIER FUNCTION

Garcia Rodenas C.; Bron P.; Kleerebezem M.; Brummer R.; Cani P.; Wells J.
Wageningen University

Introduction:
Intestinal barrier integrity is a prerequisite for homeostasis of mucosal function, which is balanced to maximized absorptive capacity, while maintaining efficient defensive reactions against chemical and microbial challenges. Evidence is mounting that disruption of epithelial barrier integrity is one of the major etiological factors associated with several gastrointestinal diseases, including infection by pathogens, obesity and diabetes, necrotising enterocolitis (NEC), irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD). The notion that specific probiotic bacterial strains can impact barrier integrity fuelled research in which in vitro cell lines, animal models and clinical trials are employed to assess whether probiotics can revert the diseased state back to homeostasis and health. This review catalogues and categorizes the lines of evidence available in literature for the role of probiotics in epithelial integrity and, consequently, their beneficial effect for the reduction of gastrointestinal disease symptoms.

Keywords: Mucosal integrity, Probiotics, Intestinal disorders, Allergy, Metabolic syndrome

REGULATORY T-CELL DEPLETION ABOLISHES THE PROTECTIVE EFFECT OF DIETARY GALACTO-OLIGOSACCHARIDES ON EOSINOPHILIC AIRWAY INFLAMMATION IN HOUSE DUST MITIE-INDUCED ASTHMA

Garssen J.; Verheijden K.; Braber S.; Kranevel A.; Folkerts G.; Willemsen L.
Utrecht University, Faculty of Science, Utrecht Institute for Pharmaceutical Sciences, Division of Pharmacology, Utrecht, The Netherlands

Introduction:
In a murine model for house dust mite (HDM)-induced asthma, dietary non-digestible galacto-oligosaccharides (GOS) have been shown to suppress allergic symptoms. Previously, CD25+ regulatory T-cells (Treg) were found to contribute to allergy protection induced by non-digestible oligosaccharides.

Aims: To examine the effect of Treg depletion in HDM-induced asthma and to study the contribution of Treg in the protective effect of dietary intervention with GOS.

Methods:
BALB/c mice were intranasally sensitized and challenged with HDM or PBS while being fed a control or a 1 w/w% GOS diet. Treg were depleted with anti-mouse CD25 antibody (PC61). T-helper (Th) cell subtypes in lung and spleen of control diet fed anti-CD25-treated mice were analyzed by flow cytometry and cytokines were measured. In all mice, leukocyte subtypes were analysed in the bronchoalveolar lavage fluid (BALF) and IL-33 and CCL5 measured in lung homogenate supernatants.

Results:
Anti-CD25 depleted CD25+Foxp3+Treg in lung and spleen of control and HDM-allergic mice, while increasing activated Th2 cells and cytokine secretion upon ex vivo lung cell restimulation. BALF leukocyte numbers and the percentage of eosinophils increased in HDM-allergic mice and remained unaffected by the anti-CD25 treatment. The GOS diet decreased airway eosinophilia and IL-33 concentrations which was abrogated by anti-CD25 treatment, CCL5 showed the same tendency.

Discussion:
Dietary GOS reduces airway eosinophilia which was abrogated by Treg depletion, indicating regulatory T-cells to contribute to the protective effect of GOS in the prevention of HDM-induced allergic asthma.

Keywords: GOS, Oligosaccharides, Prebiotics, Inflammation, Asthma
PROBIOTICS AND RESPIRATORY TRACT INFECTIONS: WHAT’S NEW?

Guillemard E.
Danone Research, Palaiseau, France.

Introduction:
Acute upper and lower respiratory tract infections (RTIs) are endemic in the general population and a major cause of morbidity and mortality resulting in significant economic and social costs. Recently a number of clinical trials showed that specific well-characterized probiotics can exert a beneficial effect in reducing the morbidity associated with RTIs. Among them, a multicenter, double-blind, randomized, placebo-controlled clinical trial (Prodeus et al., JPGN, 2016) investigated the effect of a fermented milk product containing the *Lactobacillus casei* CNCM I-1518 strain, on respiratory and gastro-intestinal common infectious diseases (CIDs) in 600 children attending day-care centers in Russia. Children consumed the product twice-daily for 3 months followed by a 1-month follow-up period. The primary outcome was the incidence of CIDs during the product consumption period. The results showed no significant difference in the incidence of CIDs between the two groups (primary outcome), however, a significantly lower incidence of rhinopharyngitis, the most frequent CID, was observed in the group consuming the probiotic product compared to the control product group (RR[95% CI]=0.82[0.69;0.96]; p=0.017) during the entire study period. This effect is supported by two studies reporting a significant effect of the same product in reducing the incidence rate of upper respiratory tract infection (URTI) in children attending day-care centers in the USA, as well as the average and cumulated duration of URTI and rhinopharyngitis in free-living elderly.

These studies were recently included in meta-analyses assessing the effectiveness of probiotics (any strains) in the prevention of acute URTIs or in the reduction of RTI duration in otherwise healthy children, adults or elderly. These meta-analyses showed promising results supporting a beneficial effect of probiotics on the clinical outcomes related to RTI and on the associated antibiotic use or absenteeism. However, some limitations have been pointed out that impacted the quality of evidence including: high or unclear risks of bias (according to Cochrane approach), a small sample size or the methods of RTI reporting used in individual studies, and the high heterogeneity between the pooled trials. These meta-analyses did not provide information on the most effective strains considering that probiotic effects are generally considered strain-specific.

The effect of probiotics on RTI is supported by plausible mechanisms of action described in both animal and humans. This field of research has recently benefited from interesting results on the role played by the immune system and the microbiota in the airways, an emerging subject of investigation which showed that a specific microbiota composition in the respiratory tract could be associated with lung diseases, and risks of respiratory infections in humans.

Therefore an increasing number of data support that probiotic interventions could be beneficial against RTI. Nevertheless, meta-analysis with high quality of evidence and less heterogeneity between the included studies are still needed to further reinforce this demonstration. This might be achieved by focusing on specific sub-populations and/or specific probiotic strains interventions. Furthermore, it is important to gain a better understanding of the associated mechanisms underlying the probiotic effects which may help in selecting the most efficacious strains.

**Keywords**: Respiratory infections, Probiotics, *Lactobacillus casei* CNCM I-1518/DN-114 001.

ENCAPSULATION OF L. RHAMNOSUS GG (LGG) IN ALGINATE-SILICATE HYBRID BEADS

Haffner F.; Diab R.; Girardon M.; Fontanay S.; Duval R.; Pasc A.
SRSMC UMR 7565, CNRS-Université de Lorraine 54506 Vandoeuvre les Nancy, France

Introduction:
One way to reestablish the microbiota equilibrium is to administrate functional food containing probiotic cells (e.g. LGG). To insure protection of the living matter during food processing and gastrointestinal transit, encapsulation is oftentimes required. Herein we propose the use of silica-based hybrid carriers as new delivery systems of probiotics. Silica matrix should offer a chemical resistance to the gastric acidic environment and to bile salts in the small intestines, which is superior to that shown by commonly used
bioadhesive polymers, such as alginate.

Methods:
Alginate-silicate beads were obtained from food grade excipients through two easy and up scalable formulation methods, which enable a superior size and topology control of the resulting capsules. Synthesis: a) Emulsification: LGG-loaded beads were synthesized by silicalization of alginate with sodium silicate in a W/O emulsion containing Polyglycerol polyricinoleate (PGPR) and miglyol; b) Electrospraying: In a first step, LGG-loaded alginate beads were recovered in a Ca2+ aqueous solution by electrospraying. Secondly, the gelled beads were dispersed in an oily solution containing PGPR where they get coated by a silica layer upon addition of the silica precursors. Characterization: SEM, FTIR and NMR were used to characterize the structure and the morphology of the beads while CLSM and TEM allow the observation of the encapsulated LGG.

Results:
Two types of alginate-silicate beads containing LGG were obtained: hybrid beads in which the bacteria are surrounded by a mixture of alginate and silica; and core-shell beads in which the bacteria are embedded in an aqueous core of alginate and thus, less exposed to silica. In addition, according to the formulation method, emulsification or electrospraying, one can tune the size of the resulting beads in the range of tens to hundreds of micrometers. Via emulsification, the resulting beads have sizes smaller than 25 µm, which are sufficiently large to encapsulate suitable amount of probiotics, but small enough to remain undetected by humans during chewing. Via electrospraying the 230 µm beads hold a potential interest in adding a ‘crunchy appeal’ to the eventual food carrier.

Discussion:
The hybrid alginate-silicate carriers were synthesized with the aim of designing potential dry probiotic delivery systems. For the first time, carriers combining within the same matrix, a bio protective polymer, alginate, and a desiccant, amorphous silica, were obtained. The later is usually added to dried formulations in order to regulate the water activity, and to insure thus, a long-term storage of the living material. The viability of the bacteria, assessed by plate counting, gave some preliminary perspectives on the impact of inorganic, rigid matrix and synthesis conditions on the living matter. Interestingly, the core-shell beads show a selective response to pH conditions, i.e. they resist to acidic gastric conditions but disintegrate in duodenal pH conditions. This behavior is clearly related to the presence of the silica shell, which generates particular interest for probiotic oral delivery carriers that target a release in the intestines.

This study offers a proof of concept for the potential use of hybrid silica/biopolymer systems in oral delivery of probiotic bacteria.

Keywords: Microencapsulation, Emulsification, Electrospraying, L. rhamnosus GG, Hybrid beads

PREBIOTIC-LIKE EFFECT OF D-FAGOMINE IN GUT MICROBIOTA

Hereu M.; Ramos-Romero S.; Moreno A.; Amézqueta S.; Torres J.
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Barcelona, Spain

Introduction:
Dietary interventions using prebiotics-like compounds could prevent or even treat a variety of metabolic disorders, such as obesity (1). D-fagomine is an iminosugar originally present in buckwheat that reduces weight gain and insulin resistance, probably by limiting the overpopulation of Enterobacteriales (2).

The main goal of this work is to evaluate the effect of D-fagomine on the major subgroups of gut microbiota in both normal and obese rats.

Methods:
Rats (n=36) were fed a high-fat high-sucrose diet (HFHS) or a standard diet (STD), both supplemented (or not) with 0.096% w/w of D-fagomine for 6 months. Blood glucose concentrations were measured by the enzyme electrode method using a blood glucose meter. Plasma insulin was measured using Milliplex technology. The levels of total bacteria, and gut microbiota subgroups were determined in fecal DNA by quantitative real-time PCR. Short chain fatty acids (SCFAs) were determined in fecal samples supernatant by gas chromatography.

Results:
In healthy conditions (STD group), rats supplemented with D-fagomine presented higher Bacteriodales percentage during the study and the populations of Lactobacilliales and Bifidobacteriales significantly
increased after 24 weeks of dietary intervention. In obese conditions (HFHS group), the animals supplemented with D-fagomine presented a proportion of Bacteroidales significantly higher (p<0.05) than those in the HFHS group from week 3 until the end of the study. Concentrations of SCFAs in feces were reduced significantly (p<0.05 vs STD) in both groups fed the HFHS diet at week 12. Rats fed HFHS supplemented with D-fagomine gained less weight (20% gain HFHS+FG) than those not supplemented (29% gain HFHS). Energy intake was independent of the D-fagomine supplementation throughout the intervention. D Fagomine prevented the increase in both fasting blood glucose (p<0.05 vs HFHS) and plasma insulin concentrations induced by the high-energy-dense diet at the end of the study.

**Discussion:**
D-Fagomine promotes the colonization of probiotics such as Lactobacilliales and Bifidobacteriales in rats fed STD diet, and also increases the proportion of Bacteroidales over time. This effect may be related to its capacity of inhibiting the adhesion of fimbriated bacteria to gut mucosa, which may facilitate the proliferation of other species (3).

D-fagomine may reduce weight gain by keeping obesity related bacterial populations close to those corresponding to a lean phenotype.

D-fagomine increases the populations of beneficial bacterial in healthy lean conditions and counteracts the effect of high fat diets on obesity related gut bacterial populations.

**Reference:**
Turnbaugh PJ. et al. Nature 2009
Ramos-Romero S. et al. Obesity 2014

**Keywords:** D-Fagomine, Obesity, Insulin resistance, Bacteroidales, Iminosugar

---

**EFFECT OF POTENTIATED PROBIOTICS ON FATTY ACID COMPOSITION IN WEANING PIGLETS**

**Hertelyová Z.**
Department of Experimental Medicine, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovak Republic

**Introduction:**
Weaning involves complex psychological, social, environmental and dietary stresses that interfere with the development and adaptation of gut. Probiotics potentiated with n-3 polyunsaturated fatty acids (PUFA) have a positive effect on digestive tract, plasma fatty acid composition, immune system and thus total adaptation of piglets after weaning.

**Methods:**
The aim of our study was to investigate the effect of Lactobacillus plantarum - BiocenolTM LP96 and Lactobacillus fermentum -Biocenol™ LF99 in combination with flaxseed as a source of n-3 PUFAs on fatty acid profile and enzymatic activity of conventional piglets in problem breed. The experiment was carried out on 36 piglets at the age of 28 days of Slovak white×Landrace cross-breed divided into control (C) and experimental (E) group. The experimental piglets in group E were supplied probiotic cheeses at a dose of 4 g/animal/day for each cheese and in the same period the feed of group E was supplemented with whole crushed flax-seed. Piglets in group C were supplied control cheese at a dose of 8 g/animal/day. Plasma fatty acids were determined using GC-FID. Results were elevated by Tukey test and Anova one way.

**Results:**
In experimental group of piglets the following processes were observed in fatty acid composition: significant increase (P<0.05) of ΣPUFA, Σn-6, Σn-3, SFA/MUFA/PUFA ratio in favour of PUFA α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid (n-3), docosahexaenoic acid and stearic acid (P<0.05) and significant decrease of ΣMUFA, ΣSFA, n-6/n-3 ratio, myristic acid, palmitic acid, palmitoleic acid, docosatetraenoic acid and docosapentaenoic acid (n-6). In control group was found increase of n-6/n-3 ratio. Significant changes between control and experimental group on Day 14 were observed of ΣMUFA, ΣPUFA, Σn-3 PUFA, linoleic acid (n-6), eicosapentaenoic acid (n-3), docosatetraenoic acid, docosapentaenoic acid (n-6) and docosapentaenoic acid (n-3) by P<0.001, of lauric acid, palmitoleic acid, stearic acid by P<0.01; of myristic acid, palmitic acid, oleic acid, arachidonic acid and docosahexaenoic acid by P<0.05.
Discussion:
The n-6/n-3 ratio in the tenderloin was significantly influenced by dietary flaxseed, which was due to increases in n-3 PUFA (especially α-linolenic and eicosapentanoic acids) and low increases in the linoleic acid and decreased n-6 PUFA contents (D’Arrigo et al., 2002; Hoz et al., 2003). Authors pointed out that n-3 PUFA potentiated the immunostimulative effect of probiotics (Kaštel et al. 2007, Valavan et al. 2006, Kaštel et al. 1999). According to Kankaanpää et al. (2001) higher concentrations of PUFA inhibited the growth and mucus adhesion of selected lactobacilli, whilst growth and mucus adhesion of Lactobacillus casei Shirota was promoted by low concentrations of γ-linolenic acid and arachidonic acid, respectively. PUFA also altered bacterial adhesion sites on Caco-2 cells. It is suggested that dietary PUFA affects the attachment sites for the gastrointestinal microbiota, possibly by modifying the fatty acid composition of the intestinal wall (Bomba et al., 2002). Dietary addition of flaxseed at dose of 10 % and probiotics in feed positively improved plasma fatty acids of conventional piglets after weaning.

Keywords: Potentiated probiotics, Omega-3 fatty acid, Fatty acid composition, Piglets, Weaning

USING A DIET-INDUCED OBESITY MURINE MODEL FOR STUDYING ACTIVE AND WHOLE MICROBIAL COMMUNITIES AND THEIR PROBIOTIC MODULATION

Holzapfel W.; Park S.; Ji Y.
Handong Global University

Introduction:
Recent research progress has confirmed the close relationship between gut microbiota and metabolic disorders. It appears that particular species may serve as indicators of a healthy gut, as opposed to dysbiotic conditions that are frequently linked with obesity and IBD. Yet, defining “indicator” microbial groups pointing to underlying patho-physiological symptoms is difficult. 16S rDNA and 16S rRNA analyses of faecal samples show wide differences in total and active microbial communities. The impact of a standard probiotic strain (Lactobacillus rhamnosus GG) and a new probiotic, Lactobacillus plantarum HAC01, on diet induced obesity (DIO) and the faecal microbial population of a C57BL/6J murine model was investigated.

Methods:
Total microbial genomic DNA was compared with ribosomal RNA data from the active microbial community of the murine model. The three groups in this study comprised two groups receiving a high fat diet resulting in DIO, while the third group, receiving a low fat diet, served as control. One DIO group was also treated with the probiotic L. rhamnosus GG. In a further study, L. plantarum HAC01, originally isolated from Korean fermented cabbage (kimchi) was also used in the treatment of DIO mice, and changes in adipose tissue accumulation modulation of the microbiota were investigated.

Results:
In addition to differences in total body weight gains, the administration of Lactobacillus strains resulted in a reduction in mesenteric fat mass, located around the intestine, but it was significant only for L. plantarum HAC01 group as compared to the control (HF-PBS). Following administration of LGG, the gDNA and rRNA based metagenomic analyses clearly showed active microbial groups associated with host DIO status such as Bacteroides spp., Oscillospira spp. and Ruminococcus spp.

Discussion:
Bacteroides spp., Oscillospira spp. and Ruminococcus spp. may serve as microbial indicators to diagnose the risk, or to target the treatment of host metabolic syndrome. In addition, Oscillospira seems to be a potential modulatory target to reduced host energy efficiency. In another study, the strain L. plantarum HAC01 was shown to significantly induce lower adipose tissue accumulation in the DIO mouse model while specific groups of gut microbiota, considered to comprise a core for host immuno-metabolic amelioration, were modulated.

Keywords: Murine model, Dysbiosis, Gut microbiota, 16S rDNA, 16S rRNA
Abstracts of Oral Presentations

ORAL ADMINISTRATION OF LACTOBACILLUS CONFRS BENEFICIAL EFFECTS AGAINST SALMONELLA INFECTION IN CHICKENS

Hong W.; Gwon G.; Kim J.; Jeong S.; Song C.
Konkuk University

Introduction:
Poultry and eggs are the biggest sources of S. enteritidis infection in the human population. Although S. enteritidis infection rarely causes symptoms in chickens, the infection leads to the colonization of S. enteritidis in the lower gastrointestinal tract followed by periodical shedding, which causes widespread S. enteritidis infection in the entire flock and represents a significant threat to public health. Moreover, a previous study identified mice found on chicken farms as the most important amplifier of the S. enteritidis bioconcentration in the chicken industry. In this study, the efficacy of a Lactobacillus strain on the inhibition of S. enteritidis was evaluated using chicken infection models.

Methods:
L. paraplantarum 6-5, a strain from Korean pickled peppers, was isolated and identified at the species level. L. paraplantarum. was cultured for 24 h at 37°C in De Man, Rogosa and Sharpe (MRS) broth (Difco, USA). One-day-old chicks were divided into four groups (30 chickens/group), and groups 1 and 3 were orally challenged with 1 mL 5 x 10^7 CFU/mL S. enteritidis. To form contact-exposure groups, groups 2 and 4 were cohoused with groups 1 and 3, respectively, immediately following the challenge. To evaluate the efficacy of L. paraplantarum in the inhibition of S. enteritidis using a chicken model, L. paraplantarum was administered in feed-additive form. The chicks in groups 1 and 2 were fed L. paraplantarum-supplemented feed, and the chicks in groups 3 and 4 were fed nonsupplemented feed.

Results:
At 7, 14, and 21 dpc, S. enteritidis growth in the small intestine was measured, and S. enteritidis environmental detection was performed. Oral administration of L. paraplantarum reduced S. enteritidis growth in the cecum, except at 7 dpc in contact-exposed chicks. Although there was no statistical significant between the Lactobacillus-treated and control groups, decreased S. enteritidis shedding was found. Similar to the S. enteritidis growth measurement results, there was no significant difference in the rate of S. enteritidis environmental detection between groups, although the rate was lower in the Lactobacillus-treated group.

Discussion:
Although the results of the chicken experiment were not statistically significant, growth of S. enteritidis in the intestine of L. paraplantarum-treated chickens was moderately inhibited. Therefore, increasing the number of animals in each group and modifying the L. paraplantarum administration method used, as discussed above, would likely confer more promising results regarding the efficacy of L. paraplantarum administration in animals. As a probiotic, L. paraplantarum has potential as a control measure against S. enteritidis infection in both mice and chickens. Our results indicate that utilization of L. paraplantarum as a probiotic might be an effective control measure for S. enteritidis-related food poisoning by reducing both S. enteritidis contamination in poultry products and S. enteritidis-related clinical symptoms in humans.

Keywords: Salmonella, Chicken, Feed-additive, Lactobacillus, Korean pickled pepper

MAJOR BREAKTHROUGH IN PROBIOTIC PRODUCTION: THE TWO-IN-ONE USE OF SWEET WHEY AFFORDS YET UNKNOWN PROBIOTIC VIABILITY AND STABILITY

Huang S.; Schuck P.; Jeantet R.; Jan G.; Chen X.
Soochow University, China

Introduction:
Probiotic efficacy relies on administration of live and active probiotic strains in adequate dose. Growth yield and stress tolerance during probiotic production and delivery thus constitute a key bottleneck. Probiotics are preferably produced, transported, stored and used under a dried form. Spray drying has been long time...
expected in producing dried probiotics due to its low cost and high productivity. Spray drying of probiotics generally comprises culture, harvesting, washing, re-suspension and drying steps in which preservation of viability remains a quest for the Holy Grail. Therefore, freeze-drying is preferred to spray-drying for producing dried probiotics, as it better preserves the cell viability although it represents subsequently higher production costs (6–10 times higher than spray drying).

Methods:
Dairy products have been widely used as delivery vehicle of probiotics. We used sweet whey, a dairy industry byproduct, as a two-in-one medium to sustain both growth of probiotics, and direct spray-drying without harvesting, washing and re-suspension steps. Moreover, hyper-concentrated sweet whey was developed to achieve one-step drying with higher production rate.

Results:
Both lactic and propionic acid bacteria were adapted to growth within sweet whey. The hyper-concentrated sweet whey produced higher final yield of bacteria population. More remarkably, growth of probiotics in hyper-concentrated sweet whey led to enhanced stress tolerance, overexpression of key stress proteins, accumulation of intracellular storage molecules and compatible solutes, consequently resulting in yet unknown survival upon heat, acid and bile challenges. The cultures were directly spray-dried with various survival rates, depending on sweet whey concentration. Specifically, both lactic and propionic acid bacteria growing in hyper-concentrated sweet whey survived better through spray-drying. The resulting spray-dried powders contained probiotic viability comparable to freeze-drying, reaching to the level of population at $10^{10}$ CFU/g. Furthermore, these powders maintained a considerable stability with constant viability during 4 month storage.

Discussion:
As a conclusion, a new 2-in-1 culture and drying process was developed, using sweet whey as a culture and drying medium. This is to the best of our knowledge the first report describing the feasibility of culturing and direct spray-drying bacteria with hyper-concentrated medium. It triggered multi-tolerance during growth, subsequently leading to the high level of probiotic viabilities remained in the powders after spray drying and following long term storage. Spray-drying being far more cost-effective than freeze-drying, this innovation opens new avenues for sustainable development of probiotic products with enhanced delivery efficiency. This patent-protected new process indeed uses a dairy industry byproduct, requires limited amounts of energy, affords high bacterial viability and protects probiotics from injury undergone within the digestive tract.

Keywords: Probiotics, Delivery vehicle, Stress tolerance, Sweet whey, Spray drying

PROBIOTICS FOR HYPER-IMMUNE DISORDERS: SELECTION AND MODE-OF-ACTION

Im S.
Institute for Basic Science (IBS) and POSTECH

Introduction:
Alteration of gut microbiota composition is associated with diverse immune disorders and restoration of dysbiosis in disease state with beneficial microorganism could confer the health benefits. As a modulator for dysbiosis Daily intake of oral probiotic preparations has been widely considered. Probiotics are non-pathogenic live microorganism that can provide a diverse health benefits on the host. Recently, many reports suggest that certain probiotic strains or mixture of them could exert potent immunomodulatory activity in diverse disorders. However, efficacy of probiotics is quite different depending on the type of strains and the amounts of doses. Definition of beneficial microorganisms (including probiotics) should be different depending on the types of diseases and heath condition of individual person. In our lab, we have been interested in developing probiotics that could suppressing hyper-immune disorders or enhancing immune system, and has developed diverse ex vivo and in vivo screening and evaluation systems.

Methods:
To selectively identify probiotic strains that could enhance the generation of CD4+Foxp3+ regulatory T cells (Tregs), we have developed ex vivo screening systems. Mesenteric lymph node cells or whole splenocytes were co-cultured candidate probiotic strains for 72 hours and then the levels of anti-inflammatory (IL-10),
pro-inflammatory (IL-12) or Treg (Foxp3+) markers were analyzed by ELISA and flow cytometry. Probiotic strains with IL-10highIL-12low Foxp3high inducing property were selected and combined to evaluate their immunoregulatory effect on hyper-immune disorders such as autoimmunity and allergic disorders.

**Results:**
Using the system, we identified several probiotic strains and a mixture of probiotics (IRT5) that up-regulates Tregs in vivo. Administration of the IRT5 induced both T-cell and B-cell hyporesponsiveness and down-regulated T helper (Th) 1, Th2, and Th17 cytokines without apoptosis induction. It also induced generation of CD4(+)Foxp3(+) Tregs from the CD4(+)CD25(-) population and increased the suppressor activity of naturally occurring CD4(+)CD25(+) Tregs. Conversion of T cells into Foxp3(+) Tregs is directly mediated by regulatory dendritic cells (rDCs) that express high levels of IL-10, TGF-beta, COX-2, and indoleamine 2,3-dioxygenase. Administration of probiotics had therapeutic or prophylactic effects in experimental disease models of inflammatory bowel disease and in non-mucosal immune disorders such as atopic dermatitis, hapten-induced contact hypersensitivity, rheumatoid arthritis, myasthenia gravis and multiple sclerosis. The immunoregulatory effect of the IRT5 probiotics is associated with enrichment of CD4(+)Foxp3(+) Tregs in the inflamed regions. In addition, monoclonization of Bifidobacterium bifidum IRT in germ free mouse significantly enhanced the generation of induced CD4(+)Foxp3(+)Helioslow Treg (iTreg) cells and upregulated CTLA4 expression. Treatment of BMDCs and CD4+ T-cells treated with Bifidobacterium bifidum IRT or capsular polysaccharides from the strain produced high amount of IL-10 in TLR-2 dependent manner. Currently we are investigating the underlying mechanism of iTreg cell generation by elucidating effector molecules from the probiotics strains. Collectively, the administration of probiotics that enhance the generation of rDCs and Tregs represents an applicable treatment of inflammatory immune disorders. This study was supported from the Institute for Basic Science (IBS; IBS-R005-G1), Republic of Korea.

**Keywords:** Hyper-Immune Disorders, Enhancing immune system, Atopic dermatitis, Hapten-induced contact hypersensitivity, Rheumatoid arthritis, Myasthenia gravis, Multiple sclerosis

**THE PROBIOTIC PROPINIBACTERIUM FREUDENREICHI AS A NEW ADJUVANT FOR TRAIL-BASED THERAPY IN COLORECTAL CANCER**

*Jan G.*
INRA, France

**Introduction:**
TNF-Related Apoptosis-Inducing Ligand (TRAIL) induces apoptosis via the extrinsic death pathway in human colon cancer cells. Short Chain Fatty Acids (SCFA) by contrast induce apoptosis of cancer cells via the intrinsic death pathway. We have previously shown that food-grade dairy propionibacteria induce intrinsic apoptosis of colon cancer cells, via SCFA metabolites (propionate and acetate) acting on mitochondria.

**Methods:**
We investigate here the possible synergistic pro-apoptotic effect between Propionibacterium freudenreichii and TRAIL.

**Results:**
Whole transcriptomic analysis demonstrated that propionibacterial supernatant or propionibacterial SCFA, in combination with TRAIL, increased pro-apoptotic gene expression (TRAIL-R2/DR5) and decreased anti-apoptotic gene expression (FLIPL, XIAP) in HT29 human colon cancer cells. The revealed synergistic pro-apoptotic effect, depending on both death receptors (TRAIL-R1/DR4, TRAIL-R2/DR5) and caspases (caspase-8, -9 and -3) activation, was lethal on cancer cells but not on normal human intestinal epithelial cells (HIEC), and was inhibited by Bcl-2 expression. Finally, milk fermented by P. freudenreichii induced HT29 cells apoptosis and enhanced TRAIL cytotoxic activity, as did P. freudenreichii culture supernatants or its SCFA metabolites.

**Discussion:**
The revealed synergy opens new applications for food-grade P. freudenreichii-containing products as new adjuvants to potentiate TRAIL-based cancer therapy in colorectal cancer.

**Keywords:** Propionibacterium, Cancer, Apoptosis, Biotherapy, Cytokine
ADJUVANT POTENTIAL OF LACTIC ACID BACTERIA WITH INACTIVATED OIL-EMULSION H9N2 VACCINE IN CHICKEN

Jeong J.; Yuk S.; Kim J.; Gwon G.; Kim K.; Song C.
Konkuk University

Introduction:
In Korea, low pathogenic avian influenza (LPAI) H9N2 was first documented in 1996 and it caused serious economic loss in the Korean poultry industry. To control the H9N2 outbreaks, since 2007, the Korean veterinary authority has permitted the use of the inactivated oil-emulsion vaccine derived from a Korean H9N2 isolate (A/chicken/Korea/01310/2001) in commercial layer and broiler breeder chickens. Despite the vaccination program, one sub-lineage group (KU114/07-like) has survived and currently circulates in Korea. Therefore, selection of more suitable vaccine strains and development of highly protective H9N2 vaccine are needed to prevent an LPAI H9N2 endemic in Korea. At present, there are few articles which have assessed the adjuvanticity of the LAB on mucosal vaccines. However, there are no investigations concerning the adjuvant effect of the LAB on the oil-emulsion vaccine. In the present study, we demonstrated that inactivated oil-emulsion H9N2 vaccine supplemented with LAB provides increased protective efficacy than the non-supplemented oil-emulsion vaccine.

Methods:
LAB were isolated from kimchi and toenjang (Korean traditional lactic fermented foods) samples obtained from Korean restaurants. Lactobacillus plantarum and Pediococcus lolli from kimchi and toenjang, respectively, were selected based on the in vitro screening assay.

Vaccine escaping H9N2 isolate (A/chicken/Korea/K040110/10) was propagated by inoculating into embryonated chicken eggs and titrated. The virus was inactivated with 0.01M BEI. Low, medium and high doses (105.7, 106.7 and 107.7EID50/0.5ml, respectively) of inactivated antigens or each doses of antigens supplemented with each LAB (108.3CFU/0.5ml) were used to formulate a water-in-oil emulsion vaccine by mixing with oil phase (Montanide ISA 71 VG) at a ratio of 30:70(v/v).

6-week-old SPF chickens were divided into 9 groups (6 birds/group), viz., low dose of vaccine only (G1), low dose+L.plantarum (G2), low dose+P.lolli (G3), medium dose only(G4), medium+L.plantarum (G5), medium+P.lolli (G6), high dose only (G7), high dose+L.plantarum (G8) and high dose+P.lolli (G9). All chickens were injected 0.5ml/birds intramuscularly in the left pectoral muscle.

The adjuvant potential of LAB was assessed by hemaglutination inhibition (HI) test at weekly intervals.

Results:
On the first week post vaccination (w.p.v), G5 showed fastest induction of humoral immune response compared to other median dose groups. From 2 w.p.v, LAB supplemented groups had significantly higher HI titer than that of vaccine only groups. Among LAB supplemented groups, groups with L.plantarum showed better protective efficacy than groups with P.lolli. In addition, the L.plantarum group had a dose-sparing effect on antibody titer.

Discussion:
To the best of our knowledge, this is the first study reporting the efficacy of inactivated oil-emulsion vaccine supplemented with LAB. In this study, the oil-emulsion vaccine combined with the LAB elicited a higher humoral immune response. It is well-known that LAB can enhance the systemic immune response by activating various immune cells. Although this study did not include responses of a variety of immune cells, our results imply that supplementation of LAB to inactivated oil-emulsion vaccine could be an attractive strategy to better control of LPAI.

Keywords: Adjuvant, Avian influenza, oil-emulsion vaccine, Lactic acid bacteria, Chicken

BILE ALT METABOLISM IN THE GUT: LESSONS FROM THE MICROBIOTA.

Joyce S.
APC Microbiome Institute, Ireland

Introduction:
The range and diversity of bile acids and their conjugated (either to Taurine or to glycine) salts is dictated by the gut microbiota. Besides their role in aiding the emulsification of lipids and the release of fat soluble vitamins, bile acids and their conjugated salts are now recognised as potent signalling molecules with the
potential to influence health and disease status. They can act as ligands binding to receptors, they can influence their own synthesis through cross-talk with the liver, they can adjust transporter systems and nutrient uptake and they also play a role in determining gut microbial residency. Therefore bile metabolizing microbes have a central role in gut metabolism and gut health. We have investigated the role and the implications of bile acid metabolism in the regulation of host physiology through bile acid alterations and microbiome signatures. Here, we present our work in correlating microbial bile acid metabolism and disease states. We profile isolated potential bespoke probiotic strains isolated on the basis of either rational (genetic targeting to isolate certain BSH types and activities) or random (non-genetic selection for any bile acid modification) selection. These strains, their effects on the gut microbiota, BA metabolism and their implications for gut health and disease status are discussed. This work was funded by Science Foundation Ireland (SFI) to APC Microbiome Institute; Grant Number SFI/12/RC/2273.

Keywords: Bile, Microbes, Disease, Microbiota, Probiotic

OBSTACLES IN FMT: METHODOLOGY OR DONOR PROPERTIES?

Karakan T.
Gazi University Department of Gastroenterology

Introduction:
Fecal microbiota transplantation (FMT) is an experimental method for restoration of dysbiosis in Ulcerative Colitis (UC). Few studies reported improvement in clinical and endoscopical response. However, route of administration, donor and patient selection, number of FMT sessions and intervals are still obscure. Steroid failure leads the patient to a next step of drugs. Immunomodulatory or biological agents have higher costs and adverse events, potential risks of infection and malignancy. Besides, these immunosuppressive agents do not have a certain end-point for duration of therapy. We have analysed the efficacy of rescue FMT for steroid dependent and/or non-responsive UC patients.

Methods:
Fourteen patients with steroid-dependent and/or non-responsive UC were enrolled, and treated with FMT. Follow-up clinical data was collected for at least 3 months (3-18 months). Donors were selected according to Amsterdam Criteria. All patients received FMT after complete colon cleansing via colon. Patient and donor clinical, demographic and laboratory data were recorded.

Results:
Eleven of fourteen (78.5 %) patients achieved clinical improvement and were able to discontinue steroids following rescue FMT. One patient was lost to follow-up. Among the 11 patients who responded, five (45.4 %) received one FMT therapy, one (9.0%) received two FMTs, and three (27.2%) received four FMTs, and two (18.1%) received six FMTs. Six (54.5 %) of the 11 patients who responded maintained long-term remission during follow-up (3–18 months). Three patients (21.4 %) failed to meet the criteria of clinical improvement and maintained steroid dependence, though one patient experienced transient or partial improvement. Eight of 11 responders had the same blood group antigen with the corresponding donor. Patient age (28±8 vs 47±11yrs, p<0.05) and disease duration (6±3 vs 35±12 months, p<0.05) were also lower in responders. Mean body mass index (kg/m2) increased in all responders (baseline: 23±3 vs post-FMT 3 months: 26±2, p<0.05). None of the patients experienced major adverse events due to FMT.

Discussion:
Rescue FMT shows promise as a therapeutic strategy for patients with steroid-dependent and/or non-responsive UC, likely due to the successful restructuring of gut microbial composition. Blood group antigen-match might be a promising research area such as in other organ transplantsations. Post-FMT weight gain might be due to cessation of inflammation or improved dysbiosis. Based on current data about FMT, we need to improve donor characteristics. Gut microbiota analysis of donor and recipient should be performed before and during follow-up periods. Repeated FMT to reinforce engraftment or different donor might to chosen to achieve therapeutic goal. Further studies are urgently needed to clarify predictive factors of success for FMT in this population.

Keywords: Fecal microbiota transplantation, Ulcerative colitis, Predictors of response, Methodology, Inflammatory bowel disease
PROBIOTICS MODULATE THE GUT-BONE-MICROBIOTA AXIS IN OVARIECTOMIZED MURINE MODEL

Kim S.
Korea University

Introduction:
Osteoporosis is a significant health burden and “silent epidemic”, affecting millions of people worldwide. It has been reported that approximately 30% of all postmenopausal women have osteoporosis in the United States and Europe. This disease is characterized by imbalance between bone formation and bone resorption, leading to an increased incidence of bone fragility and increased risk of bone fractures. The gastrointestinal tract has been initially demonstrated to affect bone metabolism and is frequently associated with the presence of inflammation. In addition, the gastrointestinal tract harbors a complex and largest microbial community, which can contribute to host health. More recently, a complex role of the gastrointestinal tract in the maintenance of bone health via “gut-bone-microbiota” axis has become a topic of intense research activity. Interestingly, the gut-microbiota composition is affected by factors, such as dietary, which can be potentially used as a means to manipulate an altered state in favour of bone health maintenance. Probiotics are defined as “living microorganisms, which when administered in adequate amounts confer health benefits on the host”. Probiotic strains, including Lactobacillus, are capable of restoring the composition of the gut microbiota, and introducing beneficial functions to gut microbiota community, leading to amelioration of gut inflammation and the other systemic disease phenotypes. Thus, probiotics may exert beneficial impact on bone health via gut-bone-microbiota axis

Methods:
In this study, the potential ameliorating effects of Lactobacillus fermentum MF 27 and Lactobacillus casei 393 on gut-bone-microbiota axis was determined using ovariectomized rat model. Female Sprague-Dawley rats were ovariectomized, and orally treated with L. fermentum MF27 and/or L. casei 393 (10⁹ CFU/mL/day) daily for 8 weeks. Intestine and liver were collected for biochemical and histopathological analysis. Meanwhile, faecal samples and bone (whole femora, cortical bone) were also collected for gut microbiota and bone turnover analyses, respectively

Results:
Oral administration of L. casei 393 significantly reduced body weight gain, serum levels of triglycerides, and cholesterol in the ovariectomized rats compared to the control. A reduction of expression of inflammatory markers in bone and intestine were also observed in the ovariectomized rats administered with L. fermentum MF 27. Meanwhile, qRT-PCR and micro-CT bone analysis further revealed that oral administration of these Lactobacillus strains modulate bone physiology and bone turnover by down-regulating osteoclastogenesis, and increasing bone mineral density in the ovariectomized rats. Fecal microbiota analysis also demonstrated that these Lactobacillus strains could modulate altered gut microbiota in the ovariectomized rats.

Discussion:
Hence, these results collectively demonstrate that oral administration of probiotics could modulate “gut-bone-microbiota” axis, thereby mitigating inflammation and improving bone health.

Keywords: Lactobacillus, Gut microbiota, Inflammation, Osteoporosis, Bone

EVALUATION OF THE INTRANASAL ADMINISTRATION OF LIVE LACTOBACILLUS SAKEI PROTECTION AGAINST DIFFERENT SUBTYPE INFLuenza VIRUS INFECTION IN MICE

Kim Y.; Kwon J.; Ju H.; Gwon G.; Song C.
Konkuk university

Introduction:
Avian influenza has high impact in public health areas. For improved therapies and preventive measures against influenza, there has been an increased tendency in modern medicine involving the use of probiotics. Lactic acid bacteria (LAB) stimulate mucosal immunity which is important to prevent influenza virus infection. However, many of recent studies are focused on screening LAB which has potent protective ability against single virus used in single experiment. There is only few articles dealing with effective LAB
selection against multiple viruses. It is required to check the capability of selected LAB inducing broadly protective immunity against various influenza subtypes.

Methods:
In this study, we compared the protective efficacy of selected LAB (Lactobacillus sakei) which isolated from Kimchi against challenge with different HA type of Influenza in mice according to the intranasal administration route. Animals were assigned to seven experimental groups; three virus (H1N1, H3 and H5N2) challenge groups after 6 times Lactobacillus sakei administration via intranasal route, positive control of each virus and a negative control. All mice except negative control were challenged with a lethal dose of AI viruses intranasally. After challenge with influenza virus at day 0 post-infection (p.i.), survival rate, weight loss and clinical signs were observed daily for 14 days p.i.

In addition, to understand the underlying mechanism behind this clinical protective effect, we performed immunologic assays including determination of lung virus titers, examination of IgA levels and cytokine profiles in the lung with two viruses which have shown difference in protective efficacy from the former experiment. The level of total and virus-specific IgA in lung homogenates was assayed using ELISA mice from each group on days 0, 2, 4, and 6 p.i. Quantitation of cytokine also measured in the same manner. To find out lung virus titers, MDCK cells were used with homogenized lung supernatants which were 10-fold serially diluted

Results:
The survival rate of mice receiving intranasal administration of LAB was higher than only virus challenged control. There were differences in protective efficacy of various influenza subtypes. All of mice of LAB administration group showed enhancement of secretory IgA production and down-regulation of pro-inflammatory cytokines in the respiratory immune system.

Discussion:
Intranasal administration route of lactic acid bacteria produced marked antiviral activity against influenza virus and activated host immune system. We found that protective effects induced by single LAB strain were distinct against various subtype of influenza infection. Therefore, for better clinical applications, selection of effective strains could be including evaluation of protection efficiency against multiple viruses.

Keywords: Influenza, Lactobacillus sakei, Lactic acid bacteria(LAB), Intranasal administration, Immune response

SMALL INTESTINE MICROBIOLOGY AND PROBIOTIC MODES OF ACTION

Kleerebezem M.
Wageningen UR, the Netherlands

Introduction:
The interest in the human intestinal microbiota has experienced a renaissance during the last decade, which is based on its prominent role in human health and disease. Most of the efforts to study this microbial ecosystem have focused on the faecal microbiota. Contrary to the faecal microbiota, very little is known about the small intestinal microbiota, while the small intestine plays a pivotal role in human immune and metabolic homeostasis and is the location where first encounters between microbiota and diet take place. Consequently, an important role of the small intestinal microbiota in the interplay between diet, microbiota and host physiology can be anticipated. Therefore, it is important to characterize the microbiota of the human small intestine and its modulation by diet, to decipher its impact on mucosal and systemic host health.

This presentation will focus on the characterisation of the composition and function of the human small intestinal microbiota, highlighting novel sampling capsules for non-invasive sampling in healthy volunteers. Additionally, the presentation will illustrate how probiotic consumption can drastically modulate this microbial ecosystem, and elicit specific molecular responses in the intestinal mucosa. These responses represent biologically coherent mucosal modulations that are specific for individual probiotic species and strains, which exemplifies the importance of identifying the probiotic effector molecules involved, in order to better understand the mode of action of these functional food ingredients. Finally, the responses to probiotics should be seen in the context of human individuality, which favours the application of probiotics and other functional foods in stratified subpopulations.

Keywords: Microbiota, Small intestine, Probiotic lactobacilli, Genomics, Mechanism of action
FUNCTIONAL DYSPEPSIA: A NOVEL FIELD FOR THE INTRODUCTION OF GASTRIC PROBIOTICS

Koga Y.
Tokai University School Of Medicine

Introduction:
Functional dyspepsia (FD) is defined as the presence of symptoms thought to originate in the gastroduodenal region (postprandial fullness, early satiation, epigastric pain or burning) in the absence of any organic, systemic or metabolic disease that is likely to explain the symptoms. FD has considerable impact on quality of life and loss of productivity. Epidemiological surveys suggest that around 30% of the general population has these symptoms over the course of a year and this percentage is reasonably constant around the world. The underlying patho-physiology in FD is incompletely understood. To date no medicine has definitely been approved for the treatment of FD. The aims of the present study are; first to examine the therapeutic effect of a probiotic strain, Lactobacillus gasseri OLL2716 (LG21), on the FD symptoms by randomized placebo-controlled clinical trial (RCT), second to investigate the mechanism how LG21 improves the symptoms.

Methods:
In the RCT, 58 and 58 adult subjects were randomly allocated to LG21- and placebo-treated groups, respectively. The LG21-treated group had 10 to the 9th CFU LG21 in yogurt everyday for 12 weeks. The placebo-treated group had yogurt without LG21 in the same way. In the analyses of the therapeutic mechanism of LG21, the volume, pH and bacterial count of the gastric fluid obtained after overnight fasting and the stomach-related serum enzymes such as pepsinogens I and II were measured in another 42 FD patients before and after LG21 treatment.

Results:
In the RCT, 54 and 52 subjects in the LG21 and placebo groups, respectively, completed the trial without any significant side effects. 10 subjects dropped out due to moving away and taking prohibited drugs during the trial. Per protocol analysis demonstrated that LG21 treatment significantly improved “postprandial fullness” after 4, 8 and 12 weeks. Moreover these improvements by LG21 treatment were significantly greater than the improvements by placebo treatment at both 8 and 12 weeks. LG21 treatment also significantly ameliorated “early satiety” after 4, 8 and 12 weeks. In the analysis of stomach-related biomarkers in the FD patients, LG21 treatment significantly decreased the volume but increased the pH value of the gastric fluid. LG21 treatment also significantly increased serum pepsinogen I level.

Discussion:
LG21 has unique properties of both acid resistance and gastric mucosa adhering ability, therefore has been used as a stomach-targeted probiotic strain. So far LG21 has been demonstrated to suppress Helicobacter pylori in the human stomach. Furthermore in the present study, LG21 was demonstrated to significantly improve the FD symptoms. While the mechanism how LG21 ameliorates the symptoms still remains elucidated, the reduction of acidity by LG21, at least, is thought to be involved in the improvement because high acidity is supposed to be one of the pathogenic factors leading to FD. The causal relationship between the changes in the gastric fluid volume/serum PGI and the amelioration of symptoms still remains to be clarified.

Keywords: Stomach, Functional, Dyspepsia, Probiotics, LG21

EFFECT OF BIFIDOBACTERIUM ANIMALIS SSP. LACTIS GCL2505, A PROBIOTIC STRAIN THAT PROLIFERATES IN THE GUT, ON VISCERAL FAT ACCUMULATION: EVIDENCE FROM HUMAN AND MICE STUDIES

Koga Y.; Nishijima T.; Aoki R.; Anzawa D.; Kamikado K.
Institute of Health Sciences, Ezaki Glico Co., Ltd., Japan

Introduction:
Gut microbiota currently has been recognized as the great contributor to the worldwide prevalence of metabolic syndrome (MS) which is based on the visceral fat accumulation. Numerous reports have indicated that gut bifidobacteria are associated with the improvement of MS, though the mechanism remains unclear. Bifidobacterium animalis ssp. lactis GCL2505 (GCL2505) was previously reported to proliferate in the gut.
and improve the fecal frequency in human study. The aims of present study were to investigate the preventive effect and its underlying mechanism of GCL2505 on visceral fat accumulation, a major risk factor of MS, in human and mice studies.

**Methods:**
The randomized, double-blind, placebo-controlled trial was carried out in 137 subjects with obese tendency. The subjects were assigned in two groups to receive fermented milk containing $8 \times 10^{10}$ CFU of GCL2505 (GCL2505 group) or fermented milk without GCL2505 as placebo (Placebo group) for 12 weeks. To investigate the mechanism, we compared the effect of oral administration of GCL2505 ($10^9$ CFU/day) to that of *Bifidobacterium longum* JCM1217 (JCM1217: $10^9$ CFU/day), a comparable Bifidobacterium strain, in a model of high-fat diet-induced C57BL/6 male mice.

**Results:**
In the human clinical study, visceral fat area in GCL2505 group at Week 8 and 12 was significantly reduced than that in Placebo group. Furthermore, GCL2505 administration significantly increased fecal bifidobacteria counts from Week 4 to the end of this study.

In the mice model study, similar preventive effect of GCL2505 on visceral fat accumulation was also observed while JCM1217 was not observed. Glucose tolerance was also improved only by GCL2505 administration. The 16S rRNA pyrosequencing analysis of mice cecal contents revealed that GCL2505 administration notably altered cecal microbiota from control and JCM1217 group mice and such alterations were characterized by the elevation of lactobacilli and bifidobacteria. GCL2505 administration resulted in an approximately 4-fold increase in cecal bifidobacteria counts as compared with JCM1217 administration. The cecal pool of acetate, a major metabolite of bifidobacteria, was also significantly elevated by GCL2505 administration, but not by JCM1217 administration. In accordance with this elevation, plasma acetate level and colonic GLP-1 expression, a gut hormone secreted by the short-chain fatty acids (SCFAs) stimulation, were significantly elevated only by GCL2505 administration.

