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SCIENTIFIC PROGRAM AND ABSTRACTS

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Monday, April 11

All times listed in central daylight time (CDT)

Special Session: Community Assembly—From Theory to Practice

Chairs: Itzhik Mizrahi, Ben Gurion University of the Negev, Israel, and
Phil Pope, Norwegian University of Life Sciences, Norway
08:30 – 12:15

Synthetic microbial communities hold the potential of enabling us to understand the basic building blocks of microbiomes, their trophic interactions, and functional consequences in gut microbial communities as well as in other environments. This special session will explore novel technologies for isolating microbial components to construct such communities, basic rules for their assembly, and their usage for answering critical questions in research and application.

08:30		**Introduction** Itzhik Mizrahi, *Ben Gurion University of the Negev, Israel.*
08:40	1	***Invited talk:*** **Ordering microbial diversity into ecologically cohesive units.** M. Polz*, *Centre for Microbiology and Environmental Systems Science, University of Vienna*, *Vienna, Austria*.
09:25	2	***Invited talk:*** **Predicting and designing synthetic human microbiomes.** O. Venturelli*, *Departments of Biochemistry, Chemical and Biological Engineering, and Bacteriology, University of Wisconsin-Madison*, *Madison, WI, USA*.
10:10		**Break**
10:30	3	***Invited talk:*** **The world of gut microbiomes through the lens of cultivation.** T. Clavel*, *Functional Microbiome Research Group, Institute of Medical Microbiology, University Hospital of RWTH Aachen*, *Aachen, Germany*.
11:15	4	***Invited talk:*** **Potential drivers of plasticity and persistence in the rumen microbiome.** I. Mizrahi*, *The Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev*, *Beer-Sheva, Israel*.
12:00		**Discussion and synthesis**
12:15		**Workshop ends**
12:15		**Lunch break**

2022 Opening Session
71st Year Anniversary Event

Invited Presentations and Bryant Memorial Lecture

Chair: Rod Mackie, University of Illinois at Urbana-Champaign, USA
13:30 – 16:30

13:30 **Welcome**
Rod Mackie, *University of Illinois at Urbana-Champaign, USA.*

13:40 5 ***Invited talk:*** **Digital optimization of the feed–microbiome–host nexus.**
S. L. La Rosa, T. O. Andersen, I. Altshuler, J. Walter, A. V. P. de Leon, M. Ø. Arntzen, L. H. Hagen, and P. B. Pope*, *Norwegian University of Life Sciences*, *Ås, Norway*.

14:25 6 ***Invited talk:*** **Unveiling the impact of host–microbiota interactions on animal biology through hologenomics.**
A. Alberdi*, *Center for Evolutionary Hologenomics, GLOBE Institute, University of Copenhagen*, *Copenhagen, Denmark*.

15:10 **Break**

15:30 7 **Bryant Memorial Lecture: A double threat of extinction: What happens to microbial diversity when soil erodes?**
J. Handelsman*, *Wisconsin Discovery Institute, Howard Hughes Medical Institute, University of Wisconsin-Madison, WI, USA*.

16:20 **Presentation of Honorary Plaque**
Rod Mackie, *University of Illinois at Urbana-Champaign, USA.*

Tuesday, April 12

All times listed in central daylight time (CDT)

Podium Presentations: Session 1

Chair: Phil Pope, Norwegian University of Life Sciences, Norway
08:30 – 12:30

08:30 16 ***Invited talk:* MAGICdb enables predictions of human cardiovascular disease from fecal microbial gene content—*or* microbial gene content in feces predicts human cardiovascular disease.**
M. A. Borton[1], M. Shaffer[1], D. W. Hoyt[2], R. Jiang[3], S. Purvine[2], C. D. Nicora[2], J. Ellenbogen[3], E. K. Eder[2], A. R. Wong[2], A. G. Smulian[4], D. J. Ferguson[5], M. S. Lipton[2], J. A. Krzycki[3], and K. C. Wrighton*[1], [1]*Department of Soil and Crop Science, Colorado State University*, *Fort Collins, CO, USA*, [2]*Environmental and Biological Sciences Directorate, Pacific Northwest National Laboratory; Richland, WA, USA*, [3]*Department of Microbiology, The Ohio State University*, *Columbus, OH, USA*, [4]*Department of Internal Medicine, University of Cincinnati*, *Cincinnati, OH, USA*, [5]*Department of Microbiology, Miami University*, *Oxford, OH, USA*.

09:15 9 **Sensing and regulation of the breakdown of dietary polysaccharides in *Bacteroides* spp.**
A. M. Abdel-Hamid*[1,2], G. V. Pereira[1], K. A. Boateng[1], N. M. Koropatkin[3], R. I. Mackie[4], and I. Cann[1,4], [1]*Carl R Woese Institute for Genomic Biology, University of Illinois*, *Urbana, IL, USA*, [2]*Department of Botany and Microbiology, Faculty of Science, Minia University*, *El-Minia, Egypt*, [3]*Department of Microbiology and Immunology, University of Michigan Medical School*, *Ann Arbor, MI, USA*, [4]*Department of Animal Sciences, University of Illinois*, *Urbana, IL, USA*.

09:35 10 **The role of TonB in polysaccharide utilization by the *Bacteroides*.**
R. M. Pollet*[1], G. Pereira[2], M. H. Foley[2], E. C. Martens[2], and N. M. Koropatkin[2], [1]*Vassar College*, *Poughkeepsie, NY, USA*, [2]*University of Michigan Medical School*, *Ann Arbor, MI, USA*.

09:55 11 **Biochemical analysis and structural prediction of a bifunctional feruloyl esterase from the ruminal anaerobic fungus *Pecoramyces* sp. F1.**
Q. Shi*[1], A. Abdel-Hamid[2], Y. Li[1], Y. Cheng[1], I. Cann[2], and W. Zhu[1], [1]*Laboratory of Gastrointestinal Microbiology, National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University*, *Nanjing, Jiangsu, China*, [2]*Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*.

10:15 **Break**

10:30 12 **Gut *Bacteroidetes* for propionate production from polysaccharides.**
S. E. Kurrer*, C. Döring, and M. Basen, *University of Rostock, Institute of Biological Sciences, Division of Microbiology*, *Rostock, Mecklenburg-Western Pomerania, Germany*.

10:50 13 **Rnf is involved in propionate production during fermentation in rumen bacteria.**
B. Zhang*, C. Lingga, H. De Groot, and T. Hackmann, *University of California, Davis*, *Davis, CA, USA*.

11:10 14 **Structure-mechanism of bacterial 20α-hydroxysteroid dehydrogenase (DesC) and CRISPR-Cas9 engineering of *desC* for constitutive expression in**

***Escherichia coli*.**
J. W. Lee*[1,2], M. C. R. Melo[3], R. M. Pollet[4], H. L. Doden[1], S. Devendran[1], S. M. Mythen[1], S. Bhowmik[5], S. A. Lesley[5], I. Cann[1,2,6,7], N. M. Koropatkin[4], R. C. Bernardi[8,9], and J. M. Ridlon[1,2,6,10,11], [1]*Department of Animal Sciences, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [2]*Microbiome Metabolic Engineering Theme, Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [3]*Departments of Psychiatry and Microbiology, Perelman School of Medicine; Department of Bioengineering, University of Pennsylvania*, *Philadelphia, PA, USA*, [4]*Department of Microbiology and Immunology, University of Michigan at Ann Arbor*, *Ann Arbor, MI, USA*, [5]*Department of Integrative and Computational Biology, Scripps Research Institute*, *San Diego, CA, USA*, [6]*Division of Nutritional Sciences, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [7]*Department of Microbiology, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [8]*NIH Center for Macromolecular Modeling and Bioinformatics, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [9]*Department of Physics, Auburn University*, *Auburn, AL, USA*, [10]*Center for Advanced Study, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [11]*Cancer Center at Illinois, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*.

11:30 15 **Effects of barley variety (hulled vs. hull-less) and head type (2 vs. 6 row) on rumen fermentation, dry matter disappearance and fermentation characteristics in batch culture.**
W. Z. Yang*[1], A. M Saleem[2,1], R. M. Bierworth[3,1], J. Nyachiro[4], L. Oatway[5], and T. A. McAllister[1], [1]*Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre*, *Lethbridge, AB, Canada*, [2]*Animal and Poultry Production Department, South Valley University*, *Qena, Egypt*, [3]*Department of Animal and Poultry Science, University of Saskatchewan*, *Saskatoon, SK, Canada*, [4]*Field Crop Development Centre*, *Lacombe, AB Canada*, [5]*Olds College*, *Olds, AB, Canada*.

