

2009 Conference on Gastrointestinal Function

Gleacher Center
Chicago IL, USA
April 20-22nd, 2009

Final Program

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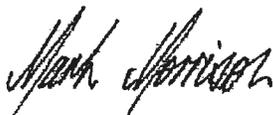
Proliant

Chair's Welcome:

It is my pleasure to welcome you to the 2009 Conference on Gastrointestinal Function (CGIF)! The CGIF (formerly Rumen Function Conference) has been held on a biennial basis in Chicago since 1951. Because the interactions between the gut microbiome, nutrition and host physiology are now more widely recognized as being influential to the health and well being of humans as well as all food animals, the scope of the Conference expanded, and in 2003 we officially changed to the Conference on Gastrointestinal Function. The Organizing Committee is especially pleased that the expanded scope of our Conference is attracting more scientists from a variety of disciplines, as evidenced again by the podium and poster presentations this year. Indeed, we are especially pleased with the range of topics being presented, which includes some of the latest advances in our understanding of gastrointestinal function. We sincerely hope you enjoy these contributions. At the end of this program booklet, you will find a brief history of the Conference, kindly prepared by Professor Burk A. Dehority.

On behalf of the CGIF 2009 organizing committee (Jane Leedle, JL Microbiology; Rod Mackie, Isaac Cann, University of Illinois; and Jeff Firkins, The Ohio State University) I wish to acknowledge the generous financial support provided by our sponsors this year. Their support is truly critical to the success of Conference's presentation of emerging areas of research, the promotion of scholarly discourse, and the exchange of research methodologies and strategies. Thanks to all of you once again. I would also like to acknowledge Springer Press for their willingness to publish the Conference abstracts in the journal *Microbial Ecology*; as well as the support of the Journal's Editor, Karen Nelson (J. Craig Venter Institute). We also appreciate the efforts of Nancy Henry (University of Illinois) and Michael Chakerian (The Ohio State University) for providing assistance with the logistical details for the Conference.

Last I thank all of you, the attendees, for selecting CGIF 2009 as one of the key venues for you to interact with colleagues and present your science this year. There are many scientific meetings that you can attend, and we sincerely hope that CGIF 2009 provides you with the professional and social interactions that warrant your continued participation and attendance at future CGIF Conferences. ENJOY!!!!



Mark Morrison
Chair CGIF 2009
CSIRO Australia and The Ohio State University

2009 CONFERENCE ON GASTROINTESTINAL FUNCTION PROGRAM AND POSTER TITLES

MONDAY, APRIL 20TH:

15:00-19:30 REGISTRATION Gleacher Center, First Floor (100 Foyer)

Please pick up your Registration Materials, Name Tag and Mixer drink tickets

17:30-19:30 WELCOME MIXER Gleacher Center, Sixth Floor (Room 621)

All attendees and guests please wear your name tag. Refreshments: Drink tickets and cash bar

TUESDAY, APRIL 21ST:

08:00-09:00 CONTINENTAL BREAKFAST AND REGISTRATION

Gleacher Center, First Floor, near the Tiered Classroom

09:00-09:10 Welcome remarks and acknowledgments. **M. Morrison**, Conference Chair, CSIRO Australia, and The Ohio State University

09:10-09:50 Seasonal Digestive Function In Arctic Ruminants: Flexible Fermentation And A Variable Food Supply. **P.S. Barboza**; University of Alaska, Fairbanks AK, USA

09:50-10:10 Biochemical Analysis of Two Glycosyl Hydrolases Provides Insight into the Utilization of Recalcitrant Polysaccharides by Rumen Bacteria. **Dylan Dodd**^{1,4}, Svetlana A. Kocherginskaya², M. Ashley Spies^{3,4}, Kyle E. Beery⁵, Charles A. Abbas^{5,2,4}, Roderick I. Mackie^{2,4}, and Isaac K. O. Cann^{1,2,4} ¹Department of Microbiology, ²Department of Animal Sciences, ³Department of Biochemistry, and the ⁴Institute for Genomic Biology, University of Illinois, Urbana, Illinois 61801 and ⁵James R. Randall Research Center, Archer Daniels Midland Company, Decatur, Illinois 62521

10:10-10:30 Diversity of Nitroreductase Genes in Ovine Rumen Under a 2,4,6-Trinitrotoluene Supplemented and Concentrate Diets. **S. Perumbakkam**, E.A. Mitchell, and A.M. Craig; Oregon State University, Oregon, USA

10:30-10:50 COFFEE BREAK First Floor, outside the Tiered Classroom

10:50-11:10 Microarray Analysis Of A Ruminal Xylanolytic Bacterium And A Methanogen Grown In Co-Culture. **C.J. Yeoman**^{1,2}, W.J. Kelly¹, J. Rakonjac², Z. Kong¹, and G.T. Attwood¹; ¹AgResearch Limited, Palmerston North, New Zealand; ²Massey University, Palmerston North, New Zealand

11:10-11:30 Analysis of Key Reductive Acetogenesis Enzymes from Microbial Enrichments from the Cattle Rumen. **S. Denman**¹, J. Padmanabha¹, E. Gagen,¹ M. Morrison^{1,2} and C. McSweeney¹; ¹CSIRO Australia, Queensland, Australia; ²The Ohio State University, Ohio, USA.

11:30-13:00 LUNCH, please make your own arrangements

13:00-13:40 Whole-Body Systems Approaches for Gut-Microbiota Targeted, Personalized Health Management. **L. Zhao**, Shanghai Jiao Tong University, Shanghai, China
Liping

13:40-14:00 Spatial And Temporal Analysis Of Intestinal Microbiota In Conventionalized Germ Free Mice In Correlation With The Host Responses, Using A High-Throughput Phylogenetic Microarray. **M. Derrien**^{1,2}, S. El Aidy^{1,2}, M. Rajilić-Stojanović², D. Molenaar³, F. Levenez⁴, E.G. Zoetendal^{1,2}, J. Dore⁴ and M. Kleerebezem^{1,2}; ¹Top Institute Food and Nutrition, Wageningen, The Netherlands; ²Laboratory of Microbiology, Wageningen University, The Netherlands; ³NIZO Food Research, Ede, The Netherlands; ⁴INRA, Jouy-en-Josas, France. Marie

14:00-14:20 High Protein Diets Impact on Microbial Metabolites and Toxicity in the Human Large Intestine. **S. Gratz**¹, S.H. Duncan², A.J. Richardson¹, A.M. Johnstone³, G.E. Lobley³, H.J. Flint², and R.J. Wallace¹; ¹Microbial Biochemistry Group, ²Microbial Ecology Group and ³Metabolic Health Group, Rowett Research Institute, Bucksburn, Aberdeen, UK
Silvia

14:20-14:40 COFFEE BREAK First Floor, outside the Tiered Classroom

14:40-15:00 Effects Of *Lactobacillus casei* Shirota On Immune Function. H. Dong¹, I. Rowland¹, K.M. Tuohy¹, L.Thomas², and P. Yaqoob¹; ¹The University of Reading, UK; ²Yakult UK Ltd.

15:00-15:20 Characterization of Human-Derived Probiotic *Lactobacillus* spp. with Anti-Pathogenic and Anti-Inflammatory Activities. **M. Taweechoatipatr**^{1,2}, C. Iyer^{3,4}, J. K. Spinler^{3,4}, J. Versalovic^{3,4} and S. Tumwasorn¹; ¹Chulalongkorn University, Thailand; ²Srinakharinwirot University, Thailand; ³Baylor College of Medicine, Texas, USA; ⁴Texas Children's Hospital, Texas, USA.
Maia

15:20-15:40 Coculture Fermentations of *Bifidobacterium* Species and *Bacteroides thetaiotaomicron* Reveal A Mechanistic Insight Into The Prebiotic Effect Of Inulin-Type Fructans. **G. Falony**¹ and L. De Vuyst¹; ¹Vrije Universiteit Brussel, Brussels, Belgium.

Gwen

16:00-18:00 POSTER SESSION/MIXER: Gleacher Center, Sixth Floor (Room 621)

WEDNESDAY, APRIL 22ND:

08:15-9:15 CONTINENTAL BREAKFAST

Gleacher Center, First Floor, outside the Tiered Classroom

09:15-10:00 A Metagenomics View On The Human Microbiota Of The Small-Intestine E.G. Zoetendal^{1,2}, C.C.G.M. Booijink^{1,2}, M. Wels^{1,3}, H. Smidt^{1,2}, W.M. de Vos^{1,2}, and M. Kleerebezem^{1,3}; ¹TI Food and Nutrition, Wageningen, The Netherlands, ²Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands, ³Nizo Food Research, Ede, The Netherlands

10:00-10:20 Towards a Complete View of the Bacterial Diversity in Poultry Intestinal Microbiota. S. Wei¹, M. Cressman¹, M. Lilburn¹, M. Morrison^{1,2}, and Z. Yu¹. ¹The Ohio State University, Ohio, USA; ²CSIRO Australia, Queensland, Australia.

10:20-10:40 Repeated Ruminant Acidotic Challenges in Sheep: Effects on pH and Microbial Ecosystem and Influence of Active Dry Yeasts. F. Chaucheyras-Durand^{1,2}, M. Silberberg², L. Commun², C. Martin², D.P. Morgavi²; ¹Lallemand Animal Nutrition, Toulouse, France; ²INRA Clermont-Ferand/Theix, France.

10:40-11:00 COFFEE BREAK First floor, outside the Tiered Classroom

11:00-11:20 Effect Of Carbohydrase Supplementation On Ileal Morphology And Gut Microbiota In Growing Pigs, Fed Different Cereal-Based Diets. J. Willamil¹, I. Badiola¹, D. Torrallardona², P. A. Geraert³ and E. Devillard³; ¹CRESA, Barcelona, Spain; ²IRTA, Constanti, Spain; ³ADISSEO SAS, Antony, France.

11:20-11:40 Characterization of Gastrointestinal Microbiota in Neonatal Piglets from Sows Supplemented with a *Bacillus* Based Direct-Fed Microbial A. Baker¹, E. Davis¹, J.D. Spencer², R. Moser², and T. Rehberger¹; ¹Agtech Products, Inc., Waukesha, WI, USA, ²JBS United, Inc., Sheridan, IN, USA.

11:40-13:00 LUNCH, please make your own arrangements

13:00-13:40 BRYANT MEMORIAL LECTURE: Synthetic Communities for Studies of Anaerobic Food Webs. David A. Stahl University of Washington, Seattle

13:40-14:00 Isolation of Fiber Degrading Bacteria from Pig Feces. C.J. Ziemer and B.J. Kerr. Agricultural Research Service, USDA, Ames, IA.

14:00-14:20 Bacterial Colonisation of the Tanniferous Forage *Lotus corniculatus* in the Bovine Rumen. J.E. Edwards, P. Morris, M. Robbins, E.J. Kim and A.H. Kingston-

Smith; Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, UK.

14:20-14:40 COFFEE BREAK First floor, outside the Tiered Classroom

14:40-15:00 *Salmonella* Diversity and Transmission Dynamics in Galapagos Iguanas. **E. Wheeler**, I. K. O. Cann and R. I. Mackie; University of Illinois at Urbana-Champaign, Illinois, USA.

15:00-15:20 Bacterial communities associated with Shiga-toxigenic *E. coli* O157:H7 (STEC O157) in beef cattle feedlot environments. **L.M. Durso**, G. Harhay, J.L. Bono, T.P.L. Smith, W.W. Laegreid, J.E. Keen, M.L. Clawson. U.S. Meat Animal Research Center, Nebraska, USA.

15:20-15:30 CLOSING REMARKS AND INVITATION TO CGIF2011

POSTER SESSION, TUESDAY APRIL 21ST
Gleacher Center, Sixth Floor (Room 621)

- ✓ 1. Nitroethane, 2-Nitro-methyl-propionate and Dimethyl-2-nitroglutarate Markedly Reduce Ruminal Methane Production Without Adversely Affecting Ruminal Fermentation. **R.C. Anderson¹**, J.K. Huwe², D.J. Smith², T.B. Stanton³, N.A. Krueger¹, T.R. Callaway¹, T.S. Edrington¹, R.B. Harvey¹ and D.J. Nisbet¹. ¹USDA/ARS, Southern Plains Agricultural Research Center, College Station, Texas, USA; ²USDA/ARS, Biosciences Research Laboratory, Fargo, North Dakota, USA, and ³USDA/ARS, National Animal Disease Center, Ames, IA, USA.
2. Molecular Properties And Transcription Of The Phosphoenolpyruvate Carboxykinase Gene In A Ruminal Bacterium *Ruminococcus albus*. **N. Asanuma**, K. Yoshizawa, K. Kanada and T. Hino; Meiji University, Japan.
3. Isolation of Mimosine Degrading Non-Pathogenic *Klebsiella pneumoniae* From Rumen Contents of German Steers. **A. Aung¹**, and H. Boehnel²; ¹University of Veterinary Science, Yezin, Myanmar; ²Institute for Applied Biotechnology in the Tropics, Georg-August-University, Germany.
- ✓ 4. Characterization Of The Duodenal Microbiota Of Commercial Layer Hens Affected By Focal Duodenal Necrosis. **J.A. Benson**, T.A. Baltzley, T.G. Rehberger, and G.R. Siragusa; Agtech Products, Inc., Waukesha, WI, USA.
- ✓ 5. Spatial and Temporal Changes in the Microbial Community in an Anaerobic Swine Waste Treatment Lagoon. **K.L. Cook¹**, M.J. Rothrock¹ and N. Lovannh¹, J.K. Sorrell², J.H. Loughrin¹. ¹USDA Agricultural Research Service, Bowling Green, KY; ²Western Kentucky University, Bowling Green, KY, USA.
- ✓ 6. Oxalate Degradation by *Lactobacillus acidophilus* and Other Probiotic Bacteria. **A.E.C. Baluka** and **S.L. Daniel**, Eastern Illinois University, Charleston, IL USA.
- ✓ 7. Differential Chemotaxis by Entodiniomorphids and Isotrichids Toward Peptides of Bacterial, Protozoal, and Soy Origin. **H.L. Diaz**, A.M. Stalford, and J.L. Firkins; The Ohio State University, Ohio, USA.
- ✓ 8. Isolation of a *Lactobacillus* sp. from Swine Lagoon Enrichments which Produces p-Ethylphenol from p-Coumaric Acid. **K.C. Doerner**, S. Hughes, T. Allen, and D. Kridelbaugh; Western Kentucky University, KY, USA.
- ✓ 9. Metabolism of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by Ovine Ruminal Microbes. **H.L. Eaton**, M. DeLorme, and A. M. Craig; Oregon State University, OR, USA.

10. Fate And Effect Of Usnic Acid In Lichen On The Bacterial Population In The Reindeer Rumen. **T. Glad**¹, **A. Falk**², **P. Barboza**², **A. Kohn**¹, **L. Brusetti**³, **S.D. Mathiesen**^{4,5}, **R.I. Mackie**⁶, and **M. A. Sundset**¹; ¹University of Tromsø, Tromsø, Norway; ²University of Alaska, Alaska, USA; ³University of Milan, Milan, Italy; ⁴The Norwegian School of Veterinary Science, Tromsø, Norway; ⁵ Saami University College, Guovdageaidnu, Norway; ⁶University of Illinois, Urbana-Champaign, USA.
11. Characteristics Of *Lactobacillus* Strains And Viability Assessment Of Microencapsulated Bacteria Using a 3-Compartment Fermentor Simulating The Gastrointestinal Tract. **K. Goderska** and **Z. Czarnecki**; Poznan University of Life Sciences, Poland.
12. Bacteriocin Compounds Produced By *Lactobacillus Plantarum* Inhibits Growth Of *Salmonella typhimurium* In The Gut Of Mice. **H. S. Gong**, **X. C. Meng**, and **H. Wang** Northeast Agricultural University, Harbin, China.
13. Adhesion to Intestinal Epithelial Cells by Domains at the N-terminus of the Lp_1643 Protein. **F. Liu**¹, **L.H. Du**^{1, 2}, **G.C. Huo**¹. ¹Northeast Agricultural University, Harbin, China; ²Nanjing University of Finance and Economics, Nanjing, China.
14. The Human Faecal Microbial Community Related To Butyrate Produced In Response To A Diet High In Resistant Starch. **S. Kang**¹, **M. Conlon**¹, **S. Denman**¹, **Z. Yu**², **M. Morrison**^{1, 2} and **C. McSweeney**¹; ¹CSIRO, Preventative Health National Research Flagship, Australia ; ²The Ohio State University, Ohio, USA.
15. Purification, Characterization, and Regulation of Glutamine Synthetase from *Prevotella ruminicola* 23. **J. N. Kim**, **I.K.O. Cann**, and **R.I. Mackie**; University of Illinois at Urbana-Champaign, Illinois, USA.
16. An *in silico* Generated Census of the Rumen Bacterial Microbiome. **M. Kim**¹, **C.S. McSweeney**², **M. Morrison**^{1,2}, and **Z. Yu**¹. ¹The Ohio State University, Ohio, USA; ²CSIRO Australia, Queensland, Australia.
17. Quantitative Comparisons of Cultured and Uncultured Bacterial Populations in the Rumen of Cows Fed Different Diets. **M. Kim**¹, **C. Shaw**¹, **M. Morrison**^{1,2}, and **Z. Yu**¹. ¹The Ohio State University, Ohio, USA; ²CSIRO Australia, Queensland, Australia.
18. Isolation and Partial Characterization of *Prevotella* Strains From Sheep Rumen. **D. Matsui**, **S. Koike**, **Y. Kobayashi**; Hokkaido University, Sapporo, Japan.

- ✓ 19. Effects of Antibodies and Glycerol as Potential Inhibitors of Ruminant Lipase Activity. **N.A. Krueger**¹, R.C. Anderson¹, T.R. Callaway¹, T.S. Edrington¹, R.C. Beier¹, W.L. Shelver² and D.J. Nisbet¹; ¹USDA/ARS, Southern Plains Agricultural Research Center, College Station, Texas, USA; ²USDA/ARS, Biosciences Research Laboratory, Fargo, North Dakota, USA.
20. Influence of Phytoecdysteroids on Digestion of Carbohydrates in Intoxication of Growing Organisms **L.S. Kuchkarova**¹, D.T. Sidikov², G.A. Dustmatova², V.N. Syrov¹, and Z. Hushbaktova²; ¹National University of Uzbekistan, Tashkent, Uzbekistan; ²Institute of Plant Substation Chemistry of Academy of Science of Uzbekistan, Tashkent, Uzbekistan.
21. Activity of Carbohydrate Digestion Enzymes in Newborn Lambs with Various Body Mass. **L.S. Kuchkarova**¹, D.T. Sidikov¹, N.A. Ergashev² The National University of Uzbekistan, Tashkent, Uzbekistan; ²Institute of Physiology and Biophysics, Tashkent, Uzbekistan.
22. Isolation and Physiological Characteristics of H⁺-ATPase Deficient Mutants of *Lactobacillus delbrueckii* subsp. *Bulgaricus*. **F. Liu**¹, L.J. Yang¹, G.C. Huo¹, Y.H. Jiao²; ¹Northeast Agricultural University, Heilongjiang, China; ²Heilongjiang University of Chinese Medicine, Heilongjiang, China.
23. Development Of A Unique Small Scale Gastrointestinal Model Simulating The Porcine Ileum And Testing The Efficacy Of The Model By Screening For Probiotic Properties Of *Lactobacillus salivarius* 241. M. S. Malema¹, C. Straker² and **L. Maré**¹; ¹Agricultural Research Council, Irene, South Africa; ²University of Witwatersrand, Johannesburg, South Africa.
24. Isolation Of Lactic Acid Bacteria (LAB) From Traditional Fermented Beverages, Prepared In Rural Areas Of Southern Africa And Screening For Probiotic Properties. M. A. Mafokoane¹, C. Straker², and **L. Maré**¹; ¹Agricultural Research Council, Irene, South Africa; ²Witwatersrand University, Johannesburg, South Africa.
- ✓ 25. Phylogenetic and Functional Specificities of Crohn's Disease Microbiota. **S. Mondot**¹, J. Tap¹, J. P. Furet¹, P. Lepage¹, J. Doré¹, P. Marteau² and M. Leclerc¹; ¹INRA, France; ²AP-HP Hôpital Lariboisière, France.
- ✓ 26. Microbial Community Analysis of Rectal Methanogens and Sulfate Reducing Bacteria in Two Non-Human Primate Species. **N. Nakamura**, S. Leigh, R. I. Mackie and H. R. Gaskins; University of Illinois at Urbana-Champaign, USA.
- ✓ 27. Characterization and Development of a Systems-level Understanding of Microbial Metabolism in the Foregut Microbiome of the Tammar wallaby (*Macropus eugenii*). P. Pope¹, S. Denman¹, S. Tringe², L. Hinds³, P. Hugenholtz², C. McSweeney¹, and **M. Morrison**^{1,4}. ¹CSIRO Australia, Queensland, Australia;

²United States Department of Energy, Joint Genome Institute, Walnut Creek CA, USA. ³CSIRO Australia, Canberra, Australia; ⁴The Ohio State University, Columbus, OH, USA.

