SCIENTIFIC PROGRAM AND ABSTRACTS

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2011 CONGRESS ON GASTROINTESTINAL FUNCTION
MEETING PROGRAM

MONDAY, APRIL 18TH:

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

Please pick up your Registration materials, Name tag and Mixer drink tickets
Mount posters on boards provided

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

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

TUESDAY, APRIL 19TH:

08:00-8:30 **Continental Breakfast** Gleacher Center, First Floor, near Tiered Classroom

Morning Session **Gleacher Center, First Floor, Tiered Classroom**

08:30-10:30 Oral Presentations

08:30-08:40 Welcome remarks and acknowledgements.
Mark Morrison, Congress Chair, CSIRO Australia and The Ohio State University

08:40-09:20 Opportunities to improve public health through the genetic optimisation of dietary carbohydrate content and composition in cereals.
Matthew Morell, CSIRO, Australia

09:20-09:40 Abundance and distribution of mucosa-associated sulfate-reducing bacteria and methanogenic archaea in the healthy colon and in inflamed and non-inflamed tissues of IBD patients
F. Carbonero, G.M. Nava, E. Greenberg, H.R. Gaskins. University of Illinois, Urbana-Champaign, IL, USA

09:40-10:00 Microecology of *Olsenella umbonata* sp. nov. and *Veillonella magna* sp. nov., two novel lactic acid-syntrophic bacterial species from the mucosal jejunum of a healthy pig
M. Kraatz, R. J. Wallace, L. Svensson, D. Taras. Free University Berlin, Berlin, Germany.

10:00-10:20 Mare prebiotic supplementation and foal fecal bacterial communities before Weaning
C. Faubladier, C. Philippeau, J. Danel, V. Julliand. AGROSUP Dijon USC INRA, Dijon, France.

10:20-10:50 **Coffee Break** Gleacher Center, First Floor, near Tiered Classroom

10:50-11:30 Oral Presentations

10:50-11:10 Genotyping of *Streptococcus bovis* isolated from the rumen of wild deer and domesticated animals
S. Koike, H. Kato, M. Sakaki, S. Iisaka, Y. Sawabe, Y. Kobayashi. Hokkaido University, Sapporo, Hokkaido, Japan.

11:10-11:30 Describing microbial population in the rumen using genomic *rrs* genes and rRNA as templates reveals greater diversity
S. E. Denman, M. Mitsumori, T. Shinkai, N. Asanuma, C.S McSweeney. CSIRO Livestock Industries, Brisbane, Queensland, Australia.

11:30-13:00 **LUNCH** Please make your own arrangements

Afternoon Session **Gleacher Center, First Floor, Tiered Classroom**

13:00-14:20 Oral Presentations

13:00-13:40 Fast and accurate taxonomic assignment of metagenomic sequences.
Alice McHardy, Max Planck Institute, Germany

13:40-14:00 Investigating the *in vivo* interactions between *Fibrobacter succinogenes* and cellulolytic ruminococci.
P. Mosoni, C. Yeoman, P. Lepercq, G. Andant, B.A. White, E. Forano. INRA, St Genes Champanelle, France.

14:00-14:20 Targeted Metagenomics/Metatranscriptomics for novel glycoside hydrolase enzyme discovery from the rumen of Arctic Muskoxen.
R.J. Forster, S. Qi, P. Wang, E. Ungerfeld, R. Teather, L.B. Selinger, A. Tsang, N. O'Toole T .McAllister, M.B. Leigh, P. Barboza, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada.

14:20-14:40 **Coffee Break** Gleacher Center, First Floor, near Tiered Classroom

14:40-15:40 Oral Presentations

14:40-15:00 Pyrosequencing sheds light on the kangaroo foregut.
L.M. Gulino, D. Ouwerkerk, A.V. Klieve. DEEDI, Dutton Park, Queensland, Australia.

15:00-15:20 Phylogenetic analysis of the intestinal microbial community in herbivorous land and marine iguanas of the Galapagos Islands using 16S rRNA-based pyrosequencing
P.Y. Hong, E. Wheeler, I.K.O. Cann, R.I. Mackie. Department of Animal Sciences, Institute for Genomic Biology, Urbana, IL, USA.

15:20-16:00 Intestinal bile acid sensing: A new player in the glucagon-like peptide story
Doug Burrin, Baylor College of Medicine, Texas, USA

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

WEDNESDAY, APRIL 20TH:

08:30-9:00 **Continental Breakfast** Gleacher Center, First Floor, near Tiered Classroom

Morning Session **Gleacher Center, First Floor, Tiered Classroom**

09:00-10:30 Oral Presentations

09:00-09:40 Tackling complexity of intestinal microbiome metabolism of dietary polyphenols
Elaine Vaughan, Unilever R&D, The Netherlands

09:40-10:00 Pyrosequencing analysis of bacterial diversity associated with the intestinal tract of Tilapia (*Oreochromis Niloticus*) supplemented with Oreganum essential oil in a Colombian tropical ecosystem
M.G. Nuñez, C.Y. Ariza, G. Afanador, D. Rodriguez, **O.L. Mayorga**. CORPOICA, Bogota, Colombia

10:00-10:20 Phytochemicals that modulate amino acid and peptide catabolism by caprine rumen microbes
M.D. Flythe, I.E. Kagan, G.L. Gellin, B.M. Goff, G.E. Aiken, P.R. Cheeke. ARS-USDA, Lexington, Kentucky, United States.

10:20-10:50 **Coffee Break** Gleacher Center, First Floor, near Tiered Classroom

10:50-11:45 Oral Presentation Bryant Memorial Lecture

Methanosarcina's contribution to the larger field of anaerobic microbiology
J. Greg Ferry, Pennsylvania State University, USA

11:45-13:00 **LUNCH** Please make your own arrangements

Afternoon Session **Gleacher Center, First Floor, Tiered Classroom**

13:00-14:20 Oral Presentations

13:00-13:20 Use of microbial genomics to understand fiber degradation and methanogenesis in the rumen environment
S. C. Leahy, W.J. Kelly, E. Altermann, C.D. Moon, A. L. Cookson, S. C. Lambie and G. T. Attwood; AgResearch, New Zealand.

13:20-13:40 Metabolomic profiling of an anaerobic fungus grown in the presence or absence of natural co-cultured methanogen
Y. Cheng, W.-Y. Zhu. Nanjing Agricultural University, Nanjing, Jiangsu, China.

13:40-14:00 Biochemical analyses of lichenin degrading enzymes from *Ruminococcus albus* 8
M. Iakiviak, R.I. Mackie, I.K.O. Cann. University of Illinois, Urbana, IL, USA.

14:20-14:40 **Coffee Break** Gleacher Center, First Floor, near Tiered Classroom

14:40-15:20 Oral Presentations

14:40-15:00 Reduction in dietary wet distillers grains alters *Escherichia coli* O157:H7 in feces of cattle
J. E. Wells, S. D. Shackelford, E. D. Berry, N. Kalchayanand, J. M. Bosilevac, T. L. Wheeler. USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA.

15:00-15:20 Intestinal microbiota of broiler chickens administered a *Bacillus* based DFM and raised on used litter from commercial farms with different disease histories
A.P. Neumann, J. A. Benson, K.W. Lee, G.D. Ritter, D.A. Bautista, H.S. Lillehoj, G.R. Siragusa. Danisco USA Inc., Waukesha, WI, USA.

15:20-15:30 CLOSING REMARKS AND INVITATION TO CGIF2013

15:30 BUSINESS MEETING

ABSTRACTS OF ORAL AND POSTER PRESENTATIONS

Microbial Physiology and Genomic Analysis:

Effects of glucose or glycerol on lipase expression by select rumen bacteria *in vitro*.

Holly Edwards*[1], Robin Anderson[2], Rhonda Miller[1], T. Matthew Taylor[1], Margaret Hardin[3], Stephen Smith[1], Nathan Krueger[2], and David Nisbet[2], [1]*Texas A&M University, College Station, TX, USA*, [2]*United States Department of Agriculture/Agricultural Research Service Southern Plains Agricultural Research Center, College Station, TX USA*, [3]*IEH Laboratories & Consulting Group, Lake Forest Park, WA, USA*.

Ruminal lipolysis has long been attributed mainly to *Anaerovibrio lipolyticus* and *Butyrivibrio fibrisolvens*. Conversely, *Propionibacterium* species *avidum* and *acnes* are also known to express lipase activity, but little is known regarding the contribution of these prominent anaerobes to rumen lipolysis. In order to characterize the lipase activity of these bacteria, each was cultured in anaerobic medium containing minerals, vitamins, yeast extract and trypticase supplemented with 5% olive oil as a lipid substrate or with non limiting amounts of glucose as a fermentable energy source. In the case of *A. lipolyticus*, glycerol was used in place of glucose due to its inability to utilize glucose. Cells harvested from mid to late log phase cultures were transferred anaerobically to a fresh set of tubes containing anaerobic assay buffer, a bed of glass beads (which served as a solid support matrix) and 0.6 ml olive oil. Free fatty acids (FFA) were measured colorimetrically after 0 and 6 h incubation at 39°C. Results from a general analysis of variance indicated that lipase activity by *A. lipolyticus* and *P. avidum* was not regulated by glucose or glycerol because lipase activity (nmol FFA/mg protein/h) by cells grown with these substrates (0.21 ± 0.03 and 0.45 ± 0.01, respectively) was not decreased ($P > 0.05$) compared to the activity by cells grown with olive oil (0.36 ± 0.37 and 0.30 ± 0.08, respectively). Regardless of growth substrate, *P. acnes* and *B. fibrisolvens* expressed lower lipase activity ($P < 0.05$) than *A. lipolyticus* and *P. avidum*. In the case of glucose-supplemented *P. acnes* and *B. fibrisolvens* cells, lipase activity (0.03 ± 0.01 and 0.01 ± 0.01, respectively) was lower ($P < 0.05$) than olive oil-grown cells (0.07 ± 0.02 and 0.05 ± 0.03, respectively), suggesting that glucose down-regulated lipase gene expression in these bacteria. Results indicate that higher and constitutively expressed lipase activity of *A. lipolyticus* and *P. avidum* probably contribute more to lipolysis of olive oil triacylglyerol in ruminants than *P. acnes* and *B. fibrisolvens*.

Function of a peptide pheromone in *Streptococcus bovis* and its genome sequence

Narito Asanuma*, *Meiji University, Kawasaki, Japan*.

In ruminants, *Streptococcus bovis* is a predominant ruminal bacterium that may be involved in rumen acidosis or bloat. In addition, *S. bovis* has also been detected in the human intestine and may be associated with deterioration of the colon wall in

colon cancer. Therefore, it is desirable to suppress the overgrowth of *S. bovis* in the human colon, as well as in the rumen. In these tracts, the closely related species, *Streptococcus gallolyticus*, was detected. It has been proposed that the peptide pheromone signaling system is a streptococcal intercellular communication mechanism. Previously, we sequenced the genes of the ComC peptide and its two components system (AB284382). In order to control the growth of *S. bovis* via the ComC peptide pheromone signaling system, we examined the function of this system. Furthermore, we show the draft genome sequence of *S. bovis* strain JB1. When*S. bovis* JB1 was grown in the presence of mature ComC peptide, the growth rate and transformation frequency increased in a dose-response manner, indicating that the ComC peptide stimulates the growth and induces genetically competent cells. Neither the growth rate nor transformation frequency was affected by the addition of *S. bovis* ComC peptide in *S. gallolyticus*, suggesting that the ComC peptide is specific to *S. bovis*. When the ComC peptide was added to the cultures of mixed ruminal microbes, the cDNA contents of *S. bovis* per total bacterial counts increased. Addition of the ComC peptide increased production of butyrate without lactate accumulation. Because the cDNA level of *Megasphaera elsdenii* increased significantly by the addition of the ComC peptide, the increase in butyrate production may reflect a conversion from lactate. Amylase activity in the mixed culture also increased; however, tannase activity slightly decreased. This result confirms that *S. bovis* ComC peptide does not function as a signal for *S. gallolyticus*. The *S. bovis* draft genome comprises approximately 1900 predicted CDSs. The genome has putative genes for glycolysis, Entner-Doudoroff pathways, and the synthesis of most essential amino acids, some vitamins, and cofactors. These results may contribute to the future development in the control of *S. bovis* overgrowth and the prevention of ruminal acidosis.