**Discussion:**
We demonstrated that GCL2505 administration resulted in the increase of the gut bifidobacteria and the prevention of visceral fat accumulation in both human and mice studies. As the results of comparison with JCM1217 administration in mice study, it is suggested that GCL2505 was superior to JCM1217 on proliferative ability in the gut, which resulted in the cecal acetate elevation. It has been reported that SCFAs receptor signaling modulates insulin resistance and GLP-1 secretion, which leads to the improvement of visceral fat accumulation and glucose tolerance. Our findings in the present study therefore indicated that gut acetate elevation by GCL2505 administration could prevent the visceral fat accumulation through the SCFAs receptor reaction, which might be caused by a high proliferative ability of this probiotics.

**Keywords:** Human and Mice studies, Acetate, Proliferative ability, Bifidobacteria, Visceral fat accumulation, 16S rRNA pyrosequencing analysis

**GROWTH AND FERMENTATIVE ACTIVITIES OF PROBIOTIC STARTER CULTURE TREATED WITH HIGH INTENSITY ULTRASOUND IN MONO- AND CO-CULTURE**

*Krizman K.; Yong Y.; Zhou W.*
National University of Singapore

**Introduction:**
Modern consumers are becoming more aware of their personal health and demand for food that provide nutritional and health benefits. Consequently, probiotic strains belonging to genera *Lactobacillus* and *Bifidobacterium* have been progressively included in various kinds of foods as a result of growing scientific evidence on their health-promoting effects when consumed in sufficient amounts. In the past few decades, dairy products containing probiotics have become a key sector in functional food development. Since growth of monocultures in milk is limited, resulting in slow fermentation, co-culturing became a common commercial approach. However, one of the major challenges faced by industrial producers is low probiotic growth when fast-acidifying starter is used. Consequently, various techniques have been investigated in order to improve starter culture viability.
Methods:
Studies conducted recently suggested high intensity ultrasound as a promising method. Ultrasound treatment is able to increase microbial viability, promote release intracellular enzymes, reduce fermentation time, and alter carbohydrate metabolism of probiotic inoculated in skim milk. However, to date no studies have been carried out on the effect of sonication on probiotic starter when cultured individually or in co-culture. In this study, the effect of high intensity ultrasound (20 kHz) on fermentative activities and viability of Bifidobacterium lactis (Bb), L.rhamnosus (Lr), L.acidophilus (La), L.paracasei (Lc) and L. delbrueckii subsp. bulgaricus (Lb) in mono and co-culture with S.thermophilus (St) was investigated. Initially, yoghurt starter was exposed to different ultrasound treatment combinations; concurrent sonication or single sonication where one of the probiotic cultures was treated and the other was added subsequently (co-culture) or not (mono-culture), and fermented until a pH of 4.70 is reached.

Results:
Results showed that survival of St immediately after the treatment was greater as compared to Lb. However, at the end of the fermentation process, sonicated yoghurts containing only Lb showed an increase in bacterial count when exposed to ultrasound waves for longer periods. Interestingly, St and Lb both release substantial amount of intracellular enzyme β-galactosidase. However, stimulated growth of the later probably resulted from higher quantities of ruptured Lb immediately after sonication, where cellular components might act as growth-promoting factor. Regardless of the treatment and probiotic combination used, fermentation time was prolonged in parallel with their exposure to ultrasound wave.

Discussion:
Similarly, different binary combinations of sonicated St co-cultured with intact counterpart probiotic (Bb, Lr, La or Lc) were assayed. It was observed that extended sonication period was able to stimulate cell growth in milk medium, with Bb population increasing approximately 1 log in the final product. Extended ultrasound treatment also increased lactose metabolism as a result of β-galactosidase released into milk medium and/or higher net growth observed, suggesting potential beneficial effect for lactose intolerant population. At the same time, yoghurt with greater amount of galactose was obtained, which might add to sweetening effect without increasing caloric content. Overall, we demonstrated that total fermentation time was significantly shorter when intact probiotic culture was co-cultured with sonicated St, than when in mono-culture fermentation. Our findings showed that high intensity ultrasound is a promising method when optimal conditions are used and can be applied in certain mono- and co-culture fermentation.

Keywords: High intensity ultrasound, Mono- and co-culture, Skim milk, Streptococcus thermophilus, Viability, Fermentation, Bifidobacterium, Lactobacillus, Lactose, β-galactosidase

IMMUNOMODULATORY EFFECTS OF NON-DIGESTIBLE POLYSACCHARIDES – A DOUBLE-BLIND, RANDOMIZED, CONTROLLED CLINICAL TRIAL

Laue C.; Soeth E.; Stolte E.; Enouf V.; Knutsen S.; Ballance S.; NOFIMA

Introduction:
Non-digestible polysaccharides were reported to have immunomodulatory properties in in vitro and clinical trials. The evidence, however, was not yet considered sufficient for substantiation of a health claim by EFSA. We aimed at assessing the immune effect in line with the guidances and opinions of the EFSA. For this purpose the effect size of 5 polysaccharides on immune parameters was assessed and the sample size for a confirmatory study was estimated in a double-blind, randomized controlled pilot trial with 6 parallel arms.

Methods:
Healthy postmenopausal woman and men (N = 239) aged ≥ 50 years maintained a low fibre diet for 7 weeks. After a wash-out period of 2 weeks, the test products were consumed for 5 weeks. Two weeks after starting consumption an influenza vaccination (2012/2013) was performed. HI titres were assessed before intervention (consumption), before vaccination and 1 and 3 weeks after vaccination. Cellular immunity was assessed before intervention, before vaccination and 1 week after vaccination by incubating full blood cells with medium, LPS, ConA and vaccine, respectively and determination of cytokine release (IL-1β, IL-2, IL-12, IFNy, TNFa, IL-
10). Respiratory and gastrointestinal infections were monitored. The test products were: Powder containing either a beta-glucan preparation from yeast (Wellmune®)(500 mg)(BG-Y), or a beta-glucan preparation from shiitake (500 mg)(BG-S), or a beta-glucan preparation from oat (Oatwell®)(10.0 g)(BG-O), or an arabinoxylan preparation from wheat (Naxus™)(10.0 g)(AX), or an exopolysaccharide preparation from L. mucosae (2.3 g)(EXO) as well as filling substances (maltodextrins) and flavour adding up to 12.0 g altogether in sachets or only filling substances (control)(CON). The products were consumed once daily.

**Results:**
Geometric mean fold increase (GMFI) of HI titres differed between the interventions in Kruskal-Wallis one way analyses of variance on ranks for influenza A H1N1 (p = 0.01), but not for A H3N2 (p = 0.198) and B (p = 0.683). Compared to control AX had higher GMFI for H1N1 (p = 0.075 in unadjusted Student t test) and BG-Y for B (p = 0.043). The increase in seroprotection rate differed between the groups for H1N1 (p = 0.003; Chi2 test) and was higher for H1N1 (p = 0.053 in unadjusted Fisher exact test) and B (P = 0.078) in AX compared to CON. Seroconversion rate differed for H1N1 between the groups (p = 0.012) and was higher for H1N1 (p = 0.062) in AX and for B (p = 0.04) in B-S compared to control. INFγ differed between the groups (p = 0.013 in Kruskal-Wallis) and was higher in cells from AX versus CON (p = 0.009). The number of respiratory tract infections was lower in AX versus CON (p = 0.04; Mann-Whitney).

**Discussion:**
The results may indicate immunomodulatory effects by arabinoxylan and beta-glucan from yeast and shiitake. The results, however, require confirmation in a pivotal trial.

**Keywords:** Beta-glucan, Arabinoxylan, Exopolysaccharides, Immunity, Respiratory tract infections

**PROBIOTIC STRAINS ALTER THE CYTOKINES PRODUCTION AT MONOSODIUM GLUTAMATE-INDUCED OBESITY IN RATS**

Lazarenko L.1, Falalyeyeva T.2, Babenko L.1, Bubnov R.1, Demchenko O.1, Boyko N.4, Spivak M.1
1Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Ukraine
2Taras Shevchenko National University of Kyiv, Ukraine
3Clinical Hospital «Pheophania» of State Affairs Department, Ukraine
4Uzhhorod National University, Ukraine

**Introduction:**
Metabolic disturbances in obesity, that becomes endemic today, it may cause a number of diseases, namely, diabetes type II, cardiovascular diseases, stroke, premature death, diseases of musculoskeletal system, hepatobiliary disease and number of tumor sites, including lung cancer, breast cancer, uterine cancer and ovarian. It has been shown that probiotic strains can improve the biomarkers of obesity, including hyperlipidemia, hyperglycemia, oxidative stress, inflammation, disbalance in gut microbiota etc [1-4]. We recently showed that in contrast to Lactobacillus casei IMV B-7280, Bifidobacterium animalis VKL and B. animalis VKB (individually) the L. casei IMV B-7280 – B. animalis VKL - B. animalis VKB composition increased the adiponectin level, decreased the body weight and the leptin concentration in adipose tissue; restored the anthropometric parameters and lipid metabolism in the cases of monosodium glutamate (MSG)-induced obesity in rats [5].

In this paper, we present a results of the study demonstrating the influence of the L. casei IMV B-7280- B. animalis VKL, B. animalis VKB, (individually) and B. animalis VKL - B. animalis VKB composition on the serum level of pro- and anti-inflammatory cytokines as IL-1β, IL-12, IL-4, IL-10, interferon-γ, TNF-α in Wistar male rats with MSG-induced obesity. Rats received 3 to 4 mg/g MSG subcutaneously on the 2nd, 4th, 6th, 8th and 10th day of life. Administration of an aqueous solution of these probiotic bacteria (individually) or L. casei IMV B-7280 – B. animalis VKL - B. animalis VKB composition at the dose of 5×10⁹ CFU/kg intragastrically was started at the age of 4 weeks just after weaning and continued for 3 months during 2-week courses.

It was established that neonatal administration of MSG into the rats led to the increase of the IL-1β and IL-12 production. In contrast, the production of the IL-4, IL-10 and TNF-α in rats with MSG-induced obesity was decreased. In the case of the treatment use of L. casei IMV B-7280 - B. animalis VKL - B. animalis VKB composition we observed a tendency to decrease of the IL-1β production and significant decrease of the IL-12
production compared with rats with MSG-induced obesity that didn’t receive probiotic composition. However, the levels of IL-4, IL-10 and TNF-α production were increased (vs levels in intact rats). But, the application of L. casei IMV B-7280, B. animalis VKL, B. animalis VKB (individually) for therapeutic purposes was less effective. These results suggested that L. casei IMB B-7280 - B. animalis VKL - B. animalis VKB composition can restore the inflammation at MSG-induced obesity in rats.

The ability altering pro- and anti-inflammatory cytokines production opens new perspectives for the development of probiotic-based treatments for obesity and metabolic syndrome.

References

Keywords: Obesity, Metabolic syndrome, Probiotic composition

LACTOBACILLI AS ALTERNATIVE FOR ANTIBIOTICS IN NON-INTESTINAL APPLICATIONS

Lebeer S.
University Antwerpen

Introduction:
Because of the increased occurrence of antibiotic resistance and the side effects of antibiotic (mis)use, probiotic bacteria with strong antimicrobial capacity form an interesting alternative for antibiotics. Topically applied probiotics hold especially potential in niches outside the gastro-intestinal tract, because these niches are generally less densely populated with a diverse microbiota and are more accessible. However, such applications require other activities and other formulation and administration strategies. Therefore, we developed a screening platform for the screening and formulation of probiotics for non-intestinal applications. Especially Lactobacillus strains from original niches were screened, because of their GRAS (generally recognized as safe)/QPS (qualified presumption of safety) state and their well-known strong antimicrobial potential.

Methods:
Various Lactobacillus strains are isolated from original niches, including fermented vegetables (carrots), human vagina, human nasopharynx and human skin and characterized at species level by 16S rRNA gene sequencing. Subsequently, their antipathogenic potential is screened against non-intestinal pathogens, mainly urogenital pathogens (incl. Candida albicans, uropathogenic E. coli, Gardnerella vaginalis), nasopharyngeal pathogens (incl. Haemophilus influenza, Streptococcus pneumoniae, Staphylococcus aureus, Moraxella catarrhalis), and skin pathogens (incl. Propionibacterium acnes, dermatophytes and Candida) via radial diffusion, spot and streak line assays as well as time course analyses of antimicrobial activity in suspension and antibiofilm activities. Lactobacillus rhamnosus GG is included as reference probiotic strain with strong antimicrobial potential. The bio-active antimicrobial molecules of the lactobacilli are characterized by genetic and biochemical approaches.

Results:
In addition to the well-known antimicrobial potential of Lactobacillus strains against gastro-intestinal pathogens, we observed that many of our Lactobacillus isolates can also inhibit pathogens from other niches including vaginal, nasopharyngeal and skin pathogens. L- and D- lactic acid are key antipathogenic
compounds, but also other bioactive molecules such as exopolysaccharides, lectins and bacteriocins were explored. For instance, we found Lactobacillus lectins with a strong antibiofilm activity against uropathogenic E. coli.

Discussion:
The Lactobacillus isolates with the strongest antimicrobial activity are further explored for topical applications, depending on their antipathogenic spectrum and strongest activity. Together with specialists from the pharmaceutical department, suitable formulations with live probiotics for the specific niche(s) of application are explored. In addition, (pre)clinical studies for vaginal and skin applications are ongoing in which the impact of the probiotics on the full microbiome (including pathogenic and commensal microbes) is investigated via 16S rRNA metagenetics (Illumina Miseq). We believe such an integrated approach is important to fully assess the potential of topically applied probiotics as alternative for antibiotics.

Keywords: Antipathogenic, Effector molecules, Exopolysaccharides, Probiotics, Lectins

ANTIMICROBIAL SUSCEPTIBILITY OF LACTOBAILLUS AND BIFIDOBACTERIUM ISOLATED FROM HEALTHY ELDERLY PEOPLE LIVING IN THE KOREAN LONGEVITY VILLAGE

Lee Y.; Paek K.; Park J.; Hong H.; Shin E.
Department of Chemistry, Soongsil UniversityR&D Center, Nam yang Dairy Produts, Co.CCARM, Seoul Womens University

Introduction:
Intestinal microflora is closely related with human health and diseases influencing intestinal immune response, obesity, and even psychology. Its composition is closely influenced by food, age, regions, and human races. We isolated various lactobacillus and bifidobacteria from healthy people over 80 years old and assayed their MICs to various antimicrobial agents as the first step to develop a safe probiotic.

Methods:
Eight villages with more than 200 residents were selected based on previous studies. More than 10 fecal samples in each village were provided by healthy people older than 80 with regular bowl movement. Lactobacillus and Bifidobacteria were isolated and identified with 16S rRNA sequencing by Bionix (Seoul, Korea). MICs of lactobacillus were performed against 15 antimicrobial agents (ampicillin, chloroamphenicol, clindamycin, ciprofloxacin, erythromycin, gentamicin, kanamycin, lincomamide, neomycin, rifampicin, synercid, streptomycin, tetracycline, trimethoprim, vancomycin) while MICs of bifidobacteria were assayed against 8 antimicrobial agents (ampicillin, clindamycin, chloramphenicol, erythromycin, gentamycin, streptomycin, tetracycline, vancomycin). Minimal inhibitory concentrations (MICs) were assayed with the liquid dilution method using LSM for lactobacillus and LSM with cysteine for bifidobacteria following the ISO guideline (2010).

Results:
Total 104 lactobacillus and 27 bifibacteria were isolated from 79 people. Isolated lactobacillus belonged to 17 species and bifidobacteria belonged to 5 species. These were L. acidicipiscis (1 isolate), L. acidophilus (1 isolate), L. animalis (1 isolate), L. brevis (3 isolates), L. crispatus (8 isolates), L. fermentum (29 isolates), L. garvieae (2 isolates), L. gasseri (3 isolates), L. graminis (1 isolate), L. paracasei (20 isolates), L. paracasei subsp. Tolerans (9 isolates), L. pentopsus (1 isolate), L. plantarum subsp. Plantarum (3 isolates), L. rhamnosus (4 isolates), L. saerimneri (4 isolates), L. sakei sub. Sakei (13 isolates), L. salivarius (1 isolates) and B. longum subsp. Infantis (13 isolates), B. longum subsp. Longum,(9 isolates), B. faecale (1 isolate), B. pseudocatenulatum (2 isolates) B. adolescentis (2 isolates). Number of susceptible isolates of lactobacillus were 14 (13%) and these were: 6 isolates (21%) of L. fermentum; 4 isolates (14%) of 29 L. paracasei; 2 isolates (50%) of L. rhamnosus; 2 isolates (100%) of L. plantarum. Resistant isolates which were resistant to at least more than one antimicrobial agents were : 2 isolates to tetracycline; 1 isolates to erythromycin, 1 isolates to clindamycin, 3 isolates to chloramphenicol; 1 isolates to ampicillin. Number of susceptible isolates of bifidobacteria were 18 (67%): 8 isolates of B. longum subsp. Infantis; 6 isolates of B. longum subsp. Longum, 1 isolates of B. faecale, 1 isolate of B. pseudocatenulatum, and 2 isolates of B. adolescentis. Number of resistant bifidobacteria were 9: 1 isolates resistant to both tetracycline and chloramphenicol; 1 isolates to tetracycline; 2 isolates to erythromycin; 1 isolates to clindamycin; 2 isolates to chloroamphenicol;
1 isolates to ampicillin. B. faecale and adolescentis were all susceptible while 1 isolate (50%) of B. pseudocatenulatum was resistant to erythromycin.

**Discussion:**
Only a small percentage (13%) of lactobacillus while a large proportion (67%) of bifidobacteria showed antimicrobial susceptibility. For development of a probiotic, antimicrobial susceptibility should be assayed to develop a safe probiotic.

**Keywords:** LAB, Antimicrobial susceptibility, Bifidobacterium Healthy elderly people, Korean longevity village

**BIFIDOBACTERIUM ANIMALIS SPP. LACTIS 420 WITH OR WITHOUT LITESSE® ULTRA CONTROLS BODY FAT MASS AND WAIST CIRCUMFERENCE IN OVERWEIGHT AND OBESITY – RANDOMIZED, DOUBLE-BLEND, MULTICENTER CLINICAL STUDY**

**Lehtinen M.; Stenman L.; Meland N.; Kloster Smerud H.; Rissanen A.; Lahtinen S.**
DuPont Nutrition and Health

**Introduction:**
The composition of gut microbiota is interlinked with energy balance, but causal evidence between its modulation and body fat mass is still very scarce. Despite intense research on probiotics, only a handful of human studies have shown benefits on improving glucose metabolism, and only tentative proof has been gathered so far for an effect on weight control. We investigated the effects of probiotic Bifidobacterium animalis ssp. lactis 420 (B420) and a dietary fiber, Litesse®Ultra polydextrose (LU), on body fat mass and other parameters related to obesity.

**Methods:**
A total of 225 healthy participants were randomized into four groups for 6 mo of treatment: 1) Placebo; 2) LU, 12 g/d; 3) B420, 1010 CFU/d; 4) LU+B420, 12 g + 1010 CFU/d. Participants maintained their habitual diet and exercise routines during the study. Body composition using Dual-Energy X-ray Absorptiometry (DEXA), anthropometric measurements, and blood samples were taken at 0, 2, 4 and 6 months, as well as one month after end of treatment. Blood samples were analyzed for markers of energy metabolism and inflammation.

**Results:**
Due to the long intervention period and several protocol violations there were marked differences in the results of Intention-To-Treat (n = 209) and Per Protocol (n = 134) groups. The Per Protocol analysis included participants who completed the intervention period with at least 80% product compliance and did not use systemic antimicrobials during the intervention, as was pre-defined. There were no significant differences in body fat mass in the Intention-To-Treat population. However, LU+B420 and B420 seemed to improve weight management in the Per Protocol population. For change in body fat mass, LU+B420 showed a -4.6% (-1.4 kg) difference to Placebo (P=0.02), whereas LU alone had no effect, and the overall ANCOVA was non-significant (P=0.095). The factorial analysis for total fat mass was significant for B420 (P = 0.002 vs. Placebo). The effects on body fat mass were most pronounced in the abdominal region, reflected by a reduction in waist circumference in the LU+B420 group -2.7% (-2.6 cm), P = 0.047 vs. Placebo, ANCOVA P = 0.10. In the factorial analysis, reduction in waist circumference was significant for B420 (P= 0.004 vs. Placebo). However changes in zonulin, a potential marker of intestinal permeability, and high sensitivity C-reactive protein (hsCRP) from baseline to 6 months were associated with corresponding changes in trunk fat mass in the total Per Protocol population. On a group-wise level this association was significant only in the LU+B420 group.

**Discussion:**
This is to date the largest and most strictly controlled study to show that a probiotic with or without a dietary fiber controls body fat mass in healthy adults. B420 and LU+B420 showed benefits for controlling body fat mass and waist circumference, whereas LU had no effect. The reduction in trunk fat mass was associated with lower levels of zonulin and hsCRP suggesting that B420 and LU+B420 could potentially influence adipose tissue metabolism by improving gut barrier and inflammatory tone. (Clinicaltrials.gov NCT01978691)

**Keywords:** Probiotics, Bifidobacterium lactis B420, Polydesxtrose, Obesity, Weight management
EFSA UPDATE ON THE SCIENTIFIC REQUIREMENTS FOR HEALTH CLAIMS RELATED TO THE IMMUNE SYSTEM, THE GASTROINTESTINAL TRACT AND DEFENCE AGAINST PATHOGENIC MICROORGANISMS

van Loveren H.
Panel on Dietetic Products, Nutrition and Allergies (NDA Panel)
European Food Safety Authority (EFSA), Parma, Italy
Maastricht University, Maastricht, the Netherlands

Introduction:
The guidance on the scientific requirements for the substantiation of health claims made on foods related to gut and immune function published in 2011 has been recently updated by the EFSA NDA Panel and published in January 2016. Since Regulation (EC) No 1924/2006 entered into force, the NDA Panel has completed the evaluation of Article 13.1 claims (except for claims put on hold by the European Commission) and has evaluated additional health claim applications submitted pursuant to Articles 13.5 and 14.
The updated guidance document reflects the views of the NDA Panel based on the experience gained to date with the evaluation of new health claim applications, and captures additional issues gathered during the consultation process with experts and stakeholders.
The guidance document has been structured to avoid overlapping with the general scientific guidance for stakeholders on health claim applications.
It is subdivided into two major sections that address: (i) function claims related to the role of a food in maintenance/improvement of a physiological function and (ii) disease risk reduction claims related to the role of a food in reducing a risk factor for disease. Specific claims addressed in the guidance include those on functions of the immune system (based or not on the essentiality of nutrients), functions of the gastrointestinal tract (e.g. discomfort, gas accumulation, normal defecation and digestion and/or absorption of nutrients), defence against pathogens, and reduction of a risk factor for infections. Claims evaluated by the Panel with a favourable opinion have been used to provide guidance to applicants on the scientific requirements for their substantiation, whereas those evaluated with an unfavourable opinion have been used to illustrate the shortcomings that prevented their substantiation.
This presentation will give an overview of the way the EFSA guidance has been re-structured in the light of the new scientific evidence available to the NDA Panel, including the outcomes of public consultations, in an attempt to provide further assistance in preparing applications for the authorisation of health claims in this area.

Keywords: Health claims, Food, Disease risk reduction

REGULATION OF PROBIOTICS IN THE EU - WHAT’S NEW?

McCartney E.
Pen & Tec Consulting Group

Introduction:
What’s New in General?
The big news this year is undoubtedly the increased pressure to reduce antibiotic use in both humans and animals. This is driving food, feed and pharmaceutical players to seek creative opportunities with probiotics. Creativity and regulation are not easy partners, so it is important to recognise and address legal obstacles and practical considerations at an early stage of project planning.

Methods:
What’s New on Strain Safety?
Most live microbial strains used in EU foods and food supplements do not require a premarket safety assessment, due to traditional and safe use in fermented foods. However, EU regulators look to EFSA (European Food Safety Authority) for guidance on strain safety, particularly for food supplements, since these must be notified prior to marketing in most Member States. All live micro-organisms used in animal nutrition must pass EFSA on strain safety prior to marketing and face a stringent safety assessment:
Strain identity – the use of modern molecular techniques to identify probiotic strains frequently results in
taxonomy updates. A change in the taxonomy of a strain can result in regulatory challenges, especially if the new strain name is not listed as QPS (Qualified Presumption of Safety), updated annually by EFSA, or is no longer recognised as having a traditional use in fermented foods.

QPS – EFSA lists absence of toxin and virulence factors and antimicrobial resistance (AMR) as key qualifiers for strain safety. Current EFSA guidance on Bacillus safety illustrates the difficulty of deciding when a toxin is a toxin. The latest EFSA update on AMR was published in 2012, and sets cut-off values based on published data. There are also technical challenges in this area, for example the difficulty of setting appropriate cut-off values for “new” taxons with relatively few members.

Full genome – Exotic probiotic strains may be classed as “novel”, and could face evaluation by EFSA. For such strains, EFSA will require the full strain genome.

Results:
Strain identity – the use of modern molecular techniques to identify probiotic strains frequently results in taxonomy updates. A change in the taxonomy of a strain can result in regulatory challenges, especially if the new strain name is not listed as QPS (Qualified Presumption of Safety), updated annually by EFSA, or is no longer recognised as having a traditional use in fermented foods.

QPS – EFSA lists absence of toxin and virulence factors and antimicrobial resistance (AMR) as key qualifiers for strain safety. Current EFSA guidance on Bacillus safety illustrates the difficulty of deciding when a toxin is a toxin. The latest EFSA update on AMR was published in 2012, and sets cut-off values based on published data. There are also technical challenges in this area, for example the difficulty of setting appropriate cut-off values for “new” taxons with relatively few members.

Full genome – Exotic probiotic strains may be classed as “novel”, and could face evaluation by EFSA. For such strains, EFSA will require the full strain genome.

Discussion:
What’s New on Strain Efficacy?
Probiotics have still not succeeded in achieving a claim under the nutrition and health regulation, other than the generic claim for yoghurt bacteria, production of lactase and aid in digesting lactose in subjects with intolerance. It is interesting that the only approved probiotic claim in the EU is generic, since EFSA rejected most other generic probiotic claims on the grounds of lack of definitive strain identity. EFSA also insisted that probiotic efficacy is strain-specific. On the other hand there are around 40 probiotic strains approved for use in animal feeds, with several strains under EFSA evaluation. Demonstrating probiotic efficacy in animals to EFSA’s satisfaction is challenging, but possible. Perhaps there is room for more coherence across EFSA food and feed panels to allow best practices from both areas – human and animal?

Keywords: EFSA, Identity, Safety, QPS, Novelty

THE ROLE OF GUT MICROBIOTA IN IMMUNE DEVELOPMENT AND HOMEOSTASIS

McCoy K.
University of Bern

Introduction:
Microbial composition and the dynamics of intestinal colonization early in life play an important role in development of the immune system and can critically influence susceptibility to a variety of immune-mediated diseases later in life. The increased susceptibility to autoimmune or autoinflammatory diseases over the past several decades often correlates with changes in the composition and diversity of the intestinal microbiota and pediatric dysbiosis may have long-term health consequences. Changes in microbial composition during early life when the immune system is still developing have been demonstrated to be particularly important, suggesting a critical window of opportunity for proper conditioning of the immune system. While live microbes are very efficiently contained to mucosal sites, systemic exposure to microbial products or metabolites is ubiquitous and exposure to maternal microbiota-derived metabolites can even occur in utero. We have found that microbial exposure during early life shapes the developing immune system and exposure to the maternal microbiota in utero and during lactation prepares the newborn for colonization with its own microbiota and sets the baseline for a regulated immune system.

Keywords: Microbiota, Immune development, Metabolites, Homeostasis, Innate immunity
ESTABLISHMENT OF A MILK-ORIENTED MICROBIOTA IN INFANTS: NEW INSIGHT INTO PROBIOTICS AND PREBIOTICS

*Mills A. D.*
University of California

**Introduction:**
Human milk contains numerous components that shape the microbial content of the developing infant gastrointestinal tract. A prominent feature of milk is an array of complex glycans and glycoconjugates that serve a passive immune function by sequestering and deflecting pathogens while simultaneously enriching a protective, milk-oriented microbiota (MOM) often dominated by bifidobacteria. Recent research suggests the timing of establishment, and proper function of, a MOM is critical for infant development. A infant’s MOM is initially established through environmental transfer to the gut and subsequently shaped by diet (milk) and host genetics. Once established, MOMs dominated by bifidobacteria exhibit low residual milk glycans and higher levels of short chain fatty acids in the feces, suggesting a strongly saccharolytic colonic microbiota.

**Methods:**
The mechanistic basis for milk glycan consumption by bifidobacteria has been the subject of active research. Different infant-borne bifidobacteria contain specific glycosidases and transport systems required to utilize free glycans or glycoconjugates. Consumption of milk glycans enhances specific bifidobacterial interaction with the infant host through both direct and indirect routes. Growth on free milk glycans results in increased bifidobacterial binding to epithelial cells and beneficially modulates intestinal function. In addition, metabolites generated during growth on milk glycans dampen inflammation and strengthen gut barrier function.

**Results:**
In aggregate, these studies suggest a co-evolutionary relationship between mammalian milk glycans, infant-borne bifidobacteria and the infant host resulting in a programmed enrichment of a protective bifidobacterial-dominant MOM during a critical stage of infant development. Importantly, disruption of this programmed enrichment, by poor environmental transfer, antibiotic use, or infection, can lead to a “poorly functioning” MOM that may pose a risk for negative health outcomes. Further analysis of this naturally evolved system will shed light on effective pre- and probiotic tools that support and ensure a protective MOM for all at risk infants.

**Keywords:** Infant gastrointestinal tract, Milk-oriented microbiota, Glycans, Glycoconjugates, Bifidobacteria

EFFECTS OF BIFIDOBACTERIUM SUPPLEMENTATION ON MILD ANEMIA WOMEN IN A RANDOMIZED CONTROLLED TRIAL.

*Minami J.*
Morinaga Milk Industry Co., Ltd.

**Introduction:**
Anemia is a condition in which there are not enough healthy red blood cells (RBC) to carry adequate oxygen to the body’s tissues. Although there are many types of anemia, which have different causes, iron deficiency anemia is the most common form. In an animal experiment using pregnant rat, supplementation of *Bifidobacterium longum* BB536 was showed to increase RBC and hemoglobin (Hb) levels. In addition, BB536 supplements suppressed the decrease of RBC and Hb levels in patients undergoing surgery for gastrointestinal diseases. Thus, administration of *B. longum* BB536 has a potential to effect RBCs’ productions. In this study, we evaluated the effect of oral *B. longum* BB536 supplements on Hb levels in adult women with mild anemia.

**Methods:**
Thirty-six young adult women with hemoglobin levels less than 13 g/dl were enrolled in a randomized, double-blind, controlled trial, and they were randomly allocated to receive either probiotic capsules or placebo capsules for 8 weeks. Subjects in the probiotic group received two capsules per day that contained...
1.0 \times 10^{10} \text{ colony-forming units of probiotic } B. \text{ longum BB536. At baseline and 4, 8 weeks after supplementations, Hb, RBC, serum ferritin and the other anemia related blood parameters together with dietary investigation (FFQ) were assessed. A comparison of treatment groups was performed by analysis of covariance at week 8 adjusted for baseline values.}

**Results:**
The effectiveness of administrating probiotics on anemia improvement was evaluated with 29 subjects (probiotics n=15, placebo n=14). In the probiotics group, Hb and RBC levels were significantly increased compared to baseline (p=0.04 and p=0.01, respectively), and Hb levels tends to be higher in the probiotics group compared with the placebo group (p=0.07). In correlation analysis, the change of blood folate values showed positive correlation with the change of Hb values (r=0.492, p=0.06). In subpopulation analysis with subjects whose baseline Hb levels ranging 12 to 12.9 g/dl (probiotics n=11, placebo n=13), the Hb levels of the probiotics group were significantly higher compared with the placebo group after 8 weeks intervention (probiotics: 13.3, placebo: 12.6; p=0.03). In this study, average intake of nutrition did not differ between groups based on dietary investigation.

**Discussion:**
Although we did not observed significant inter-group differences, probiotic group showed significant increase of Hb and RBC levels after 8 weeks intervention. Besides, B. longum BB536 supplements ameliorated the blood Hb levels in the subject with mild anemia (Hb level 12 to 12.9 g/dl). It was reported that administration of B. longum BB536 increased fecal folate concentration and blood hemoglobin level in an animal experiment using germ free mice. In this study, we observed a weak positive correlation with the change of blood folate level and the change of Hb values (r=0.492, p=0.06), therefore supplementation of B. longum BB536 might have a potential to increase blood Hb level via folate supply especially for mild anemia women. Further investigation with large number of subjects is needed to confirm the efficacy of probiotics on improving anemia and to reveal the mechanisms.

**Keywords:** Bifidobacterium, Anemia, Randomized controlled trial, Hemoglobin , Probiotic capsules

**BACTERIOCINOGENIC LACTIC ACID BACTERIA ISOLATED FROM DIFFERENT LOCAL SOURCES IN UZBEKISTAN AND THEIR ANTIMICROBIAL ACTIVITY**

Miralimova S.; Ogay D.; Kutlieva G.; Sokhibnazarova K.; Elova N.
Institute of microbiology of AS of Uzbekistan

**Introduction:**
Antimicrobial resistance of microorganisms become a global problem and without urgent, coordinated action, the world is heading towards a post-antibiotic era, in which common infections and minor injuries, which have been treatable for decades, can once again kill. This stipulates necessity to search for new infection treatment options and other tools (WHO, 2015). Bacteriocins of lactic acid bacteria have a great potential for application as preservatives in food industry and as alternative to antibiotics in medicine (Arques J. et al., 2015).

The aim of this work was to screen for bacteriocin-producing lactic acid bacteria among the strains isolated from a range of traditional fermented products, to study their spectrum of antimicrobial activity and their probiotic properties such as acid-, bile- and salt-tolerance, susceptibility to antibiotics.

**Methods:**
Forty two strains of lactic acid bacteria were isolated from the kumis (fermented horse milk), shubat (fermented camel milk), sauerkraut and screened for antagonistic activity, 31 of them showed antagonistic activity against several gastrointestinal disorder agents and foodborne pathogens, such as Escherichia coli, Enterococcus faecalis, Enterococcus faecium, Candida albicans, Pseudomonas aeruginosa, Proteus morganii, Citrobacter freundii, Serratia marcescens, Staphylococcus aureus, Helicobacter pylori. Based on the loss of antimicrobial activity after treatment with protease K and pepsin (Klaenhammer T. et al., 2012) the proteinaceous nature of antimicrobial substances of 6 isolates have been established. Identification of the isolates producing bacteriocins have been performed based on carbohydrate fermentation pattern and 16S rRNA gene sequence.
**Results:**

According to phenotypic and genotypic identification 2 isolates are Lactobacillus plantarum, one is Lactobacillus rhamnosus and the remaining Lactobacillus isolates are not identified to species yet. Among these strains, L. plantarum 42 produce a bacteriocin which inhibits the growth of E. faecalis and E. faecium strains, L. plantarum 44 produces a bacteriocin against P. morganii strain, L. rhamnosus bacteriocin inhibits the growth of H. pylori strains and other isolates have protease eliminated inhibition against E. coli, P. aeruginosa, S. marcescens, P. morganii, S. aureus strains.

All identified Lactobacilli were found to be resistant to bile salts (0.6%), NaCl (8%) and pH 3, susceptible to most antibiotics.

**Discussion:**

Several conditionally-pathogenic bacteria appear to be sensitive to bacteriocins produced by the isolates: E. faecalis and E. faecium, which are cause gastrointestinal infections; P. morganii - urinary tract infections; H. pylori - gastric and duodenal ulcer agent; S. marcescens - urinary tract infections, sepsis and pneumonia; P. aeruginosa, E. coli and S. aureus infections can involve any part of the body and the latter two bacteria are identified as a food-borne pathogens. Taking into account the clinical importance of susceptible strains, tolerance to bile, salts and low pH these Lactobacillus isolates have a great potential for application in pharmaceutical and food industry as a bio therapeutic agents and probiotic products.

**Keywords:** Lactic acid bacteria, Bacteriocins, Antimicrobial activity, Probiotics, Food-borne pathogens

**PANCREATIC LIPASE INHIBITORY ACTIVITIES OF LACTIC ACID BACTERIA ISOLATED FROM RAW CAMEL MILK**

*Mudgil P.; M.K.Y. Mustafa N.; Al Kaabi M.; Al Ketbi M.; Abubaid A.*
United Arab Emirates University, UAE

**Introduction:**

Obesity, as an ever-growing epidemic, remains the top contributor to global burden of disease. It not only strains the healthcare systems but also has profound effects on economy and psychology of people suffering. Pancreatic lipase is the primary lipase that hydrolyzes dietary fat molecules in the human digestive tract, converting triacylglycerol substrates to monoacylglycerols and free fatty acids. As obesity is primarily a disorder of lipid metabolism, hence, selective inhibition pancreatic lipase could be targeted for its management. Lipstatin (Orlistat), a FDA approved pancreatic lipase inhibitor obtained from fermentation broth of Actinomycetes remains the most celebrated and successful anti-obesity drug till now. However, excessive inhibition of pancreatic lipase leads to certain discomforts like steatorrhea and isolated cases of organ toxicity. Therefore, in quest of natural and safe inhibitors researchers are screening a lot nutritional products that could potentially possess lipase inhibitory abilities. Till today, the ability of lactic acid bacteria (LAB) towards inhibition of pancreatic lipase remains to be unexplored. Hence, the present study was undertaken to explore the pancreatic lipase inhibitory potential of lactic acid bacteria isolated from camel milk.

**Methods:**

Streptococcus and Lactobacillus isolates were isolated from raw milk samples collected from local camel farms of Al Ain, Abu Dhabi, UAE. For screening of lipase inhibitory activity, cell free extracts (CFE) were prepared by incubating washed bacterial cell pellets (250 mg/mL; ww/v) in phosphate buffer saline (PBS) for 12 hours. Inhibitory activities were determined by comparing the release of p-Nitrophenol from p-Nitrophenyl palmitate through porcine pancreatic lipase type VI, in presence and absence of CFE using Orlistat as positive control while PBS, as negative control.

**Results:**

Overall, Lipase inhibitory profile of 97 LAB isolates and 11 reference strains was achieved. A wide variation in inhibitory activities were observed among isolates and reference cultures. Among Streptococcus isolates (52) inhibition ranged from 3.0-99%. 11 isolates possessed potent inhibitory activities comparable or higher than Orlistat (≥ 83%; 2 mg/ mL). Among 45 isolates of Lactobacilli negative inhibition was observed among 13 isolates, while for rest 32 isolates it ranged from 4.0-81%. Inhibition percentage among reference cultures ranged from 3.0-37%. L. acidophilus DSMZ 9126 showed strongest inhibition while L. gasseri 20243 showed lowest inhibition.
**Discussion:**
These preliminary results definitively demonstrate that LAB isolated from raw camel milk have pancreatic lipase inhibitory activities and can reduce the hydrolysis of dietary fats in vivo. Results obtained also presents the opportunities in the future to use probiotic bacteria in the management of obesity and hyperlipidaemia. However, as the nature of bacterial metabolites responsible and their fate in gastrointestinal tract is still unclear, hence, further work towards identification of responsible molecules and to better understand its fate under in vivo condition is needed.

**Keywords:** Pancreatic Lipase Inhibition, Camel milk, Lactic acid Bacteria, Probiotics, Obesity

**MICROBIOTA MANAGMENT FOR THE PREVENTION OF RECURRENT EAR AND THROAT INFECTIONS**

*Mulder L.*
Wincllove Probiotics

**Introduction:**
Upper respiratory tract infections (URTIs) are caused by an (acute) infection of a region in the upper respiratory tract. This commonly includes diseases like tonsillitis, sinusitis, otitis media and the common cold. URTIs can be caused by a variety of microorganisms including viruses and bacteria. Due to the combination of high colonization rates of potential pathogens and an immature immune system especially children are prone to the development of URTIs. Recurrent infections can have even more serious consequences, like hearing loss, and speaking- and learning disability. Treatments vary between countries and include to wait until the infections over, antibiotic prescription or in more severe cases surgery. A main disadvantage of traditional treatment options is that they only relieve the symptoms but do not address the underlying cause. The extensive use of antibiotic for acute otitis media (AOM) has obvious public health and economic consequences. Only very recently studies are being published researching the microbial communities that inhabit the (upper) respiratory tract. These studies show that the bacterial players in the respiratory tract are quite different to the key players in the gastro-intestinal tract. Data indicate that just like the gastro-intestinal tract, diversity in the microbiota seems to play an important role in the susceptibility to infections. Alternative therapies that support the commensal microbiota in the upper respiratory tract are highly desired. Recent studies have suggested that probiotics may reduce the risk of various symptoms of URTIs. The mechanisms behind the possible beneficial effect of probiotics on URTIs are not completely clear but may be related to colonization of the probiotic bacteria in the upper respiratory tract.

**Methods:**
A probiotic formulation (Ecologic® ENT) was developed to enhance the natural respiratory microbiota. The composition of the probiotic formulation was done based on a small collection of bacterial strains that already have shown their efficacy to be able to prevent the recurrence of ear and throat infection in several clinical trials. Especially the strains Streptococcus oralis 89a and Lactobacillus rhamnosus LB21 showed positive effects in URTIs in children without any reported side-effects.

**Discussion:**
The probiotic formulation Ecologic® ENT stimulates the commensal upper respiratory tract microbiota and inhibits the specific pathogens involved in recurrent ear and throat infections. Probiotic bacteria can have local beneficial effect by competition with pathogens for space and nutrients. In addition they can directly inhibit pathogens by local secretion. In this way they can function as a biological barrier against pathogens and their presence can contribute to the restoration of the natural microbiota in the upper respiratory tract. Probiotics thus have the potential to be a safe alternative therapy in the prevention of URTIs.

**Keywords:** Probiotics, Upper respiratory tract infections, Microbiota, Streptococcus oralis , Medical device
EFFECT OF FRUCTANS WITH DIFFERENT DEGREE OF POLYMERIZATION AND STRUCTURE ON GROWTH OF SELECTED PROBIOTIC STRAINS AND FORMATION OF SHORT CHAIN FATTY ACIDS

Mueller M., Viernstein H., Loeppert R., Praznik W.
University of Vienna, Department of Pharmaceutical Technology and Biopharmaceutics, Vienna, Austria;

Introduction:
Fructans are well known prebiotics which are accumulated by a great variety of plants including composites (e.g. chicory, Jerusalem artichoke) or agavaceae (div. agave species) (1). The influence of structure and polymerization degree (dp) of fructans on the prebiotic potential is not fully elucidated yet. Thus, we compared the growth of selected probiotic strains with fructans from different sources including chicory and agave related to diverse structures such as unbranched inulin-type (only β2-1 linkages), mixed-type (combined β2-1 and β2-6 linkages with branching) and levans (β2-6 linkages) with branching characteristics (1). Furthermore we tested the influence of branching and dp on the formation of short chain fatty acids (SCFAs).

Methods:
Fructans from chicory or agaves were separated into 3 to 5 fractions using size exclusion chromatography and the polymerization degree of the fructan fractions was determined using ions exchange chromatography. The influence of the fructan samples on the growth curve of selected probiotic strains was determined including Lactobacilli spp. and Bifidobacteria spp. based on a turbidity measurement. The degradation of fructo-oligosaccharides by probiotics and the formation of short chain fatty acids (SCFAs) were studied.

Results:
Fructan samples with lower polymerization degree and branching induced the growth of the probiotics faster than those with higher polymerization degree. The correlation between growth induction and polymerization degree was strain dependent. The degradation process of the fructo-oligosaccharides by probiotics correlated well with the growth curves. Some strains grew only with fructans of low dp, some with fructans from all dp, but faster with fructans from low dp and a few strains grew fast even with higher molecular fructans (2,3). The formation of SCFAs by selected prebiotic strains or by a mixture of gut bacteria was dependent on the polymerization degree and branching (4).

Discussion:
Fructans from agave and chicory significantly induce the growth of probiotic bacteria. In contrast to a previous study, all our tested probiotics could use fructans as sole carbon source (5) with strain dependency on the usage of higher molecular fructans. The study also elucidates the correlation of the growth of the bacteria with the degradation process of the oligosaccharides and the formation of SCFAs. Unbranched and branched fructans led to the formation of butyrate which plays a major role in the prevention of colon cancer and other colonic diseases.

In conclusion, this study contributes to elucidate the fermentation behavior of selected prebiotic strains dependent on the molecular structure and polymerization degree of the fructans and on their formation of SCFAs which play a major role in health for usage in Functional Food industry and pharmaceutical applications.

References:

Keywords: Fructans, Short chain fatty acids, Polymerization degree

POTENTIAL USE OF LACTOCOCCUS LACTIS KA-FF 1-4 SUPPLEMENT WITH NON-DIGESTIBLE OLIGOSACCHARIDE AGAINST VANCOMYCIN-RESISTANT ENTEROCOCCI

Nakphaichit M.; Plupjeen S.; Phumsombat P.; Nakayama J.; Nitisinp rasert S.
Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University

Introduction:
Vancomycin-resistant enterococci (VRE) is a major opportunistic pathogen in immunocompromised populations. VRE treatment with antibiotics remains challenging due to the robustness and ability of the pathogen to mutate under harsh conditions. The close relationships between gut microbiota and health and disease have increased interest in the use of probiotics and prebiotics to positively modulate the gut microbiota to prevent or treat infections. Lactococcus lactis KA-FF 1-4 isolated from Thai fermented fish produces a bacteriocin which is tolerant to temperatures of 121°C for 15 min, and stable at pH 3 to 8. The purified bacteriocin has a molecular weight of 4703 Da, and inhibitory activity against a wide range of lactic acid bacterial strains and pathogens, especially VRE.

Methods:
The in vitro gastrointestinal assay of strain KA-FF 1-4 was performed by following the method described by Ranadheera (2012) with some modification. The stimulated gastric juice was prepared by suspending pepsin in sterile filtered 0.5% (w/v) NaCl solution to a final concentration of 3 g/l, with the pH adjusted to 2.0. The simulated small intestinal juices were prepared by suspending pancreatin in filter sterile 0.5% NaCl (w/v) solution to a final concentration of 1 g/l and adjusting pH to 8.00 with sterile 0.1 mol/l NaOH. The probiotic strain was incubated in gastric juice at 37°C for 1 hour and then was transferred to small intestine juice for 2 hours. To evaluate the efficiency of KA-FF 1-4 in eliminating VRE, the large intestine system was performed under anaerobic batch fermentation. The temperature was maintained at 37°C and pH was controlled between 6.65 and 6.95. The optimum concentration of KA-FF 1-4 against VRE was investigated. In addition, commercial prebiotics including inulin, fibersol (maltodextrin), fructo-oligosaccharide (FOS), and xylo-oligosaccharide (XOS) were applied to improve the inhibition activity in gut model. The inhibition activity was determined by spot on lawn and culture based technique.

Results:
The strain KA-FF 1-4 survived 65% (6.03 Log CFU/ml) in human gastrointestinal conditions. A concentration of 108 CFU/ml of KA-FF 1-4 showed the highest activity, decreasing VRE from 104 CFU/ml to zero in 9 h in human gut model. Moreover, the KA-FF 1-4 supplement with fibersol showed the greatest inhibition and improved cell growth. The highest inhibition activity again VRE was determined after 18 hours; higher than the non-prebiotic supplement for 150 times by spot-on-lawn technique. The optimum concentration of fibersol was determined at 2%, and decreased VRE from 104 CFU/ml to zero in 6 hours.

Discussion:
However, probiotic and prebiotics work largely through direct or indirect effects on the gut microbiota in host function. The combination of KA-FF 1-4 and fibersol requires further study for fecal anaerobic batch culture. The molecular base technique as qPCR will be performed for gut microbiota analysis, and bacteriocin production will be analysed by enzyme-linked immunosorbent assay (ELISA). This research will be useful to apply synbiotics for therapeutic products or functional food in the future.

Keywords: Gut Microbiota, Synbiotic, Vancomycin resistance enterococcus, Gut modelling, Probiotic
RESEARCH AND DEVELOPMENT FOR AN EFFECTIVE AVIAN PROBIOTIC

Oakley B.; Cox N.; Berrang M.; Collett S.; Aggrey S.
University of Georgia, USA

Introduction:
There now exists a large body of evidence that GI microbes make significant contributions to host nutrition and pathogen resistance. In food animals, many studies now support the idea that the gut microbiota is a logical target to improve performance and food safety. In the poultry industry, the cost of feed represents a majority of production costs, and therefore any improvement to feed conversion ratios that can be achieved by optimizing the microbiota could have significant value to the industry. We have recently completed work on a number of different areas with a general objective of generating defined and transferable microbiota that can improve poultry nutrition and reduce broiler colonization by human pathogens.