28. True Ileal Amino Acid Digestibility of Partially Hydrolyzed and Normal Infant Formulas. **D.X. Ren**, C.C. Duan, and G.C. Huo; Northeast Agricultural University, Harbin, China.
29. Diversity of Lactic Acid Bacteria in the Equine Stomach and Small Intestine. **A. H. Smith** and T.G. Rehberger. Agtech Products, Wisconsin, USA.
30. Group Specific qRT-PCR Analysis of Methanogenic Archaea in Stored Swine Manure. **C. Spence**, T.R. Whitehead and M.A. Cotta National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL, USA.
31. Impact Of Different Prebiotics On In-Vitro Fermentation And Microbial Ecology Of Infant Stool Samples. **J. Stiverson**¹, L. Wang¹, T. Williams^{1,2}, C. Shaw¹, D. Husted², P. Price², J. Guerrieri³, J. Deacon³, M. Morrison¹, and Z. Yu¹. ¹The Ohio State University, Columbus, OH, USA; ²Abbott Nutrition, Columbus, OH, USA; ³Institute of Clinical Research, Mayfield Heights, OH, USA.
32. The Differential Impact of Lactose/ Lactase Phenotype on Colonic Microflora. **A. Szilagyi**¹, I. Shrier¹, D. Heilpern¹, J.-S. Je², S. Park², G. Chong¹, C. Lalonde¹, L.-F. Cote¹, B. Lee². Sir M.B. Davis; ¹Jewish General Hospital and ²Macdonald Campus, McGill University, QC, Canada.
33. Towards The Healthy Human Intestinal Microbiota Phylogenetic Core? **J. Tap**¹, S. Mondot¹, F. Levenez¹, E. Pelletier², C. Caron³, J.-P. Furet¹, E. Ugarte², R. Muñoz-Tamayo^{1,4,6}, D. Le Paslier², R. Nalin⁵, J. Dore¹ and M. Leclerc¹; ¹INRA, UEPSD, UR910, France, ²CEA-Genoscope, France, ³INRA, MIG, UR1077, France, ⁴INRA, MIA, UR341, France, ⁵Libragen, France, ⁶L2S UMR 8506, University Paris-Sud, CNRS-Supelec, France.
34. Effects of Gluconic Acid and Methionine Hydroxy Analog on Growth and Intestinal Characteristics of Rats. **H. Uchihori**, S. Koike and Y. Kobayashi; Hokkaido University, Hokkaido, Japan.
35. The Effect of Diet and the Inclusion of Active Dry Yeast on the Population Size and Dynamics of Key Ruminal Bacteria, as Determined by Classical and Molecular Techniques. **N. D. Walker**¹, M.E. Quintino Cintora¹, A. Ameilbonne², F. Chaucheyras-Durand² and H. Durand³; ¹Lallemand Animal Nutrition, Montreal, Canada; ²Lallemand Animal Nutrition, Clermont Ferrand, France; ³Lallemand Animal Nutrition, Toulouse, France.
36. The Effect of an Active Dry Yeast on the Digestibility of Different Forages and Feed Components During *in vitro* Incubations with Mixed Rumen Contents. **N. D.**

Walker¹, M. E. Quentino Cintora¹, J. Bax¹, F. Chaucheyras-Durand² and H. Durand³; ¹Lallemand Animal Nutrition, Montreal, Canada; ²Lallemand Animal Nutrition, Clermont Ferrand, France; ³Lallemand Animal Nutrition, Toulouse, France.

37. Environmental and Maternal Influence on Colonization of Infant Gastrointestinal Tract by Microorganisms. **L. Wang**¹, J. Stiverson¹, T. Williams^{1,2}, C. Shaw¹, D. Hustead², P. Price², J. Guerrieri³, J. Deacon³, M. Morrison¹, and Z. Yu¹. ¹The Ohio State University, Columbus, OH, USA; ²Abbott Nutrition, Columbus, OH, USA; ³Institute of Clinical Research, Mayfield Heights, OH, USA.
38. Prevalence and Level of *Escherichia coli* O157:H7 in Feces and on Hides of Feedlot Steers Fed Diets With or Without Wet Distillers Grains With Solubles. **J.E. Wells**, S.D. Shackelford, E.D. Berry, N. Kalchayanand, M.N. Guerini, V.H. Varel, T.M. Arthur, J.M. Bosilevac, H.C. Freetly, T.L. Wheeler, C.L. Ferrell, and M. Koochmarai. USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA.
39. Safety And Tolerance Of Infant Formulas Containing Prebiotics In Healthy, Term Infants. **T. Williams**, Y. Choe, P. Price, G. Katz, J. Boff, F. Suarez, and A. Mackey. Abbott Nutrition, Columbus, OH, USA.
40. Diversity of Fiber-Adherent Bacteria on Three Potential Bioenergy Feedstocks During Digestion in the Cow Rumen. **A.C. Yannarell**¹, M. Hess², P. Hugenholtz², and R.I. Mackie¹; ¹University of Illinois at Urbana-Champaign, Illinois, USA; ²DOE Joint Genome Institute, California, USA.
41. The Multi-Replicon Genome of *Butyrivibrio proteoclasticus* B316^T. **C.J. Yeoman**^{1,2}, W.J. Kelly¹, J. Rakonjac², S.C. Leahy¹, E. Altermann¹, and G.T. Attwood¹; ¹AgResearch Limited, Palmerston North, New Zealand; ² Massey University, Palmerston North, New Zealand.
42. Diet-Disrupted Gut Microbiota Dwarfs a Genetic Defect in Metabolic Syndrome Development. **C. Zhang**¹, M. Zhang¹, S. Wang³, R. Han², Y. Cao¹, W. Hua¹, Y. Mao¹, X. Zhang¹, X. Pang¹, C. Wei¹, G. Zhao³, Y. Chen², L. Zhao¹; ¹Shanghai Jiao Tong University, Shanghai, China; ²Chinese Academy of Sciences, Shanghai, China; ³Chinese National Human Genome Sequencing Centre, Shanghai, China.

A Brief History of the Conference on Rumen/Gastrointestinal Function 1951-2007

Burk A. Dehority
Department of Animal Sciences
Ohio Agricultural Research and Development Center, The Ohio State University
Wooster, Ohio 44691

The first Conference on Rumen Function, held on November 27-28, 1951 in Chicago, was primarily in response to a letter from P. V. Cardon, Administrator of ARS, USDA. His letter was addressed to the Deans of 17 different Schools of Veterinary Medicine, outlining the problems with bloat which were being observed in both cattle and sheep grazing improved pastures containing legumes. He suggested that it might be desirable for workers in the area of rumen function to meet and evaluate possible strategies for utilizing these improved pastures without digestive complications. The majority of individuals working in this area would probably be attending either the meeting of the American Society of Animal Production (name subsequently changed to the American Society of Animal Science in 1962) or the Conference of Research Workers in Animal Diseases in North America, both of which meet in Chicago in late November. Thus, he arranged for a meeting to be held on November 27 and 28, 1951 at the Congress Hotel. The program was divided into five panels, with a chairman designated for each as follows:

Rumen Physiology – Dr. C. F. Huffman
Physio-Pathology – Dr. R. W. Dougherty
Agronomic – Dr. W. K. Kennedy
Animal Management – Dr. H. H. Cole
Microbiology – Dr. W. D. Pouden

At least four individuals were chosen for each panel, to aid in guiding the discussion with the audience. Dr. H. W. Marston, Research Coordinator, Agricultural Research Administration, U. S. D. A. served as overall chairman and recorded notes for subsequent distribution to all participants. A total of 71 persons were in attendance sometime during the two day meeting. By the end of the meeting, each panel had listed several suggestions (4-9) for potential areas to be researched for the control of bloat. The group decided that another meeting about this problem should be held in approximately two years and the overall chairman and panel chairman were charged with the responsibility of planning the next meeting.

The second Conference on Rumen function was held at the Congress Hotel, Chicago, Illinois, on December 2 and 3, 1953. A total of 76 researchers attended. The program consisted of same five panels and panel Chairmen; however, the format was changed in that two or more research presentations were given in each panel area by various investigators. A general discussion followed each presentation. Examples of the topics covered in the different panels are:

Animal Management panel – The role of animal management in the prevention of bloat. J. E. Foster, University of Maryland.

Rumen Physiology panel – Chemical and physical factors in the etiology of acute bloat on legume pastures. H. H. Cole, University of California

Physio-pathology panel – Cardiovascular and blood gas changes in experimental bloat. C. D. Meredith and R. B. Barrett, Cornell University

Microbiology panel - The bacteria of the rumen and some observations on their possible relationship to bloat. M. P. Bryant, ARS, USDA.

The group agreed that a similar Conference should be held in 1955.

The third conference was held in the same location on November 30 and December 1, 1955, with 70 participants. The five panel chairman organized presentations of research in the various areas, i.e., six presentations in the Microbiology, Physio-Pathology and Agronomic panels, four in Rumen Physiology and three in Animal Management. Most of the presentations dealt with some aspect of bloat; however, a few papers were more general in the area of rumen function, i.e., “The physiological response to ammonium carbamate” by L. C. Payne, Iowa State College and “Influence of nitrogen and phosphate fertilizers upon the yield and chemical composition of smooth brome grass” by K. L. Anderson, Kansas Agricultural Research Station.

Location of the fourth Conference on Rumen Function was shifted to the Maryland Hotel in Chicago, and was held on December 4 and 5, 1957. Sixty eight persons attended. All chairmen continued except for Dr. Kennedy, who was replaced by Dr. J. N. Scholl as chairman of the Agronomic panel. A total of 25 presentations were made in the five different panels, most of which dealt with various aspects of bloat. Dr. Marston continued to serve as the overall chairman.

The fifth conference was again held at the Maryland Hotel in Chicago, December 2 and 3, 1959, and was chaired by Dr. N. R. Ellis from the Animal husbandry Research Division, Agricultural Research Center, Beltsville, Maryland. Programs for the individual panels were again organized by the individual panel chairmen. The chairman of all panels remained the same, except Dr. E. H. Hollowell assumed chairmanship of the Agronomic panel. A total of 35 presentations were given, with the highest number, 13, in the microbiology panel. Bloat continued to be the primary focus in all of the panels. Attendance was similar to that in previous years, 68 persons.

In 1961, the sixth conference was again held at the Maryland Hotel and Dr. Marston returned as Chairman. Three panels had new chairmen: J. M. Boda in Animal Management; N. L. Jacobson in Rumen Physiology; and D. E. McCloud in the Agronomic panel. Most of the 32 presentations were related to bloat, principally dealing with the contribution of saliva, prevention and treatment with antibiotics and rumen microbial activity. There were 68 participants in attendance, which included three international visitors: Dr. K. J. Hill, Institute of Animal Physiology, Babraham, England; Dr. C. S. Reid, Plant chemistry Division, Palmerston North, New Zealand; and Dr. Alan Dobson, The Rowett Research Institute, Aberdeen, Scotland.

Site of the seventh Conference, December 4 and 5, 1963, was moved to the Hamilton Hotel in Chicago. Dr. C. K. Smith took over chairmanship of the Microbiology panel. In general, the Conference appeared to be moving away from its focus on bloat and was more concerned with rumen function. Only nine of the 30 presentations had a connection to bloat. Some of the more divergent subjects were: “Survival of selected exogenous microorganisms in the rumen of cattle”, “Intestinal obstruction in cattle”, “Transport of sodium and chloride by the isolated rumen epithelium” and “Estimation of

the digestibility and nutritive value of forages by cellulose and dry matter solubility methods". Seventy five persons were in attendance.

In 1965, the eighth Conference on rumen function was held on December 1 and 2 at the Midland Hotel in Chicago, Illinois. Dr. Marston was again the overall chairman, with only one new panel chairman, Dr. P. R. Henson for the Agronomic panel. Seventeen of the 31 papers dealt with some aspect of bloat. It was of interest that seven of the bloat papers were concerned with the use of poloxalene, a non-ionic surfactant, which in the correct amount completely prevents pasture bloat. Attendance was similar to previous years, 74 participants.

A number of personnel changes were involved for the ninth Conference. Dr. C. R. Richards replaced Dr. Marston as overall Chairman. Dr. R. F. Barnes substituted for Dr. G. E. Carlson as chairman of the Agronomic panel and Dr. A. D. McGilliard chaired the Physiology panel. The meeting was held at the Midland Hotel on November 29 and 30, 1967. A major shift was observed in subject areas of the program. There were 19 presentations, all concerned with rumen function but none of which specifically dealt with bloat. Fifty nine persons were in attendance. A committee was formed to investigate the possibility of formalizing the Group participating in the Rumen Function Conference into a national organization

An additional panel on Nutrition, chaired by J. T. Huber, was added for the tenth Conference held at the Midland Hotel in Chicago on December 3 and 4, 1969. Dr. Clyde Richards assumed duties as overall Chairman and Drs. M. P. Bryant and J. C. Burns took over as Chairman of the Microbiology and Agronomic panels, respectively. The panel chairmen served as a program committee, responsible for soliciting and choosing the 35 research studies presented. Similar to the previous Conference, the reports focused on rumen function with none directly related to bloat. Attendance increased to 104, and for the first time a Business Meeting was held at the end of the Conference. It was decided to hold another conference at the same time in 1971. No report was given by the committee on formalizing the group.

Seventy one persons attended the eleventh Rumen Function Conference in Chicago at the Midland Hotel, December 1 and 2, 1971. Overall and panel chairman remained the same as in 1969, with a slight program change in that the physiopathology and physiology panels were combined. A total of 33 reports were presented.

The twelfth Conference on Rumen Function was moved to the LaSalle Hotel in Chicago, and met on November 28 and 29, 1973. Conference and panel chairmen remained from 1971. Details of the eleventh through twentieth Conferences are listed in Tables 1, 2 and 3. At the twentieth Conference, poster as well as podium presentations were given and each of the panels began their programs with an invited paper. Up to this point, the Conference had been held on an informal basis, with no memberships or dues involved. A steering committee was formed to consider questions about time, location, structure and future of the Conference. Members of the committee were: Dr. M. T. Yokoyama (Michigan State), Dr. R. H. Dunlop (Univ. Minnesota), Dr. R. Hatfield (U.S.D.A.) and Dr. F. Owens (Oklahoma State). In addition, a Treasurer (J. R. Russell) and Arranger (S. F. Kotarski) were appointed to help facilitate the Conference. For the first time, several companies provided financial support for a mixer and coffee breaks (American Cyanamid; Hoffman-LaRoche, Inc; Lilly Research Lab.; Purina Mills, Inc.; Moorman Manufacturing Co.; The Upjohn Co.)

Table 1. Details of the Eleventh through Fifteenth Conferences on Rumen Function.

	Eleventh	Twelfth	Thirteenth	Fourteenth	Fifteenth
Location ^a	Midland Hotel	LaSalle Hotel	LaSalle Hotel	Pick Congress Hotel	Pick Congress Hotel
Dates	Dec. 1-2, 1971	Nov. 28-29, 1973	Dec. 3-4, 1975	Nov.30–Dec.1, 1977	Nov. 28-29, 1979
General Chairman	C. R. Richards	C. R. Richards	C. R. Richards	C. R. Richards	C. R. Richards
Agronomic Chair	J. C. Burns	J. C. Burns	J. C. Burns	J. C. Burns	J. C. Burns
Microbiology Chair	M. P. Bryant	M. P. Bryant	M. P. Bryant	M. P. Bryant	R. B. Hespell
Nutrition Chair	J. T. Huber	J. T. Huber	J. T. Huber	R. M. Cook	J. T. Huber
Physiology Chair	A. D. McGilliard	Panel omitted	Panel omitted	A. D. McGilliard	A. D. McGilliard
Physiopathology Ch.	R. W. Dougherty	R. W. Dougherty	W. M. Wass	W. M. Wass	W. M. Wass
No. of Presentations	33	35	31	49	44
No. of Attendees	71	64	87	Not reported	Not reported

^aAll hotels are located in Chicago, Illinois

Table 2. Details of the Sixteenth through Twentieth Conferences on Rumen Function^a

	Sixteenth	Seventeenth	Eighteenth	Nineteenth	Twentieth
Location ^b	Americana Congress Hotel	Americana Congress Hotel	Americana Congress Hotel	Americana Congress Hotel	Congress Hotel
Dates	Nov. 11-13, 1981	Nov. 16-17, 1983	Nov. 13-14, 1985	Nov. 17-19, 1987	Nov. 7-9, 1989
General Chairman	C. R. Richards	C. R. Richards	M. J. Allison	M. J. Allison	M. J. Allison
Agronomic Chair	J. C. Burns	No presentations	J. C. Burns	J. C. Burns	J. C. Burns
Microbiology Chair	M. P. Bryant and R. B. Hespell	M. P. Bryant	J. B. Russell	K. A. Dawson	J. B. Russell
Nutrition Chair	J. T. Huber	J. T. Huber	J. T. Huber	J. T. Huber	J. T. Huber
Physiology Chair	A. D. McGilliard	A. D. McGilliard	No presentations	No presentations	No presentations
Physiopathology Ch.	W. M. Wass	Combined with Physiology	W. M. Wass	W. M. Wass	R. H. Dunlop
No. of Presentations	61	40	54	62	39 – podium 41 – poster

^aAttendance was not reported after the Thirteenth Conference.

^bAll hotels are located in Chicago, Illinois.

Table 3. Details of the Twenty first through Twenty fifth Conferences on Rumen Function

	Twenty first	Twenty second	Twenty third	Twenty fourth	Twenty fifth ^a
Location ^b	Congress Hotel	Congress Hotel	Congress Hotel	Congress Hotel	Congress Hotel
Dates	Nov. 12-14, 1991	Nov. 9-11, 1993	Nov. 14-16, 1995	Nov. 11-13, 1997	Nov. 14-16, 2000
General Chairman	J. B. Russell	J. B. Russell	J. B. Russell	M. S. Allen	M. S. Allen
Treasurer	J. R. Russell	J. R. Russell	M. A. Rasmussen	M. A. Rasmussen	R. I. Mackie
Secretary	S. F. Kotarski	S. F. Kotarski	J. A. Z. Leedle	J. A. Z. Leedle	J. A. Z. Leedle
Agronomic Chair	D. R. Buxton and R. Hatfield	M. S. Allen	M. S. Allen	J. L. Firkins	J. L. Firkins
Microbiology Chair	M. T. Yokoyama and D. E. Akin	M. T. Yokoyama	M. A. Cotta	M. A. Cotta	M. A. Cotta
Nutrition Chair	F. N. Owens and R. S. Swingle	M. S. Allen	M. S. Allen	J. L. Firkins	J. L. Firkins
Physiopathology Ch.	R. H. Dunlop and E. C. Crinchlow	M. T. Yokoyama	M. A. Cotta	M. A. Cotta	M. A. Cotta
No. of Presentations	31 podium 39 posters	30 podium 31 posters	30 podium 51 posters	24 podium 52 posters	17 podium 41 posters
Sponsors	Alltech; Lilly; Monsanto; Moormans; Upjohn; Purina Mills	Alltech; Church & Dwight; Monsanto; Smith, Kline & Beecham; Syntex; Upjohn; Purina Mills	Major donors: USDA and Purina Mills. Other donors: Abbot Labs; ANKOM; Chr. Hansen; Church & Dwight; Diamond V; Monsanto; Pioneer Hi-Bred	Major donors: USDA and Purina Mills. Other donors: Chr. Hansen; Diamond V; Fort Dodge; Hoffman-LaRoche; Purina Mills	Pioneer; Purina Mills; Chr. Hansen; Diamond V; Fort Dodge, Abbott – Ross Products.

^aThe 25th Conference on Rumen function was dedicated to the memory of Marvin P. Bryant. (1925-2000).

Prior to the Conference scheduled for 2000, expenses, including printing of Abstracts, were covered by the sponsors listed in the Tables. Meeting rooms were provided by the hotel, based on their increased business from the lodging of Conference participants. However, in making arrangements for the 2000 meeting, all costs for services and meeting rooms had increased to a point that necessitated the start of charging a Registration fee. This has continued up the present time. Increased expenses such as renting the Meeting site and travel for International speakers have been met with Conference grants and greater Sponsor support.

During the period 2001-2002, a number of new problems in different areas of animal agriculture became apparent. Thus, it was decided to broaden the Rumen Function Conference to include such topics as: reduction or elimination of antibiotics for prophylaxis and growth promotion in both livestock and poultry; microbiology in pre- and post-harvest food safety; and animal agriculture's impact on the environment, i.e. manure and odor control. In addition, numerous studies in the fields of human and non-ruminant animal gastrointestinal microbiology have direct association with rumen microbiology. On this basis, the name of the Rumen Function Conference was changed to Conference on Gastrointestinal Function (CGIF), to reflect the expanded scope of the meetings. Also the time of the meeting was changed from November to Spring. The 2003 CGIF was held at the Congress Hotel in Chicago on March 10-12, 2003. M. A. Cotta served as Chairman, with J. L. Firkins, M. Morrison and T. R. Whitehead as members of the Organizing Committee. R.I. Mackie served as Treasurer and Jane Leedle as Secretary. The Conference was partially funded by a grant from the NRI Competitive Grants Program/CSREES/USDA. Other donors were Purina Mills, LLC, Chr. Hansen, Abbot Labs and Abbott – Ross Products. The conference included 24 podium presentations and 26 posters. The Marvin P Bryant Memorial Lecture was initiated at the 2003 conference and was delivered by M. J. Allison, a student and colleague of Dr. Bryant.

The second CGIF was again held at the Congress Hotel in Chicago on April 11-13, 2005. General Chairman, organizing committee and officers remained from 2003. The Bryant Lecture was presented by Joël Doré on "Reassessment of the human intestinal microbiota-from phylogenetics to metagenomics". The program included twenty three podium presentations and 31 posters. In addition to six commercial sponsors (Alltech, Diamond V, Elanco, Chr. Hansen, Inc., DSM Nutritional Products. and Land O' Lakes Purina Feed) the Conference was partially funded by the French General Consulate, Office for Science and Technology.

In 2007, The CGIF was held in Chicago on April 16-18 and the venue was changed to the Gleacher Center. Mark Morrison was Chair, with Jane Leedle, Rod Mackie, Isaac Cann and Jeff Firkins serving on the Organizing Committee. The program consisted of 21 podium presentations and 66 posters. For the first time, The Conference Abstracts were published in the journal *Microbial Ecology in Health and Disease*, 19:25-55 (2007). The Marvin P. Bryant Memorial Lecture was given by Michael McInerney from The University of Oklahoma on "Syntrophy: The Lifestyle of the Thermodynamically Challenged." Although a number of presentations and posters dealt with the rumen and its microorganisms, studies on gut microbiology in humans and non-ruminant animals received considerable attention. A competitive grant from USDA-NRI provided major support plus additional funding was received from 16 commercial sponsors and the French Ministry of Foreign Affairs.