Keywords: genome sequence, peptide pheromone, rumen bacteria, *Streptococcus*, transcription

Production and Degradation of 4-Ethylphenol in Blended Swine Lagoon Enrichments

C. Copp*[1] and K.C. Doerner[2], [1]*Western Kentucky University, Bowling Green, KY, USA*, [2]*Western Kentucky Universityq, Bowling Green, KY, USA*.

4-Ethylphenol (4EP) is a malodorant of swine waste and derived from p-coumaric acid (pCA), a known component of lignin. While production of 4EP from pCA is established whether lignin yields 4EP in swine waste is unclear. Fe(III) addition to swine lagoon enrichments increase levels of swine lagoon malodorants, namely 3-methylindole and 4-methylphenol, but the effect of Fe(III) on 4EP levels is also unclear. Blended swine lagoon slurries were tested for 4EP production using the following conditions: 1), anaerobic tryptone-yeast media (TY); 2), TY+0.2% (w/v) sulphite lignin; 3), TY+10mmol l^{-1} Fe(III); 4), TY+10mmol l^{-1} Fe(III)+ 0.2% sulphite lignin; 5), TY + 1mmol l^{-1} pCA; and 6), TY+10mmol l^{-1} Fe(III)+1mmol l^{-1} pCA. Samples were collected on 0, 3, 7, 14, 21, & 28 days (d) and 4EP was quantified using reverse-phase HPLC. Maximum concentration of 4EP for each condition was observed on 7d. By 21d, 4EP levels decreased to near undetectable levels. Condition 1 and 2 yielded maximum 4EP levels of 626.2 ±45.42 (S.E.) and

487.6±20.7 μmol l^{-1}, respectively. Conditions 3 and 4 yielded 499.3±50.5 and 496.7±27.2 μmol l^{-1} 4EP, respectively. Conditions 5 and 6 yielded the 4EP levels of 1026.4±18.1 and 969.4±64.7 μmol l^{-1}, respectively. Data indicate that swine lagoon slurries, when supplemented with pCA, as expected, produces 4EP but addition of sulphite lignin failed to yield stimulate 4EP production. Also, Fe(III) failed to stimulate 4EP production from pCA or sulphite lignin. Data also indicate blended swine slurries are capable of degrading endogenously produced 4EP or 4EP produced from exogenous pCA.

Determining the nutritional requirements of the bile acid-metabolizing gut bacterium *Clostridium hylemonae*

S. Daniel* and J. Huckaba, *Eastern Illinois University, Charleston, IL, USA.*

Clostridium hylemonae is an obligate anaerobe that resides in the human gut and is involved in the conversion (7a-dehydroxylation) of primary bile acids to secondary bile acids. Increased levels of secondary bile acids have been associated with an increased risk of gallstone disease and colon cancer. Resolving the nutritional requirements of *C. hylemonae* will provide insight into the metabolic capabilities of this bacterium and the factors which may regulate its survival and activities in the human gut. The goals of our study were to determine the vitamin and amino acid requirements of *C. hylemonae*. *C. hylemonae* TN-271 was grown in an anaerobic undefined medium (UM; 0.1% yeast extract, 20 mM glucose, minerals, metals, bicarbonate, 100% CO_2 gas phase, and cysteine [reducing agent]) at 37°C. When *C. hylemonae* was transferred from UM to a defined medium (DM; UM without the yeast extract), growth was negligible. Only when DM was supplemented with a vitamin mix (*p*-aminobenzoate, biotin, cyanocobalamin, folate, lipoate, nicotinate, pantothenate, pyridoxal, riboflavin, and thiamine) and an amino acid mix (alanine, arginine, asparagine, aspartate, cystine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) was growth observed. The leave-one-out technique was subsequently used to determine the specific vitamin(s) and amino acid(s) required for growth by supplementing DM with the complete amino acid mix and vitamin mixes which lacked one of the vitamins and by supplementing DM with the complete vitamin mix and amino acid mixes which lacked one of the amino acids, respectively. With this technique, three vitamins (pyridoxal, riboflavin, and pantothenate) and three amino acids (methionine, valine, and tryptophan) were found to be required for growth by *C. hylemonae*. This approach is now being employed to resolve the nutritional requirements of two other bile acid-metabolizing gut clostridia, *Clostridium scindens* and *Clostridium hiranonis*.

Describing microbial population in the rumen using genomic rDNA and rRNA as templates reveals greater diversity.

Stuart E Denman*[1], Makoto Mitsumori[2], Takumi Shinkai[2], Narito Asanuma[3], and Chris S McSweeney[1], [1]*CSIRO Livestock Industries, Brisbane, Queensland, Australia*, [2]*National Institute of Livestock and Grassland Science,, Tsukuba, Japan*, [3]*College of Agriculture, Meiji university, Meiji, Japan.*

Environmental microbial studies have long targeted the 16S rRNA gene as a phylogenetic marker to describe diversity and observe population shifts within these samples. However, the use of 16S rRNA as template may provide additional information about metabolic activity of microbial populations in these environments. Now with the advent of next generation sequencing and "bar-coded" primer sets, it is possible to gather 1000's of sequences per sample. Software packages have been developed that allow for the efficient analysis and statistical interpretation of these large datasets. In this study, we have extracted both gDNA and total RNA from the goat rumen microbiome to be used as template material for 16S gene diversity studies. An animal metabolism trial using goats fed a maintenance diet of roughage and concentrate (1:1) with increasing levels of a methanogen inhibitor (bromochloromethane) were used. Measurements of digestion, fermentation, microbial populations, expired gases and ruminal gas concentrations were performed. Bromochloromethane (BCM) reduced methane production by >80% and dramatically increased ruminal H2 concentration (> 2000 ppm), but dry matter intake and digestibility were unaffected. Microbial population studies targeting gDNA showed that the animals were dominated by Bacteroidetes, in particular *Prevotella* species and that these populations increased in numbers with a concurrent decrease in total microbial diversity in relation to the level of BCM administered. Increases in *Selenomonas* species were also observed with the highest dose of BCM. Beta diversity metrics grouped microbiome samples from goats in relation to the level of BCM dosing with the largest variance between the control and high BCM dosing. A similar variance effect between treatments was observed when using total RNA as a template. However, the greatest variance in the entire diversity data was between the two template types. Bacteroidetes were still the most dominant members when using RNA based analysis; however, it was *Porphyromonas* species that were the dominant members of the population in the high BCM samples from RNA template compared with gDNA template. In addition to this shift, populations of *Treponema*, which were either not detected or only detected at low numbers when using DNA template, were found to be in high abundance in the BCM samples based on RNA analysis.

Phagocytosis on *Entodinium caudatum* and *Epidinium caudatum* as affected by the concentration of fluorescent beads, glucose and feeding regime.

H.L. Diaz*, K. Barr, K. Godden, and J.L. Firkins, *The Ohio State University, Department of Animal Science, Columbus, Ohio, USA.*

We hypothesized that carboxylate-modified, fluorescent blue latex beads (0.5 mm) could assess rates of feed and(or) bacteria uptake by cultures of *Entodinium caudatum* (EN) and *Epidinium caudatum* (EPI) to avoid the complication that uptake and digestion occur simultaneously. In Experiment 1, cultures of EN were fed at two feeding regimes (Fed vs Unfed) with 10^7 or 10^8 beads/ml in a 2 x 2 factorial arrangement of treatments. Samples were taken at 0.017, 0.33, 1 and 3 h of time (t). Data were analyzed with PROC NLIN using the model, % of cells ingesting beads = $A(1-e^{-kt})$. The fitted values for A and k were assessed in a randomized complete block design (blocked for 2 replicate runs). Rate of uptake (k) tended (P = 0.08) to have a feeding x bead concentration interaction (1.25, 1.27,

1.91, and 2.86/h for UNFED-10^7, UNFED-10^8, FED-10^7, and FED-10^8, respectively). Asymptotic bead uptake (A) had no feeding x bead concentration interaction ($P > 0.40$). Feeding was not affected ($P > 0.10$), but the main effect was lower ($P < 0.05$; 41.5 vs. 94.8%) for 10^7 than 10^8 beads/ml. Increasing bead concentration increased the A and the k for EN, probably due to an increased frequency of contact with the oral region. In Experiment 2, EN and EPI were fed or not fed without (saline vehicle, Sal) or with saline plus glucose (Glc, 58 m*M* final concentration, glucose equivalent to fed starch) to potentially prevent a chemotactic glucose gradient. Using 10^8 beads/ml, A and k were assessed in a randomized complete block design (2 replicate runs) with a 2 x 2 factorial arrangement. For EN, k had no feeding x Glc ($P > 0.60$) nor main effect of glucose ($P > 0.30$), but the main effect of FED (6.35/h) was less than ($P < 0.01$) UNFED (34.2/h). There was a feeding x Glc interaction ($P < 0.05$) for A (90.3, 94.7, 97.6, and 96.8% for UNFED-Sal, UNFED-Glc, FED-Sal, and FED-Glc, respectively). For EPI, neither k nor A had a feeding x Glc interaction ($P > 0.10$). Main effect means for k were lower ($P < 0.05$) for UNFED (2.44/h) vs FED (3.38/h), but Glc (3.12/h) only tended ($P = 0.09$) to be greater vs Sal (2.70/h). Neither feeding nor Glc affected ($P > 0.10$) the A for EPI. With minor response for asymptotic bead uptake, feeding decreased k by over 5 fold for EN but increased k moderately for EPI. Further research is necessary to assess protozoal feeding behavior and chemotaxis to drive nutrient uptake and subsequent growth.

Keywords: rumen protozoa, glucose, phagocytosis

Biochemical characterization of Cel9B, an exo-cellulase from *Ruminococcus albus* 8

A. Evans*, M. Iakiviak, B. Mayer, R.I. Mackie, and I.K.O. Cann, *University of Illinois, Urbana, IL, USA*.

Ruminal microorganisms are generally known to be efficient utilizers of recalcitrant polysaccharides. Cellulose, the main structural polymer of the plant cell wall, is composed of glucose molecules, linked by a β1,4 glycosidic bond, with hydrogen bonding between glucan chains that confers a crystalline character. To degrade cellulose, the concerted actions of 3 classes of enzymes are required. Endo-glucanases, randomly cleave internal β1,4-linkages in amorphous regions. Exo-glucanases utilize the glucan chain ends and processively cleave off repeating cellobiose units from the reducing or non-reducing end. Finally, β-glucosidases or cellodextrinases cleave the cello-oligosaccharides produced into glucose that can then be fermented by the bacterium. *Ruminococcus albus* is a major fibrolytic bacterium found in the rumen, and it has been shown to degrade cellulose as well as hemicellulose. Several endo-glucanases from *R. albus* have been previously characterized, as well as a β-glucosidase, but there have been no exo-glucanases reported.

In the present study, we determine the biochemical activity of a putative exo-glucanase, Cel9B, an enzyme shown to be absent from mutants of *R. albus* 8 deficient in cellulose adhesion and deconstruction. This enzyme contains one catalytic domain belonging to GH family 9, two carbohydrate binding domains belonging to CBM family 4 and 37, as well as an Ig-like domain. The enzyme was

cloned and expressed in *E. coli* BL-21 Codon PLUS. The recombinant protein was purified through metal affinity chromatography, followed by size exclusion chromatography, and finally anion exchange chromatography. Enzymatic activity was tested on cellulosic substrates and cello-oligosaccharides. Cel9B was able to produce cellobiose as the main product from cello-oligosaccharides hydrolysis with a degree of polymerization of 3 or higher, but was unable to produce a significant amount of reducing ends from carboxymethyl cellulose or Whatman filter paper. This work provides evidence for the exo-cellulolytic properties of Cel9B and provides biochemical evidence for a critical component of the cellulolytic machinery of *R. albus* 8.