11:50 **Break**

12:00 **Business meeting: CGIF 2022 (open to all registrants)**

13:00 **Lunch break**

Podium Presentations: Session 2

Chair: Isaac Cann, University of Illinois at Urbana-Champaign, USA
13:30 – 16:40

13:30 8 ***Invited talk:* Opposing roles for mucin-degrading bacteria and bacterial protein metabolites in the development of intestinal inflammation.**
E. Martens*, *Microbiology and Immunology, University of Michigan*, *Ann Arbor, MI, USA*.

14:15 17 **Common viral inactivation techniques affect 16S rRNA amplicon-based analysis of the gut microbiota.**
Z. McAdams*, K. Gustafson, and A. Ericsson, *University of Missouri*, *Columbia, MO, USA*.

14:35 18 **Biogeography may be key to microbial anti-inflammatory production using dietary precursors in inflammatory bowel disease mouse models.**
S Ishaq*[1], Y Li[1], J Holman[1], T Zhang[2], G Mawe[3], M Hurd[3], B Lavoie[3], G Chen[4], D Baudewyns[2], L Colucci[2], J Balkan[1], and P Moses[5], [1]*University of Maine*, *Orono, ME, USA*, [2]*Husson University*, *Bangor, ME, USA*, [3]*University of Vermont*, *Burlington, VT, USA*, [4]*University of Michigan*, *Ann Arbor, MI, USA*, [5]*Finch Therapeutics*, *VT, USA*.

14:55 20 **Creation of a gut microbial and viral genome catalog exposes gene content and taxonomic variation in inflamed and noninflamed mice.**
I. Leleiwi*[1], M. Schaffer[1], M. A. Borton[1,2], A. Sabag-Daigle[2], J. Rodriguez-Ramos[1], R. A. Daly[1], R. Flynn[1], B. Ahmer[2], and K. C. Wrighton[1], [1]*Colorado State University*, *Fort Collins, CO, USA*, [2]*Ohio State University*, *Columbus, OH, USA*.

15:15 **Break**

15:40 21 **Fecal matter transplantation restores the gastrointestinal microbial populations following a traumatic brain injury in a pediatric porcine model.**
C. B. Welch*, M. M. Fagan, S. E. Sneed, K. M. Scheulin, J. H. Jeon, M. E. Golan, H. J. Park, T. R. Callaway, K. J. Duberstein, T. D. Pringle, J. M. Lourenco, and F. D. West, *University of Georgia*, *Athens, GA, USA*.

16:05 22 **The maternal oral microbiota significantly contributes to the neonatal gut microbiota composition in CD-1 mice.**
A. L. Russell*, E. Donovan, N. Seilhamer, K. Gustafson, and A. C. Ericsson, *University of Missouri*, *Columbia, MO, USA*.

Wednesday, April 13

All times listed in central daylight time (CDT)

Podium Presentations: Session 3

Chair: Itzhik Mizrahi, Ben Gurion University, Israel
09:00 – 11:30

09:00 23 ***Invited talk:*** **Enrichment of gut microbiomes from herbivores: Engineering carbon flux through microbial diversity and selection.**
M. A. O'Malley*, *University of California*, *Santa Barbara, CA, USA*.

09:50 24 **Genome-centric metaproteomics reveals metabolic influence of the ciliate *Entodinium caudatum* within the rumen microbiome.**
T. O. Andersen*[1], I. Altshuler[1], A. V. P. de Leon[2], C. Martin[3], L. Bernard[3], H. Fougere[3], D. P. Morgavi[3], J. L. Firkins[4], Z. Yu[4], T. R. Hvidsten[2], M. Popova[3], M. Ø. Arntzen[2], L. H. Hagen[2], and P. B Pope[1], [1]*Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences*, *Ås, Norway*, [2]*Faculty of Chemisty, Biotechnology and Food Science, Norwegian University of Life Sciences*, *Ås, Norway*, [3]*Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores*, *Saint-Genes-Champanelle, France*, [4]*Department of Animal Sciences, The Ohio State University*, *Columbus, OH, USA*.

10:10 25 **Polyclonal antibodies inhibit growth of key cellulolytic rumen bacterial species.**
S. M. Tondini*, R. I. Mackie, and J. C. McCann, *University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*.

10:30 **Break**

10:50 26 **Supplementation of slow-release urea affects rumen microbial community in an artificial rumen system.**
Y. Guo[1,2], L. Jin[2], L. Xiao[3], S. Yan[1], and W. Yang*[2], [1]*Inner Mongolia Agricultural University*, *Hohhot, Inner Mongolia, China*, [2]*Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre*, *Lethbridge, AB, Canada*, [3]*Hangzhou King Techina Feed Co. Ltd.*, *Hangzhou City, China*.

11:10 27 **Impacts of irrigating triticale with dairy lagoon wastewater before harvest and treating with a bacterial inoculant at harvest on the chemical composition, fermentation quality, and bacterial community of silage.**
O. Y. Koyun*, J. M. Lourenco, T. R. Callaway, S. Tao, and J. K. Bernard, *Department of Animal and Dairy Science, University of Georgia*, *Athens, GA, USA*.

11:30 **Lunch break**

Podium Presentations: Session 4

Chair: Jeff Firkins, The Ohio State University, USA

13:00 – 15:30

13:00 28 ***Invited talk:*** **The scientific accomplishments of Dr. Milton J. Allison (1931–2022)—Microbiologist.**
M. Rasmussen*[1], S. Daniel[2], and R. Anderson[3], [1]*Iowa State University*, *Ames, IA, USA*, [2]*Eastern Illinois University*, *Charleston, IL, USA*, [3]*USDA/ARS*, *College Station, TX, USA*.

13:50 29 **Branched-chain volatile fatty acid conversion into branched-chain amino acids and lipid synthesis in dual-flow cultures varying in forage and polyunsaturated fatty acid concentrations.**
K. E. Mitchell*[1], S. L. Kienzle[1], B. A. Wenner[1], C. Lee[2], D. H. Kleinschmit[3], M. T. Socha[3], and J. L. Firkins[1], [1]*Department of Animal Sciences, The Ohio State University*, *Columbus, OH, USA*, [2]*Department of Animal Sciences, The Ohio State University*, *Wooster, OH, USA*, [3]*Zinpro Corporation*, *Eden Prairie, MN, USA*.

14:10 30 **Effects of dosing nontoxigenic *Clostridia* on bacterial populations and immunological responses in the intestinal tract of lactating dairy cows.**
H. W. Kim*[1], A. H. Smith[2], J. S. Thompson[2], T. G. Rehberger[2], M. N. Griffin[2], F. F. Cardoso[1], F. Cardoso[1], and R. I. Mackie[1], [1]*University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [2]*Church & Dwight Co. Inc.*, *Waukesha, WI, USA*.

14:30 31 **The equine microbiome's subtle responses to dietary challenges can be seen over time.**
A. C. B. Johnson* and A. S. Biddle, *University of Delaware*, *Newark, DE, USA*.

14:50 **Break**

15:10 **Presentation of Russell Awards**
Best oral presentations by graduate students and young investigators

15:15 **Closing remarks and invitation to CGIF 2024**

Special Session: Community Assembly—From Theory to Practice

1 ***Invited talk:*** **Ordering microbial diversity into ecologically cohesive units.** M. Polz*, *Centre for Microbiology and Environmental Systems Science, University of Vienna, Vienna, Austria.*

Populations are fundamental biological units that form the basis of our understanding of evolution, ecology, and systematics. They are defined as local representatives of species that share a gene pool in which adaptive mutations can spread, leading to shared ecological niche space. Yet for bacteria and archaea, widespread and frequent horizontal gene transfer has raised questions about whether classical definitions of populations and species can be applied. We show that despite high gene turnover, it is possible to define genotypic clusters, which represent ecologically cohesive populations. These display hallmarks of animal and plant populations, including speciation, shared gene pools, and social interactions. By developing a new method to identify recent recombination events among genomes, we generalize these insights by showing that clusters in gene-flow networks correspond to previously defined ecologically differentiated populations among diverse bacteria and archaea. We propose a reverse ecology approach where gene-flow clusters serve as hypotheses of population structure, allowing for rapid identification of adaptations and mapping onto microbiomes to differentiate associations with health and disease.