Microbial Ecology

Volume 57 Number 3

April 2009



 Springer

57(3) 391–588 • 248 ISSN 0095-3628

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Proceedings of the 2009 Conference on Gastrointestinal Function, Chicago, USA, April 20–22

Nitroethane, 2-Nitro-methyl-propionate and Dimethyl-2-nitroglutarate Markedly Reduce Ruminal Methane Production Without Adversely Affecting Ruminal Fermentation. **R.C. Anderson¹**, J.K. Huwe², D.J. Smith², T.B. Stanton³, N.A. Krueger¹, T.R. Callaway¹, T.S. Edrington¹, R.B. Harvey¹ and D.J. Nisbet¹. ¹USDA/ARS, Southern Plains Agricultural Research Center, College Station, Texas, USA; ²USDA/ARS, Biosciences Research Laboratory, Fargo, North Dakota, USA, and ³USDA/ARS, National Animal Disease Center, Ames, IA, USA

Ruminal methanogenesis is considered a digestive inefficiency that results in the loss of 2–12% of the host's gross energy intake and accounts for nearly 20% of the United States' annual CH₄ emissions. The objective of the present experiment was to evaluate the effects of the known CH₄ inhibitor, nitroethane (NE) and two synthetic nitrocompounds, 2-nitro-methyl-propionate (NMP) and dimethyl-2-nitroglutarate (DNG) on ruminal CH₄ production and fermentation *in vitro*. Ruminal fluid (10 ml/tube) obtained from a pasture fed cow was incubated ($n=3$ /treatment) at 39°C under 100% CO₂ with 0.2 g alfalfa. After 24 h, incubations supplemented with 2.5 or 10 mM NE, NMP and DNG reduced ($P<0.05$) CH₄ production >92% compared to controls incubated without added nitrocompound (10.3±2.2 μmol CH₄ ml⁻¹ ruminal fluid). Amounts of acetate, propionate, isobutyrate, isovalerate and valerate produced were unaffected ($P>0.05$) by nitro-treatment, averaging 33.4±14.4, 16.8±4.9, 0.4±0.2, 0.6±0.3 and 1.0±0.4 μmol ml⁻¹, respectively. Incubations containing 2.5 or 10 mM NE produced more butyrate (11.3±0.7 and 12.7±1.6 μmol ml⁻¹, respectively) than controls (4.3±1.8 μmol ml⁻¹; $P<0.05$). Measurable amounts of lactate and formate did not accumulate in control or nitro-treated incubations. Accumulations of NH₃ were increased ($P<0.05$), albeit slightly, in incubations containing 10 mM NE or 2.5 mM NMP (1.8±0.1 and 2.0±0.2 μmol ml⁻¹,

respectively) compared to controls (1.4±0.1 μmol ml⁻¹) but not in any of the other nitro-treated incubations. Stoichiometric estimates of amounts of hexose fermented were unaffected ($P>0.05$) by nitro-treatment and averaged 35.5±12.4 μmol ml⁻¹. Electron balance estimates of reducing equivalents produced were also unaffected by nitro-treatment, averaging 122.9±45.2 μmol H₂ ml⁻¹. Greater than 96% of the reducing equivalents produced in the control incubations were recovered in fermentation products. However, because CH₄ production was reduced by nitro-treatment amounts of reducing equivalents recovered in fermentation products from nitro-treated incubations were consequently reduced >36% ($P<0.05$). We hypothesize, but have yet to confirm, that microbial reduction of the added nitrocompounds may consume some of the reducing equivalents not accounted for in measured fermentation products.

Keywords: Nitroethane, 2-nitro-methyl-propionate, dimethyl-2-nitroglutarate, CH₄-inhibitor

Molecular Properties And Transcription Of The Phosphoenolpyruvate Carboxykinase Gene In A Ruminal Bacterium *Ruminococcus albus*. **N. Asanuma**, K. Yoshizawa, K. Kanada and T. Hino; Meiji University, Japan.

Ruminococcus albus plays an important role in ruminal cellulolysis. The primary fermentation endproducts include acetate, CO₂, hydrogen, ethanol and succinate. The hydrogen (H₂) produced by *R. albus* may be used to produce methane in the rumen. Because methanogenesis in the rumen results in both a loss of feed energy for the host animal and a positive burden on greenhouse gas accumulation, reducing methanogenesis is desirable. Fortunately, reduction in methanogenesis by decreasing H₂ and formate production can be accomplished without necessarily inhibiting the growth of *R. albus*. Most of the succinate produced in the rumen is fermented secondarily to

propionate by succinate-utilizing bacteria. Since production levels of methane are generally reciprocal to formation of propionate, our aim is to manipulate *R. albus* growth and increase succinate production, with a concomitant decrease in H₂ production. Thus, molecular properties and transcriptional control of phosphoenolpyruvate carboxykinase (PCK) in *R. albus* were examined. The putative 537-amino acid PCK polypeptide has a predicted mass of 59.4 kDa and an isoelectric point of 4.82. Both RT-PCR and Northern blot analyses of *pck* mRNA suggest that the transcript is monocistronic and that *pck* transcription is not affected by changes in sugar sources present in growth medium. The *R. albus* PCK phosphorylated ADP more readily than GDP. Apparent *K_m* values of PCK for PEP and ADP were considerably lower than those for OAA and ATP, suggesting that the reaction from PEP to OAA is favored in *R. albus*. The enzyme properties of PCK in *R. albus* appear more similar to *Selenomonas ruminantium* PCK than *Ruminococcus flavefaciens*. The specific activity of PCK, representing the amount of enzyme per cell, in *R. albus* was much lower than that in *S. ruminantium*. The amount of succinate produced in *R. albus* from one unit of cellobiose was also much lower than the sum of succinate and propionate produced in *S. ruminantium*. Based on these results, we propose enhancement of PCK activity by stimulating PCK transcription might be a method to positively alter *R. albus* metabolism and H₂ production, without suppressing growth.

Keywords: metabolism; rumen bacteria; *Ruminococcus*; phosphoenolpyruvate carboxykinase; transcription

Isolation of Mimosine Degrading Non-Pathogenic *Klebsiella pneumoniae* From Rumen Contents of German Steers. A. Aung¹, and H. Boehnel²; ¹University of Veterinary Science, Yezin, Myanmar; ²Institute for Applied Biotechnology in the Tropics, Georg-August-University, Germany

Mimosine is known to be a goitrogenic toxin for ruminants animals not adapted to the consumption of plant materials that contain this compound. Much of our knowledge concerning mimosine metabolism in ruminants comes from the study of *Synergistes jonesii*. The goal of this study was to isolate other mimosine-degrading bacteria that might be present in ruminal contents. Rumen liquor was collected from a German steer and a gradually increasing amount of mimosine was added to the liquor, with the goal of enriching mimosine-degrading bacteria. Every second day the amount of mimosine was increased by 25 mg mimosine per day, with 200 mg mimosine added on day 16. Samples were collected daily and mimosine degradation was measured, and samples of the cultured rumen liquor were plated after 16 days on selective medium. The morphology,

biochemical properties and phylogenetic characterization of the isolates were then studied. The candidate mimosine-degrading isolates were tentatively classified as *Klebsiella pneumoniae* by phylogenetic analysis, although the biochemical properties measured for the isolates were not the same as *K. pneumoniae*. More detailed studies were conducted with isolate 3948. The isolate was assessed to be non-pathogenic using a lethality test with 15 albino rats. *In vivo* mimosine degradability of isolate 3948 was subsequently measured in Myanmar sheep. According to results, isolate 3948 is non-pathogenic and mimosine-degrading, suggesting this bacterium might be used as a probiotic in animals not adapted to consuming mimosine rich forages.

Keywords: bacteria, degradability, mimosine

Characterization of Gastrointestinal Microbiota in Neonatal Piglets from Sows Supplemented with a *Bacillus* Based Direct-Fed Microbial A. Baker¹, E. Davis¹, J.D. Spencer², R. Moser², and T. Rehberger¹; ¹Agtech Products, Inc., Waukesha, WI, USA, ²JBS United, Inc., Sheridan, IN, USA.

Supplementation of animal diets with direct fed microbials (DFMs) can have several benefits for the host, including enhanced health status, immune regulation, and increased performance factors. In the swine industry, DFMs are being investigated as antibiotic replacements in diets, and supplementation of a DFM in the sow diet may reduce pathogen shedding, reduce pathogens in the environment, as well as confer additional health benefits in the piglet through fecal-oral transmission. In this study we evaluated the effect of a *Bacillus subtilis* based DFM on the gastrointestinal microbiota of neonatal piglets. A total of 30 sows were divided into two treatments: a control diet and the control diet supplemented with a *Bacillus* based DFM (3.75 × 10⁵ cfu/g feed). Twenty-one piglets sampled from each sow treatment group were euthanized on day 3 of lactation as well as an additional 15 piglets per treatment on day 10 of lactation. Terminal restriction fragment length polymorphism (T-RFLP) was used to assess gastrointestinal microbial populations in the ileum and proximal colon of the piglets. Terminal restriction patterns derived from three enzymes (*MspI*, *BstUI*, and *HaeIII*) were compared between control and DFM treated piglets using multivariate analysis of variation (MANOVA) and ordination plots in Canoco. The TRF peaks correlated with treatment were presumptively identified using the Microbial Community Analysis III (MiCA 3) database. Several of the discriminate peaks associated with DFM treatment in both day 3 and day 10 piglets were putative *Lactobacillus* species including *L. gasseri*, *L. reuteri*, and *L. delbrueckii*. Discriminate peaks associated with the control piglets included several putative

species of *Clostridium*, *Streptococcus*, and *Eubacterium*. Results of this study suggest that the developing gastrointestinal microbiota of a neonatal piglet can be influenced by DFM supplementation to the sow. In addition, the correlation of beneficial microbes in gastrointestinal community to DFM supplementation could provide health benefits to the neonatal piglet.

Keywords: T-FRLP, probiotic, gastrointestinal microbiota, direct fed microbial

Seasonal Digestive Function In Arctic Ruminants: Flexible Fermentation And A Variable Food Supply. **P.S. Barboza**; University of Alaska, Fairbanks AK, USA

Arctic herbivores such as muskoxen (*Ovibos moschatus*), caribou and reindeer (*Rangifer tarandus*) must alter food intake and digestion to use the short season of plant growth at high latitudes. Consequently, ruminal fermentations accommodate large changes in the flow and composition of substrates through the year. Muskoxen and reindeer are born with large rumens that grow quickly to support an active fermentation before 60 days of age. Ruminal volumes also increase with food intake during lactation and mass gain in adult caribou during summer. Muskoxen increase intakes of the same diet of grass by 79% between spring and autumn as they gain body fat for winter. The time for digestion is conserved between spring and autumn by increasing the volume of the ruminal fermentation by 33% and by increasing the rate of ruminal degradation of fiber by over 100%. Microbial ureolysis minimizes N demands by degrading 68% of urea produced by the host; 50% of that degraded urea is returned to body protein. Low bacterial counts in the rumen are associated with low rates of water turnover and food intake in spring and winter. Declines in ruminal bacteria are not associated with cold shocks even though ruminal temperatures drop to 26°C from an average of 39°C after ingestion of snow or water. Increases in food intake and microbial activity reduce ruminal pH and increase the concentrations of fermentation acids before and after feeding. However, ruminal conditions are more variable when food intakes are low, that is, diurnal variations of ruminal pH and osmolarity are greater in spring than in autumn. Sequences of 16S rDNA indicate that the bacterial community in the particulate phase of digesta is more diverse during autumn than during spring (460 vs. 129 estimated phylotypes). Seasonal shifts in the microbial community allow muskoxen to fatten quickly during autumn and to minimize the costs of digestion during winter. Flexibility in fermentative digestion allows these large herbivores to subsist on forages that are often low in both quality and abundance.

Keywords: fermentation, temperature, grazer, browser, bacterial diversity

Characterization Of The Duodenal Microbiota Of Commercial Layer Hens Affected By Focal Duodenal Necrosis. **J.A. Benson**, T.A. Baltzley, T.G. Rehberger, and G.R. Siragusa; Agtech Products, Inc., Waukesha, WI

Focal Duodenal Necrosis (FDN) is a poorly understood disease affecting the poultry egg layer industry, resulting in characteristic lesions in the duodenal loop of the gastrointestinal tract (GIT). Lesions vary in severity from a slight grey discoloration to grossly calloused crusty lesions. Although mortality is low, the disease can lead to decreased egg production in layer hens and lower egg weights. Initial research indicates that there may be a bacterial component to the disease. To examine the role bacterial communities of the duodenum have in the development of the disease, the duodenal microbial communities of layer hens with FDN lesions and asymptomatic birds were characterized. A total of 60 duodenal samples were collected from layer hens from a commercial egg production facility between March–September, 2008. Duodena were scored for FDN lesion severity on a scale of increasing severity from 0–4. Genomic DNA was isolated from the mucosal bacteria from the duodenum of each bird and analyzed by T-RFLP. Terminal restriction fragment patterns from diseased and healthy birds derived from three enzymes (*MspI*, *HaeIII*, and *BstUI*) were compared by multivariate analysis (MANOVA), revealing peaks correlated with each health status. Presumptive identification of peaks was made using the Microbial Community Analysis III database. Peaks strongly correlated with FDN affected birds were presumptively identified as *Clostridium colinum*. Peaks correlated with health were presumptively identified as *Enterococcus* or *Lactobacillus* species. The correlation of these species with health suggests that these organisms may have a role in disease prevention resistance or as a resistance barrier against the FDN condition.

Keywords: T-RFLP; gastrointestinal microbiota; *Clostridium*; layer chickens; *C. colinum*

Repeated Ruminal Acidotic Challenges in Sheep: Effects on pH and Microbial Ecosystem and Influence of Active Dry Yeasts. **F. Chaucheyras-Durand**^{1,2}, M. Silberberg², L. Commun², C. Martin², D.P. Morgavi²; ¹Lallemand Animal Nutrition, Toulouse, France; ²INRA Clermont-Ferrand/Theix, France.

In ruminants, the use of readily fermentable carbohydrates is known to increase the risk of rumen acidosis. Animals may encounter several acidotic episodes, lasting for long

periods of time. The decrease in rumen pH has been reported to be linked to changes in the fermentative profile and shifts in the balance of microbial communities. The aim of this work was to follow the evolution of pH and microbial ecosystem during repeated acidotic challenges, and also to investigate the effects of Active Dry Yeasts (ADY). Rumen-cannulated sheep ($n=12$) were divided into one control group and one receiving the ADY CNCM I-1077. They were fed during 3 weeks with a basal diet (hay: wheat, 80:20), then they received an acidotic diet (hay: wheat, 40:60) during 5 days. This sequence was repeated 3 times. Throughout the experiment, rumen pH was continuously monitored using indwelling probes. Rumen samples were collected for determination of bacterial and protozoal counts, quantification by qPCR of specific bacterial populations (*Streptococcus bovis*, *Megasphaera elsdenii*, *Allisonella histaminiformans*), and VFA concentrations. The 1st acidotic challenge induced sharp decline in pH. Subsequent acidotic bouts led to a significant increase in time spent under pH 5.6 in the control group, whereas pH was stabilised in the ADY group from the 2nd episode. The 1st acidotic bout deeply modified the microbial balance (increase in lactate-producing bacteria, decrease in cellulolytic bacteria and protozoa numbers) and probably selected the more acidotic-resistant microbes. For example, *A. histaminiformans*, a histamine-producing species possibly involved in laminitis, was detected only sporadically from the 2nd challenge, but was more consistently quantified during the last challenge. Moreover, ADY induced a sharp decline in streptococci and lactobacilli, and in *Streptococcus bovis* during the last acidotic episode. VFA profile shifted from propionate, during the 1st challenge, to butyrate during the 2nd and the 3rd challenges. As intended, ADY appears a useful tool to limit the onset of acidosis, but also to help recovery from repeated acidotic bouts. The dietary history of the animal appears important to partly explain differences observed between animals, with the same potential in feeding practices including high levels of readily fermentable carbohydrates.

Keywords: rumen, acidosis, microbiota, pH, Active Dry Yeasts

Spatial and Temporal Changes in the Microbial Community in an Anaerobic Swine Waste Treatment Lagoon. K.L. Cook¹, M.J. Rothrock¹ and N. Lovannh¹, J.K. Sorrell², J.H. Loughrin¹. ¹USDA Agricultural Research Service, Bowling Green, KY; ²Western Kentucky University, Bowling Green, KY.

Swine slurry is stored in pits beneath confinement buildings or in adjacent lagoons. This slurry is a valuable resource for crop fertilization and soil conditioning, but may also be a

source of unpleasant odors. Microorganisms are crucial to all of the important processes that occur in anaerobic storage systems. Therefore, understanding more about how the microbial community functions should aid in developing better strategies for management and use of stored slurries. In this study, both quantitative (real-time polymerase chain reaction) and qualitative (denaturing gradient gel electrophoresis, cloning, sequence analysis) molecular analyses were used to track spatial and temporal changes in the microbial community of swine slurry from a 0.4 ha anaerobic lagoon over a period of one year and at four depths within the lagoon. Sub-populations within the bacterial community may be responsible for many important processes in the swine lagoon. Therefore, in addition to monitoring changes in the broader lagoon population (total cells, *Clostridium/Eubacterium* and *Bacteroides*), microbial groups important to odor production were specifically targeted including sulfate-reducing bacteria (SRB), methanogens, acetogens, photosynthetic organisms, and others. The concentration and diversity of *Bacteroides* sp. was seasonal (up to 90% decrease between March and June). *Hespellia* sp. and other clostridial species, on the other hand, were endemic in the slurry (concentrations up to 1.0×10^7 cells mL⁻¹ slurry) regardless of time of the year or lagoon depth. Similarly, some genes targeted to specific functional groups were detected throughout the year (i.e., those for urea and methane production), while others were seasonal (acetogens, phototrophs and nitrifiers). The effect of depth was only seen for phototrophs and acetogens (both absent in the bottom of the lagoon). These results suggest that there were seasonal effects on the microbial community in the swine lagoon, while the effect of depth was not as pronounced. Seasonal changes in the microbial community in stored wastes may be (directly or indirectly) correlated with changes in malodor emissions from lagoons.

Keywords: Waste Treatment, Denaturing Gradient Gel Electrophoresis (DGGE), Lagoon, Odor, Swine

Oxalate Degradation by *Lactobacillus acidophilus* and Other Probiotic Bacteria. A.E.C. Baluka and S.L. Daniel, Eastern Illinois University, Charleston, IL USA

Lactobacillus and *Bifidobacterium* spp. are the active components of commercial probiotics and have been recently shown to consume oxalate, a major risk factor for kidney stone formation in humans. The goals of this study were to examine the oxalate-consuming activities of probiotic microorganisms. Commercial probiotics and pure cultures of probiotic bacteria were grown anaerobically at 37°C in 5.5% Difco Lactobacilli MRS broth (pH 6.5) containing 5 mM oxalate. Oxalate consumption and fermentation end products were quantitated using HPLC

analysis. Of the 7 commercially available probiotics tested, 5 (Oxadrop, VSL#3, Florajen-Bifido Blend, Florajen-Acidophilus, and 4×6 Acidophilus) totally consumed the oxalate after 120 h of incubation. Of the 17 pure cultures tested, all displayed growth in the presence of 5 mM oxalate but only *Lactobacillus acidophilus* NCFM, *L. acidophilus* LA-14, and *Bifidobacterium lactis* Bl-07 totally consumed the oxalate after 120 h of incubation. Little, if any, oxalate was consumed by *B. lactis* Bl-04, *Lactococcus lactis* LL-23, *Lactobacillus brevis* LBR-35, *Lactobacillus rhamnosus* LR-32, *Bifidobacterium longum* BL-05, *Bifidobacterium breve* BB-03, *Lactobacillus paracasei* LPC-37, *Streptococcus thermophilus* ST-21, *Lactobacillus salivarius* LS33, *Lactobacillus plantarum* LP-115, *Lactobacillus bulgaricus* Lb-64, *Lactobacillus casei* LC-11, *Pediococcus acidilactici* P751, and *Lactobacillus helveticus* Lh-138. Time-course analysis of oxalate consumption and growth indicated that oxalate consumption by *L. acidophilus* NCFM occurred after active growth. Increased amounts of oxalate (25 mM) were also consumed and, with cells previously exposed to oxalate, additional amounts of oxalate (5 and 25 mM) were consumed more rapidly. Formate levels increased when oxalate was consumed, suggesting that oxalate was converted to formate. *L. acidophilus* NCFM did not consume oxalate when grown in 0.55% MRS or in an undefined medium (glucose, minerals, metals, CO₂/bicarbonate, 0.1% yeast extract) with 5 mM oxalate. Understanding the factors that regulate oxalate consumption by probiotic bacteria such as *L. acidophilus* NCFM will be important in the development of commercial probiotics for the prevention and treatment of kidney stones.

Keywords: oxalate degradation, probiotics, kidney stones, gut

Analysis of Key Reductive Acetogenesis Enzymes from Microbial Enrichments from the Cattle Rumen. **S. Denman**¹, **J. Padmanabha**¹, **E. Gagen**¹, **M. Morrison**^{1,2} and **C. McSweeney**¹; ¹CSIRO Australia, Queensland, Australia; ²The Ohio State University, Ohio, USA.