Contributions of *Methanosarcina* to the Larger Field of Anaerobic Prokaryotic Biology

J. Ferry*, *Penn State University, University Park, Pennsylvania.*

Methanosarcina species are the most diverse of all methanogens, utilizing a broad range of growth substrates. Remarkably, *Methanosarcina* species have the largest genomes of all methanogens that are annotated, with a high proportion of homologs from the domain Bacteria. For example, the pathway for conversion of acetate to methane by *Methanosarcina acetivorans* employs no fewer than six enzymes and enzyme complexes common to anaerobic microbes of the domain Bacteria. The biochemical characterization of several of these enzymes from *Methanosarcina* species has contributed to advances in understanding the enzymology and physiology of diverse anaerobes from both prokaryotic domains.

Targeted Metagenomics/Metatranscriptomics for novel glycoside hydrolase enzyme discovery from the rumen of Arctic Muskoxen

RJ Forster*[1], S Qi[1], P Wang[1], E Ungerfled[1], R Teather[1], LB Selinger[2], A Tsang[3], N O'Toole[3], T McAllister[1], MB Leigh[4], and P Barboza[4], [1]*Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada*, [2]*University of Lethbridge, Lethbridge, Alberta, canada*, [3]*Concordia University, Montreal, Quebec, Canada*, [4]*University of Alaska-Fairbanks, Fairbanks, Alaska, USA.*

The availability of novel, highly active fibrolytic enzymes for lignocellulosic biomass conversion into ethanol or value added products is limited and may restrict the development of these new technologies. Arctic muskoxen are ruminants that have developed novel nutritional strategies to cope with long periods of cold, low feed intake with poor quality forage and short seasonal periods of high intake of higher quality forage. They also live in isolation from other domestic ruminants and have been found to harbour novel rumen microbes. A comparative rumen metagenomics study was conducted using a crossover design comparing two diets (based on brome hay or triticale straw as fiber sources) and two seasons (early spring, low intake and late fall, high intake). DNA was extracted from rumen solids content from each treatment and subjected to phylogenetic and metagenomic analysis. RNA was extracted and used for eukaryotic metatranscriptomic analysis. 18S-27S internal transcribed spacer analysis revealed a diverse population of rumen fungi, including clusters possibly representing several new genera. Comprehensive bacterial phylogenetic analysis demonstrated differences in microbial communities

between diets and seasons. Analysis of metagenomic glycoside hydrolase (GH) families reveals families not previously found or in low abundance in other published metagenomes, as well as evidence of dockerins and cohesins associated with cellulosomal structures. Analysis of the metatranscriptome reveals a large enrichment in endoglucanases, including GH families 6 and 48, that are absent in other metagenomes. These findings indicate that muskoxen are a rich source of novel microbes and enzymes that will extend the repertoire of fibrolytic enzymes beyond those found in domesticated ruminants.

Keywords: metagenome, metatranscriptome, rumen, muskoxen, glycoside hydrolases

Reductive acetogen and methanogen diversity in a gnotobiotic lamb model

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Reductive acetogenesis is not competitive with methanogenesis in adult ruminants, whereas acetogenic bacteria appear to be the dominant hydrogenotrophs in the early establishing rumen microbiota. In order to understand the ecology of hydrogenotrophs in the developing rumen, acetogen diversity was investigated in a gnotobiotic lamb model before and after methanogen establishment, and in 2-year-old conventional sheep. Two 17-hour-old lambs lacking cultivable methanogens were inoculated with cellulolytic bacteria and reared to maturity before inoculation with *Methanobrevibacter sp.* 87.7. Diversity of the hydrogenotrophic community was investigated by functional gene analysis (acetyl-CoA synthase and formyltetrahydrofolate synthetase genes for acetogens; methyl coenzyme reductase A gene for methanogens). Methanogens were enumerated using most probable numbers and qPCR (*rrs* target gene). Surprisingly, methanogens were present in gnotobiotic lambs that had been isolated at 17 hours old, although were undetectable using cultivation techniques. Methanogen numbers were low in these lambs ($< 10^4$ *rrs* copies per mg DNA) however *mcrA* diversity was not dissimilar to that found in 2-year-old conventional sheep. In contrast, putative acetogen diversity in gnotobiotic lambs and conventional sheep was significantly different. In sheep, novel putative acetogens affiliated as an undefined taxonomic group between the *Lachnospiraceae* and *Clostridiaceae*, while in gnotobiotic lambs they affiliated with the *Blautia* genus and the *Lachnospiraceae*. The establishment of *Mbb. sp.* 87.7 (1000-fold increase in methanogens) did not substantially affect acetogen diversity in this animal model. These findings indicate that early colonising methanogens may persist in the developing rumen. The early rumen acetogen population is co-incident to methanogen colonisation to some degree and is not affected by methanogen numbers alone. Molecular, quantitative monitoring tools for rumen acetogens are needed to better resolve the factors that affect these populations during rumen development. Continued rumen colonisation studies are necessary to refine our understanding of the ecology of hydrogenotrophs in the developing rumen in order to identify key factors that lead to the prevalence of methanogens in the mature rumen.

Key words: rumen, gnotobiotic, acetogen, methanogen, diversity

Pyrosequencing sheds light on the kangaroo foregut.
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Enteric methane produced by cattle and sheep in Australia accounts for 53% of the total methane emissions and 14% of the total greenhouse gas emissions. Kangaroos are herbivores that often graze on the same pastures as cattle, yet produce little or no methane. It is believed that reductive acetogenesis is the predominant pathway for hydrogen utilisation in the kangaroo foregut (i.e., end product of acetate), whereas, in the rumen of cattle, methanogenesis predominates, even though it is a less efficient use of feed energy. An understanding of the microbial ecology in kangaroos may help establish reductive acetogenesis as a dominant mechanism in cattle, to mitigate methane production and improve productivity. The objective of this research was to describe the bacterial microbiome of the kangaroo foregut and identify species common to kangaroos that may be of importance to the functioning of this ecosystem. A total of 35 kangaroos, from five Queensland locations were examined. Three species (Eastern Grey, *Macropus giganteus*; Red, *Macropus rufus*; and Wallaroo, *Macropus robustus*) grazing a variety of native pastures were sampled. DNA was extracted from fresh foregut samples using a bead-beating protocol. Partial 16S rRNA gene fragments were amplified using individual 10bp barcoded forward primers (341F) and the reverse primer 787R. Pooled amplicons were subjected to 454-amplicon pyrosequencing (Australian Genome Research Facility). The analysis pipeline Quantitative Insights into Microbial Ecology (QIIMEs) was used to analyse the data (Caporaso et al. 2010). Samples displayed inter and intra sample diversity. In all samples examined, except Red Buck 22 (RB22) and Grey Buck 35 (GB35), the Phyla Firmicutes and Bacteroidetes predominated (>80% of the community). In RB22 and GB35, the Phylum Proteobacteria dominated (>40% of the community). Over 2500 operational taxonomic units (OTU) were identified (at the 97% level) across the 35 samples. Five predominant OTUs were subject to BLAST analysis, and these OTUs matched uncultured clones from the Tammar wallaby (Pope et al. 2010). Results from the Ribosomal Database Project (RDP release 10) identified the OTUs as Bacteroidales, Psuedobutyrivibrio, Ruminococcaceae (x2), and Clostridiales, respectively.

Keywords: kangaroo, 454/pyrosequencing, reductive acetogenesis, methane

Biochemical analyses of lichenin degrading enzymes from *Ruminococcus albus* 8
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Ruminant animals have coevolved with a microbial consortium in the rumen. The resident microbes metabolize diverse plant matter into microbial biomass and short chain fatty acids that are subsequently metabolized by the host animal. Plant matter entering the rumen contains a large proportion of lignocelluloses, the

degradable components which are cellulose and hemicelluloses. Cellulose is a crystalline polymer of glucose units joined together by β-1,4 glycosidic bonds. Hemicellulose refers to non-crystalline sugar polymers, including xylan, mannan, arabinan, and galactan. Aside from cellulose, there are other glucose polymers including mixed linkage β-1,3-1,4 glucans such as lichenin, or a β-1,3-1,6 glucan called laminarin. Lichenin is found in lichens (winter feed in reindeer) and Poales order of plants including barley, sorghum, and wheat.
Ruminococcus albus 8 is a major cellulolytic and hemicellulolytic ruminal bacterium. A partial genome sequence of *R. albus* 8, generated by 454 sequencing, yielded 61 genes encoding putative glycosyl hydrolases (GH) that can be classified into 22 different families within the CAZy database. In the present study, we demonstrate that four gene products, two from GH family 5 and one each from families 3 and 16 function synergistically to degrade lichenin into glucose and cello-oligosaccharides. The genes encoding Ra0453 and Ra2830 (family 5), Ra1595 (family 3) and Ra505 (family 16) were amplified by PCR and cloned into pET46b for overexpression in *E. coli*. The recombinant proteins were purified to near homogeneity by affinity chromatography and size exclusion chromatography. The proteins were tested for activity on a wide range of substrates. The Ra0453 and Ra2830 proteins showed hydrolytic activities on cellulosic substrates; however, each enzyme possessed a much greater capacity to degrade lichenin. The protein Ra0505 was able to rapidly degrade lichenin, and to a smaller extent laminarin, but this enzyme exhibited no activity on cellulosic substrates. The Ra1595 protein, annotated as a putative β-1,4 glucosidase, showed a much higher specific activity on laminaribiose (β-1,3 linked glucose units) than cellobiose (β-1,4 linked glucose units). When the enzymes were tested for synergistic activity in the degradation of lichenin, they were found to cooperative to release more glucose, cellobiose, and cellotriose. This work demonstrates the versatility of *R. albus* 8 in capturing nutrients from diverse plant matter.

Ribosomal RNA based analysis of microbial communities in the rumen identifies metabolically active groups underrepresented by genomic DNA detection

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Diversity of microbial communities is commonly ascertained by targeting total community DNA (16S rRNA gene). By focusing on RNA (16S rRNA) we gain an additional insight into the metabolic contributions that some bacteria play in these communities. Here we compare total rumen microbial diversity from five animals fed two different diets using DNA and RNA derived material. Bacterial nucleic acids from three cattle fed 40-70% Leucaena (L) legume and Rhodes grass (LR) and two animals fed 100% L were extracted. The extracted DNA, and cDNA synthesised by reverse transcription from the RNA were both amplified using bacterial or archaeal universal primers. Microbial diversity and abundance were observed by hybridization to a 16S rRNA gene-based custom microarray. Amplicons were also generated from the V1 to V3 regions of 16S rRNA genes using bacterial DNA and cDNA extracted from five rumen samples and pyrosequenced. A total of 117

probes, which were different ($P<0.05$, ANOVA test) in abundance between individual animals, were identified by microarray analysis. Each individual sample based on diet and nucleic acid (RNA vs. DNA) exhibited distinct microbial profiles that were separated on a principal components analysis (PCA). Evaluation of metabolically active bacteria based on comparative analysis of DNA and RNA showed a significantly higher abundance of some species from the genera *Prevotella* and Proteobacteria in RNA samples, but some species of the Firmicutes phylum were relatively higher for DNA samples especially in the LR diet. When we compared microbial responses to diets based on RNA, it appeared that *Methylocella* sp. (methanotroph) and *Desulfovibrio* sp. (SRB) may have a higher metabolic activity on the L diet compared to LR diet. The RNA based results also suggest that some of the Proteobacteria group that appear to be in low abundance in the rumen when assessed using genomic DNA might make a greater contribution to metabolism than previously recognized. The functions of this group in the rumen require further investigation.