2 ***Invited talk:*** **Predicting and designing synthetic human microbiomes.** O. Venturelli*, *Departments of Biochemistry, Chemical and Biological Engineering, and Bacteriology, University of Wisconsin-Madison, Madison, WI, USA.*

The human gut microbiome is a complex and dynamic microbial ecosystem that shapes human physiology, nutrition, and behavior. Key functions performed by gut microbiota include the production and degradation of health-relevant metabolites and colonization resistance to intestinal pathogens. However, in response to perturbations, the composition and functions of gut microbiota can be shifted to alternative states that negatively impact human health. The functions performed by gut microbiota are determined by an unknown web of interactions linking species and environmental factors. Developing the capability to predict and design the functions of gut microbiota hold tremendous therapeutic potential. By combining bottom-up assembly of human gut communities with computational modeling, we decipher the networks shaping community dynamics and health-relevant functions. We exploit the data-driven models to design communities with desired behaviors including specific metabolite profiles, resistance to invasion and community diversity. Our work elucidates the molecular and ecological mechanisms shaping community assembly, response to perturbations and community-level metabolic functions of the human gut microbiome.

3 ***Invited talk:*** **The world of gut microbiomes through the lens of cultivation.** T. Clavel*, *Functional Microbiome Research Group, Institute of Medical Microbiology, University Hospital of RWTH Aachen, Aachen, Germany.*

The gut microbiome is important for health and the development or treatment of chronic diseases. Because of this, it has attracted a great deal of attention over the last 2 decades, mainly based on new sequencing technologies. However, more than half of the microbes colonizing the intestine of mammals remain unknown, which hampers both our understanding of molecular mechanisms underlying microbe–host interactions and the implementation of microbiome-based applications. In this context, the laboratory uses cultivation approaches to study the diversity, taxonomy, and functions of gut bacteria. I will talk about multiple collections of isolates that we have established from

different host species, share new biological and functional insights within these collections, and show examples of how to use the isolates, as single strains or synthetic communities, in experimental in vivo models. **Main related publications:** Afrizal et al., bioRxiv 2022, https://doi.org/10.1101/2022.03.07.483007; Afrizal et al., *Environ Microbiol*. 2022, https://doi.org/10.1111/1462-2920.15935; Clavel et al., *Microb Biotechnol*. 2022;15:164–175, https://doi.org/10.1111/1751-7915.13970; Hitch et al., *ISME Commun*. 2021;1, https://doi.org/10.1038/s43705-021-00017-z; Zenner et al., *mSystems*. 2021;6:e01300-20, https://doi.org/10.1128/mSystems.01300-20; Kumar et al., *Microb Biotechnol*. 2021;14:1757–1770, https://doi.org/10.1111/1751-7915.13845; Wylensek et al., *Nat Commun*. 2020;11:6389, https://doi.org/10.1038/s41467-020-19929-w; and Lagkouvardos et al., *Nat Microbiol*. 2016;1:16131, https://doi.org/10.1038/nmicrobiol.2016.131.

4 ***Invited talk:*** **Potential drivers of plasticity and persistence in the rumen microbiome.** I. Mizrahi*, *The Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel.*

In recent years, the mammalian gut has emerged as a fundamentally important microbial environment. Intriguingly, in special cases, complete obligatory dependence exists between the host and its associated microorganisms, whereby the microbial communities perform fundamental processes, such as digestion of the feed for the host. Among the most representative and ecologically relevant examples are ruminants—foregut fermenters that rely critically on their associated gut microbes to digest their plant feed. Due to this paradigmatic obligatory dependence of the host on its microbiome, such systems serve as excellent models to understand fundamental aspects of the ecological and evolutionary relationships between hosts and their microbes. In my lecture, I will discuss some of our recent findings of the rumen microbiome ecosystem stability, development, and interaction with the host.

Invited Presentations and Bryant Memorial Lecture

5 ***Invited talk:*** **Digital optimization of the feed–microbiome–host nexus.** S. L. La Rosa, T. O. Andersen, I Altshuler, J. Walter, A. V. P. de Leon, M. Ø. Arntzen, L. H. Hagen, and P. B. Pope*, *Norwegian University of Life Sciences, Aas, Norway.*

As the human population expands toward 10 billion, pressure is being applied to food production systems to develop nutritious, efficient and sustainable practices, such as optimizing health benefits from food, improving feed conversion and animal welfare and mitigating harmful byproducts such as greenhouse gases (GHG). One promising route to achieve this is combining the use of functional dietary components with a deeper understanding of the intimate genetic and physiological connection between animals and their microbiomes. However, first we must unlock critical and poorly understood microbiota and their biological pathways that control digestion, as well as identify exploitable interactions that exist within the complexity of gut microbiomes. Our research seeks to combine high-resolution genome-guided meta-omics technologies with enzymology, bacteriology, bioinformatics, and phenotyping of relevant digestive eco-systems from human and production animals (pigs, fish and ruminants). Herein we highlight how such an integrated approach can visualize how distinctive dietary fibers stimulate known model microorganisms within a complex endogenous microbiome. We further reveal the metabolic influence of uncharacterized bacterial and eukaryotic populations that are surprisingly conserved across diverse dietary conditions and host species. Importantly, as our work develops with technological advancements, we are actively expanding our analyses further across the “holobiont,” with the long-term objective

being to understand, monitor, and ultimately manipulate host–microbiome interactions.

Key Words: microbiome, microbiota-directed fiber, multi-omics

6 ***Invited talk:*** **Unveiling the impact of host–microbiota interactions on animal biology through hologenomics.** A. Alberdi*, *Center for Evolutionary Hologenomics, GLOBE Institute, University of Copenhagen, Copenhagen, Denmark.*

Variation of phenotypic traits across individuals is essential for both natural and artificial selection processes to occur, and it thus plays a central role in evolution of wildlife as well as the optimization of farm animals. The phenotypic variation of animals has been historically attributed to the interplay between genomic properties and environmental factors. However, the intense molecular work conducted on animal-associated microorganisms has recently revealed that microorganisms can also shape or even determine animal phenotypes, mainly those related to gastrointestinal function. Although individually, both animal genomic and microbial metagenomic approaches have proven useful for understanding many biological processes, each approach has typically ignored the effect of the other domain and, critically, their interplay. Hence, the knowledge gained through such approaches is, at the very least, incomplete. The recognition of the importance of these host–microbiota interactions has recently opened up new research avenues based on the integrated analysis of coupled animal genomic and microbial metagenomic data; namely, hologenomics. Hologenomic thinking and methodologies are relevant to address a wide range of biological questions, spanning biomolecular interactions between microbial and animal cells to evolutionary interactions between microorganisms and animals. In my talk, I will propose a journey through the different approaches we are using to identify the impact of host–microbiota interactions on animal biology using hologenomic techniques. This journey will include (1) the host–microbiota multi-omic analyses we are conducting in the international project HoloFood to unveil hidden factors that determine performance of broiler chickens; (2) the micrometer-scale 3-dimensional reconstructions of the animal-microbiota interactions we are performing in the international project 3D'omics to understand how animals and microbes interact at their scale of action; and (3) the global-scale hologenomic analyses we are conducting in the Earth Hologenome Initiative to address a variety of ecological and evolutionary questions. I will provide an overview of methodological approaches, highlight the main hurdles to implement hologenomics, and provide hints to optimize study designs and analytical procedures, aimed at aiding researchers to design new fully powered studies to address some of the most challenging and insightful questions about the interconnectedness of life forms on Earth.

Key Words: microbiome, performance, phenotype, symbiosis

7 Bryant Memorial Lecture: A double threat of extinction: What happens to microbial diversity when soil erodes? J. Handelsman*, *Wisconsin Discovery Institute, Howard Hughes Medical Institute, University of Wisconsin-Madison, WI, USA.*

Soil is a precious resource for planet Earth and the humans who inhabit it. Ninety-five percent of our food supply is dependent upon soil. More than three-quarters of the antibiotics used in the clinical medicine are derived from soil bacteria. And soil stores 3 to 4 times as much carbon as the entire atmosphere. Despite the importance of soil to human survival and the health of the Earth, we are letting it slip away. Soil erosion exceeds the rate of soil genesis by at least 10-fold. This talk will explore the forces that are eroding soil and what we can do to save it.