Reductive acetogenesis is an alternative hydrogen-utilising pathway to methanogenesis in the rumen and has potential as a strategy for reducing ruminant greenhouse gas emissions. The obligately anaerobic bacteria responsible for reductive acetogenesis are known as homoacetogens. Homoacetogens use the acetyl-CoA pathway to reduce carbon dioxide to acetate, and most are able to use this pathway for growth on hydrogen and carbon dioxide as the sole energy source. Homoacetogens are present in the rumen and molecular tools are necessary to further investigate their ecology. The aim of the present work

was to identify DNA sequences of genes present in homoacetogens that may be suitable as targets for the development of functional-group molecular tools for these microorganisms. The genes investigated were the key enzymes in the acetyl-CoA pathway: carbon monoxide dehydrogenase (CODH), acetyl-CoA synthase (ACS) and formyltetrahydrofolate synthetase (FTHFS). Several different batch fermentation systems inoculated with rumen microbes from either pasture or grain fed animals were established using ¹³C-sodium carbonate as the principal carbon and energy source, and in the presence or absence of bromoethanesulfonate (BES) to inhibit methanogenesis. Both VFA and gas analyses were performed to characterize and monitor fermentative changes, hydrogen and carbon balances. Metagenomic DNA was extracted and Isotope ratio mass spectrometry (IRMS) was used to confirm the uptake of ¹³C into the DNA, followed by the separation of ¹³C-labeled DNA via isopycnic gradient ultracentrifugation. The ¹³C-labeled DNA was used as a template for the production of 16S rDNA phylogenetic and FTHFS clone libraries, as well as metagenomic (fosmid) libraries and 454 short-read sequencing. Results from 16S analysis from both PCR based and 454 sequencing of the metagenomic DNA revealed similar results with the most dominate numbers aligning with the Lachnospiracea then Enterobacteraceae followed by Bacteroidaceae. The 454 sequence binning of the FTHFS gene revealed greater diversity than that generated from PCR cloning, with sequences from the Bacteroidaceae not being represented in the PCR clone library. The majority of FTHFS sequences grouped within the homoacetogenic cluster were dominated by clostridial representatives. There was a high degree of similarity between the pasture fed and grain fed enrichments, although the pasture fed enrichments also were highly represented by a group that clustered close to *Proteus vulgaris*, a bacterium not known to produce acetate. Newly developed degenerate primers for ACS and CODH were used to generate clone libraries, which revealed limited diversity of these functional genes with respect to pasture or grain based enrichments. Clusters of ACS and CODH sequences aligned closely with *Ruminococcus obeum*, and similar results were obtained from the 454 sequencing analysis.

Keywords: Acetogenesis, metagenomics, microbiology

Spatial And Temporal Analysis Of Intestinal Microbiota In Conventionalized Germ Free Mice In Correlation With The Host Responses, Using A High-Throughput Phylogenetic Microarray. **M. Derrien**^{1,2}, **S. El Aidy**^{1,2}, **M. Rajilić-Stojanović**², **D. Molenaar**³, **F. Levenez**⁴, **E.G. Zoetendal**^{1,2}, **J. Dore**⁴ and **M. Kleerebezem**^{1,2}; ¹Top Institute Food and Nutrition, Wageningen, The Netherlands; ²Laboratory of

Microbiology, Wageningen University, Wageningen, The Netherlands; ³NIZO Food Research, Ede, The Netherlands; ⁴INRA, UR910, Jouy-en-Josas, France.

The intestinal microbiota profoundly influences the host. The mouse intestinal microbiota has not been investigated intensively. In this study, we aim to analyze the spatial and temporal change of the microbiota of colonized germ-free mice using a high-throughput phylogeny diagnostic microarray, called Mouse Intestine Tract Chip, (MITChip) in parallel to host gene responses, enabling microbiota development pattern correlation with cognate host responses. The MITChip was designed based on public available 16S rRNA sequences (>9,000) associated with the mouse intestinal microbiota. Oligonucleotide probes (24±6 nucleotides) were designed based on two regions of the 16S rRNA (V1 and V6) for all unique OTUs (sharing <98% similarity). MITChip microbiota profiling was applied on intestinal samples from germ-free Black6 mice (n=30) sacrificed at different time points following conventionalization (days 1, 2, 4, 8 and 16). Luminal contents of jejunum, ileum, cecum, and colon as well as feces, were processed for microbiota analyses by the MITChip. Cognate host intestinal mucosal tissue samples were used for gene expression profiling (Affymetrix mouse microarrays) and (immuno)histochemistry. The MITChip comprises 3500 unique probes targeting 1,885 OTUs covering 10 bacterial phyla. Its application on intestinal samples showed that bacteria gradually colonized the mouse intestine during the first days and that the microbiota reached a high level of similarity compared to the inoculum used after eight days, in which host-microbe communication shapes the ecosystem. Analogously, mouse mucosal tissue morphology, and host gene expression data go through drastic changes during the same period, and subsequently stabilize. Moreover, the study of the microbial colonization along the GI tract showed that specific bacterial groups colonize the different intestinal regions, which we aim to correlate to transcriptomic and histochemical data, related to pathways such as epithelial turnover. Overall, the MITChip provides a suitable platform for rapid determination of microbiota profiles in murine intestinal samples, and in this study sequential microbial community patterns can be associated with developmental processes occurring in the intestinal host tissues.

Keywords: Microbiota; gastrointestinal tract; phylogenetic microarray; conventionalization; host responses

Effect Of Carbohydrase Supplementation On Ileal Morphology And Gut Microbiota In Growing Pigs, Fed Different Cereal-Based Diets. J. Willamil¹, I. Badiola¹, D. Torrallardona², P. A. Geraert³ and E. Devillard³; ¹CRESA,

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This experiment was designed to study the changes in ileal and caecal microbiota induced by a carbohydrase preparation (Rovabio™ Excel) in growing pigs fed with diets based on corn (C) or wheat-barley-rye (WBR). Thirty-six pigs (25 kg body weight) were fed during 28 days with one of 4 different treatments based on the type of diet (WBR or C) with or without enzyme supplementation (+ or -). The pigs were slaughtered to collect samples of ileal and caecal digesta and mucosa. Morphometric analysis of ileum mucosa was performed. Differences in the microbiota composition of both digesta and mucosal populations, within the ileum and caecum were examined by comparing electrophoretic profiles obtained by Restriction Fragment Length Polymorphism (RFLP-PCR). Morphological changes of the ileum mucosa were observed when enzymes were added to the WBR diet: the ratio of villi height: crypt depth was significantly increased and the number of intraepithelial lymphocytes tended to decrease. Biodiversity in ileal and caecal digesta samples appeared greater in WBR + diets, when compared to the same samples collected from animals fed the WBR- diet. The most diverse RFLP profile from ileal mucosal samples was produced from animals fed the C + diet, while the caecal mucosal samples from animals fed the WBR + diet showed more biodiversity than the other treatments. In conclusion, enzyme supplementation of the WBR diet led to changes in bacterial community structure at all sites examined. Some modifications of the bacterial populations in animals fed the C diet were also observed when enzymes were added, especially those associated with the gut mucosa. Our results clearly showed that pig digestive microbiota can be modified by enzyme supplementation of the diet. These changes are more important with high non-starch polysaccharide containing diets. Further analysis will allow us to identify the bacterial species that are affected by enzyme supplementation.

Keywords: pig digestive microflora, PCR-RFLP, carbohydrases

Differential Chemotaxis by Entodiniomorphids and Isotrichids Toward Peptides of Bacterial, Protozoal, and Soy Origin. H.L. Diaz, A.M. Stalford, and J.L. Firkins; The Ohio State University, Ohio, USA

Because of their role in proteolysis, we hypothesized that entodiniomorphids (EN) would be more chemotactic toward bacterial peptides than the sugar-fermenting isotrichids (IS). From ruminal fluid, protozoa were harvested and dialyzed in 10-µm filter bags, and bacteria were recovered and washed using differential centrifugation.

After hydrolysis in proteinase K, incubation in 5% v/v of perchloric acid, and centrifugation, supernatants (after removal of perchloric acid) and soy protein isolates (soy) were analyzed for protein. Two blood capillary tubes (75 mm) were not filled (positive control, POS) or filled with 0 (SAL), 125, 250, 500, 750 or 1000 mg/L of soluble peptides from soy, bacteria protein (BP) or protozoal protein (PP). After capping, the tubes were placed in 3 replicate beakers with 20 mL of rumen fluid recovered from a dairy cow 3 h after feeding. After 20 min, protozoa in the capillary tubes were counted and corrected for their 0-min controls. The experiment was replicated 3 times. There was no interaction ($P > 0.10$) between peptide source and concentration for EN. Main effect means were greater ($P < 0.05$) for soy than BP or PP. Main effect means for log₁₀ counts in the capillary tubes were 0.74, 1.84, 1.94, 2.22, 2.38, and 2.49 for saline through 1000 mg/L, respectively, with linear, quadratic, cubic, and quartic contrasts all $P < 0.05$. POS was 3.09. Explaining the interaction ($P < 0.05$) between protein source and peptide concentration, the log₁₀ counts of IS increased linearly ($P < 0.05$) from 0.74 to 1.15 for soy and from 0.74 to 1.20 for BP, respectively, when peptide increased from 0 to 1000 mg/L. For PP, log₁₀ IS counts followed a cubic ($P < 0.05$) response, peaking at 1.11 for 125 through 500 mg/L. POS was 1.61. In contrast with prior results showing lower chemotaxis toward glucose for EN vs. IS, there is more extensive chemotaxis by EN than IS toward peptides, regardless of source, explaining their niche for proteolysis and bacterial predation.

Keywords: Rumen protozoa, chemotaxis, peptide hydrolysis

Isolation of a *Lactobacillus* sp. from Swine Lagoon Enrichments which Produces p-Ethylphenol from p-Coumaric Acid. K.C. Doerner, S. Hughes, T. Allen, and D. Kridelbaugh; Western Kentucky University, KY, USA.

The compound p-Ethylphenol (pEP) is a prominent malodorant emanating from cattle and swine feedlots and detected at considerable distances from the source. Little is known of the microbial components of swine lagoons which produce pEP. Primary swine lagoon sediment was collected, transported to the laboratory, and blended for 10 min. Ten mls of slurry was added to 100 mls of anaerobic tryptone-yeast extract (TY) broth containing: water (Control), 10 mM p-coumaric acid (TYC), 10 mM p-ferulic acid, 1 mM L-tryptophan, and 1 mM L-tyrosine. Enrichments were incubated at room temperature for five weeks and analyzed for pEP using GC-MS. Enrichment cultures were serially-diluted in TYC, incubated for 1 week and screened for pEP production using HPLC. pEP-positive cultures were anaerobically plated for isolation on TY agar containing 1 mM p-coumaric acid (pCA).

Following three successive platings, isolated colonies were cultured in TYC and screened for pEP production. A p-coumaric acid decarboxylase (*pd*c) fragment (340 bp) was amplified using primers derived from the *Lactobacillus plantarum* *pd*c gene. Only the pCA-amended swine lagoon enrichment culture produced appreciable levels of pEP (580 μM). The pCA-amended enrichments yielded positive pEP cultures through 10⁻⁷-fold dilutions. Six colonies were isolated from the pEP-positive dilutions: strain pEP8 produced the highest levels of pEP (827 μM) in TYC. Comparative 16S-rDNA analysis indicated pEP8 was a *Lactobacillus* sp.; Gram-staining indicated Gram-positive rods consistent with lactobacilli. Amplification and sequencing of the *pd*c fragment revealed pEP8 contained a target 100%- identical to the *L. plantarum* *pd*c. Amplifications using the *pd*c primers and subsequent cloning, from pCA or control-enrichment cultures, revealed 9/9 and 2/10 clones, respectively, were identical in nucleotide sequence to the *L. plantarum* *pd*c. Swine lagoon enrichments in TY medium produced pEP from pCA and a *Lactobacillus* sp. was isolated with such activity and contained a gene identical to a *L. plantarum* *pd*c gene, known to encode a pCA decarboxylase. The *pd*c gene was also found in swine lagoon and the control cultures of swine lagoon enrichments.

Keywords: *Lactobacillus* sp., malodorant, p-ethylphenol, p-coumaric acid, p-coumaric decarboxylase

Effects Of *Lactobacillus casei* Shirota On Immune Function. H. Dong¹, I. Rowland¹, K.M. Tuohy¹, L.Thomas², and P. Yaqoob¹; ¹The University of Reading, UK; ² Yakult UK Ltd.

Modulation of host immunity is an important potential mechanism by which probiotics confer health benefits. This study was designed to study the effect of a probiotic strain *Lactobacillus casei* Shirota (LcS) on the immune function using human peripheral blood mononuclear cells (PBMC) in vitro. The PBMC were obtained from 11 healthy adults and LcS was grown in MRS broth anaerobically and harvested in exponential phase. Then PBMC (2 × 10⁶ cells/ml) were exposed to three different concentrations of viable LcS for 24 h with or without 2.5 μg/ml ConA, or to three different concentrations of ConA with or without 10⁶ CFU/ml viable LcS. The activation markers, CD69 and CD25, on T cell subsets were assessed by flow cytometry. Additionally, whole blood (WB) or PBMC were stimulated for 24 h by three different concentrations of viable LcS with or without 1 μg/ml LPS and cytokines were measured by ELISA. In the absence or presence of ConA, LcS induced expression of both CD69 and CD25 on CD8 + T cell subset, but had a less effect on CD4 + T cells. The maximal effect was seen with 10⁶ CFU/ml, representing an LcS: PBMC ratio of 1:1.

Treatment with LcS alone induced production of IL-1 β , IL-6, TNF- α and IL-10. In the presence of LPS, LcS promoted IL-1 β production, but greatly inhibited LPS-induced IL-10 and IL-6 production. Cytokine production was also measured in WB. Production of cytokines in response to LcS and /or LPS by PBMC and WB was highly correlated ($P < 0.01$, $r = 0.69$ – 0.83). Based on these results we conclude that in the absence and presence of mitogenic stimulation, LcS enhances lymphocyte activation, particularly that of cytotoxic T lymphocytes (CD8 + T cells). This is the first time such an effect of LcS has been shown, and suggests that LcS could potentiate the destruction of infected cells in the body. LcS also induces both pro-inflammatory and anti-inflammatory cytokine production in the absence of LPS, indicating that LcS could promote cell mediated immunity and also down-regulate inflammation. Further research to investigate mechanisms is required and human trials are needed to confirm immunomodulation of LcS in vivo.

Keywords: probiotic, *Lactobacillus casei*, immunomodulation

Bacterial communities associated with Shiga-toxigenic *E. coli* O157:H7 (STEC O157) in beef cattle feedlot environments. L.M. Durso, G. Harhay, J.L. Bono, T.P.L. Smith, W.W. Laegreid, J.E. Keen, M.L. Clawson. U.S. Meat Animal Research Center, Nebraska, USA.

The life cycle of Shiga-toxigenic *E. coli* O157:H7 (STEC O157) in beef cattle feedlots involves two habitats: the warm, nutrient rich primary habitat of the cattle lower gastrointestinal tract, and the generally cool, nutrient limiting secondary habitat outside of the animal, including the feedlot surface material. In both of these habitats STEC O157 must interact with other microorganisms, and compete for resources. We used 16S ribosomal RNA (*rrs*) gene sequence analysis to characterize the bacteria present in the STEC O157:H7 primary and secondary habits. Fecal samples were collected from three STEC O157 culture-positive, and three STEC O157 culture-negative beef cattle. All 6 animals were housed in the same pen and fed the same diet. Additionally, three feedlot surface material samples were collected from the pen in which the animals were housed. The *rrs* genes were amplified using “universal” bacterial primers, cloned into TOPO4 vectors, and sequenced using an ABI 3730. Sequences were screened for chimeras using Bellepheron, and aligned using a NAST alignment tool. Then DOTUR was used to generate rarefaction curves and sequences were assigned taxonomies using the RDP classify tool, and BLAST. Near full length 16S rDNA sequence was obtained for 14,944 clones from cattle feces and the corresponding feedlot pen surface material. The Firmicutes were the predominant phylum in

feces (54%), followed by the Bacteroidetes (40.5%), and the Proteobacteria (5.5%). The predominant phylum in the feedlot surface material was Actinobacteria (42%), followed by Firmicutes (24%), Bacteroidetes (24%), and Proteobacteria (9%). There were 139 genera identified in the STEC O157 primary and secondary habitats, 25 of which were present in both habitats. Eleven genera had different frequencies of occurrence in STEC O157 culture-positive and -negative cattle. Our results demonstrate 1) there are differences in the microbial frequencies between STEC O157 culture-positive and culture-negative animals, and 2) while feces may be the source of much of the mass of the feedlot surface material, the microbial profile of the feces and that of the feedlot pen differ greatly.

Keywords: *Escherichia coli* O157:H7, STEC O157, feedlots, environment

Metabolism of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by Ovine Ruminant Microbes. H.L. Eaton, M. DeLorme, and A. M. Craig; Oregon State University, OR, USA.

Bioremediation is of great interest in the detoxification of soil contaminated with residues from explosives such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Whole ovine rumen fluid was inoculated into five different enrichments to isolate and select for archaeal and bacterial consortia able to metabolize RDX. Enrichments containing 33 $\mu\text{g mL}^{-1}$ RDX were incubated and transferred each time the RDX concentration decreased to 5 $\mu\text{g mL}^{-1}$ or less. Daily time point samples were analyzed for RDX metabolism by HPLC. The two fastest degrading enrichments, in which RDX was reduced to 0.53 or 2.63 $\mu\text{g mL}^{-1}$ in three days, were in methanogen-specific media (RDX8MM) and low basal nitrogen media (RDX5BN), respectively. After 22 days, DNA was extracted from all enrichments able to degrade RDX in seven days or less. To understand the microbial diversity, 16S rRNA-gene-targeted denaturing gradient gel electrophoresis (DGGE) fingerprinting was conducted. Based on the banding patterns, six culturable archaeal and five to seven culturable bacterial species may be involved in RDX metabolism in the RDX5BN and RDX8MM consortia from the ovine rumen. Cloning and sequencing were performed on both RDX5BN and RDX8MM consortia targeting the 16S rRNA gene marker. Phylogenetic analysis revealed homology matches (99–100%) to five different bacterial species.

Keywords: Bioremediation, RDX, rumen, 16S rRNA, Archaea, DGGE

Bacterial Colonisation of the Tanniferous Forage *Lotus corniculatus* in the Bovine Rumen. J.E. Edwards, P.

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The tanniferous forage *Lotus corniculatus* generally has a beneficial effect on ruminal fermentation, protecting dietary protein from excessive degradation. In *L. corniculatus*, condensed tannins (CTs) are normally concentrated within plant cell vacuoles, in subsets of plant cells within the spongy and palisade mesophyll. However, the effect of these concentrated pockets of CT on the rumen bacteria that colonise *L. corniculatus* is not clear. In order to investigate this, two *L. corniculatus* varieties with differing CT content in leaf (0.64% v 2.3% dry matter) and stem (0.34% v 0.77% dry matter) were incubated in the rumen of two cows over a 72 h period. For each cow duplicate polyester bags containing fresh, chopped samples of each variety were incubated per time point (1, 2, 4, 8, 12, 24, 48 and 72 h), with 0 h bags processed directly. At the end of their incubation, bag residues were hand washed and then frozen. DNA was extracted from the residues and the population composition and size of bacteria colonising the plant material was assessed using 16S rDNA denaturing gradient gel electrophoresis and quantitative PCR. The size of the colonising bacterial population was significantly affected by ruminal incubation time ($P < 0.001$), with no effect of variety ($P > 0.05$). The population size initially increased during 0–1 h of incubation, and then increased again during 2–4 h of incubation; thereafter the population size remained stable for the remainder of the incubation period. Bacterial population composition was also significantly affected by time, with no effect of variety. The bacterial population composition of the rumen incubated samples were distinct from the 0 h samples, and separated into two main clusters containing 1–8 h and 12–72 h time points. Within the latter cluster a further separation was evident after 24 h, which was characterised by a substantial increase in the intensity of one band. The sequence from the dominant band had 98% identity with *Butyrivibrio hungatei*. The dominance of this bacterium in latter time points is intriguing as its ecological niche is thought to be in the utilisation of oligo- and monosaccharides, as it does not possess proteolytic or fibrolytic activity. Further work is needed to clarify the basis of this observation, and the change in the colonising bacterial population composition after 8 h.

Keywords: condensed tannins, rumen bacteria, *Lotus corniculatus* colonisation

Coculture Fermentations Of *Bifidobacterium* Species And *Bacteroides thetaiotaomicron* Reveal A Mechanistic Insight Into The Prebiotic Effect Of Inulin-Type Fructans. G.

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Studies concerning the bifidogenic effect of inulin-type fructans tend to consider the bifidobacterial colon population as a whole, not taking into account the interspecies differences that exist between various bifidobacteria. As not all bifidobacteria are able to degrade inulin or even oligofructose, it seems likely that not all bifidobacterial species benefit in the same way of the presence of inulin-type fructans as growth substrates in the colon. Also, not all bifidobacteria might prove equally fit to compete with other large-intestinal inulin-degrading species, such as *Bacteroides* spp.. In this study, four bifidobacterial strains, each representing a cluster with specific inulin-type fructan degradation capacities, were grown in coculture fermentations with *Bacteroides thetaiotaomicron* LMG 11262, a strain able to metabolize both oligofructose and inulin, to assess their *in vitro* competitiveness. In a medium for colon bacteria with inulin as the sole added energy source, the ability of the bifidobacteria to compete for this substrate reflected phenotypical variation. *Bifidobacterium breve* Yakult, a strain that was not able to degrade oligofructose or inulin, was outcompeted by *Ba. thetaiotaomicron* LMG 11262. *B. adolescentis* LMG 10734, a strain that could degrade oligofructose (displaying preferential breakdown of its short chain-length fractions) but that did not grow on inulin, managed to become competitive when oligofructose and short fractions of inulin started to accumulate in the fermentation medium. *B. angulatum* LMG 11039^T, a strain that was previously shown to degrade all chain-length fractions of oligofructose simultaneously and to be able to partially break down inulin, was competitive from the beginning of the fermentation, consuming short fractions of inulin from the moment they appeared. *B. longum* LMG 11047, representing a cluster of bifidobacteria that shared both high fructose consumption and oligofructose degradation rates and were able to perform partial breakdown of inulin, was the dominating strain in a coculture with *Ba. thetaiotaomicron* LMG 11262. These observations indicate that distinct subgroups within the large-intestinal *Bifidobacterium* population will be stimulated by (different groups of) prebiotic inulin-type fructans. As health effects of (probiotic) *Bifidobacterium* spp. are considered strain-dependent, the question which bifidobacteria are stimulated by which inulin-type fructans appears crucial for further development of prebiotics.