Methods:
First, chicks from low feed efficiency (HRFI) and high-efficiency (LRFI) genetic lines were reared and samples collected at bi-weekly intervals to characterize and compare the GI microbiome. Second, groups of chicks from each genetic line received microbiome transplants from the two donor lines. Birds were weighed at weekly intervals and feed conversion efficiency calculated on a per-bird basis for the last two weeks of the experiment. Next, we took two parallel approaches. First, we sequenced microbial DNA and compared the taxonomic and genomic composition of the cecal microbiome of birds in the experiment. Second, we used a selective cultivation scheme to enrich for specific taxonomic groups present in the chicken GI tract. The targets of these enrichments were based on the sequencing data from this and other projects. Using this approach, approximately 40 strains were brought into axenic culture, some of which appear to represent previously undescribed taxa.

Results:
Feed-efficiency status of the donor birds (high or low efficiency) made little difference to the performance of the recipient. However, there was a dramatic difference between inoculated versus uninoculated chicks. Over a 6 week growth period, inoculated chicks showed significant improvements in body weight gain and feed efficiency relative to uninoculated chicks. This difference appeared to be mediated by the microbiota: by several different metrics inoculated versus uninoculated birds had significantly different microbial communities at the end of the experiment. Characterizations of communities and individual strains are ongoing.

Discussion:
The strategy taken here strongly supports the use of microbial consortia as probiotics with significant effects on poultry growth performance. Optimizing the microbiota of commercial poultry has great potential to provide value to the industry by improving feed conversion efficiency and thus reducing feed costs, improving food safety, reducing the carbon footprint of the industry, limiting regulatory burdens, and providing new probiotic products as alternatives to antibiotics. Further development is required to better characterize and propagate inocula and to better understand the mechanisms behind the observed effects.

Keywords: Poultry microbiome, 16S rRNA sequencing, Metagenomics, Food Safety, Food Animals

THE DOUBTS AND TRUTHS OF PLANT-ORIGIN PROBIOTICS: IS IT REALLY MORE BENEFICIAL TO HUMAN HEALTH THAN HUMAN (ANIMAL)-ORIGIN?

Oh S.
Chonnam National University

Introduction:
Probiotics are used as the health foods or the starters in the fermentation of foods. Among probiotics, the use of Lactobacillus species to enhance intestinal health in dairy or plant products has been proposed for many decades. There have been hundreds of publications describing the use of Lactobacillus species to prevent and treat a variety of gastrointestinal disorders. The scientific basis of Lactobacillus in dairy products has been firmly established, however, clinically beneficial effect of plant-origin Lactobacillus begun to be published
recently. The best-studied Lactobacillus species are Lactobacillus acidophilus, L. casei, L. rhamnosus, and L. plantarum. A common belief that many people believe is that plant based-fat is much healthier than animal (dairy) fat. However, there is a misconception when it comes to probiotics. Many companies insist that fermented-plant origin probiotics are superior to animal origin probiotics without any scientific data that supports their claim. How are plant proteins much better than animal proteins for human health? The simple answer is that it is not. Such claims are no different from a form of social gossip that is made by companies for commercial benefit. Many people think this claim is true because many companies advertise their products in such matter. The advertisements are misleading and spread false ideations that animal based probiotics are nowhere near healthy as plant based. In a recent report, my research group measured viability of commercial probiotics during long-term storage. Lactobacillus casei Shirota and L. rhamnosus GG, a famous animal-origin probiotics in dairy products, maintained at 107–108 CFU/mL for up to 35 ~ 52 weeks of storage. In kimchi fermentation, Lactobacillus plantarum was detected up to 25 days. Lactobacillus plantarum, one of the facultatively hetero-fermentative group of lactobacilli, is a heterogeneous and versatile species that is encountered in a variety of environmental niches, including dairy, meat, fish, and many vegetable or plant fermentations. Therefore, this species easily detected in plant-fermentation products and dairy foods. It has proven ability to survive gastric transit and colonize the intestinal track of humans and other mammals.

Methods:
L. plantarum strain isolated from infant feces has a high survival rate in low pH conditions. Proteins isolated from L. plantarum L67 could stimulate the apoptotic signals and then consequently induce programmed cell death in HT-29 cells. The glycoprotein (18kDa) isolated from L. plantarum L67 inhibited degranulation and histamine release in the BPA stimulated RBl-2H3 cells, while amount of Ca2+ and iNOS were diminished.

Results:
My research group discovered that L. plantarum strain isolated from infant feces has a high survival rate in low pH conditions. Proteins isolated from L. plantarum L67 could stimulate the apoptotic signals and then consequently induce programmed cell death in HT-29 cells. The glycoprotein (18kDa) isolated from L. plantarum L67 inhibited degranulation and histamine release in the BPA stimulated RBl-2H3 cells, while amount of Ca2+ and iNOS were diminished. Also the 18kDa glycoprotein inhibits the phosphorylation of ERK and p3 MAPK, and the activation of AP-1(c-jun and c-Fos). Additionally, TNF-α, IL-1β, IL-4, IL-6 and IL-10 of allergy-related cytokines were suppressed by the 18kDa glycoprotein. Pretreatment of 18 kDa glycoprotein inhibits the cytotoxicity, intracellular Ca2+ mobilization following exposure to cadmium chloride. Also pretreatment of glycoprotein (18 kDa) significantly suppressed the expression of MAPKs (ERK and JNK), and Ap-1 (c-Fos and c-Jun). And also, activities of PCNA and cyclin D1/CDK4 were suppressed on pretreatment with glycoprotein (18 kDa) in the presence of Cd.

Discussion:
Furthermore, L. plantarum L67 could be used as a probiotic culture for the production of dairy or vegetable fermented foods. In conclusion, we need to focus on defining what types of probiotics are beneficial to us despite its origin. Plant probiotics is not bad; but it is hard to say that it is better than human-, animal-, dairy-origin probiotics due to the cyclic processed of bacterial circulation (soil-plant-human/animal-dairy-feces). The positive effects on human health can be heightened if probiotics is consumed, whatever its source.

Keywords: Probiotics, Dairy, Plant-origin, Lactobacillus, Intestinal health

LACTOBACILLUS PENTOSUS STRAIN S-PT84 ATTENUATES LIPOTOXICITY-INDUCED HEPATIC INSULIN RESISTANCE AND STEATOHEPATITIS BY MAINTAINING GUT PERMEABILITY AND INDUCING MACROPHAGE ALTERNATIVE ACTIVATION IN MICE

Ota T.
Brain/Liver Interface Medicine Research Center, Kanazawa University, Japan

Introduction:
Nonalcoholic fatty liver disease (NAFLD), a form of lipotoxic liver injury that can impair systemic insulin resistance, and can progress to nonalcoholic steatohepatitis (NASH). We previously demonstrated that excessive hepatic lipid accumulation promotes the activation of macrophages/Kupffer cells, resulting in exacerbated insulin resistance and hepatic inflammation (Ota T et al, Gastroenterology 132:282, 2007,
Introduction:
Incretin hormones are gastrointestinal insulin-releasing peptides involved in the regulation of postprandial nutrient homeostasis. The incretin hormones have been the basis for a number of clinically approved pharmaceutical compounds with good efficacy for the treatment of human type 2 diabetes and its complications. The two established incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinogetic polypeptide (GIP) and they are produced by enteroendocrine cells lining the intestine. Exploiting the effects of human commensal microbiota on intestinal cells could provide novel,

Results:
After 22 weeks of feeding, histological examination revealed hepatic steatosis and inflammation in mice fed the CL diet. The mice exhibited hyperinsulinemia and glucose intolerance, indicating that the development of NASH associated with insulin resistance. S-PT84 administration improved hepatic steatosis by decreasing TG and FFA levels by 34% and 37%, respectively. S-PT84 also inhibited the development of hepatic inflammation and fibrosis, lowering F4/80+ macrophage/Kupffer cell infiltration and the hydroxyproline content of the liver. S-PT84 administration in mice fed a CL diet led to improved glucose intolerance and hyperinsulinemia as well as enhanced hepatic insulin signal assessed by phospho-Akt. These changes were associated with attenuated excess lipid peroxidation (TBARS) and MAPK (JNK/p38MAPK) and NF-kB activation in the liver. FACS analysis revealed that CD11c+CD206- M1 or ‘classically activated’ pro-inflammatory macrophages were not significantly altered in the CL + S-PT group compared with mice fed the CL diet. However, mice fed the CL + S-PT had 71% more CD11c-CD206+ M2 or ‘alternatively activated’ non-inflammatory macrophages than mice fed the CL diet, resulting in a predominance of the M2 over the M1 macrophage population. S-PT84, however, showed little effect on NK/NKT cell and T regulatory cell populations in the liver. Furthermore, S-PT84 induced significant improvements in intestinal epithelial barrier permeability. Importantly, plasma lipopolysaccharide binding protein (LBP) levels were markedly increased in mice fed the CL diet compared with normal chow, while S-PT84 markedly diminished plasma LBP levels.

Discussion:
A CL diet enhanced intestinal permeability and metabolic endotoxemia, contributing to hepatic insulin resistance and inflammation in NASH. S-PT84 treatment attenuated lipotoxicity-induced hepatic insulin resistance and steatophepatitis by maintaining gut permeability and inducing alternative activation of liver macrophages. Further studies are required to determine whether S-PT84 can directly influence the gut immune system.

Keywords: Lactobacillus pentosus strain S-PT84, Nonalcoholic steatohepatitis (NASH), Insulin resistance, Lipopolysaccharide binding protein (LBP), Macrophage

PROBIOTIC STIMULATION OF INCRETIN HORMONE SECRETION AS A POTENTIAL THERAPEUTIC STRATEGY FOR TYPE 2 DIABETES MELLITUS: EXAMINING THE MECHANISM OF ACTION

Panwar H.; Calderwood D.; Gillespie A.; Graham S.; Wylie A.; Grant I.; Grover S.; Green B.
Institute for Global Food Security, School of Biological Sciences, Queen’s University Belfast, Northern Ireland, UK

Introduction:
Incretin hormones are gastrointestinal insulin-releasing peptides involved in the regulation of postprandial nutrient homeostasis. The incretin hormones have been the basis for a number of clinically approved pharmaceutical compounds with good efficacy for the treatment of human type 2 diabetes and its complications. The two established incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinogetic polypeptide (GIP) and they are produced by enteroendocrine cells lining the intestine. Exploiting the effects of human commensal microbiota on intestinal cells could provide novel,
natural, safe and cost effective strategies for diabetes therapy. The present study examined the ability of human gut Lactobacillus isolates along with reference probiotic strains to modulate the expression and secretion of incretin hormones besides examining the potential mechanisms involved.

**Methods:**
Live bacteria (approximately 1x10^9) were co-cultured with STC-1pGIPneo murine intestinal cells (2x10^6) for 3h and incretin hormone secretion was assessed. GLP-1 and GIP concentrations were assessed in cell supernatant using specific and selective immunoassays. Data were analysed by One-Way ANOVA with the Tukey post hoc test. Co-incubation was followed by RNA isolation, cDNA synthesis and RT-qPCR SYBR green based expression study for GLP-1, GIP and free fatty acid receptors. Studies were also conducted with L. rhamnosus alone or in combination with either a Myd88 blocking peptide or an anti-CD14 antibody. Amino acid profiling of cell supernatant was done by GC-MS. Cell cytotoxicity was determined by LDH based cytotoxicity detection kit (Roche). RT2profiler PCR arrays were used to detect the expression of 84 genes implicated in regulating TLR pathways, in STC-1pGIPneo cells induced with L. rhamnosus.

**Results:**
Acute co-culture of LAB with enteroendocrine cells showed that certain LAB strains elicit GLP-1 and GIP secretion (13-194fold) and upregulate their gene expression. Maximum secretion of GLP-1 and GIP was recorded with L. plantarum subs. argentototensis (Lb-3), L. johnsonii and L. rhamnosus as determined by RIA and ELISA respectively. However, a varied response for RT-qPCR based expression of respective genes was recorded at transcriptional level with Lb4, Lb6, L. casei, L. plantarum and L. rhamnosus significantly upregulating expression of both GLP-1 and GIP. However, Lb8, Lb9, L. acidophilus and B. bifidum could activate only GIP. LAB induced incretin hormone secretion did not appear to involve nutrient mechanism nor was there any evidence of cytolysis. Instead PCR array studies implicated signalling agents of the toll-like receptor system e.g. L. rhamnosus downregulated MyD88 (23fold) along with over-expression of cell surface antigen, CD14 (17fold). Mechanistic studies found that blockade of MyD88 triggered significant GLP-1 secretion. Furthermore, blocking of CD14 completely attenuated LAB-induced secretion. Besides this, upregulated expression of free fatty acid receptors in presence of selected isolates i.e. GPR-41 and 120 by Lb-6, GPR-40 and 41 by L. casei and only GPR-40 by L.rhamnosus was observed. GC-MS based profiling revealed enhanced level of alanine, proline and histidine.

**Discussion:**
Shortlisted strains could be explored as prospective candidates for management of diabetes through incretin hormone stimulation, after ensuring their safety and efficacy in animal models and human clinical trials.

**Keywords:** GIP, Probiotics, Incretin hormones, Type 2 Diabetes, GLP-1

**AUTOINDUCER-2 SIGNALLING IN PROBIOTICS: A MECHANISM OF GUT MICROBIOTA MODULATION**

*Park H.; Lee K.; Yeo S.; Shin H.; Holzapfel W.*
Handong Global University, South Korea

**Introduction:**
Bacterial symbioses are vital in the human host. Gut microbiota, in particular, are closely associated with physiological traits and several diseases. Therefore, a major focus has shifted to the potential of probiotics as bacterial therapeutics for modulating the gut microbiota. Several recent studies on the impact of microbiota modulation by selected functional strains in specific disease models are supporting this concept. However, mechanisms of the modulation by probiotics administration have not been fully elucidated, thus severely limiting prediction and control of modulatory effects on the bacterial community.

Bacteria-bacteria interactions are primary events in any bacterial community. Among these, bacterial quorum sensing, cell-density dependent and secretory signalling systems provide valuable opportunities for unveiling mechanisms of the bacterial community construction. In particular, the LuxS-mediated autoinducer-2 (AI-2) signalling system is found in a wide variety of Gram-positive and Gram-negative bacteria, including Lactobacillus spp. It has been reported to regulate the expression of genes associated with specific adaptation and resistance to the environment. The signalling system also correlates with bacterial colonisation and
adhesion to the host epithelial cell. Furthermore, recent studies suggest that AI-2 activity can support restoration of the balance between the phyla Firmicutes and Bacteroidetes in antibiotic-induced dysbiosis. It is therefore expected that the AI-2 signalling status may influence a change in bacterial survival and even modulation of the gut microbial composition.

**Methods:**
AI-2 signalling properties of 104 probiotic lactobacilli were analysed using a modified AI-2 bioluminescence assay for lactic acid bacteria. In order to evaluate the influence of AI-2 signal status on bacterial community changes, a synthesized AI-2 molecule and non-specific quorum signalling inhibitor were applied the human stool and C57BL/6J mouse experimental models. The bacterial community of each sample was analysed using qRT-PCR and 454 GS-FLX platinum pyro-sequencing based on the 16S rRNA gene.

**Results:**
Treatment of the human stool with AI-2 molecules resulted in a dose dependent change in composition ratio of Firmicutes/Bacteroidetes compared to the control. The tendency was also shown in shifts at the level of families and genera. Also the bacterial community distance matrix showed diverting changes in microbiota following AI-2 addition and inhibition. In the in vivo mouse model, modulatory effects differed significantly from the human stool model.

Our investigations showed Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus brevis, and Lactobacillus lactis to be AI-2 producing species, while Lactobacillus johnsonii and Lactobacillus sakei showed AI-2 inhibition features. In the case of Lactobacillus acidophilus, AI-2 production and inhibition were strain-specific. The differences in AI-2 signalling properties of probiotic Lactobacillus species or strains infer widely different bacterial interactions in the human gastrointestinal tract.

**Discussion:**
Although the AI-2 signalling in gut bacteria is still insufficiently understood, our results suggest the AI-2 signalling property of probiotics to be a key mechanism by which gut microbiota are modulated.

**Keywords:** Gut microbiota modulation, Probiotics, Quorum sensing, Autoinducer-2, Bacterial interaction

---

**STUDY ON THE FUNCTIONALITY OF TWO DIFFERENT LACTOBACILLUS RHAMNOSUS STRAINS IN A MURINE MODEL**

Park S.; Ji Y.; Choi S.; Holzapfel W.
Handong Global University, South Korea

**Introduction:**
With widening interest in the use of probiotics and beneficial bacteria, requirements and expectations are increasing for deeper and better understanding on particular modes of action in support of appropriate use. Even though lactic acid bacteria (LAB), a major group of probiotic strains, are considered generally as safe and beneficial, mechanisms basic to positive impacts on various health conditions are still ambiguous. Vagueness in these issues may open debatable questions on the definition of a beneficial feature. Investigations to clarify the underlying basis for beneficial effects of LAB have pointed to key metabolites or/and components of specific strains. In our study, we focused on two different Lactobacillus rhamnosus strains and their strain-specific effect on lowering cholesterol level and on comparison of their complete genome sequences to trace potential mechanisms of the specific beneficial effect. We used Lb. rhamnosus GG as a “standard” and strain BFE5264, isolated from Maasai fermented milk. Their cholesterol-lowering effect was monitored in a murine model receiving a high-cholesterol diet. As people of the Maasai tribe in Kenya are well known for their excellent health conditions and low cholesterol levels although the fermented milk, containing around 3.4-4.0 % (W/V) of animal fat, serves as their primary food. We therefore expected BFE5264 strain to have a special influence on lipid metabolism

**Methods:**
Lb. rhamnosus strains BFE5264 and GG were administered to C57BL6 male mice receiving an atherogenic (high-cholesterol) diet. Blood and hepatic cholesterol levels were analysed after nine weeks. Lovastatin, inhibitor of HMG-CoA reductase, was used as control to compare the impact of LAB strains on cholesterol metabolism in the murine model. To assess the influence of two different strains on intestinal environment, composition of gut microbiota and short-chain fatty acids from mice faecal samples were analysed. Complete
genome sequencing of \(Lb.\ rhamnosus\) BFE5264 opened the scope for comparison with the genome of the \(LGG\) strain, and of possible candidate gene(s), responsible for strain-specific effects.

**Results:**

Even belonging to the same species, \(Lb.\ rhamnosus\), these two strains showed differences in lipid metabolism and effects on gut microbiota. Comparison of whole genome of the two strains opened understanding of some key differences between these strains.

**Discussion:**

It is generally agreed that probiotic functions are strain specific. Insights into intra-species diversity are opened by comparing full genome sequences of phenotypically different strains of a species. Advantages of this approach are obvious, thereby providing a solid scientific basis for differences in key functions thus also giving added valuable to functional claims in support of commercial applications. In this study, we have shown the strain-specific functionality of BFE5264 strain and identified several possible genes as potential targets of a cholesterol-lowering treatment. Improved understanding of underlying mechanisms may enforce future research on selected functional characteristics, and towards deepening insight into LAB interactions in complex ecosystem such as the GIT. This may also open new possibilities for developing and validating of in vitro models.

**Keywords:** Lactobacillus rhamnosus, Cholesterol-lowering, Gene comparison, Fermented milk, Strain-specific effect

---

**USE OF MICROORGANISMS FOR CAROTENOID DELIVERY: NEXT GENERATION OF PROBIOTICS FOR CARDIOVASCULAR DISEASE**

**Possemiers S.**

ProDigest

**Introduction:**

Functional foods provide a buoyant growth sector. A particular dynamic area is the use of carotenoids, not only as colorants but also as food additives. One issue with these products is their instability both on the shelf and upon digestion. Specific bacteria from the Bacillus species were shown to produce carotenoids, which have been shown to exert superior stability throughout the gut and higher antioxidant activity and bioavailability than common dietary carotenoids. Furthermore, several Bacillus species have been shown to have probiotic properties, making that the administration of specific carotenoid-producing Bacilli can have unique health benefits. Based on initial promising data, the EU FP7 CARODEL project aimed to reach complete development of an efficient oral delivery strategy of this concept and to evaluate health-beneficial activities of both the carotenoids and the Bacillus delivery vehicle, with the ultimate goal to improve biomarkers associated with cardiovascular disease (CVD).

**Methods:**

In practice, effective delivery of the carotenoids in the human body was compared upon administration as Bacillus vegetative cells, spores or extracted bacterial carotenoids. In parallel, the ability of the Bacillus strain to exert bona fide effects (i.e. effects on the host microbiota, metabolism and beyond) was investigated using in vitro gut models and in vivo rat studies. Based on this, the best delivery strategy was selected and validated in a 6-week human study, in which carotenoid bioavailability was assessed as well as endpoints related to CVD and probiotic activity. These efforts were combined with a full safety assessment, including 2 phase I human studies, a proof-of-concept production strategy and exploitation plan, to provide a framework for efficient further commercialization of a well-characterized Bacillus carotenoid product.

**Results:**

Full genomic screening, in vitro and in vivo safety assessment confirmed that the specific Bacillus spores used in this product are safe for human consumption and have an even better safety profile as compared to other Bacilli. Furthermore, safety of oral intake was confirmed in two phase I safety studies conducted in healthy individuals. By combing in vitro gut models and in vivo animal studies, the CARODEL project efforts resulted in an efficient oral delivery strategy of highly active carotenoids, in the form of Bacillus spores, with the additional ability to exert probiotic effects (i.e. effects on gut microbiota, metabolism and beyond). In direct comparison with plant carotenoids, this strategy resulted in superior bioavailability of the bacterial carotenoids, in combination with potent biological effects. The delivery strategy was validated in a 6-week phase II efficacy study in healthy, but overweight individuals. Beneficial effects were observed on biological endpoints after intake of the formulation.
Discussion:
As it was the first time that such effects were shown in humans, the results provide compelling evidence for the further development and commercialization of the CARODEL product as a unique and versatile probiotic.

Keywords: Probiotic, Bacillus, Carotenoids, Cardiovascular Disease, Gut Health

PEPTIDOGLYCAN FROM LACTOBACILLUS RHAMNOSUS PROMOTES AVIAN BETA-DEFENSIN 9 GENE EXPRESSION IN IMMUNE CELLS AND INTESTINE OF CHICKEN

Qu M.; You J.; Li G.; Ouyang K.; Huang J.
College of Animal Science and Technology/Jiangxi Province Key Laboratory of Animal Nutrition, Jiangxi Agricultural University

Introduction:
Defensins serve as crucial components of the innate immune system and play an essential role in the host defense against infection. In addition to their direct broad-spectrum antimicrobial activities against Gram-positive and Gram-negative bacteria, fungi and some enveloped viruses, defensins can function as potent immune regulators and act as a bridge between innate and adaptive immunity. AMPs can act as signal molecules between cells to play multiple functions in immune defense against infection. The avian beta-defensin 9 (AvBD9), an important antimicrobial peptide expressed in chicken tissues, especially in the digestive tract and immune tissues, plays a crucial role in maintaining the homeostasis of gastrointestinal microflora and shows multiple functions in immune defense against infection. Our previous study has demonstrated that probiotic Lactobacillus rhamnosus (L. rhamnosus) MLGA and the whole cell wall peptidoglycan (WPG) from L. rhamnosus MLGA induced AvBD9 expression in primary cultured chicken small intestinal epithelial cells.

Methods:
In this study, the effects of WPG on AvBD9 mRNA expression in immune cells from different tissues and intestine of chicken were investigated, and the different effects of peptidoglycan from pathogenic bacteria and probiotics on AvBD9 gene expression were compared. The proinflammatory cytokines expression in immune cells in response to WPG stimulation were determined to define whether or not the AvBD9 expression induced by WPG was inflammatory cytokine-dependent. Moreover, the antibacterial effect of cell lysates from coculture of immune cells with Lactobacillus rhamnosus-derived WPG was determined.

Results:
WPG from L. rhamnosus MLGA promoted AvBD9 mRNA expression with different potential in chicken peripheral blood mononuclear cells (PBMCs), splenocytes, thymocytes, liver cells and chick embryo jejunum, ileum, cecum explants in a dose-dependent manner. Differences in the magnitude of up-regulation of AvBD9 expression triggered by WPG between different cells or different intestinal segments were observed. In contrast to the effect of L. rhamnosus-derived WPG, Staphylococcus aureus-derived peptidoglycan down-regulated AvBD9 mRNA expression in chicken PBMCs and splenocytes. Pro-inflammatory cytokines IL-1β, IL-8 and IL-12p40 mRNA expressions in chick PBMCs and splenocytes were not significantly affected by L. rhamnosus MLGA WPG, whereas these cytokine expressions in chick PBMCs and splenocytes were significantly suppressed by hydrolysate of Lactobacillus rhamnosus MLGA WPG. The L. rhamnosus-derived WPG and its hydrolysate by lysozyme promoted AvBD9 expression without being accompanied by inflammatory response. The growth of Salmonella enteritidis (S. enteritidis) was significantly inhibited by cell lysates from coculture of PBMCs or splenocytes treated with L. rhamnosus-derived WPG. The antimicrobial effect of cell lysates was increased with the increase of WPG concentrations. L. rhamnosus-derived WPG itself showed no inhibitory effect on the growth of S. enteritidis.

Discussion:
Collectively, the results in the present study indicated that L. rhamnosus MLGA derived-peptidoglycan and its hydrolysate can exert beneficial effects to the host and enhance the innate defense response against infection through the upregulation of β-defensin such as AvBD9 expression in tissues especially in immune cells of chicken.

Keywords: Innate immunity, Proinflammatory cytokines, Avian Beta-Defensin 9, Peptidoglycan, Chicken
CHANGES IN GUT MICROBIOTA SUBGROUPS DIFFERENTLY RELATE TO PROGRESSION OF OBESITY, HYPERTENSION AND INSULIN RESISTANCE IN RATS

Ramos-Romero S.1,2; Hereu M.1; Atienza L.3; García N.1; Amézqueta S.2; Torres J. L.1
1 Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Barcelona, Spain;
2 University of Barcelona, Barcelona, Spain
3 Department of Pathology, Puerta del Mar University Hospital, Cadiz, Spain

Introduction:
WHO global estimates reported that 39% of the world population is overweight, from them 13% is obese and 9% is diabetic (1). Obesity and its related factors such as insulin resistance and hypertension, have been linked to an imbalance in the proportions of the major subgroups of distal gut microbiota (2, 3). To establish possible relationships between each of these risk factors and particular types of microorganisms we have used rat models of dietary induced alterations.

Methods:
Rats (n=29) were fed a high-sucrose diet (HS), a high-fat high-sucrose diet (HFHS), or a standard diet (STD) for 6 months. Insulin resistance was assessed by the oral glucose tolerance test (OGTT) and determinations of plasma insulin by the Milliplex technology. Hypertension was assessed by measuring arterial pressure using indirect tail detection by a non-invasive blood pressure system; and uric acid concentration was measured by colorimetry. The levels of total bacteria, and Bacteroidales, Clostridiales, Lactobacilliales, L. acidophilus, Enterobacteriales and E.coli were determined in fecal and cecal DNA by quantitative real-time polymerase chain reaction (qRT-PCR). Short-chain fatty acids (SCFA) were determined by GS-FID.

Results:
Compared to the STD and HS groups, rats fed HFHS had significantly higher body weight and lower percentage of Bacteriodales already at week 1 and until the end of the study. The concentration of short chain fatty acids (acetic, propionic, isobutyric and butyric acid) was significantly lower in the HFHS group than in the STD and HS groups. Systolic and diastolic pressures and urine uric acid concentration were significantly higher in the HS group compared to STD and HFHS. The percentages of gut Enterobacteriales and E.coli significantly increased in the HFHS group with respect to the other two groups already after one month. Plasma insulin and the area under the curve from the OGTT were significantly higher in HFHS fed animals than in animals fed the STD diet after 2-3 months of experiment. HS diet increased the Enterobacteriales and E. coli after 6 months of consumption. In this group, insulin resistance was evident only after 6 month of HS intake.

Discussion:
Body weight gain inversely relates to the percentage of bacteriodales and the concentrations of SCFA, in agreement with the literature (2). Hypertension induced by a high sucrose diet does not relate to changes in bacteriodetes/firmicutes ratio. Other authors have show that this ratio is altered in spontaneously hypertense rats (3). Taken together these results suggest that variations in bacteriodetes/firmicutes ratio may be a consequence rather that a cause of hypertension. The progression of diet induced insulin resistance differs between high fat and high sucrose models and may be associated to changes in the populations of other groups of bacteria such as Enterobacteriales and E.coli, probably through the activation of inflammatory pathways (4).

Reference:
1 http://www.who.int/mediacentre/factsheets/fs311/en/
2 Turnbaugh PJ et al. Nature 2009
3 Yang T et al. Hypertension 2015
4 Caricilli A.M. & Saad M.J.A. Nutrients 2013

Keywords: Obesity, Hypertension, Insulin resistance, Bacteriodales, Enterobacteriales
IMPACT OF GUT MICROBIOME MODULATION ON QUALITY OF LIFE IN PEOPLE WITH CHRONIC KIDNEY DISEASE (CKD) USING PRO/PREBIOTICS - RESULTS OF A SURVEY

Ranganathan N.; Pechenyak B.; Vyas U.; Ranganathan P.; DSilva H.; Weinberg A.
Mount Sinai School of Medicine. New York, NY, USA

Introduction:
Probiotics and Prebiotics are attracting much greater interest for various applications in health and disease conditions. They are generally used in gut disorders like digestive and immune health. Recent scientific advances, in the field of “Gut Microbiome” and its modulation beyond gut health is garnering greater exposure in several other diseases like obesity, cardiovascular diseases, autism, colon cancer, asthma and allergies including gut-brain axis. As yet unrecognized field is the R&D on gut microbiome, dysbiosis and its modulation with Pro/Prebiotics towards CKD (Gut-Kidney connection) patients worldwide. We at Kibow Biotech have been working over a decade in our R&D with a Pro/Prebiotic dietary supplement product formulation for the removal of uremic toxins diffusing from the circulating blood into the bowel. A pharma like validation – in vitro and in vivo both in animal and human clinical trials have demonstrated its usefulness towards CKD applications. This case study is primarily aimed to collect information on the impact of gut microbiome modulation using Renadyl™, a specifically formulated Pro/Prebiotic probiotic supplement product for kidney health, on quality of life and health status of patients with CKD.

Methods:
Survey questionnaires were mailed out to 951 patients using Renadyl™. The final sample size was n=834. Results were tabulated and analyzed using SAS V9.2 and MS Excel software tools.

Results:
A total of 168 responses were received (20% response rate, 42% female, 47% male, aged 12-98 years). A majority (85%) was over 51 years of age, in stage III or IV of kidney disease (58%) with at least one comorbid condition (77%), and almost half (48%) were retired. A greater number (61%) reported experiencing at least some or even great improvement since they started taking the supplement. Statistical analysis indicated a significant difference (p<0.0001) in distributions of quality of life responses when comparing responses before and after taking the product. Multivariate analysis indicated that the duration of administration (p<0.0001), employment (p<0.012), comorbidity (p<0.012), and GFR (p<0.0015) were significant factors influencing the reported quality of life. Even the disabled respondents all reported significant improvement.

Discussion:
Based on the patient/consumer survey and the results of our randomized clinical trials, Kibow’s product-Renadyl™ is well documented to provide benefit to patients in all stages of CKD and with a variety of comorbid conditions. It does not interfere with any other medical treatments, including dialysis. It appears to have a stabilizing effect on the overall health status and quality of life, maintaining or improving kidney health in particular. Further, adequately powered studies that could establish a clearer correlation between this supplement and its impact on GFR are warranted.

Keywords: Probiotics, Gut microbiome, Chronic kidney disease, Uremic toxins, Quality of life

COMPARATIVE GENOMICS AND FUNCTIONAL ANALYSIS OF LACTOBACILLUS PLANTARUM PROBIOTIC CANDIDATES HIGHLIGHTED A STRAIN-DEPENDENT CAPABILITY TO PRODUCE BUTYRIC ACID BY FATTY ACID BIOSYNTHESIS PATHWAYS

Rantsiou K.; Cocolin L.; Bertolino M.; Acquadro A.; Barchi L.; Greppi A.; Botta C.
Department of Forestry, Agriculture and Food Sciences. University of Turin, Italy

Introduction:
The genome-scale analysis of health-promoting bacteria is a fundamental approach to investigate their physiological behaviour or foresee potential probiotic and postbiotic features. Among the intensively studied LAB species, Lactobacillus (L.) plantarum is one of the most characterized, since it is a natural inhabitant of human gut with a wide genome rich of probiotic traits. The production of butyric acid is a metabolic activity often sought in new probiotic candidates, but the investigations of this pathway in L. plantarum species are few and limited to physiological observations. Therefore, the aim of the present work was to study the
genomes of three L. plantarum strains (S2, S11 and O2) in order to understand the genetic determinants of their probiotic features. In particular, L. plantarum S2 and S11, but not O2, were previously shown to produce butyric acid and to inhibit the colonisation of Listeria monocytogenes in human gut models.

Methods:
The three genomes were de novo assembled and reconstructed with the Mauve suite. Subsequently, they were structurally annotated with the Prokka platform, functionally annotated with Interproscan 5 suite, and compared with OrthoMCL software. An analysis of SNPs was performed and functional damages were predicted for genes of interest and their regulators. The assumptions generated from the in silico reconstruction and analysis were validated with targeted physiologic tests, such as the investigation of growth dynamics in different substrates and consequent production of short chain fatty acids (SCFAs).

Results:
A pool of unique gene families was shared by S2 and S11 whereas it was not present in the genome of O2, which confirmed to be phylogenetically different from the other two. Among these common genes a highly conserved region of plantaricin operons was detected. However, the reconstruction of this genomic region highlighted functional damaging and therefore the inability to actually produce plantaricins; such inability was further confirmed in vitro. Concerning the butyric acid, we observed that production was maximal after 48 hours of incubation (4 mM) and exerted only in presence of glucose. Interestingly, the strain O2 did not produce significant amounts of butyrate and showed a lower capability to consume glucose. Genes associated with terminal steps of common bacterial butanoate pathways were not observed in the three LAB genomes. However, we identified a type II fatty acid synthase (FASI) gene as the gene most likely responsible for the butyric acid production in S2 and S11 strains; a search in O2 genome, for SNPs present in FASI pathway and glucose transport system revealed some functional damages.

Discussion:
In light of the results achieved, the inhibition of Listeria monocytogenes by S2 and S11 was mainly related to the production of butyric acid. This metabolic activity was correlated to the complexity and amount of sugar and nitrogen sources available, whereas the functional SNPs observed in the genome of strain O2 could explain its inability to produce butyric acid. To conclude, this study may represent a first step for understanding the complexity of the butyrate biosynthesis pathway in L. plantarum and provides the bases for guiding further transcriptomic investigations.

Keywords: Probiotics, Genome-wide analysis, Butyric acid, Fatty acid biosynthesis, Plantaricins

PREBIOTIC MANIPULATION OF THE HUMAN GUT MICROBIOME FOR HEALTH

Rastall R.
The University of Reading, UK

Results:
Prebiotics are food ingredients and supplements, currently all carbohydrates, which bring about selective changes in the microbiome and metabolome of the gut with positive consequences for health. The meaning of the term prebiotic is still being debated and new definitions are being proposed. Since 1995 when the concept was first defined, there has been an evolution of molecular microbiological and metabonomic methods that allow us to take a highly detailed view of the microbiota and what it is doing. Concomitantly with this, our view of what is meant by a prebiotic is also evolving. Early studies in the field tended to focus on selectively increasing bifidobacteria and lactobacilli relative to other microbial groups. This simplistic view is no longer tenable as we identify new microbial functionalities and new targets for prebiotic intervention. However, even with a more detailed understanding of the microbial changes occurring on prebiotic consumption, it is still clear that prebiotics do not impact all populations equally and still have a markedly selective effect.

Early studies also focused on the changes in microbial populations rather than changes in the metabolites produced. This too has changed over the years as we learn more. Microbial metabolites clearly have a wide ranging impact on health, together with co metabolites resulting from both microbial and human metabolic activities. Although we do not have anywhere near a comprehensive understanding of the complex cross-feeding relationships that occur in the gut ecosystem, we can see a selective effect on metabolites of
prebiotics; we can identify substrates that produce distinct metabolic profiles in faecal cultures. Relating these to specific health benefits is, however, a considerable challenge. Whilst there are many studies on health benefits of prebiotics, too few of them are designed to probe the mechanistic relationships between gut microbiome, metabolome and health outcome.

Traditionally the health benefits of prebiotics were thought to be mainly focussed on effects in the gut and on immune function since the large gut is the major site of prebiotic fermentation. Whilst there are data to support positive effects on gut health, recent studies have focussed more on metabolic health. Studies have shown positive effects on inflammation, insulin response and postprandial glucose levels. The role of prebiotics in lipid metabolism, obesity and satiety continues to be of interest, albeit with mixed results at the present time.

This presentation will evaluate how our understanding of prebiotics has evolved and will examine recent studies on benefits to gut, immune and metabolic health.

**Keywords:** Prebiotics, Inulin, Gut Microbiome, Gut Metabolome, Gut Fermentation

---

**LONG-TERM PROBIOTIC IMPLEMENTATION TO RE-CREATE A BALANCED VAGINAL ECOSYSTEM: A PROMISING BOOST AGAINST HPV-INFECTION**

**Recine N.; Palma E.; Domenici L.; Giorgini M.; Pierangeli A.; Benedetti Panici P.**
Department of Gynecological, Obstetrics and Urologic Sciences, University "Sapienza" of Rome, Italy

**Introduction:**

An ever-increasing interest has developed in microbiota, with the belief that probiotics could be able to promote women’s well-being and illnesses in several ways. Commonly, human vaginal microbiota is lactobacilli-dominated but if not other microorganisms may grow reducing anti-bacterial defence mechanisms, promoting disorders such as bacterial vaginosis and yeast vaginitis, and then endorsing the occurrence of sexually transmitted diseases. We have guessed that this event might be the result of a transitional process, beginning by compromising the physiological vaginal eubiosis, increasing lactobacilli-mediated cytolysis and then reaching the stage of pathobiosis, when the vaginal ecosystem start to be defenceless and so vulnerable to a huge variety of infections. The aim of our study was to confirm that Lactobacillus rhamnosus BMX 54 long-lasting vaginal application in women with dysbiosis and concomitant HPV-infections might be able to have an advantageous effect on viral infection control by re-establishing the natural balanced ecosystem.

**Methods:**

This is a prospective study, performed between February 2012 and December 2015 at Department of Gynecological Obstetrics and Urologic Sciences, "Sapienza" University of Rome. A total of 117 patients with BV/vaginitis and associated HPV-infection documented as PAP-smear abnormalities (ASCUS, L-SIL or H-SIL histologically demonstrated as CIN1) and/or positive HPV-DNA were included in the study. Patients were consecutively randomized in two groups, standard treatment plus short-term lactobacillus implementation (group 1, n=60) vs standard treatment plus long-lasting probiotic treatment (group 2, n=57). Standard initial treatment for bacterial or yeast infections was metronidazole 500 mg (orally twice a day for 7 days) or fluconazole 150 mg (orally once a day for two consecutive days), respectively. Probiotic implementation (as Lactobacillus rhamnosus BMX54 vaginal tablets) was performed as follows: once a day for 10 days, once every 3 days for a month and then once every 5 days for another month in all patients. Then, patients belonging to long-term treatment arm (group 2) continued using probiotic vaginal tablets once a week for a 6-month period. All patients followed a strict follow-up (every 3 months) including, when indicated, PAP-smear, bacterioscopic exam and colposcopy check. HPV-DNA test was repeated at the end of the study period.

**Results:**

After a mean follow up of 15.6 months (range 9.6-24), probiotic long-term users demonstrated a chance twice higher to solve HPV-related cytological anomalies (71.9% vs 36.6%, p=0.04). Moreover, a total HPV-clearance was shown in 13.3% of control patients comparing with a percentage of 33.3% in probiotic users (p=0.05), assessed as negative HPV-DNA test documented at the end of the study period.

**Discussion:**

In this study, we used cytological and viral endpoints to evaluate a potential effect of probiotic long-term implementation in solving cervical abnormalities, through the re-creation of the physiological vaginal
balance (eubiosis). The consistent percentage of clearance of PAP-smear abnormalities obtained in probiotic users was incredibly high and encouraging. Obviously, larger and randomized studies are warranted to confirm these boosting results, but we believe that eubiosis re-establishment is the key to tackle effectively even HPV-infection.

**Keywords:** Sexually transmitted diseases, Probiotics Implementation, Lactobacillus rhamnosus BMX54, HPV infection, PAP-smear abnormalities

**BACTERIOCINS FROM LAB**

*Saharan B.*

KUK

**Introduction:**

Lactic acid bacteria (LAB) produce bacteriocins having antimicrobial activity against pathogenic microorganisms causing food spoilage. They are involved in food fermentations and Produce lactic acid as the major metabolic end product of carbohydrate fermentation. Lactic acid and other metabolic products contribute to the organoleptic and textural profile of a given food item. They are generally recognized as safe (GRAS), due to their ubiquitous appearance in food and their contribution to the healthy micro flora of human mucosal surfaces. They are found in milk products and decomposing plants. The genera that comprise the LAB include Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and Streptococcus, as well as Weissella, Vagococcus, Tetragenococcus, Sporolactobacillus, Oenococcus, Enterococcus, Carnobacterium and Aerococcus.; these belong to the order Lactobacillales. They are acid-tolerant, rod/cocci-shaped, normally non-sporulating, Gram-positive, with low-GC content, microaerophilic bacteria. Bacteriocins are similar to Paramaecium and Yeast killing factors. They are ecologically, functionally and structurally very diverse biomolecules. A. Gratia called his first discovery a colicine because it killed E. coli. Bacteriocins are proteinaceous compounds produced by bacteria to inhibit the growth of similar or closely related bacterial strains. They were first discovered by A. Gratia in 1925. He was involved in the process of searching for ways to kill bacteria, which also resulted in the development of antibiotics and the discovery of bacteriophage, all within a span of a few years. Applications of bacteriocins are being tested to assess them as narrow-spectrum antibiotics. LAB also produce bacteriocins which are heat-stable ribosomally synthesized antimicrobial peptides. Future perspectives and potential applications of these novel bacteriocins will be discussed during the presentation. The reports of new bacteriocins with unique properties indicate that there is still a lot to learn about peptide antibiotics. The system of fast tracking the discovery of novel bacteriocins, belonging to different classes, and isolated from various sources will also be discussed in detail. These bacteriocins have huge potential as both food preservatives, and as next-generation antibiotics targeting the multiple-drug resistant pathogens. Current updates regarding the structural characterization, mode of antimicrobial action, and biosynthetic mechanisms of various novel bacteriocins will be highlighted during the presentation. Bacteriocins are antimicrobial peptides produced by a large number of bacteria, including lactic acid bacteria, acting against closely related and some spoilage and disease-causing pathogens. Biosafety, biotechnological applications in food and pharmaceutical industries, mode of action, purification techniques and recent classification of bacteriocins as well as recent attempts to generate custom-designed bacteriocins using genetic engineering techniques will be discussed in detail. Consumers are very much aware of the health concerns regarding food additives. Demand for new antibacterial compounds has brought great interest for new technologies able to enhance food microbiological safety. Nisin is one of the bacteriocins which exhibits a wide-spectrum antimicrobial action against many pathogens including Bacillus cereus, Listeria monocytogenes and Staphylococcus aureus. Milk or whey can be supplemented with ex situ produced bacteriocin preparations obtained by fermentation.

**Methods:**

All the standard methods have been followed.

**Results:**

Bacteriocins with great potential have been obtained and characterized

**Discussion:**

Bacteriocins so obtained have great potential in food and pharmaceutical industry.

**Keywords:** Lactic acid bacteria, Bacteriocin, Antimicrobial, Peptides, Bacteriocidal
SNAPSHOTS ON RECENT PREBIOTIC FIBER RESEARCH

Sailer M.
BENEÖ-Institute

Results:
A prebiotic is defined as “a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” according to the most recent definition elaborated by the International Scientific Association for Probiotics and Prebiotics in 2008. Inulin-type fructans such as inulin and oligofructose derived from chicory root are amongst the very few that are accepted as “proven” prebiotic fibers and are amongst the best studied prebiotics.

Since the prebiotic concept was first defined intensive research has been carried out, and prebiotic research has continued at a rapid pace with more than 2000 research articles published over the past 5 years. In particular the effect of prebiotic fibers on the gut ecosystem and digestive health was in the research focus. Just recently the positive effect of chicory-derived inulin-type fructans on bowel motor function has been confirmed in a meta-analysis. After a positive scientific opinion by the European Food Safety Agency, the European Commission authorized the Art. 13(5) claim for Orafti® Inulin and “improved bowel function” end of 2015. The health claim can now be used in the food industry market.

Various human intervention studies have been conducted in order to investigate research topics beyond the effects on the gut ecosystem and digestive health. Such health benefits of prebiotic inulin-type fructans include the effects on energy intake and body weight management, metabolic benefits like blood glucose management and improvements in obesity related disorders. Also with respect to these health benefits, new systematic reviews and meta-analyses confirmed that prebiotics could reduce the rate of infections in infants and children and have beneficial effects on lipid metabolism, satiety and postprandial glucose and insulin response.

Prebiotic inulin-type fructans are characterized as fermentable dietary fibers. There is more and more evidence emerging that in particular the fermentation process and hence the generation of short-chain fatty acids is important for the beneficial prebiotic fiber effects. Elaborating the mechanisms underlying these effects is in the scientific focus. Just recently, the application of stable-isotope methods allowed for the first time to estimate the in vivo production of short-chain fatty acids after ingestion of prebiotic inulin intake in humans. Data obtained with imaging technologies provide evidence that acetate derived from colonic fermentation has a direct role in central appetite regulation. Such kind of data will emerge in future and will help to understand how fermentation processes act via the Gut-Brain Axis.

This talk will focus on latest findings obtained by novel systematic reviews, human intervention trials as well as mechanistic studies that continue to support the benefits of prebiotic fibers.

Keywords: Inulin, Prebiotic, Health benefits, Fermentation, Short-chain fatty acids

DEVELOPMENT OF NEW FUNCTIONAL YOGURTS USING PROBIOTIC LACTIC ACID BACTERIA (LAB) AND/OR BIFIDOBACTERIA AND THE FUTURE STRATEGY IN JAPAN

Saito T.
Tohoku University

Introduction:
R.Fuller (1989) introduced definition of probiotics as live microorganism which beneficially affects the host by improving its intestinal microbial balance. Now, probiotics defined as live microorganisms, which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2002). As probiotics, lactic acid bacteria (LAB) and Bifidobacteria (BF) have been investigated as starter strains for functional yogurts. In Japan (1991), the Food for Specified Health Uses (FOSHU, Japanese name TÖKUHO) system was inaugurated as the world’s first approval system on health claim labeling for food products. There are 13 health claim categories and the foods to modify gastrointestinal conditions (400 products) including probiotic yogurt are most popular in Japan. In total, 1,250 products are approved under FOSHU as of Feb 2016. The market scale of fermented milk products including functional yogurt from 2006 to 2016 (estimate) was compared by the data of a sum of money and category of yogurt. In Japan, the
second probiotic boom has been started. As the market scale of yogurt increase in these 5 years, the breakthrough of 700 billion yen must be true in 2016. The market of drink-type yogurt increased 12% and become 300 billion yen. The market size of plain fat 0 yogurts has been 50 billion yen and will be more increase in the future.

Many functions are considered in the selection of probiotics including competitive adhesion and exclusion of enteric pathogens, cholesterol lowering effects, and positive and negative immuno-modulatory effects. Adhesive activity to the human intestine is one of the most important characteristics of probiotic LAB/BF. Recently, we developed a new screening assay using the BIACORE and found “blood type LAB” expected to improve gastrointestinal health by continuous proliferation in colon and removal of harmful bacteria causing inflammatory bowel disease (IBD) such as UC/CD.