Genotyping of *Streptococcus bovis* isolated from the rumen of wild deer and domesticated animals

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Streptococcus bovis is known as an amylolytic bacterial species in the rumen. Recently, we have isolated *S. bovis* strains from the rumen of wild deer, domesticated cattle and sheep. In order to assess the genotypic diversity of ruminal streptococci, we analyzed genomic fingerprints of the newly isolated *S. bovis* strains by PCR-based approaches. Rumen digesta were collected from wild deer (*Cervus nippon yesoensis*), Holstein cow, Japanese Black cattle and Corriedale sheep. Three deer living in Hokkaido, Japan were culled for research purposes at the end of a long winter (March) in which deer had been exposed to snow-covered land with limited access to foods. The domesticated animals were fed corn silage, concentrate-based or hay-based diets. We isolated 13 stains from the wild deer and 40 strains from the domesticated animals fed different diets. All of 53 strains had more than 98% similarity in 16S rDNA sequence with *S. bovis*. Four different banding patterns among the *S. bovis* strains were detected by BOX-PCR, meanwhile 32 and 26 patterns were found by RAPD analysis and $(GTC)_5$-PCR, respectively. The banding patterns were similar among the strains isolated from the same animal species/breed. Cluster analysis of the banding patterns detected 4 major clusters having 60 to 80% similarity of the banding patterns in each cluster, and the clusters were separated by the host animal species/breed. On the other hand, the amylase activity was different among the strains, and high- or low-amylase activity groups were detected by cluster analysis of the amylase activity. However, clear correlations between the amylase activity and the genotype or the isolation source were not found. These findings suggest that genotypically and physiologically heterogeneous strains of *S. bovis* were distributed in the rumen of wild deer and domesticated animals. Although the genotypic diversity of *S. bovis* strains can be related to the different dietary conditions or individualities, each animal species/breed might harbor unique genotypic member of *S. bovis*.

Key words: rumen, *Streptococcus bovis*, genotypic diversity

Microecology of *Olsenella umbonata* sp. nov. and *Veillonella magna* sp. nov., two novel lactic acid-syntrophic bacterial species from the mucosal jejunum of a healthy pig

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Despite its importance for host nutrition and health, the metabolic functioning of individual bacterial species in the jejunal mucosa-associated microhabitats of pigs is largely unknown. In the course of polyphasic taxonomic studies, the present work aimed at elucidating the ecotypes of four isolates of novel *Olsenella* and *Veillonella* spp. from porcine mucosal jejunum. Genomic studies included 16S rRNA and *dnaK* gene sequence analysis and DNA-DNA hybridizations. Ecological information was further gained from cellular fatty acid (CFA), physiological and biochemical analyses. The *Olsenella* strains formed a genomically coherent group with the sheep rumen isolate A2 which, therefore, was included in the description of *O. umbonata* sp. nov. A2 differed mainly in its CFA profile. The strain of *V. magna* sp. nov. was closely related only to *V.* sp. MY-P9 from porcine intestine. The novel species are microaerotolerant anaerobes with growth at O_2 levels of up to ca. 5 %. Their temperature and pH growth optima also correspond to the conditions of the pig mucosal jejunum. They grow on porcine gastric mucin and in 20 % bile (*O. umbonata*). *O. umbonata* is markedly peptidolytic and ferments epi-, mono- and dimers of a-glucose and fructose. It produces D-lactic acid and minor amounts of ammonia, formic and acetic acid. *V. magna* is weakly peptidolytic, asaccharolytic and forms propionic and acetic acid from lactate and also H_2S. The novel species are apparently indigenous to the pig jejunum. *V. magna* exhibits a greater degree of host species specificity, while *O. umbonata* may be in a process of diversification into specific subpopulations. *O. umbonata* is well-adapted to the utilization of dietary ¢fast food¢ and may negatively interfere with the pig endogenous N and carbohydrate metabolism. *V. magna* exerts less direct nutrient competition. During digestion, *O. umbonata* qualifies as ¢moderately antagonistic parasite¢ and *V. magna* as ¢protective commensal¢. In the interdigestive jejunum, they likely sustain ecostability by peptidolysis (*O. umbonata*) and lactate removal (*V. magna*) in the cooperative bacterial fermentation of mucins. In this respect, they are mutualists with protective benefits. The competition of *O. umbonata* during digestion exemplifies how the microbiota-host relationship is context-dependent and a mixed blessing.

Keywords: jejunum, lactic acid, pigs, symbiosis

Novel Bacteroidetes Dominate the Gut Microbial Community of Wild Manatees Revealed Using 454-pyrotag Sequencing

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Manatees and dugongs (Order Sirenia) are the only known herbivorous aquatic mammals. They are hindgut fermenters and possess a high cellulose digestion efficiency, which may be attributable to resident gut microorganisms. To understand the composition of gut microbial communities of manatees as well as its relationship to geographic location, a comparative study between wild manatees (T. manatus) from Belize and Florida was carried out. Fecal bacterial and archaeal (methanogenic) communities were analyzed by barcoded pyrosequencing and cloning of 16S rRNA genes, respectively. In total, 4543 OTUs were observed within all the manatees by pyrosequencing; 290 OTUs, mainly belonging to the Bacteroidetes phylum, were unclassifiable at the class level while 92 of the OTUs had low similarity (<90%) with any yet known 16S rRNA gene sequences. Bacteroidetes (63.5±9.5%) rather than Firmicutes (32.3±8.5%) were found to be the most predominant bacterial phyla in all manatees. T-RFLP, DGGE and pyrosequencing results showed that gut bacterial communities of these two groups of manatees were clearly different. Rarefaction analysis revealed that the bacterial diversity of Belize manatees (BMs) was consistently lower than that of Florida manatees (FMs). The methanogenic communities of these two groups were also significantly different (P=0.001). Belize and Florida manatees were dominated by methanogens related to *Methanobrevibacter ruminantium* and *Methanobrevibacter smithii*, respectively. Unifrac analysis revealed that the gut bacterial community of manatee was very different to that of the dugong and other phylogenetically related Afrotherian mammals. These results demonstrate that manatees harbor unique gut bacterial populations that are shaped by this endangered host species and likely contribute to their efficient digestion and utilization of aquatic herbage.

Biochemical characterization of multiple cellulose degrading enzymes from the rumen bacterium *Ruminococcus albus* 8

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Ruminant animals rely on the microorganisms inhabiting the rumen to metabolize ingested feed to short chain fatty acids that constitute the majority of their energy demands. *Ruminococcus albus* 8 is a bacterium that was isolated from the rumen and shown to efficiently utilize cellulose, as well as hemicelluloses, as the sole carbon source. Cellulose, a polymer of glucose units linked by β-1,4 glycosidic bonds, is resistant to hydrolysis due to the extensive hydrogen bond network that leads to formation of microfibrils of high tensile strength. Several classes of enzymes are required to work in concert to effectively deconstruct cellulose. Of importance are the endoglucanases, exoglucanases (cellobiohydrolases) and β-glucosidases. The endo-glucanases (EC 3.2.1.4) randomly cleave internal linkages that are found in amorphous (non-crystalline) regions to generate reducing end and non-reducing ends. These regions are attacked by exoglucanases (EC 3.2.1.91)

that processively cleave cellobiose or longer cello-oligosaccharides. The endproducts of the exo-acting enzymes are then further hydrolyzed by β-glucosidases (EC 3.2.1.21) or cellodextrinases (EC 3.2.1.74) into glucose. A detailed bioinformatics search of the *R. albus* 8 genome identified 15 genes that were predicted to be involved in cellulose degradation. Several of these genes (9 in total) have been cloned and overexpressed in *E. coli*. The gene products include seven putative GH family 5 endo-glucanases, and two putative exo-glucanases from GH families 5 and 9. The endo-glucanases vary in the modular structure of the polypeptide, with several enzymes including one or more carbohydrate binding modules. The seven GH5 enzymes have been shown to degrade cellulosic substrates such as the model crystalline cellulose Avicel, phosphoric acid swollen cellulose, and carboxymethyl cellulose (CMC). Final products of hydrolysis include mainly cellobiose and cellotriose. The exo-glucanases release cellobiose as the major product during hydrolysis of cello-oligosaccharides. When all of the enzymes were co-incubated on Avicel or phosphoric acid swollen cellulose, synergistic release of reducing ends was observed. Our results provide insights into how R. albus 8 effectively degrade recalcitrant cellulose into cello-oligosaccharides that can be subsequently fermented by the bacterium.

Fast methods for taxonomic assignment of metagenome sequences.
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Next generation sequencing allows to extensively survey the genome-wide genetic diversity of microbial communities, including those from the human gastrointestinal system. A major challenge is the development of computational methods for the analysis of the large-scale data sets resulting from shotgun sequencing of complex microbial communities. In our research we focus on the development of such techniques, using statistical learning and phylodynamic methods.

An important problem in metagenome analysis is identification of the taxonomic origin for the fragments of the sequenced community. This allows to subsequently analyze the resulting taxonomic "bins" in terms of their functional capabilities and to generate hypotheses about the capabilities and interactions of individual populations and higher-level evolutionary groups within the respective system. For this problem we have developed a novel new state-of-the-art machine learning method with sequence composition based features. The proposed method allows the rapid analysis of large datasets, which is a critical advantage for the analysis of the next generation sequencing datasets. Furthermore, we are working on methods for inference of functional and phenotypic relationships between protein families and seeking to detect and understand the imprint of selection from population-level genomics data.

Keywords: Metagenomics, computational biology, taxonomic sequence assignment, binning, functional inference

Xylan degrading enzymes of *Ruminococcus albus* 8

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Ruminants depend on microbial breakdown and metabolism of polysaccharides from plant matter, such as grasses, weeds, hay and silage to generate for their utilization nutrients in the form of microbial biomass and short chain fatty acids. Thus, ruminants have evolved a microbial consortium that works in concert to degrade a large variety of plant cell wall polysaccharides. Whereas some rumen bacteria degrade only one type of polysaccharide, *Ruminococcus albus* 8 has evolved the ability to degrade and utilize cellulose and hemicellulose, the two major components of plant cell wall.
A partial genome sequence of *R. albus* 8 revealed that this bacterium harbors multiple genes encoding polypeptide sequences with similarities to glycoside hydrolase (GH) family 10 and family 11 endoxylanases. Seven of these genes were overexpressed in *E. coli* BL21 codon plus and their biochemical activities were experimentally verified. The GH10 and GH11 enzymes exhibited different patterns of hydrolysis with soluble wheat arabinoxylan, oat spelt xylan, and birchwood xylan as substrate. An α-L-arabinofuranosidase, a β-xylosidase, and an α-glucuronidase were expressed from hitherto uncharacterized genes from *R. albus* 8. These three gene products were predicted to act as accessory enzymes of the endoxylanases to completely breakdown xylan substrates. By coupling hydrolysis of the three enzymes to the *R. albus* 8 endoxylanases we demonstrated synergistic activities that led to extensive hydrolysis of the three model xylans. The GH10 endoxylanases functioned together with the accessory enzymes to release more endproducts from the simpler substrate soluble wheat arabinoxylan compared to the family GH11 endoxylanases. In contrast, the GH11 endoxylanases working together with the accessory enzymes released more products from the more complex substrates oat spelt xylan and birchwood xylan compared with the GH10 endoxylanases. Our data, therefore, suggest that the multiple endoxylanases found in the rumen bacterium R. albus 8 aid the microorganism in maximizing its capture of nutrients from complex plant cell wall polysaccharides.

Investigating the *in vivo* interactions between *Fibrobacter succinogenes* and cellulolytic ruminococci

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Fibrobacter succinogenes, Ruminococcus albus and *R. flavefaciens* are the three major cellulolytic species of the rumen. *In vitro* studies have shown that these species compete for adherence to, and growth upon, cellulose. Yet the relationship of these studies to their interactions *in-vivo* remains unknown. Therefore, we sought to determine how these species interact within the rumen among a constrained microbiome lacking protozoa and fungi. Seventeen hours following

birth, two lambs were removed from their mother and reared on sterile alfalfa pellets under standard gnotobiotic conditions over a seven-month period. Initial experiments revealed no cellulolytic activity, and the lambs were subsequently inoculated with *F. succinogenes* S85. At five months of age, the lambs were inoculated with *R. albus* 8 and *R. flavefaciens* FD-1. Fibrolysis was estimated *in sacco* using both alfalfa and wheat straw. Select samples were also subjected to metagenome sequencing and RNAseq using an Illumina Hi-Seq 2000. Metatranscriptome data was sorted to the metagenome and the available genomes of each inoculated organism. *F. succinogenes* was found at high levels initially but was gradually outcompeted following the inoculation of the *Ruminococcus* strains. This shift resulted in a 100-fold increase in methanogen density and alterations in the VFA profile. By 33 weeks, *R. albus* 8 was the dominant cellulolytic strain followed by *R. flavefaciens* FD-1. These densities reflected their relative contributions to the mRNA pool. In all ~0.1% of mRNA transcripts detected at week 33 matched cellulases from each of the inoculated organisms, with 87% matching cellulases of *R. albus*. This caused increases in rumen CMCase and xylanase specific activities of 5 fold and 3 fold respectively. The estimated *in sacco* colonization of alfalfa and wheat straw reflected the distribution of the three cellulolytic strains. Alfalfa degradation was maximal in the presence of all three strains, whereas maximal wheat straw degradation depended on high levels of *F. succinogenes*. In conclusion, this study provides a comprehensive view across multiple levels of the *in vivo* symbioses among the three major ruminal cellulolytic species and physiological information on how these interactions modify rumen fermentation parameters and affect plant cell wall digestion.