Keywords: *Bifidobacterium*, *Bacteroides thetaiotaomicron*, inulin-type fructans, bifidogenic effect, competitiveness

Fate And Effect Of Usnic Acid In Lichen On The Bacterial Population In The Reindeer Rumen. T. Glad¹, A. Falk², P.

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Reindeer and their symbiotic rumen microorganisms have interacted with lichens through millions of years. We propose that the reindeer rumen microbiota can tolerate high concentrations of, and even utilize the lichen phenolic compound usnic acid (UA), well known for both its antibiotic and toxic effects. The purpose of this study was to (1) investigate if UA is subjected to rumen microbial degradation in reindeer; (2) search for UA-resistant rumen bacteria; and (3) determine the effect of UA on rumen bacterial diversity to identify dominant strains in reindeer supplemented with UA. *In sacco* digestibility of UA was determined by rumen incubation (48 hours) of *Cladonia stellaris* (0.63 mMol UA/g DM) and *Cetraria nivalis* (0.40 mMol UA/g DM) in rumen fistulated male reindeer (n=2) given *ad libitum* access to lichens (mainly *Cladonia stellaris*) for four weeks. After incubation, bags were collected, adherent material removed and dried before determining the UA disappearance, which was as much as 56–70% from *C. stellaris* and 38–61% from *C. nivalis*. A total of 66 rumen bacterial isolates were isolated from sacrificed reindeer consuming natural winter pastures with lichens, and were screened for their ability to grow in the presence of lichen secondary compounds including UA, atranoric acid, fumarprotocetraric acid and lobaric acid, using the agar diffusion method. Both UA and lobaric acid showed a potent antimicrobial effect on 63 of the isolates, which were determined to be Bacilli, Clostridia, Enterococci and Streptococci. Three Clostridial isolates were found to be resistant to all lichen acids tested, one showing a 98.1% 16S rRNA sequence identity to *Butyrivibrio hungatei*, and the other two 99.5–100% 16S rRNA identity to *Pseudobutyrvibrio ruminis*. The resistant isolates were all motile, curved rods and stained Gram negative. For the rumen bacterial diversity studies, rumen fistulated female reindeer (n=3) were given *ad libitum* access to a pelleted diet coated with a gelatin solution to provide UA at 10 g/kg diet for 17 days. Consumption of 1.78 kg/d was equivalent to daily doses of 170–267 mg UA/kg body mass. Sampling of the rumen was performed prior to the UA treatment, after 9 and 17 days of treatment, and 7 days after withdrawal of UA. Preliminary DGGE analysis indicated that the bacterial population profiles were altered in response to UA supplementation.

Keywords: Rumen; usnic acid; digestion; resistance; diversity

Characteristics Of *Lactobacillus* Strains And Viability Assessment Of Microencapsulated Bacteria Using a 3-Compartment Fermentor Simulating The Gastrointestinal Tract. K. Goderska and Z. Czarniecki; Poznan University of Life Sciences, Poland

The widespread interest in probiotic bacteria results from their presumed beneficial effects on health and nutrition, both in humans as well as animals. The present study aimed to characterise chosen strains of *Lactobacillus* and other candidate probiotic bacteria, as well as evaluate their biochemical properties and antagonistic effect on *Helicobacter pylori*. Additionally, we assessed the influence of different microencapsulation on *Lactobacillus* cell viability *in vitro* using a 3 compartment fermentor system simulating the stomach, small intestine and large intestine. The chosen probiotic strains were encapsulated according to the previously published methods, and the isolates of pathogenic bacteria used in this study were recently isolated from patients. The evaluation of the antagonistic effect between microorganisms was performed using the agar slab method, and the minimal inhibitory concentration (MIC) of different antibiotics was determined by microdilution methods. The ability of bacteria to produce hydrogen peroxide was also investigated. The number of cfu/ml was determined on modified Garcke's medium for *Bifidobacterium*, on MRS for *Lactobacillus*, on medium with kanamycin, esculin and sodium azide for *Enterococcus*, and on McConkey medium for *Enterobacteriaceae*. All the examined probiotic strains were characterised by diverse biochemical properties, resistance to antibiotics and showed antagonistic action against *Helicobacter pylori*. Microencapsulation was shown to increase the viability of both *Lactobacillus* strains *in vitro*. It was observed that *Lactobacillus* strains had an antagonistic effect on growth of all strains of *H. pylori* tested, although the two *L. acidophilus* strains examined did not appear to produce hydrogen peroxide. Given the obtained results we determine that the gastrointestinal tract model applied in this work can be successfully used to study how different microencapsulation methods may affect the viability of potential probiotic bacteria.

Keywords: probiotic, *Lactobacillus*, microencapsulation, cell viability

Bacteriocin Compounds Produced By *Lactobacillus Plantarum* Inhibits Growth Of *Salmonella typhimurium* In The

Gut Of Mice. **H. S. Gong**, X. C. Meng, and H. Wang
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Lactobacillus plantarum KLDS1.0391 was isolated from “Jiaoke”, a traditional fermented cream from China, and produces a bacteriocin named plantaricin MG. Plantaricin MG showed a broad inhibitory activity against Gram-positive and Gram-negative bacteria including *Listeria monocytogenes* and *Salmonella typhimurium*. This study was designed to determine whether plantaricin MG and (or) other bacteriocins produced by strain KLDS1.0391 inhibit the growth of *Salmonella typhimurium* in the gut of mice. Bacteriocins were recovered from MRS cultures of *L. plantarum* KLDS1.0391 by means of centrifugation, precipitation in 70% saturated ammonium sulfate and freeze-drying. The 8-week old KM mice (SPF) received 2×10^7 cfu *Salmonella typhimurium* ATCC14028. After inoculation, these mice were randomly placed into 5 groups each consisting of sixteen mice and received by oral gavage once per day one of the following treatments: (a) phosphate-buffered saline (negative control), (b) 80 mg/kg bodyweight (BW) cefazolin sodium (positive control), (c) 80 mg/kg BW bacteriocin compounds, (d) 400 mg/kg BW bacteriocin compounds, and (e) 2000 mg/kg BW bacteriocin compounds. After seven days of treatment, the surviving mice from each group were euthanized. The liver, spleen, duodenum, ileum, colon were aseptically removed and homogenized. All samples were processed (i.e. diluted and plated on Hektoen Enteric Agar) within 3 h. Mouse survival was 18.75% (3/16) and 100% (16/16) for the negative and positive controls, respectively. Increasing quantities of bacteriocins also improved mouse survival, relative to the negative controls: 80 mg/kg: 37.50% (6/16), 400 mg/kg: 56.25% (7/16), and 2000 mg/kg: 62.50% (10/16). The viable cells of *S. typhimurium* in liver, spleen, duodenum, ileum, colon and feces of mice receiving 2000 mg/kg bacteriocins were 1.55×10^5 cfu/ml, 1.45×10^4 cfu/ml, 3.70×10^2 cfu/ml, 1.43×10^2 cfu/ml, 3.9×10^1 cfu/ml and 1.01×10^2 cfu/ml, respectively. These counts were all significantly lower than those measured from the surviving mice in the negative control group ($p < 0.05$); and there were no significant differences to cell counts produced from mice in the positive control group, with the exception of the ileal and fecal samples. In conclusion bacteriocin compounds from strain KLDS1.0391 can inhibit growth of *S. typhimurium* ATCC14028 in the gut of mice.

Keywords: bacteriocin; *Lactobacillus plantarum*;
Salmonella typhimurium, mice

High Protein Diets Impact on Microbial Metabolites and Toxicity in the Human Large Intestine. **S. Gratz**¹, S.H. Duncan², A.J. Richardson¹, A.M. Johnstone³, G.E. Lobley³, H.J. Flint², and R.J. Wallace¹; ¹Microbial Biochemistry Group, ²Microbial Ecology Group and ³Metabolic Health Group, Rowett Research Institute, Bucksburn, Aberdeen, UK

High-protein, low carbohydrate (CHO) diets have been shown to be effective in weight loss regimes, and have gained increasing popularity. Nonetheless, negative effects on gut health might be expected if less carbohydrate and more protein reach the large intestine, due to changes in the composition of the intestinal microbiota and their metabolites. This study compared concentrations of bacterial metabolites (short chain fatty acids (SCFA), ammonia, N-nitroso compounds) in faeces of volunteers consuming diets with differing carbohydrate and protein content and their impact on genotoxicity in vitro. After a period of one week on a weight maintenance diet (53% as CHO), seventeen obese men were randomised, in a cross-over study design, to receive for four week periods each two high-protein (30% calories) weight loss diets with either moderate (35%) or low carbohydrate (4%) contents. Faecal water was extracted by high-speed centrifugation (20,000 g, 2 h) and aliquots stored at -80°C until analysis. SCFA and ammonia concentrations were determined from whole faecal samples. Intestinal toxicity was assessed in human colonocytes cell lines using a genotoxicity (Comet) assay. N-nitrosocompounds were analysed by denitrosation and expressed as apparent N-nitrosocompounds (ATNC). Ammonia concentrations were lowest in the faecal samples collected from the volunteers on the low CHO, high protein diets. Total SCFA concentrations and butyrate concentrations in particular decreased as a result of low CHO and high protein dietary intakes. Concentrations of ATNC were increased in faecal samples of subjects receiving both high-protein diets compared with the maintenance (high CHO) diet. Furthermore, increased DNA damage in the Comet assay was observed in volunteers consuming the high-protein diets. In conclusion, high-protein diets designed to promote weight loss lead to changes in the concentrations of microbial metabolites in faecal samples from human volunteers. In particular the concentrations of total SCFA and butyrate decreased. In addition N-nitrosocompound concentrations and genotoxicity potential of faecal water towards intestinal cells increased. Decreased butyrate concentrations in combination with the formation of adverse protein degradation products suggests that consuming high protein diets long-term poses a risk to gut health.

Keywords: high-protein diet; human; faeces; N-nitroso- compounds; comet assay; SCFA; ammonia

Adhesion to Intestinal Epithelial Cells by Domains at the N-terminus of the Lp_1643 Protein. F. Liu¹, L.H. Du^{1, 2}, G.C. Huo¹. ¹Northeast Agricultural University, Harbin, China; ²Nanjing University of Finance and Economics, Nanjing, China.

The cell-surface protein Lp_1643 of *Lactobacillus plantarum* WCFS1 was deduced to have mucin-binding ability, but its adhesion to intestinal epithelial cells is not completely characterized. The gene encoding the Lp_1643 protein was amplified from *Lactobacillus plantarum* KLDS 1.0320's genomic DNA and sequenced. Two domains at the N terminus of Lp_1643 protein were reproduced in *E. coli* BL21 as His-tagged proteins and purified using HisTrap FF columns, and are designated N1 and N2. The adhesion capacity of N1 and N2 to Caco-2 cell was then studied using BSA as a negative control. It was found that the counts of KLDS 1.0320 adhering to Caco-2 cell pretreated with N1 and N2 decreased dramatically ($p < 0.05$) compared with the control, and the cells pretreated by N2 gave the lowest adhesion values for KLDS 1.0320. These results suggests the recombinant N1 and N2 domains could adhere to Caco-2 cells and thereby reduce the adhesion of intact cells. The gene encoding the signal peptide of Usp45, the two domains at the N terminus of Lp_1643 and the anchoring region at the C terminus of Lp_1643 were linked together using fusion PCR, and cloned into pNZ8148 to construct a cell wall anchoring vector pNZ8148/usp/N2/ar. A *L. lactis* NZ9000 strain carrying plasmid pNZ8148/usp/N2/ar induced with nisin adhered to Caco-2 cell line, whereas the control NZ9000 strain did not exhibit this phenotype. The nisin-induced strain had a weak protein band appeared near 66 kDa after SDS-PAGE electrophoresis. These means the two domains at the N terminus of Lp_1643 can be expressed on the surface of *L. lactis* NZ9000 and significantly strengthen the adhesion ability of the host. This study verified that certain domains at the N-terminus of Lp_1643 protein have the ability to adhere to intestinal epithelial cells. It was also demonstrated that by heterologous expression of certain domains of this protein borne on a plasmid non-adhesive strains could be made to adhere to Caco-2 cells.

Keywords: *Lactobacillus plantarum*, Lp_1643, Caco-2 cell, adherence

The Human Faecal Microbial Community Related To Butyrate Produced In Response To A Diet High In Resistant Starch. S. Kang¹, M. Conlon¹, S. Denman¹, Z. Yu², M. Morrison^{1, 2} and C. McSweeney¹; ¹CSIRO,

Preventative Health National Research Flagship, Australia; ²The Ohio State University, Ohio, USA

The colonic microbiota acts as a complex metabolic interface between dietary components and the host and by doing so ultimately influence colonic health. Resistant starch (RS), which is abundant in foods such as grains and legumes, may contribute to a decreased risk of colorectal disease by increasing faecal bulking and short-chain fatty acid (SCFA) levels. Among the SCFA produced, butyrate is considered beneficial to the colonocytes and bowel health. However, the impact of diet on the bacterial community and butyrate production in the colon is poorly understood. In this study we examined the effect of a diet high in resistant starch (RS) (22 g/d for 4 weeks) on faecal butyrate levels and the composition of the faecal microbial community in 46 healthy adults. The bacterial community was estimated using a phylogenetic custom microarray with specific probes targeting 766 unique 16S rRNA gene sequences that represent the major genera and species of bacteria currently identified in the human colon. The RS diet increased faecal butyrate concentrations relative to those of faeces collected during the consumption of habitual diets (at entry) in a majority of individuals. However, concentrations tended to fall in response to the RS diet in individuals with the highest entry butyrate levels (20–30 mM). The microarray analysis showed that *Faecalibacterium prausnitzii* was approximately 10 times more abundant in the RS group than in the habitual group after RS was consumed. Interestingly, in the individuals with a high entry butyrate concentration there was a high abundance of polyhydroxybutyrate accumulating and hydrolysing bacteria (*Ralstonia sp.*, *Acidovorax sp.*, *Alcaligenes sp.*). These species may be involved in the production and metabolism of butyrate, thus playing a role in gut health. The effects of RS on the colonic microbiota may be central to the beneficial activity of these organisms in the colon.

Keywords: Resistant starch, custom microarray, butyrate, colonic microbiota, poly hydroxybutyrate

Purification, Characterization, and Regulation of Glutamine Synthetase from *Prevotella ruminicola* 23. J. N. Kim, I.K.O. Cann, and R.I. Mackie; University of Illinois at Urbana-Champaign, Illinois, USA.

Prevotella ruminicola 23 contains three genes encoding different glutamine synthetase (GS) enzymes; Glutamine synthetase I (GSI; ORF02151), GSIII-1 (ORF01459), and GSIII-2 (ORF02034). GSI shares 77% identity with the GSI of *Bacteroides fragilis* YCH46, while GSIII-1 and GSIII-2 are 81% and 77% identical, respectively, to orthologs found in *Prevotella bryantii*. Alignments of GS type I family proteins has previously shown five conserved

regions (motifs I, II, III, IV, and V) and GS type III family proteins have five conserved regions as well as four GSIII specific conserved region (motifs A, B, C, and D). Alignment of GSI with characterized orthologes from *Mycobacterium tuberculosis*, *E. coli*, *Salmonella typhimurium*, and *Synechocystis* sp. PCC 6803 revealed seven insertions in motif II and substitutions in the ATP binding site (motif III). In contrast alignment of GSIII-1 and GSIII-2 with GSIII-family enzymes of *Ruminococcus albus* 8 and *Synechococcus* sp. PCC 7942 revealed strong sequence conservation through all motifs. GSI, GSIII-1 and GSIII-2 have each been heterologously expressed and purified from *E. coli*. The molecular weight of GSI was 56 kDa and GSIII-1 and GSIII-2 were both 83 kDa. The conditions for optimum γ -glutamyl transferase activity were found to be 35°C at pH 5.6 with 0.25 mM Mn^{2+} ions (GSI) or 37°C at pH 6.0 (GSIII-1 and GSIII-2) with 0.50 or 1.00 mM Mn^{2+} ions, respectively. GS biosynthetic activity was found to be optimal at 60°C at pH 7.0 with 10 mM Mn^{2+} ions (GSIII-1) or 50°C at pH 6.8 with 10 mM Mn^{2+} ions (GSIII-2), while GSI showed no GS biosynthetic activity. Kinetic analysis revealed K_m values for glutamate and ammonium as well as the hydrolysis of ATP, to be 8.579, 0.482, and 1.906 mM, respectively, for GSIII-1 and 1.721, 0.432, and 2.652 mM, respectively, for GSIII-2. Overall, the results show that both GSIII enzymes were functional *in vitro* and may be involved in ammonia acquisition and metabolism under ammonia-limiting conditions by *P. ruminicola*. This work suggests that only type III GS enzymes are required for growth during ammonia-limiting conditions.

Keywords: *Prevotella ruminicola*; Glutamine synthetase; biosynthetic activity

An *in silico* Generated Census of the Rumen Bacterial Microbiome. **M. Kim**¹, C.S. McSweeney², M. Morrison^{1,2}, and Z. Yu¹. ¹The Ohio State University, Ohio, USA; ²CSIRO Australia, Queensland, Australia.

A large number of 16S rRNA gene (*rrs*) sequences have been generated from numerous studies of rumen microbiomes, which have advanced our perspective of the microbial diversity resident in these habitats. However, each study has principally been conducted using only a few individuals of a single ruminant species, fed on one or two specific diets. Additionally, the prevailing technologies (and costs) at the time these studies were performed placed restrictions on the number of *rrs* gene clones that were analyzed. A meta-analysis of the 10,451 *rrs* eubacterial gene sequences of rumen origin retrieved from the RDP (Release 10) showed that 10,192 of these sequences could be assigned to one of 17 bacterial phyla. The large number of bacterial phyla identified indicates great diversity at a

higher taxonomic rank, and thus presumably a diverse range of metabolism and interactions in the rumen. Approximately half (5,043 sequences) of these sequences have been assigned to existing individual genera for which there is a cultured representative, while the rest (5,233 sequences) could not. The *Firmicutes* and *Bacteroidetes* are the most predominant phyla with respect to either total number of sequences (64% and 26%, respectively) or sequences that have been assigned to existing genera (52% and 20%, respectively). Approximately 77% of the deposited sequences are longer than 500 bp and were selected for more detailed phylogenetic analysis. Based on the classified sequences, the ruminant microbiome is predicted to have as many as 3,303 species (97% sequence identity), 2,279 genera (95% sequence identity), and 1,122 families/classes (90% sequence identity). The *Lachnospiraceae* and *Prevotellaceae* are the most dominant families within the *Firmicutes* and *Bacteroidetes*, respectively. The current coverage at the species, genus, and family/class level by the deposited sequences are estimated to be 77%, 83%, and 93% respectively. These results show that the ruminant eubacterial microbiome has been properly sampled and catalogued by *rrs* sequences, but nearly 50% of the total bacterial diversity still remains uncultured and much remains to be learned about their functions.

Keywords: 16S rRNA; rumen diversity; bacteria; RDP; meta-analysis

Quantitative Comparisons of Cultured and Uncultured Bacterial Populations in the Rumen of Cows Fed Different Diets. **M. Kim**¹, C. Shaw¹, M. Morrison^{1,2}, and Z. Yu¹. ¹The Ohio State University, Ohio, USA; ²CSIRO Australia, Queensland, Australia.

Extensive research has been conducted to characterize the populations of microbes perceived to be important to ruminant nutrition. However, much remains unknown regarding the partitioning of different bacterial populations throughout the rumen milieu (e.g. biofilm-associated vs. planktonic) and the magnitude of impact from the diets. In this study we quantified the populations of the well-characterized bacterial species/groups, as well as uncultured bacteria represented by novel 16S rRNA sequences, in fractionated rumen samples of cows fed different diets (grass hay vs. corn silage plus grains and hay) as well as the same type of diet (corn silage) with different source (wheat straw, corn stover, alfalfa hay) and content of fiber. The microbial communities recovered from these fractions were also compared using PCR-DGGE. Real-time qPCR assays showed that the numbers of *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* in total ruminal contents were similar ($1.1 \times$

10^6 – 1.2×10^7 and 1.9×10^6 – 3.1×10^7 *rrs* copies/ μ g community DNA, respectively), and much more abundant than *Ruminococcus albus* (3.5×10^4 – 9.7×10^5 *rrs* copies/ μ g DNA). When the digesta was fractionated into liquid and particulate fractions, *R. flavefaciens* was 10-fold higher in the particulate fractions. However, the abundance of *F. succinogenes* and *R. albus* in both fractions were similar. Cows fed grain:corn silage:hay were shown to have greater numbers of *Prevotella* and *Ruminobacter amylophilus* than the hay-fed cows in both the adherent and liquid fractions. Some of the uncultured bacteria appeared to be as abundant as, if not more so, than the three species of classic cellulolytic bacteria in the hay-fed cows, especially in the adherent fraction. As major members of the biofilm adherent to the plant particles in the rumen, some of these uncultured bacteria may be important to fiber digestion and plant biomass degradation. Similar to previous findings, it seems that *F. succinogenes* and *R. flavefaciens* are numerically more predominant in ruminal contents than *R. albus*. Furthermore, the relative abundances of the uncultured bacteria measured in this study by qPCR suggest that the adherent biofilms are not only quite diverse, but bacteria other than *F. succinogenes*, *R. flavefaciens* and *R. albus* play a key, yet cryptic role in plant biomass degradation.