Podium Presentations: Session 1

8 *Invited talk:* Opposing roles for mucin-degrading bacteria and bacterial protein metabolites in the development of intestinal inflammation. E. Martens*, *Microbiology and Immunology, University of Michigan, Ann Arbor, MI, USA.*

Inflammatory bowel disease (IBD) is a chronic condition characterized by periods of spontaneous intestinal inflammation and is increasing in industrialized populations. Combined with host genetic predisposition, both diet and gut bacteria are thought to be prominent environmental contributors to IBD, but precise mechanisms are still emerging. We show that low dietary fiber promotes increased bacterial erosion of the protective colonic mucus layer, leading to inflammation in mice lacking interleukin-10 and this effect is dependent on mucin-degrading bacteria. Onset of diet-induced inflammation is preceded by expansion of natural killer T cells and reduced high-affinity immunoglobulin A coating of specific bacteria species. Low fiber, exclusive enteral nutrition reduced disease in part by increasing bacterial production of the metabolite isobutyrate, which was dependent on the presence of soy protein in the diet. Our results highlight a mechanistic framework to unravel the complex web of potentially opposing diet, host and microbial factors that combine to influence IBD development.

9 Sensing and regulation of the breakdown of dietary polysaccharides in *Bacteroides* spp. A. M. Abdel-Hamid*[1,2], G. V. Pereira[1], K. A. Boateng[1], N. M. Koropatkin[3], R. I. Mackie[4], and I. Cann[1,4], [1]*Carl R Woese Institute for Genomic Biology, University of Illinois, Urbana, IL, USA,* [2]*Department of Botany and Microbiology, Faculty of Science, Minia University, El-Minia, Egypt,* [3]*Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, USA,* [4]*Department of Animal Sciences, University of Illinois, Urbana, IL, USA.*

Dietary fibers form components of the human diet and have major impact on gut microbiota composition and diversity. Common dietary fibers such as arabinoxylans, arabinans, and pectins are energy-rich polysaccharides. However, they are generally nonmetabolizable by the human host because humans lack the genes encoding the requisite degradative enzymes in the host genome. Within the human colon are, however, microbiota endowed with a wide range of enzymes for fiber breakdown. These carbohydrate-active enzymes (CAZymes) include glycoside hydrolases, polysaccharide lyases and carbohydrate esterases. In the major colonic bacterial phylum *Bacteroidota*, the CAZymes for the breakdown of a specific polysaccharide are encoded in a gene cluster known as polysaccharides utilization locus (PUL). The genomes of *Bacteroides* spp. contain numerous PULs that facilitate degradation and uptake of different carbohydrates; and in general, each of these PULs is controlled by a large polypeptide designated hybrid 2-component system (HTCS). Using RNaseq analyses, we identify the PULs responsible for the degradation of arabinan, pectin, and arabinoxylans when *Bacteroides intestinalis* is grown on the respective polysaccharide and thus their associated HTCS. Each HTCS protein is composed of a sensor and Y_Y_Y domains (Sensor_Y_Y_Y) in the N-terminal half and a histidine kinase, response regulator and helix-turn-helix domains (HK_RR_HTH) in the C-terminal half. By raising polyclonal antibodies that target the C-terminal and N-terminal halves, we demonstrate by immunostaining that the Sensor_Y_Y_Y is membrane associated whereas the HK_RR_HTH is localized in the cytoplasm. We further expressed and purified recombinant forms of several Sensor Y_Y_Y polypeptides from different colonic *Bacteroides* spp. and determined by isothermal titration calorimetry that these polypeptides bind uniquely to components of the polysaccharides cognate to their respective PULs. In addition, using untargeted metabolomics, we identified metabolites associated with PULs of interest during their upregulation in members of the colonic *Bacteroidota* and thus link polysaccharide sensing and degradation to potential health

and nutritional benefits to the host. The results presented herein provide mechanistic insights to manipulating human health and nutrition through probiotic, prebiotic, or synbiotic administration.

10 The role of TonB in polysaccharide utilization by the *Bacteroides*. R. M. Pollet*[1], G. Pereira[2], M. H. Foley[2], E. C. Martens[2], and N. M. Koropatkin[2], [1]*Vassar College, Poughkeepsie, NY, USA,* [2]*University of Michigan Medical School, Ann Arbor, MI, USA.*

The human gut microbiota is required for the degradation of otherwise undigestible polysaccharides. These polysaccharides are a key energy source for the gut microbiota and fermentation products such as short chain fatty acids are beneficial to the human host. The *Bacteroides* are prominent contributors to polysaccharide degradation and TonB-dependent transporters (TBDT) are a key component responsible for uptake of polysaccharides. Transport through TBDT is energized by an inner membrane complex composed of TonB, ExbB, and ExbD which harnesses proton motive force and contacts the TBDT through TonB. *Bacteroides thetaiotaomicron* (*B. theta*) encodes 11 TonB homologs but it is not clear which homologs are important for polysaccharide uptake. To address this question, we have generated *B. theta* strains in which each of the 11 *tonB* genes are deleted. Using these single deletion strains, we have shown that TonB4 (BT2059) is important but not essential for proper growth on starch. Using membrane proteomics, we have shown an increase in abundance of TonB6 (BT2762) when TonB4 is absent, suggesting redundancy in function of these TonB proteins. Additionally, growth of the single deletion strains, on pectic galactan, chondroitin sulfate, arabinan, and levan suggest a similar redundancy in TonB function. Comparison of the *B. theta* TonB proteins to those encoded by other *Bacteroides* species suggests that TonB4 is widely conserved and may play a common role in polysaccharide uptake as recently shown in *B. fragilis*. However, conservation of TonB6 is much more limited suggesting that this redundancy of TonB function may be unique to *B. theta* and closely related species. This mechanistic understanding of how *B. theta* and related *Bacteroides* use polysaccharides to establish their niche in the microbiota will allow us to design noninvasive approaches for optimizing the gut community and improving patient outcomes caused by a lack or overgrowth of *Bacteroides*.

Key Words: *Bacteroides*, TonB-dependent transporters, TonB, polysaccharide utilization

11 Biochemical analysis and structural prediction of a bifunctional feruloyl esterase from the ruminal anaerobic fungus *Pecoramyces* sp. F1. Q. Shi*[1], A. Abdel-Hamid[2], Y. Li[1], Y. Cheng[1], I. Cann[2], and W. Zhu[1], [1]*Laboratory of Gastrointestinal Microbiology, National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University, Nanjing, Jiangsu, China,* [2]*Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.*

The present study was designed to express a novel feruloyl esterase (FE) derived from *Pecoramyces* sp. F1, analyzing the relevant biochemical functions, ultimately predicting the spatial structure of this enzyme with AlphaFold. The gene sequence of feruloyl esterase was codon optimized, which could only be efficiently expressed in Arctic Express (DE3) competent cells. When the protein expression was completed, this enzyme was purified by consecutive steps including Ni-affinity chromatography, ionic exchange chromatography and size exclusion chromatography. After obtaining purified proteins, determining its biological characteristics and biochemical functions. The theoretical molecular weight of this enzyme is 67.80 kDa, and its optimum pH and temperature are 7.5 and 37°C, respectively. Size exclusion chromatography indicated that the enzyme is a monomer in solution. Online annotation demonstrated that this feruloyl esterase possess both carbohydrate esterase and xylanase activities. This enzyme showed higher specific activity and catalytical efficiency on *p*NP-acetate in comparison with

methyl-ferulate. For the degradation of several feruloyl oligosaccharides by the enzyme, FAXX was depolymerized to produce the highest concentration of ferulic acid (151.12 ± 3.46 μM). Moreover, this feruloyl esterase had significant activities on wheat arabinoxylan and xylan. The predicted structure of this enzyme contained 625 amino acids with one CE1, GH11, and 2 CBM10 families; the CE1 region was very closed to the GH11 domain in stereo view.