Key words: rumen, gnotobiotic lambs, cellulolytic bacteria, metatranscriptomics.

Vitamin Requirements of the Bile Acid-Dehydroxylating Intestinal Bacterium *Clostridium scindens*

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The human gastrointestinal tract harbors a complex microbiota compared to the other parts of the body. The impact of the indigenous microbiota on host physiology is most pronounced in the colon, where the primary bile acids chenodeoxycholic acid and cholic acid are converted, via 7a-dehydroxylation, to the toxic secondary bile acids lithocholic acid and deoxycholic acid, respectively, by *Clostridium scindens*, an obligate anaerobe that resides in the human gut. Interestingly, other than bile acid dehydroxylation, little is known about the basic physiology of *C. scindens*. Understanding the metabolism of *C. scindens* will hopefully provide much-needed information as to how this gut bacterium impacts human health and disease. The goal of our study was to determine the vitamin requirements of *C. scindens*. *C. scindens* VPI 12708 was routinely maintained in anaerobic BHI broth medium at 37°C. When *C. scindens* was transferred from the BHI medium to a defined medium (DM; 25 mM glucose, minerals, metals, bicarbonate, 100% CO_2 gas phase, and cysteine), growth was negligible. Only when DM was supplemented with a vitamin mix (p- aminobenzoate, biotin, cyanocobalamin, folate, lipoate, nicotinate, pantothenate, pyridoxal, riboflavin, and thiamine) and an amino acid mix (alanine, arginine, asparagine, aspartate, cystine, glutamate, glutamine,

glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) was growth observed. The leave-one-out technique was subsequently used to determine the specific vitamin(s) required for growth. With this technique, three vitamins (biotin, riboflavin, and pantothenate) were found to be essential for the growth of *C. scindens*. Growth was also reduced when pyridoxal or lipoate was absent from DM. This approach is now being employed to resolve the amino acid requirements of *C. scindens*.

Investigating rumen microbial community structure by molecular fingerprinting methods.

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Terminal-restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis (DGGE) were compared and contrasted to determine the sensitivity, consistency and compatibility of these methodologies on subsequent data interpretation. Microbial community structure of both the solid and liquid phases of rumen content were compared under two contrasting diets. Samples were obtained from four ruminally cannulated heifers fed both a high-concentrate control diet (83:15:0) barley grain: barley silage: wheat-based distillers grains (DM basis) and high-concentrate, no-forage diet (63:0:35) in a randomized crossover design. Fingerprinting protocols were developed to optimize the separation and detection of less abundant ribotypes. The two PCR-based methods, performed with differing 16S rRNA universal primer sets, revealed very different assemblage compositions. T-RFLP was less discriminatory as it generated less ribotypes for each sample compared to DGGE. Cluster analysis of binary coefficient data using Jaccard's and UPGMA showed no significantly similar clusters between the two fingerprinting techniques. DGGE profiles tended to group within an animal on a specific treatment, whereas T-RFLP showed higher similarities between liquid or solid fractions within an animal regardless of diet. One-way ANOVA analysis on Pielou's, Shannon-Weiner and Simpson's indices showed significantly higher values for DGGE compared to the T-RFLP results. Use of principle component analysis (PCA) to assess the compatibility of fingerprinting techniques with identification of key environmental parameters showed that a greater percent of variance in T-RFLP community profiles could be explained by the first four principle components as compared to DGGE. In conclusion, these two microbial fingerprinting methods gave statistically different results, although samples were processed following the same procedure and results were analyzed using similar statistical methods. The results of this study suggest a degree of caution should be used when interpreting ribotype community analysis techniques without additional quantitative analysis.

Keywords: DGGE, T-RFLP, cattle, rumen, forage.

Biochemical characterization of Bi4213, an endoxylanase from the human colonic bacterium *Bacteroides intestinalis*

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Many mammalian species have co-evolved with a microbial consortium, including the phylum Bacteroidetes, in their digestive tract. *Bacteroides intestinalis*, a Gram-negative rod, is a strictly anaerobic bacterium present in the human intestine, and recent genomic data shows that this bacterium contains enzymes that are involved in the degradation of polysaccharides, including xylan. Xylan, a polymer of mostly xylose and found in plant cell walls, contains β-1,4 glycosidic linkages with side-chain substitutions of arabinose, glucuronic acid, acetic acid, and feruloyl esters. The complete hydrolysis of xylan, therefore, requires multiple enzymes, including endoxylanases, arabinofuranosidases, α- glucuronidases, acetyl xylan esterases, and ferulic acid esterases.
The gene product of the *B. intestinalis* gene designated Bi4213 was originally annotated as a hypothetical protein, and its homologs in B. cellulosilyticus and *B. eggerthii* were also similarly annotated. Functional assignment of a related gene product in Prevotella bryantii B14 as an endoxylanase provided insight into the function of the protein encoded by *B. intestinalis* Bi4213. The gene was cloned into pET46b and the plasmid was designated pET46b/Bi4213. The recombinant plasmid (pET46b/Bi4213) was transformed into BL21 Codon PLUS cells. The recombinant *E. coli* cells containing the plasmid were selected on LB plates containing ampicillin and chloramphenicol. A single colony was cultured to an optical density (O.D) of 0.3 and gene expression was induced with isopropyl β-D-thiogalactopyranosyl (IPTG) at 1 mM. Metal affinity chromatography and ion-exchange chromatography were used to purify the recombinant Bi4213 protein, and the purity was examined with SDS-PAGE analysis. The enzymatic activity of the recombinant protein, with a molecular mass of 73.3 kDa, was investigated in different buffers of varying pH. The enzyme showed endoxylanase activity with an optimum pH of 5.5. In a citrate buffer at the optimum pH, the enzyme exhibited a temperature optimum at 35°C. The predicted protein architecture suggested two modules, a GH5 and Ig2-like module, are present in the polypeptide of the endoxylanase. Mutational analysis that examines the contribution of the two domains and studies on kinetic parameters are currently underway and will be discussed.

Nutrition of Livestock, humans and Companion Animals

Exploration of fiber-associated rumen *Prevotella* community and isolation of previously uncultured strains.

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Global analysis of the 16S rRNA gene sequences of the rumen microbiota of ruminants revealed the dominance of the genus *Prevotella*. Previously, we reported the involvement of diverse uncultured *Prevotella* in the degradation of hay and

concentrate diet. In this study, fiber-associated *Prevotella* were targeted to demonstrate the contribution of the genus to fiber degradation in the rumen. Comparison of *Prevotella* 16S rRNA gene clone libraries from rumen contents of sheep fed orchardgrass hay supplemented with 10% concentrate and from in sacco incubated orchardgrass hay stems indicated that the in sacco fiber-associated community was similar to the larger community in rumen contents of hay-fed sheep. On the basis of 95% sequence identity criterion, 101 cloned *Prevotella* 16S rRNA gene sequences that were obtained from the two libraries were grouped into 41 operational taxonomic units (OTUs). Only 17 clones (16.8%) showed 95% or more sequence similarity with *P. ruminicola* and the remaining majority (84 clones, 83.2%) were related to the uncultured *Prevotella*. These results indicated the predominance of the fiber-associated uncultured *Prevotella*. Attempts were made to obtain functional information by cultivating the uncultured *Prevotella*. Freshly collected rumen contents from hay-fed sheep were inoculated into a medium containing glucose, cellobiose or the natural plant material as a sole carbon source. Bacterial strains were obtained from discrete colonies formed in roll tubes and the 16S rRNA gene for each strain was PCR amplified and sequenced. The strains were genotyped by the (GTG)5- and RAPD-PCR, and tested for their fibrolytic enzyme activities. We successfully isolated 41 novel *Prevotella* strains which were distributed in nine OTUs. Among these, seven OTUs (13 strains) were phylogenetically novel. The remaining 28 strains were related to either *P. ruminicola* (22 strains) or *P. brevis* (6 strains). The genetic fingerprints and enzymatic profiles of the novel *Prevotella* were distinct from that of the commonly cultivated species. In particular, the strains belonging to novel OTUs tended to have higher xylanase or CMCase activity than that of the known *Prevotella* species. These findings indicate that the novel *Prevotella* strains have superior fiber degrading potential compared with previously characterized prevotellas.

Keywords: fiber degradation, rumen, novel, *Prevotella*

Mare Prebiotic Supplementation And Foal Fecal Bacterial Communities Before Weaning

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Very few data are available on gut bacterial communities in the foal. It is established that there is a rapid colonization after birth (Sakaitani *et al* 1999; Julliand *et al*, 1996), but it is unknown whether the whole complexity of the digestive population is fully established until weaning. The aim of our work was to describe the bacterial communities in foal feces from birth to preweaning and to test the impact of the prebiotic supplementation to mares. Eight mares and their foals were used. Mares received a daily basal diet of meadow hay *ad libitum* and concentrates (commercial pelleted feed and soybean meal) and were assigned into two groups: supplemented (S) or not (NS) with the prebiotic ingredient (product of lactic fermentation and germination of cereals, F.A.P. technology, Original Process, France) from day 285 of gestation until day 60 after foaling. Foals couldn't access their dams' concentrates. Microbial counts of total anaerobes, lactate-utilizers, amylolytics and cellulolytics using culture-based methods and quantification of total

bacteria and *Fibrobacter succinogenes* (FS) using real time PCR were performed on foal feces from birth (d0) to 180 days of life (d180). Surprisingly, the meconium was not sterile (4.9 log10 cells of total bacteria/g). The supplementation of mares affected the early establishment of total anaerobes (p=0.029). The increase of total anaerobes was higher the first day of life in S group compared to NS group (+6.35 vs +3.76 log10 CFU/g). The counts of lactate-utilizers and amylolytics increased rapidly within the first three days of life to reach a high level plateau (9.9 and 6.8 log10 CFU/g, respectively) and then slowly decreased from d10 for lacticolytics and from d45 for amylolytics. From 2-3 months of life, these bacterial groups were stable but were present in different proportions compared to adults, suggesting that the bacterial community was not yet comparable to those of adult horses. Cellulolytics were first detected around d4 at the dilution 10-1, increased slowly until d30 (5.6 log10 MPN/g at d30) and then were stable until d180. FS was first detected between d3 and d7 (0.06% of total bacteria number). Our results confirmed the early establishment of bacterial communities and suggested that the maternal supplementation with a prebiotic could affect the microbial establishment in the gut of the foal.