Keywords: cellulolytic species; PCR-DGGE; real-time qPCR; rumen; uncultured bacteria

Isolation and Partial Characterization of *Prevotella* Strains From Sheep Rumen. D. Matsui, S. Koike, Y. Kobayashi; Hokkaido University, Sapporo, Japan.

Molecular biological approaches revealed the predominance and genetic diversity of the genus *Prevotella* in the rumen. Through the process for analyzing this group, the presence of uncultured *Prevotella* strains has been recognized. In the present study, we attempted isolation and characterization of uncultured *Prevotella* strains to determine the functional contribution of this genus to rumen fermentation. Rumen contents were collected from sheep and inoculated into the medium containing glucose/cellobiose, orchardgrass hay or a natural plant material as a sole carbon source. In order to inhibit the growth of Gram positive bacteria and to enrich Gram negative prevotellas, benzylpenicillin or amoxicillin were added to the medium. After incubation, the enrichment was serially diluted and inoculated into prewarmed agar medium for making roll tubes. Colonies that formed in the roll tube were transferred to slant medium to obtain bacterial isolates. The DNA was extracted from each bacterium, and the gene encoding 16S rRNA was PCR amplified using bacterial universal primers, cloned and

sequenced. The isolates were grown in the medium containing cellobiose as a carbon source and bacterial cells were harvested by centrifugation. The cells were suspended in 50 mM potassium buffer and disrupted by sonication. The centrifuged supernatant of sonicated material was used to quantify carboxymethylcellulase (CMCase), xylanase, cellobiohydrolase, β -glucosidase, β -xylosidase, arabinofuranosidase and acetylcetate activities. We obtained 28 strains that were phylogenetically affiliated with *Prevotella* group. Three out of 28 strains had <94% similarity with known *Prevotella* isolates, while rest of the strains showed >97% similarity with either of *Prevotella ruminicola* L16 or *Prevotella brevis* GA33. Four strains possessed measurable CMCase activity, which was comparable to or higher than that of *P. ruminicola* 23. On the other hand, 16 strains including 3 strains belonging to phylogenetically new group had xylanase activity, the extent of which was comparable to or higher than that of *P. ruminicola* 23. These findings indicate that new *Prevotella* isolates may possess superior fibrolytic ability compared with common ruminal *Prevotella* isolates such as *P. ruminicola* 23.

Keywords: uncultured bacteria, rumen, *Prevotella*, fiber digestion

Effects of Antibodies and Glycerol as Potential Inhibitors of Ruminal Lipase Activity. N.A. Krueger¹, R.C. Anderson¹, T.R. Callaway¹, T.S. Edrington¹, R.C. Beier¹, W.L. Shelver² and D.J. Nisbet¹; ¹USDA/ARS, Southern Plains Agricultural Research Center, College Station, Texas, USA; ²USDA/ARS, Biosciences Research Laboratory, Fargo, North Dakota, USA.

Ruminant-derived foods contain high proportions of saturated fats, a result of ruminal biohydrogenation which rapidly saturates and thus limits the availability of free unsaturated fatty acids for assimilation. Strategies to enrich ruminant-derived foods with unsaturated fatty acids are desired as these are considered beneficial for good human health. Lipolysis is a prerequisite for biohydrogenation because saturase enzymes act only on free fatty acids. This study aimed to evaluate the effects of bacteria specific IgY antibodies and glycerol on rates of free fatty acid (FFA) accumulation during *in vitro* incubation of mixed rumen microbes. Whole cells of four lipase positive ruminal bacteria; *Anaerovibrio lipolytica* 5S, *Butyrivibrio fibrisolvens* strain H17C, *Clostridium chauvoei* and *Propionibacterium acnes* were harvested and used for preparation of IgY antibodies. The developed antibodies were tested against the lipolytic activity expressed by mixed populations of rumen bacteria obtained from a cannulated cow receiving a 50% grain: 50% dried distillers grains diet. Additionally, two levels of glycerol were tested for their

potential inhibitory effect against ruminal lipolysis by mixed rumen microbes. Antibodies raised against the whole cell preparations reduced ($P<0.05$) the rate of FFA accumulation as compared to controls (normalized to 100%), with antibodies raised against *P. acnes* and *C. chauvoei* being less effective in reducing the rate of FFA accumulation (73.7 and 73.6% that of controls, respectively) than those raised against *A. lipolytica* 5S and *B. fibrisolvens* H17c (50.3 and 51.2% of controls, respectively). Results from *in vitro* incubations of ruminal contents with glycerol support, but do not yet prove, our hypothesis that glycerol may exhibit end product inhibition of lipolysis as evidenced by 48 and 77% reductions ($P<0.05$) in rates of FFA accumulation in incubations supplemented with 2 or 20% added glycerol, respectively, as compared to controls (5.06 ± 0.06 $\mu\text{mol FFA/mL per h}$). These results provide evidence that inhibiting bacterial fat degradation may promote ruminal passage of total lipid thereby providing greater proportions of beneficial unsaturated fat for incorporation into beef products.

Keywords: Biohydrogenation, Lipolysis, rumen bacteria, *Anaerovibrio*, *Butyrivibrio*

Isolation Of Lactic Acid Bacteria (LAB) From Traditional Fermented Beverages, Prepared In Rural Areas Of Southern Africa And Screening For Probiotic Properties. M. A. Mafokoane¹, C. Straker², and L. Maré¹; ¹Agricultural Research Council, Irene, South Africa; ²Witwatersrand University, Johannesburg, South Africa.

Traditional fermented beverages constitute an integral part of dietary culture in Southern Africa and they contain LAB which can be utilized as probiotic bacteria. This study aimed to isolate LAB from traditionally fermented beverages and screen for probiotic properties. Isolates from these beverages including Maas (traditional fermented milk), Madilla (traditionally fermented milk product from Botswana), distilled beer, KB (fermented sorghum beer products) and Ginger beer (fermented maize meal and sugar beer), were screened for probiotic properties (low pH and bile resistance). Selected Gram positive and catalase negative isolates were cultured in MRS broth, with adjusted pH ranging from 6.5 to 2.0 (0.5 pH unit decrements) and MRS broth supplemented with oxgall to 0.3%; 0.6%; 0.8%; 1.0% and 1.2% (w/v) to screen for low pH and bile resistance. Growth at 37°C was monitored by measuring absorbance at 590 nm over 30 minute intervals. The average lowest pH level at which viability was recorded for selected isolates was 3.0. In the presence of oxgall, some isolates were able to grow with 1.0% bile added to the medium. Sixteen isolates were selected that indicated some probiotic potential (low pH and bile

resistant). These isolates were identified using Analytical Profile Index® (API®). Results revealed *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus brevis* species isolated from most beverages, while *Lactococcus lactis* and *Pediococcus pentosaceus* were identified from Madilla only. Unique (resistant to very low pH and bile) indigenous potential probiotic bacteria were isolated in this study. These properties suggest that the selected bacteria may survive the harsh environmental conditions of the gastrointestinal tract and with further investigation these bacteria can be developed as unique probiotics. Rural fermented drinks could be utilized in the treatment of diarrhoea in HIV/AIDS patients and with the correct consumer awareness campaigns, might play a role in improving the quality of life for these patients.

Keywords: Probiotics; Rural Fermented Beverages; *Lactobacillus*; *Pediococcus*

Development Of A Unique Small Scale Gastrointestinal Model Simulating The Porcine Ileum And Testing The Efficacy Of The Model By Screening For Probiotic Properties Of *Lactobacillus salivarius* 241. M. S. Malema¹, C. Straker² and L. Maré¹; ¹Agricultural Research Council, Irene, South Africa; ²University of Witwatersrand, Johannesburg, South Africa.

The aim of the study was to develop a gastrointestinal (GI) model simulating the porcine ileum and to test the efficacy of the model by screening for probiotic properties of *Lactobacillus salivarius* 241. Bacteria selected as probiotics should be studied in an environment that resembles their natural environment. To date, *in vitro* models proved to be a popular tool in probiotic research. The ileum model developed in this study was based on a human intestinal model. In the first experiment, *L. salivarius* 241 was inoculated into the model containing fresh MRS broth. Sample collection was done hourly to determine the survival and growth of *L. salivarius* 241 by calculating the colony forming units (CFU). The second experiment introduced bile salts and low pH in the model. The same vessel was utilized to simulate the complete gastrointestinal tract. For the first period the vessel was set up as the stomach (pH 4) followed by the duodenum (bile and pancreatic juices were added) followed by a simulation period of the jejunum and ileum. The sample was inoculated into the stomach simulation and the survival and growth of *L. salivarius* 241 was determined through all the different simulations by CFU count. The CFU indicated survival of *L. salivarius* at pH 5.5–6.0 in the ileum and pancreatic juices and bile in the duodenum at pH 6.5–7.0. Results indicated survival of *L. salivarius* throughout the simulation of the porcine gastrointestinal tract. A unique

small scale porcine GI model simulating the complete gastrointestinal tract in one vessel was developed in this study. The model can be utilized to test potential probiotic bacteria before exposure to testing in animals.

Keywords: Gastrointestinal Model, probiotics

Phylogenetic and Functional Specificities of Crohn's Disease Microbiota. S. Mondot¹, J. Tap¹, J. P. Furet¹, P. Lepage¹, J. Doré¹, P. Marteau² and M. Leclerc¹; ¹INRA, France; ²AP-HP Hôpital Lariboisière, France.

Crohn's disease (CD) is an inflammatory bowel disease with a still unclear etiology. Several factors are involved in its development and maintenance, such as individual genetic background, environment and endogenous microbiota. The aim of this study was to highlight ecological and functional specificities of CD microbiota. First, we analyzed four CD/healthy 16S rRNA genes libraries (3,302 sequences) in order to point out commensal bacterial species which could be related to CD, healthy or common to both microbiota. Six molecular bacterial species phylogenetically close to *Escherichia coli*, *Oscillibacter valericigenes*, *Ruminococcus bromii*, *Faecalibacterium prausnitzii*, *Parabacteroides distasonis*, *Bacteroides vulgatus* were identified, and validated on 44 DNA samples as being statistically affiliated to CD (1/6) or healthy (4/6) microbiota, or common (1/6). This analysis confirmed that CD microbiota diversity was deeply disturbed, as indicated by a low number of common microorganisms between CD and CD/healthy microbiota. Conversely, healthy microbiota, with more bacterial species in common, appeared to be more conserved among individuals. Furthermore, using a metagenomic approach, we quantified the bacterial genomic content dissimilarities between one CD and four healthy sequence libraries. We found that genes classified in cell wall/membrane/envelope biogenesis and transcription pathways were differentially represented between CD and healthy samples. Analysis of cell wall/membrane/envelope biogenesis pathway indicated that COG0793 (periplasmic protease) and COG0668 (small-conductance mechanosensitive channel) were enriched in CD microbiota. Finally, the sequencing of cDNA libraries obtained from CD patients undergoing surgery will provide a validation of these *in silico* predictions, by identifying the overexpression of expected, or new, pathways. As a whole these results indicated that the disease modified the CD microbiota diversity, and reorganized its metabolism toward cell wall biogenesis. It also suggests that CD microbiota is highly dynamic and seems to be constantly adapting to environmental changes, which might include host- and (or) microbial-centric perturbations.

Keywords: Crohn's disease; microbiota; 16S rRNA genes; metagenomics

Microbial Community Analysis of Rectal Methanogens and Sulfate Reducing Bacteria in Two Non-Human Primate Species. N. Nakamura, S. Leigh, R. I. Mackie and H. R. Gaskins; University of Illinois at Urbana-Champaign

Methanogenesis by methanogenic Archaea and sulfate reduction by sulfate reducing bacteria (SRB) are the major hydrogenotrophic pathways detected in the human colon. A competitive relationship between these two groups of hydrogenotrophic microbes has been demonstrated in environmental ecosystems according to the availability of sulfate. However, it remains unclear whether a similar competitive relationship exists in the human colon or that of other primate species. It has been suggested that the methanogenic status of mammalian species is under control of evolutionary rather than dietary constraints. However, detailed information is lacking regarding the dynamics of hydrogenotrophic microbial communities among different primate species. The objective of this study was to examine the diversity and abundance of methanogens and SRB communities of two non-human primate species by using PCR-denaturing gradient gel electrophoresis (DGGE) and real-time quantitative PCR (qPCR). DNA was isolated from rectal swabs collected from 10 *Papio* baboons (*Papio hamadryas*) and 10 sooty mangabeys (*Cercocebus atys*) in captivity (4 – 11 years old). The animals were fed with monkey chow supplemented with fruits. Functional genes for methanogens (methyl coenzyme-M reductase; *mcr*) and SRB (dissimilatory (bi)sulfite reductase; *dsr*, dissimilatory adenosine-5'-phosphosulfate; *aps*) were selected as target molecules for the molecular techniques, as well as the 16S rRNA gene of Archaea and Desulfovibrionales-related SRB. The DGGE data indicated greater diversity and inter-individual uniformity of Archaea and SRB communities in mangabeys than in baboons. Principal component analysis of DGGE data revealed that rectal Archaea and SRB communities differ between baboons and mangabeys. The qPCR data indicated that the numbers of *mcrA* and *dsrA* genes in these primate samples ranged 10–1.3×10³ and 50–4.0×10² copies/10 ng DNA, respectively. Methanogens and SRB were detected for each animal of the two host species, and the two hydrogenotrophic microbial groups coexisted with no apparent dominance of either group in these primates.

Keywords: methanogens, sulfate reducing bacteria, non-human primates

Diversity of Nitroreductase Genes in Ovine Rumen Under a 2,4,6-Trinitrotoluene Supplemented and Concentrate Diets.

S. Perumbakkam, E.A. Mitchell, and A.M. Craig; Oregon State University, Oregon, USA

A NADPH dependent nitroreductase enzyme initiates bacterial metabolism of 2,4,6-Trinitrotoluene (TNT) in anaerobic conditions. Previous work has shown the sheep rumen microbes can metabolize TNT to its polar constituents. In this study, we evaluated the dietary effects on the diversity of the nitroreductase enzyme in the sheep rumen contents. Rumen samples were collected from sheep fed diets with TNT (TNT +, n=3), or without TNT (TNT-, n=1). Rumen samples were also collected from sheep that were fed a concentrate/forage diet (OSU, n=3). Genomic DNA was extracted by published methods and degenerate primer sets were developed using 17 reference nitroreductase protein sequences from the NCBI database. The CODEHOP program was used to develop four sets of degenerate primers (Groups 1, 2, 3, and 4) amplifying various nitroreductases. Using touchdown PCR, the nitroreductase genes were amplified, cloned, and sequenced. BLAST hits of the sequences were used to construct a phylogenetic tree, employing a Bayesian approach. The initial results from the PCR indicated that primers designed for the *E. coli nfsA* gene (Group 4) produced no amplification product from any of the rumen samples tested. Conversely, the Group 1-based primers successfully amplified genes from all three rumen samples. Of the 100 clones that were sequenced, more than 94 of them were most closely related to the nitroreductase gene from *Staphylococcus saprophyticus* ATCC 15305. The remaining 6 sequences formed very loose associations to the other reference genes. The Group-2 based primers amplified nitroreductase genes from both TNT + and TNT- sheep, but there was no amplification using DNA recovered from OSU Sheep. The BLAST results indicate that these Group-2 produced sequences are closely related to dihydrodipicolinate reductases. The nitroreductase sequences amplified with Group 3-based primers were most similar to the reference sequences from *Streptococcus pyogenes* MGAS 8232 and *Clostridium perfringens* str. 13. The phylogenetic analysis showed 95% of the sequenced clones were most similar to *Streptococcus pyogenes* and 4% were assigned to a new clade. Finally, DOTUR analysis was performed to enumerate the collector's curves. Diet appears to have some influence on the diversity of nitroreductases in ovine rumen microbiomes.

Keywords: Nitroreductase; rumen; community; Bayesian; phylogenetics

Characterization and Development of a Systems-level Understanding of Microbial Metabolism in the Foregut Microbiome of the Tammar wallaby (*Macropus eugenii*). P.

Pope¹, S. Denman¹, S. Tringe², L. Hinds³, P. Hugenholtz², C. McSweeney¹, and M. Morrison^{1,4}. ¹CSIRO Australia, Queensland, Australia; ²United States Department of Energy, Joint Genome Institute, Walnut Creek CA, USA. ³CSIRO Australia, Canberra, Australia; ⁴The Ohio State University, Columbus, OH, USA

The microbial biology underpinning lignocellulose hydrolysis and anaerobic fermentation within the foregut of Australian marsupials is gaining greater attention, in part because methane production by this microbial consortium is widely considered to be less than what occurs in cattle and sheep. We have utilized metagenomic approaches to characterize the foregut microbiome of a member of the Kangaroo family considered to be Australia's "model" marsupial: the Tammar wallaby (*Macropus eugenii*). Total community DNA from pooled foregut contents was purified, cloned and sequenced. A total of 54 million base pairs (Mb) of Sanger sequence data was generated and assembled. An analysis of 663 bacterial 16S rRNA gene sequences amplified from the community DNA revealed a broad diversity of bacteria representing 6 phyla and 293 phylotypes (99% sequence identity threshold) consisting of numerous novel bacterial clades that appear to be unique to the host. The partial genome extraction of 2 dominating chemoheterotrophs and the predictive reconstruction of their metabolism has led to new insights and hypothesis into the host animal's nutrition and other important symbiotic functions such as carbohydrate metabolism, energy production and conversion, and nitrogen metabolism. Further community analysis of the resident microbiome also suggests the presence of a large, diverse set of bacterial genes for cellulose, xylan and pectin hydrolysis. The present study provides a much needed initial understanding of microbial metabolism in the foregut microbiome of the Tammar wallaby, and provides a platform from which future investigations in pursuit of methane mitigation and improved lignocellulose hydrolysis can be developed.

Keywords: Microbiome, Metagenomics, Foregut, Kangaroo

Diversity of Lactic Acid Bacteria in the Equine Stomach and Small Intestine. A. H. Smith and T.G. Rehberger. Agtech Products, Wisconsin, USA.

There is very little information in the literature on the normal microbiota of the equine gastrointestinal tract. The information that is available is often from a limited number of animals and frequently fecal bacteria are studied as being representative of the microbiota of the intestinal tract. A study analyzing the molecular diversity of microbiota in the horse intestine indicated that 89% of the 16S ribosomal

gene sequences were not present in the 2001 public databases indicating that novel bacterial species may be found in the equine intestine. As lactic acid bacteria are thought to play a role in intestinal homeostasis in many animals and known to colonize the epithelium of the non-glandular stomach area of the horse this study was performed to determine the diversity of culturable lactic acid bacteria isolated from the non-glandular stomach and small intestine of horses. Sections of the non-glandular region of the stomach and small intestine were collected from ten horses at a slaughter facility. Tissue sections were washed with sterile peptone to remove stomach and luminal contents. Tissue sections were then placed in bags with sterile peptone and stomached to release adherent bacteria. The bacterial cells were collected by centrifugation, diluted and plated on de Man, Rogosa, Sharpe (MRS) plus 0.4% cysteine agar plates. Plates were incubated at 37°C under anaerobic conditions for 48 hours after which well-isolated colonies were transferred to MRS plus 0.4% cysteine broth. Random amplified polymorphic DNA (RAPD) analysis was used to compare genomic diversity of the 818 isolated strains. A total of 87 isolates representing the diversity from each horse, were selected for sequencing of the 16S gene. EzTaxon: a web-based tool for the classification of bacteria based on 16S ribosomal RNA gene sequences was used to identify the isolates. The majority of the isolates belonged to the genus *Lactobacillus* (34) and the other genera represented were *Enterococcus* (18), *Streptococcus* (14), *Pediococcus* (13), *Lactococcus* (4) and *Weissella* (4). Using 98% similarity of the 16S gene for the identification of species all, but three, of the isolates grouped with one or more type strains. The remaining three were most similar to *Streptococcus gallolyticus* subsp. *macedonius*.

Keywords: Equine, lactic acid bacteria, non-glandular stomach, small intestine, RAPD

Group Specific qRT-PCR Analysis of Methanogenic Archaea in Stored Swine Manure. **C. Spence**, T.R. Whitehead and M.A. Cotta National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL.

Consolidated storage of swine manure is associated with the production of a variety of odors and emissions which result from anaerobic digestion of materials present in the manure. Methanogenic archaea are a diverse group of anaerobic microorganisms responsible for the production of methane. In the United States, methane emissions from lagoons and manure storage pits are estimated to be over 40 Tg/year, accounting for approximately 30% of the total methane emissions from agriculture. Little is known about the populations or concentrations of methanogens in stored swine manure and surprisingly few methanogens have been

isolated from this environment. The final reaction of methanogenesis involves reduction of the methyl group bound to coenzyme-M, and results in the release of methane gas. In all methanogens this step is ubiquitously catalyzed by methyl coenzyme-M reductase (MCR). The *mcrA* gene encodes the alpha subunit of the MCR holoenzyme. The subunits of MCR are phylogenetically conserved and *mcrA* can be used as a phylogenetic marker for methanogen populations. In this study, total DNA was isolated from stored swine manure from local swine facilities and used as a template for direct PCR. Clone libraries were created using degenerate primer sets that amplified a 464–491 bp variable region of the *mcrA* gene. Comparative phylogenetic analysis identified three major clusters of methanogenic archaea in stored swine manure representative of *Methanomicrobiales*, *Methanobacteriales* and *Methanosarcinales* orders. Many cloned *mcrA* sequences showed little similarity to any known methanogens suggesting these sequences represent novel, as yet unidentified methanogens. qRT-PCR primer sets were developed to specifically target approximately 150 bp of the *mcrA* gene of the major groups of methanogenic archaea in stored swine manure. Standard curves were generated from different concentrations of cloned target *mcrA* genes representative of each group, and the linear range of detection was at least six orders of magnitude from 1×10^2 to 1×10^8 copies per PCR. This newly developed qRT-PCR assay will provide a sensitive and specific new method for detection and enumeration of methanogenic archaea in environmental samples.