Japanese functional yogurts are classified into 3 big categories such as Functional LAB type yogurt, Functional materials type yogurt and others. As the present topic yogurt in Japan, Probio Yogurt LG21, R-1 yogurt and PA-3 yogurt (Meiji HD) and anti-allergy yogurt. We are proposing “Blood Type Yogurt ABO” as a new type of yogurt in the future. H. pylori and norovirus are known to combine to the sugar portion of ABO blood type antigen of host in the first stage of infection. As we found many strains of blood type LAB, we may be able to use them to remove harmful bacteria which also recognize the human blood type antigen. Moreover, new yogurts in the future were introduced such as anti-obesity yogurt, anti-IBD yogurt and decontamination yogurt. Most recently, we have started the new study about the removal of Cesium 137 from human intestine by using LAB. One hundred LAB strains mainly isolated from fermented vegetables were tested to 1) strong binding ability with Cesium and 2) no-binding ability to human colon mucus. A few LAB strains which showed both abilities at the same time could isolate and are expected the removal of the dietary Cs137 from human intestine with quite safe.

**Keywords:** Functional yogurt, Probiotics, Lactic acid bacteria (LAB), Bifidobacteria, Prebiotics

---

**DESIGNING SPECIFIC AND EFFICIENT PROBIOTICS FOR BROILER CHICKENS BASED ON NATIVE LACTOBACILLUS STRAINS**

*Salehi Jouzani G.*

Agricultural Biotechnology Research Institute of Iran

**Introduction:**

The objective of the present study was to design native specific probiotic bacteria for poultry applications.

**Methods:**

To do this, about 700 lactobacilli and bacilli bacteria were isolated from ileum of some Iranian native chickens and ducks by using selective media.

**Results:**

selected. Finally, 6 Lactobacillus and 4 Bacillus subtilis strains with high probiotic potentials were selected and used in the farm experiments. The farm studies was performed to evaluate effects of the selected Lactobacillus and Bacillus strains on the yield (weight, feed conversion ratio (FCR), breast and thigh yield and feed consumption ratio) and immunological parameters (antibody titers against bronchitis and Salmonella, cholesterol and triglycerides contents, and also mucin gene (muc2) expression rate) of broilers (Ross strain) challenged or non challenged with Salmonella. The research was performed during 42 days at the framework of Factorial statistical design (5 (Six Lactobacillus strains (106 cfu/g feed), Six B. subtilis strains (106 cfu/g feed), Lactobacillus and B. subtilis strains (106 cfu/g feed), Commercial probiotic, and Control (feed without probiotics)) x 2 (challenged or non challenged with Salmonella) by using 300 one-day old roosters fed by a defined feed regime containing one of the above mentioned probiotics. The maximum increase in the broilers yield and immunological indexes was observed for those chickens fed by feed supplements containing the Lactobacillus strains. The application of the selected Lactobacillus strains could significantly reduce the feed consumption rate (FCR) and cholesterol and triglycerides contents, and significantly increased the total broiler, breast and thigh weight in both challenged and non challenged broilers. This also significantly increased the antibody titers against bronchitis and Salmonella. The real time PCR results, also showed that the presence of Lactobacillus strains significantly increased the mucin2 (muc2) gene expression rate in the broilers in comparison to the control and other treatments.
Discussion:
So, the presence of probiotics (Lactobacillus, B. subtilis or commercial probiotic) could significantly increase yield and immunological parameters in the living challenged broilers. These results confirmed that the selected lactobacilli strains could be effectively used as specific probiotic bacteria for broilers at commercial level.

Keywords: Broiler chicken, Feed consumption rate (FCR), Immunological parameters, Probiotic, Mucin gene (muc2), Salmonella challenge

EFFICIENT SYNTHESIS OF SPECIALTY CARBOHYDRATES AND HUMAN MILK OLIGOSACCHARIDES THROUGH INDUSTRIAL BIOTECHNOLOGY

Salvador P.; Beauprez J.; Soetaert W.
Inbiose N/V

Introduction:
Rare sugars such as L-fucose and sialic acid or human milk oligosaccharides such as fucosyllactose and sialyllactose are very difficult to synthesize. They play vital roles in animal and human health processes and are considered as an untapped source of innovation within the wellness, biomedical, Pharmaceutical, cosmetics High-end areas. Due to the lack of availability and lack of efficient technology to produce them, these products are consequently very expensive. For the synthesis of such highly complex carbohydrates, organic synthesis is not an efficient production method because of the high chirality and excessive presence of hydroxyl-groups in the carbohydrate building blocks. Also extraction of these compounds from natural sources is often hampered by the substrate availability and extraction cost.

Methods:
Inbiose solved this problem via a highly efficient specialty carbohydrates production method which dramatically reduces their production costs. The method is based on the use of cell factories in which a natural pathway has been expressed for the synthesis of the target specialty carbohydrate.

Results:
Using our versatile platform technology, Inbiose can produce any naturally occurring specialty complex carbohydrate. Depending on the target, a biochemical pathway will be designed and expressed in one of the Inbiose proprietary base strains. Through synthetic biology and metabolic engineering, the production strains are optimized. The target carbohydrate is then produced by fermentation and is efficiently excreted in the culture medium. After fermentation, the target carbohydrate is recovered from the fermentation broth in high yield and purity using a simple down-stream processing. The production method is generic and has already been proven on an industrial scale.

Discussion:
The presentation will explain the features of the Inbiose technology and will highlight a number of concrete examples where a process to produce pure human milk oligosaccharides has been developed and commercialized in collaboration with an industrial partner towards infant nutrition.

Keywords: L-fucose, fucosyllactose, sialyllactose, complex carbohydrate, synthetic biology, metabolic engineering

SYNTHESIS OF PREBIOTIC ISOMALTOOLIGOSACCHARIDES BY WEISSELLA CONFUSA DEXTRANSUCRASE

Shi Q.; Juvonen M.; Maaheimo H.; Katina K.; Tenkanen M.
University of Helsinki

Introduction:
Ismaltoooligosaccharides (IMO) containing predominantly α-(1→6) glucosidic linkages have successfully served as functional oligosaccharides in Asia. Industrial IMO production involves the action of α-
transglucosidase on maltose as a substrate; however, the proportion of digestible carbohydrates (e.g. glucose and isomaltose) in commercial IMO differs substantially. IMO with a higher degree of polymerization (DP) are preferred for a longer persistence in the colon, and thus a greater prebiotic potential. Dextransucrases can synthesize IMO by successive transfer of glucosyl residues from sucrose on the non-reducing end of maltose and the oligosaccharides produced, forming a series of linear IMO. The size distribution of the IMO can be controlled by varying the sucrose/maltose ratio. This work aimed at optimizing the synthesis of longer IMO by Weissella confusa dextranseucrase and characterizing the unknown minor IMO produced.

Methods:
The effects of substrate concentrations and enzyme dosage on IMO profile was studied by response surface modeling. The minor trisaccharide was isolated by gel filtration and characterized by MSn and NMR spectroscopy analysis.

Results:
A series of linear maltose-based IMO were the principal products. Quadratic regression models were established for the yield of individual IMO of DP3–6 as well as the total IMO and the maltose consumption ratio. The yield for each IMO was maximized with the highest sucrose input concentration but varying maltose inputs. The optimal maltose concentration for DP3–4, DP5, and DP6 was at its highest, medium, and lowest level, respectively. The dextransucrase dosage had smaller effects on these responses. According to the modeling results, medium maltose (~0.5 M) and high sucrose (1 M) concentrations would be optimal to produce a good yield of IMO with higher DP.

The minor series of products were observed for typical dextransucrases. The minor IMO were characterized for the first time to be a homologous series of novel IMO with a single unit branch α-(1→2) linked to the reducing end of maltose. The higher members of the series were probably formed by the attachment of a single unit branch to linear IMO.

Discussion:
This study enhances the current understanding of the specificity of dextranseucrase and demonstrates the use of dextranseucrase in the size-controlled synthesis of IMO. The minor IMO, which have previously been noticed in sourdough fermented with Weissella bacteria, could have additional health benefits due to the high resistance of α-(1→2) linkage to gastrointestinal digestion.

Keywords: Isomaltooligosaccharides, Dextranseucrase, Maltose acceptor reaction, Response surface modeling, α-(1→2) linkage

---

CLINICAL EFFICACY OF PROBIOTIC LYSATE DEL-IMMUNEV® FOR THE TREATMENT OF GASTROINTESTINAL MANIFESTATIONS ASSOCIATED WITH FOOD ALLERGY IN PRESCHOOL CHILDREN

Sichel L.
Pure Research Products, LLC

Introduction:
Probiotic lysates are cellular structures that can be administered therapeutically without any of the potential adverse side effects associated live microbial cultures. Both in vitro and in vivo studies have demonstrated a high therapeutic potential for probiotic lysates, which are particularly oriented towards immune system regulation. The objective of the present study was to determine the effectiveness of the lysate of Lactobacillus rhamnosus V8 in preschool children with gastrointestinal manifestations associated with food allergy.

Methods:
55 patients aged from 2 to 6 years with a demonstrated history of food allergies were recruited for this open-label investigation. Subjects were randomized into two groups; the primary group (n=30) who as part of a complex therapy received 1 capsule of Del-Immune V® daily (30 minutes prior to food) and; the control group (n=25) who received standard therapy without the inclusion of Del-Immune V®.

Gastrointestinal, respiratory and skin clinical manifestations were evaluated using questionnaires and the SCORAD (SCORing AD) index at baseline and 2 months. Paraclinical evaluations included serum total Immunoglobulin E (IgE), serum eosinophil cationic protein (ECP), salivary secretory IgA (sIgA) and stool culture at baseline and 2 months. Obtained data was statistically evaluated using the t-Student criterion, and a
Abstracts of Oral Presentations

Results:
The primary group showed a statistically significant (p < 0.05) reduction in the frequency and severity of skin and respiratory syndromes associated with food allergy. The beginning score on the SCORAD index averaged 32 ± 2.3 points, and following treatment with Del-Immune V®, values reduced to 10 ± 1.2 points, compared with 22 ± 2.1 points in the control group.

Serum levels of ECP reduced from 78.5 ± 0.6 ng/ml at baseline to 28.6 ± 1.5 ng/ml following 2 months supplementation of Del-Immune V® and to 46.2 ± 2.8 ng/ml in control. In subjects taking Del-Immune V®, concentrations of salivary slgA increased by 25% from baseline (94.6 ± 1.07) to reach 236.2 ± 5.3 mg/l. In the comparison group, concentrations of salivary slgA increased from 91.3 ± 1.01 mg/l at baseline to 148.8 ± 5.1 mg/l. These diagnostic findings indicate a more intense activation of endogenous immune protective factors following Del-Immune V® supplementation.

Findings indicate Del-Immune V® has a positive impact on the microbiota profile, evidenced by a significant increase in Bifidobacteria and Lactobacilli counts and significant reduction on the colonization of pathogens as Staphylococcus aureus, Candida spp., Citrobacter spp., Proteus spp., Klebsiella spp. and Enterobacter spp. Assessment of the frequency and duration of acute respiratory disease (ARVI) demonstrated a 2-fold reduction in the frequency (2.3 ± 0.32 vs. 4.3 ± 0.54 episodes, p < 0.05) and the duration of ARVI episodes (4.1 ± 0.15 vs. 9.2 ± 0.51 days, p < 0.05) in the Del-Immune V® group.

Discussion:
Results support previous data regarding the therapeutic potency and bifidogenic activity of probiotic lysates and allows us to recommend the inclusion of Del-Immune V® as a part of an integrated therapy for preschool children who present with gastro-intestinal, respiratory and skin manifestations associated with food allergy.

Keywords: Live cells, Probiotic lysate, L.rhamnosus V, Del-ImmuneV®, Food allergy, Open-label investigation, Therapeutic pothency, Immune regulation, Bifidogenic activity

PREBIOTIC POTENTIAL OF KAMUT® KHORASAN WHEAT: FROM IN VITRO STUDIES TO HUMAN CLINICAL TRIALS

Simonetti E.; Dinelli G.; Gianotti A.; Marotti I.; Taneyo Saa D.
Department of Agri-Food Sciences and Technologies, University of Bologna, Cesena, Italy

Results:
Wheat grains are a rich source of dietary fibres, particularly in the western human diet. Many of the health effects attributed to dietary fibres are believed to be related to their microbial fermentation in the gut. An in vitro study investigated the ability of two potentially probiotic strains, Lactobacillus plantarum L12 and Bifidobacterium pseudocatenulatum B7003, to ferment soluble dietary fibres (SDFs) as sole carbon source from modern and ancient durum-type wheat varieties. Although no significant differences in SDF content were observed, the tested wheat varieties showed different qualitative fiber composition resulting in a high variability of prebiotic activity scores. Among tested wheat SDF fractions, the ancient grain KAMUT® khorasan wheat and the modern variety Solex have the most promising potential to promote the growth of both tested strains in the gastrointestinal tract.

The in vitro findings related to KAMUT® khorasan wheat fibres have been further assessed in vivo in 30 healthy volunteers. The study was a randomized, parallel arm study designed to test whether a replacement diet with grain products (pasta, bread, biscuits, crackers, crisp toasts) made from the ancient organic whole KAMUT® khorasan wheat would impact the gut microbiome ecology and metabolic profiling of the participants, compared with a similar replacement diet using as a control grain products made from organic modern whole durum wheat. Participants were randomly divided into two groups (15 individuals/group), each assigned to consume either the KAMUT® khorasan wheat or the control products, respectively, for a period of 4 months. The whole KAMUT® khorasan wheat-based diet was mainly characterized by a tendency towards a reduction in Bacteroides/Prevotella and an increase in members of Clostridium cluster XIVa in fecal microbiota in comparison to whole durum wheat adopted as a control diet.

The metabolic profile of subjects administered with the whole KAMUT® khorasan wheat-based diet, in comparison to the control, was mainly characterized by phenol, nonanol and short chain fatty acids (SCFA),...
whereas alcohols, such as oleyl alcohol and isopropyl alcohol, better discriminated the whole durum wheat intake. Co-abundance analysis of microbiota and metabolome data evidenced the presence of a potentially health-promoting co-abundance group (CAG), which was more abundant in the whole KAMUT® khorasan wheat-based diet group.

These results may contribute to support recent findings from other human clinical trials where a replacement diet with KAMUT® khorasan wheat-based products was effective in reducing markers of oxidative stress and inflammation, as well as cardiovascular risk factors in healthy volunteers and in patients suffering from non-infectious chronic diseases (Irritable Bowel Syndrome, Acute Coronary Syndrome, Type-2 Diabetes).

Cited bibliography:

Keywords: KAMUT® khorasan wheat, Soluble dietary fibres, Prebiotic index, Gut microbiota, Metabolome, Diet intervention study

CHARACTERIZATION OF CELLULOSE BASED HYDROGELS AS PROBIOTICS DELIVERY VEHICLES

Singh P.1; Medronho B.2; Miguel M.G.1,3,4; Lindman B.1,3,4,5
1 Department of Chemistry, University of Coimbra,
2 Faculty of Sciences and Technology, University of Algarve,
3 Division of Physical Chemistry, Lund University
4 Materials Science and Engineering, Nanyang Technological University, Singapore
5 FSCN, Mid Sweden University

Introduction:
Cellulose is abundantly found in nature with unique properties such as biocompatibility and biodegradability. As an amphiphilic polysaccharide it can be used to form hydrogels which may undergo swelling or de-swelling depending on media conditions. Among vast range of possible systems to be encapsulated and delivered, probiotics emerge as a very interesting class receiving a lot of attention nowadays. This study is based on development and characterization of cellulose based hydrogels and encapsulation of probiotic bacteria.

Methods:
Three different cellulose derivatives with positive modified HEC (SoftCAT Polymer SK-M, da Amerchol, lote: TC2550GRA1), negative sodium CMC (Mw 70,000 Sigma Aldrich) and no charge HPMC (2,600-5,600 mPa.s, 2 % in H2O) were selected in combination of different polysaccharides. Chitosan (20-300 cP, 1 wt. % in 1% acetic acid) was studied cross linking with genipin.

Systems were characterized by rheology using a HAAKE MARS III rheometer (Thermo Fisher Scientific, Germany) controlling the temperature (at 25 and 37 ºC). Swelling Analysis using fresh sample, freeze dried and oven dried in a neutral aqueous solution and continuous readings were taken every five minutes.

Cryo-scanning electron microscopy (Cryo-SEM) was carried out using a JEOL JSM-6301F (Tokyo, Japan), Oxford Instruments INCA Energy 350 (Abingdon, UK.)

Thermogravimetric analyses (TGA) of pure samples and composite gel membranes, prepared with different amounts were carried out on thermo-microbalance thermogravimetric analyzer TG 209 F3 Tarsus®.

lactobacillus rhamnosus GG (LMG 18243) was revived by inoculating in MRS media at 37°C for 2 days in a CO2 incubator. The most promising hydrogels were dried in oven for 10 hours at 60°C. 1 mg of dried gel was soaked in bacterial cultured media for 30 minutes, after that serial dilution was performed on MRS Agar media using spread plate method. The plates were further incubated in anaerobic conditions for 48 hours for viability count. Analysis through Fluorescence microscope (Olympus BX51M microscope) was performed

Results:
Several phase diagrams were developed for physical gels but essentially it was found that most of the systems formed stiff hydrogels at high concentration of polymers. After freeze drying, the systems showed the maximum swelling. Moreover, it was found that different cellulose derivatives showed almost the same
degradation temperature in the TGA analysis. For the chemical gels the addition of genipin demonstrated high efficiency in cross-linking the chitosan based systems. Viability: The hydrogels showed a promising results using diffusion method as the viability of bacteria counted was in the limit mentioned by the regulations to be effective in the gut.

**Discussion:**
Development of encapsulation matrix is not an easy task. Most of the probiotics products in the market are based on dairy; this encapsulation strategy will open new choice of food products to opt for.

**Keywords:** Cellulose, Rhamnosus GG, Hydrogels, Characterization, Delivery

### EFFICIENT SYNTHESIS OF SPECIALTY CARBOHYDRATES AND HUMAN MILK Oligosaccharides THROUGH INDUSTRIAL BIOTECHNOLOGY

**Soetaert W.**, **Beauprez J.**, **Salvador P.**  
Inbiose NV

**Introduction:**
Rare sugars such as L-fucose and sialic acid or human milk oligosaccharides such as fucosyllactose and sialyllactose are very difficult to synthesize. They play vital roles in animal and human health processes and are considered as an untapped source of innovation within the wellness, biomedical, Pharmaceutical, cosmetics High-end areas. Due to the lack of availability and lack of efficient technology to produce them, these products are consequently very expensive. For the synthesis of such highly complex carbohydrates, organic synthesis is not an efficient production method because of the high chirality and excessive presence of hydroxyl-groups in the carbohydrate building blocks. Also extraction of these compounds from natural sources is often hampered by the substrate availability and extraction cost.

**Methods:**
Inbiose solved this problem via a highly efficient specialty carbohydrates production method which dramatically reduces their production costs. The method is based on the use of cell factories in which a natural pathway has been expressed for the synthesis of the target specialty carbohydrate.

Using our versatile platform technology, Inbiose can produce any naturally occurring specialty complex carbohydrate. Depending on the target, a biochemical pathway will be designed and expressed in one of the Inbiose proprietary base strains. Through synthetic biology and metabolic engineering, the production strains are optimized. The target carbohydrate is then produced by fermentation and is efficiently excreted in the culture medium. After fermentation, the target carbohydrate is recovered from the fermentation broth in high yield and purity using a simple down-stream processing. The production method is generic and has already been proven on an industrial scale.

**Results:**
The presentation will explain the features of the Inbiose technology and will highlight a number of concrete examples where a process to produce pure human milk oligosaccharides has been developed and commercialized in collaboration with an industrial partner towards infant nutrition.

**Keywords:** Human Milk oligosaccharides, Industrial biotechnology, Infant formula, Sialyllactose, fucosyllactose, Medical Nutrition

### HUMAN MILK OLIGOSACCHARIDES (HMOS): FROM OBSERVATION TO CLINICAL INTERVENTION

**Sprenger N.**  
Nestec S.A.

**Introduction:**
“Nothing in biology makes sense, except in the light of evolution” (Dobzhansky, 1973) is of particular bearing for milk, the sole and presumably adapted nutrition of newborn mammals. Besides nutrients, human
milk contains a plethora of components without nutritive function and amongst them HMOs, the third largest solid constituent of breast milk by mass. HMOs are extensions of lactose by galactose, N-acetylglucosamine, sialic acid and fucose leading to great number and diversity of distinct structures with over 130 described and about 20 covering the majority. As for blood group glyco-types, HMOs depend on the mother’s genotype leading to distinct HMOs phenotypes like those dependent on fucosyltransferase-2 (FUT2, secretor gene) and -3 (FUT3, Lewis gene).

What drives this HMO diversity and what is the role of HMOs for the suckling newborn are questions fascinating scientists and pediatricians for over a century. We hypothesize that HMOs may provide health benefits to newborn infants likely and largely through effects on the newly establishing gut microbiota.

Methods:
Possible relations between HMOs and clinical measures in infants are increasingly explored in clinical observational studies. FUT2-dependent HMOs are especially suited for such association studies, because a significant number of mothers lack FUT2-HMOs in their milk. Observational studies generate hypothesis towards functions that are further substantiated in preclinical or clinical intervention trials. With the advent of HMOs becoming available such hypothesis can now be tested in randomized, placebo-controlled, blinded intervention trials.

Results:
In clinical observational studies, FUT2-dependent HMOs in breast milk were related with the establishment of a bifidobacteria dominated gut microbiota (Lewis, 2015, Microbiome3:13), with reduced gastrointestinal infections (Newburg, 2004, Glycobiology 14:253-263), and morbidity and mortality (Kuhn, 2015, JON 145:66-72) in infants. In C-section born infants FUT2-HMOs are possibly related with a delayed onset of atopic eczema (Sprenger, 2016, EJON). Likely, most such observations are due to a microbiota modulating effects of specific HMOs, yet some direct HMO effects on pathogens and gut epithelia might explain part of the observations. In a randomized placebo-controlled clinical intervention trial infants fed infant formula with 2′fucosyllactose and lacto-N-neotetraose, shown to be safe and well tolerated, were less likely to experience reported medication (e.g. antibiotic) use and morbidity, especially lower respiratory tract infections (Puccio, 2016, Nutrition & Growth conference). At 3 months the microbiota profile of the HMOs-supplemented group was shifted away from that of infants fed infant formula without HMOs and towards that of breastfed infants (Berger, 2016, Experimental Biology conference).

Discussion:
Clinical observational studies are an interesting option to help decipher the biology of HMOs. Yet, the infant genotype for glycosyltransferases such as FUT2 needs to be considered as it might bias clinical findings. Further, in breast milk HMOs are in a different matrix compared to HMOs as supplement or in an infant formula, which might impact their effect. Still such association studies provide hypotheses for prospective intervention trials for which synthetic HMOs are now becoming available. A first intervention trial showed that such synthetic HMOs are safe, well tolerated and it advances our understanding of HMO biology.

Keywords: Oligosaccharides, Infant, Microbiota, Milk, Infections

INFLUENCE OF GUT MICROBIAL METABOLISM ON THE BIOCHEMICAL PROFILE OF DIETARY COMPOUNDS

Swann J.; Rowland I.; Gibson G.; Kristiansen K.; Scott K.; Tuohy K.
Division of Computational and Systems Medicine, Imperial College London

Introduction:
The biotransformation capabilities encoded in the gut microbiome vastly exceed that of the host genome. This includes functions essential for host digestion. As such, the gut microbiota is a key factor in shaping the biochemical profile of the diet and therefore, its impact on host health and disease. Importantly, the gut microbiome is highly host-specific, shaped by a myriad of intrinsic (host genome) and extrinsic (nutrition, lifestyle) host factors, and variation in this population may contribute towards differences in individual responses to the diet. This critical role of the gut microbiota in human metabolism has stimulated research into the identification of the specific microorganisms involved in different processes and a deeper understanding of their metabolic pathways, particularly those associated with metabolism of dietary

INFLUENCE OF GUT MICROBIAL METABOLISM ON THE BIOCHEMICAL PROFILE OF DIETARY COMPOUNDS

Swann J.; Rowland I.; Gibson G.; Kristiansen K.; Scott K.; Tuohy K.
Division of Computational and Systems Medicine, Imperial College London

Introduction:
The biotransformation capabilities encoded in the gut microbiome vastly exceed that of the host genome. This includes functions essential for host digestion. As such, the gut microbiota is a key factor in shaping the biochemical profile of the diet and therefore, its impact on host health and disease. Importantly, the gut microbiome is highly host-specific, shaped by a myriad of intrinsic (host genome) and extrinsic (nutrition, lifestyle) host factors, and variation in this population may contribute towards differences in individual responses to the diet. This critical role of the gut microbiota in human metabolism has stimulated research into the identification of the specific microorganisms involved in different processes and a deeper understanding of their metabolic pathways, particularly those associated with metabolism of dietary
components. Here, the main gut microorganisms and microbial pathways associated with the metabolism of dietary carbohydrates (to short chain fatty acids and gases), proteins, fats, bile acids, plant polyphenols and vitamins will be discussed. In addition, novel and existing methodologies used to study these diet-gut microbial interactions will also be discussed including mathematical modeling, systems biology approaches, isolated microbes, and enzyme assays. By studying the gut microbial processing of dietary inputs at a higher resolution we will advance our understanding of the relationship between diet and health.

**Keywords:** Biotransformation, Digestion, Gut microbiota, Diet

---

**PROBIOTICS IN CHILDREN. DO THEY WORK? A CRITICAL REVIEW OF THE PEDIATRIC USE OF PROBIOTICS FROM A PRACTITIONER'S POINT OF VIEW**

*Szajewska H.*
Department of Paediatrics, The Medical University of Warsaw

**Introduction:**
Although causality remains to be confirmed, current evidence supports the view that the gut microbiota play role in human health and disease. If so, it is logical to assume that manipulation of the gut microbiota, such as through the administration of probiotics and/or prebiotics, could potentially be a preventive and/or therapeutic measure in the evolution of disease states. Here, some examples of current research related to probiotics are described.

The best documented is the efficacy of certain probiotics for the treatment of acute gastroenteritis, for the prevention of antibiotic-associated diarrhoea and nosocomial diarrhoea, and for the prevention of necrotising enterocolitis; however, in the latter condition it is not clear which probiotic(s) should be used. There is some evidence to support the use of certain probiotics to prevent or treat other conditions, such as infantile colic, *H pylori* infection, and atopic eczema, but further studies are needed.

Not all probiotics are equal. The clinical effects and safety of any single probiotic or combination of probiotics should not be extrapolated to other probiotics. It is reasonable to use the regimens proven to be effective in well-designed and executed RCTs in a given population. The use of products with no documented health benefits should be discouraged.

**Keywords:** Probiotics in children, Gut microbiota, Antibiotic-associated diarrhoea, Acute gastroenteritis, *H pylori* infection

---

**REAL-TIME MONITORING OF SPECIFIC OXYGEN UPTAKE RATES OF ADHERENT CELLS IN A MICROFLUIDIC DEVICE**

*Szita N.*
University College London

**Introduction:**
Microfluidic cell culture devices offer precise control over the soluble, physical and mechanical microenvironment of the cells, and cells can be perfused with a variety of different compounds; yet monitoring in these devices is often limited to end-point analyses only. Oxygen plays a key role for cell growth and as an indicator of cell energy metabolism. Quantification of cellular oxygen kinetics, i.e. the determination of specific oxygen uptake rates (sOURs) provides invaluable insight. Combining the precise control over the cellular microenvironment afforded by microfluidic culture devices with real-time monitoring of oxygen tensions and cell culture confluency, will thus help to unravel kinetics of cell cultures, and provide new insight into the impact of natural bio-active compounds.

**Methods:**
Monitoring in the microfluidic culture device consisted of two alternating sequences: For the imaging sequence, a motorized stage of an inverted fluorescence microscope scanned the entire culture chamber. Sequentially acquired phase-contrast microscopy (PCM) images were then stitched together and processed to determine global and local cell confluency. For the oxygen monitoring, an optical fibre (attached to the
Abstracts of Oral Presentations

microscope objective with a custom-built adapter) aligned with a planar oxygen sensor recessed in the bottom of the culture chamber (for peri-cellular oxygen measurement). Additionally, oxygen was monitored at the inlet and outlet of the device using optical flow-through oxygen sensors. Stage positioning and data acquisition routines were controlled via LabVIEW. Using a bespoke pressure pump to create gas-driven flow (5% CO2, 21% O2), embryonic stem cells and Chinese Hamster Ovary cells were cultured under continuous perfusion (300 µL/h) and oxygen concentrations and cell density (cell number) monitored. Image processing further enabled the quantification of local growth and cell migration patterns in the culture chamber. The device was sterilized by autoclaving.

Results:
Mouse emryonic stem cells and Chinese Hamster Ovary cells were successfully cultured until they reached confluency, i.e. 6 days and 2 days, respectively. Our image processing algorithm enabled the real-time estimation of the number of cells in the microfluidic culture chamber without any disruption of the culture. The oxygen sensors enabled real-time detection of oxygen uptake rates (OUR) of the culture. By dividing the OUR with the cell density, we estimated the specific oxygen uptake rate. All data was generated non-invasively, i.e. did not require the disruption of the culture, and only phase-contrast microscopy images were required.

Discussion:
We demonstrate a label-free, non-invasive approach to monitor cell density, oxygen levels, specific oxygen uptake rates from a tiny cell culture. Therefore, we can determine cellular oxygen kinetics, and we can quantify the kinetics without disrupting the culture. Furthermore, the cell culture can be continuously perfused. Whilst we demonstrated perfusion with growth media for the expansion of two cell lines (mouse embryonic stem cells and Chinese Hamster Ovary cells), the same technology is applicable to culture other adherent cells and to perfuse them with a variety of compounds. Such compounds could be nutritional or bioactive substances. Currently we are working on expanding our multiplexed system for 3 cultures in parallel to a system with 6 or 12 culture wells.

Keywords: Microfluidic cell culture, Cell growth kinetics, Specific oxygen uptake rate, Cell energy metabolism, Real-time monitoring

IMPACT OF L.BULGARICUS-151 FERMENTED BUTTERMILK ON INTESTINAL MICROBIOTA COMPOSITION OF ALLERGIC AND NON-ALLERGIC MICE MODEL

Szyc A.; Fotschki J.; Markiewicz L.; Laparra M.; Wróblewska B.
Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Department of Immunology and Food Microbiology; Olsztyn, Poland

Introduction:
The composition of the intestinal microbiota has a significant impact on the intestinal homeostasis and ensures the balance between the defense immune function of the mucosa and systemic tolerance. An imbalance can be triggered by food allergy.

Many factors, especially fermented product, reach diet and probiotic supplementation have a significant influence on the microbiota structure. Buttermilk in the properly balanced diet of healthy people provides a rich source of essential proteins, phospholipids and bio-available minerals and vitamins and remains a frequent source of lactic acid bacteria The main aim of study was to determine the effect of diet supplementation with buttermilk beverage fermented with L.bulgaricus- 151 on the mice intestinal microbiota composition of allergic and non-allergic organisms.

Methods:
The female BALB/c mice at 8 weeks of age, were randomly divided into four groups (n=8/group). Two of groups were intra-peritoneally sensitized with cows’ milk protein (β lactoglobulin, α casein with aluminum adjuvant) to imitate an allergic reaction to milk proteins. One group of animals after sensitization procedure (A) and one of non-sensitized group (B) were fed with standard diet supplemented with fermented L. bulgaricus- 151 buttermilk. The remaining two groups: control-sensitized (C) and control non-sensitized (D) were fed with standard diet with PBS. After 3-week feeding period the mice were sacrificed. Blood, feces and intestine samples were collected. IgE, IgG and cytokines secretion was evaluated by ELISA method. In intestine mucosa the expression (mRNA) immune markers (TNF-α, IL-4, MCP-1 and TLRs) were measured.
In caecal digesta the microbiota structure using qPCR method was determined. The experiment was approved by the Ethical Committee (75/2011/N).

**Results:**
The desensitization by oral administration of L. bulgaricus-151 fermented buttermilk caused a significant decrease of total IgE (P=0.012) and antigen specific IgG (P=0.0038) in serum of sensitized group animals. A slight but insignificant increase of total IgE level relatively to the control group was observed in non sensitized animals treated with fermented product. The administered beverage caused the expression reduction of pro-inflammatory biomarkers especially IL-4, MCP-1 and TNF-α (P<0.005) in sensitized group of animals. There were also observed significant increase of TLR-2 and TLR-4 expression (P<0.005) in sensitized group of mice contrary to non-sensitized group that revealed only the TLR-4 expression increase (P=0.037). Fermented buttermilk administration resulted in increase of quantity of intestinal microbiota. Differences in composition were observed in sensitized and non-sensitized groups subjected the supplementation. In sensitized group the abundance increase was observed for Lactobacilli, Bifidobacteria, Enterococci and Atopobium sp. (P<0.05). In non-sensitized group there were also noticed a significant quantity reduction of Bacteroides-Prevotella-Porphyromonas group and Clostridium coccoides (P<0.05).

**Discussion:**
This study showed that applied dairy product caused discrepancy in quantitative and qualitative composition of intestinal microbiota in allergic and healthy individuals that is reflected in different secretion pro-inflammatory biomarkers and expression of innate immune responses markers. Different response of the immune system associated with the gut mucosa to the fermented buttermilk confirms the need of personalized nutrition and individual modulating properties assessment of used bacteria strain.

**Keywords:** Intestinal microbiota, Personalized nutrition, Individual predisposition, Strain-level dependent properties, Allergy

**AN ATP-BINDING CASSETTE (ABC) UPTAKE SYSTEM GOVERNS THE PREFERENCE OF PROBIOTIC BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS FOR B-GALACTOSIDES**

**Theilmann M.**
Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Denmark

**Introduction:**
The human gastrointestinal tract is colonized by a vast amount of microbes forming a diverse and dynamic ecological niche. This microbial community, which is established upon birth, develops rapidly in the first two years of life to a more constant state that persists for most of the adult life of the human host. A diversity of β-galactosides of different monosaccharide composition and glycosidic linkages is abundantly present in the human gut either from dietary intake, e.g. in fruits, vegetables and milk, or from the host mucin glycoprotein layer coating the intestinal epithelial cells. With the exception of lactose, β-galactosides are not hydrolyzed by human digestive enzymes presenting an attractive nutritional resource to human gut adapted bacteria. Probiotic bifidobacteria are preferentially stimulated by various β-galactosides, which is exploited in the manufacturing of galactooligosaccharides (GOS) prebiotics. A degree of species-specific β-galactoside utilization by bifidobacteria is observed, but the molecular basis of this selectivity remains unexplored. The objective of the present study is therefore to evaluate the role of a Bifidobacterium oligosaccharide transport system in the preference for specific β-galactosides.

**Methods:**
A β-galactoside utilization gene cluster from probiotic Bifidobacterium animalis subsp. lactis BI-04 (BI-04) encoding a transcriptional regulator, an ATP-binding cassette (ABC) importer and an intracellular β-galactosidase of glycoside hydrolase family 42 (GH42) has previously been identified from whole genome microarrays transcriptional analysis. The specificity determining solute-binding protein (SBP) of the ABC transporter was recombinantly produced and ligand binding of the SBP with 16 different β-galactosides varying in monosaccharide composition, degrees of polymerization (DP), and glycosidic linkage was evaluated using surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC). Selected β-galactosides were evaluated for their ability to support growth of B. animalis subsp. lactis BI-04 in mono-cultures.
Results:
The SBP showed a strong preference for β-6'-galactosides and the highest affinity binding was observed for β-6'-galactobiose and β-6'-galactotetraose with dissociation constants (KD) in the 100 nM range. The binding of β-6'-galactobiose was enthalpy-driven, whereas the interaction of β-6'-tetraose was driven by both entropy and enthalpy. The SBP interactions with β-3’- and β-4’-galactosides were 300- and 1700-fold weaker, respectively. Growth curves of B. animalis subsp. lactis BI-04 on three β-galactobiase isomers (β-3’-, β-4’, and β-6-galactobiose) reflected the preference of the SBP.

Discussion:
The GH42 enzyme has previously been shown to exhibit broad specificity hydrolyzing β-1,6, β-1,3 and β-1,4 galactosidic bonds with only a modest preference for β-1,6 bonds. The results of the present study represent a unique case allowing the comparison of selectivities of a transport protein and a glycoside hydrolase involved in the metabolism of the same glycan type highlighting the important role of ABC-mediated glycan transport in establishing the metabolic preference of a physiologically important taxon of the gut microbiota. Bioinformatic analyses suggest that the ABC transporter endows B. animalis sp. lactis subspecies with a unique metabolic capability, and hence the presented results provide a methodological framework for the design of highly selective and potent prebiotics that target specific probiotic taxa.

Keywords: Bifidobacterium, Galactooligosaccharides, Oligosaccharide uptake, Prebiotic, Solute-binding protein

ARE BACTERIOCINS WILL BE AN ANSWER/SOLUTION IN INCREASED PROBLEM WITH ANTIBIOTIC RESISTANCE?

Todorov S.
Universidade Federal de Viçosa, Brazil

Introduction:
The horizon of the application of bacteriocins have been enlarged and based on intensive research worldwide, novels applications of bacteriocins have been addressed. From the discovery of nisin produced by Lactococcus lactis in 1933, more than 7,000 papers published in different scientific journal around the world until 2015: this is a short story of the bacteriocins. From the application in the biopreservation in the control of spoilage organisms, prevention of the foodborne pathogens to the innovative application in the control of human and other animals’ diseases, this is the future of these antimicrobial peptides. Described as antimicrobial agents, active against closely related microorganisms, nowadays we have knowledge on the inhibitory effect and potential application of bacteriocins against some Gram-negative microorganisms, viruses, Mycobacterium spp., fungus, etc. However, what about application of bacteriocins as an alternative or synergetic compound/s in human and veterinary medicine? Is this will be an answer/solution in increased problem with antibiotic resistance? New horizons in the application of bacteriocins were reached and the life story of these antimicrobial peptides is just begun.

Keywords: Bacteriocins, LAB, Antimicrobials, Pathogens, Medical application

GARVIEACIN Q - THE WIDE-SPECTRUM CLASS IIID BACTERIOCIN THAT TARGETS MAN-PTS

Tymoszewska A.; Aleksandrzak-Piekarczyk T.; Diep D.; Bardowski J.
Institute of Biochemistry and Biophysics Polish Academy of Sciences

Introduction:
Bacteriocins are currently the subject of intensive study worldwide due to their great potential as a second-generation antibiotics or natural food preservatives. Bacteriocin-producing bacteria (such as lactic acid bacteria) can also be use as probiotics with health-giving effects for human and animals. As bacteriocins are highly diverse in terms of their primary and secondary structures, physico-chemical properties, inhibitory spectra, etc, it is therefore expected that they likely recognize different receptors on target membrane. So far, 5 receptors are known. Among them, the mannose phosphotransferase system (Man-PTS) serves as a
receptor for all class IIa bacteriocins as well as class IIId bacteriocin - lactococcin A (lcnA). In this study, we searched for genes encoding proteins engaged in conferring the sensitivity to the class IIId bacteriocin - garvieacin Q (garQ) produced by Lactococcus garvieae BCC 43578. Among them, we expected to specify gene(s) coding for potential garQ receptor(s).

Methods:
The activity spectrum of garQ was determined by the spot-on lawn inhibition spectrum assay. Spontaneous mutants resistant to garQ were obtained through the induction of sensitive L. garvieae strain by the presence of the bacteriocin in the solid medium. The degree of resistance in the garQ resistant mutants was estimated by the microtiter plate assay. Whole genome sequencing of mutants was carried out by the method of Illumina sequencing. The deletion of identified genes was performed through a double cross-over between the chromosome (target genes) and the targeting plasmid. The deleted genes were complemented using nisin-controlled expression system involving pNZ9530 and pNZ8037 plasmids.

Results:
The results showed wide activity spectrum of garQ, which was active against several bacterial species from the Carnobacterium, Enterococcus, Lactobacillus, Lactococcus and Leuconostoc genus. The obtained 20 L. garvieae garQ resistant mutants were over 1024-fold more resistant than the wild-type strain. Analyses of the 9 sequenced mutants genomes revealed mutations in genes encoding subunits IIC (4 mutants) or IID (5 mutants) of Man-PTS and included nonsense or nonsynonymous mutations. Deletion of the Man-PTS operon resulted in the full resistance to garQ. Complementation of the membrane located IIC and IID components was sufficient to restore the sensitivity to garQ.

Discussion:
The results of this study enable us to propose IIC and IID components of Man-PTS as receptors for garQ. This is second to date documented case, that the Man-PTS is a receptor for class IIId bacteriocin. Importantly, no similarity in primary structures and in the activity spectrum between the garQ and lcnA bacteriocins suggest that they both may interact with the Man-PTS by different mechanisms, however confirmation of this hypothesis requires further research.

Keywords: Bacteriocin biology, Resistance to bacteriocins, Receptors for bacteriocins, Man-PTS, Garvieacin Q

USE OF MICROBIAL NETWORKS AS A STRATEGY TO RECOVER FROM GUT DYSBIOSIS

Van den Abbeele P.
ProDigest bvba

Introduction:
Disruption of the normal composition and function of the gut microbiome (i.e. dysbiosis) is involved in a number of gastrointestinal pathologies such as infections, chronic inflammation and the metabolic syndrome. Attempts to treat these pathologies are frequently conducted with antibiotics or by means of various functional ingredients (i.e. probiotics). Recent years have been revolutionized by the application of so-called fecal microbial transplants, in which fecal microbiota from a healthy donor is transferred to a diseased individual. In this work we propose an alternative strategy by making use of a well-characterized mixture of microorganisms. Careful selection of candidate strains within such mixture can guarantee the desired functionality and stability under fluctuating environmental conditions and thereby assist in recovering from the negative effects of dysbiosis. Dysbiosis recovery may then in turn result in normalized host-microbiota interactions and ultimately improvement of disease symptoms.

Methods:
In practice, a set of specific microbial strains was selected for their interdependence in metabolic capacities and beneficial effects on biomarkers of gut health and inflammation. Different mixtures of bacterial candidates were first tested in a screening approach for their capacity to restore situations of dysbiosis (i.e. Inflammatory Bowel Diseases, Clostridium difficile infection, antibiotics-induced dysbiosis) in the in vitro Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). The SHIME was coupled with the HMITM module (Host-Microbiota Interaction module) to assess the effect of dysbiosis recovery on host microbiota cross talk and disease biomarkers. The best mix (i.e. PD-V4G-002) has then been evaluated in different dysbiosis-associated in vitro disease models such as TNBS-induced colitis and antibiotic-associated dysbiosis mouse models.
**Results:**
Initial screening experiments showed strong variation in the networking capacities and functional stability within the different synthetic mixtures of candidate strains. This also resulted in strong differences in efficacy to restore the intestinal microbiome upon dysbiosis induction. Based on this, a specific composition, designated as PD-V4G-002, was selected and tested both in vitro and in vivo in different dysbiosis models. PD-V4G-002 was shown to be highly effective in restoring functionality profiles in dysbiosed microbiomes and, upon comparison with competitor approaches, this resulted in superior protection against inflammation and disease induction.

**Discussion:**
Summarized, the approach followed in this work to create active and robust synthetic bacterial mixes was shown to be a promising alternative strategy for enhanced recovery from the negative effects of gut microbiota dysbiosis. Further research is currently ongoing to turn these promising early-stage concepts into functional biotherapeutics for different target areas.

**Keywords:** Synthetic bacterial mixtures, Dysbiosis, SHIME, Fecal transplants, Inflammation, Antibiotic, Clostridium difficile

**FERMENTATION OF $^{13}$C-LABELED 6'-SIALYL LACTOSE BY MICROBIOTAS ORIGINATING FROM BABIES, ADULTS AND ELDERLY – TRACING THE LABEL**

Venema K.
Beneficial Microbes® Consultancy, Wageningen, the Netherlands; koen.venema@outlook.com

**Introduction:**
Over the past two decades it has become clear that the gut microbiota is heavily involved in health and disease, not only for the gut itself, but also for the rest of the body. Correlations have been found between the presence/absence of certain bugs and many diseases and disorders. One of the major activities of the gut microbiota is the fermentation of carbohydrates. In breast-fed infants these are the human milk-oligosaccharides (HMO). Oligosaccharide amount and composition vary between women and over the course of lactation and vary from 5-15 g/L human breast milk. Breast-feeding is considered the ‘golden standard’ for infants, and hence infant-formula producing companies are trying to mimic the composition of HMO with prebiotics. Prebiotics are defined as non-digestible carbohydrates that specifically stimulate one or a few members of the gut microbiota, leading to improved health for the host.

**Methods:**
We have developed tools making use of $^{13}$C-labeled substrates to trace fermentation of these by the gut microbiota. These were used in a validated, dynamic, computer-controlled in vitro model of the large intestine, nick-named TIM-2, inoculated with microbiotas originating from babies, adults or elderly. This presentation will discuss use of the $^{13}$C-labeled HMO 6'-sialyl lactose (6'-SL) by these microbiotas, in comparison to the commercial prebiotics galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS). Due to the 13C label, metabolites that were produced from the substrates by the three different microbiotas could be analyzed by gas-chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR).

**Results:**
Clear differences were observed between the microbiotas. For instance, in the elderly microbiota label was incorporated in the amino acid alanine. Also label incorporation in ethanol was observed. Breakdown of 6'-SL could be traced using NMR, and was shown to be different between the three microbiotas. Also label incorporation in microbial biomass and gene-expression were analyzed, using stable-isotope-probing and a customized gene-microarray, respectively. This indicated that bifidobacterial species were important in fermentation of all three substrates that were compared. The data obtained gives a nice indication about similarities and difference in metabolism of HMOs by the different microbiotas.

**Discussion:**
Significance and impact of the results, as well as limitations will be discussed. E.g. development of novel prebiotics that mimic even better the metabolism of HMO could be tested using the current set-up. If time allows results of experiments performed with Lacto-N-neotetraose (LNnT) and mixtures of other HMO will be shown as well. The latter experiments are based on differences in secretor stage of lactating women.

**Keywords:** Human milk oligosaccharides
TOWARD MICROBIAL FERMENTATION METABOLITES AS MARKERS FOR HEALTH BENEFITS OF PREBIOTICS (AND PROBIOTICS)

Verbeke K.; Boobis A.; Chiodini A.; Edwards C.; Franck A.; Kleerebezem M.; Natua A.; Raes J.; Tuohy K.; Tol R.
1 Translational Research Center for Gastrointestinal Disorders, KU Leuven, Belgium
2 Imperial College London, UK
3 ILSI Europe, Brussels, Belgium
4 University of Glasgow, Scotland
5 Cargill, Belgium
6 Wageningen University, The Netherlands
7 FrieslandCampina, The Netherlands
8 KU Leuven, Belgium and Vrije Universiteit Brussels, Belgium
9 Fondazione Edmund Mach, Italy
10 Mead Johnson Nutrition, The Netherlands.

Introduction:
The intestinal gut microbial ecosystem produces a wide range of metabolites that interact with the host’s cells and in this way influence the physiological processes in the colon.

Objectives:
To evaluate the available evidence on the bioactive, nutritional and putative detrimental properties of gut microbial metabolites to support a more integrated view of how prebiotics might affect host health throughout life.

Methods:
A literature inventory was performed that targeted evidence for the physiological and nutritional effects of metabolites, e.g. short chain fatty acids (SCFA), the potential toxicity of other metabolites and attempted to determine normal concentration ranges. Furthermore, the biological relevance of more holistic approaches like faecal water toxicity assays and metabolomics and the limitations of faecal measurements were addressed.

Results:
Existing literature indicates that protein fermentation metabolites (phenol, p-cresol, indole, ammonia), typically considered as potentially harmful, occur at concentration ranges in the colon such that no toxic effects are expected either locally or following systemic absorption. The end products of saccharolytic fermentation, SCFA, may have effects on colonic health, host physiology, immunity, lipid and protein metabolism and appetite control. However, measuring SCFA concentrations in faeces is insufficient to assess the dynamic processes of their nutrikinetics. Existing literature on the usefulness of faecal water toxicity measures as indicators of cancer risk seems limited.

Conclusions:
At present there is insufficient evidence to use changes in individual faecal bacterial metabolite concentrations as markers of prebiotic effectiveness. Integration of results from metabolomics and metagenomics holds promise for understanding the health implications of prebiotic microbiome modulation but adequate tools for data integration and interpretation are currently lacking. Similarly, studies measuring metabolite fluxes in different body compartments to provide a more accurate picture of their nutrikinetics are needed.

Keywords: Microbial metabolites, Prebiotic health benefits, Metagenome, Nutrikinetics

INTESTINAL MICROBIOTA SCREENING PLATFORM (I-SCREEN) PROVIDES INSIGHTS INTO THE EFFECTS OF FOOD INGREDIENTS AND MEDICATION ON GUT MICROBIOTA COMPOSITION AND - ACTIVITY

Vossen J.; Pálková L.; Keijser B.; Montijn R.; Schuren F.
TNO

Introduction:
The relation between ingested products and health has been acknowledged widely. Ingested food and medicine exert their influence on the microbiota of the gastrointestinal tract. Possibilities to study these
effects in human subjects are limited. We developed the i-screen platform that allows for studying effects of compounds on the gut microbiome. This contribution describes the features of the system as well as its application for screening ingredients and studying effects of medication on the gut microbiome.