Keywords: bacterial communities, foal, maternal supplementation, prebiotic

Long-term defaunation increases the abundance of cellulolytic ruminococci and methanogens but does not affect the bacterial and methanogen diversity in the rumen of sheep

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Protozoa are commensal eukaryotes in the rumen of herbivores. They are high producers of hydrogen which is utilized by methanogenic archaea to produce methane, a greenhouse gas. The removal of protozoa from the rumen (defaunation) decreases methanogenesis, but also negatively affects fiber digestion, which is the main function of the rumen. The aim of this study was to examine the effect of long-term defaunation on the structure of the microbiota and particularly methanogenic archaea and fibrolytic bacteria in order to better understand the microbial mechanisms responsible for the decrease in methanogenesis and fibrolysis. The trial was conducted in five adult sheep subjected successively to long-term defaunation (2 years), refaunation (12 weeks) and short-term defaunation (10 weeks). Methanogens were enumerated by quantitative PCR targeting the *rrs* and *mcrA* genes. The *rrs* gene was used to quantify the three major culturable rumen cellulolytic bacterial species, *i.e. Fibrobacter succinogenes, Ruminococcus albus*, and *Ruminococcus flavefaciens*, and total bacteria. Bacterial and methanogen diversity was also examined by PCR-DGGE analysis targeting the *rrs* and *mcrA* genes, respectively. Total rumen bacterial density estimated as *rrs* copies per gram DM of rumen content increased in response to long- and short-term defaunation (+ 1 Log, $P < 0.001$), but without noticeable shifts in diversity. Defaunation increased the *rrs* copies per gram DM of rumen content of *R. albus* and *R. flavefaciens* (+ 2 Log, $P < 0.001$), but did not affect that of *F. succinogenes*. Despite a 20% reduction in methane emission in the two defaunated periods, the *mcrA* and *rrs* copies of methanogens per gram DM of

rumen content increased (+ 1 Log, $P < 0.001$) in the absence of protozoa, whereas the diversity of the dominant methanogenic community was not modified. This study shows no major difference between long- and short-term defaunation in abundance and diversity of bacteria and archaea. It also provides evidence that monitoring the abundance and diversity of methanogens is not sufficient to comprehend the microbial mechanisms leading to a reduction in methane emissions by ruminants. This study also reports for the first time in sheep a selective effect of defaunation on the abundance of cellulolytic bacterial species.

Keywords: rumen, defaunation, cellulolytic bacteria, methanogens.

Effect of wet distiller's grains with solubles on rumen bacterial community profiles in individually fed cattle

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The production of ethanol for use as a biofuel from corn crops throughout the US has led to a decreased availability of corn for use in the beef cattle industry. The coproduct of the ethanol industry, wet distiller's grains with solubles (WDGS) is being used as a replacement feed; however, very few data are available that describe potential rumen fermentation and microbial ecology alterations that may occur by feeding high concentrations of WDGS. These potential changes can alter the health and performance of the host animal; therefore, the objective of this experiment was to evaluate the effects of WDGS on ruminal bacterial communities. Twenty-three steers (average BW = 340 ± 29.6 kg) were acclimated to steam-flaked corn finishing ration, then randomized and assigned to one of three treatment groups. Cattle were individually fed once per day. Treatments were replacement of steam-flaked corn with 0, 30, or 60% WDGS (dry matter basis; n = 7, 8, and 8, respectively). Ruminal fluid was collected once per wk for 5 wk via esophageal tubing before feeding. Ruminal fluid community DNA was extracted, 16S rDNA was amplified using PCR and analyzed by denaturing gradient gel electrophoresis (DGGE). Clustering of DGGE banding patterns was normalized to an external standard and compared based on binary and numerical coefficients of Dice and Pearson, respectively. Binary banding patterns for all samples were 59.8% similar and total number of bands per sample was not influenced by treatment (P = 0.96). Analysis of treatment dendrograms for binary banding pattern revealed a decrease in similarity from 30 to 60% WDGS, with 0% being intermediate (68.5, 71.2, and 59.2 ± 7.09% for 0, 30, and 60% WDGS, respectively). Banding pattern similarity decreased during the duration of the experiment (73.4, 80.4, 72.8, 60.0, and 61.9 ± 8.65% for wk 1-5, respectively). Construction of dendrograms based on band intensity resulted in a dramatic reduction (19.2 ± 12.32%) of similarity across treatments. These results indicate feeding high levels of WDGS does not decrease the richness of the bacterial population but shifts in individual bacterial community members do occur.

Keywords: cattle, wet distiller's grains, rumen bacteria

Associations between fiber digestion kinetics and bacterial genera in muskoxen rumen

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The objective of this study was to relate fiber digestion in muskoxen to the abundance of bacterial genera, in order to identify novel organisms and enzymes for ruminal digestion. Muskoxen were fed brome hay (medium quality diet) or triticale straw (low quality diet) in a crossover design during both spring and fall 2009. Each experimental period consisted of 3 weeks adaptation, followed by 1 week of measurements and sampling. Kinetics of fiber digestion were measured by incubating milled (1-mm mesh) hay or straw in polyester bags to determine *in situ* rate and 120 h extent of digestion for NDF, cellulose and hemicellulose. Ruminal samples were taken before daily feeding, solid and liquid fractions were separated, and DNA extracted from the solids. We used 16S rRNA gene amplicon 454 pyrosequencing and a customized Silva database to assign 145,000 bacterial sequences to genera. Fiber digestion and bacterial populations were affected by season and diet. Partial least squares regression was used to identify bacterial genera associated with fiber digestion. Out of 79 genera, 41 (hay) and 39 (straw) were deemed important (variable importance > 0.8) in models for rate of cellulose digestion. Similarly, 41 genera were important for hemicellulose digestion rate both in hay and straw. The most important genera positively associated with fiber digestion were *Catabacter*, *Catenibacterium*, *Clostridium*, *Papillibacter*, RC9 gut group, *Ruminobacter* and *Barnesiella*, whereas *Roseburia*, *Coprococcus*, *Pseudubutyrivibrio*, *Thalassospira* and *Quinella* were the most important genera negatively associated with fiber digestion. Simple linear regression confirmed these relationships. Digestion of fiber in muskoxen is therefore associated with a complex bacterial community with both positive and negative interactions. Because this analysis does not demonstrate causal relationships, the biological basis of these interactions would need to be elucidated. Further analysis of genomic sequences could be used to explore functional relationships among the organisms and their substrates.

Reduction in dietary wet distillers grains alters *Escherichia coli* O157:H7 in feces of cattle

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Cattle fed finishing diets with distillers grains have been shown to more often have *Escherichia coli* O157:H7 in the feces and on the hides. The objectives of this study were to determine if pathogen levels and prevalence differ at higher level of wet distillers grains with solubles (WDGS) in the diet, if switching to a predominantly corn diet reduces pathogen load, and how long corn diets should be fed before reductions in pathogen load are observed. To accomplish these objectives, 608 heifers (20 pens, 27-33 animals per pen) were sorted into 1 of 5 treatments, and fed 0, 40, or 70% WDGS (DM basis). For 3 of the treatments, WDGS was reduced

midway through the study. Treatment 0W0W (positive control) was corn grain fed continuously, and 40W40W (negative control) was 40% WDGS fed continuously. Treatments 40W0W, 40W15W and 70W15W were fed either 40% or 70% WDGS for the first 56 days, and switched to 0% or 15% WDGS, respectively, for the last 56 days. Samples of feces from each animal were collected 56 days before, day of, and up to 56 days after the dietary switch for the enumeration and detection of *E. coli* O157:H7. After the dietary switch, animals fed 0W0W diets had the lowest fecal prevalence and percent enumerable samples (10.2 and 3.23%), whereas animals fed 40W40W had the highest fecal prevalence and percent enumerable samples (70.1 and 29.2%; $P < 0.05$). Animals switched to lower levels of WDGS with the 40W0W, 40W15W, or the 70W15W, had lower fecal prevalence and percent enumerable samples (33.4 and 6.3%, 31.03 and 9.7%, 34.9 and 8.4%; $P < 0.05$) compared to the animals fed the 40W40W diet. Time after dietary switch was important, as animals fed lower levels did not have significantly lower fecal prevalence and percent enumerable samples until 56 days after the dietary switch. The study indicates that cattle can be switched to lower levels of dietary WDGS (15% or less) 56 days prior to slaughter to significantly reduce the *E. coli* O157:H7 load.

Keywords: cattle, diet, distillers grains, *Escherichia coli* O157:H7

Prebiotic, probiotic and DFM Development

Fibre colonization and degradation by rumen microbiota in the presence of a live yeast, *Saccharomyces cerevisiae* I-1077.

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The inclusion of live yeasts in ruminant diets can have significant effects on ruminal fermentation, host productivity and health. One of the expected benefits is improved fibre digestion, which may be achieved through increased growth and activity of the fiber-degrading community. We investigated the effect of live yeast on microbial colonization and degradation of alfalfa hay in 3 rumen-cannulated cows. Nylon bags containing 5 g of alfalfa hay were incubated in the rumen in the absence of yeast (control period, 3 weeks) and in the presence of *Saccharomyces cerevisiae* I-1077 (Levucell SC20, 10^{10} cfu/day/cow, SC period, 3 weeks). During the last week of each period, triplicate bags per incubation time were introduced into the rumen at the morning feeding and removed after 2, 6, 12 and 24 hours, with this sequence being repeated once. For each incubation time and each cow, residues from two bags were analysed for DM and NDF content. Total DNA was extracted from the residues recovered from the last bag and from total rumen contents. *Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens* and total fungi were quantified by qPCR. Fiber-degrading bacteria colonized alfalfa hay particles more rapidly than fungi, which were not very numerous at the early phase of incubation. Fibrolytic species were more abundant on alfalfa particles than in rumen contents, but the same species hierarchy was preserved (*F. succinogenes* > *R. flavefaciens* >> *B. fibrisolvens*). In the presence of

SC, the abundance of *B. fibrisolvens* and *R. flavefaciens* associated with alfalfa hay particles increased (P £0.05). No significant effect was observed on colonization by *F. succinogenes*. Adherent fungal biomass was numerically increased in the presence of SC after 6 and 12 h of incubation. In parallel, DM and NDF degradation were improved (P £0.05), the rate of degradation being particularly stimulated (3-fold increase in NDFd after 2 hours of incubation). Our study shows that the live yeast *S. cerevisiae* I-1077 enhanced colonization of fibrous materials, which could explain its positive impact on NDF degradation. Several factors promoting fibrolytic microorganisms (supply of nutrients or growth factors, improvement of anaerobic conditions) could be involved.

Keywords: rumen, live yeast, microbial colonization, fibre digestion, qPCR.

Effect of pH and temperature on the shelf life of *Megasphaera elsdenii* NCIMB 41125.

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Microbial products (probiotics, direct-fed microbials) often depend on extended shelf life for convenient distribution. Various preservation methods are used to extend shelf-life, the most common being low temperature (£ 5 °C), freeze-drying and pH adjustment. Due to the lack of a suitable freeze drying protocol for *Megasphaera elsdenii*, alternative methods to extend shelf-life (i.e., temperature and pH) were investigated in this study. A batch culture of *M. elsdenii* NCIMB 41125 was grown in a fermenter, with working volume set to 2 liters. After 24 h of batch cultivation, continuous cultivation was initiated. The culture was allowed to reach a steady state after which it was continuously harvested for 5 h into a vessel allowing control of pH and temperature. Seven hours after harvesting commenced, samples were removed from the harvest vessel and placed in glass bottles and stored anaerobically. When investigating the effect of different pH values, the temperature of the harvest vessel was controlled at 22 °C. Conversely, when temperature was investigated, the pH was controlled at 5.5. The temperatures investigated were 5°C, 10°C, 22°C and 30°C. The pH range investigated was from 4 to 7 with 0.5 increments. Viable cell counts were determined with the pour plate technique during the shelf life period. Only pH 6.0 resulted in acceptable survival (21%) of *M. elsdenii*. Survival was only 12.4% at pH 5.5 and was less than 1.5% for the remaining pH values. Only a temperature of 22°C gave acceptable survival (12.3%), with other temperatures having survival rates below this. It is concluded that maintaining *M. elsdenii* NCIMB 41125 at pH 6.0 after harvesting is beneficial for survival. Furthermore, in contrast to the general notion that low storage temperatures slow down microbial activity, leading to increased shelf life, *M. elsdenii* NCIMB 41125 appeared to maintain viability better at around room temperature.

Keywords: pH, temperature, probiotic, *Megasphaera elsdenii*, survival.