Keywords: Methanogens; swine manure; *mcrA*; waste treatment;

Impact Of Different Prebiotics On In-Vitro Fermentation And Microbial Ecology Of Infant Stool Samples. **J. Stiverson**¹, L. Wang¹, T. Williams^{1,2}, C. Shaw¹, D. Husted², P. Price², J. Guerrieri³, J. Deacon³, M. Morrison¹, and Z. Yu¹. ¹The Ohio State University, Columbus, OH, USA; ²Abbott Nutrition, Columbus, OH, USA; ³Institute of Clinical Research, Mayfield Heights, OH, USA.

The microbiota and its metabolism in the infant intestinal tract are determined to a large extent by the mode of post-natal feeding. Breast feeding has been shown to foster a microbiota abundant with bifidobacteria and other beneficial bacteria that is believed to have long-term benefits to infant health and development. Oligosaccharides present in human milk are believed to be the major components that stimulate the growth of bifidobacteria. A number of oligosaccharides have been examined *in vivo* for their bifidogenic effect when added to infant formula, but conclusions drawn are sometimes inconsistent with respect

to the extent of the bifidogenic effects. In this study we attempted to systematically compare the bifidogenic and prebiotic effects of common oligosaccharides, or combinations thereof, using fresh stool samples cultured *in-vitro*. The prebiotics selected and doses tested were galacto-oligosaccharides (GOS) at 7.2 g/L, inulin (IN) at 0.8 g/L, GOS at 7.2 g/L in combination with IN at 0.8 g/L, and oligofructose enriched with inulin (OF-IN) at 4 g/L. A basal medium containing each of the above prebiotics was inoculated with fresh stool sample of either breast-fed or formula-fed infants and incubated at 37°C. The growth of bifidobacteria was determined during the *in-vitro* incubation using selective agar plates and a specific real-time PCR assay. The total bacteria, *Bacteroides*, *Bifidobacterium longum*, and *E. coli* in the cultures were also quantified using respective real-time PCR assays. Culture broth was analyzed for short chain fatty acid (SCFA) profiles and pH. No significant differences were observed between GOS and the GOS-IN blend in their effect on the growth of any of the bacterial groups analyzed, SCFA production, or culture pH at either the 12 or 24 hours time point. Relative to the negative controls that received no prebiotics, IN alone at 0.8 g/L concentration did not significantly affect the measured bacterial groups or SCFA. However, the OF-IN at 4 g/L did stimulate the growth of bifidobacteria and the production of SCFA, although to a lesser extent than GOS. The IN at 0.8 g/L may be too small a dose to have any significant bifidogenic effect. These results may be useful for the future formulation of infant formulas.

Keywords: GOS, inulin, oligofructose, prebiotics, infants.

The Differential Impact of Lactose/ Lactase Phenotype on Colonic Microflora. A. Szilagy¹, I. Shrier¹, D. Heilpern¹, J.-S. Je², S. Park², G. Chong¹, C. Lalonde¹, L.-F. Cote¹, B. Lee². Sir M.B. Davis; ¹Jewish General Hospital and ²Macdonald Campus, McGill University, QC, Canada

We hypothesize that prebiotic effects of lactose may be more prominent in maldigesters (LNP) than digesters (LP) and this may alter risk of some diseases through different effects in the two populations. There is currently little or no data to evaluate this hypothesis, so the effect of a fixed dose of lactose on fecal *bifidobacteria* and *lactobacilli* in healthy LNP and LP individuals was measured. A prospective unblinded, 4 week interventional study was carried out in 23 LNP and 18 LP subjects classified by genetic testing (RT-PCR, Roche Diagnostics). After 2-weeks of dairy food withdrawal (maintained throughout the 4 weeks), subjects ingested a 25 g aqueous lactose solution twice a day for 2 weeks (the intervention). Using selective culture media we measured *bifidobacteria* and *lactobacilli* numbers in pre- and post-intervention periods as the primary outcome.

For secondary outcomes, we measured (pre and post intervention) stool samples for total anaerobes, *Enterobacteriaceae*, β -galactosidase (β -gal), N-acetylglucosaminidase (NAG) (using ONPG-labeled substrates), breath hydrogen, and symptoms following acute 50 g lactose challenge tests after overnight fast. The LNP group had a statistically significant change in *bifidobacteria* compared with LP (0.8 log₁₀ cfu/g stool versus 0.11, $p=0.04$). In LNP group 35% had ≥ 1 log cfu change and overall, 61% of the subjects had ≥ 0.5 log cfu change, compared with 11% ($p=0.14$) and 22% ($p=0.02$) in the LP subjects, respectively. The mean log cfu change for *lactobacilli* was 0.5 in LNP versus 0.02 in LP ($p=0.2$). Colonic adaptation in the LNP group was partial (total breath hydrogen difference between test 3-test 2, $p=0.13$). Changes in β -gal and NAG pre and post intervention (log Units, LNP versus LP, 21 and -22, $p=0.02$ and 6 and -17, $p=0.04$ respectively). Reduction in total hydrogen between test 3 and test 2 correlated with increase in *lactobacilli* ($r^2=0.3$, slope -74, $p=0.007$). These results suggest that lactose exerts a differential impact on the colonic microbiota, depending on lactase genetic status. This may be important for defining certain disease risks.

Keywords: Prebiotic; lactose; digestion phenotype; colonic bacteria

Towards The Healthy Human Intestinal Microbiota Phylogenetic Core? J. Tap¹, S. Mondot¹, F. Levenez¹, E. Pelletier², C. Caron³, J.-P. Furet¹, E. Ugarte², R. Muñoz-Tamayo^{1,4,6}, D. Le Paslier², R. Nalin⁵, J. Dore¹ and M. Leclerc¹; ¹INRA, UEPDS, UR910, France, ²CEA-Genoscope, France, ³INRA, MIG, UR1077, France, ⁴INRA, MIA, UR341, France, ⁵Libragen, France, ⁶L2S UMR 8506, University Paris-Sud, CNRS-Supelec, France.

The human fecal microbiota has been described as host specific, but is also characterized by global functionalities conserved between healthy humans. This apparent paradox led us to explore the existence of a phylogenetic core, a set of bacterial molecular species that would be altogether dominant and prevalent within the fecal microbiota of healthy humans. A total of 10,456 non chimeric bacterial 16S rRNA sequences were obtained after cloning of PCR amplified rRNA genes from 17 human fecal DNA samples. Using alignment or tetranucleotide frequency based methods, 3,180 Operational Taxonomic Units were detected. As expected, the 16S rRNA sequences mainly belonged to the phyla Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia. Interestingly, while most of OTUs appeared individual-specific, 2% were present in more than half of the samples and accounted for 35.8% of the total sequences. These 66 dominant and prevalent OTUs included members of the genera *Faecalibacterium*, *Rumi-*

nococcus, *Eubacterium*, *Dorea*, *Bacteroides*, *Allistipes* and *Bifidobacterium*. Furthermore, 24 OTUs had cultured type strains representatives which should be subjected to genome sequence with a high degree of priority. Strikingly, 52 of these 66 OTUs were detected in at least 3 out of 4 recently published human fecal microbiota datasets, obtained with very different experimental procedures. A statistical model confirmed these OTUs prevalence. Despite the important species richness and a high individual specificity, a limited number of OTUs are shared among individuals and might represent the phylogenetic core of the human intestinal microbiota. Its role in human health appears critical to investigate. Using qPCR and microarray techniques, the modulation of the structural dynamic of this phylogenetic core is now being tested through processing of 170 samples from a randomized, cross-over double-blind clinical study; where individuals received 10 g and 40 g of dietary fibers per day.

Keywords: Human GIT; microbiota; 16S rRNA; microbial diversity; phylogenetic core.

Characterization of Human-Derived Probiotic *Lactobacillus* spp. with Anti-Pathogenic and Anti-Inflammatory Activities. M. Taweechoitpatr^{1,2}, C. Iyer^{3,4}, J. K. Spinler^{3,4}, J. Versalovic^{3,4} and S. Tumwasorn¹; ¹Chulalongkorn University, Thailand; ²Srinakharinwirot University, Thailand; ³Baylor College of Medicine, Texas, USA; ⁴Texas Children's Hospital, Texas, USA.

Lactobacillus species have beneficial effects as probiotics. In this study, the bacteria were isolated from feces of healthy human volunteers to investigate two main probiotic properties: inhibition of gastrointestinal pathogens and modulation of tumor necrosis factor (TNF) production. Almost 500 *Lactobacillus* isolates were tested for antagonistic activity against ten gastrointestinal pathogens. The results demonstrated that 4 isolates displayed weak inhibitory activities against *Vibrio cholerae* non O1 by agar well diffusion assay. Forty-six isolates were randomly selected and investigated for the modulation of TNF production in THP-1 monocytic cells activated with lipopolysaccharide (LPS). The results revealed that 12 isolates significantly inhibited TNF production in varying magnitude. *Lactobacillus* strain TH58 displayed the most potent TNF inhibitory activity (70–80%). However, this strain had no effect on nuclear factor kappa B (NF- κ B) activation. On the basis of phenotypic and genotypic characteristic including API 50 CHL, 16S rRNA gene sequencing, pyrosequencing and rep-PCR genotyping, the 4 anti-pathogenic strains were identified as *Lactobacillus plantarum*. Of the 12 TNF inhibitory strains, 10 were identified as *Lactobacillus plantarum*, one as *Lactobacillus salivarius* and the TH58

strain as *Lactobacillus saerimneri*. *Lactobacillus saerimneri*, *Lactobacillus plantarum* and *Lactobacillus salivarius* displayed 60–78% similarity to each other by rep-PCR. It is interesting to note that the *Lactobacillus* TH58 strain with the most potent TNF inhibitory activity was identified as *Lactobacillus saerimneri*, which has never been reported as the isolate from human origin and exhibited TNF inhibitory activity.

Keywords: *Lactobacillus*, probiotics, tumor necrosis factor, antagonistic activity.

Effects of Gluconic Acid and Methionine Hydroxy Analog on Growth and Intestinal Characteristics of Rats. H. Uchihori, S. Koike and Y. Kobayashi; Hokkaido University, Hokkaido, Japan

Although gluconic acid (GA) is known to stimulate butyrate production in the intestine of monogastric animals, its effect on intestinal bacteria and tissue morphology remains uncharacterized. In the meantime, liquid methionine hydroxy analog (LM) is becoming attractive as an alternative to methionine (M) that is one of the limiting amino acids. In the present study, we investigated the effect of GA and LM on growth performance and intestinal characteristics of rats. Twenty-four male rats individually housed in cages were divided into four groups. Each group was assigned to diets supplemented with either M, LM, M plus GA (MGA), or LM plus GA (LMGA). After 4 weeks all the rats were sacrificed to take intestinal samples. Numbers of total bacteria, lactobacilli and bifidobacteria were monitored by real-time PCR assays. Analyses by denaturing gradient gel electrophoresis (DGGE) and *rrs* gene clone libraries were carried out to detect any changes in bacterial community structure. Profiles of intestinal short chain fatty acids (SCFA) and mucosal tissue were also measured. Effects of GA, LM and their interactions were not detected on growth performance such as body weight gain, feed intake, or feed conversion. The GA treatment tended to increase the number of bifidobacteria ($P < 0.10$) in cecal contents, though it did not affect total bacteria and lactobacilli. Relative proportions of bifidobacteria were significantly increased by GA ($P < 0.05$). Although GA did not influence total SCFA levels it did increase butyrate levels ($P < 0.05$). The length of villi was significantly increased in the small intestine, cecum and colon of rats fed GA ($P < 0.05$). The DGGE banding profiles suggested the cecal microbiome is changed by the consumption of GA. Enhanced growth of butyrate-producing bacteria by GA was indicated by the clone library analysis. Of 180 clones, 9 clones closely related to genus *Roseburia* were detected in MGA, while only one of 173 clones in M had a high similarity with *Roseburia*. LM did not show any significant effect on intestinal metabolites,

bacteria and mucosal morphology. Judging from these results, GA can be a prebiotic that improves the intestinal environment of monogastric animals.

Keywords: gluconic acid; liquid methionine; intestinal environment; butyrate

The Effect of an Active Dry Yeast on the Digestibility of Different Forages and Feed Components During *in vitro* Incubations with Mixed Rumen Contents. N. D. Walker¹, M. E. Quentino Cintora¹, J. Bax¹, F. Chaucheyras-Durand² and H. Durand³; ¹Lallemand Animal Nutrition, Montreal, Canada; ²Lallemand Animal Nutrition, Clermont Ferrand, France; ³Lallemand Animal Nutrition, Toulouse, France.

The inclusion of Active Dry Yeasts (ADYs) in ruminant diets has been shown to have significant effects upon ruminal fermentation, host productivity and health. One of the positive results that may be observed is improved fibre digestion due to increased growth and metabolic activity of the fibre degrading community. This may occur due to several different reasons: improved ruminal pH stabilization; improved anaerobiosis; as well as supply of stimulatory nutrients and (or) co-factors by the yeast. Together, all these factors combine to promote the fibrolytic population and increase fibre digestion. The aim of the current work was to determine the effect of including an ADY CNCM I-1077 on the *in vitro* true digestibility (IVTD) of different forages by mixed rumen contents in the DaisyII incubator system. Rumen fluid was removed from 3 lactating dairy cows, pooled and strained (SRF). The SRF was mixed with anaerobic buffer (1:4) and added to the incubation jars under CO₂. To each jar, a pre-weighed filter bag containing a known amount of substrate was added. To the treatment jars, ADY, CNCM-1077 was added to give the normal dose rate for dairy cows and the jars were incubated at 39°C in the DaisyII system. Triplicate bags were removed at different time points under CO₂, washed with detergent to remove any attached microbes and dried. The filter bags were then weighed and the resulting IVTD calculated. A variety of different substrates with differing Neutral Detergent Fibre (NDF) content were tested. Each substrate was tested on 3 different days and the mean rate of digestion calculated. In most instances, particularly for ensiled forages, the IVTD was significantly increased in the presence of the ADY. Digestibility of highly lignified substrates, e.g. straw was also significantly increased upon ADY addition. For some substrates the end point of digestion was the same, however, the addition of the ADY increased the initial rate of digestion. Interestingly, the digestion of rapidly digestible carbohydrate sources eg ground corn was slowed upon the addition of the ADY. To conclude, ADYs can significantly improve the digestibility

of different forages. The digestibility of ensiled forages, e.g. corn silage was particularly enhanced, indicating that the ADY had a positive stimulatory effect upon the fibrolytic population and overall fibre digestion.

Keywords: Fibre digestion; Active dry yeast; rumen

The Effect of Diet and the Inclusion of Active Dry Yeast on the Population Size and Dynamics of Key Ruminal Bacteria, as Determined by Classical and Molecular Techniques. N. D. Walker¹, M.E. Quentino Cintora¹, A. Ameilbonne², F. Chaucheyras-Durand² and H. Durand³; ¹Lallemand Animal Nutrition, Montreal, Canada; ²Lallemand Animal Nutrition, Clermont Ferrand, France; ³Lallemand Animal Nutrition, Toulouse, France.

Significant effects upon the health and productivity of ruminants have been seen upon the addition of Active Dry Yeasts (ADYs) to the diet, due to positive interactions with key members of the rumen microbial ecosystem. Previous studies have shown ADYs increased populations of cellulolytic and lactate-utilizing bacteria. Unfortunately, although differences in the total number of these organisms were determined, individual sub-populations could not be differentiated using classical cultivation techniques. The aim of the current work was to quantify key ruminal bacterial populations by both classical and molecular techniques and to evaluate the effect of including an ADY, CNCM I-1077 on these populations in animals fed a diet increasing in concentrate. Cannulated cows (4) were split into control or ADY groups and initially fed 100% hay for 20 days then switched to increasing concentrate with a step up of 10% concentrate every 2 days. Rumen samples were removed on different days during the trial and selective growth media were used to enumerate the populations of the total, cellulolytic, lactolytic bacteria and *S. bovis* present. In order to determine the effect on sub-populations, DNA was extracted from the samples and total bacteria, *S. bovis*, *F. succinogenes*, *R. albus*, *R. flavefaciens*, *M. elsdenii*, *S. ruminantium* populations were enumerated by qPCR. Changes in the microbial profile were monitored using TTGE. Classical cultivation techniques showed that increasing concentrate increased the *S. bovis* present, decreased the cellulolytics and initially increased the amount of lactolytics, however these then declined. Ruminal lactate concentration also increased. The ADY reduced these adverse events. *S. bovis* was decreased, lactate-utilisers increased and the ADY helped to protect and stabilize the cellulolytic population. Similar results were seen with qPCR except that it was possible to differentiate which sub-populations had been affected. *S. ruminantium* was the main lactolytic organism present and was stimulated by the ADY, as were *F. succinogenes* and *R.*

flavefaciens. Dietary changes caused changes in the microbial profiles. Thus, an ADY can have a positive effect on key ruminal bacteria. Molecular techniques may be used as a way of monitoring and quantifying these changes without the need for cultivation.

Keywords: Active Dry Yeast, qPCR, TTGE, rumen

Environmental and Maternal Influence on Colonization of Infant Gastrointestinal Tract by Microorganisms. L. Wang¹, J. Stiverson¹, T. Williams^{1,2}, C. Shaw¹, D. Husted², P. Price², J. Guerrieri³, J. Deacon³, M. Morrison¹, and Z. Yu¹. ¹The Ohio State University, Columbus, OH, USA; ²Abbott Nutrition, Columbus, OH, USA; ³Institute of Clinical Research, Mayfield Heights, OH, USA.

The gastrointestinal tract of infants is sterile at birth, but is colonized at birth by bacteria from the mother and then by bacteria from the environment. It is also generally accepted that human milk promotes an intestinal microbiota predominant with bifidobacteria, which is believed to have long-term benefits to infant health and development, while formula-fed infants typically do not have such an “ideal” intestinal microbiota. It is desirable to help formula-fed young infants to establish an intestinal microbiota similar to that present in breast-fed infants using functional foods such as prebiotics and or probiotics. However, a host of factors can significantly affect the colonization of the intestinal tract, especially during early infancy when the intestinal microbiota undergoes rapid successions. Better understanding of these factors and their effects can help guide efforts towards improving infant formula. In this study we attempted to assess the impact of maternal gut microbiota on that of the infants. This study involved two groups of subjects: one group consisting of mothers and their exclusively breast-fed infants (n=22), and the other consisting of mothers and their exclusively formula-fed infants (n=20). Fresh fecal samples were obtained from the mothers within 5 days after the infant’s birth and the infants between 15 and 28 days of age. Microbial community DNA was then extracted from each of the samples, and specific denaturing gradient gel electrophoresis (DGGE) was carried out to analyze *Bifidobacterium*, *Lactobacillus*, and *Bacteroides*. Bacteria represented by intense DGGE bands were identified by DNA sequencing. *Bifidobacterium* appears to be diverse in both feeding groups, corroborating previous finding that *Bifidobacterium* is highly variable in both abundance and diversity among different individuals. As judged by the DGGE profiles, maternal microbiota seems to have some impact on the infants’ microbiota, and certain mothers had more impact on the gut microbiota of their infant than other mothers. There is also evidence for maternal and feeding impact on colonization of lactobacilli.

Keywords: intestinal microbiota, maternal impact, bifidobacteria, lactobacilli, DGGE.

Towards a Complete View of the Bacterial Diversity in Poultry Intestinal Microbiota. S. Wei¹, M. Cressman¹, M. Lilburn¹, M. Morrison^{1,2}, and Z. Yu¹. ¹The Ohio State University, Ohio, USA; ²CSIRO Australia, Queensland, Australia.

The intestinal microbiota in poultry is known to be complex, but the scope of the diversity and species richness remains undefined. The objective of this study was to examine the intestinal bacterial diversity in chickens and turkeys by a combination of metadata analysis and next generation sequencing technology. The current coverage of gut bacterial diversity in poultry was first assessed by analyzing the 16S rRNA gene (*rrs*) sequences available in the Ribosomal Database Project (Release 10). Rarefaction analysis of the 886 and 1,610 bacterial *rrs* sequences recovered from the intestinal tracts of turkeys and chickens, respectively, suggested that only ~60% of the bacterial diversity in the gut of these birds has been revealed. Microbial community DNA was extracted from both cecal mucosal and digesta samples collected from flocks of both broiler chickens and turkey poults. The V3 region of the *rrs* genes present in these different samples was amplified by PCR using universal primers, tagged, and then subjected to pyrosequencing using a Genome Sequencer FLX (GS-FLX) unit. Preliminary analysis of the sequence data suggested the coverage was greater than 96% at the species level (defined by 97% sequence identity). A library comparison also identified predominance by different bacteria among the four habitats (mucosa vs digesta, chicken vs turkey). Although more than 88% of the sequences belong to *Firmicutes*, *Proteobacteria* or *Bacteroidetes* in each of the habitats, the numbers of OTUs shared by any two of the habitats were relatively small. For instance, only 25.7% and 43.5% of the OTUs found in the mucosal and the digesta fractions from turkeys, respectively, are represented in similar locations within chickens. Reciprocally, only 2.8% and 25.2% of the OTUs found in the mucosal and digesta fractions from chickens, respectively, can be found in similar locations within turkeys. These results revealed the differences in microbial diversity between these two species of birds, as well as between the mucosa and digesta fractions. The pyrosequencing data may also be used to design a high density microarray for comparative nutritional studies in poultry.