Key Words: anaerobic fungi, feruloyl esterase, bifunctional enzyme, AlphaFold

12 Gut *Bacteroidetes* for propionate production from polysaccharides. S. E. Kurrer*, C. Döring, and M. Basen, *University of Rostock, Institute of Biological Sciences, Division of Microbiology, Rostock, Mecklenburg-Western Pomerania, Germany.*

Bacteria of the phylum *Bacteroidetes* are important representatives in the gut microbiome. Propionate, an important precursor chemical in a variety of industries, is a major fermentation product of many *Bacteroidetes* species. Beyond that, they are equipped with a large repertoire of carbohydrate-active enzymes that target plant polymers. These abilities may allow to develop *Bacteroidetes* sp. toward the utilization of complex plant biomass as a substrate for renewable propionate production. Ten species known to produce propionate were initially grown in complex media and fermentation products were measured by HPLC-analysis. Among all tested strains, *Parabacteroides johnsonii* produced the highest amount of propionate (11 m*M*), whereas *Bacteroides graminisolvens* showed the highest propionate:acetate ratio (1.7). In defined minimal medium with glucose (DMMG), growth and propionate production of both strains were negatively affected. This could be overcome by adding vitamin B_{12} to the medium, which is an important co-factor for propionate formation. Addition of yeast extract to DMMG increased the cell density of *P. johnsonii* even further but had only a minor effect on *B. graminisolvens*. These results make clear that the medium composition significantly affects growth and product formation. Finding appropriate culture conditions, which result in high propionate yields in a range of *Bacteroidetes* is therefore a crucial step for the selection of model strains. To meet the goal of sustainability, one of the next steps is to select strains that utilize plant-based polysaccharides. In that regard, new isolates have been obtained with xylan or cellulose as substrate. Screening of these isolates for propionate formation in complex media revealed one isolate of the genus *Dysgonomonas* that outcompeted the previous tested type strains in propionate production (19 m*M*) as well as in propionate/acetate ratio (2.4). Although product formation in minimal medium has yet to be examined, propionate production by the isolate is highly promising. In conclusion, we compared propionate production of 72 type strains and isolates, and utilization of polymeric substrates has been investigated. Currently, the physiology of the strains with the highest propionate titers and yields is studied, toward the development of platform strains for conversion of polymers to propionate.

Key Words: *Bacteroidetes*, propionate, isolates

13 Rnf is involved in propionate production during fermentation in rumen bacteria. B. Zhang*, C. Lingga, H. De Groot, and T. Hackmann, *University of California, Davis, Davis, CA, USA.*

Propionate is a valuable carboxylic acid that can be produced by microbial fermentation, including in the cow's rumen. The fermentation routes for its production had been extensively investigated. However, it is unclear what enzymes recycle redox cofactors, ferredoxin and NAD^+, during fermentative propionate formation. We hypothesize that Rnf, an ion pump, can regenerate redox cofactors by oxidizing ferredoxin and reducing NAD^+. Analysis of genomes sequences shows many bacteria encode Rnf. Here we studied 2 such bacteria isolated from rumen, *Prevotella ruminicola* 23 and *Prevotella brevis* GA33. We performed fermentation products analysis, enzymatic assays, and shotgun proteomic analysis. We confirmed both bacteria fermented glucose

into succinate (precursor of propionate) or propionate, and acetate. The ferredoxin:NAD^+ oxidoreductase activity of Rnf was 8.68 (0.85) milliunits per milligram (mU/mg) protein in *P. ruminicola* 23 and 29.82 (5.72) mU/mg protein in *P. brevis* GA33. Both values are higher than zero ($P = 0.031$ and $P = 0.009$). The activities of other enzymes for regenerating redox cofactors were detected. These enzymes were glyceraldehyde-3-phosphate dehydrogenase (phosphorylating), malate dehydrogenase, pyruvate:ferredoxin oxidoreductase, NADH:ubiquinone oxidoreductase, and fumarate reductase. They had activities higher than 0 mU/mg protein ($P < 0.05$) in both strains. Our shotgun proteomic analysis confirmed that both bacteria expressed these enzymes. In silico analysis found that Rnf is prevalent in many other fermentative bacteria that form succinate or propionate. Our study demonstrates the importance of Rnf during fermentative propionate formation in bacteria. Along with other enzymes, Rnf could be an important target for manipulating fermentation.

Key Words: rumen bacteria, propionate, ferredoxin:NAD^+ oxidoreductase

14 Structure-mechanism of bacterial 20α-hydroxysteroid dehydrogenase (DesC) and CRISPR-Cas9 engineering of *desC* for constitutive expression in *Escherichia coli*.

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Cortisol, the major glucocorticoid in humans, is converted to numerous derivatives by enzymes throughout our body, altering biological function. Local tissue-specific concentrations of cortisol are regulated by enzymes that reversibly modify functional groups on the steroid nucleus and side chain, saturate the steroid rings, and conjugation to sulfate or glucuronide (Schiffer et al., J Steroid Biochem Mol Biol 2019; 194:105439). In this way, ratios of active versus inactive glucocorticoids can be locally and rapidly altered in peripheral tissues. Intriguingly, gut microbes possess multiple cortisol-altering enzymes that have co-evolved separately from eukaryotic counterparts (Ly et al., Mol Cell Endocrinol 2021; 525:111174). Microbes are therefore expected to play an important role in the complex steroid metabolome of the host. We previously discovered a cortisol-inducible gene cluster (desABCD) in *Clostridium scindens* ATCC 35704, encoding NADH-dependent 20α-hydroxysteroid dehydrogenase (20α-HSDH, DesC) (Ridlon et al., J Lipid Res2013; 54:2437-2349) and heterotetrameric steroid-17,20-desmolase (DesAB) (Devendran et al., J Lipid Res 2018; 59:1005-1014), which produce 20α-dihydrocortisol and 11β-OHAD from cortisol, respectively. Furthermore, the 20α-dihydrocortisol DesC product is not a substrate for DesAB (Devendran et al., J Lipid Res 2018; 59:1005-1014), suggesting DesC acts as a metabolic "switch" regulating side-chain cleavage of cortisol. Here, we combine multiple experimental and computational approaches to investigate the enzymatic mechanism of *C. scindens* ATCC 35704 DesC. Our findings reveal the structure of this enzyme with atomistic detail as

well as its interaction with substrates NADH and cortisol. Classical and hybrid QM/MM molecular dynamics simulations elucidate the intricate reaction mechanism including a hydride transfer from NADH to cortisol and a proton relay that finally leads to a 20α-dihydrocortisol enzymatic product. Moreover, we developed and validated a CRISPR-Cas9 strategy for integrating *desC* into the chromosome of *Escherichia coli* allowing for constitutive expression of recombinant DesC for future in vivo determinations of the biological function of 20α-dihydrocortisol.

Key Words: cortisol, DesC, CRISPR-Cas9 engineering, *Clostridium scindens* ATCC 35704, *Escherichia coli*

15 Effects of barley variety (hulled vs. hull-less) and head type (2 vs. 6 row) on rumen fermentation, dry matter disappearance and fermentation characteristics in batch culture. W. Z. Yang*[1], A. M Saleem[2,1], R. M. Bierworth[3,1], J. Nyachiro[4], L. Oatway[5], and T. A. McAllister[1], [1]*Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, AB, Canada,* [2]*Animal and Poultry Production Department, South Valley University, Qena, Egypt,* [3]*Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada,* [4]*Field Crop Development Centre, Lacombe, AB Canada,* [5]*Olds College, Olds, AB, Canada.*

This study was conducted to evaluate the effects of barley variety (hulled vs. hull-less) and head type (2 vs. 6 row) on kernel characteristics, particle size distribution (PSD), gas production (GP) kinetics, and dry matter disappearance (DMD) in in vitro batch cultures. Barley grain was dry-rolled to a constant processing index (PI) of 80%. Kernel uniformity and PSD on 3.35-, 2.36-, 1.18-, 0.85-mm sieves, GP kinetics, and DMD at 6 and 48 h of incubation were measured. For both hull-less and hulled barley, the 2-row types tended to exhibit higher test weight, plumpness, ether extract, and starch and β-glucan contents than the 6-row types. A hull type × head type interaction ($P < 0.01$) was noted for kernel uniformity and particles retained on 3.35- and 2.36-mm sieves. For both hulled and hull-less barley, more ($P < 0.05$) kernels were retained on the 3.35-mm sieve for the 2-row versus 6-row type, but this pattern was the opposite for both barley types on the 2.36-mm sieve where the 6-row exceeded the 2-row type. For rolled-hulled barley, more particles ($P < 0.05$) were captured on the 3.35-mm sieve for 2 versus 6 row, whereas the opposite response was observed for the 2.36-mm sieve. In contrast, for rolled hull-less barley, more ($P < 0.01$) particles were captured on the 1.18- and 2.36-mm sieves, with a similar distribution between head types. The accumulative gas volume (GV, mL/g of organic matter), GP rate, and average GP rate (AGPR) were higher ($P < 0.01$) for hull-less than for hulled barley. The DMD after 6 and 48 h were also higher ($P < 0.01$) for hull-less than for hulled barley. In general, GP kinetics and DMD at 6 and 48 h were lower ($P < 0.01$) for 6- than 2-row types, with the exception of $T_{1/2}$, which was greater ($P < 0.01$) for the 6-row type. The GP and DMD at 6 and 48 h were strongly correlated with finer particles. These results suggest that hull and head types can have a significant effect on rate and extent of barley grain digestion. The differences in PSD of rolled barley, along with the correlation between fine particles and rumen fermentability, presents an opportunity to select barley varieties on ruminal fermentation responses in addition to agronomic traits.