**Methods:**
In the i-screen platform the ex-vivo gut microbiota can be simultaneously maintained in multiple wells and individually exposed to up to hundreds of different conditions covering different ingredients and compounds. The effect of different food ingredients, probiotics, prebiotics and antibiotics, on the microbiota composition and -activity can be analyzed and described by applying a polyphasic analysis. This analysis consists of next generation 16S rDNA sequencing for microbiota composition, metagenomics sequencing, RNA-sequencing for the metatranscriptome and short chain fatty acid (SCFA) analysis as part of the metabolome.

**Results:**
Effects of food fibers and prebiotics including inulin, xylobiose are presented. Xylobiose showed a strong concentration dependent effect on the proliferation of Bifidobacterium spp. in the gut microbiome. By using metatranscriptome analysis the specific upregulation of genes involved in the xylose metabolic pathway in Bifidobacterium was detected. In the presence of xylobiose, the microbiota produces SCFA including butyrate. Butyrate was produced at a higher level than in the absence of xylobiose. Apart from beneficial bacteria, the fate of potential pathogenic bacteria in the i-screen is also shown. When challenging the microbiota with antibiotics, significant changes in the microbiota composition are noticed. Introducing ESBL Enterobacteriaceae in the microbiota in presence and absence of beta lactam antibiotics resulted in typical effects of bacterial overgrowth. The type of beta lactam antibiotic is rather dictating the effect. Amoxicillin has a general Enterobacteriaceae stimulating effect on the expense of other microbiota members while cefotaxime only stimulate the relative abundance of ESBL bacteria. Mitigating effects of prebiotics in case of antibiotic use are observed and will be shared.

**Discussion:**
From these results we conclude that prebiotic effects in the presence of food fibers can be identified and dysbiosis in case of a gastrointestinal infection with ESBL while using beta lactam antibiotics can be simulated. Although the i-screen platform is a model representing the large intestine in this study, it clearly simulates effects on the gut microbiota as observed in vivo. This result implies that the i-screen platform has predictive value and can be used to screen and study initial effects of different compounds on a complex ecosystem in the absence of the mammalian immune system.

**Keywords:** Microbiota, Prebiotics, Screening model, Polyphasic analysis, Antibiotics, Metagenomics, Metatranscriptomics

---

**HUMAN INTESTINAL BARRIER FUNCTION IN HEALTH AND DISEASE**

*Wells J. M.; Brummer R. J.; Derrien M.; MacDonald T. T.; Troost F.; Cani P. D.; Theodorou V.; Dekker J.; Méheust A.; de Vos W. M.; Mercenier A.; Nauta A.; Garcia-Rodenas C. L.*

1 Host-Microbe Interactomics Group, Animal Sciences, Wageningen UR, The Netherlands;
2 Nutrition-Gut-Brain Interactions Research Centre, School of Medicine and Health, Örebro University, Sweden;
3 Centre Daniel Carasso, Danone Research, 91767 Palaiseau, France;
4 Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Whitechapel, London, U.K.;
5 Division of Gastroenterology-Hepatology, Department of Internal Medicine, University Hospital Maastricht, Maastricht University Medical Centre, The Netherlands;
6 Louvain Drug Research Institute, WELBIO, Metabolism and Nutrition Research Group, Université Catholique de Louvain, Brussels, Belgium;
7 Neuro-Gastroenterology and Nutrition Group, INRA UMR1331 Toxalim Toulouse, France;
8 The International Life Sciences Institute, European Branch, Brussels, Belgium;
9 Laboratory of Microbiology, Wageningen UR, Building 316, Dreijenplein 10703 HB Wageningen, The Netherlands; 10 Nutrition & Health Research, Nestlé Research Center, Lausanne, Switzerland;
11 FrieslandCampina, Amersfoort, The Netherlands.
Introduction:
This introductory lecture will give an overview of the factors maintaining intestinal barrier function and the role of a ‘leaky gut’ in the pathophysiology of a variety of gastrointestinal disorders. The gut barrier plays a crucial role by spatially compartmentalising bacteria to the lumen through the production of secreted mucus and is fortified by the production of slgA and antimicrobial peptides and Reg3 family proteins. With exception of slgA the expression of these protective barrier factors is largely controlled by innate immune recognition of microbial molecular ligands. Several specialised adaptations and checkpoints are operating in the mucosa to scale the immune response according to the threat and prevent overreaction to the trillions of symbionts inhabiting the human intestine.

Increased permeability of the intestinal epithelium is now recognised as having a role in the pathophysiology of a variety of gastrointestinal disorders. Deterioration of the intestinal barrier function may lead to increased and prolonged mucosal immune activation and consequently to increased afferent sensory signalling and abdominal complaints. In turn, neuronal mechanisms affect the intestinal barrier partly by activation of the HPA-axis and both mast cell-dependent as well as mast cell- independent mechanisms. Aging significantly increases the vulnerability to gastrointestinal disorders but there are few studies investigating the key factors in aging that affect the GI tract. The age-associated changes in barrier function will also be presented and the impact this could have on vulnerability to gastrointestinal disorders, the systemic immune system and organs.

Nutrition and microbiome-gut interactions can have a substantial and clinically relevant impact on the development of the immune system and intestinal barrier function with consequences for the resistance to pathogens, development of gut inflammation, and abdominal complaints. This has major implications for the prevention and therapy of a variety of ‘modern’ diseases called ‘immune mediated disorders of affluence’, such as IBS, IBD, and other non-intestinal disorders, such as allergy and type-1 diabetes, which have all dramatically increased in incidences over the past five decades. Several biomarkers have been used to measure gut permeability and loss of barrier integrity in intestinal diseases but for many markers there is still a need for standardization, establishment of normal ranges and their predictive values in the general population. Ultimately such markers and assays could be used to assess associations between particular nutritional traits and gut barrier function or experimentally assess the impact of a specific nutritional intervention.

Keywords: Intestinal Barrier Function, Gastrointestinal disorders, Mucosal immune activation, Age-associated changes

MILK FERMENTATION AS A PREVENTATIVE INTERVENTION IN THE SPREAD OF TUBERCULOSIS IN SUB-SAHARAN AFRICA

Witthuhn R. C.; Macuamule C. L.
University of the Free State, Republic of South Africa

Introduction:
We will present data on the potential of milk fermentation in controlling the growth and multiplication of Mycobacterium bovis, the causal agent of zoonotic tuberculosis (TB), in Africa where large varieties of indigenous fermented milk products are traditionally manufactured from raw milk. The main aims of the study were to evaluate different culturing and molecular methods for the isolation and accurate quantification of M. bovis in dairy products, to determine the effect of the fermentation process on the survival of M. bovis in milk, and to evaluate the use of the food additive, Ethyl pyruvate (EP), as a means of increasing the antimicrobial effect of lactic acid fermentation on M. bovis.

Three sample treatments namely 0.75% (m/v) hexadecylpyridinium chloride (HPC), N-acetyl-L-cysteine-sodium hydroxide (NALC-1% NaOH), and direct plating onto 7H10 - 2% (m/v) PANTA agar were evaluated for recovery of M. bovis from contaminated milks. The 7H10 agar enriched with PANTA was found to be an effective method for suppressing natural microorganisms present in both raw milk and fermented milk, while allowing the enumeration of M. bovis. The established culture method was used to monitor the survival of M. bovis during fermentation using kefir grains or single strains of lactic acid bacteria (LAB) isolated from African indigenous fermented milks. Ethyl pyruvate (EP) at concentrations of 10 mM, 20 mM and 80 mM was added at the beginning of the fermentation of the contaminated milks using kefir grains as starter. A PCR-based method that accurately detects bacterial species belonging to the Mycobacterium tuberculosis complex (MTBC) in raw and fermented milk was also established.
The study has demonstrated milk fermentation decreases *M. bovis* cell concentration and a fermentation period of 60 h is required for elimination of *M. bovis*. The organic acids produced during fermentation are the most important antimicrobial factor. Addition of EP resulted in higher mortality of *M. bovis* and was found to be a potential candidate to be used as an antimicrobial against *M. bovis* during milk fermentation. Therefore, the fermentation process has potential in ensuring the safety of traditional fermented milk produced by some African communities.

**Keywords:** Milk fermentation, Mycobacterium bovis, Zoonotic tuberculosis, Raw milk
Abstracts of Poster Presentations

THE OPTIMUM CONDITION OF MILK BEVERAGE SUPPLEMENTED WITH COFFEE BY RESPONSE SURFACE METHODOLOGY

Ahn S.; Park J.; Yoon J.; Park S.; Kim G.; Jhoo J.
Kangwon National University

Introduction:
Recently, it is tendency to increase the consumption of milk beverage more than milk or other milk products. However, its precipitation becomes issue during storage or distribution because it has various ingredients, and it is needed to determine suitable emulsification condition to solve the issue. Therefore, this study was carried out to determine the optimum condition for emulsification of milk beverage supplemented with coffee.

Methods:
Four kinds of emulsifiers were used in this study, such as sucrose fatty acid ester F110, sucrose fatty acid ester F160, Almax 9280, Almax 9080, and its HLB value were 11, 15, 7.5, and 14.5, respectively. To manufacture the milk beverage, some materials were used as follows: raw milk, coffee extracts, skim milk powder, cream, emulsifier, and so on. High speed homogenizer was used for primary emulsification, and high pressure homogenizer was used for secondary emulsification to disperse into nano size. To determine the most efficient conditions for the emulsification, the speed of primary homogenization and the amount of emulsifiers were investigated by response surface methodology. Particle size distribution and z-potential were measured to select suitable emulsifier and to investigate the manufacturing condition of the beverage. To observe the distribution and shape of the particle, the milk beverage was observed with optical microscope.

Results:
It was revealed that the particle size of the group added sucrose fatty acid ester F160 was significantly bigger than others (p<0.05). Also, there were not significant differences in z-potential. Sucrose fatty acid ester F110 and F160 were powder type, whereas, Almax 9080 and Almax 9280 were gel type emulsifiers which had strong viscosity. Because of it, they were not easy to handle in the manufacturing process. Therefore, sucrose fatty acid ester F110 was selected as suitable emulsifier in this study. With the results from response surface methodology, as speed of primary homogenization was decreased, particle size was increased, and z-potential was decreased. At the amount of emulsifier with 0.2071%, it was predicted that z-potential was the highest with approximately -32.62 mV, and particle size showed a tendency to decrease. Finally, the optimum conditions of primary homogenization speed and amount of emulsifier to manufacture the milk beverage were found to be 5,000 rpm and 0.2071%, respectively. After optimization of the condition, the beverage was manufactured to confirm it. The average particle size of the beverage was about 176.65 nm, and z-potential was -32.56 mV under the optimum condition. Every particles of the beverage in the optimum condition was round shaped, and evenly distributed in the micro photograph of the milk beverage. Generally, it is known that the absolute value of z-potential greater (30 mV or more), a colloidal system has better dispersibility.

Discussion:
Based on the data obtained from this study, it was considered that dispersibility of the beverage under the optimum condition was good enough to maintain its disperse system, and it is respected that the milk beverage supplemented with coffee can effectively be produced with this optimized condition.

Keywords: Response surface methodology, Emulsification, Milk beverage, Z-potential, Coffee

LACTOBACILLUS SPP. PROTECTS THE BARRIER FUNCTIONALITY OF CACO-2 CELLS EXPOSED TO INFLAMMATORY STIMULI

Alarcón P.; Mellado J.; Cofré J.; González M.; Aguayo M.; Castro E.
Facultad de medicina, Instituto de políticas públicas en salud IPSUSS, Universidad San Sebastián, Concepción, Chile.

Introduction:
Lactobacillus bacteria are able to make contact with the immune system of the mucosae. A loss of the normal
and homeostatic interaction between Lactobacillus spp. and gut cells can lead to develop several diseases, ranging from dysbiosis to chronic inflammation, and even to intestinal cancer. The Caco-2 cell line of human colon adenocarcinoma is a model for study of enterocytes. These cells differentiate into enterocytes after 15 days, and show the same morphological and functional characteristics than those obtained from humans. This study aims to determine the effects of bacterial lysates, named soluble protein homogenates (SPHs), obtained from the probiotic strains Lactobacillus gasseri LPV31 and LPV22, (extracted from healthy women’s vaginal microbiota) and Lactobacillus salivarius LPLM-O1, (extracted from healthy breast milk) in Caco-2 cells that have been exposed to inflammatory stress.

Methods:
The Lactobacillus strains were subjected to mechanical disruption in order to obtain the SPH, resulting in 22 protein bands for strains LPV31 and LPV22, and 27 bands for LPLM-O1 after a 10% SDS-PAGE analysis. Three Caco-2 cell cultures (400,000/well) were differentiated to enterocytes Group 1 was stimulated with Salmonella typhimurium lipopolysaccharide (LPS) (100 ng mL-1) for 21 hours. After this, the transepithelial electric resistance (TEER) was measured. Group 2 was stimulated with 100 ng mL-1 of LPS for 21 hours and then washed with 10 mM PBS (pH = 7.3); after that, 150 µg mL-1 of SPH of the three probiotic strains were added to three different wells in triplicate. For Group 3, Caco-2 cells were stimulated with 150 µg mL-1 of each bacterial SPH in three different wells, and 100 ng mL-1 of LPS were applied after washing.

Results:
The results showed for G1 TEER value of the cells descended by 20% in respect to cells without LPS. For G2 all three strains reduced TEER values recorded after adding the LPS. For Group 3, SPH obtained from LPLM-O1 increased TEER values over base levels, showing a better performance than the other strains. When stimulating with LPS, there are no statistically significant differences in the increase of TEER by SPHs. Also, there was no production of IL-1β, TNF-α, IL-6, and IL-10.

In Group 1, IL-8 passed its basal level, and in Group 2, it went significantly over the basal level (p<0.05) after stimulating with SPH of LPLM-O1, and in Group 3, the SPHs of both LPV31 and LPV22 reduced it, but the SPH of LPLM-O1 increased it significantly (p<0.05). When restimulating Groups 2 and 3 with LPS, IL-8 concentration is similar to basal levels.

Discussion:
In conclusion, it is proposed that the SPHs of probiotic strains L. gasseri LPV31 and LPV22, and of L. salivarius LPLM-O1 have a different protective effect on barrier function on Caco-2 cells that have been exposed to inflammatory stress.

Keywords: Probiotic, Inflammatory Stimuli, Lactobacillus, Caco-2 cell, Bacterial lysates

PRODUCTION OF INTERLEUKINS BY WHOLE-BLOOD CELL CULTURE FROM INFLAMMATORY BOWEL DISEASE PATIENTS IN THE PRESENCE OF SEVERAL YEASTS

Antoine P.; Fonteyn F.; Didderen I.; Weekers F.; Thonart P.; Louis E.
Department of gastroenterology, CHU of Liège, Sart Tilman, B35, 4000 Liège, Belgium

Introduction:
Inflammatory bowel diseases are characterized by both a dysbiosis and a disturbance of the mucosal inflammatory homeostasis. Probiotics may favorably influence these two aspects. Beside bacteria, some yeast strains may also be considered as probiotics. However, apart from Saccharomyces boulardii no other yeast has been tested clinically for the treatment of IBD or IBS. Furthermore, their ability to modulate the immune response has been little studied.

Our aim was to test several yeast strains for their ability to modulate the production of interleukin-1β, interleukin-10, interleukin-12+p40 and interleukin-17 by whole blood cells cultures.

Methods:
Patients affected with inflammatory bowel disease were recruited and their blood was put in whole blood cells culture with lipopolysaccharide, Saccharomyces boulardii ATY-SB-101, Saccharomyces cerevisiae Sigma 1278b, Yarrowia lipolytica ATY-YL-103, Brettanomyces bruxellensis Van der Walt MUCL 27700, Debaryomyces hansenii ATY-DH-110 or Candida albicans ATCC 14053. A negative control was also performed without any stimulus. Interleukin-1β, interleukin-10, interleukin-12+p40 and interleukin-17 were
measured in the supernatants. Results were compared using Mann-Whitney U test. Differences were considered to be significant when p < 0.05.

**Results:**
Eleven patients with Crohn’s disease and six patients with ulcerative colitis were recruited. As compared to negative controls, all yeast strains were able to stimulate production of significantly higher amount of interleukin-1β, interleukin-10, interleukin-12+p40 and interleukin-17 with the exception of Brettanomyces bruxellensis Van der Walt MUCL 27700 regarding the levels of IL-12+p40 and IL-17.

IL-10 concentrations varied between 10.43 (± 15.88) pg/ml and 131.94 (± 113.27) pg/ml depending on the yeast. The highest production of interleukin-10 was obtained with Yarrowia lipolytica ATY-YL-103 and Debaryomyces hansenii ATY-DH-110. These strains were also among those that stimulate the highest production of interleukin-1β, while the lowest concentrations of this pro-inflammatory cytokine were obtained with Saccharomyces boulardii ATY-SB-101 and cerevisiae Σ 1278b (respectively p<0.001 and p<0.01 compared to LPS).

Saccharomyces cerevisiae Σ 1278b was also among the strongest inducers of interleukin-10 and was the strongest inducer of interleukin-17.

The production of interleukin-17 was also highly stimulated with Yarrowia lipolytica ATY-YL-103 and Saccharomyces boulardii ATY-SB-101.

About interleukin-12+p40, the strongest inducers were Yarrowia lipolytica ATY-YL-103 and Saccharomyces cerevisiae Σ 1278b, however the observed values were much lower than those obtained with LPS. The lowest interleukin-12+p40 concentrations were obtained with Brettanomyces bruxellensis Van der Walt MUCL 27700 and Saccharomyces boulardii ATY-SB-101.

**Discussion:**
Different yeast strains show highly significant differences in their ability to stimulate interleukins by whole blood cell cultures in patients with inflammatory bowel disease. This suggests very different probiotic properties which have to be taken into account when contemplating probiotic treatment for inflammatory bowel disease.

According to these results, Saccharomyces cerevisiae Σ 1278b and Saccharomyces boulardii ATY-SB-101 may be considered as having a favorable profile to be tested in a disease like IBD or IBS, while the profile of Yarrowia lipolytica ATY-YL-103 may be interesting in infectious diseases.

**Keywords:** Yeast, Probiotics, Crohn’s disease, Ulcerative colitis, Cytokine

---

**IMPROVING THE VIABILITY OF LACTOBACILLUS SALIVARIUS SPP. SALIVARIUS IN APPLE SNACKS BY ADDING Trehalose AND/OR APPLYING HIGH PRESSURE HOMOGENIZATION**

**Barrera C.; Calabuig-Jiménez L.; Balau M.; Seguí L.; Betoret N.**
Instituto Universitario de Ingeniería de Alimentos para el Desarrollo. Universitat Politècnica de València.
Valencia, Spain

**Introduction:**
There is an increasing interest in developing probiotic foods alternative to the dairy ones commonly found on the market. In agreement with this, the research group running this study succeeded in incorporating mandarin juice inoculated with Lactobacillus salivarius spp. salivarius in the structural matrix of apple slices by vacuum impregnation (Betoret et al., 2012). However, the number of viable cells in such samples felt sharply after their convective drying at 40 °C and became below 103 CFU/g after 7 days of storage. Probably, the water activity of the dried product (aw ≈ 0.45) was not low enough to ensure the probiotic stability (Vesterlund et al., 2012). Apart from reducing the water activity, this study poses the incorporation of trehalose and the homogenization in order to increase the probiotic viability in the snack.

**Methods:**
Probiotic apple snacks were obtained by vacuum impregnation and further convective drying. Commercial mandarin juice prepared as reported by Betoret et al. (2012) was employed as impregnating solution. A peculiar thing about this study involved adding different concentrations of food grade trehalose (0 and 0.1 g/g) to the juice before its inoculation with 4 mL/L of MRS Broth containing 109 CFU of Lactobacillus salivarius.
salivarius spp. salivarius per mL. Moreover, fermented juices were subjected to different homogenization pressures (0 and 100 MPa) before being incorporated into the porous structure of apple slices (var. Granny Smith) by the vacuum impregnation technique. Once impregnated, apple samples were dried at 40 °C until they reached a water activity value around 0.45 or 0.35. Dried samples were placed in hermetic opaque bags and the number of viable cells was analyse at different times over 30 days of storage at room temperature.

**Results:**
Due to the longer exposure to the air stream, samples reaching lower activity value at the end of the drying process had lower counts at the beginning of the storage. However, the microbial population remained more stable and in concentrations of around 106 CFU/g during the storage than the set of samples with a water activity of around 0.35. Thus confirming the decisive role of water activity in the survival of the probiotic microorganism. Even the effect of the trehalose content and the homogenization pressure on the microbial population was dependent on the water activity of the stored samples. Under adverse conditions (aw ≈ 0.45), adding 10% (w/w) of trehalose to the impregnating solution or homogenizing the juice at 100 MPa improved the viability of Lactobacillus salivarius spp. salivarius. Under optimal conditions (aw ≈ 0.35), the highest counts were obtained when neither trehalose nor pressure were applied to the impregnating solution.

**Discussion:**
The study shows the protective effect separately exerted by trehalose and the homogenization pressure on the survival of Lactobacillus salivarius spp. salivarius during the storage of apple snacks with a water activity of around 0.45. However, these treatments do not improve the viability of the microorganism when the water activity snack reaches lower values around 0.35.

**Keywords:** Probiotic, Apple-snack, Vacuum impregnation, Trehalose, Homogenization, Convective drying

---

**NOVEL METHOD BASED ON CHROMOGENIC MEDIA FOR DISCRIMINATION AND SELECTIVE ENUMERATION OF LACTIC ACID BACTERIA IN FERMENTED MILK PRODUCTS**

Boyé M.; Galat A.; Fourmestraux C.; Combrisson J.; Boumghar-Bourtchai L.
Danone Nutricia Research

**Introduction:**
Microbial analyses of fermented milk products require selective methods to discriminate between close species simultaneously present in high amounts. A culture-based method combining novel chromogenic agar media and appropriate incubation conditions was developed to enumerate lactic acid bacteria (LAB) strains in fermented milk.

**Methods:**
M1 agar, containing two chromogenic substrates, allowed selective enumeration of Lactobacillus rhamnosus, two strains of Lactobacillus paracasei subsp. paracasei and Streptococcus salivarius subsp. thermophilus based on differential β-galactosidase and β-glucosidase activities. Depending on the presence of some or all of the above strains, M1 agar was supplemented with l-rhamnose or vancomycin and incubations were carried out at 37 °C or 44 °C to increase selectivity. A second agar medium, M2, containing one chromogenic substrate was used to selectively enumerate β-galactosidase producing Lactobacillus delbrueckii subsp. bulgaricus at 47 °C.

**Results:**
By contrast with the usual culture media, the chromogenic method allowed unambiguous enumeration of each species, including discrimination between the two L. paracasei, up to 109 CFU/g of fermented milk. In addition, the relevance of the method was approved by enumerating reference ATCC strains in pure cultures and fermented milk product.

**Discussion:**
The method could also be used for enumerations on non-Danone commercial fermented milk products containing strains different from those used in this study, showing versatility of the method. To our knowledge, this is the first description of a chromogenic culture method applied to selective enumeration of LAB.

**Keywords:** Lactic acid bacteria, Chromogenic agar, Strain enumeration, Strain discrimination, Fermented milk, Food analytical microbiology
EFFECT OF PECTINS AND STARCHES ON BACTERIAL SURVIVAL IN THE GASTRO-INTESTINAL JUICES AND MECHANISMS OF SYNBIOTIC INTERACTIONS

Cahu T.; Larsen N.; Blennow A.; Saad S.; Jespersen L.
University of Copenhagen

Introduction:
The ability of bacterial strains to exert probiotic properties depends on their survival in the gastro-intestinal (GI) tract. There is scientific evidence that prebiotic carbohydrates exhibit a protective effect on bacterial tolerance to gastric conditions by interacting with bacterial cells. An interesting approach to characterize pro-/prebiotic interactions is based on determination of Zeta potential (Zp)-the electrical potential in the shear plane of particles in suspensions. The objective of the study was (i) to investigate the ability of selected pectins and starches to improve survival of probiotic bacteria during co-incubation in simulated GI juices and (ii) to characterize the factors involved in synbiotic interactions, such as functional groups, hydrodynamic diameter, Zp and polydispersity.

Methods:
Probiotic Lactobacillus and Bifidobacterium strains (6 in total) were obtained from Chr.Hansen A/S. Survival of the strains was determined in the simulated stomach solution (SS) in the presence of nine pectins or three starches and compared to control SS without carbohydrates. Differences in reduction of viable counts (CFU) between treatments and controls were assessed by the paired two-tailed Student s t–test. Zeta potential of carbohydrates and bacterial cells was determined at pH 2.5 and pH 6.5 by dynamic light scattering with a ZetasizerZS (Malvern Instruments, UK).

Results:
L. acidophilus LA-5, L. fermentum PCC and L. reuteri RC-14 were most resistant to acid stress, followed by B. animalis BB-12. Viability of these strains in SS was either improved or consistent in the presence of carbohydrates. The highest increase of viability (10 fold) was obtained for PCC and RC-14 in combination with sugar beet pectin, harsh extracted pectin from orange, differentially extracted HM pectin from lemon and potato fiber. L. rhamnosus LGG and L. paracasei F-19 were less tolerant to SS conditions and their survival was negatively affected by pectins. Zeta-potential of bacterial cells and carbohydrates was highly affected by pH. The values of Zp in bacteria were reduced with lower pH, due to protonation of cell surface molecules; the largest shifts were observed for L. acidophilus LA-5. Zp values were positive or close to zero for Lactobacillus strains and negative for BB-12 at acidic conditions. Pectins, especially low-methoxyl, were characterized by more negative Zp than starches due to the polygalacturonic acid residues in their structure.

Discussion:
Viability of bacterial strains in SS incubated in the presence of pectins and starches correlated significantly (P<0.5) with Zp values of carbohydrates. Generally, carbohydrates with intermediate to lower negative Zp were more efficient to improve bacterial survival. The results of this study indicated that Zp approach might be useful to predict the extent of electrostatic interactions between polysaccharides and bacteria, thereby, promoting the ability to survive at GI conditions.

Keywords: Probiotics, Survival, Synbiotic Interacions, Zeta Potential, Prebiotics

MODIFICATION OF ADHESION AND SURFACE PROTEOME BY CARBOHYDRATES AND MUCIN-SUPPLEMENTED GROWTH OF PROBIOTIC BACTERIUM LACTOBACILUS ACIDOPHILUS NCFM

Celebioglu H.; Prehn K.; Lahtinen S.; Brix S.; Abou Hachem M.; Svensson B.
Department of Systems Biology, Technical University of Denmark

Introduction:
Lactobacillus acidophilus NCFM (NCFM) is a probiotic bacterium isolated from a human source and has been used extensively in foods and dietary supplements. It is a Gram-positive lactic acid-producing, well studied bacterium and its genome has been sequenced. Surface layer (S-layer) is a crystalline array of proteins forming the outermost part of the bacterial cell wall. In addition to S-layer proteins, extracellular proteins secreted by probiotics can have roles in interactions with the host as they are available for direct contacts with the mucosa.
and epithelial cells, which can involve bacterial adhesion to intestinal cells (HT-29 and Caco-2) and mucus layer. Prebiotics are non-digestible components in food, which beneficially affect host health by selective stimulation of growth of probiotic bacteria in the gastrointestinal tract (GIT). Fructooligosaccharides (FOS), galactooligosaccharides (GOS), and lactulose fulfill prebiotic criteria as approved in clinical trials. By contrast, a number of emerging prebiotics; cellobiose; melibiose; isomaltulose; polydextrose; and trehalose await approval.

**Methods:**
Following the growth of NCFM on different carbohydrates or supplemented with mucin and labeling with 5(6)-carboxyfluorescein diacetate, in vitro adhesion to mucin and HT-29 cells were performed by measuring the fluorescence. Surface proteomes were prepared by using 5 M lithium chloride, followed by 2-dimensional gel electrophoresis (2-DE), image analysis, and mass spectrometric identifications of differentially abundant proteins.

**Results:**
While growth on FOS only stimulated adhesion of NCFM to intestinal HT-29 cells, cellobiose and polydextrose also increased the adhesion to mucin. No significant change in adhesion to mucin and HT-29 cells was found for NCFM grown on GOS, lactulose, melibiose, palatinose, or trehalose. Remarkably, addition of mucin to cultures on glucose only stimulated adhesion to HT-29 cells. Comparative 2DE-based surface proteome analysis of NCFM showed higher relative abundance for cultures with added mucin of oxalyl-CoA decarboxylase co-migrating with pyruvate kinase and fructose-bisphosphate aldolase. Reduced relative abundant spots contained elongation factor G, phosphoglycerate kinase, BipAEFTU family GTP-binding protein, ribonucleoside triphosphate reductase, adenylosuccinate synthetase, 30S ribosomal protein S1, manganese-dependent inorganic pyrophosphatase. Comparative surface proteome analysis of cellobiose- and glucose-grown NCFM indicated phosphate starvation inducible protein stress-related, thermostable pullulanase, and elongation factor G to increase fold in abundance, while GAPDH, elongation factor Ts, and pyruvate kinase diminished fold in abundance when grown on cellobiose.

**Discussion:**
The alteration in adhesive properties can lead to easier bacterial colonization and thus exert more positive health effects. Higher abundant surface proteins in different conditions (i.e. growth on cellobiose or mucin-supplemented growth media) may indicate these proteins are responsible for the increased adhesion. Therefore, combination of NCFM with some carbohydrates gives insight into potential determinants of the synbiotic interactions.

**Keywords:** Subproteomes, Adhesion, Synbiotics, Mucin, HT-29 cells

---

**QUALITY AND FERMENTATION CHARACTERISTICS OF GREEK-STYLE YOGURT ADDED WITH HERB EXTRACT**

Chai C.; Kim H.; Park S.; Hwang J.; Jhoo J.; Kim G.
Kangwon National University

**Introduction:**
Substitution of sugars generally added to Greek-style yogurt with a noncaloric natural sweetener such as Stevia rebaudiana extract may be a good way to promote the value of Greek-style yogurt. Greek-style yogurt where sucrose was substituted with Stevia rebaudiana extract was prepared, and its quality and fermentation characteristics during storage at 4 °C were investigated in this study.

**Methods:**
Stevia rebaudiana extracts were prepared in two different methods. Dried Stevia rebaudiana leaves (100 g) were put into water (3 L) and heated at 100 °C for 6 h. Hot water extract was concentrated using a rotary evaporator and freeze-dried. Ethanol extract from Stevia rebaudiana leaves (100 g) was prepared via three consecutive treatments in 70% ethanol (3 L). Ethanol extract was concentrated and freeze-dried in a same manner applied to hot water extract.

Greek-style yogurt was manufactured via incubation of reconstituted milk inoculated with yogurt starter. Hot water extract or ethanol extract (0.5% w/v) was added to prior to the incubation, which were used as treatments. Greek-style yogurt with sucrose (0.5% w/v) without any supplement were prepared and used as
controls. Prepared Greek-style yogurts were stored at 4 °C for 15 days.
Number of lactic acid bacteria in Greek-style yogurt during the incubation and storage was enumerated using plate counts on MRS agar. The pH of Greek-style yogurt was also monitored.
Sensory properties of Greek-style yogurt were evaluated after the storage for 3 days. Sensory properties were color, flavor, sweetness, bitterness, sourness, stickiness, after taste, and overall acceptability in 5 point scale.

Results:
The numbers of lactic acid bacteria and pH of all treatments were gradually increased and decreased, respectively, during the incubation. Although there was no significant difference in the numbers of lactic acid bacteria between treatments and controls, the pH of Greek-style yogurt supplemented with Stevia rebaudiana extracts and sucrose were likely to be less than that of Greek-style yogurt without any supplement. Especially, the pH of Greek-style yogurt supplemented with hot water extract after the incubation for 12 h reached to 4.50 that was least value of pH than those of others. During the storage, the pH of all treatments and controls decreased slightly and reached to 4.38-4.56, but the difference in the pH between day 0 and day 15 of storage was not exceed 0.3. Sensory evaluation reveals that Greek-style yogurt supplemented with hot water extract scored greatest in sweetness. However its overall acceptability scored least. Poor evaluation of Greek-style yogurt supplemented with hot water extract was probably due to unfamiliar herb flavor.

Discussion:
Inclusion of Stevia rebaudiana extracts in fermentation of Greek-style yogurt promoted the production of lactic acid, which resulted in less value of pH than those of controls. But the difference in the pH was not obvious. As Greek-style yogurt supplemented with Stevia rebaudiana extracts scored better in sweetness than controls, Stevia rebaudiana extracts may be a good substitute with sucrose for Greek-style yogurt.

Keywords: Greek-style yogurt, Sugar substitute, Fermentation characteristics, Herb extract, Sensory evaluation

EFFECTS OF OXIDATIVE STRESS EXPOSURE ON MUCUS BINDING PROPERTIES AND ANTIBIOTIC RESISTANCE IN L. CASEI GROUP STRAINS

Comi G.; Ginaldi F.; Camprini L.; Iacumin L.
Department of Food Science, University of Udine, via Sondrio 2, 33100 Udine, Italy

Introduction:
Adherence to mucus is one of the features required for a strain to be considerate as probiotic. Also antibiotic resistance is a fundamental character that needs to be investigated. In this study, 44 potentially probiotic strains, belonging to the species Lactobacillus casei, L. paracasei, and L. rhamnosus, were tested for their capability to bind mucin, as well as for their antibiotic resistance before and after being exposed to aerobic stress.
The bacterial cells capability to adhere to porcine gastric mucin type III was tested after a preliminary cell adaptation under anaerobic and respiratory conditions. In the first assay, some strains with interesting adhesion properties were found, but oxidative stress did not improve their binding features. Other strains, which did not show to have relevant binding capability when cultured under anaerobic conditions, increased their capability in binding mucin after the adaptation under respiratory conditions.
Results allowed the selection of strains showing good adherence to mucus. The oxidative stress seems not to be directly correlated to an increase in the binding mucin capability. The effect resulted strain specific, in fact for some strain a 2 fold increase in binding capacity was observed, conversely, some strains were not affected from the oxidative stress, and some others decreased their binding capability. On this bases, a selection of the more suitable strains, for exploitation in probiotic industry or in several food industry applications related to the production of probiotic-fermented food, can be made in order to proceed with further characterization studies.
As far as the antibiotic resistance is concerned, several changes in the behavior of the strain have been observed after oxidative stress exposure, but the effects were strain-specific and no general considerations can be deduced.

Keywords: Mucin; Adhesion; Antibiotic resistance; Aerobic stress;
Abstracts of Poster Presentations

ANTIOXIDANT, ANTI-INFLAMMATORY AND GUT EPITHELIAL REGENERATIVE ACTIVITIES OF PREBIOTICS, PROBIOTICS AND BIOGENIC PROPOLIS

De Marco S.; Piccioni M.; Pagiotti R.; Pietrella D.
University of Perugia

Introduction:
The most widely used probiotic strains in humans are Bifidobacteria and Lactobacilli, but other microorganisms, such as the yeast Saccharomyces boulardii, have been reported to have some beneficial effects. Probiotics can be administrated with specific prebiotics and biogenic compounds in a combination called synbiotic.
The aim of the present work was to assess the probiotic potentiality of Lactobacillus casei, Lactobacillus reuteri, Lactobacillus acidophilus, Lactococcus lactis and Saccharomyces boulardii and the effect of propolis extracts, as biogenic, in terms of antioxidant and anti-inflammatory activities. Successively, the effects of cell-free supernatants on regeneration of colon epithelial HT-29 cell line have been determined. Prebiotics inulin, fructo-oligosaccharide (FOS) and isomaltose were added to the cultures to observe if they could improve probiotic activities.

Methods:
Cell-free culture supernatants of probiotics incubated overnight in presence or absence of prebiotics were used in this study. Bee and bud poplar propolis samples were obtained by ethanol extraction.
The antioxidant activity of probiotic supernatants and propolis was measured by means of neutralizing the free DPPH radical and reducing ROS production by neutrophils activated with phorbol-12-myristate-13-acetate. The anti-inflammatory activity has been determined by testing the IL-8 secretion by intestinal cells (HT-29), IL-1β, TNF-α and IL-10 by peripheral blood mononuclear cells (PBMC) stimulated with LPS and/or probiotic supernatants or propolis extracts by immune assay ELISA.
Regeneration of HT-29 cells treated with cell-free supernatants was assessed by determining cell proliferation by bioluminescent detection of cellular ATP and by in vitro wound healing assay.

Results:
A dose-dependent DPPH radical scavenging activity of probiotic was observed for all strains with the exception of S. boulardii supernatant. Propolis showed a good anti-oxidant activity in cell-free and neutrophil-based assays.
Regarding anti-inflammatory activity, supernatants were able to down-regulate the basal production and the LPS-induced secretion of pro-inflammatory IL-8 in HT-29 cells in a dose dependent manner. Moreover, supernatants of L. acidophilus, L. lactis and L. reuteri were able to increase significantly the basal production of anti-inflammatory IL-10 in PBMC. Propolis extracts were able to inhibit the TNF-α and IL-1β secretion by human leukocytes stimulated with LPS. It is important to highlight that cell viability was not affected by LPS-stimilation and/or cell-free supernatants or propolis treatments.
All strains, except for L. reuteri, affect HT-29 cell proliferation: cell-free supernatants significantly increased the growth rate of cells after 48-h of incubation.
Furthermore, we observed that the addition of prebiotics did not promote probiotic effects in any activity tested.

Discussion:
Our results indicated that probiotic supernatants and propolis could effectively scavenge free radicals. Both propolis and probiotics have shown anti-inflammatory property. The modulation of pro- and anti-inflammatory cytokines expression is probably due to metabolites produced by probiotics during their growth. This activity suggests that a synbiotic combination of metabolites of cell-free supernatants and propolis could be used as adjuvant therapy in gut inflammatory diseases.

Keywords: Probiotics, Prebiotics, Propolis, Anti-inflammatory activity, Anti-oxidant activity, Regenerative activity
A CONSERVED SOLUTE BINDING PROTEIN RECOGNIZES A-(1,6)-OLIGOSACCHARIDES AND PROVIDES EVIDENCE OF ATP- TRANSPORTER MEDIATED UPTAKE OF THESE DIETARY GLYCANS IN BIFIDOBACTERIUM

Ejby M.; Fredslund F.; Andersen J.; Svensson B.; Slotboom D.; Abou Hachem M.
Technical University of Denmark

Introduction:
Introduction-Glycan utilization plays a key role in establishing and modulating the composition of the highly diverse and dynamic microbial community referred to as the human gut microbiota. Oligosaccharide transporters are likely to play an important role in the competition for energy sources, as the intracellular (or periplasmic) accumulation of oligosaccharides prevents loss to competing organisms. Despite the competitive advantages of specialized oligosaccharide-uptake by gut bacteria, molecular insight for most oligosaccharide transporters in the gut niche is largely unexplored. Here we characterize the solute binding protein (BIG16BP) of an α-(1,6)-oligosaccharide ABC-transporter from Bifidobacterium animalis subsp. lactis BI-04 and discuss the utilization preference of α-(1,6)-oligosaccharides derived from diet and the role of this ABC-transporter in competition within other gut microbiota taxa.

Methods:
Methods-The gene encoding the solute binding protein BIG16BP (Balac_1599) was cloned and the resulting recombinant protein was produced in Escherichia coli and purified to electrophoretic homogeneity. The Affinities of immobilized BIG16BP towards oligosaccharide ligands were measured using surface plasmon resonance (SPR). Binding energetics of raffinose and panose were also measured using isothermal titration calorimetry. The structures of BIG16BP in complex with either panose or raffinose were determined to a maximum resolution of 1.4 Å by X-ray crystallography using single-wavelength anomalous diffraction (SAD) experimental phasing of selenomethionine labelled BIG16BP in complex with raffinose.

Results:
Results- BIG16BP is able to recognize α-(1,6)-linked glucosides and galactosides of varying size, glycosidic linkage and monosaccharide composition, with a preference for trisaccharides. This preference corresponds well to the in vivo uptake preference of Bifidobacterium animalis subsp. lactis BI-04. Crystal structures of BIG16BP in complex with the tri-saccharides raffinose and panose provide an explanation for this remarkable plasticity. BIG16BP recognizes the non-reducing α-(1,6)-diglycoside motif in its ligands, while an open binding-site architecture lacking further interactions allows promiscuous ligand accommodation beyond this motif.

Discussion:
Discussion- Diet is a major effector of the composition of the gut microbiota and the metabolism of glycans has been highlighted as extremely important in maintaining a healthy bacterial community. Raffinose family oligosaccharides (RFO), containing α-(1,6)-galactosides are abundant in soybeans and other legumes and seeds, but are non-digestible by humans. Similarly, isomalt-o-oligosaccharides (IMO) comprising α-(1,6)-gluco-oligosaccharides derived from breakdown of starch and the bacterial exopolysaccharide dextran are resistant to degradation by humans. Both these classes of α-(1,6)-oligosaccharides are selectively fermented by bifidobacteria and gut-adapted lactobacilli. Homologs of BIG16BP occurs predominantly in gut adapted bifidobacteria and distinct Firmicutes providing a possible explanation for the selective utilizations of α-(1,6)-linked glucosides and galactosides by these taxa. These findings highlight the role of oligosaccharide-transport in defining the metabolic specialisation of gut bacteria and offer a framework for design of prebiotics based on mapping of the optimal ligands for oligosaccharide transport systems.

Keywords: Glycan utilization, Bifidobacteria, Carbohydrate transport, Prebiotics, Molecular microbiology

DEVELOPMENT OF A MOLECULAR QUALITY CONTROL FOR A COMMERCIAL PROBIOTIC FORMULA

Ferreira S.; Vauvy G.
GENOSCREEN

Introduction:
The quality control of probiotic in foods traditionally has relied solely on tests to ensure that an adequate
number of viable bacteria are present in the products throughout their shelf lives. Viability is an important factor, but not the only criterion for quality assurance. The literature has already shown that many commercial probiotic preparations lack necessary quality control, as often the probiotic preparations are not only non-viable but also incorrectly identified or, worse, contain microorganisms not recognized as probiotics. Thus, there is a need for strain-specific methodologies to assure consumers of product quality. We here report the development of a method for the strain-specific identification and quantification of five different probiotics mixed in a unique marketed formulation.

**Methods:**
The five strains of probiotics (L. casei, L. acidophilus, L. rhamnosus GG, B. lactis and S. boulardii) constituting the marketed formulation were obtained separately from suppliers as well as the commercial capsules with their exact probiotic composition. Five strain-specific designs were carried out to detect and quantify the strains of interest through a qPCR method. Using an internal optimized protocol, gDNA was isolated from each lyophilized probiotic, from the commercial caps and from an artificial mix made according to the exact composition of the commercial caps. Each separate strain-specific DNA was then used to verify the proper PCR amplification, build and validate each calibration range and ensure the absence of cross-reactivity of the qPCR between strains.

**Results:**
Four assays distinguished its specific strain from the other bacteria regardless of whether the DNA was isolated from pure lyophilized strain, the artificial mix or the commercial caps. The calibration curves for each four strain could be established and therefore used for the quantitative control of the strains present in the commercial formulation. On the contrary, the qPCR assay specific for L. acidophilus strain was not effective at all, even on the pure gDNA extracted from the lyophilized form of the probiotic. A second qPCR assay was then designed on another genomic region and tested. Similarly, no detection was obtained for this specific probiotic strain. Two unsuccessful strain-specific qPCR designs being unexpected, we performed two additional molecular controls: the Sanger sequencing of the qPCR targeted region and 16S gene to further confirm strain identity. Surprisingly, we discovered that the qPCR targeted region was much mutated (~19%) compared to an L.acidophilus reference. Comparison of this qPCR specific region and 16S gene sequences to public database highlighted that the strain identified as L. acidophilus is actually a L. johnsonii.

**Discussion:**
This study further confirm the efficiency of strain-specific molecular methods in the quality control of marketed formulation of mixtures of probiotics as it allows strain-specific identification, detection and quantification but also detection of misspelled bacteria.

**Keywords:** Molecular Biology, Quality control, Probiotic quantification, Probiotic identification, qPCR

---

**IN VITRO SELECTION OF A PROBIOTIC POTENTIALLY USEFULL IN THERAPY OF INFLAMMATORY BOWEL DISEASES**

**Fonteyn F.; Didderen I.; Thonart P.; Louis E.**
Department of gastroenterology, CHU of Liège, Sart Tilman, B35, 4000 Liège, Belgium

**Introduction:**
Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the gastrointestinal tract including ulcerative colitis and Crohn’s disease. The exact causes of these immune-mediated inflammatory diseases are unclear. It is thought that several factors may play a part, including immune dysregulation against host intestinal microflora.

Probiotics have been tested in several trials. Results have generally been disappointing with the exception of the treatment of pouchitis with VSL-3 a broad mix of highly dosed probiotics and E coli Nissle 1917 to treat ulcerative colitis.

The previous inconsistent results of probiotics approach may partly be linked to insufficient characterisation of the strain used leading to an inappropriate choice of these strains. The aim of this study is to evaluate a range of lactic acid bacteria (LAB) for the development of a combination of probiotic strains with demonstrated properties.
Methods:
Sixteen Lactobacillus strains, Lactococcus lactis and Streptococcus thermophilus coming from THT s.a. collection (Isnes, Belgium) were tested for acid resistance, bile salts resistance, adhesion of Caco-2 cell and cytokines production by whole blood cell cultures from patients with IBD. Statistical analysis of results was performed.

Results:
A large variability was observed among the strains tested with regard to their viability after incubation at low pH, their ability to grow in the presence of bile salts and adhere to human intestinal epithelial Caco-2 cells. The acid resistance of strains Lb. rhamnosus LR02 and Lb. plantarum LP03 is 51.91% ± 39.61 and 81.53% ± 9.81 respectively at pH2.5 after 3 hours of incubation. These same strains tolerate the maximum concentration of 20 g/L of bile salts for growth. The highest levels of adherence to human intestinal epithelial Caco-2 cells have been observed with four strains including LP03. LR02, Lb. casei LCa and LP03 also significantly increased production of IL-10 cytokine from whole blood cell cultures from patients with IBD.

Discussion:
Despite the development of medications to treat Crohn’s disease and the ulcerative colitis, there are currently no medical cures for IBD. LAB have emerged as serious candidates to fill a role as therapeutic option. Nevertheless, the evaluation of potential probiotics to influence the human immune system has to be considered carefully. Validation of probiotic contents in commercial products is a preliminary step to the realization of a clinical trial and is needed to generate data confirming the benefits of using these products. Our results demonstrate that ProbioTer might have beneficial effect on IBD, suggesting a possibility for a clinical trial.

Keywords: Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus, Acid resistance, Bile salts, Caco-2 cells, Cytokines

COMPARATIVE ASSESSMENT OF DIFFERENT MODEL-METHODS USED FOR THE ESTIMATION OF PREBIOTIC EFFECTS OF IN VITRO DIGESTED PASTAS

Füstös Z.; Antal O. T.; Naár Z.; Kiss A.; Némedi E.
NARIC FSRI (National Agricultural Research and Innovation Center Food Science Research Institute)

Introduction:
Nowadays consumption of healthy foodstuffs are becoming widespread, but a lot of people frequently consume ready-to-eat or semi-ready-to-eat foods. Among these products pastas play a key role, thus it is of great significance to provide pasta-based foodstuffs with justified health-promoting effect. Prebiotic index (PI) is considered as a reliable measure of prebiotic effect, however several distinctive methods are available. Our major goal was to accomplish comparative assessment of the most frequent PI-estimating methods, and point out the most relevant one. Hence an efficient tool is gained for estimation of the prebiotic content of selected foodstuffs, whose prebiotic effect is featured in highly authentic way. In this study prebiotic effects of pastas prepared from different flour-combinations were evaluated by using 5 distinctive methods, subsequent to in vitro digestion, modelling the real human metabolism.