Keywords: poultry; intestinal microbiota; mucosa; 16S rRNA; PCR; pyrosequencing

Prevalence and Level of *Escherichia coli* O157:H7 in Feces and on Hides of Feedlot Steers Fed Diets With or Without

Wet Distillers Grains With Solubles. **J.E. Wells**, S.D. Shackelford, E.D. Berry, N. Kalchayanand, M.N. Guerini, V.H. Varel, T.M. Arthur, J.M. Bosilevac, H.C. Freetly, T.L. Wheeler, C.L. Ferrell, and M. Koochmaria. USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933–0166, USA.

Studies have indicated distillers grains in cattle diets may alter the shedding of *Escherichia coli* O157:H7 in feces thereby increasing the *E. coli* O157:H7 load on the hide and ultimately on the carcass. To determine if wet distillers grains with solubles (WDGS) in the diet affected *E. coli* O157:H7 in growing and finishing cattle, weaned steers (n=603) were blocked by genetic lines and weaning weight, and randomly assigned to dietary treatments (with and without WDGS). At approximately 53 days after weaning, steers were sorted into 8 pens (4 pens per treatment) with 75 to 77 steers per pen. Growing diets with 0% or 13.9% WDGS (as %DM) were fed on d 0 and finishing diets with 0% or 40% WDGS were fed on d 78. Hide and fecal samples were collected from each animal for enumeration and enrichment of *E. coli* O157:H7 from October through June. In the growing phase, the fecal prevalence for *E. coli* O157:H7 was twice that in animals fed diets with 13.9% WDGS ($P<0.001$), but neither the percentage nor the distribution of *E. coli* O157:H7 enumerable samples in feces was different for the two diets. In the finishing phase, average fecal prevalence for cattle fed 40% WDGS was 14.9% compared to 1.5% for animals fed corn ($P<0.001$), and hide prevalence was 32.8 and 9.2% ($P<0.001$), respectively. The average percentage of fecal *E. coli* O157:H7 enumerable samples during the finishing phase for cattle fed 40% WDGS was 2.7% compared to 0.1% for corn ($P<0.001$). The average percentage of *E. coli* O157:H7 enumerable hide samples was not different (1.9 vs 0.0%, $P<0.06$), but the distributions of these counts were different ($P<0.05$). Animals fed 40% WDGS had higher levels of generic *E. coli* ($P<0.001$), higher pH ($P<0.001$), and lower concentrations of L-lactate ($P<0.001$) in their feces. Within cattle fed 40% WDGS, fecal shedding of *E. coli* O157:H7 was not associated with fecal pH. However, fecal shedding of *E. coli* O157:H7 from the cattle fed 40% WDGS was associated with lower levels of fecal generic *E. coli* (6.94 vs 7.06 \log_{10} CFU per g, $P<0.05$) and higher fecal concentrations of L-lactate (1.87 vs 1.39 mM, $P<0.01$). These results indicate that feeding 40% WDGS (DM basis) may increase the level and prevalence of *E. coli* O157:H7 in feedlot cattle, although the magnitude of the difference in this study may have been affected by low prevalence in the control pens.

Salmonella Diversity and Transmission Dynamics in Galapagos Iguanas. **E. Wheeler**, I. K. O. Cann and R. I.

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Patterns of *Salmonella* carriage in wild and captive reptiles suggest that host ecology and biogeography are both important for determining strain transmission among populations. In this study, we explore the relative importance of ecological similarity versus spatial proximity of hosts on the transmission of *Salmonella* among populations of free-living Galapagos iguanas. We isolated *Salmonella* from marine iguanas (*Amblyrynchus cristatus*) on two islands (South Plazas and Santa Fe), from land iguanas (*Conolophus subcristatus*) on South Plazas and from land iguanas (*Conolophus pallidus*) on Santa Fe. Genomic similarity among isolates was evaluated by cluster analysis of repetitive extragenic palindromic PCR (rep-PCR) fingerprinting. Genetic similarity of a subsample of these isolates was also evaluated using multi-locus sequence typing (MLST) of four coding loci (two housekeeping and two virulence genes). The Rep-PCR fingerprinting revealed high strain diversity within and among the four iguana populations, and clustering of isolates by iguana population of origin. However, these patterns were not evident in the sequence analysis, suggesting either high migration rates among the populations or strong stabilizing selection or convergence in these gene sequences. Our findings suggest that both ecological and geographical barriers may contribute to *Salmonella* population dynamics in the Galapagos. In addition, our findings highlight how disparate time scales of evolutionary effects on different genomic elements can result in conflicting models when using various molecular approaches to explore bacterial transmission patterns.

Keywords: *Salmonella* bacteria, transmission dynamics, microbial biogeography, Rep-PCR, MLST

Safety And Tolerance Of Infant Formulas Containing Prebiotics In Healthy, Term Infants. **T. Williams**, Y. Choe, P. Price, G. Katz, J. Boff, F. Suarez, and A. Mackey. Abbott Nutrition, Columbus, OH, USA.

Gastrointestinal (GI) microbiomes impact the maturation of intestinal and systemic immunity, and therefore, human health. Human milk (HM) is recognized as promoting the colonization and persistence of bifidobacteria and lactobacilli in the infant GI tract. The types and amounts of HM oligosaccharides are contributing factors that have a prebiotic effect. To make infant formula (IF) composition more like HM, non-digestible oligosaccharides could be added. This randomized, double-blinded, clinical study assessed the safety and GI tolerance of IFs containing galacto-oligosaccharides (GOS) compared to infants fed an IF without GOS or HM.

Full-term healthy infants ($n=180$) were enrolled by 8 days of age and were to receive their assigned feeding until 119 days of age. Study visits were conducted at enrollment, 14, 35, 56, 84, and 119 days of age. The control formula (CF) was Similac® Advance®. The experimental formulas were Similac Advance-based IFs with 8.0 g/L of GOS (EF8) or 4.0 g/L of GOS (EF4). Statistical significance was declared at $p<0.05$. The groups were similar at enrollment in age, gender and birth weights. In general, there were no significant differences between the groups in the relative frequencies of subjects with adverse events (AEs), specific or within a system organ class, or growth except nasopharyngitis (HM>CF) and length gain for males during 14 to 35 days of age (CF>EF4). Premature discontinuations of study product due to AEs were not significantly different between the IF groups. The total number of serious AEs was CF=1, EF8=6, EF4=1, HM=3; with EF8 significantly greater than CF and EF4. The CF group had the highest observed Mean Rank Stool Consistency (MRSC) (1=Watery, 5=Hard) at all study periods and the difference was significant between CF vs. EF8 and CF vs. HM for all study periods and CF vs. EF4 during 15–35 days of age. The MRSC for EF4 was significantly greater than HM only during enrollment-14 days of age. There were no significant differences in average stool frequencies between the formula groups during any study period. Similac Advance IF with added GOS at 4.0 g/L is safe and well tolerated by healthy term infants when fed from the first week of life through 4 months of age. The addition of GOS to Similac Advance infant formula results in softer stools more like those of HM-fed infants.

Keywords: Prebiotics, fecal mass, infants, colonic function

Diversity of Fiber-Adherent Bacteria on Three Potential Bioenergy Feedstocks During Digestion in the Cow Rumen. A.C. Yannarell¹, M. Hess², P. Hugenholtz², and R.I. Mackie¹; ¹University of Illinois at Urbana-Champaign, Illinois, USA; ²DOE Joint Genome Institute, California, USA

The large-scale use of grains for bioenergy (ethanol) production is undesirable because it detracts from human and animal food supplies. Efficient fermentation of plant cell walls will facilitate the shift to “second generation,” cellulose-based bioenergy feedstocks, such as perennial grasses. Rumen microorganisms, which constitute a highly efficient system for cellulose fermentation, may point the way to economically feasible bioethanol production. The goal of this work is to characterize the diversity of fiber-adherent rumen bacteria on three potential bioenergy feedstocks: corn stover, switchgrass (*Panicum virgatum*), and *Miscanthus X giganteus*. These feedstocks were incubated in live cows for 72 h, followed by DNA extraction (fibrous material and rumen fluid) and microbial

community characterization by bacterial ARISA (automated ribosomal intergenic spacer analysis) and T-RFLP (terminal restriction fragment length polymorphism). In addition, subsamples of switchgrass were removed periodically over the course of a 72-h live incubation to investigate bacterial colonization dynamics. Fiber-adherent microbial biomass (indicated by total extractable DNA) increased rapidly over the first hour of incubation, and then slowly doubled over the course of the following 71 h. Final microbial biomass varied between the feedstocks and between cows. The richness of bacterial phylotypes, assessed with ARISA, also varied by feedstock and cow, but the fiber-adherent communities were consistently more diverse than that of rumen fluid. The colonization of switchgrass fibers occurred in several stages. Fiber-associated bacteria increased from an initial 9 phylotypes to over 150 phylotypes after 2 h, but then richness and evenness dropped dramatically, fluctuating between 30 and 90 phylotypes for the duration. Both T-RFLP and ARISA indicated that fiber-associated communities were distinct from those of rumen fluid. Within each cow, different feedstocks accumulated different communities, but these differences tended to be smaller than differences observed between cows. Thus, the colonization of plant fibers in the rumen is a dynamic process, and the composition of the fiber-associated microbiota depends both on the feedstock and on factors specific to each individual cow rumen.

Keywords: fermentation; bioenergy feedstock; colonization; rumen

The Multi-Replicon Genome of *Butyrivibrio proteoclasticus* B316^T. C.J. Yeoman^{1,2}, W.J. Kelly¹, J. Rakonjac², S.C. Leahy¹, E. Altermann¹, and G.T. Attwood¹; ¹AgResearch Limited, Palmerston North, New Zealand; ²Massey University, Palmerston North, New Zealand

The genome of the rumen-inhabiting Gram-positive bacterium *Butyrivibrio proteoclasticus* B316^T has been completely sequenced and was found to be spread across 4 replicons including two chromosomes of 3500 and 302 Kb, and two megaplasmids of 361 and 186 Kb. Both megaplasmids are largely cryptic in function with around 75% of their open reading frames (ORFs) showing no significant similarity to any gene currently sequenced. In contrast, 75% of the proteins encoded on the two chromosomes are homologous to known proteins and can be assigned functions. The 302 Kb secondary chromosome, designated BPC2, possesses 2 tandemly arrayed ribosomal RNA operons that are >99% identical to the remaining 4 rRNA operons found on the main chromosome. BPC2 also encodes proteins that appear essential to the uptake of biotin, important to the uptake and biosynthesis of

nicotinamide adenine mononucleotide and the ability to utilise fumarate as the terminal electron acceptor during anaerobic respiration. The replicon also makes extensive contributions to carbohydrate and nitrogen metabolism as well as cellular detoxification. The smallest megaplasmid, designated pCY186, was the only *B. proteoelasticus* replicon that was able to be displaced *in-vitro* using standard curing techniques without the loss of cell viability. Furthermore, the loss of the pCY186 replicon was found to occur spontaneously during *in-vitro* culture. Analysis of pCY186 codon usage and %G + C content suggest it is not transient but rather has co-resided with the host chromosome for a significant period of time. This would imply its function is specific to, and important within its natural environment. One potential explanation for this is its possession of several restriction modification systems that may be important to *B. proteoelasticus* in the defence against invasion by foreign DNA. Analysis of megaplasmid copy number by qPCR revealed the largest megaplasmid, designated pCY360, at a copy number of ~5 while the other replicons exist at a copy number of ~1. Therefore the 389, mostly cryptic, ORFs encoded by pCY360 are likely to impose a significant burden to the translational efficiency of *B. proteoelasticus*. This along with its retention during classical curing techniques suggests pCY360 imparts some competitive advantage to *B. proteoelasticus*.

Keywords: rumen bacteria; xylan degradation; replicon; genomics; *Butyrivibrio proteoelasticus*

Microarray Analysis Of A Ruminant Xylanolytic Bacterium And A Methanogen Grown In Co-Culture. C.J. Yeoman^{1,2}, W.J. Kelly¹, J. Rakonjac², Z. Kong¹, and G.T. Attwood¹; ¹AgResearch Limited, Palmerston North, New Zealand; ²Massey University, Palmerston North, New Zealand

Butyrivibrio proteoelasticus is a Gram-positive bacterium that is found in the rumen at significant concentrations. It was initially isolated due to its high xylanolytic and proteolytic characteristics and has more recently been implicated in biohydrogenation. Due to the biologically-rich nature of the rumen it is likely *B. proteoelasticus* interacts with a diverse range of organisms. One interaction that can be observed microscopically is that with the ruminal methanogenic archaeon, *Methanobrevibacter ruminantium*. Using a specially constructed microarray containing the genomes of both *B. proteoelasticus* and *M. ruminantium*, the gene expression levels of the organisms grown in co-culture were compared to those of each grown in monoculture. Increased expression was seen in many *B. proteoelasticus* ORFs whose products are predicted to have roles in exopolysaccharide biosynthesis and export, flagella formation, the phosphoenolpyruvate sugar import system (PTS),

particularly as it relates to fructose, glycogen biosynthesis and storage, RNA catabolism and the conversion of glutamate to NAD⁺. Decreased expression was seen in many of the *B. proteoelasticus* ORFs whose products are predicted to be glycosyl hydrolases, except those predicted to be involved in the degradation of xylan; non-PTS sugar transporters; and fatty acid biosynthesis, except those predicted to have a role in butyrate production. Increased expression was seen in many of the *M. ruminantium* ORFs whose products are predicted to have roles in adherence, glutamate production and methanogenesis. *B. proteoelasticus* is known to produce formate as an end product of fermentation. The up-regulation of many critical components of methanogenesis pathway by *M. ruminantium* during co-culture suggests it can utilise H₂ and formate more efficiently when supplied by *B. proteoelasticus*. Analysis of the *B. proteoelasticus* genome suggests it lacks two enzymes critical to *de-novo* biosynthesis of NAD⁺, and instead relies on scavenging exogenous nicotinamide riboside or the conversion of glutamate for NAD⁺ production. The up-regulation of genes involved in the production of glutamate by *M. ruminantium* and those involved in the conversion of glutamate to NAD⁺ by *B. proteoelasticus* may suggest a mutualistic relationship has evolved between these two co-inhabitants of the rumen.

Keywords: *Butyrivibrio*, *Methanobrevibacter*, Rumen, Mutualism, Microarray

Diet-Disrupted Gut Microbiota Dwarfs a Genetic Defect in Metabolic Syndrome Development. C. Zhang¹, M. Zhang¹, S. Wang³, R. Han², Y. Cao¹, W. Hua¹, Y. Mao¹, X. Zhang¹, X. Pang¹, C. Wei¹, G. Zhao³, Y. Chen², L. Zhao¹; ¹Shanghai Jiao Tong University, Shanghai, China; ²Chinese Academy of Sciences, Shanghai, China; ³Chinese National Human Genome Sequencing Centre, Shanghai, China.

With its compositional plasticity, gut microbiota can remain either a partner for health or can result in the development of some diseases. Specifically, gut microbiota may be pivotal in the development of metabolic syndromes (MS). However, the relative contributions of host genetics and diets in determining the gut microbiota structure and the modulation of MS-relevant phenotypes remain unknown. Using a knockout mouse with impaired glucose tolerance (IGT), and its wild-type counterpart fed two differing diets (high fat diet, or normal chow) for 25 weeks, we show that diet plays a much more profound role than host genotype in shaping gut microbiota related to MS development. All three IGT/obesity groups had significantly different gut microbiota compared with the healthy wild-type/normal chow-fed animals. Through a combination of pyrosequencing and multivariate statistics, we identified 65 species-level phylotypes showing differential responses to changes in diets, genotypes,

and MS phenotypes. Most notably, gut barrier-protecting *Bifidobacterium* spp. were almost completely diminished in all animals on high fat diet regardless of genotype. Sulfate-reducing, endotoxin-producing bacteria in the family *Desulfovibrionaceae* were enhanced in all animals with IGT/obesity, particularly in the wild-type/high fat diet group, which had the highest calorie intake and the most serious MS phenotypes. Taken together, a long-term, unlimited intake of high fat diet had thus transformed gut microbiota of the wild-type animals into a pathogen-like entity for development of metabolic syndromes despite a complete host genome. This finding implies a possibility for combating metabolic diseases via diet interventions with the key phylotypes in the altered gut microbiota as a primary target.

Keywords: mouse; gut microbiota; metabolic syndrome; obesity

Whole-Body Systems Approaches for Gut-Microbiota Targeted, Personalized Health Management. L. Zhao, Shanghai Jiao Tong University, Shanghai, China

Human beings are superorganisms with two genomes, the genetically inherited human genome (25,000 genes) and the environmentally acquired human microbiome (over 1 million genes). The two genomes need to work in harmonious integration to maintain our health. Nutrition plays a crucial role in shaping our gut microbiomes and keeping them as partner for health or turning them into pathogen for diseases. Many chronic diseases such as obesity, diabetes and cardiovascular diseases are developed as a result of a low grade, systemic and chronic inflammation most probably induced by the altered balance between these two genomes under poorly managed diets. Molecular profiling of gut microbiomes and urine metabolites can provide us new systems approaches for quantitative assessment and monitoring of our health at the whole-body levels. Based on a Chinese family cohort, we were able to develop a new functional metagenomics method in which population variations of gut microbiota and compositional changes of urine metabolites are correlated with each other by a multi-variate statistical tool. Key functional species in the gut microbiota for health or diseases can be identified based on the scope and profiles of host metabolic pathways affected by their population changes. These approaches measure human health based on the results of interactions between the two genomes and the environments. They are non-invasive and molecular-based and can provide global, dynamic, quantitative and non-targeted assessment of human health at both individual and population levels. Large scale cohort studies with these whole-body systems methods will generate pre-disease biomarkers with predictive power; thus make preventive

health management of populations possible through individualized re-engineering of gut microbiome with designed foods and natural drugs. Gut microbiota-targeted, whole-body systems biology may change the landscape of health management technology and help build a new bridge for the convergence between traditional and western medicines.

Keywords: gut; microbiomes; human health; systems biology; genomics

Isolation of Fiber Degrading Bacteria from Pig Feces. C.J. Ziemer and B.J. Kerr. Agricultural Research Service, USDA, Ames, IA.

In order to better understand fiber digestion in the pig, two types of selective enrichment to isolate cellulose, xylan and pectin utilizing bacteria. Rapid flow isolations utilized a chemostat with a flow rate of 0.25 per hour, combined with substrate immobilized in Dacron bags. Substrate depleted isolations utilized substrate depleted rumen fluid medium as a base with single carbohydrate additions. Both methods used carbohydrate specific agars to assist in bacterial isolations. A total of 332 isolates were recovered using the rapid flow isolation method while 59 were recovered from the substrate depleted method. All isolates had approximately 700 bp of 16S rRNA genes initially sequenced. Based on initial sequence groupings, 150 isolates had nearly full 16S rRNA genes sequenced resulting in 87 unique sequences. Isolates which had both low sequence similarity to cultured bacteria and those which were closely related to more than one bacterium were biochemically characterized. Bacteria with the genera classification *Clostridium* were isolated with the highest frequency, 24.1% of isolates, followed by 11.5% *Lactobacillus*, 11.5% *Enterococcus* and 9.2% *Escherichia*. Isolates most closely related to uncultured sequences comprised 6.9% of total isolates. *Clostridium* species were most often isolated from cellulose and pectin while *Lactobacillus* species were most frequently isolated from xylan. These isolates will be used to gain a better understanding of fiber digestion in the pig.

Keywords: swine; bacteria; fiber hydrolysis

A Metagenomics View On The Human Microbiota Of The Small-Intestine E.G. Zoetendal^{1,2}, C.C.G.M. Booijink^{1,2}, M. Wels^{1,3}, H. Smidt^{1,2}, W.M. de Vos^{1,2}, and M. Kleerebezem^{1,3}; ¹T1 Food and Nutrition, Wageningen, The Netherlands, ²Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands, ³Nizo Food Research, Ede, The Netherlands

The human gastrointestinal (GI) tract harbors a diverse microbial community consisting of various phylogenetic

groups. Since only a minority of GI tract microbes has successfully been cultured to date, culturing approaches are insufficient to describe the microbial diversity and functionality. Therefore, 16S ribosomal RNA (rRNA) and its encoding genes have been used as markers for describing the phylogeny and diversity of microbes. However, most GI tract studies have focused on the colon, leaving our insight into the small intestinal microbiota in its infancy. To study the microbial ecology of the small intestine we applied a functional metagenomics approach to answer the following questions: which microbes are in the small intestine, what is their potential and what are they doing? Samples from ileostomy subjects were collected; in these individuals small intestinal effluent leaves their body at the end of the ileum via a stoma since their colon has been removed. Thereby, these subjects provide a continuous non-invasive access to small intestinal content. DNA and RNA were isolated and subsequently used for phylogenetic profiling using the Human Intestinal Tract Chip (HITChip) and a functional metagenomics approach. The HITChip analysis indicated that the microbiota composition of ileostomy effluent is significantly less diverse and more dynamic over time as

compared to that of the colon of healthy adults. It is dominated by *Streptococcus*- and *Veillonella*-related species, while typical colonic bacteria such as those belonging to *Bacteroidetes* and *Clostridium* clusters IV and XIVa are hardly detected. Sequencing surveys chain terminator and pyrosequencing of the metagenome consisting of more than 25,000 Fosmids (700 Mbp) indicated that the small intestinal microbiota harbors an enormous genetic diversity from which the majority of sequences obtained do not have a significant hit to gene sequences in the public databases. The sequences that have a homologue in the database could be assigned to all 20 COG-groups (clusters of orthologous genes). Remarkably, only a few of the observed COGs were found to be dominantly expressed, especially those involved in carbohydrate transport and metabolism, which made up one fourth of the expressed genes. Overall, our data demonstrated that the functional metagenomics approach applied provides insight into the ecology of the microbiota in the small intestine.

Keywords: Small Intestine, Human Intestinal Tract Chip, metagenomics, metatranscriptomics