Key Words: hulled and hull-less barley, dry-rolling, particle size distribution, in vitro gas production and dry matter disappearance, batch culture

Podium Presentations: Session 2

16 *Invited talk:* MAGICdb enables predictions of human cardiovascular disease from fecal microbial gene content—*or* microbial gene content in feces predicts human cardiovascular disease. M. A. Borton[1], M. Shaffer[1], D. W. Hoyt[2], R. Jiang[3], S. Purvine[2], C. D. Nicora[2], J. Ellenbogen[3], E. K. Eder[2], A. R. Wong[2], A. G. Smulian[4], D. J. Ferguson[5], M. S. Lipton[2], J. A. Krzycki[3], and K. C. Wrighton*[1], [1]*Department of Soil and Crop Science, Colorado State University, Fort Collins, CO, USA,* [2]*Environmental and Biological Sciences Directorate, Pacific Northwest National Laboratory; Richland, WA, USA,* [3]*Department of Microbiology, The Ohio State University, Columbus, OH, USA,* [4]*Department of Internal Medicine, University of Cincinnati, Cincinnati, OH, USA,* [5]*Department of Microbiology, Miami University, Oxford, OH, USA.*

The gut microbiome processes protein-rich foods to trimethylamine (TMA), a metabolite that promotes atherosclerotic cardiovascular disease, the leading global cause of human mortality. The microbial biochemical processes controlling gut TMA concentrations were recently identified. However, the development of diagnostic and therapeutic interventions is hampered by limited knowledge of the microorganisms that contain the genes encoding these enzymes. Here, we mined over 200,000 gut-derived genomes from cultivated and uncultivated microbial lineages obtained from humans spanning diverse ages, geographies, and health conditions. From these microbial genomes, we created the Methylated Amine Gene Inventory of Catabolism database (MAGICdb), an open access resource containing 6,341 microbial genomes scored for atherogenic contribution based on gene composition, as well as a corresponding catalog of the 8,721 genes capable of regulating gut TMA concentrations. We then evaluated diverse human fecal metatranscriptome and metaproteome data sets to reveal global expression patterns in methylated amine metabolism. Last, we demonstrated that methylated amine gene content in human feces was as effective as currently used lipid blood markers for predicting cardiovascular disease status in a human cohort. By revealing the microorganisms and genes contributing to gut TMA metabolism, MAGICdb shows promise for diagnosing cardiovascular disease and empowering the development of therapeutic interventions.

17 Common viral inactivation techniques affect 16S rRNA amplicon-based analysis of the gut microbiota. Z. McAdams*, K. Gustafson, and A. Ericsson, *University of Missouri, Columbia, MO, USA.*

The gut microbiome plays a key role in shaping the host's response to viral infection and can dramatically influence clinical outcomes. 16S rRNA sequencing of the gut microbiome is often used to investigate these mechanisms; however, to protect those handling virus-laden samples, the infectious agents must first be inactivated. Here, we assessed how 5 common viral inactivation techniques including a sodium-dodecyl sulfate (SDS)-based lysis buffer, Holder pasteurization, TRIzol, and 2 Buffer AVL-based methods affect 16S rRNA-based analysis of the gut microbiome. The inactivation techniques were applied to cross-matched fecal samples from 16 female CD-1 mice of the same vendor-derived gut microbiome before fecal DNA extraction. Control DNA extractions were performed with a QIAGEN PowerFecal Pro Kit. The V4 region of the 16S rRNA gene was amplified and sequenced from extracted DNA. Inactivation method-dependent effects on DNA yield, genus-level taxonomic abundance, and α and β diversity metrics were assessed. The SDS-based inactivation method and Holder pasteurization had no effect on measures of microbial richness, while 2 Buffer AVL-based inactivation methods resulted in a decrease in detected richness. SDS inactivation, Holder pasteurization, and the AVL-based inactivation methods had no effect on measures of α diversity within samples or β diversity between samples. Fecal DNA extracted with TRIzol-treated samples failed to amplify and sequence, making it unsuitable for microbiome analysis. These results provide guidance for inactivating viral species in fecal samples for 16S rRNA-

based genomic analysis of the gut microbiome in efforts to improve our understanding of how the gastrointestinal microbial community influences the host response to viral infection.

Key Words: gut microbiome, viral inactivation, 16S rRNA

18 Biogeography may be key to microbial anti-inflammatory production using dietary precursors in inflammatory bowel disease mouse models. S Ishaq*[1], Y Li[1], J Holman[1], T Zhang[2], G Mawe[3], M Hurd[3], B Lavoie[3], G Chen[4], D Baudewyns[2], L Colucci[2], J Balkan[1], and P Moses[5], [1]*University of Maine, Orono, ME, USA,* [2]*Husson University, Bangor, ME, USA,* [3]*University of Vermont, Burlington, VT, USA,* [4]*University of Michigan, Ann Arbor, MI, USA,* [5]*Finch Therapeutics, VT, USA.*

Inflammatory bowel disease (IBD) is a chronic gastrointestinal (GI) tract condition characterized by aberrant immune responses to gut microbiota. Broccoli sprouts contain inactive precursors (glucoraphanin, GLR) that can be converted to bioactive anti-inflammatories (sulforaphane, SFN). Plant enzymes usually create nonfunctional end products, and humans lack the necessary enzymes, but some gut microbes robustly create SFN. We previously demonstrated that different broccoli sprout preparations alter how much SFN can be produced and the fecal bacterial composition; that SFN reduces colitis and colon tumorigenesis in mice; and that there is anatomical specificity to this action: SFN was only present in colon tissues, implying localized conversion there, and in high enough concentration to reduce inflammation at the site of IBD symptoms. We used 2 complementary mouse models to study the interaction of diet and gut bacteria in IBD. Our established DSS mouse model of ulcerative colitis used C57BL/6 mice, fed a purified AIN93G diet ± 10% steamed broccoli sprouts, and ± DSS. The broccoli-fed mice did not weigh less than the control mice. Bacterial community and biochemistry data from 4 locations along the GI tract are pending. We isolated >800 gut bacteria using selective media and anaerobic culturing, which are being screened for β-thioglucosidase activity and capacity to convert GLR to SFN. We used IL-10 knockout (ko) mice as an immunological model of Crohn's disease, but in a novel way to study dietary bioactives. IL-10ko mice raised in clean housing were colonized by conventional mice microbiota to trigger inflammation and an immune response. Mice were fed a purified AIN93G diet ± 10% steamed sprouts. Broccoli-fed mice did not exhibit weight stagnation, had very little fecal blood, better stool consistency, and collectively a much lower Disease Activity Index, implying a protective effect from diet. Histological results are still being processed but appear to confirm the protective effect. The control mice displayed more hyperplasia of gut epithelia, more inflammatory cell infiltrate, and more damage at the apical surface of epithelia where the cells encounter microbes. Bacterial community and biochemistry data from multiple locations along the GI tract are pending.

Key Words: broccoli sprouts, dietary bioactive, sulforaphane, gut microbiota

20 Creation of a gut microbial and viral genome catalog exposes gene content and taxonomic variation in inflamed and noninflamed mice. I. Leleiwi*[1], M. Schaffer[1], M. A. Borton[1,2], A. Sabag-Daigle[2], J. Rodriguez-Ramos[1], R. A. Daly[1], R. Flynn[1], B. Ahmer[2], and K. C. Wrighton[1], [1]*Colorado State University, Fort Collins, CO, USA,* [2]*Ohio State University, Columbus, OH, USA.*

The gut microbiome is linked to human diseases like atherosclerotic cardiovascular disease, and irritable bowel syndrome, and to mental illnesses such as schizophrenia and depression. Increasing evidence links intestinal microbiota to host physiology and enteric disease progression, with mouse models often used to inform how the gut microbiome modulates human disease. Despite the CBA/J mouse model being widely used to study immunology, metabolism, and enteric inflammation, its microbiome remains understudied with current murine gut bacterial catalogs lacking CBA/J mice. Here, we present the first culture-independent microbial and viral

genomic catalog of the CBA/J gut microbiome that utilizes deep metagenomic sequencing (>30 Gbp/mouse) combined with custom assembly methods to reconstruct the microbial genomes from feces of healthy and inflamed CBA/J mice. This genomic catalog includes 2,281 bacterial and 114 viral metagenome assembled genomes and over 139,000 corresponding unique microbial genes. Genome refinement and dereplication based on completion and contamination criteria resulted in 504 medium- or high-quality MAGs and 113 unique microbial genomes. These dereplicated genomes span 7 phyla, with over 86% (n = 98) representing novel microbial species that lacked prior genomic sampling. 16S rRNA genes from fecal CBA/J communities revealed that the genome database inventoried the most prevalent and abundant microbial genera in noninflamed and inflamed mouse guts. Further, this resource is the first genomic investigation in mice where inflammation reduced overall microbial community diversity by 40% but enhanced the recovery of novel, inflammation-specific, genomes otherwise missed in healthy communities. Functional analysis of these genomes showed significant increases in respiratory metabolic capabilities and propionate production in inflamed mice, concomitant with decreases in relative butyrate and acetate production potential. Importantly, the CBA/J database contains microbes previously unsampled in existing microbial databases from other mice, demonstrating the value of model-specific microbiota resources for enabling precision microbiome manipulations in the future.