Intestinal microbiota of broiler chickens administered a *Bacillus* based DFM and raised on used litter from commercial farms with different disease histories

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The gastrointestinal tract (GIT) is inhabited by a substantial population of microorganisms that contribute to the health and development of their animal host. This relationship is primarily mutualistic, with the host providing colonization sites and substrate while the resident microbiota helps prevent the establishment of pathogens and hydrolyze otherwise inaccessible substrates. Reports demonstrating the beneficial effect of probiotics, or direct-fed microbials (DFMs) in the case of livestock, on health and the GIT microbiota have begun to accumulate. The purpose of this study was to determine whether GIT microbial communities were altered in broiler chickens fed a *Bacillus* based DFM and raised in a controlled setting, but on used litter from farms with and without a history of clostridial gangrenous disease (GD). Four dietary treatments were included; 1)basal corn-soy diet, 2) basal diet plus DFM, 3) basal diet plus salinomycin, and 4) basal diet plus DFM and salinomycin. Three different types of litter were utilized; 1) used GD positive, 2) used GD negative, and 3) unused fresh pine shavings. At days 14, 28, and 45 post-hatch, intestines from five broilers were sampled from each of the 12 treatment combinations. Intestinal mucosa was extracted from each bird and pooled by treatment and age prior to molecular characterization. Bacterial tag-encoded FLX amplicon pyrosequencing targeting the variable 16s rDNA regions immediately downstream of the 8F conserved sequence tag was utilized to characterize the mucosa associated microbial communities of the broiler small intestine. Family level classification of the 270,944 total bacterial sequences analyzed indicated a predominance of *Clostridiaceae* in most 14 day old samples with a shift toward a greater abundance of *Lactobacillaceae* in the intestinal mucosa of broilers at days 28 and 45. While most sequences classified as *Lactobacillaceae* were of the genus *Lactobacillus*, the majority of sequences classified as *Clostridiaceae* were representative of the segmented filamentous bacteria, *Candidatus arthromitus*. Interestingly, the broilers receiving the Bacillus DFM consistently had higher proportions of *Lactobacillus* sequences at day 14 compared with treatments receiving the basal diet alone or supplemented with ionophore only. Differences in GIT microbiota were also observed among broilers raised on the different types of litter.

Pyrosequencing Analysis of Bacterial Diversity Associated With The Intestinal Tract Of Tilapia (*Oreochromis niloticus*) Supplemented With Oreganum Essential Oil in a Colombian tropical ecosystem

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Evidence indicates that the gastrointestinal microflora of tilapia (*Oreochromis niloticus*) is highly variable and is a reflection of their aqueous environment, which can introduce excreted gastrointestinal bacteria in with their food. Antibiotics commonly used to control bacterial diseases in fishes have generated resistance in pathogens. In Colombia, the oregano essential oil (OEO) has been explored as a new additive and an alternative instead of using antibiotics; because OEO contains antioxidants and antimicrobial properties, it can help boost the immune system. In this study, the effect the OEO on the bacterial diversity associated with the intestinal tract of tilapia in a Colombian tropical ecosystem was evaluated by supplementing with different ratios of carvacrol: thymol, which are the main chemical components from OEO. The experimental design was carried out in an experimental farm of the Colombian National University, located at 467 m. s.l.m. (Villavicencio, Colombia). The animals were fed with a basal diet with 200 ppm of OEO with different ratio of carvacrol:thymol as following: T1 = 0.15, T2 = 3.25, T3= 6.5, and a treatment control without OEO. The intestinal tracts were aseptically sampled from 24 adult tilapias. The total DNA was extracted, followed by amplification of the 16S rDNA using PCR and total bacteria quantification using Q-PCR. The microbial diversity was described by 454 pyrosequencing analysis targeting the hypervariable v8-v9 regions of the 16S rRNA genes. Q-PCR data revealed no difference ($P > 0.287$) between treatments, with an overall average of 3.91 ± 0.62 [Log_{10} bacterial DNA (ng/g dry matter)]. However, the approach of 16S rDNA amplicon pyrosequencing showed clusters associated with different communities between treatments, identifying 10 phyla, 63 families and 158 genera of bacteria. The phylum Fusobacteria was the most representative in all treatments, with 54.5%, followed by the phylum Proteobacteria, with 38.6% of the total community. In terms of families, Fusobacteriaceae represented 54.6%, followed by the family Enterobacteriaceae, with 26.5%. The most representative genera were Plesiomonas and Cetobacterium, being 45.9 and 15.1%, respectively. The results demonstrated that the bacterial community of the intestinal tract of tilapia was effecting by carvacrol: thymol ratios from OEO and that barcoded pyrosequencing is a promising and cost-effective method for analyzing microbial diversity compared with conventional approaches.

Evolution of the equine gut bacterial community according to dietary changes and live yeast supplementation as assessed by Automated Ribosomal Intergenic Spacer Analysis (ARISA)

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To satisfy energy requirements of exercised horses, increased amounts of concentrate are fed. By modifying the gut microbial balance these feeding practices increase the risk of gastrointestinal diseases incidence. Therefore, new feeding

strategies including probiotic supplementation are proposed, in order to maintain gut health and integrity. The effect of live yeast probiotics was investigated on bacterial community structure of horses fed a high concentrate diet. Six fistulated adult horses were divided into three groups in an experimental design during 9 weeks. Each group was first fed a forage (F) diet during one week. The following two weeks, group G1 was fed F diet, group G2 : F diet and probiotics (FP; Levucell SC20), and group G3 : high concentrate diet (C; 50% forage: 50% concentrate). At weeks 4 and 5, G1, was fed diet C, but G2 and G3 were both fed the C diet containing probiotics (CP). For the last fourth weeks all groups switched back to F diet. Colonic and fecal samples were collected during the last week before each dietary shift. Diet and probiotic effects were investigated with ARISA, on DNA pools constituted with colonic or fecal DNA extracts of each sampling time per group. Irrespective of the group and diet considered, no sampling site effect was observed, indicating that bacterial structure was similar between colon and feces ($P=0.08$). For each group a diet effect was measured: the bacterial community structure of all F diet periods was significantly different from those of C and CP diet ($P<0.0001$ and $P=0.002$, respectively). That of FP diet could not be analyzed. No difference was observed between the bacterial community structures of diets C and CP ($P>0.05$), suggesting that probiotic supplementation did not limit the changes of bacterial community structure during the period of concentrate feeding. However, the dendrogram generated by clustering analysis showed the grouping of the ARISA pattern obtained from colonic CP sample of G3 with bacterial profiles. As G2 horses received live yeasts before and during the concentrate feeding period, our data suggest that probiotic supplementation may prevent imbalance of the bacterial community that can occur with high concentrate diets. However, more molecular biological studies are needed to validate a potential benefit of live yeasts on the horse gut microbial balance.

Keywords: equine gut bacteria, automated ribosomal intergenic spacer analysis, live yeast probiotics

Research

Determination of the Concentration of the Efflux Pump Inhibitor, Pheophorbide in the Feces of Animals

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Background. Efflux pumps are vital components of antimicrobial resistance in bacteria. When bacteria are exposed to antimicrobials, these pumps often serve as an initial defense mechanism. Efflux pump inhibitors (EPI) are recognized as important therapeutic adjuvants, and discovery of new inhibitors is an active area of research. Recent screening work has demonstrated that some plant compounds and their digestive metabolites also possess EPI activity. In particular, pheophorbide a (php), a metabolite of chlorophyll possesses EPI activity. In studies with *Staphylococcus aureus*, php reduced the minimum inhibitory concentration of

ciprofloxacin several fold (10-100x), indicating increased susceptibility. Given that php is a normal metabolite of chlorophyll in the G.I. tract of animals, we wished to determine if the quantity of php present in feces would be of sufficient concentration for EPI activity. Methods. Feces were collected from several individuals (n>3) of different species of laboratory and farm animals being fed a wide variety of animal feeds. The fecal material was dried, ground and extracted with 2-propanol. The chlorophyll metabolites, php and pyropheophorbide (pyp) were determined in the fecal extracts using fluorescent spectroscopy (excitation = 420 nm; emission = 673 nm). Peak emission (673 nm) of authentic standards of php and pyp was used for preparation of a calibration curve and for quantification. Results. The average concentration of php/pyp in feces were as follows: guinea pig, 185 µg/g DM feces; goat, 155; rabbit, 151; cow, 122; rat, 2.5; pig, <1. Concentrations were in part diet dependent, and low chlorophyll diets produced correspondingly low levels of php and pyp in feces. Conclusion. These data indicate that animals consuming diets with adequate levels of green plant material will excrete feces with concentrations of php that exceed levels demonstrated to be inhibitory to bacterial efflux pumps (0.5 µg/ml). The natural presence of php and its properties as an EPI may exert a selection pressure upon microbial populations dependent upon efflux pump activity. The EPI activity of php may also influence the level of antimicrobial resistance expressed in the GI tract environment. Given these data, further research is warranted.

Keywords: efflux pump, pheophorbide, antimicrobial resistance

The phylogeny of hydrogenotrophic microbes distinguishes native Africans from African Americans

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Compared to other U.S. racial and ethnic groups, African Americans (AA) have the highest incidence and mortality from sporadic colorectal cancer (CRC), the second leading cause of cancer death in Western countries, while native Africans (NA) are rarely diagnosed with the disease. Reduced susceptibility to CRC in NA is associated with low consumption of animal products and greater microbial production of colonic methane. In this context, two hydrogenotrophic microbial groups are of interest, methanogenic archaea (MA) utilizing H2 to produce methane and sulfate-reducing bacteria (SRB) generating hydrogen sulfide, which has been linked with chronic inflammatory disorders of the colon. The present study characterized and compared MA and SRB in stool from 12 NA and 12 AA. Molecular approaches targeting the SRB genera *Desulfovibrio*, *Desulfobulbus*, *Desulfobacter* and *Desulfotomaculum* and Archaea 16S rRNA gene sequences as well as the functional genes dissimilatory sulfite reductase (*dsrA*) and methyl-coenzyme M reductase (*mcrA*) were used. Sulfate reducing bacteria sequences were amplified from all subjects while MA sequences were amplified more often in NA (75%) than in AA (25%). Significantly distinct SRB and MA dominant genotypes were observed in each group. *Desulfotomaculum* spp. were significantly more abundant in AA. Sequences from clone libraries belonging to the *Methanosarcina* genus were

detected for the first time from stool samples. These data further support our prior observations of host genetic background and/or diet influencing hydrogenotrophic community structure. We are in the process of designing taxa-specific qPCR assays targeting the distinct SRB and MA groups observed in the clone libraries. These assays will be used to confirm the differences between subjects of the two groups, and to assess the potential effects of a diet exchange.

Keywords: sulfate-reducing bacteria, methanogenic archaea, African Americans, Native Africans, colorectal cancer

Abundance and distribution of mucosa-associated sulfate-reducing bacteria and methanogenic archaea in the healthy colon and in inflamed and non-inflamed tissues of IBD patients

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Stool samples have been used extensively for gut microbiota composition studies, but they do not adequately represent the mucosa-associated microbiota. An even greater challenge is to characterize low abundant microbial communities from colonic tissues such as hydrogenotrophic microbiota. Here, biopsies from healthy and inflammatory bowel disease (IBD) patients were used to examine the mucosa-associated sulfate-reducing bacteria (SRB) and methanogenic archaea (MA), which have been linked to chronic inflammation and intestinal homeostasis, respectively. The abundance of the two groups was quantified through qPCR targeting the functional genes dissimilatory sulfite reductase (*dsrA*) and methyl-coenzyme M reductase (*mcrA*). Sulfate reducing bacteria were ubiquitously associated with the colonic mucosa in right-colon, left-colon or rectum of twenty-four healthy subjects. The abundance of SRB was similar among colonic regions ranging from $1.1x10^3$ to $6.9x10^{16}$ gene copies.g^{-1}. On the other hand, twenty of the twenty-four subjects harbored significant *mcrA* gene copy numbers in at least one colonic region ranging from $1.1x10^3$ to $1.5x10^{11}$ gene copies.g^{-1}. The two functional genes were detected more often in rectum than right-colon and left-colon. These data indicate that there may not be a direct correlation between the presence of mucosal MA and detection of breath methane since detectable breath methane concentrations greater than 1 ppm have historically been detected in 30-60% of Caucasians, which comprised our subjects. Among these 24 subjects, 5 provided replicate biopsies less than 1 cm apart. Variable *dsrA* and *mcrA* quantities were detected for most of the replicates indicating the likelihood of microheterogeneity in mucosal hydrogenotrophs. Biopsies of inflamed and adjacent non-inflamed tissue from 3 Crohn¢s disease and 2 ulcerative colitis patients were compared for *dsrA* abundance. A greater abundance of *dsrA* was detected in inflamed tissue (4 of 5 patients), consistent with the proinflammatory properties of hydrogen sulfide, the end product of sulfate respiration. Together the data confirm the prevalence of both SRB and MA in colonic mucosa of healthy subjects and are consistent with a possible influence of SRB on chronic inflammation.