Methods:
In total 8 distinctive pastas and 21 pasta-fillings were analysed. Pastas were prepared from wheat, buckwheat, amaranth flour, supplemented with CaCO3, MgCO3, and vitamin D. Self-developed in vitro digestion model was applied to implement the studies (NAIK-ÉKI model) based on Versantvoort et al. (2005) protocol, but supplemented with a colon modelling phase. Colon phase was constructed by inoculating the digested samples with a bacterial mixture made of Bifidobacterium, Lactobacillus, Escherichia coli, Clostridium and followed by a 24-hour-long anaerobic incubation. Changes in bacterial populations were measured by plate counting on selective media. PIs were calculated by using the Palframan et al. (2003), logarithmic values of Palframan et al. (2003), Olano-Martin et al. (2002), Vulevic et al. (2004) and NAIK-ÉKI model equations.
Results:
According to PIs, with the exceptions of Palframan et al. (2003) equation based PIs, it can be observed that pastas supplemented with buckwheat and amaranth flours have positive prebiotic indices (0.28-0.33). Similar results were obtained for fruit jam fillings, supplemented with vitamin D (0.33). Rest of the samples showed mainly negative values (-0.06 - -1.09) or close to zero (0.03-0.09). PI values calculated with Palframan et al. (2003) and logarithmic values of Palframan et al. (2003) equations were higher in absolute terms (0.07-13) than that of the others (0.01-1.09). Likewise, in some cases Palframan et al. (2003) equation gave negative values for samples showed positive values when using the other equations. The remaining three equations provided nearly the same values for all samples.

Discussion:
Our results suggested that pastas prepared from different flours and filled with fruit jam, could exert considerable prebiotic effects in the human colon. Taking into account the different equations used for PI calculation, it can be concluded that most of them, except Palframan et al. (2003) equation, can be used for assessment of experimental data gained by the NAIK-ÉKI digestion model, since they are showing similar trends for all samples. We suggest that Palframan et al. (2003) equation should be used with precaution, as with this model PI can not be significantly influenced by the other three bacteria if one bacterial strain is represented in high ratio at the end of the experiments.

This work was supported by the project AGR_PIAC_13-1-2013-0084

References:

COMPARISON OF A MIXTURE OF PROBIOTICS AND ITS COMBINATION WITH BUCKWHEAT BRAN AND HERBALS FOR PIGLET IMMUNE SYSTEM, INTESTINAL MICROFLORA AND PREVALENCE OF RESISTANT ESCHERICHIA COLI

Gåliņa D.; Valdovska A.
Faculty of Veterinary Medicine, Latvia University of Agriculture

Introduction:
For young piglets, probiotics stimulate the development of healthy microbiota, prevent colonization of enteric pathogens, improve mucosal immunity (Lange et al., 2010) and the antagonistic impact on the transfer of antibiotic resistance genes (Pomorska-Mól et. al., 2013). The aim of our study was to find out impact of probiotics, prebiotics (buckwheat bran) and herbs on piglets’ immune system, intestinal microflora and prevalence of resistant Escherichia coli.

Methods:
A total of 44 piglets from 14 to 56 days of age were allocated to four groups (11pigs/pen): basal diet contained 803mg zinc from analytical grade ZnOkg⁻¹feed (group C), basal diet + ProbioHelp (group P), basal diet + ProbioHelp + 3% buckwheat bran (group PB) and basal diet + 1.5% herbal mixture: Urtica dioica : Plantago major : Hypericum perforatum (group H). On day 56, piglets (four from each group) were slaughtered. During the experiment blood and serum (n=45), faecal (n=67) and intestinal content from jejunum were collected. Faeces and digestive contents were used for enumeration of Enterobacteriaceae, E.coli and Lactobacillus spp. A total of 90 E.coli isolates (80 isolates from faeces and 10 isolates from jejunum) were tested for phenotypic of resistance screening to 12 antibiotics via agar disc diffusion and screened for zinc tolerance using an agar dilution assays.
Results:
The count of WBC and monocyte was significantly decreased in group PB, ASAT - in groups P and PB, but ALP - only in group PB compared to group C. The level of CRP in groups C, P, PB, and H were 15.22±6.22; 19.03±2.85; 10.24±2.05; 10.35±2.25mg/mL, respectively. The supplement ProBioHelp significantly increased the counts of Lactobacillus in group P (lg7.75±0.24) in the jejunum of piglets compared to the group C (lg6.26±0.81), but the total count of Enterobacteriaceae and E.coli was lower in the groups P, PB and H. In the faeces of piglets, the count of Enterobacteriaceae and E.coli significantly decreased only in the group H (lg3.07±0.46 and lg3.05±0.45) compared to the group C (lg5.19±0.70 and lg4.94±0.60). After feeding of 6 weeks the highest resistance to tetracycline was observed in groups P, PB and H (50%;50%;44%, respectively), trimethoprim in group P (50%) and chloramphenicol - in group C (38%). Resistance to cefotaxime (6%) was observed only in group C. All E.coli isolates showed MIC between Zn >4 and <8mM/mL.

Discussion:
Piglets fed with ProBioHelp had significantly increased populations of Lactobacillus in the jejunum, despite the fact that the basal diet contained high levels of zinc. The significant reduction of the count of E.coli in the faeces of group H can be explained by antibacterial effect of herbals. Also E.coli could be inhibited by buckwheat (Coman et al., 2013). It is known, that zinc promotes the spread of antimicrobial resistance (Bednorz et al., 2013) that explained the high prevalence of E.coli resistance in all study groups. Probiotics and herbals could have the role of resistant gene transfer mechanism, based on the range of different resistance profile of different study groups.

Keywords: Piglet, Probiotic , Herbals, Intestinal microflora, Immunity, Resistance, Zinc

THE PROCESS OF PREPARING MICROBIOLOGICAL MEDIUM CONTAINING BENEFICIAL MICROORGANISMS ENSURING PRODUCT QUALITY AND STABILITY

Gebler I.; Plaza G.; Powlowski S.; Chojniak J.
Institute for Ecology of Industrial Areas

Introduction:
Nowadays more and more attention is paid to natural products, ecological lifestyle and healthy food. Probiotics play an increasingly important role in healthy eating, body care and now in agriculture. Together with growing public awareness one can observe an increasing demand for biologically active products containing beneficial microorganisms. The present study focuses on describing the process of preparing a product containing live microorganisms.

Methods:
In this experiment there have been used three strains of microorganisms (E41, E42, R85), isolated, examined and identified in the project called acronym: Phyto2Energy (call identifier: FP7-PEOPLE-2013-IAPP). Having selected and chosen proper microorganisms, the next step of preparing a innoculum was adjusting appropriate substrate that would enable the growth of all the microorganisms used and ensure their stability. The experiment used waste from the agri-food industry such as: sugar cane molasses, sugar beet molasses, apple extract and original combinations of microbiological medium. The choice of substrates was dictated by the efficient growth of the microorganisms as well as economic aspects. As an additional component, 1-2% inulin was used as the form of a prebiotic. High-quality microbiological product preparation required conducting a number of bioreactor cultures controlling the following parameters: temperature, stirring speed, aeration, pressure and liquid volume. In order to assess the quality of the preparations, the bacteria growth was evaluated by the following methods: measurements of optical density, cytometry analysis and classical plating cultures. During cytometry analysis cells count and their liveliness using BacLight™ RedoxSensor™ Green Vitality Kit and propidium iodide was determined.

Results:
The conducted analysis showed that the presence of inulin in bacterial cultures as a prebiotic affected the quality and stability of the produced inoculum positively in all cases. The use of agri-food industry waste can only be applied in this case after the sterilization process because indigenous microflora tends to displace introduced microorganisms. The research showed that sugar cane molasses can be successfully used to prepare inoculum.
Discussion:
It is certain that the use of dietary products containing pro- and prebiotics has a positive effect on human health. However, the surrounding environment is essential for life and health of living organisms. That is why the use of beneficial microbes in agriculture and environmental protection can have a huge impact on improving the health of humans and animals. Therefore, the use of waste substrates, for instance from agri-food industry, to produce microbiological preparations dedicated to agriculture while ensuring their high quality and stability appears to be a huge chance for balanced economic development having a direct impact on human health.

Keywords: Quality Assurance and Stability, Inoculum, Prebiotic, Bioreactor cultures, Cytometer analysis

CELL-SURFACE HYDROPHOBICITY AND ADHESION ABILITY TO HUMAN EPITHELIAL CELL LINE OF INDUSTRIALLY IMPORTANT LACTIC ACID BACTERIA AND BIFIDOBACTERIA

Georgieva R.; Danguleva A.; Stefanova Todorova N.; Karapetkov N.; Rumyan N.; Karaivanova E. Lactina Ltd.

Introduction:
The rapid growth of probiotic market stimulates more profound examination of already existing probiotic strains or searching of new ones for different applications. Adhesion to host tissues, in particular to epithelial cell line, is a well established probiotic criteria as it provides prolonged persistence in the gut and more efficient host-microbial interaction. Cell-surface hydrophobicity is often considered as the primary factor influencing the strength of bacterial adhesion. In the present study the cell-surface hydrophobicity of 40 lactic acid bacteria and bifidobacteria was measured and the bacteria’ capacity to adhere to human epithelial cells was assessed.

Methods:
Two groups of Lactobacillus, Bifidobacterium and Streptococcus thermophilus strains from the microbial collection of Lactina Ltd were tested: (i) 22 industrially important strains included in different health formulas and (ii) 18 new potentially probiotic strains interesting for future application. Bacterial cell-surface hydrophobicity was assessed by measuring microbial adhesion to hydrocarbons (MATH) using hexadecane and the percentage of hydrophobicity (H%) was calculated. Human adenocarcinoma cell line HT-29 (ATCC® HTB-38TM) was used for the adhesion assay. The results were expressed as: (i) percentage of adhesion (A%) calculated after determination of cfu/ml of initial and adherent cells on appropriate agar media and (ii) adhesion index- average number of adherent cells in 20 microscopic fields. Bacterial strains were scored as nonadhesive (<40 bacteria), moderate adhesive (41-100), highly adhesive (101-200) and strongly adhesive (>200 bacteria).

Results:
On base of cell surface-hydrophobicity and the adhesion to HT-29 cell line most of the strains were scored as adhesive (77 %), as strains with moderate to high adhesion were the dominant part of the group. Four L. casei/rhamnosus (LLC-J31; LLC-115; LLC-4k; LLRh-V410;), two L. fermentum (LLF-01; LLF-43) strains and 1 Str. thermophilus (LST-Rt4) demonstrated strong adhesion index. Weak adhesion was registered for most L. bulgaricus and L. reuteri strains. All strains (35%) characterized as hydrophobic (H>45%) adhered well to the cell line, but 47 % of the hydrophilic strains (H<17%) including L. helveticus, L. bulgaricus, L. lactis and Str. thermophilus strains were also estimated as good adhesive. Three of the strains into the latter (hydrophilic) group (LLH-108; LLL-14 and LLB-02) showed among the highest percentages of adhesion.

Discussion:
The combined approach for studying the adhesive ability of selected lactic acid bacteria and bifidobacteria gave us good differentiation between nonadhesive and adhesive strains, and also allows discrimination between intermediate (less and more) adhesive strains. Good correlation between adhesion capacity and cell-surface hydrophobicity for hydrophobic strains could be used as precondition for selection of adhesive probiotic cultures in future work. However, the high adhesion capacity of certain hydrophilic strains demonstrates that surface hydrophobicity is not the sole crucial determinant of bacteria-gut epithelium interaction. Therefore, for hydrophilic strains the MATH method should not be used as an estimate for adhesion capability. The obtained valuable information for the possible colonization of human intestine will help for optimization the composition of different probiotic products including strains with more beneficial effects.

Keywords: Probiotic, Adhesion, Hydrophobicity, Lactic acid bacteria, Bifidobacteria
EFFECTS OF EPS-PRODUCING LACTOBACILLUS PARAPLANTARUM BGCG11 ON STREPTOTOTOZIN INDUCED DIABETES IN RATS

Golić N.; Živković M.; Djordjević M.; Rajić J.; Tolić A.; Tolinački M.
Laboratory for Molecular Microbiology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia

Introduction:
Diabetes mellitus type 1 (T1D) is a group of metabolic disease which main characteristic is hyperglycaemia. It has recently been shown that T1D develops only in genetically predisposed infants that have dysregulated lipid metabolism. Some of the significantly disturbed metabolic markers (succinic acid and choline) are metabolised by GI microbes, and it has been hypothesized that in addition to genetic factors, GI microbiota plays a crucial role in the onset of T1D. It is believed that intestinal colonization with certain bacteria strongly influences systemic immune responses early in life and may play a significant role in modulating the development of various chronic diseases. Experiments with animal models, have shown that GI microbiota is an important factor in development of T1D. The strain Lactobacillus paraplantarum BGCG11 produces the exopolysaccharide EPS-CG11 with high immunomodulatory potential. Specifically, the purified polymer EPS-CG11 elicited the increased production of the IL-10 and IL-1β, downregulating the immune response.

Methods:
Experiments were performed on 2.5-month-old adult albino Wistar rats. All animal procedures were approved by the local Ethical Committee (IBISS). Diabetes was induced by streptozotocin. Rats were randomly divided into four groups: (i) non-diabetic group (n=8); (ii) non-diabetic group (n=8) with orally administered L. paraplantarum BGCG11; (iii) diabetic group (n=8); (iv) the diabetic group (n=4) with orally administered L. paraplantarum BGCG11. After 4 weeks of treatment with probiotic, the rats fasted overnight and blood serum was collected to determine serum total cholesterol, triglyceride, blood glucose levels. Liver function was evaluated by determining the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Kidney function was evaluated by measuring blood urea nitrogen (BUN). The level of DNA damage in liver and kidney was examined using the alkaline Comet assay. The pancreas from all experimental groups were removed and fixed in 10% buffered formalin for histological and immunohistological examination.

Results:
With the aim to develop novel strategies for prevention and/or therapy of T1D, we tested the influence of probiotic strain L. paraplantarum BGCG11 on the health parameters of streptozotocin-induced diabetic rats. The results obtained in this study showed that consumption of the strain BGCG11 regulates glucose levels in serum and restores pancreatic islets in diabetic rats. The level of blood sugar, BUN, as well as AST and ALT levels from liver were reduced when the rats were fed with BGCG11 strain resuspended in milk. Moreover, the improvements in the crypts architecture in duodenum and the Langerhans islets in pancreas were noticed, as well as less damage in DNA level of liver and kidney cells in the diabetic rats fed with BGCG11. The results indicate that BGCG11 is able to ameliorate the effect of induced diabetes in rats.

Discussion:
Lactobacilli may help in the prevention and treatment of diabetes by immunomodulation, preventing the inflammation and thus the destruction of insulin-producing beta cells of the pancreas.

Keywords: Diabetes mellitus type 1, Lactobacillus paraplantarum BGCG11, Exopolysaccharide EPS-CG11, Wistar rats, Immunomodulation

IN VITRO SCREENING OF LACTIC ACID BACTERIA ISOLATED FROM KOREAN FERMENTED FOODS TO CONTROL SALMONELLA ENTERITIDIS

Gwon G.; Jeong S.; Hong W.; Kim K.; Song C.
Konkuk university

Introduction:
Salmonella has been significant cause of food poisoning in humans and contaminated eggs and chickens have been thought to be an important source of its infection. The use of Lactic acid bacteria (LAB) has been
suggested as an effective strategy to reduce salmonella infection in poultry industry. In this study we tested Lactic acid bacteria isolated from Korean fermented foods for their viability in the gastrointestinal tract and their inhibitory capacities against salmonella enteritidis.

**Methods:**
LAB were isolatd from Korean fermented foods (Kimchi and Doenjang). Kimchi and Doenjang samples were homogenized with MRS broth and cultured anaerobically for 48h at 37°C. The colonies that showed LAB morphologies were selected. For gastric juice assay, 4mg/ml pepsin was added into phosphate buffered saline adjusted to pH2.5. to test bile tolerance, 0.3% bile salt was added to the MRS broth. Each isolated lactic acid bacteria was inoculated into the artificial gastric juice and bile solution and incubated at 37°C. Viable bacteria cells were counted at 0 and 3h for the gastric juice tolerance and 0 and 24h for the bile tolerance. Disk diffusion method was followed for susceptibility test. Mueller Hinton agar was swabbed with trypic soy broth containing Salmonella enteritidis. A paper disc (diameter, 6mm) was soaked with filtered supernatant of lactic acid bacteria and the paper disc were placed on the surface of plate. The plates were incubated at 37°C for 24h and were examined for clear inhibition zones around discs. The 16S rDNA sequencing was used to identify the genus and species of the LABs, the sequences were analyzed with the BLAST program of the National Center for Biotechnology Information.

**Results:**
A total 22 of LABs were isolated from Korean fermented foods (11 isolates : Kimchi, 11 isolates : Doenjang). 9 of 11 LABs isolated from Doenjang showed tolerance to both gastric acid and bile salts. Whereas 3 of 11 LABs originated from Kimchi showed tolerance to them. In antimicrobial activity assay, Several LABs showed antimicrobial activity against Salmonella enteritidis. The species of the LABs were distinctively classified depending on the Kimchi and Doenjang origin. Kimchi isolates belonged to genus Lactobacillus and Leuconostoc. Whereas from the Doenjang, genus Bacillus, Enterococcus, Sporolactobacillus, Pediococcus were identified.

**Discussion:**
In this study, depending on the origin (Kimchi or Doenjang), various LABs were isolated and showed different characteristics. LABs isolated from Doenjang were more resistant to gastric acid and bile salts than those from Kimchi. It is possible that genus of LABs isolated from doenjang may have more resistance to bile and gastric juice than those from kimchi. Further study about relationship between genus of LAB and viability in the gastrointestinal tract is needed.

This study also showed that Lactic acid bacterias isolated from Korean fermented foods (Kimchi and Doenjang) have antimicrobial activity inhibiting salmonella spp.

We think that it can be used as supplementation to reduce salmonella infection in poultry industry.

**Keywords:** Antimicrobial activity, Korean fermented foods, Salmonella enteritidis, Lactic acid bacteria, Poultry

---

**CHRONIC TRICHURIS MURIS INFECTION DRAMATICALLY ALTERS THE MURINE INTESTINAL MICROBIOTA**

**Holm B. J.** 1, Sorobetea D. 2, Kiilerich P. 1, Ramayo Caldas Y. 3, Jordi Estelle J. 3, Ma T. 1, Madsen L. 1, 4, Karsten Kristiansen K. 1, Svensson-Frej M. 2

1 Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, Copenhagen, Denmark.
2 Immunology Section, Department of Experimental Medical Sciences, Medical Faculty, Lund University, Lund, Sweden.
3 UMR1313 Génétique Animale et Biologie Intégrative (GABI) Equipe Génétique Immunité Santé (GIS), INRA, Jouy-en-Josas, France.
4 National Institute of Nutrition and Seafood Research, Bergen, Norway.

**Introduction:**
The parasitic worm *Trichuris muris* is the murine-specific counterpart to the human pathogen *Trichuris trichuria* that infects approximately 500 million people globally. Given that *T. muris* larvae inhabit the same niche as the majority of the intestinal microbiota it is plausible that these organisms influence each other, which may
subsequently impact on the intestinal microenvironment of the host. In the present study we have investigated the effect of chronic *T. muris* infection on the gut microbiota and immune response in mice. C57BL/6 mice were either infected with a low dose of *T. muris* eggs by oral gavage or left uninfected. Colon content was sampled after 0, 13, 20, 27, 35 days of chronic infection corresponding to the larvae molting stages. Microbiota analysis was performed using 16S-rRNA gene-based sequencing of the V4 region. *T. muris* infection induced clear changes in the microbiota by decreasing alpha- and gamma diversity, and increasing beta diversity. Multiple bacteria families were affected by the infection, most notably with a marked increase in the relative abundance of *Lactobacillaceae*, supporting a previously suggested mutualism between nematodes and *Lactobacillaceae*. In addition, chronic *T. muris* infection induced an early T helper cell type 1 (Th1) response and affected the balance between inflammatory and regulatory immune cells in the intestinal mucosa. Taken together, our data demonstrate that chronic infection with the nematode *T. muris* results in a significantly altered intestinal microbiota. These findings are of importance and relevance given the high incidence of helminth infections worldwide and the current efforts to use parasitic nematodes to treat immune-associated diseases.

**Keywords:** Trichuris muris, Intestinal microenvironment, Lactobacillaceae, Chronic *T. muris* infection

### DEVELOPMENT OF PUTATIVE PROBIOTICS WITH TARGET-SPECIFIC VALIDATION

**Huh C.; Yeo S.; Lee S.**
Institute of Green-Bio Science & Technology, Seoul National University

**Introduction:**
Since it was revealed the growth-promoting effect of sub-therapeutic levels of antibiotics in 1940s, antibiotics have been widely used in livestock production. However, the extensive use of antibiotics caused the occurrence of antibiotic-resistant pathogenic bacteria and the risk of transference of antibiotic-resistance genes from animal to human. Consequently, antibiotics as growth promoters (AGPs) in animal production has been banned in the European Union since 2006, and the development of alternatives to AGPs as feed additives has increased.

Pig is a very influential livestock in the world food market and economy as 2 billion of pigs are supplied per every year. The pig industry was a representative farming influenced by the AGPs, however, after the prohibition, the world average loss and its ratio to the total distribution has been decreased even without AGPs. To the consequence, many of nutritional strategies and additives have been suggested as alternative. Probiotic lactic acid bacteria (LAB) which is currently a major alternative feed additive for livestock has been reported to be able to inhibit non-commensal microbes and modulate the microbiota in the human gastrointestinal tract (GIT). With several reports about that the gastrointestinal flora is related to animal health and growth performance, LAB has scientific basis for treatment of various disease of animals such as diarrhea caused by pathogen colonization.

However, many of the previous probiotic strains applied for AGPs alternative were isolated from human, and verified by human-based in vitro screening methods which are not enough to reflect the physiology, immune system, and gastrointestinal microbial community of the host animal. Therefore, it is necessary to consider and develop a host target-specific probiotics and its adaptive in vitro screening, thereby the compatibility of the characterized strain is expected to show enhanced in vivo efficacy when it is administered to livestock as feed additives.

**Methods:**
In the present study, we have developed a pig host target probiotic strain. We confirmed the availability of two conventional methods for selecting putative probiotic strain as feed additives, using *Lactobacillus salivarius* strains isolated from fed-pig feces and *Lactobacillus rhamnosus* GG commercial-probiotics originated from human intestine. We compared the viability of the strains during passage through the in vitro human and porcine GIT models. We also observed the different adhesion ability of each strain on a porcine intestine cell line (PSI) and a human intestine cell line (Caco-2).

**Results:**
The results showed significant differences in GIT survival and adhesion ability when comparing the probiotic strains for human and porcine with target different in vitro model conditions. We suggest that the
target-specific screening and validation are important to develop probiotic feed additives, and this approach may support future-oriented agriculture.

**Discussion:**
In conclusion, although our suggested in vitro models are not enough to represent the physiology of human and pig, but it may be postulated that it is needed to develop target-specific validation method for screening probiotic candidates depending on the host of application. The applicability of our suggested models may provide a basis for the selection of pig probiotic candidates to be tested in vivo.

**Keywords:** Probiotics, Feed additives, Porcine, Gastrointestinal tract, Adhesion ability

---

**IN VITRO SCREENING OF LACTIC ACID BACTERIA ISOLATED FROM WILD BIRDS FOR FUTURE PROBIOTIC DEVELOPMENT**

**Jeong S.; Kwon J.; Gwon G.; Kim K.; Song C.**
Konkuk University

**Introduction:**
Intestinal microbiota plays a crucial role in maintaining health by helping nutrient utilization, inhibiting enteric pathogen colonization, and generating greater immune capacity. Since 2011, antibiotic supplement in animal feed has been completely banned in South Korea, and as a consequences, the need for probiotic agent with exceeding ability of promoting immune activity has been rapidly growing. Lactic acid bacteria isolated from fermented food is widely used for animal consumption in South Korea, but animal-originated LAB also has possibility of better host colonization and immune activation. The aim of present work is to evaluate the probiotic potential of lactic acid bacteria (LAB) strains isolated from wild duck and to select probiotic candidates for animal, especially for poultry use.

**Methods:**
Thirtyfour (34) lactic acid bacteria strains (11 Lactobacillus, 3 Enterococcus, 2 Weissella, 1 Pediococcus, 1 Streptococcus) were isolated from the intestinal tract (crop, proventriculus, small intestine and cecum) of wild duck (2 mallards, 2 common teal, 1 pin-tailed duck, 2 widgeons). 5 in vitro tests for screening probiotic potential were conducted - survival test in 2 artificial gastrointestinal conditions (pH 2.5 gastric acid with 4mg/ml pepsin, and 0.3% bile acid), antibiotic susceptibility test to 9 antibiotics (ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, tetracycline, chloramphenicol, clindamycin), antimicrobial activity test against Salmonella Enteritidis, and Caco-2 cell (human colon adenocarcinoma cell line) surface adhesion assay.

**Results:**
1 Lactobacillus strains survived at highest final population (>10^7 cfu/ml) and 2 survived at moderate high final population (>10^5 cfu/ml) after 3 hours of exposure at low pH. Most of the tested strains displayed partial to complete resistance to bile salt even after 24 hours of exposure. Tested strains exhibited variable abilities of adhesion to Caco-2 cells and variable susceptibility to 9 antibiotics. Some of the strains had considerable anti-Salmonella activity.

**Discussion:**
Totally, most of the wild bird intestinal tracts-originated lactic acid bacteria strains tested in this study exhibited poor resistance to low pH compared to early studied lactic acid bacteria strains isolated from fermented food with low pH condition like Kimchi or Toenjang (soy bean paste), traditional foods of Korea. But survival at low pH, bile acid tolerance, and intestinal cell adhesion ability of some strains are indicating that these strains can colonize in harsh condition of animal gastrointestinal tract successfully. Also, antimicrobial activity against Salmonella, which is one of the most causal agent of economic loss and public health problems in poultry markets, is indicating the great possibility of future probiotic use which can partially substitute antibiotics. Selected strains are in need of further investigations including in vivo studies about their probiotic functions in target animal to confirm health benefits and describe the mechanisms of action.

**Keywords:** Lactic acid bacteria, Probiotics, In vitro screening, Intestinal microbiota, Wild bird
PROTECTIVE EFFICACY OF PEDIOCOCCUS LOLLI AGAINST INFLUENZA A VIRUS

Ju H.; Youn H.; Kwon J.; Hong W.; Song C.
KCAV Co., Ltd.

Introduction:
Influenza virus infections continue to be a significant public health problem. Generally, the probiotics are considered as immune-modulator and increasing resistance against infectious disease. For improved therapies and preventive measures against influenza, there has been an increased tendency in modern medicine involving the use of probiotics. In particular, oral administration of lactic acid bacteria was proven to be effective in preventing influenza virus infection in mice, but there have been concerns about intact arrival of live bacteria at the intestine after passing through acidic condition of the stomach. Therefore, it has been suggested that intranasal administration of probiotics could be effective on protection against respiratory infection due to direct augment of the respiratory immune system. In this study, we compared the protective efficacy of various live lactic acid bacteria isolated from Korean fermented food (soybean paste [doenjang]) collected from different regions of Korea, and evaluated for their protective efficacy against the influenza virus infection in mice according to the administration route. Moreover, to understand the underlying mechanism behind this clinical protective effect, we performed immunologic assays including examination of IgA levels and cytokine profiles in the lung.

Methods:
We compared protective efficacy of probiotics from soybean paste against Influenza virus on mice administered by intranasal rout. Among probiotics which showing good result, we compared against the protective efficacy by oral administration against same virus. Finally, we selected probiotics showing good protective efficacy against Influenza virus on both of administration rout. In addition, to understand the underlying mechanism behind this clinical protective effect, we performed immunologic assays including examination of IgA levels and cytokine profiles(TNF-α, IFN-γ, IL-6, and IL-12) in the lung.

Results:
The survival rate of mice receiving intranasal administration of lactic acid bacteria was higher than after oral administration. The interleukin (IL)-12 and IgA levels in lung were significantly increased after intranasal administration. Conversely, the levels of the proinflammatory cytokines, including tumor necrosis factor-alpha and IL-6, were decreased. Interestingly, the protective efficacy of lactic acid bacteria on influenza virus infection was variable among different strains.

Discussion:
In conclusion, intranasal administration of live lactic acid bacteria provided higher protection against influenza virus infection by enhancement of secretory IgA production and down-regulation of pro-inflammatory cytokines in the respiratory immune system. Therefore, selection of effective strains could be critical and individually optimized application regimens of the selected strains are required. Further studies should consider effective strain selection in certain species because each lactic acid bacteria strains could provide a protective efficacy in a strain-dependent manner.

Keywords: Influenza, Mouse, Pedicoccus, Doenjang, Immune

EFFICACY AND TOLERANCE OF SIX LACTIC ACID BACTERIA STRAINS IN TRIALS WITH RABBITS

Karapetkov N.; Karaianova E.; Georgieva R.; Surdjiiska S.
Lactina Ltd., Bulgaria

Introduction:
The aim of the study was to demonstrate the efficacy and tolerance of a probiotic preparation based on viable cells of the strains Lactobacillus acidophilus NBIMCC 8242, Lactobacillus bulgaricus NBICCM 8244, Lactobacillus helveticus NBIMCC 8269, Lactobacillus lactis NBIMCC 8250, Streptococcus thermophilus NBIMCC 8253 and Enterococcus faecium NBIMCC 8270 by targeting sensitive parameters in comparison to a negative control group and by evaluating the short-term toxicity of the strains. This broad range of bacteria was selected hopping to achieve an extensive beneficial effect in the target animals.
**Methods:**
The study included the offspring of twelve rabbit's mothers (Californian rabbit breed), which were aligned by age and weight. The experimental design consisted of four groups: one control without supplementation of the probiotic feed additive and three experimental units (300, 500 and 700 g probiotic mixture/t feed). The weaning of the rabbits took place on the 35-th day, when their gender was determined and they were sorted in cages for fattening. The duration of the experiment was 77 days. The tolerance study included three groups: a control group and tolerance groups with the ten-fold of the hundred-level of the optimal recommended dose. During the trial, the following indicators were controlled:
  - Survival rate of the rabbits - daily by groups;
  - Bodyweight on first day, seventh day, 35-th day, at weaning, on 56-th day and on 77-th day;
  - Feed consumption per unit of growth;
  - Pathoanatomical examination of the carcasses and the internal organs.

**Results:**
The addition of six lactic acid bacteria strains to the feed of the animals led to a significantly higher bodyweight when applied at 700 g/t in the pelleted feed from birth to the weaning on 35-th day compared with the control group by 8.5% (P <0.05). The bodyweight of the rabbits at 56 days of age was also positively influenced. The difference of 7.3% compared to the control group was statistically significant (P <0.05). The feed consumption per 1 kg live weight gain in all experimental groups was lower than the control group by 12% to 14% (P <0.01). The tenfold and hundredfold increase of the recommended optimal dose of the feed additive did not adversely affect the taste of the feed, the appetite of rabbits and the daily feed consumption. The pathological examination conducted with the slaughtered rabbits showed no changes and negative effects. The use of the probiotic mixture is therefore presumed safe for the target species. During the test period there were no dropped out rabbits neither in the control group, nor in the other experimental groups.

**Discussion:**
The experiment showed that supplementation of the feed with the mixture of six lactic acid bacteria strains to the fodder of the animals has a potential to increase the live weight of the rabbits, to improve the feed conversion and decrease morbidity. All these effects are probably due to the modulation of the intestinal microbiota of the animals. Further studies are required to clarify the exact mechanism of action.

**Keywords:** Probiotic feed additive, Rabbits, LAB, Feed conversion, Live weight gain
osmotic pressure of LAB culture broth was analyzed. Also, we evaluated the survival rate of LAB during downstream processes as well as the stability of freeze-dried LAB.

**Results:**

As a results, we observed a correlation between the osmotic pressure and proline concentration of LAB culture environments. Also, when an amino acid such as proline were added to medium during or after the culturing process, it was shown that the supplemented amino acid could significantly increase not only the survival rate of LAB during freeze-drying but also anti-oxidant activity, storage stability and resistance to acid and bile salt of freeze-dried LAB.

**Discussion:**

It is considered that the increasing stability by addition of an amino acid especially proline is associated with high osmotic pressure adaptation and/or the protective effect of amino acid. Therefore, it seems that our improved technology is economic and advanced tool for a high productivity of LAB.

**Keywords:** Lactic acid bacteria (LAB), Amino acid, Proline, Freeze-drying, Storage stability

---

**ENTEROCOCCUS FAECIUM AL41 AND ITS APPLICATION IN HORSES**

**Kubašová I.**, **Lauková A.**, **Styková, E.**, **Plachá I.**, **Strompfová V.**, **Gancárčiková S.**

†Institute of Animal Physiology Slovak Academy of Sciences, Košice, Slovakia

‡University of Veterinary Medicine and Pharmacy, Košice, Slovakia

**Introduction:**

Horses microbiota as well as their optimization by the use of e.g. probiotic bacteria to maintain a good health condition is still open area for researchers but also for breeders or owners. In horses, disorders due to pathogenic bacteria involve ehrliosis, salmonellosis, clostridiosis or disorders caused by B group streptococci (Lavoie et al., 2000). It is well-known that probiotic bacteria can provide benefits in animals. Our Laboratory of Animal Microbiology has dealt with probiotic bacteria (especially those bacteriocins-antimicrobial substances producing) for years. Based on benefits achieved previously after application of Enterococcus faecium AL41 (our probiotic, Enterocin M-producing strain) in food-producing animals e.g. in rabbits or poultry (Lauková et al., 2012, 2014, 2015), we decided for its experimental application in horses.

**Methods:**

Eight mares and three horses-geldings of different breeds (Norik breed Muráň Plain, English hot-blooded, Slovak hot-blooded, Hucul breed) were involved in the experiment. They were fed twice a day with hay and oats, alternatively grazed. Experiment lasted for 14 days during autumn 2014. Faeces and blood were sampled at the start of experiment (day 0/1), then on day 14 (after 2 weeks of E. faecium AL41 application). Blood was sampled from vena jugularis. E. faecium AL41 was prepared as previously indicated Lauková et al. (2012)-109 cfu/ml. The dose of strain (1g per 1 animal per day) was applied into the diet, in the small part-bolus. Animals had the attitude to water ad libitum. Microbial analyses were performed according to ISO methods and media. AL41 strain was marked by rifampicin to differ it from the other enterococci on M-Enterococcus agar with rifampicin, confirmed by PCR and MALDI-TOF spectromerty. Phagocytic activity (PA), biochemical parameters were also tested.

**Results:**

E. faecium AL41 sufficiently colonized digestive tract of horses; on day 14 its average count was 2.35 (0.70) cfu/g (log10). The total enterococci reached 3.52 (0.73) cfu/g (log10) in average; lactic acid bacteria counted 5.62 (0.38) cfu/g. The counts of other bacteria were not high and they were not influenced by AL41 strain, except Aeromonas sp.; their significant decrease was noted (p<0.001). On day 14, PA increasing tendency was detected; an average PA value on day 0/1 was 73.13 (8.55) and on day 14 75.11 (8.66) %. Biochemical parameters were not influenced by AL41 strain or they were optimized in the physiological range.

**Discussion:**

In spite of the preliminary results, they have importance from the basic point of view (stability and colonization of strain AL41, PA). The originality of our study is own isolate, bacteriocin–producing, probiotic E. faecium strain applied in horses. VEGA 2/0004/14, VEGA 2/0012/16.

**Keywords:** Horses, Probiotic, Enterococcus faecium, Effect
ALGINITE AS SUITABLE COMPOUND FOR COMBINATION WITH LACTOBACILLUS FERMENTUM CCM 7421 IN DOGS

Kubašová I.; Strompfová V.; Farbáková J.; Maďari A.; Gancarčíková S.; Mudroňová D.; Lauková A.
Institute of Animal Physiology Slovak Academy of Sciences

Introduction:
Alginité is a loam-like material formed by an accumulation of organic material (algae) and inorganic material (mainly clays and volcanic materials) rich in minerals and trace elements (Litavec and Barančíková, 2013). So far it has been used to improve soil structure, moisture and nutrient content as well as growth of plants within agriculture and forestry (Kulich et al., 2001). Alginité has not been studied as feed additive in animals until now and therefore there are no information on its antimicrobial or physiological effects in organism. Our aim was to test effects of this organic rock alone or in combination with probiotic strain to avoid possible side-effects observed after long-term probiotic application resulting from overproduction of organic acids (Ku et al. 2006; Munakata et al. 2010).

Methods:
Healthy dogs (n=40, 25 males, 15 females) were devided in 4 experimental groups, 10 animals in each (C-control, A-alginite 0.3 g/kg BW/day, LF-Lactobacillus fermentum CCM 7421 1.2x108 CFU/dog/day, A+LF-both additives). The application period lasted 14 days; faecal samples were taken at day 0, 7, 14 and 35. For microbial analysis, standard ten-fold dilution method and selective media were used (MRS, MacConkey agar, M-Enterococcus agar, Becton and Dickinson; TOS-propionate agar, Merck; Clostridium difficile agar, Oxoid). Plates were incubated at 37 °C for 24-48 h, clostridia and bifidobacteria anaerobically for 48-72 h. Faeces was scored visually (from 1-hard to 5-liquid). Organic acid analysis was measured by capillary isotachophoresis (ZKI 01, Slovakia).

Statistic: repeated-measures ANOVA - Dunnett’s post test.

Results:
Oral supplementation with alginité for 14 days led to a significantly higher population of Clostridium-like bacteria during the treatment period compared to initial numbers at day 0 (P<0.05). In contrast, a decrease of Clostridium-like bacteria was detected in combined A+LF group (P<0.05) and in LF group (P<0.01). The population of coliform bacteria was decreased only in the A+LF (P<0.01) and with trend noted in the LF group (P=0.08) at day 14. The population of lactic acid bacteria increased in all experimental groups (P<0.05 in the A and LF group, P=0.09 in A+LF) at day 7 or 14. The faecal level of probiotic strain ranged from 103-106 CFU/g in the A+LF and in levels of 104-106 in the LF group. No effect of alginité or probiotic supplementation was noted on the consistency of faeces and faecal dry mater. The faecal pH values indicated a trend to decrease only in the LF group (P=0.08) with the lowest values detected at day 14.

Discussion:
It seems alginité has no antimicrobial rather the opposite stimulatory effect on the abundance of certain intestinal bacterial groups. Although no evidence on antimicrobial or stimulatory effect of alginité on the abundance of intestinal bacteria exist, an improvement of microbial activity indicators of forest soil (basal respiration, catalase activity) was reported by Gömöryová et al. (2009). Buffering or alkalinizing effect of alginité in combined group was observed and thus could contribute to maintenance of acid-base balance.

Keywords: Lactobacillus, Probiotic, Alginité, Microflora, Dog

RANDOMIZED CONTROLLED TRIALS TO STUDY THE EFFICACY OF PROBIOTIC LACTOBACILLUS RHAMNOSUS GG IN RESPIRATORY INFECTIONS

Kumpu M.; Pitkäranta A.; Korpela R.
University of Helsinki

Introduction:
Respiratory infections in healthy population account for significant part of total illnesses and thus incur significant personal and socio-economic burden. Probiotics have shown promise in reducing the respiratory infections, but data is still limited regarding individual strains and mechanistic aspects.
Methods:
A PhD project consisting of three double-blinded randomized controlled clinical trials (RCTs) in healthy children and adults was conducted with the aim of understanding potential of Lactobacillus rhamnosus GG (GG) in respiratory infection reduction, and to explore factors that might affect GG’s efficacy. In the first RCT, tonsillar recovery of GG was evaluated in young adult tonsillectomy patients after three weeks’ oral consumption of GG as a single strain or as a part of a multispecies combination, using strain-specific PCR. The second RCT, lasting 28 weeks, studied the effects of milk supplemented with GG on respiratory symptoms and on nasopharyngeal presence of 14 respiratory viruses in children attending day care. Third RCT, a 6-week pilot study using an experimental rhinovirus model, assessed the efficacy of live and inactivated GG on viral infections in adults. After three weeks intervention, subjects were intranasally inoculated with experimental rhinovirus.

Results:
GG was recovered in the tonsil tissue of 40% of the subjects in the GG group, 41% in the multispecies group and 30% in the control group. Most of the subjects in the control group with GG harboured from tonsil tissue had GG recovered from the fecal sample already at the start of the intervention. In children attending day care, occurrence of respiratory symptoms was similar in GG and control groups. Explorative analysis on the completed cases subgroup based on fecal GG recovery suggested that children in the GG group had one day less with respiratory symptoms per month compared to the control group. In a subgroup of children who visited study physician due to infection during the intervention, number of days with respiratory symptoms was lower in the GG group, but the presence of respiratory viruses and number of study physician visits did not differ between the groups. In the experimental rhinovirus trial, occurrence and severity of cold symptoms and number of subjects with positive rhinovirus culture and rhinovirus infection were lowest in the group receiving live GG, but differences between the groups were not statistically significant.

Discussion:
GG was overall ineffective in reducing respiratory symptoms or viral occurrence in the nasopharynx, but appeared to reduce symptoms in specific subgroups within the study cohort of children attending day care. Experimental rhinovirus model was demonstrated a potential controlled approach to studying effectiveness of probiotics in viral respiratory infections. Live strain of GG showed more promise compared to inactivated strain in reducing respiratory infection in adults, but further research is needed to confirm the trend observed in the pilot trial. There might be individual variation in the ability of GG to colonize tonsil tissue. Persistence of tonsillar colonization and its potential role in respiratory infection occurrence should be further investigated.

Keywords: Respiratory tract infection, Clinical trials, Lactobacillus rhamnosus GG, Respiratory virus, Respiratory symptoms

CROSS-REACTIVITY OF IGA ANTIBODIES INDUCED BY INTRANASALLY ADMINISTERED LACTOBACILLI AGAINST INFLUENZA VIRUSES

Kwon J.; Kim Y.; Kim K.; Yuk S.; Song C.
Konkuk university

Introduction:
Influenza virus infections continue to be a significant public health problem. Recently new subtypes of influenza virus represent a global pandemic threat. For improved therapies and preventive measures against influenza, the need for a broad-spectrum antiviral therapeutics such as probiotics has been increased. In the our previous study, the secretory IgA antibodies induced by intranasally administered Lactobacillus showed cross-reactivity with H1N1 influenza virus and seem to play a role in preventing the entry and replication of the influenza virus in the respiratory tract. In this study, we evaluated the cross-reactivity of induced by Lactobacillus against H1N1, H3N2 and H5N2 influenza viruses.

Methods:
Female specific pathogen-free (SPF) BALB/c mice (Orient Bio Laboratories, Seoul, Korea) weighing 18–20 g were used. For determination of secretory IgA concentration, mice were immunized intranasally with L. rhamnosus (108 colony forming units) or PBS for 10 days and bronchoalveolar lavage fluid (BALF) samples were collected. The level of influenza-specific IgA against each virus were measured by ELISA.
For evaluate the cross-protective immunity, mice were assigned to 7 experimental group (n=10 per group). Mice of 3 group received L. rhamnosus as described above before the viral challenge and challenged with H1N1, H3N2 and H5N2 influenza virus. Mice of 4 group received PBS in the same manner and challenged with H1N1, H3N2 and H5N2 as positive control and PBS as normal control. After challenge with influenza virus, survival rate and weight loss were observed daily for 14 days post-infection (p.i.) (30% loss in body weight as the IACUC endpoint).

**Results:**
Total IgA concentrations and anti-influenza virus IgA concentration in BALF from mice treated with 108 cfu of L. rhamnosus were significantly higher than that from the control group. In the mouse challenge study, administration of L. rhamnosus reduced the mortality in mice challenged with 3 subtype of influenza virus

**Discussion:**
Mucosal secretory IgA antibodies in the respiratory tract provide cross-protection against variant respiratory virus infections, which may confer higher antiviral effects than systemic IgG antibodies. Particularly, mucosal secretory IgA antibody is essential and crucial for immune protection against influenza virus infection. Previous studies demonstrated that Lactobacillus spp. characteristically stimulate the production of IgA antibodies and prevent invasive infection of pathogens. In the present study, intranasal administration of L. rhamnosus also elicited high levels of anti-influenza virus-specific IgA in the lungs. Such secretory IgA antibody responses seem to play a role in broad-spectrum protection against influenza virus infection, although the precise underlying mechanism remains unclear.

**Keywords:** Influenza virus, Immunogloblin A, Braod-spectrum protection, Lactobacillus, Anti-viral

---

**A NOVEL VITAMIN B2-OVERPRODUCER, LACTOBACILLUS PLANTARUM HY7715**

**Lee J.; Kim J.; Lee H.; Kang H.; Ahn Y.; Sim J.; Lee J.**
Korea Yakult

**Introduction:**
Vitamin B2 (riboflavin), a water soluble vitamin which belongs to the B group, plays a significant role in cellular metabolism of living organisms, participating in various redox reactions of flavoenzymes as a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). In recent years, the use of lactic acid bacteria as natural vitamin B2 suppliers was proposed, because some bacteria including certain Lactobacilli strains are able to synthesize the riboflavin by expression of rib operon. The transcription of rib operon, a best studied system for the riboflavin biosynthesis in bacteria, is regulated by RFN element. In this study, we carried out to develop and characterize a spontaneous riboflavin-overproducing lactobacilli strain.

**Methods:**
The strategy for development of riboflavin-overproducing Lactobacilli was described as follows. First, candidates for riboflavin-producing lactobacilli strains were prescreened by growth in the riboflavin free chemically defined medium (CDM). And then, prescreened strains were cultured in riboflavin free CDM supplemented with various concentrations (10 ~ 150 ug/ml) of roseoflavin, a structural analog of riboflavin, for selection of riboflavin-overproducer. The concentrations of synthesized total vitamin B2 from selected strains in screening procedure were determined by bioassay method using a L. rhamnosus strain, incapable of growing in CDM, as a biosensor. To quantify accurately the total vitamin B2 including FMN and FAD, final concentrations of vitamin B2 group were determined by HPLC.

**Results:**
14 strains in Korea Yakult culture collection isolated from various food sources were able to grow vigorously in riboflavin free CDM. Among them, 6 roseoflavin resistant strains are obtained from riboflavin free CDM supplemented with roseoflavin. We induced spontaneous riboflavin-overproducing mutant strains by successive subculture in CDM with roseoflavin. The best roseoflavin resistant lactobacillus strain, characterized to be L. plantarum by both 16S rRNA sequencing and API test was able to increase more than about 8 times the riboflavin production compared with a wild type strain in riboflavin free CDM. In optimized culture condition, the L. plantarum synthesized the riboflavin more than about 22 times compared with a mutant strain grown in CDM. To investigate the significant differences for riboflavin synthesis
between the wild type and spontaneous mutant strain, putative RFN element of rib operon were analyzed by cloning, sequencing and real time qPCR. Transcription of rib operon in riboflavin-overproducing strain was dramatically increased more than about 30 times compared with a wild type. There was single base mutation of “A” to “G” in putative RFN element.

**Discussion:**
It suggested that regulation of rib operon was changed to constitutive expression by single base mutation, which resulted in a dramatic increase of riboflavin production. Taken together, results presented here not only show an effective way to develop of vitamin B2-overproducing strains for the probiotics industry, but also contribute the clues to elucidate mechanisms for overproduction of vitamin B2 in biosynthesis system.

**Keywords:** Lactobacillus plantarum, Probiotics, Overproduction, Riboflavin, Vitamin B2

---

**INHIBITORY EFFECT OF LACTOBACILLUS REUTERI LDTM 7503 AGAINST S. MUTANS ON RAT DENTAL CARIES**

*Lee S.; Yeo S.; Hong D.; Huh C.*
Graduate School of International Agricultural Technology, Seoul National University

**Introduction:**
Dental caries are one of the most common bacterial infections in oral environment. Streptococcus mutans and Porphyromonas gingivalis are responsible for these infection diseases in mouth. Several researches indicate that S. mutans easily colonizes in mouth by biofilm formation which is the main cause of dental caries. Cariogenic property of S. mutans relies on the expression of extracellular glucosyltransferases(GTFs). Glucosyltransferases are responsible for the synthesis of insoluble glucans. Insoluble glucan biosynthesis enhances biofilm formation by increasing the attachment of S. mutans on enamel surface.

Probiotics are living microorganisms that are of benefits to the host. Lactic acid bacteria exists as a part of the microflora in the mouth. They play occasionally an opportunistic role of dental caries through their lactic acid formation and extracellular polysaccharides production. However, a recent study indicates that several lactic acid bacteria effectively inhibits cariogenic bacteria and helps oral health by preventing colonization of S. mutans. For these reasons, many industrial application of probiotics was increased to prevent dental caries.

**Methods:**
The object of this study was to evaluate potential roles of lactic acid bacteria strain by comparing L. reuteri KCTC 3594 which had profound effects on production of reuterin and already have been used in commercial market. In vitro and in vivo activities of isolates from pig feces were evaluated by agar diffusion method, co-cultivation, calcium releasing assay and cariogenic rat model.