Key Words: metagenomics, database, CBA/J mouse, gut, genome

21 Fecal matter transplantation restores the gastrointestinal microbial populations following a traumatic brain injury in a pediatric porcine model. C. B. Welch*, M. M. Fagan, S. E. Sneed, K. M. Scheulin, J. H. Jeon, M. E. Golan, H. J. Park, T. R. Callaway, K. J. Duberstein, T. D. Pringle, J. M. Lourenco, and F. D. West, *University of Georgia, Athens, GA, USA.*

Annually, 400,000 children in the United States are treated for traumatic brain injury (TBI), with 3,000 dying. Still, there is no FDA-approved therapeutic. Due to the bidirectional communication between the brain and gut microbiota, the microbiome–gut–brain axis (MGBA) may be harnessed as a novel therapeutic to mitigate critical TBI-induced inflammatory cascades, thus lessening neural damage. The objective of this study was to determine the potential of fecal matter transplant (FMT) to restore TBI-induced gut microbiota alterations to preinjury populations utilizing a pediatric porcine model. Moderate or severe controlled cortical impact TBI was induced in 4-week-old crossbred piglets that were then administered an FMT (n = 6) or saline solution (CON, n = 6) by oral gavage 2 h after injury and every 24 h for 7 d. Sham (S, n = 6) control animals received a craniectomy and daily saline. Magnetic resonance imaging data were collected at 1 and 7 d post-injury. Fecal samples were collected preinjury and 1, 3, and 7 d post-injury. Cecal samples were collected 7 d post-injury. Fecal and cecal microbial populations and short-chain fatty acids (SCFA) were identified using 16S rRNA gene sequencing and gas chromatography, respectively. Lesion and intracerebral hemorrhage volumes decreased in FMT piglets from d 1 to 7 ($P \leq 0.031$). Cecal *Lactobacillus coleohominis* and *Lactobacillus pontis* increased in FMT piglets ($P \leq 0.018$) compared with CON and S piglets. Fecal *Actinobacillus*, *Actinobacillus indolicus*, *Actinomyces howellii*, and *Bifidobacterium* increased in CON pigs at d 1 ($P \leq 0.048$). *Erysipelotrichaceae_UCG-006* and *Streptococcus hyointestinalis* increased on d 1 and 3, respectively, in CON piglets ($P \leq 0.034$) before returning to preinjury abundances on d 7. Total SCFA in the feces of FMT piglets were comparable to those of S piglets, unlike CON piglets ($P = 0.014$). These results suggest that an acute increase in potentially pathogenic bacteria following a TBI that can be corrected by FMT, thus lessening the severity of pediatric TBI.

Key Words: traumatic brain injury, fecal matter transplant, pediatric porcine model, dysbiosis

22 The maternal oral microbiota significantly contributes to the neonatal gut microbiota composition in CD-1 mice.
A. L. Russell*, E. Donovan, N. Seilhamer, K. Gustafson, and A. C. Ericsson, *University of Missouri, Columbia, MO, USA.*

There is limited understanding of how compositional differences in the maternal gut microbiota (GM) influence vertical transfer of microbes colonizing various maternal tissue, and the subsequent development of the neonatal GM. In our study, we utilize *Mus musculus* outbred CD-1 dams to determine the contribution of various maternal microbiome sites to the GM composition of pups' upper and lower gastrointestinal tract (GIT) over time. Four time points (day of age 9, 10, 11, and 12) were selected based on the GM shift seen between wk 1 and 2 of life in mice. Dams seeded at birth with either a low-richness GM (GM1) or a high-richness GM (GM4) were utilized to determine whether richness has a significant effect on neonatal GM development. Two dams per GM and their pups were euthanized, and tissue samples were collected for each time point, resulting in 16 dams and their pups. Samples representative of oral and vaginal microbiota were collected from dams, whereas samples representative of the upper GIT (feces) and lower GIT (ileum) were collected from all mice. Sample were analyzed using 16S rRNA amplicon sequencing. Bray-Curtis similarity of pup–dam matched samples were used to determine the contribution of each maternal source to the pup fecal and ileal composition at each time point. Results showed that pup fecal similarity to dam feces only increased significantly over time within GM1. Within GM4 mice, pup feces increased in similarity to dam oral samples, and oral similarity to pup feces was higher in GM4 compared with GM1. In contrast, there was no overall effect of GM on pup ileal similarity to maternal tissues. However, there were significant interactions between GM, age of pups, and maternal tissue at both GIT locations. To determine the relationship between maternal tissue and overall pup GM composition, Spearman correlations of pup fecal and ileal similarities for dam oral and vaginal tissue were used. In conclusion, maternal oral composition had the highest correlation coefficient and, therefore, was the maternal tissue with the overall greatest contribution to pup GM composition. However, interactions between all factors tested were found, highlighting the importance of considering environmental factors such as maternal GM composition on future study outcomes.

Key Words: gut microbiota, maternal transfer

Podium Presentations: Session 3

23 *Invited talk:* Enrichment of gut microbiomes from herbivores: Engineering carbon flux through microbial diversity and selection. M. A. O'Malley*, *University of California, Santa Barbara, CA, USA.*

Anaerobic microbes work together in complex communities that decompose and recycle carbon biomass throughout the Earth—from our guts to landfills and compost piles. Despite their importance, little information exists to parse the role of each microbial member within their dynamic community. To address these knowledge gaps, we pioneered new techniques to isolate anaerobes from biomass-rich environments (e.g., guts and fecal materials of herbivores), characterize their shared metabolism, and build synthetic microbiomes to drive biomass to renewable chemicals. Herbivore fecal samples were challenged by different types of biomass during cultivation to identify important microbial partnerships; 10 billion metagenomic reads spread across 402 enrichment samples tracked biological diversity as the cultures converged to a set of stable microorganisms. Nearly 200,000 carbohydrate-active enzymes were identified from the fecal samples, and 724 genomes were assembled for previously uncultured microbes. Surprisingly, consortia dominated by anaerobic fungi generated more than twice the amount of methane compared with prokaryotic consortia, suggesting that fungi accelerate biomass breakdown and methane release in herbivores. Overall, our analysis points to natural compartmentalization between anaerobes as a means to degrade crude biomass, which can be exploited to harness nature's microbes for sustainable chemical production using synthetic systems.

Key Words: anaerobic fungi, lignocellulose, metabolism, enrichment, metagenomics

24 Genome-centric metaproteomics reveals metabolic influence of the ciliate *Entodinium caudatum* within the rumen microbiome. T. O. Andersen*[1], I Altshuler[1], A. V. P. de Leon[2], C. Martin[3], L. Bernard[3], H. Fougere[3], D. P. Morgavi[3], J. L. Firkins[4], Z. Yu[4], T. R. Hvidsten[2], M. Popova[3], M. Ø. Arntzen[2], L. H. Hagen[2], and P. B Pope[1], [1]*Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences,* Ås*, Norway,* [2]*Faculty of Chemisty, Biotechnology and Food Science, Norwegian University of Life Sciences,* Ås*, Norway,* [3]*Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, Saint-Genes-Champanelle, France,* [4]*Department of Animal Sciences, The Ohio State University, Columbus, OH, USA.*

The rumen constitutes a specialized ecosystem composed of a dense and complex mixture of anaerobic bacteria, archaea, protozoa, fungi and phages, that interact closely in the degradation and fermentation of complex plant material into volatile fatty acids (VFAs), utilized for host energy metabolism, and methane gas. The metabolic functions carried out by the rumen microbiome are of scientific and industrial interest, because they contribute to feed efficiency and production of an important human food source (meat and dairy), but additionally contribute significantly to global methane emissions. Therefore, extensive efforts are needed to mitigate enteric methane emissions from ruminant animals without compromising livestock production. While the protozoal populations in the rumen microbiome can comprise up to 50% of the microbial biomass, their biological and metabolic features remain largely unsettled. Advances in culture-independent "meta-omics" approaches continue to increase our understanding of microbiomes. By integrating multiple culture-independent meta-omics techniques, we can obtain a detailed real-time and in situ molecular portrait in which organisms occupy specific metabolic niches. By resolving (meta)genome-centric metaproteome data sets from rumen fluid samples originating from dairy cows and goats fed diets supplemented with different lipid sources, we explore protozoal function in the rumen microbiome. We specifically leveraged these data to investigate

their active metabolic genes and pathways that are responsible for polysaccharide digestion, generation of hydrogen, and production of VFAs. Our results illustrate the significant metabolic influence that these under-explored eukaryotic populations have in the rumen toward both fiber and hydrogen metabolism.