Keywords: sulfate-reducing bacteria, methanogenic archaea, Crohn's disease, ulcerative colitis, colonic biopsies

Metabolomic profiling of anaerobic fungus grown in the presence or absence of natural co-cultured methanogen

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The rumen anaerobic fungi are one of the most important microorganisms in the primary digestion of plant fiber and produce formate, acetate, lactate, most of which could be used by methanogens to produce methane. This study aimed to investigate the metabolomic profiling of anaerobic fungi and the effects of natural co-cultured methanogens on the metabolism of anaerobic fungi by nuclear magnetic resonance (NMR) spectroscopy, a powerful analytical technique for metabolomic approaches. Three cultures were used for NMR analysis: 1) mixed co-culture of anaerobic fungi and methangoens enriched from rumen digesta, 2) simple natural co-culture of anaerobic fungus and associated methanogen isolated from the mixed co-culture, and 3) anaerobic fungal culture obtained from the simple natural co-culture. Unsupervised principle components (PC) analysis revealed a good separation between anaerobic fungal culture and co-cultures when investigating the first two PC, but it could not separate the two co-cultures. The data were then analyzed with partial least squares discriminatory analysis (PLS-DA) and orthogonal-PLS (O-PLS). Only O-PLS showed good clustering of the two co-cultures. The variable importance plot constructed from the O-PLS analysis showed that the component at 1.34 ppm was the most important one, followed by the components at 1.94 ppm, 3.94 ppm, 3.50 ppm and 3.42 ppm. The corresponding metabolites for all components were identified based on their chemical shifts and multiplicities with the assistance of publicly available metabolites libraries. The identified metabolites in this study were ethanol, lactate, acetate, succinate, citrate, formate, glucose and sugars. Statistical analysis showed that the mean values of the metabolites in co-cultures were significantly higher than those in the anaerobic fungal culture. Meanwhile, glucose, which was detected in the anaerobic fungal culture, was not observed in co-cultures. The concentrations of sugars in co-cultures were much lower than that in the fungal culture, which demonstrated that the metabolism of anaerobic fungi was improved by addition of co-cultured methangens. However, some of the components from the media were detected in the co-cultures, whereas they were not detected in the anaerobic fungal culture. In conclusion, the water soluble metabolites of anaerobic fungi were formate, acetate, lactate, ethanol and an unknown metabolite ($\hat{I}'$=4.54). The metabolism of anaerobic fungus was shifted by co-cultured methanogens to more formate, acetate, lactate, ethanol and succinate, and especially, citrate was produced only by anaerobic fungus co-cultured with methangens.

Remediation of Swine Slurry Odor and Methane Using a Partitioning Bioreactor

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Large amounts of animal waste are generated in confined animal feeding operations that can potentially pollute air as well as ground and surface waters. Usually these wastes are treated in large earthen lagoons as a means of effecting reductions in wastewater strength before application to fields as fertilizer. More promising technologies developed to remediate volatile and semi-volatile organics from water include two phase partitioning bioreactors and permeable membranes. Here we describe a hybrid bioreactor that removes small molecules by re-circulating slurry though a permeable silicone membrane located in an aerobic tank that also contains a silicone sink that acts as a buffer for potentially toxic molecules. Mal-odorants and methane diffuse through the silicone and are degraded by microbial populations that utilize these molecules as their sole carbon source. In the anaerobic treatment tanks (TT) receiving re-circulated waste, 14.5, 0.8, 0.15 and 0.7 mg L^{-1} d^{-1} of p-cresol, p-ethylphenol, indole, and skatole, respectively, were removed. On the other hand, an average of 2.8 mg L^{-1} d^{-1} more phenol was produced in these tanks than in the control tank (CT). Although the relative reduction in VFA concentrations were not as great as was the case for the aromatic malodors, acetate, propionate, 2-methylpropanoate, butyrate, and 3-methylbutyric removal rates were 1.46, 0.9, 0.8, 0.2 g L^{-1} d^{-1} and 0.01 g L^{-1} d^{-1}, respectively. Microbial community composition was also effected by treatment; although both the CT and TT were dominated by common swine slurry species (*Bacteroides* sp. and *Clostridia* sp.), sequenced clones from the TT included more diverse species such as (*Ochrobactrum* sp., streptococci and iron-reducing bacteria. The system also raises slurry pH (8.0 in TT) and bicarbonate buffering (average 2,850, 3,470, 7,340 mg, and 8,860 mg L^{-1}, respectively, in TT), thereby lowering carbon dioxide concentrations in the slurry tanks biogas. Therefore, even though methane production is similar in both tanks, as are concentrations of methanogenic organisms (1.25 to 2.75 X 10^8 cells ml^{-1} slurry), biogas quality in the slurry tanks is enhanced due to a favorable shift in the carbon dioxide/bicarbonate equilibrium.

Phytochemicals that modulate amino acid and peptide catabolism by caprine rumen microbes

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Background. Microbe-derived ionophores and macrolide antibiotics are often added to ruminant diets, and growth promotion and feed efficiency are among the benefits. One mechanism of action is inhibition of microbes that catabolize amino acids or peptides and produce ammonia. Plants also produce antimicrobial compounds. These experiments were initiated to determine the effects of three categories of phytochemicals on amino acid and peptide fermentation by microbes from the rumina of goats. Methods. Rumen fluid was collected from forage-fed Kiko goats (n=8 wethers) and used to inoculate either peptide- or amino acid-rich

media. Amendments included the following extracts: hops (*Humulus lupulus*), soapbark tree (*Quillaja saponaria*), or red clover (*Trifolium pratense*), which are rich in β-acids, saponins, or isoflavones, respectively. Biochanin-A, a dominant red clover isoflavone, was also tested. Ammonia concentrations were determined at zero and 48 hours. Experiments were performed in triplicate and analyzed with Student□s t-tests. Results. Ammonia production was inhibited by hops β□acids (30 ppm), clover isoflavones (20 ppm), biochanin A (30 ppm) and saponins (0.5%). The combined effect of biochanin A with either saponins or β-acids was similar to that of biochanin A alone, but only when peptides were the fermentation substrate. When amino acids were fermented, the addition of saponins counteracted the inhibitory effect of biochanin A. Furthermore, low concentrations (200 ppb) of saponin/biochanin A mixture caused an increase in the final ammonia concentration (P=0.02). There was a similar trend with the β-acid/biochanin A mixture (P=0.08). Conclusions. These three categories of phytochemicals have the potential to control wasteful ammonia production in the goat rumen. However, there was an apparent hormesis at low doses of phytochemical mixtures, and a differential effect on amino acid and peptide fermentation was observed in one case. Plant metabolites are quite diverse even in conventional forages, and these results indicate that potential interactions among metabolites cannot be ignored

Phylogenetic analysis of the intestinal microbial community in herbivorous land and marine iguanas of the Galapagos Islands using 16S rRNA-based pyrosequencing

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Herbivory in reptiles is rare but herbivorous iguanas can digest 54% of cell wall constituents and supply 30-40% of their energy needs from hindgut fermentation, implicating a symbiotic gut microbiota capable of effectively degrading complex polysaccharides. Marine (*Amblyrynchus cristatus*) and land iguanas (*Conolophus subscristatus* and *C. pallidus*) are endemic to the Galapagos Islands and have contrasting diets. Marine iguanas are sea-going and are the only iguanas that feed on soft macrophytic algae, whereas land iguanas feed on terrestrial plants with high fiber content. We hypothesize that these two types of iguanas would have distinct differences in their gut microbiota. To address this hypothesis, a total of 41 fecal samples were collected from marine and land iguanas from different islands in the archipelago. High-throughput 16S rRNA-based pyrosequencing was utilized to provide a comparative analysis of fecal microbial diversity. Our findings revealed that at the phylum level, the intestinal microbial community was predominated by Firmicutes (69.5±7.9%), Bacteroidetes (6.2±2.8%) and unclassified Bacteria (20.6±8.6%), suggesting that a large group of bacterial targets present in the iguana fecal microbiota was novel and could be involved in unknown functions. Further evaluation revealed differences in the abundance of some bacterial groups. *Bacteroides* spp., Lachnospiraceae, and Clostridiaceae were significantly more abundant in the marine iguanas (P-value>1E-9). In contrast, Ruminococcaceae was present at >5-fold higher abundance in the land iguanas than marine iguanas (P-value>6E-14). The number of operational taxonomic units (OTUs) in the land

iguanas (356-896 OTUs) was >2-fold higher than in the marine iguanas (112-567 OTUs), and this increase in OTU diversity could be related to the complexity of the resident bacterial population and their gene repertoire required breakdown the recalcitrant polysaccharides prevalent in terrestrial plants. Our findings suggest that dietary differences can drive differentiation in the gut microbiota of herbivorous lizards. Most importantly, this study provides a better understanding of the microbial diversity in the iguana gut, therefore facilitating future efforts to discover novel bacterial-associated enzymes that can effectively breakdown a wide variety of complex polysaccharides, as well as microbial-associated pathways that help herbivorous iguanas overcome plant defense mechanisms.

The Improvement of *in vitro* Culture Conditions for Human Gastrointestinal Tract Microbiota

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A stable intestinal microbiota is important in maintaining human physiology and health. Although there have been a number of studies using *in vitro* and *in vivo* approaches to determine the impact of diet and xenobiotics on intestinal microbiota, there is no consensus for the best *in vitro* culture conditions for growth of the human gastrointestinal microbiota. To investigate the dynamics and activities of intestinal microbiota, it is important to cultivate representative intestinal microbiota from the fecal inoculum and optimize *in vitro* culture growth conditions. Here we compared the *in vitro* bacterial community in three culture media, Brain Heart Infusion broth (BHI), high- and low- carbohydrate with or without fecal supernatant to determine optimal inoculum concentration and growth conditions. The bacterial community was analyzed using denaturing gradient gel electrophoresis (DGGE), pyrosequencing, and quantitative real-time PCR. Based on the molecular analysis, this study indicated that the bacterial community of 3% fecal inoculum in low-concentration carbohydrate medium with the addition of 1% fecal supernatant provided optimal growth conditions to conduct in vitro studies representative of the human intestinal microbiota in the gastrointestinal tract.

Keywords: *in vitro* culture growth condition, human gastrointestinal microbiota, pyrosequencing

Temporal stability of bacterial community structure from equine feces

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Different factors such as feeding practices or stress of transport are known to modify the composition of equine gut bacterial community and challenge the healthy gut status of the host. Despite its potential interaction with experimental effects currently observed, the changes of this community have never been investigated over time. The aim of this study was to investigate the temporal stability of equine fecal bacterial community. Five adult Arabian horses, at rest, were fed the same diet (90 forage : 10 concentrate) during the five experimental weeks. At the end of each week, feces from every horse were spot-sampled from the rectum two hours after the morning meal. DNA from fecal samples was

extracted by a bead-beating procedure (Yu and Morrison 2004). The fecal bacterial community structure was studied using a fingerprint technique: the Automated Ribosomal Intergenic Spacer Analysis (ARISA). Data were analyzed by a clustering analysis and a permutation test. Irrespective of the sampling period considered, an individual effect was observed by clustering analysis of ARISA profiles ($P < 0.05$). Whatever the individual, the structure of fecal bacterial community was unchanged along the five weeks of the experiment ($P > 0.05$). To conclude, results suggests that, in equine experimental design, time should not interfere with other experimental effects. Further investigations are necessary to monitor this temporal effect on equine cecal and colonic bacterial community structure. In parallel, these data could be completed by studying the stability of equine microbial activity over time.

Keywords: equine, feces, bacterial structure, ARISA