**Results:**
In this study, isolates from pig feces were evaluated by co-cultivation with S. mutans to assess inhibitory effect of isolates. L. reuteri LDTM 7503 and LDTM 7504 significantly suppressed higher bacterial growth of S. mutans than L. reuteri KCTC 3594. Also, L. reuteri LDTM 7503 and LDTM 7504 exhibited greater inhibitory activities against biofilm formation of S. mutans. Furthermore, L. reuteri LDTM 7503 and LDTM 7504 prevented better calcium degradation from hydroxyapatite than L. reuteri KCTC 3594. We evaluated in vivo activity of L. reuteri LDTM 7503 against S. mutans ATCC 25175 in cariogenic rat model. L. reuteri LDTM 7503 and LDTM 7504 showed more enhanced antibacterial activity against S. mutans than NaF administration group. These results indicated that L.reuteri LDTM 7503 has protective factors against dental caries development. These strain was identified as Lactobacillus reuteri by 16S rDNA sequencing.

**Discussion:**
In conclusion, L. reuteri LDTM 7503 and LDTM 7504 isolates from pig feces had enhanced inhibitory effects on biofilm formation and bacterial growth of S. mutans. L.reuteri LDTM 7503 was more effective in reducing viability of S. mutans and protecting rat from dental caries.

**Keywords:** Biofilm, Streptococcus mutans, Lactobacillus reuteri, Cariogenic rat model, Dental caries
PROBIOTIC STRAINS OF BIFIDOBACTERIUM ADOLESCENTIS

Leser T.; Myling-Petersen D.; Wellejus A.; Brockmann E.; Olsen J.; Rehdin S.
Chr Hansen A/S

Introduction:
Bifidobacteria are natural inhabitants of the gastrointestinal tract possessing genetic adaptations that enable colonization of this habitat. Bifidobacteria contribute to maintaining gastro-intestinal homeostasis through strain-dependent interactions with the host. Mechanisms involved include reducing mucosal antigen load, improving the intestinal barrier, and inducing regulation of local and systemic immune responses. In this study we investigated the taxonomic diversity and functional effects of strains of Bifidobacterium adolescentis to identify new isolates with a probiotic potential.

Methods:
B. adolescentis strains were isolated from healthy humans and characterized taxonomically by 16S rDNA sequencing, DNA-DNA relatedness, and genomic sequencing. Carbohydrate utilization was determined using the API 50 CH assay. Potential improvement of the intestinal barrier was assessed in vitro, by measuring the ability of strains to increase trans-epithelial electrical resistance (TER) across Caco-2 cell monolayers. Potential immune-regulatory effects of the B. adolescentis strains were investigated by determining the induction of cytokines in human PBMC derived dendritic cells (DC). In vivo effects of one selected strain were determined in a dextran sodium sulphate (DSS) colitis rat model. Rats were dosed with freeze-dried bacteria suspended in PBS for 2 weeks prior to and during colitis-induction with 3% DSS in the drinking water for additionally 9 days. During the study, disease activity index (body weight, stool consistency score and fecal bleeding score), whole gut permeability, colonic macroscopic and microscopic scoring were measured and compared to the DSS control group.

Results:
Four taxonomic subgroups of B. adolescentis different from the type strain were identified by specific 16S rDNA signatures, coding DNA sequences and carbohydrate utilization profiles. A subset of strains was found to increase TER up to 150% compared to untreated controls. Some strains strongly induced IL-10 (>1000 pg/ml) resulting in a high IL-10:IL-12 ratio. B. adolescentis BIF038 strongly increased TER and reduced IL-10 secretion. This strain was further studied in the DSS colitis model. DSS induced body weight loss (19 g), whereas BIF038 treatment reduced DSS-induced weight loss to 14 g (P = 0.002). Whole gut permeability as determined by CrEDTA urinary secretion decreased by 30% (P = 0.057) in treated animals compared to DSS alone. BIF038 reduced stool consistency scores by 10% (P = 0.048), reduced the number of animals with a fecal bleeding score at termination by 30%, reduced histological scoring by 26% (P = 0.049), and macroscopic scoring at termination by 19%.

Discussion:
In vitro data indicated that B. adolescentis BIF038 could be a new probiotic candidate that improves the intestinal barrier and induces a regulatory mucosal immune response. The DSS colitis model showed that BIF038 prevented and/or attenuated inflammation and tissue damage in the gastrointestinal tract and ameliorated DSS-induced body weight loss, thus confirming the beneficial effects of this strain demonstrated in vitro.

Keywords: Bifidobacterium, Adolescentis, Barrier, Immune, Colitis

DEVELOPMENT OF NOVEL PROBIOTIC FOR PREVENTION AND TREATMENT OF INTESTINAL INFECTIONS IN ANIMALS

Mrvaljević I.; Veljović K.; Teržić-Vidojević A.; Dinić M.; Popović N.; Golić N.
Invetlab Ltd., Adaševci, Serbia; Laboratory for Molecular Microbiology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia

Introduction:
Exposure of cubes to environmental bacteria in the first days after birth is prerequisite for development of the immune system. Nevertheless, newborns could be infected by pathogens causing the intestinal infections accompanied with diarrhea and weight loss resulting in significant economic loss in the livestock production.
Increased interest in use of probiotics in livestock has arisen from European regulations related to the use of antibiotics in livestock production. Since probiotics are considered as an appropriate alternative, number of studies deal with development of probiotics for animals based on use of lactic acid bacteria, Bacillus sp., or yeasts.

Methods:
The strains Streptococcus thermophilus BGVLJ1-44, Lactobacillus fermentum BGHI14 and Lactobacillus helveticus BGRA43 were determined by nucleotide sequencing of 16S rDNA and pulse-field gel electrophoresis (PFGE). The main probiotic features recommended by EFSA were followed: susceptibility to antibiotics, antimicrobial activity, survival in simulated gastrointestinal tract (GIT) conditions, adhesion of the strains to Caco-2 epithelial intestinal cells, adhesion of Escherichia coli ATCC25922 and Salmonella 654/7E (veterinary isolate) to Caco-2 cells in the presence and absence of probiotic strains, as well as in the presence of probiotic combination, and the effect of UV-inactivated strains, live strains and probiotic combination on GALT proliferation. The 25 gravid sows and 10 gravid goats treated with probiotic culture for 10 days before farrowing and 25 non-treated sows and 10 goats were followed. The general health of treated and control animals and the total number of LAB, Enterobacteriaceae, and Clostridium perfringens in faeces was followed.

Results:
Tested strains successfully survived in the simulated GIT conditions. The strains were sensitive to the recommended levels of antibiotics according to the EFSA recommendations. The strain BGRA43 exhibited broad spectrum of antimicrobial activity on Staphylococcus sp. (clinical isolate), S. aureus ATCC25923, Yersinia enterocolitica O3 (clinical isolate), Shigella sonnei (Hamburg IO5), Sh. flexneriae (serotype 5 and 7), Streptococcus pneumoniae ATCC276, St. pneumoniae ATCC496, Pseudomonas sp., E. coli C600, E. coli ATCC25922 while probiotic combination shows a synergistic effect and exhibits the strongest antimicrobial effect on C. sporogenes, C. perfringens (veterinary isolate) and C. difficile (clinical isolate). The strain BGRA43 exhibited the highest level of adhesion to Caco-2 cells and significantly reduced the number of attached E. coli ATCC25922 (p<0.5). The probiotic combination exerts a synergistic effect and a significantly (p <0.1) reduced the number of attached E. coli ATCC25922. The strains BGVLJ1-44 (p<0.1) and BGRA43 (p<0.5) significantly reduce the number of attached Salmonella 654/7E. The live strains significantly increase the GALT lymphocytes proliferation. Besides, results of farm trial revealed reduction of Enterobacteriaceae in neonatal piglets after the probiotic treatment of pregnant sows, as well as reduction of C. perfringens in goats (below the detection threshold) after the probiotic treatment.

Discussion:
A novel improved probiotic culture in treatment of intestinal infections in animals was developed. The novel probiotic culture represents an alternative to use of antibiotics in treatment of intestinal infections, based on wide range of antimicrobial activity, specifically for severe hardly curable infections caused by C. perfringens and C. difficile.

Keywords: Probiotic, Antimicrobial activity, Intestinal infections, Streptococcus thermophilus, Lactobacillus fermentum, Lactobacillus helveticus

LACTOBACILLUS FERMENTUM CECT5716, A HUMAN MILK PROBIOTIC STRAIN, REDUCES STAPHYLOCOCCUS COUNTS IN BREAST MILK AND PREVENTS LACTATIONAL MASTITIS IN WOMEN.

Olivares M.; Hurtado J.; Gil M.; Fonollá J.
Biosearch Life

Introduction:
During breastfeeding period, women can experience a range of breast related problems, such as breast and nipple pain, nipple cracks and mastitis. Mastitis is an inflammatory condition of the breast that is usually associated with lactation. The reported incidence is around 15% but can reach values as high as 35% when any clinical mastitis case is considered. Human mastitis is characterized by a mammary bacterial dysbiosis, a process in which the population of mastitis agents such as Staphylococcus, increases at the expense of the normal mammary microbiota.
Previous studies have demonstrated that L. fermentum CECT5716, a probiotic strain previously isolated
from breast milk, can be used as an effective treatment of mastitis and painful breastfeeding by reducing pathogen counts in breast milk.

The objective of the present study is to evaluate the preventive effect of the consumption of Lactobacillus fermentum CECT5716 on mastitis incidence in lactating women.

Methods:
A randomized double blinded controlled study including 600 women was conducted. Women were recruited 1-6 days after childbirth and randomly assigned to a group.

Intervention: Probiotic group received 1 capsule/day containing L.fermentum 3x10^9 cfu; Control group received 1 placebo capsule/day containing maltodextrin. The intervention period was 16 weeks.

The primary outcome of the study was the incidence of clinical mastitis defined as: at least two out of the three breast symptoms (pain, redness, lump) and at least one of fever or flu-like symptoms (shivering, hot sweats or aches). Secondary outcome was bacterial load in breast milk. Secondary outcomes were also growth and health of infants of infants.

This study was carried out according to the Helsinki declaration, and the protocol was approved by the Regional Ethics Committee of the Sistema Andaluz de Salud based in Seville (Spain).

Results:
Two hundred and ninety-four women completed 16 weeks of treatment. Incidence rate of mastitis in Probiotic group was significantly lower than in Control group (IR=0.130 in Probiotic group vs IR=0.263 in Control group; p=0.021) (Table 3). Specifically, the consumption of Lactobacillus fermentum CECT5716 during lactation decreased by 51% the incidence rate of clinical mastitis. The probiotic treatment induced a significant decrease in Staphylococcus load in breast milk of women (p=0.013). No significant differences were detected in Staphylococcus and Lactobacillus load. Growth of infants were similar in both groups.

Values of incidence of total diseases, respiratory infections and diarrhea were lower in probiotic group than in control group but differences were not significant.

Discussion:
Staphyloccus has been identified as the main causal agent of lactational mastitis. Probiotic treatment might modulate breast milk microbiota reducing the load of potentially pathogens as Staphylococcus.

Keywords: Lactobacillus, Human milk, Staphylococcus, Mastitis, Breastfeeding

THE IMMUNOMODULATORY PROPERTIES OF PROBIOTIC STRAINS ON DENDRITIC CELL FUNCTIONS

Pang V.; Chen Y.; Chen M.
Department of Animal Science and Technology, National Taiwan University

Introduction:
Lactobacillus kefiranofaciens M1 and Lb. mali APS1, previously isolated from different kefir grains in our lab, are potential probiotic strains. Both strains demonstrated an anti-colitis effect in vitro and in vivo. Lb. kefiranofaciens M1 was also proven to possess immunoregulatory ability on sustaining intestinal homeostasis, anti-allergic and anti-asthmatic effects. In further mechanism study, we found that the putative receptor for recognition of Lb. kefiranofaciens M1 in intestinal epithelial layers was Toll-like receptor (TLR)-2. Dendritic cells (DCs), also existing in the epithelial barrier, are professional antigen-presenting cells and act as messengers between innate and adaptive immune systems. When receiving foreign antigen and becoming mature, they migrate to the draining lymph nodes and mediate to convert naïve lymphocytes into effector T cell or regulatory T (Treg) cell. The relationship between DCs and benefits of our probiotic strains are still remain unclear.

Methods:
Thus, we isolated BMDCs from C57BL/6 mice and co-culture with different dosages of Lb. kefiranofaciens M1 or Lb. mali APS1 to understand the role of DCs on health benefits of the probiotic strains. To further investigate the immunomodulatory activity of DCs activated by these two probiotic strains, we evaluated their capacity to regulate T cell functions, including cytokine profiles as well as proliferation of allogenic T cell in a mixed lymphocytes reaction. Furthermore, we investigated the signal transduction pathways engaged by these two strains, including pattern recognition receptor TLRs and mitogen-activated protein kinases (MAPK) pathway using anti-TLR2 and specific inhibitors of p38MAPK, JNK and MEK1/2.
Results:
Results indicated that both live and heat-killed bacteria of these two strains could stimulate phenotypic maturation of DCs and up-regulate surface expression of CD40, CD80, CD86 which are co-stimulatory molecules of DC maturation, as well as major histocompatibility complex (MHC) class II. These two strains markedly enriched production of IL-12, TNF-α, IL-6 and IL-10 secreted by BMDCs in a dose-dependent manner. On the other hand, their supernatant may not interact with DCs. We found that probiotic-stimulated DC could induce CD4+ T cell proliferation when DC: T cell = 1:10. Besides, live Lb. mali could promote T cells to secrete more IL-10 rather than IFN- production, indicating that CD4+ T cells may differentiate into Treg cells when receiving Lb. mali, as consistent to the result of alleviating inflammation symptoms in DSS-induced colitis mice from our previous studies. With p38MAPK and JNK inhibitors, CD80 and CD86 mfi of probiotic-induced DC maturation were downregulated while MEK1/2 was not. However, there was a large decrease of IL-10 and IL-6 production by blocking JNK of DC among all the other inhibitor groups, thus JNK pathway is most likely involved in modulating DC biological functions by our strains.

Discussion:
As both of the live and heat-killed bacteria differentially modulate DC activation, the following step is to know which components participate in immunoregulation. This study elucidates that DCs may be the important route for Lb. kefiranofaciens M1 and Lb. mali APS1 to interact with immune system and influence T cell shaping, resulting in amelioration of various immune disorders.

Keywords: Dendritic cell, Lactobacillus, Kefir, Immunoregulation, MAPK pathway

ANTIOXIDANT EFFECT OF GREEK-STYLE FERMENTED MILK ADDED WITH HERB EXTRACTS

Park J.; Hwang J.; Yoon J.; Kim H.; Jhoo J.; Kim G.
Kangwon National University

Introduction:
The consumption of fermented milk products are steadily increased in the world. Especially, it is revealed that Greek-style yogurt has many nutritional benefits, and many people are interested in it for healthy food. It is yogurt that has been strained to remove its whey, resulting in a thicker consistency than unstrained yogurt. Meanwhile, stevia is a sweetener and sugar substitute extracted from the leaves of the plant species Stevia rebaudiana Bertoni and has 200~300 times sweeter than table sugar, and it is known that it has various polyphenol compounds. Therefore, this study was carried out to evaluate the antioxidant activities and total polyphenol content of Greek-style yogurt increased in 12% solid content added with stevia leaf extracts for sugar substitute.

Methods:
Stevia leaf extracts as a sugar substitute for Greek-style yogurt were prepared by hot water (100°C) for 6 h 3 times, and by 70% fermented ethanol for 24 h at room temperature 3 times. The antioxidant activities were measured by radical scavenging effect of DPPH, ABTS and FRAP assay during storage. DPPH radical scavenging test was determined according to the method from Blois (1958). ABTS radical scavenging test was determined according to the modified from ABTS cation decolorisation assay method. Ferric antioxidant potential ability (FRAP) was measured according to the method from Benzi and Strain (1996). And also to identify the compound of substances, total polyphenol content was experimented using Folin-Dennis assay.

Results:
It was tendency to increase the DPPH radical scavenging ability as increase the added amount of stevia extracts in yogurt. Especially, DPPH radical scavenging ability was the highest with 58.21±0.37% when the extracts by fermented ethanol were added 1% in the yogurt at 15th day of storage. And DPPH radical scavenging ability was increased during storage. ABTS radical scavenging ability of extract added group were higher than control, and it increased as the addition of stevia increased. Also, the extracts by fermented ethanol added group was higher than that by water added group. However, it was not increased during storage unlike that of DPPH. FRAP was the highest with 3.96±0.31% on 1% extract by fermented ethanol added group in 5th day of storage. It is considered that the stock solution was diluted with water, and soluble
antioxidant ingredients were more measured than DPPH and ABTS. The capability to scavenge free radical and total polyphenol content were the highest at GFE (Greek-style yogurt added with stevia extract 1% by fermented ethanol 70%) 1%.

**Discussion:**
According to the results on the experiments, antioxidant activities were significantly increased when high concentration of stevia extracts as functional source in Greek-style yogurt. Therefore, using stevia extracts gave antioxidant effects to fermented milk, and the fermented ethanol extraction was effective method to obtain it.

**Keywords:** Stevia extracts, Greek-style yogurt, Antioxidant effect, Fermented ethanol Extraction, DPPH

---

THE PREBIOTIC EFFECT OF GLUCO-OLIGOSACCHARIDE ON SKIN MICROFLORA, STAPHYLOCOCCUS

**Park T.**
Amorepacific

**Introduction:**
Diverse bacterial communities can be found on the surface of skin, but relatively there were few comprehensive studies, because the normal and commensal communities of human skin compromises a complex communities.

Staphylococcus epidermidis (S. epidermidis), the most prevalent of many cutaneous resident microflora, is generally innocuous [1]. One of its role is competitive protection of the skin against all forms of pathogenic bacteria. Staphylococcus aureus (S. aureus), a opportunistic human and animal pathogen, is a gram-positive bacterium that commonly lives or colonizes on human skin and causes skin diseases such as atopic dermatitis [2]. Therefore, we assumed that S.epidermidis is a beneficial skin microflora, but S. aureus is pathogenic. The aim of this study is to investigate the prebiotic effect of α-Glucan on skin microflora, Staphylococcus.

**Methods:**
Chemicals and Strains
The test material, α-Glucan, is an oligosaccharide obtained by enzymatic synthesis from natural sugars. (INCI: α-Glucan oligosaccharide)
S. epidermidis (ATCC 12228) and S. aureus (ATCC 6538) was grown in tryptic soy broth (TSA) (Difco, USA) in 32°C incubator for 24 h to reach the exponential phase. Formation of the microorganisms was checked by optical microscope
Study of the Metabolisation
Strains were incubated in a culture medium containing a carbonated substrate (α-Glucan vs glucose), at a concentration of 0.5%. After 24 hours of culture, the residual substrate content was quantified by means of ion chromatography (IC) systems.
Study of the Growth rate
To distinguish the growth rate of two strains, we measured the number of colonies for each time. 1X106 CFU/mL S. epidermidis was inoculated to PBS with 0.5% α-Glucan in sterile condition, and counted the number colonies in 0 h, 4 h, 8 h and 24 h by agar dilution susceptibility test. S. aureus proceed to the same way. After checking the number, we expressed as a Log10 value.

**Results:**
In order to evaluate the prebiotic effect of α-Glucan, we compared the growth rate and metabolism of each strains.
According to the result, S. epidermidis had a higher growth rate with 0.5% α-Glucan than S. aureus.
The initial inoculation was 6.342 in S. epidermidis and 6.079 in S. aureus. After incubating for 24 h, a final concentration was 8.491 in S. epidermidis and 6.944 in S. aureus. According to the metabolism study, we found that residual substrate was about 34.05% in S. epidermidis and 8.18% in S. aureus. The α-Glucan was metabolized faster and better by the beneficial microflora (S epidermidis), but undesirable flora (S.aureus)
Discussion:
Our study provided that α-Glucan stimulated the growth of beneficial resident flora, and its bio-selectivity as a substrate will enable to restrict the growth of pathogenic flora. Ecological balance is very important to prevent invasion of external pathogen and maintain the barrier function of skin. Therefore, we concluded that α-Glucan had the possibility of prebiotics effect, and expected that the product containing this material has a potential benefit for skin health.

Keywords: Prebiotics, α-Glucan oligosaccharide, Staphylococcus epidermidis, Staphylococcus aureus, Skin microflora

STUDY OF THE EFFECT OF PROBIOTIC MICROORGANISMS AND THEIR ASSOCIATIONS ON THE CELLULAR IMMUNITY AND MORPHOLOGICAL COMPOSITION OF THE BLOOD OF RABBITS

Ratnikova I.; Gavrilova N.; Sadanov A.; Bayakyshova K.; Turlybayeva Z.
1RSOE

Introduction:
The published data testify to a huge role of normal intestinal microflora as factor of nonspecific resistance which is realized not only due to microbic antagonism, but also nonspecific activation of fagositarny and cytostatic activity of macrophages, stimulation of lymphoid fabric, impact on immunocompetent T- and B-cells.

Research objective - studying of the immunomodelling properties of probiotic microorganisms and their associations.

Methods:
In experients in vivo the the immunostimulating activity of 10 preparations from lactic and propionic acid bacteria is studied. Researches were conducted on 44 rabbits weighing 3-3,2 kg., from calculation on four rabbits on a preparation. Prior to experiment blood of all animals was studied (hematological parameters are defined, T- and B-lymphocytes, tests with loading: NST-test, phagocytosis with latex). Examinees preparations set in a dose 10 ml on an animal in the morning in 20 minutes before feeding for 7 days. Blood for researches was taken on day 8 of the experiment from an ear vein in the morning on an empty stomach.

Results:
As a result of research of parameters of the immune status and a hematologic profile of blood of laboratory animals prior to experiment it is established that all indicators were in limits of physiological norms for this species of animals.

For the 8th day after reception of probiotic preparations the maintenance of the T-lymphocytes has increased in all experimental groups, and more with P. shermanii 2/10, L. plantarum 2v/A-6, plantaferminy and A-5 association with wheat bran. The quantity of the B-lymphocytes has practically not changed in all cases.

The maintenance of leukocytes has slightly increased in the groups accepting P. shermanii 2/10, L. plantarum 2v/A-6 and A-5 association with wheat bran. Increase in percent of lymphocytes is noted in the groups receiving L. acidophilus 27w, L. plantarum 14d/A-24, L. plantarum – 22, A-5 association with wheat bran, monocytes – P. shermanii 2/10, L. acidophilus 27w, L. plantarum 14d/A-24, A-5 association, granulocytes - P.shermanii 2/10, L. plantarum 2v/A-6, L. fermentum 127/A-4, B. longum 7w, L. plantarum – 22, plantapherminy, A-5 association. Also after giving the preparations P. shermanii 2/10, L. plantarum 2v/A-6, L. fermentum 127/A-4, plantapherminy, L. plantarum – 22 has increased fagocytarny activity of neutrophils, as in the spontaneous test (except for P. shermanii 2/10), and in the presence of the activator of a fagocytosis. In other studied indicators between control and experimental groups of a significant difference it was not observed.

The stated data confirm stimulation with the help of tested preparations of cellular factors of immunity within the upper bounds of physiological norm, more at the rabbits receiving the preparations P.shermanii 2/10, L. plantarum 2v/A-6, L. fermentum 127/A-4, B. longum 7w, L. plantarum – 22, plantaferminy, A-5 association with wheat bran.

Thus, preparations of lactic acid and propionic acid bacteria possess immunostimulating activity, increase
the proliferation of T-lymphocytes, granulocytes, monocytes, activate bactericidal function of phagocytic cells (neutrophils) increasing their functional reserve.

**Keywords:** Probiotics, Lactic-acid bacteria, Bifidobacteria, Propionic-acid bacteria, Immunomodulatory properties, Cellular immunity

HUMAN MILK OLIGOSACCHARIDES; NOW AS SUBSTANTIAL MODULATORS OF THE ADULT GUT MICROBIOTA

**Salomonsson E.; Vigsnaes L.; Sommer M.; Hennet T.; Bytzer P.**
Department of Medicine, Zealand University Hospital

**Introduction:**
Intensive research over the past decade has established the gut microbiota as an important player influencing many aspects of human physiology including energy metabolism, hormonal balance and immunity. Breast milk, the first diet for an infant, contains human milk oligosaccharides (HMOs) that shape the infant’s gut microbiota by selectively stimulating the growth of specific bacteria, especially bifidobacteria. After weaning, diet, together with genetics and environmental influences, is one of the main factors contributing to the composition of the human gut microbiota. Hence, dietary manipulation represents a strategy to promote a beneficial gut microbiota. In addition to their bifidogenic activity, the ability of HMOs to modulate immune function and the gut barrier make them prime candidates to restore a beneficial microbiota in dysbiotic adults and provide health benefits. However, the effects of HMOs on the adult gut microbiota and gastrointestinal tract have never been examined.

**Methods:**
We conducted a parallel, double-blind, randomized, placebo-controlled, HMO supplementation study in 100 adult healthy volunteers. Participants were randomized into 10 groups, each consuming chemically produced HMOs at various daily doses (5, 10 or 20 grams), or 2 grams of glucose as placebo for 2 weeks. Safety was assessed through physical examination, hematology and blood chemistry analysis. Tolerance was recorded by a self-administered Gastrointestinal Symptoms Rating Scale (GSRS) questionnaire covering symptoms related to abdominal pain, indigestion, reflux, diarrhea and constipation, and stool consistency by the Bristol Stool Form Scale (BSFS). Adverse events were monitored from intake of first dose and throughout the intervention period. The composition of the intestinal microbiota was determined by 16S rRNA sequencing of faecal samples before and during HMO intervention.

**Results:**
All participants completed the study without premature discontinuation. Physical parameters, including pulse rate and blood pressure, remained unchanged during and after HMO uptake. Routine clinical chemistry and haematology analyses also remained stable over the course of the study. The GSRS scores were low at baseline, and remained low after intervention, and only a minor change was observed for stool frequency and consistency. Adverse events recorded throughout the intervention period were all characterized as mild. The 16S rRNA sequencing analysis revealed that HMO supplementation specifically modified the adult gut microbiota with the primary impact being substantial increases in abundance of Actinobacteria and Bifidobacterium in particular, and a reduction of Firmicutes and Proteobacteria. The increase in Bifidobacterium, reaching more than 25% in some individuals, was dose-dependent but was not dependent on the initial Bifidobacterium abundance.

**Discussion:**
This study provides the first data on safety, tolerance and impact of HMOs on the gut microbiota of healthy adult subjects. Collectively, the results from this study show that supplementing the diet with HMOs is a valuable strategy to shape the gut microbiota and specifically promote the growth of beneficial bifidobacteria and health.

**Keywords:** HMOs, Safety, Tolerance, Sequencing, Bifidobacterium
BIOCENOSIS AND HISTAMINE INDICATORS IN ORAL FLUID AS BIOMARKERS OF THE EFFICIENCY OF PROBIOTICS CHOICE IN TREATMENT OF ORAL MUCOUS MEMBRANE DISEASES

Saygusheva L.; Dudko E.; Kuyarov A.; Yevtushenko E.
Surgut State University

Introduction:
The majority of diseases of oral mucous membrane proceed against the background of the violation of intestinal microbiocenosis. The maintenance of histamine as one of the mediators of inflammation and pain has a pathogenetic value in case of inflammatory processes. The use of probiotics realizing ecological mechanisms of microbiosenosis normalization defines the relevance of the chosen subject in theoretical and practical aspects.

The purpose of the present research is the assessment of intestinal biocenosis and histamine indicators in oral fluid as biomarkers of the efficiency of probiotics choice in treatment of oral mucous membrane diseases.

Methods:
Research was conducted on the basis of Stomatogy N1 in Surgut city and the laboratory "Fundamental problems of healthcare of the indigenous community and Northern migrants" in Surgut State University.

Two groups were under supervision, they included 67 people aged from 19 till 52 years (men - 30, women – 37) with the diagnosis recurrent aphthous stomatitis. After professional hygienic measures we used the traditional scheme of treatment of recurrent aphthous stomatitis in the comparative group (group 1; 32 persons). Patients of the main group (group 2; 35 people) received a probiotic mix orally and in the form of oral trays as a complex medical treatment.

All the patients had a clinical examination and the level of histamine in oral fluid was determined before and after treatment by the immunoenzymometric method (IEM). Examinees of the main group had a bacteriological examination of intestinal microflora for dysbacteriosis before the treatment and 30 days after it.

During the choice of probiotic strains for the increase of the efficiency of correction of violations of microflora strains of Lactobacillus bacteria were prescribed to the investigatated group, they have a certain decarboxylase and antagonistic activity. The efficiency criterion of the conducted treatment was the reduction of the inflammatory activity, reduction of the period of the existence of aphthas, and also the maintenance of histamine in oral fluid.

Results:
As a result of the conducted research it was established that a positive effect, but with various degrees of expressiveness was observed in both groups. In group 2 morbidity reduction of aphthas was observed during the first two days. The period of the presence of aphthas was reduced by 2-4 days. After the conducted treatment in group No. 1 the maintenance of free histamine in saliva decreased in 30% of the investigated people and this reduction wasn’t reliable according to the average indicators (P>0,05). In group No. 2 decrease of histamine was observed in more than 90,0% of the examined people, which is a reliable indicator (P<0,05).

During the dysbacteriosis analysis 82,8% of patients of group No. 2 tended to restore the quantity of lactobacilli, bifidobacteria and typical colibacillus. In case of 42,8% of the examined the quantity of opportunistic microorganisms has decreased by 1-2 levels.

Discussion:
Thus, the assessment of biocenosis and histamine indicators in oral fluid as biomarkers of the efficiency of probiotic treatment of oral mucous membrane diseases has shown a sufficient degree of informational content, and the use of probiotics with a certain decarboxylizing activity in complex treatment of recurrent aphthous stomatitis has considerably reduced the terms of aphthas presence. Morbidity reduction of aphthas due to the decrease in maintenance of free histamine in saliva in association with normalization of indicators of intestinal microflora can be explained by the combined intake of probiotic preparations, - both locally, and orally. It dictates the need of inclusion of probiotic preparations in a complex treatment of recurrent aphthous stomatitis. A positive tendency of intestinal microflora normalization testifies to the need of a long intake of probiotic preparations at treatment of recurrent aphthous stomatitis for the improvement of organismal resistance.

Keywords: Biocenosis , Histamine, Probiotics, Stomatitis, Decarboxylase
EVALUATION OF TOLERANCE TO GASTRO-INTESTINAL STRESS FACTORS, ANTIMICROBIAL ACTIVITY, ANTIBIOTIC SUSCEPTIBILITY AND CURD FORMING POTENTIAL OF PRESUMPTIVE PROBIOTIC LACTOBACILLUS STRAINS ISOLATED FROM RURAL VICINITY OF PUNJAB, INDIA

Sharma C.; Gulati S.; Thakur N.; Panwar H.
Department of Dairy Microbiology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana – 141004, Punjab, INDIA.

Introduction:
Probiotic bacteria are live microorganisms which when administered in adequate amount, confers health benefits to the host. Nowadays, the consumption of probiotic products is growing intensively in developing countries. The milk flora of Punjab (India), a state recognized for its milk production and consumption, remains unexplored. This study was aimed at selecting novel strains of Lactobacillus from rural background of Punjab for their establishment as potential probiotic candidate.

Methods:
Lactobacillus strains were isolated from curd samples, collected from rural background of Punjab; following standard serial dilution method. Gram positive, catalase negative, rods were characterized as lactobacilli using genus specific PCR. Lactobacillus isolates were subjected to gastrointestinal stress i.e. acid and bile conditions. Antibiotic susceptibility of isolates was determined against 26 common antibiotics, following disc diffusion assay. Antimicrobial activity of cell supernatants was evaluated against gram+ve and gram-ve pathogens through agar well diffusion assay. Auto-aggregation potential of LAB isolates was also determined. Antibiotic susceptibility, Antimicrobial activity and auto-aggregation potential were compared to reference probiotic LAB strains. Product viz. Curd forming potential of selected isolates was also evaluated.

Results:
A total of twenty curd isolates were confirmed as Lactobacillus sp. on basis of 250bp PCR product. When subjected to low pH (1.5, 2, 2.5, 4.5) and bile (0.5, 1, 1.5, 2 %) for different time intervals (0 to 4 hrs), varied responses were recorded. Curd isolates, D4 and D6 could resist acid and bile stress most remarkably, as observed with only 2 and 2.5 log (cfu/ml) reduction at pH2.5 and around 2 log (cfu/ml) reduction at 2% bile after 4hr exposure. Among the 26 antibiotics screened, most of the Lactobacillus isolates showed resistance pattern towards cefoxitin (30mcg), vancomycin (30mcg) and teicoplanin (30mcg). In contrary, isolates were intermediate to moderate susceptible towards other antibiotics with highest zone of inhibition (>40mm) recorded with imipenem (10mcg). Culture supernatants from the Lactobacillus isolates exhibited varying degrees of inhibitory activity pathogens. S. aureus, S. mutans, L. monocytogenes, E. coli and K. pneumoniae were resistant towards all the tested isolates. However, moderate level of antimicrobial activity was exhibited against B. cereus, S. Typhii, P. mirabilis, S. flexneri and P. aeruginosa. None of the isolates exhibited β- haemolytic activity. Highest cell auto-aggregation (6h, %) was observed with standard probiotic, Lactobacillus fermentum (NCDC 214) (50.44% ±0.39) followed by D7 (36.94 ±0.68), D10 (33.38 ±0.43) and D18 (31.40 ±0.40). Isolate D6 exhibit remarkable curd forming potential as observed with good texture and high water holding capacity.

Discussion:
The study highlighted a noticeable heterogeneity in the ability of the strains to withstand harsh growth conditions and some strains meet the criteria for being a potential probiotic candidate. Studies are going on to further establish and validate their probiotic and safety attributes under in vitro and in vivo conditions.

Keywords: Lactobacillus, Probiotic, Acid tolerance, Bile tolerance, Antimicrobial activity, Auto-aggregation
pathogenesis of both forms of inflammatory bowel disease (IBD), Crohn’s disease and ulcerative colitis. The development of chemokine binding proteins as potential therapeutic agents in IBD is therefore of great interest. Recombinant lactic acid bacteria (LAB) are considered interesting candidates for use in therapy, and could be engineered to bind chemokines on their surface, and thereby prevent chemokine proinflammatory action. We used model LAB Lactococcus lactis for the surface display of chemokine binding proteins, produced by the salivary gland of the brown tick Rhipicephalus sanguineus, named evasins. Evasins are small proteins, which have the ability to bind and neutralize chemokines of different families (CC and CXC) and inhibit the chemokine-mediated recruitment of leukocytes.

Methods:
We designed genes for evasin-1, evasin-3 and evasin-4. Evasin genes were cloned into lactococcal surface display vector pSDLBA3b in fusion with secretion signal, B domain and surface anchor, and over-expressed in L. lactis NZ9000. Expressed fusion proteins were detected with SDS-PAGE and Western blot. The surface localization of evasins was assessed with flow cytometry and confocal microscopy, using fluorescein (FITC)-conjugated human IgG, or anti-protein A antibody, both recognizing B domain part of the fusion protein. Evasin fusion proteins functionality was tested by the ability to remove the individual chemokines from the solution with ELISA and Luminex. The influence of evasin-3-displaying L. lactis on the CXCL8 secretion by Caco-2 intestinal epithelial cells was evaluated.

Results:
Flow cytometry of evasin-displaying cells showed a distinct shift in mean fluorescence intensity in comparison to the control which indicates that evasins were successfully displayed on the surface. The significant binding of chemokines after incubation with 2×10⁹ evasin-displaying L. lactis cells was observed for CCL3 by evasin-1, CCL5 by evasin-4 and murine CCL1, CXCL2, murine CXCL2 and CXCL8 by evasin-3. The binding depended on the number of the bacterial cells, and was decreased by lowering the number of cells. By decreasing the concentration of the chemokine that was added to the constant number of the evasin-displaying bacterial cells, the portion of removed chemokine also decreased. The ability of evasin-3-displaying L. lactis to decrease IL-1β-induced secretion of CXCL8 from Caco-2 cells was demonstrated. Significant time-dependent decrease of CXCL8 secretion was observed by increasing the bacterial concentration.

Discussion:
In the present study we developed evasin-displaying L. lactis with the ability to bind chemokines as a promising approach for the treatment of IBD. Developed bacteria will be tested in an animal model of IBD.

Keywords: Lactococcus lactis, Evasins, Chemokine binding, Inflammatory bowel disease, Caco-2 cell model

CLINICAL EVALUATION OF A SYNBIOTIC IN PEDIATRIC PATIENTS WITH ACUTE VIRAL DIARRHEA

Suárez Almarza J.; García Marín F.; García Menor E.; Vecino López R.; Horcajo Martínez G.; Nieto Magro C.
ITF Research Pharma S.L.U, Madrid- Spain

Introduction:
Viral gastroenteritis is a common illness that occurs worldwide. In resource-limited settings, it is associated with considerable mortality and in developed countries, with substantial health-care costs. It is a clinical syndrome often defined by increased stool frequency, with or without vomiting. It usually lasts less than one week and not longer than two weeks and occurs throughout the year with a fall and winter predominance. Systematic reviews and meta-analyses of randomized trials of some probiotics have demonstrated a benefit in reducing stool frequency and the duration of diarrhea (by approximately one day), without increasing the risk of adverse effects. Synbiotics may be used for the management of acute viral diarrhea however, it was concluded in the 2014 Guidelines for the Management of Acute Gastroenteritis in Children in Europe that none of the synbiotics studied so far could be recommended until confirmatory data were available. The aim of this study was to evaluate the additional benefit of a synbiotic (Prodefen®) in the clinical management of acute viral diarrhea in children between 6 months and 12 years of age, attending to outpatient clinics or
emergency departments. This synbiotic contains a combination of prebiotics (fructooligosaccharides) and 7 probiotic-strains (L.casei-PXN37, L.rhamnosus-PXN54, S.thermophilus-PXN66, B.breve-PXN25, L.acidophilus-PXN35, B.infantis-PXN27, L.bulgaricus-PXN39) 1x10^9 Colony Forming Units/sachet.

Methods:
Multicenter, prospective, randomized and controlled study. Patients were randomized into two groups: control group receiving supportive treatment based on diet and oral rehydration therapy and synbiotic group receiving, in addition Prodefen®, 1 sachet/day for 7 days. Evolution of the diarrhea, tolerability and acceptance were evaluated after 7 days of treatment.

Results:
101 children between 6 months and 12 years of age were recruited; 43 children that received the synbiotic and 42 controls successfully completed the study. 79% of children in the synbiotic group vs 64% of controls (p=0.07) and 95% of children in the synbiotic group vs 78% of controls (p<0.001) did not present with diarrhea after 4 and 5 days of treatment, respectively. The shortening of the duration of diarrhea was of 1 day in children receiving synbiotic compared to controls (3vs4, p=0.377), and of 2 days in the subgroup of children from 6 months to 2 years of age (3vs5 p=0.034). During the study, 14% of children in synbiotic group and 26% of controls (p=0.15) had to reconsult to the physician. Significant differences on efficacy’s perception and tolerability’s evaluation were observed in children receiving the synbiotic vs controls, as 68% vs 28% of them found the treatment very or quite efficacious in the synbiotic vs control group, respectively and 67% vs 23% of them tolerated very well the treatment in the synbiotic vs control group, respectively. 95% of parents of children receiving the synbiotic reported being very satisfied/satisfied with the treatment.

Discussion:
Overall, the results of this study indicate that the addition of the synbiotic Prodefen® is a well-tolerated and well-accepted approach that provides an additional clinical benefit to the standard supportive therapy in the management of acute viral diarrhea in children.

Keywords: Synbiotic, Diarrhea, Gastroenteritis, Children, Probiotic

EFFECTS OF DIETARY FAT INTAKE AND AGE ON GUT MICROBIOTA AND COLONIC INFLAMMATION IN C57BL/6J MICE

Sung M.
Sookmyung Women’s University

Introduction:
Gut microbiota has emerged as one of the key environmental factors closely related with obesity. Recent studies have showed that diet-induced alterations of gut microbiota composition play a pivotal role in the development of metabolic diseases. However, studies also suggest that the relationship between the microbiota and obesity is complex with contradictory findings relating to the nature of shift in the relative contribution of phyla to the microbiota composition in obesity. Also, it is not still clear whether changes in gut microbiota are induced by dietary perturbations. In this study, we investigated whether dietary fat intake and age would affect gut microbiota, permeability, and inflammation.

Methods:
C57BL/6J mice were randomly assigned to either normal fat diet or high-fat diet group. After 10 weeks, a half of mice in each group were switched to either high-fat diet or normal fat diet feeding for additional 10 weeks. Microbiome composition and diversity were analyzed by 16S rRNA-based pyrosequencing. Colonic mRNA expressions of tight junction proteins and inflammatory cytokines were measured by real-time quantitative polymerase chain reaction. We determined the association between the alterations of gut microbiota composition and colonic inflammatory cytokines.

Results:
The main bacterial phyla of mice were Frimicutes, Bacteroidetes, and Actinobacteria. Diversity of gut microbiota was reduced in mice fed high-fat diet compared to those of mice fed normal fat diet. In mice fed high-fat diet for 20 weeks, the proportions of Actinobacteria and Firmicutes increased while the proportion of Bacteroidetes decreased compared to mice fed normal fat diet for 20 weeks. The proportions of Firmicutes and Bacteroidetes were significantly associated with age whereas the proportion of Actinobacteria was
Abstracts of Poster Presentations

significantly associated with dietary fat content and age. The correlation data indicated that the relative abundance of Actinobacteria and Firmicutes was positively associated with body weight. Actinobacteria was positively associated with colonic inflammatory cytokines. In addition, we found that high-fat diet was negatively associated with the expressions of tight junction proteins while it was positively associated with the expression of inflammatory cytokines. These results indicate that changes in the expression of tight junction protein and inflammatory cytokines followed changes in fat content of the diet.

Discussion:
Our data showed that both dietary fat intake and age affect gut microbiota composition as well as colonic membrane integrity and inflammation. Future studies are needed to investigate whether gut microbiota associated with dietary fat intake would have a modulatory effect on obesity-induced diseases [This study was supported by the Mid-Career Research Program 2012R1A2A01046228 NRF].

Keywords: Gut microbiota, High-fat diet, Age, Gut permeability, Colonic inflammation

BIFIDOBACTERIUM ANIMALIS SSP. LACTIS GCL2505 REDUCES ABDOMINAL VISCERAL FAT, A KEY FACTOR ASSOCIATES WITH METABOLIC DISORDERS.

Takahashi S.
Institute of Health Sciences, Ezaki Glico Co., Ltd., Japan

Introduction:
Gut microbiota currently has been recognized as an important factor for causes of overweight, obesity and excess accumulation of abdominal visceral fat, which is a known underlying component of metabolic syndrome (MS). Several studies indicated that gut bifidobacteria elicited beneficial effects on obesity and excess accumulation of fat mass, and also reported that the number of Bifidobacterium in feces was lower in overweight and obese subjects than in lean subjects. Bifidobacterium animalis ssp. lactis GCL2505 (B. lactis GCL2505) as a probiotics, which was previously shown to reach the gut, proliferates there and subsequently increases the total number of gut bifidobacteria. We previously showed that B. lactis GCL2505 proliferated in the gut and improve visceral fat accumulation in animal study. The aim of the present study was to investigate the impact of B. lactis GCL2505 on abdominal visceral fat storage in overweight and mildly obese Japanese adults.

Methods:
A multicenter, randomized, double-blind, placebo-controlled intervention trial was performed for 12 weeks. Healthy Japanese subjects (N=137) with body mass index ranging 23 - 30 kg/m² consumed either fermented milk containing 8 × 10¹⁰ CFU of B. lactis GCL2505 (GCL2505 group) or placebo (placebo group) every day. Abdominal visceral fat area (VFA) and subcutaneous fat area (SFA) were measured by computed tomography. Furthermore, the number of fecal bifidobacteria was measured.

Results:
Compared with the placebo group, the VFA in the GCL2505 group was significantly reduced from baseline at 8 and 12 weeks (-6.8 cm² and -5.1 cm², respectively). However there was no reduction in SFA. The total number of fecal bifidobacteria was significantly increased in the GCL2505 group, compared with in the placebo group during the treatment period (p < 0.001). There were no significant differences in nutrient intake (dietary energy, protein, carbohydrate, and fat) and steps walked during treatment period. No adverse events were also observed throughout this study in any subjects either.

Discussion:
In the present study, we revealed that the intake of B. lactis GCL2505 led to increase in the gut bifidobacteria and reduce visceral fat accumulation in overweight and mildly obese adults. Several studies showed that a higher abundance of gut bifidobacteria was associated with suppressed excess accumulation of fat mass. These results suggest that increasing gut bifidobacteria by ingesting B. lactis GCL2505 might play an important role in reducing abdominal visceral fat. Therefore, our findings in the present clinical study suggest that a specific strain of B. lactis GCL2505 may be useful for the reduction of abdominal visceral fat, and improve metabolic disorders in overweight or mildly obese individuals.

Keywords: Randomized trial, Visceral fat, Bifidobacterium, Probiotics, Overweight
IN VITRO EVALUATION OF SELECTED PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM THAI TRADITIONAL FERMENTED VEGETABLE

Thongaram T.
Silpakorn University

Introduction:
Probiotics are proposed to have a wide range of health benefits. Their benefits have been proven effective in supporting immune function and digestive system. Currently, much attention is paid to the role of probiotics in human health improvement and treatment of human disease. Researches effort allowed to obtain sufficient numbers of well-characterized probiotic strains available for application in functional foods and health-associated products. However, the isolation and characterization of new strains are still needed. The aim of this study was to characterize the probiotic characteristics of lactic acid bacteria from traditional fermented vegetable.

Methods:
Isolation of lactic acid bacteria
Sixteen samples of Thai traditional fermented vegetable products were obtained from the local markets of Thailand central region. 10 g of sample were weighed aseptically and homogenized for 2 min in stomacher containing 90 ml of peptone water. The homogenized samples were serially diluted and pour-plated on MRS (de Man, Rogosa and Sharpe) agar and were incubated at 37°C for 48-72 h.

Resistance to low pH
Cultures of all lactic acid bacteria strains were cultivated for 24 h in MRS medium, centrifuged, washed twice with PBS buffer and re-suspended to a final concentration 10^7-10^8 CFU/ml in PBS. Bacteria were incubated in 0.85% NaCl at pH 2.5 for 4 h at 37°C.

Antimicrobial activity
Inhibition of pathogens of the selected lactic acid bacteria strains was analyzed in the cell-free supernatant (CFS), obtained from cultures of the isolates in MRS broth. After 24 h of incubation at 37°C, the CFS were recovered by centrifugation (10,000 rpm for 10 min). The nutrient agar plates were inoculated with pathogenic bacteria, air-dried and 6 mm diameter wells were punctured in each plate and 50 µl of cell free supernatant (CFS) were placed in each well and incubated for 24 h at 37°C.

Results:
A total thirty-two lactic acid bacteria strains were isolated from fermented vegetable products and twelve isolates were evaluated for probiotic potential with the resistance to low pH and antimicrobial activity. The studies on resistance to gastrointestinal condition showed that isolate MCSU4 exhibited high tolerance to the simulated gastric juice (pH 2.5) and high concentration of NaCl (10 and 15%). Interestingly, while in the simulated gastric condition isolate MCSU4 could survive 4 h at pH 2.5. All of the selected lactic acid bacteria strains showed various antibacterial activities against five pathogens; Bacillus subtilis ATCC 6633, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimurium ATCC 13311, and Staphylococcus aureus ATCC 25953, however only isolate MCSU4 possessed the highest activity of inhibiting pathogens.

Discussion:
Among the selected strains, isolate MCSU4 showed the best probiotic potential with the high tolerance to simulated gastrointestinal juice and high concentration of NaCl and also broad antimicrobial activity. Results revealed that isolate MCSU4 is ideal probiotic candidate and requires further in vitro and in vivo safety studies.

Keywords: Probiotics, Lactic acid bacteria, Gastrointestinal condition, Low pH, Antimicrobial activity

EVALUATION OF – AMINOBUTYRIC ACID (GABA) – PRODUCING LACTOBACILLI AS A POTENTIAL PROBIOTICS

Tolinacki M.; Sokovic S.; Djokie J.; Zivkovic M.; Popovic D.; Mihajlovic S.
Institute of Molecular Genetics and Genetic Engineering (IMGGE), University of Belgrade, Serbia

Introduction:
G-aminobutyric acid (GABA), a nonprotein amino acid, possesses several physiological functions such as
neurotransmission, induction of hypotension, and diuretic and tranquilizer effects. GABA is synthesized from glutamate by the activity of glutamic acid decarboxylase (GAD). GABA-producing lactobacilli are promising candidates as starters for manufacture of GABA-rich foods and to synthesize GRAS (generally recognized as safe)-grade GABA. Given that LMM-IMGGE possesses a unique collection of natural lactic acid bacteria (LAB) isolates we screened the collection for the best GABA-producing candidates with probiotic potential.

Methods:
In silico analysis, conserved CoreF/CoreR primers as well as a degenerate primer GAD typing method were used to screen the presence of a glutamate decarboxylase gene, gadB in the LMM collection of autochthonous LAB isolates. Qualitative and quantitative assessment of GABA in the supernatants of selected LAB isolates was performed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Susceptibility to various antibiotics was determined according to EFSA recommendations. Antimicrobial activity was determined by deferred antagonism method. The survival of the selected LAB during the passage through the gastrointestinal tract (GIT) was studied in an in vitro model. The adhesion and pathogens’ exclusion abilities of the GABA producing LAB were assessed with the epithelial intestinal cell line Caco-2 and HT-29 MTX.

Results:
The 81% of lactococci, 64% of leuconostocs, 52% of lactobacilli, 33% of streptococci and 17% of enterococci from LMM collection were positive for the presence of the gadB gene. Preliminary screening of LAB carrying the gadB gene by TLC methodology demonstrated that the best GABA producers are among lactobacilli. The highest GABA producers were determined by HPLC among Lactobacillus brevis isolates. In order to evaluate probiotic potential of selected L. brevis isolates numerous in vitro tests were carried out. All tested lactobacilli were sensitive to all tested antibiotics. L. brevis BGLMM10 strain inhibited 12 indicator microorganisms. All tested lactobacilli strains successfully survived in the simulated GIT conditions and showed decreases lower than 0.5 log cycle with respect to their initial cell density (8.6 to 8.9 log CFU/ml). L. brevis BGZLS10-17 showed the best adhesion ability to epithelial cell lines (15%). The exclusion assay showed that the highest reduction of the growth of Escherichia coli ATCC 25922 and Salmonella Enteritidis C2 9039 was observed in the presence of L. brevis BGZLS10-17 (80%) and L. brevis BGZLS30-2 (60%), respectively.

Discussion:
The selection of a microbial strain to be used as a probiotic is a rather complex process. A degenerate primer GAD prescreening typing method combined with TLC and reversed-phase HPLC confirmation was an efficient and cost-effective method to identify high-GABA-producing LAB. Numerous in vitro tests stressed five L. brevis isolates as good probiotic candidates that could be eventually used in formulation of functional starter cultures for production of the innovative foods.

Keywords: GABA, Lactobacillus brevis , Probiotic, TLC, gadB

MICROBIOTA-GUT-BRAIN AXIS AND PSYCHOBIO蒂CS: LACTOBACILLUS PLANTARUM PS128 AS AN EXAMPLE

Tsai Y.
National Yang-Ming University

Introduction:
Increasing evidence indicates that the gut microbiota influences brain development and host behavior through the microbiota-gut-brain axis, which is a bidirectional communication system between the gut microbiota and the brain. This communication system integrates neural, hormonal, and immunological signaling, and can be modulated by a class of probiotics called “psychobiotics”; therefore, these probiotics may be exploited to treat a broad spectrum of complex central nervous system diseases, including irritable bowel syndrome (IBS), mental illness, and neurological disorders. In our previous screening for novel psychobiotics, a candidate strain, PS128, was isolated from Fu-Tsai, a traditional fermented mustard product in Taiwan. Strain PS128, identified later as Lactobacillus plantarum, can increase the locomotor activity of naïve mice in the open field test, and exerts a high anti-inflammatory activity in the dextran sulphate sodium-induced colitis animal model. Here we describe the psychobiological effects of PS128 in germ-free mice, a maternal separation (MS) mouse model of depression, and a rat model of IBS.
Methods:
In the MS mouse model, C57BL/6J neonates were separated from their mothers and littermates for 3 hours per day between postnatal day (PD) 2 and PD 14. The MS mice were administered with PS128 from the age of 4 to 8 week old and then underwent behavioral tests. Moreover, blood and specific brain regions were collected immediately after sacrifice for further analysis. Eight-week-old C57BL/6J germ-free male mice were daily administered with PS128 for 2 weeks and then subjected to behavior tests and analysis as the MS mice. The IBS-like model was established by 5-Hydroxytryptophan (5-HTP), which was injected subcutaneously into awake Sprague-Dawley rats to induce visceral hypersensitivity. The rats were orally administered with PS128 for 2 weeks. The visceromotor responses were measured after colorectal distension (CRD) to assess visceral hypersensitivity. CRD with simultaneous electromyography recording was done 30 minutes before and 30 minutes after 5-HTP injection. After finishing CRD test, rats were sacrificed in order to take out brain tissue, distal colon, L6-S1 spinal cord, dorsal root ganglion and blood for further analysis.

Results:
In both germ-free and MS mice, PS128 administration significantly reduced depression-like behaviors; PS128-treated mice also showed increased contents of dopamine and serotonin in the prefrontal cortex, elevated serum IL-10, and reduced levels of serum corticosterone, TNF-α, and IL-6. In the IBS model, PS128 administration significantly reduced the 5-HTP-induced visceral hypersensitivity, and alleviated the problem of alteration of the stress-related neuromodulator repertoire, including SP, BDNF, CGRP and NGF in dorsal root ganglia and the spinal cord, dopamine and serotonin in the prefrontal cortex, glucocorticoid receptor and mineralocorticoid receptor in the amygdala, and corticosterone in the serum.

Discussion:
Our data suggest that Lactobacillus plantarum PS128 is a psychobiotic. Daily intake of PS128 can help lessen stress-induced neuromodulator and behavioral dysfunctions. Administration of PS128 may be a promising therapeutic approach for neuropsychiatric disorders.

Keywords: Microbiota-Gut-Brain Axis, Psychobiotics, Depression, Irritable Bowel Syndrome, Dopamine

IDENTIFICATION OF KEY GUT MICROBES INVOLVED IN AO AND DA RATS’ SUSCEPTIBILITY TO EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Veljović K.; Stanisavljevic S.; Lukić J.; Mihajlović S.; Mostarica Stojković M.; Miljković D.
Department of Immunology, Institute for Biological Research “Siniša Stanković”, University of Belgrade

Introduction:
The development of effective therapy for multiple sclerosis (MS) that could alleviate the symptoms and stop the disease progression is the ultimate goal of MS research. It is becoming clear that gut microbiota represents an essential factor in development of gut-associated lymphoid tissue (GALT). Studies on experimental autoimmune encephalomyelitis (EAE) indicated a role of altered gut microbiota in the MS development. Recent comparative studies on gut microbiota composition provided evidence of a moderate dysbiosis in the structure of gut microbiota in MS patients. Furthermore, 21 species showed significant differences in relative abundance between MS patients and healthy controls. These taxa comprised primarily of clostridial species belonging to Clostridia clusters XIVa and IV and Bacteroidetes. Moreover, it was found that Faecalibacterium was found to be lower abundant in MS patients.

Methods:
In this study Albino Oxford (AO) and Dark Agouti (DA) rats were compared for their mesenteric lymph nodes (MLN) and Peyer’s patches (PP) cellular composition and their gut microbiota. AO rats are highly resistant to EAE induction, as they do not develop EAE in response to harsh immunization protocols that are efficient in other relatively resistant rat strains. On the other side, DA rats develop EAE after mild immunization, i.e. in the absence of any adjuvant. Housing of the rats was performed under conventional conditions. At least 6 rats were analyzed from each of the tested groups. EAE was induced with rat spinal cord homogenate in phosphate buffer saline mixed with equal volume of complete Freund’s adjuvant. GALT isolated cells were analyzed by cytofluorimetric analysis, while cytokine concentration was determined by sandwich ELISA. The microbial composition was determined by DGGE of PCR amplicons of 16S rRNA followed by nucleotide sequencing of bands of interest.
**Results:**
Differences between AO and DA rats within GALT, including lower percentage of CD4+ T cells and generation of interleukin-17 and interferon-g in MLN and PP of AO rats were found. Microbial analyses showed higher diversity of Lactobacillus spp. in EAE-resistant AO rats. In addition, an uncultivated species of Turicibacter genus was exclusively present in feces of non-immunized AO rats, but not in gut tissue samples. Some members of Firmicutes and Proteobacteria (Undibacterium oligocarboniphilum) were detected only in feces of DA rats between 12 and 15 days after induction (peak of the diseases). Interestingly, the members of Lachnospiraceae were detected in feces of healthy non-immunized DA rats, as well as in DA rats that remained healthy 16 days after induction together with an uncultivated species of Turicibacter genus.

**Discussion:**
It was previously shown that members of the bacterial family Lachnospiraceae were dominantly present in gut microbial community of animals with symptoms of various diseases. In addition, it is tempting to assume that Turicibacter sp. contributes to the resistance of AO rats to EAE induction.

**Keywords:** Multiple sclerosis, Gut microbiota, Albino Oxford rats, Dark Agouti rats, Experimental autoimmune encephalomyelitis

**EFFECT OF POTENTIAL PROBIOTICS ON ALLEVIATING CHRONIC KIDNEY DISEASE PROGRESSION USING CISPLATIN-INDUCED LANYU PORCINE MODEL**

**Wang P.**
National Taiwan University

**Introduction:**
Chronic kidney disease (CKD), characterized by a gradual loss of kidney function, is a global health issue that has a substantial impact on affected individuals. The symptoms of CKD are diverse and include uremia syndrome. Hemodialysis (HD) is unable to effectively eliminate protein-bound solutes as opposed to small water-soluble solutes. The accumulation of protein-bound uremic toxins, including p-cresol and indoxyl sulfate (IS), has been suggested to be related to complications and mortality in HD patients. Our previous study clearly demonstrated that the combination of three strains, Pm-1 which includes Lactobacillus plantarum subsp. plantarum, Lb. paracasei subsp. paracasei and Streptococcus salivarius subsp. thermophilus, have the better ability to reduce uremic toxin, indoxyl sulfate (IS) in MRS broth than individual strains. In vivo, we observed that oral administration of Pm-1 in cisplatin-induced acute kidney injury model significantly suppressed the accumulation of IS in the serum.

**Methods:**
Since swine shares similar anatomic and physiologic characteristics with humans, in the present study, we developed a cisplatin-induced CKD model using Lanyu pig, a Taiwan indigenous breed, and investigated the effects of Pm-1 on preventing CKD using this model. Eight-month-old Lanyu pigs were oral administrated with either control diet, or Pm-1 in different dosages (1×10^9 and 10^10 CFU/kg) from 90 days before cisplatin injection and throughout the whole experiment period. Cisplatin was injected intravenously via auricular vein for nine consecutive times every two days.

**Results:**
The results indicated that the pigs fed with high dosage of Pm-1 group showed the trend to reduce both creatinine and blood urea nitrogen (BUN) when compared with cisplatin group. The high dosage Pm-1 group also demonstrated the lower incidence of lesions including atrophy, mononuclear inflammation, cell infiltration and interstitial fibrosis in renal tubules in H&E and masson’s trichrome stain. In fecal microbiota, Pm-1 administration group had more Lactobacterium and Bifidobacterium proportion while less Clostridium when compared with cisplatin group. The high dosage Pm-1 group also decreased TNF-α production and increased level of catalase activity in plasma. However, results of IS in the blood showed no difference among treatments. According to the above results, administration of high dose Pm1 could alleviate cisplatin-induced CKD in a porcine model.

**Discussion:**
The possible mechanisms of Pm-1 action may due to competitive exclusion of pathogenic bacteria, or
modulate immune system of the host as it ameliorates inflammation. Other than that, Pm-1 may possess antioxidant properties that resulted in alleviation of oxidative stress which caused by cisplatin administration.

**Keywords:** Lanyu pig, Cisplatin, Chronic kidney disease, Probiotics, Anti-oxidative

---

**LACTOBACILLUS RHAMNOSUS GG ENHANCES INTESTINAL MUCUS BARRIER UPON ESCHERICHIA COLI INFECTION**

*Yung C.; Wan M.; Shah N.; El-Nezami H.*

School of Biological Sciences, The University of Hong Kong

**Introduction:**
Foodborne infection is a major food safety issue in both developing and developed countries. The use of probiotics has been emerging as a tool for the control of foodborne infections and gastrointestinal disorders such as diarrhea. Our study aims to investigate potential protective effects of probiotics on intestinal epithelial cells against invasion of foodborne pathogens and to elucidate the mechanisms of such effects. We hypothesize that Lactobacillus rhamnosus GG (LGG) can either be used as prevention against or as an antagonist of foodborne pathogens, and such protective effects can be conferred through the upregulation of mucins in the intestinal epithelial cells.

**Methods:**
Two strains of Escherichia coli (E. coli), including an enteroinvasive strain and a control strain, were used. The direct inhibitory effect of LGG on the growth of E. coli was studied using inhibition assay. LGG and E. coli were mixed and incubated for 2 hours. In order to test the protective ability of LGG against foodborne pathogens in the intestine, HT29-MTX cells, a human colon cell line well-characterized for the production of secretory mucins (e.g., MUC5AC and MUC5B), was used as an in vitro model. HT29-MTX cells were pre-treated with LGG for 1 hour followed by the addition of E. coli for another hour. In another set of experiment where LGG was used as an antagonist, LGG and E. coli were incubated simultaneously with HT29-MTX cells for 2 hours.

**Results:**
The presence of LGG inhibited the growth of both strains of E. coli at LGG:E. coli ratio of 10:1 to 1:10. LGG showed stronger inhibitory effect on enteroinvasive E. coli than the control strain. The addition of LGG, either as a prevention or as an antagonist of both strains of E. coli, upregulated the expression of MUC5AC and MUC5B genes of HT29-MTX cells. Such effect was observed when LGG was present at multiplicity of infection (MOI) from the range of 1000:1 to 10:1. LGG induced higher level of MUC5AC gene expression when added as an antagonist than as a prevention. In simultaneous incubation of LGG and E. coli, level of MUC5B gene expression upon infection of enteroinvasive strain was higher than that of control strain.

**Discussion:**
The inhibitory effect of LGG on E. coli showed the potential of probiotics as a way to control the growth of pathogenic bacteria in food. The upregulation of MUC5AC and MUC5B genes when LGG and E. coli were added at different conditions further demonstrate the protective effect of LGG on HT29-MTX cells, possibly through bacterial-cell interactions. These results would lead us to further investigations of effects of probiotics on strengthening of intestinal mucus barrier against foodborne pathogens, and possibly the development of preventive measures of foodborne infections.

**Keywords:** Intestinal Mucus Barrier, Mucin, Foodborne infection, Intestinal integrity, Lactobacillus rhamnosus GG, Escherichia coli
### Author Index

<table>
<thead>
<tr>
<th>Author Name</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aboú Hachem M.</td>
<td>87, 91</td>
</tr>
<tr>
<td>Abubaid A.</td>
<td>49</td>
</tr>
<tr>
<td>Acquaro A.</td>
<td>61</td>
</tr>
<tr>
<td>Aggrey S.</td>
<td>53</td>
</tr>
<tr>
<td>Aguayo M.</td>
<td>83</td>
</tr>
<tr>
<td>Ahn S.</td>
<td>83</td>
</tr>
<tr>
<td>Ahn Y.</td>
<td>106</td>
</tr>
<tr>
<td>Al Kaabi M.</td>
<td>49</td>
</tr>
<tr>
<td>Al Kethi M.</td>
<td>49</td>
</tr>
<tr>
<td>Alarcón P.</td>
<td>83</td>
</tr>
<tr>
<td>Aleksandrzak-Piekarczyk T.</td>
<td>76</td>
</tr>
<tr>
<td>Amézqueta S.</td>
<td>28, 60</td>
</tr>
<tr>
<td>Andersen J.</td>
<td>91</td>
</tr>
<tr>
<td>Antal O. T.</td>
<td>93</td>
</tr>
<tr>
<td>Antoine P.</td>
<td>84</td>
</tr>
<tr>
<td>Anzawa D.</td>
<td>38</td>
</tr>
<tr>
<td>Aoki R.</td>
<td>38</td>
</tr>
<tr>
<td>Asenjo S.</td>
<td>17</td>
</tr>
<tr>
<td>Atenia L.</td>
<td>60</td>
</tr>
<tr>
<td>Auglaulet M.</td>
<td>21</td>
</tr>
<tr>
<td>Awati A.</td>
<td>14</td>
</tr>
<tr>
<td>Babenko L.</td>
<td>41</td>
</tr>
<tr>
<td>Balau M.</td>
<td>85</td>
</tr>
<tr>
<td>Ballance S.</td>
<td>40</td>
</tr>
<tr>
<td>Bardowski J.</td>
<td>76</td>
</tr>
<tr>
<td>Barchi L.</td>
<td>61</td>
</tr>
<tr>
<td>Barrera C.</td>
<td>85</td>
</tr>
<tr>
<td>Barrera Puigdollers C.</td>
<td>17</td>
</tr>
<tr>
<td>Bastiaans J.</td>
<td>24</td>
</tr>
<tr>
<td>Bayakhyshova K.</td>
<td>113</td>
</tr>
<tr>
<td>Beauprez J.</td>
<td>67, 71</td>
</tr>
<tr>
<td>Benedetti Panici P.</td>
<td>62</td>
</tr>
<tr>
<td>Berlee A.</td>
<td>15, 116</td>
</tr>
<tr>
<td>Berang M.</td>
<td>53</td>
</tr>
<tr>
<td>Bertolino M.</td>
<td>61</td>
</tr>
<tr>
<td>Betoret N.</td>
<td>85</td>
</tr>
<tr>
<td>Betoret Valls N.</td>
<td>17</td>
</tr>
<tr>
<td>Blennow A.</td>
<td>87</td>
</tr>
<tr>
<td>Bomba A.</td>
<td>16</td>
</tr>
<tr>
<td>Boobis A.</td>
<td>79</td>
</tr>
<tr>
<td>Bórquez R.</td>
<td>17</td>
</tr>
<tr>
<td>Botta C.</td>
<td>61</td>
</tr>
<tr>
<td>Boumghar-Bourtchali L.</td>
<td>86</td>
</tr>
<tr>
<td>Boyer M.</td>
<td>86</td>
</tr>
<tr>
<td>Braber S.</td>
<td>26</td>
</tr>
<tr>
<td>Brinch K.</td>
<td>22</td>
</tr>
<tr>
<td>Brix S.</td>
<td>87</td>
</tr>
<tr>
<td>Brockmann E.</td>
<td>108</td>
</tr>
<tr>
<td>Bron P.</td>
<td>26</td>
</tr>
<tr>
<td>Brummer R.</td>
<td>26</td>
</tr>
<tr>
<td>Brummer R. J.</td>
<td>80</td>
</tr>
<tr>
<td>Bubnov R.</td>
<td>41</td>
</tr>
<tr>
<td>Burca C.</td>
<td>17</td>
</tr>
<tr>
<td>Bustos A.</td>
<td>17</td>
</tr>
<tr>
<td>Bytzer P.</td>
<td>114</td>
</tr>
<tr>
<td>Cahu T.</td>
<td>87</td>
</tr>
<tr>
<td>Calabuig-Jiménez L.</td>
<td>85</td>
</tr>
<tr>
<td>Calderwood D.</td>
<td>55</td>
</tr>
<tr>
<td>Campri D.</td>
<td>89</td>
</tr>
<tr>
<td>Cani P.</td>
<td>26</td>
</tr>
<tr>
<td>Cani P. D.</td>
<td>80</td>
</tr>
<tr>
<td>Castro E.</td>
<td>17, 83</td>
</tr>
<tr>
<td>Celebioglu H.</td>
<td>87</td>
</tr>
<tr>
<td>Cernat R.</td>
<td>18</td>
</tr>
<tr>
<td>Cesi V.</td>
<td>19</td>
</tr>
<tr>
<td>Cocolin L.</td>
<td>61</td>
</tr>
<tr>
<td>Cofré J.</td>
<td>83</td>
</tr>
<tr>
<td>Colantoni E.</td>
<td>19</td>
</tr>
<tr>
<td>Collett S.</td>
<td>53</td>
</tr>
<tr>
<td>Combrisson J.</td>
<td>86</td>
</tr>
<tr>
<td>Comi G.</td>
<td>89</td>
</tr>
<tr>
<td>Constable A.</td>
<td>21</td>
</tr>
<tr>
<td>Costanzo M.</td>
<td>19</td>
</tr>
<tr>
<td>Coulet M.</td>
<td>21</td>
</tr>
<tr>
<td>Cox N.</td>
<td>53</td>
</tr>
<tr>
<td>Cucchiara S.</td>
<td>19</td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
<tr>
<td>Danguleva A.</td>
<td>96</td>
</tr>
<tr>
<td>DaSilva H.</td>
<td>61</td>
</tr>
<tr>
<td>De Marco S.</td>
<td>90</td>
</tr>
<tr>
<td>de Vos W. M.</td>
<td>80</td>
</tr>
<tr>
<td>Dekio I.</td>
<td>21</td>
</tr>
<tr>
<td>Dekker J.</td>
<td>80</td>
</tr>
<tr>
<td>Demchenko O.</td>
<td>41</td>
</tr>
<tr>
<td>Derrien M.</td>
<td>80</td>
</tr>
<tr>
<td>Devillard E.</td>
<td>22</td>
</tr>
<tr>
<td>Diab R.</td>
<td>27</td>
</tr>
<tr>
<td>Dideren I.</td>
<td>84, 92</td>
</tr>
<tr>
<td>Dtep D.</td>
<td>23, 76</td>
</tr>
<tr>
<td>Dinelli G.</td>
<td>69</td>
</tr>
<tr>
<td>Dinic M.</td>
<td>108</td>
</tr>
<tr>
<td>Djokic J.</td>
<td>120</td>
</tr>
<tr>
<td>Djordjević M.</td>
<td>97</td>
</tr>
<tr>
<td>Domenici L.</td>
<td>63</td>
</tr>
<tr>
<td>Dudko E.</td>
<td>115</td>
</tr>
<tr>
<td>Durán D.</td>
<td>17</td>
</tr>
<tr>
<td>Duval R.</td>
<td>27</td>
</tr>
<tr>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Edwards C.</td>
<td>79</td>
</tr>
<tr>
<td>Ejby M.</td>
<td>91</td>
</tr>
<tr>
<td>El-Nezami H.</td>
<td>124</td>
</tr>
<tr>
<td>Elova N.</td>
<td>48</td>
</tr>
<tr>
<td>Enouf V.</td>
<td>40</td>
</tr>
<tr>
<td>F</td>
<td></td>
</tr>
<tr>
<td>Falaluyeveya T.</td>
<td>41</td>
</tr>
<tr>
<td>Farbáková J.</td>
<td>104</td>
</tr>
<tr>
<td>Ferreira S.</td>
<td>91</td>
</tr>
<tr>
<td>Fischer M.</td>
<td>24</td>
</tr>
<tr>
<td>Folkerts G.</td>
<td>24, 25, 26</td>
</tr>
<tr>
<td>Fonollá J.</td>
<td>109</td>
</tr>
<tr>
<td>Fontanay S.</td>
<td>27</td>
</tr>
<tr>
<td>Fonteyn F.</td>
<td>92</td>
</tr>
<tr>
<td>Fotschki J.</td>
<td>74</td>
</tr>
<tr>
<td>Fourmestraux C.</td>
<td>86</td>
</tr>
<tr>
<td>Franek A.</td>
<td>79</td>
</tr>
</tbody>
</table>
Author index

Lindman B........................................ 70
Loopeert R........................................ 51
Louis E........................................... 84, 92
Lukić J............................................. 122
M
Ma T.............................................. 98
Maheimo H...................................... 67
MacDonald T. T................................. 80
Macuamule C. L................................ 81
Maďari A......................................... 104
Madsen L......................................... 98
Markiewicz L................................... 74
Marotti I.......................................... 69
McCartney E..................................... 45
McCoy K.......................................... 46
Medronho B..................................... 70
Méheust A........................................ 80
Meland N.......................................... 44
Mellado J.......................................... 83
Mercenier A...................................... 80
Miguel M.G...................................... 70
Mihajlovic S..................................... 120
Mihajlovic S..................................... 122
Miljkovic D...................................... 122
Mills A. D......................................... 47
Minami J.......................................... 47
Miralimova S.................................... 48
Montijn R......................................... 79
Moreno A......................................... 28
Mostarica Stojkovic M......................... 122
Mrvaljević I...................................... 108
Mudgil P.......................................... 49
Mudroňová D.................................... 104
Mueller M........................................ 51
Mulder L.......................................... 50
Mustafa N........................................ 49
Myling-Petersen D.............................. 108
N
Naár Z............................................. 93
Nakayama J...................................... 52
Nakthaichit M................................... 52
Nato A............................................. 24
Natua A........................................... 79
Nauta A............................................ 80
Negroni A......................................... 19
Nelson A.......................................... 22
Nemedi E.......................................... 93
Nielsen B........................................ 18
Nielsen P.......................................... 22
Nieto Magro C.................................. 117
Nishijima T...................................... 38
Nitisinprasert S................................ 52
O
Oakley B.......................................... 53
Ogay D............................................ 48
Oh S................................................ 53
Olivares M....................................... 109
Olsen J............................................ 108
Ota T............................................... 54
Ouyang K........................................ 59
P
Pack K............................................. 43
Pagiotti R........................................ 90
Palková L......................................... 79
Palma E.......................................... 63
Palma E.......................................... 19
Pang V............................................. 110
Panwar H......................................... 55, 116
Park H............................................. 56
Park J.............................................. 43, 83, 111
Park S.............................................. 30, 57, 83, 88
Park T.............................................. 112
Pasc A............................................. 27
Pechenyak B..................................... 61
Penard L.......................................... 21
Phumsombat P................................... 52
Piccioni M........................................ 90
Pierangelii A..................................... 63
Pierdomenico M................................. 19
Pietrella D........................................ 90
Pitkäranta A...................................... 104
Plachá I............................................ 103
Plaza G............................................ 95
Pluipjeen S....................................... 52
Popovic D........................................ 120
Popovic N......................................... 108
Possemiers S.................................... 58
Powalski S....................................... 95
Pražnik W........................................ 51
Prehn K............................................ 87
Q
Qu M............................................... 59
R
Raes J............................................. 79
Rajic J............................................. 97
Ramuyo-Caldas Y............................... 98
Ramos-Romero S............................... 28, 60
Ranganathan N................................. 61
Ranganathan P................................. 61
Rantisio K........................................ 61
Rastall R......................................... 62
Ratnikova I...................................... 113
Rayat L............................................ 22
Recine N.......................................... 63
Rehdin S......................................... 108
Rissani N......................................... 44
Röhrig C.......................................... 21
Rowland I........................................ 72
Rumyan N........................................ 96
S
Saad S............................................ 87
Sadanov A......................................... 113
Saharan B........................................ 64
Sailer M.......................................... 65
Saito T............................................. 65
Salehi Jouzani G............................... 66
Salomonsson E................................. 114
Salvador P....................................... 67, 71
Saygusheva L................................. 115
Scott K............................................ 72
Segui Gil L....................................... 17
Segui I............................................ 85
Shah N............................................ 124
Sharma C......................................... 116
Shrestha R......................................... 116
Shi Q.............................................. 67
Author Index

S
Shin E.................................................43
Shin H.................................................56
Schilte B.............................................21
Schrezenmeir J..................................40
Schuck P.............................................31
Schuren F...........................................79
Sichel L.......................................68, 106
Sim J.................................................134
Simonetti E.........................................69
Singh P..............................................70
Slotboom D......................................91, 342
Soetaert W......................................67, 71
Soeth E................................................40
Sokhbnbazarova K...........................48
Sokovic S..........................................120
Sommer M........................................114
Song C...........................................31, 34, 36, 97, 100, 101, 105
Sorobeta D........................................98
Spivak M.............................................41
Sprenger N.........................................71, 351
Stanišavljevic S..................................122
Stefanova Todorova N.;...................96
Stenman L...........................................44
Stolte E...............................................40
Strojnić L...........................................16
Strompfová V....................................103, 104
Stronati L............................................19
Styková, E.........................................103
Suárez Almarza J.................................117
Sung M...............................................118
Surdjiška S.......................................101
Svensson B.......................................87, 91
Svensson-Frej M.................................98
Swann J...............................................72
Szajewska H.......................................73
Szita N...............................................73
Szyć A.................................................74
S
Škrlec K.............................................15, 116
Strukelj B..........................................15
Strukelj B..........................................116
T
Takahashi S......................................119
Taneyo Saa D.....................................69
Tenkanen M........................................67
Teržič-Vidojević A.............................108
Thakur N............................................116
Theilmann M......................................75
Theodorou V.......................................80
Thonart P...........................................84, 92
Thongaram T.....................................120
Todorov S..........................................76
Tol R...................................................79
Toljč A...............................................97
Tošinački M.......................................120
Tošinački M.......................................97
Torres J............................................28, 60
Tsai Y...............................................121
Tuohy K............................................72, 79
Turk B...............................................15
Turlybayeva Z....................................113
Tymoshewska A..................................76
V
Valdvoska A.......................................94
Van den Abbeele P.............................77
van Loveren H....................................45
Vauvy G..............................................91
Vecino López R.................................117
Veljović K.........................................108, 122
Venema K..........................................78
Verbeke K..........................................79
Verheijden K......................................26
Viermeinstein H................................51
Vigsnaes L.........................................114
Vitali R...............................................19
Vos A..................................................24
Vossen J.............................................79
Vyas U.................................................61
W
Wan M...............................................124
Wang P..............................................123
Weekers F.........................................84
Weinberg A........................................61
Wellejus A.........................................108
Wells J...............................................26
Wells J, M...........................................80
Willemsen L......................................26
Withuhn R. C.....................................81
Wróblewska B.................................74, 139
Wylie A...............................................55
X
Xiao L...............................................24
Y
Yeo S...............................................56, 99, 107
Yevtushenko E.................................115
Yong Y...............................................39
Yoon J...............................................83, 111
You J..................................................59
Youn H...............................................101
Yuk S.................................................34, 105
Yung C..............................................124
Z
Zadravec P.........................................15
Zhou W.............................................39
Zivkovic M........................................120
Z
Živković M.........................................97
**Keyword index**

1

16S rDNA ........................................... 30
16S rRNA ........................................... 30
16S rRNA pyrosequencing analysis ............ 39
16S rRNA sequencing ................................ 53

2

2'-O-Fucosyllactose .................................. 21

A

Acetate ............................................. 39
Acid resistance .................................... 93
Acid tolerance ..................................... 116
Acute gastroenteritis ................................ 73
Adhesion ........................................... 88, 89, 96
adhesion & invasion assay ........................... 20
Adhesion ability ..................................... 100
Adjuvant ........................................... 34
Adolescents ......................................... 108
Aerobic stress ....................................... 89
Age .................................................. 119
Age-associated changes .............................. 81
Albino Oxford rats .................................. 123
Alginate ............................................. 104
Allergy ............................................... 26, 75
Amino acid .......................................... 103
Anemia ............................................. 48
Animal performance ................................ 23
Animal’s gut health .................................. 15
Antibiotic ............................................ 78
Antibiotic resistance .................. .......................... 89
Antibiotic-associated diarrhoea .................. 73
Antibiotics ........................................... 24, 80
Anti-inflammatory activity ......................... 90
Antimicrobial ........................................ 64
Antimicrobial activity .................. .......................... 49, 98, 109, 116, 120
Antimicrobial development ......................... 24
Antimicrobial susceptibility ......................... 44
Antimicrobials ....................................... 76
Anti-oxidant activity ................................ 90
Antioxidant effect .................................... 112
Anti-oxidative ........................................ 124
Antipathogenic ...................................... 43
Anti-viral ............................................ 106
Apoptosis ........................................... 33
Apple-snak .......................................... 86
Arabinoxylan ...................................... 41
Asthma .............................................. 26
Atopic dermatitis .................................... 33
Auto-aggregation .................................... 116
autoimmune diabetes ................................ 25
Autoinducer-2 ...................................... 57
Avian Beta-Defensin 9 ................................ 59
Avian influenza ..................................... 34

B

Bacillus .............................................. 59
Bacillus spp ........................................ 19
Bacterial interaction ................................ 57
Bacterial lysates .................................... 84
Bacteriocidal ........................................ 64
Bacteriocin .......................................... 24, 64

Bacteriocin biology .................................. 77
Bacteriocin receptor ................................ 24
Bacteriocins ........................................ 49, 76
Bacterioidales ...................................... 29, 60
Barrier .............................................. 108
Beta-glucan ........................................ 41
Bifidobacteria ........................................ 39, 47, 66, 91, 96, 114
Bifidobacterium ................................... 40, 48, 76, 108, 114, 119
Bifidobacterium Healthy elderly people ........ 44
Bifidobacterium lactis B420 ......................... 44
Bifidogenic activity .................................. 69
Bile .................................................. 35
Bile salts ............................................... 93
Bile tolerance ....................................... 116
Bioenosis ............................................ 115
Biofilm ............................................... 107
Bioreactor cultures .................................. 96
Biotherapy ........................................... 33
Biotransformation ................................... 73
Bone ............................................... 36
Broad-spectrum protection ......................... 106
Breastfeeding ........................................ 110
Broiler chicken ...................................... 67
Butyric acid .......................................... 62

C

C. difficile ........................................... 24
Caco-2 cell .......................................... 84
Caco-2 cell model .................................... 117
Caco-2 cells .......................................... 93
Camel milk ......................................... 50
Cancer ............................................... 33
Carbohydrate transport .............................. 91
Cardiovascular Disease ............................... 59
Cariogenic rat model ................................ 107
Carotenoids .......................................... 59
Cell energy metabolism ............................. 74
Cell growth kinetics ................................ 74
Cellular immunity ................................... 114
Cisplatin ............................................ 124
Clinical trial ......................................... 22
Clinical trials ........................................ 105
Clostridium difficile ................................ 78
Coffee ............................................... 83
Colitis ............................................... 108
Colonic inflammation ................................ 119
Complex carbohydrate .............................. 67
Convective drying .................................... 86
Crohn’s disease ..................................... 85
Cytokine ............................................. 33, 85
Cytokine binding .................................... 16
Cytokines ............................................ 93
Cytometer analysis ................................... 96

D

Dairy .................................................. 54
Dark Agouti rats .................................... 123
Decarboxylase ....................................... 115
Del-ImmuneV® ...................................... 69
Delivery ............................................. 71
Delivery vehicle ...................................... 32
Dendritic cell ......................................... 111
Dental caries ......................................... 107
<table>
<thead>
<tr>
<th>Keyword Index</th>
<th>IPC 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td>122</td>
</tr>
<tr>
<td>Dextranase</td>
<td>68</td>
</tr>
<tr>
<td>D-Fagomine</td>
<td>29</td>
</tr>
<tr>
<td>Diabetes type 1</td>
<td>97</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>118</td>
</tr>
<tr>
<td>Diet</td>
<td>73</td>
</tr>
<tr>
<td>Diet intervention study</td>
<td>70</td>
</tr>
<tr>
<td>Digestion</td>
<td>73</td>
</tr>
<tr>
<td>Disease</td>
<td>35</td>
</tr>
<tr>
<td>Disease prevention and treatment</td>
<td>16</td>
</tr>
<tr>
<td>Disease risk reduction</td>
<td>45</td>
</tr>
<tr>
<td>Doenjiang</td>
<td>101</td>
</tr>
<tr>
<td>Dog</td>
<td>104</td>
</tr>
<tr>
<td>DPHM</td>
<td>122</td>
</tr>
<tr>
<td>DPPH</td>
<td>112</td>
</tr>
<tr>
<td>Dysbiosis</td>
<td>30, 78</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>38</td>
</tr>
<tr>
<td>Early life nutrition</td>
<td>25</td>
</tr>
<tr>
<td>Effect</td>
<td>103</td>
</tr>
<tr>
<td>Effector molecules</td>
<td>43</td>
</tr>
<tr>
<td>EFSA</td>
<td>46</td>
</tr>
<tr>
<td>Electrospaying</td>
<td>28</td>
</tr>
<tr>
<td>Emulsification</td>
<td>28, 83</td>
</tr>
<tr>
<td>Enhancing immune system</td>
<td>33</td>
</tr>
<tr>
<td>Enterobacterioides</td>
<td>60</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>103</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>124</td>
</tr>
<tr>
<td>Evasin</td>
<td>117</td>
</tr>
<tr>
<td>Exogenous enzymes</td>
<td>15</td>
</tr>
<tr>
<td>Exopolysaccharide EPS-CG11</td>
<td>97</td>
</tr>
<tr>
<td>Exopolysaccharides</td>
<td>41, 43</td>
</tr>
<tr>
<td>Experimental autoimmune encephalomyelitis</td>
<td>123</td>
</tr>
<tr>
<td>Fatty acid biosynthesis</td>
<td>62</td>
</tr>
<tr>
<td>Fatty acid composition</td>
<td>30</td>
</tr>
<tr>
<td>Fecal microbiota transplantation</td>
<td>35</td>
</tr>
<tr>
<td>Fecal microbe transplantation</td>
<td>24</td>
</tr>
<tr>
<td>Fecal transplants</td>
<td>78</td>
</tr>
<tr>
<td>Feed additives</td>
<td>100</td>
</tr>
<tr>
<td>Feed consumption rate (FCR)</td>
<td>67</td>
</tr>
<tr>
<td>Feed conversion</td>
<td>102</td>
</tr>
<tr>
<td>Feed-additive</td>
<td>31</td>
</tr>
<tr>
<td>Fermentation</td>
<td>65</td>
</tr>
<tr>
<td>Fermentation characteristics</td>
<td>89</td>
</tr>
<tr>
<td>Fermented ethanol Extraction</td>
<td>112</td>
</tr>
<tr>
<td>Fermented milk</td>
<td>58, 86</td>
</tr>
<tr>
<td>Fluorescence animal imaging</td>
<td>16</td>
</tr>
<tr>
<td>Food</td>
<td>45</td>
</tr>
<tr>
<td>Food allergy</td>
<td>69</td>
</tr>
<tr>
<td>Food analytical microbiology</td>
<td>86</td>
</tr>
<tr>
<td>Food Animals</td>
<td>53</td>
</tr>
<tr>
<td>Food Safety</td>
<td>53</td>
</tr>
<tr>
<td>Foodborne infection</td>
<td>124</td>
</tr>
<tr>
<td>Food-borne pathogens</td>
<td>49</td>
</tr>
<tr>
<td>Freeze-drying</td>
<td>103</td>
</tr>
<tr>
<td>Fructans</td>
<td>52</td>
</tr>
<tr>
<td>Fucosyllactose</td>
<td>67, 71</td>
</tr>
<tr>
<td>Functional</td>
<td>38</td>
</tr>
<tr>
<td>Functional yogurt</td>
<td>66</td>
</tr>
<tr>
<td>GABA</td>
<td>121</td>
</tr>
<tr>
<td>gadB</td>
<td>121</td>
</tr>
<tr>
<td>Galactooligosaccharides</td>
<td>76</td>
</tr>
<tr>
<td>Garviecin Q</td>
<td>77</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>118</td>
</tr>
<tr>
<td>Gastrointestinal condition</td>
<td>120</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>81</td>
</tr>
<tr>
<td>Gastrointestinal simulation</td>
<td>17</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>100</td>
</tr>
<tr>
<td>Gene comparison</td>
<td>58</td>
</tr>
<tr>
<td>Genome-wide analysis</td>
<td>62</td>
</tr>
<tr>
<td>Genomics</td>
<td>37</td>
</tr>
<tr>
<td>GIP</td>
<td>56</td>
</tr>
<tr>
<td>GLP-1</td>
<td>56</td>
</tr>
<tr>
<td>Glycan utilization</td>
<td>91</td>
</tr>
<tr>
<td>Glycans</td>
<td>47</td>
</tr>
<tr>
<td>Glycoconjugates</td>
<td>47</td>
</tr>
<tr>
<td>GOS</td>
<td>26</td>
</tr>
<tr>
<td>Greek-style yogurt</td>
<td>89, 112</td>
</tr>
<tr>
<td>Gut</td>
<td>16</td>
</tr>
<tr>
<td>Gut Fermentation</td>
<td>63</td>
</tr>
<tr>
<td>Gut health</td>
<td>19, 59</td>
</tr>
<tr>
<td>Gut Metabolome</td>
<td>63</td>
</tr>
<tr>
<td>Gut microbeime</td>
<td>61, 63</td>
</tr>
<tr>
<td>Gut microbiota</td>
<td>24, 30, 36, 52, 70, 73, 119, 123</td>
</tr>
<tr>
<td>Gut microbiota modulation</td>
<td>57</td>
</tr>
<tr>
<td>Gut modelling</td>
<td>52</td>
</tr>
<tr>
<td>Gut permeability</td>
<td>119</td>
</tr>
<tr>
<td>H</td>
<td>73</td>
</tr>
<tr>
<td>H pylori infection</td>
<td>73</td>
</tr>
<tr>
<td>Hapten-induced contact hypersensitivity</td>
<td>33</td>
</tr>
<tr>
<td>Health benefits</td>
<td>65</td>
</tr>
<tr>
<td>Health claims</td>
<td>45</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>17</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>48</td>
</tr>
<tr>
<td>Herb extract</td>
<td>89</td>
</tr>
<tr>
<td>Herbs</td>
<td>95</td>
</tr>
<tr>
<td>High intensity ultrasound</td>
<td>40</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>119</td>
</tr>
<tr>
<td>Histamine</td>
<td>115</td>
</tr>
<tr>
<td>HMOs</td>
<td>114</td>
</tr>
<tr>
<td>Homeostasis</td>
<td>46</td>
</tr>
<tr>
<td>Homogenization</td>
<td>17, 86</td>
</tr>
<tr>
<td>Horses</td>
<td>103</td>
</tr>
<tr>
<td>Host response</td>
<td>23</td>
</tr>
<tr>
<td>HPV infection</td>
<td>64</td>
</tr>
<tr>
<td>HT-29 cells</td>
<td>88</td>
</tr>
<tr>
<td>Human and Mice studies</td>
<td>39</td>
</tr>
<tr>
<td>Human milk</td>
<td>110</td>
</tr>
<tr>
<td>Human Milk Oligosaccharides</td>
<td>21, 25, 71, 78</td>
</tr>
<tr>
<td>Hybrid beads</td>
<td>28</td>
</tr>
<tr>
<td>Hydrogels</td>
<td>71</td>
</tr>
<tr>
<td>Hydrophobicity</td>
<td>96</td>
</tr>
<tr>
<td>Hyper-Immune Disorders</td>
<td>33</td>
</tr>
<tr>
<td>Hypertension</td>
<td>60</td>
</tr>
<tr>
<td>C</td>
<td>71</td>
</tr>
<tr>
<td>Characterization</td>
<td>71</td>
</tr>
<tr>
<td>Chemokine binding</td>
<td>16, 117</td>
</tr>
<tr>
<td>Chicken</td>
<td>31, 34, 59</td>
</tr>
<tr>
<td>children</td>
<td>18, 118</td>
</tr>
<tr>
<td>Cholesterol-lowering</td>
<td>58</td>
</tr>
<tr>
<td>Chromogenic agar</td>
<td>86</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>61, 124</td>
</tr>
<tr>
<td>Chronic T. muris infection</td>
<td>99</td>
</tr>
<tr>
<td>M</td>
<td>Immune response</td>
</tr>
<tr>
<td>L</td>
<td>Lactobacillus fermentum</td>
</tr>
<tr>
<td>K</td>
<td>Lectins</td>
</tr>
<tr>
<td>K</td>
<td>Lectins</td>
</tr>
<tr>
<td>I</td>
<td>Lectins</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>I</td>
<td>Lactic acid bacteria</td>
</tr>
</tbody>
</table>
**Keyword Index**

<table>
<thead>
<tr>
<th>N</th>
<th>Non starch polysaccharide</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonalcoholic steatohepatitis (NASH)</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Non-Obese Diabetic Mice</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Novel Bacillus subtilis</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Novelty</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Nutrigenetics</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>Obesity</td>
<td>18, 29, 42, 44, 50, 60</td>
</tr>
<tr>
<td>Oligosaccharide uptake</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>26, 72</td>
<td></td>
</tr>
<tr>
<td>Omega 3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Omega-3 fatty acid</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Open-label investigation</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Overproduction</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>Pancreatic Lipase Inhibition</td>
<td>50</td>
</tr>
<tr>
<td>PAP-smear abnormalities</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Pathogens</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Pediococcus</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Peptides</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Personalized nutrition</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Piglet</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Piglets</td>
<td>19, 30</td>
<td></td>
</tr>
<tr>
<td>Plantaricins</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Plant-origin</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Polysaccharose</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Polymerization degree</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Polyphasic analysis</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Porcine</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Potentiated probiotics</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Poultry microbiome</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Powder</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Prebiotic</td>
<td>65, 76, 96</td>
<td></td>
</tr>
<tr>
<td>Prebiotic health benefits</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Prebiotic index</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Prebiotics</td>
<td>26, 63, 66, 80, 87, 90, 91, 113</td>
<td></td>
</tr>
<tr>
<td>Preclinical studies</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Predictors of response</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Prevention</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Probiotic18, 35, 52, 59, 67, 84, 86, 96, 103, 104, 109, 116, 118, 121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probiotic capsules</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Probiotic composition</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Probiotic feed additive</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Probiotic identification</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>Probiotic lactobacilli</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Probiotic lyase</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Probiotic quantification</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>Probiotics19, 23, 26, 27, 32, 38, 43, 44, 49, 50, 54, 56, 57, 61, 62, 66, 85, 87, 90, 100, 107, 114, 115, 119, 120, 124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probiotics Implementation</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Probiotics in children</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Proinflammatory cytokines</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Proliferative ability</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Propionibacterium</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Propionic-acid bacteria</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Propolis</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Psychobiotics</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>qPCR</td>
<td>92</td>
</tr>
<tr>
<td>QPS</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Quality Assurance and Stability</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Quality control</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>Quality of life</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Quorum sensing</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Rabbits</td>
<td>102</td>
</tr>
<tr>
<td>Randomized controlled trial</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Randomized trial</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>Raw milk</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>Real-time monitoring</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Receptors for bacteriocins</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Regenerative activity</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Resistance</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Resistance development</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Resistance to bacteriocins</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Respiratory infections</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Respiratory symptoms</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Respiratory tract infection</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Respiratory tract infections</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Respiratory virus</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Responsive surface modeling</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Response surface methodology</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Rhamnosus GG</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>Safety</td>
<td>21, 46, 114</td>
</tr>
<tr>
<td>Salmonella</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Salmonella enteritidis</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Salmonella challenge</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Screening model</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Sensory evaluation</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>Sequencing</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Sexually transmitted diseases</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>SHIME</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Short chain fatty acids</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Short-chain fatty acids</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Sialyllactose</td>
<td>67, 71</td>
<td></td>
</tr>
<tr>
<td>Skin milk</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Skin microflora</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Skincare product</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Soluble dietary fibres</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Solute-binding protein</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Somaltooligosaccharides</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Specific oxygen uptake rate</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Spray drying</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>22, 113</td>
<td></td>
</tr>
<tr>
<td>Stevia extracts</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Stomatitis</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Storage stability</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Strain discrimination</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Strain enumeration</td>
<td>86</td>
<td></td>
</tr>
</tbody>
</table>

---

Strain-level dependent properties .................................. 75
Strain-specific effect .................................................. 58
Streptococcus mutans .................................................. 107
Streptococcus oralis .................................................... 50
Streptococcus thermophilus ......................................... 40, 109
Stress tolerance .......................................................... 32
Subproteomes ............................................................. 88
Sugar substitute ......................................................... 89
Survival ................................................................. 87
Swine ................................................................. 19
Symbiotic ............................................................. 52, 118
Symbiotic Interactios .................................................. 87
Symbiotics ............................................................... 88
Synbiotic bacterial mixtures ......................................... 78
Synbiotic ............................................................... 52, 118
Synbiotic Interactios .................................................. 87
Synbiotics ............................................................... 88
Synthetic bacterial mixtures ......................................... 78
synthetic biology .......................................................... 67

T
Therapeutic potency .................................................. 69
TLC ................................................................. 121
Tolerance ............................................................. 114
Transplantation ....................................................... 16
Trehalose ............................................................... 17, 86
Trichuris muris ......................................................... 99
Type 2 Diabetes .......................................................... 56

U
Ulcerative colitis ...................................................... 35, 85
Upper respiratory tract infections .................................. 50
Uremic toxins ........................................................... 61

V
Vacuum impregnation .................................................. 86
Vancomycin resistance enterococcus ................................ 52
Viability, Fermentation ................................................ 40
Visceral fat ............................................................. 119
Visceral fat accumulation ........................................... 39
Vitamin B2 .............................................................. 107

W
Weaning ................................................................. 30
Weight management .................................................. 44
Wild bird ............................................................... 100
Wistar rats .............................................................. 97

X
Xylanase ................................................................. 15

Y
Yeast ................................................................. 85

Z
Zeta Potential .......................................................... 87
Zinc ................................................................. 95
Zoonotic tuberculosis .................................................. 82
Z-potential ............................................................. 83

α
α-(1→2) linkage .......................................................... 68
α-Glucan oligosaccharide ............................................ 113
β
β-galactosidase .......................................................... 40