Key Words: metagenomics, metaproteomics, rumen, protozoa, methane

25 Polyclonal antibodies inhibit growth of key cellulolytic rumen bacterial species. S. M. Tondini*, R. I. Mackie, and J. C. McCann, *University of Illinois at Urbana-Champaign, Urbana, IL, USA.*

The rumen microbial ecosystem plays an essential role in the nutrition of ruminants, as they are responsible for degrading and fermenting plant biomass into energy sources for the host. However, many rumen microorganisms have overlapping functions, so inhibiting the activity of targeted bacterial species may help elucidate their dynamic contributions to rumen fermentation. Therefore, our objective was to develop efficacious polyclonal antibodies to inhibit growth of targeted cellulolytic rumen bacterial species. Egg-derived polyclonal antibodies were developed against whole-cell cultures of *Ruminococcus albus 7* (anti-RA7), *Ruminococcus albus 8* (anti-RA8), and *Fibrobacter succinogenes S85* (anti-FS85). Antibodies were added to a cellobiose-containing growth medium of pure cultures of the 3 targeted species. Antibody efficacy was determined via an inoculation time (0 and 4 h) and dose response. Antibody doses included: 0 (CON), 0.013 (LO), 1.3 (MD), 13 (HI) mg of antibody per 10 mL of medium. Each targeted species inoculated at 0 h with HI of their respective antibody had decreased ($P < 0.01$) final optical density and total volatile fatty acid (VFA) concentration after a 52-h growth period compared with CON or LO. Live/dead stains of *R. albus 7* and *F. succinogenes S85* dosed at 0 h with HI of their respective antibody indicated a decrease (≥96%; $P < 0.05$) of live bacterial cells during mid-log phase compared with CON or LO. Addition of HI of anti-FS85 at 0 h in *F. succinogenes S85* cultures reduced ($P < 0.01$) total substrate disappearance over 52 h by at least 48% compared with CON or LO. Cross-reactivity was assessed by adding HI at 0 h to nontargeted bacterial species. Addition of anti-RA8 or anti-RA7 to *F. succinogenes S85* cultures did not affect ($P \geq 0.45$) total VFA accumulation after 52 h of incubation. Overall, polyclonal antibodies were more efficacious at inhibiting growth of targeted cellulolytic bacteria than nontargeted bacteria. Specific polyclonal antibodies can be used to further characterize functional contributions of bacterial species to rumen fermentation.

Key Words: rumen fermentation, cellulolytic bacteria, *Fibrobacter succinogenes*

26 Supplementation of slow-release urea affects rumen microbial community in an artificial rumen system. Y. Guo[1,2], L. Jin[2], L. Xiao[3], S. Yan[1], and W. Yang*[2], [1]*Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China,* [2]*Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, AB, Canada,* [3]*Hangzhou King Techina Feed Co. Ltd., Hangzhou City, China.*

Adding slow-release urea (SRU) in the ruminant diets appears not only for providing a steady nitrogen (N) supply to synchronize energy availability for supporting the microbial protein synthesis, but may also affect rumen microbial diversity and community composition. The objective of this study was to evaluate the effects of SRU on microbial protein synthesis and their diversity and profiles using an artificial rumen system. The experiment was a completely randomized design with 4 treatments and 4 replications of each treatment. The treatments were control diet (no SRU), control plus 0.28% SRU (U28), control plus 0.56% SRU (U56), and control diet that was modified substituting a part of soybean meal equivalent to 0.35% SRU (MU35). The diets were formulated to be isoenergetic, but protein concentration of U28 and U56 increased due to SRU addition and isonitrogenic between control and U35 diets. Experimental period consisted of 8 d of adaptation and 7 d of

sampling. Rumen inoculum was obtained from 3 ruminally fistulated Angus cows fed the same diet to the substrate incubated. Particle-associated bacteria (PAB) and liquid-associated bacteria (LAB) samples were used for high-throughput sequencing to assess the microbial community. Microbial protein synthesis was greater (P = 0.03) with SRU treatments (average, 83.9 mg of N/d) than control (78.9 mg of N/d). Observed operational taxonomic units, Shannon diversity index, and β diversity of the microbial community did not differ among treatments. Taxonomic analysis revealed no effect of adding SRU on the relative abundance (RA) of bacteria at the phylum level. Whereas at the genus level, the RA of *Megasphaera* linearly (P < 0.04) increased and the RA of *Rikenellaceae* group quadratically (P < 0.05) changed with increasing SRU addition in FPA sample. *Prevotellaceae* group in LAB samples tended (P = 0.06) linearly increased with increasing SRU supplementation. In comparison with control, the MU35 had greater (P = 0.01) RA of *Rikenellaceae* and *Prevotellaceae* in LAB. These results demonstrated the benefits of adding SRU in dairy cow diets for improving the synchronization of fermentable carbohydrates and N in rumen and the beneficial impact of SRU on RA of rumen microbial profiles.

Key Words: slow-release urea, rumen microbial diversity, artificial rumen system

27 Impacts of irrigating triticale with dairy lagoon wastewater before harvest and treating with a bacterial inoculant at harvest on the chemical composition, fermentation quality, and bacterial community of silage.

O. Y. Koyun*, J. M. Lourenco, T. R. Callaway, S. Tao, and J. K. Bernard, *Department of Animal and Dairy Science, University of Georgia, Athens, GA, USA.*

We evaluated the combined effects of irrigating triticale with lagoon wastewater before harvest and including a bacterial inoculant (containing *Lactobacillus buchneri* and *Pediococcus pentosaceus*) at harvest on the chemical composition, fermentation profile, and bacterial community of the resulting silage. Treatments were arranged as a 3 × 2 factorial to include 3 time points (21, 14, or 7 d before harvest) for the last application of wastewater to the growing forage, and application of inoculant at harvest (treated vs. control). The statistical model included the effects of day of wastewater application, presence of inoculant, and the interaction between them. Results were considered significant when $P \leq 0.05$. After 60 d of storing ensiled triticale in vacuum sealed bags, samples were analyzed for DM, ash, crude protein, ether extract, NDF corrected for ash, ADF, pH, ammonia, total VFA, and lactic, acetic, and butyric acids. The bacterial community of silage samples were identified using next-generation sequencing technology. Timing of wastewater irrigation resulted in significant but minor differences in chemical composition and fermentation quality of ensiled triticale, yet the differences were not consistent for any time point. Inoculation of the forage with an inoculant resulted in more consistent, desirable effects on fermentation end products. Neither inoculation of the forage nor application of wastewater affected the concentration of select pathogens in the silage, but did affect its microbial composition. The addition of the inoculant reduced microbial richness, diversity, and evenness. Additionally, it increased the abundance of specific microbial taxa such as *Lactobacillus* and *Pediococcus*, at the expense of other taxa such as *Enterococcus*, *Leuconostoc*, *Weissella*, and several other minor genera. Application of wastewater 21 d before harvest increased microbial richness and tended to increase microbial diversity, but the effects on specific taxa were less evident. In contrast, utilization of an inoculant changed the microbial diversity by selecting for microorganisms that are considered more beneficial.

Key Words: triticale, *Lactobacillus buchneri*, silage inoculant, silage microbiota, lagoon wastewater

Podium Presentations: Session 4

28 *Invited talk:* The scientific accomplishments of Dr. Milton J. Allison (1931–2022)—Microbiologist. M. Rasmussen*[1], S. Daniel[2], and R. Anderson[3], [1]*Iowa State University, Ames, IA, USA,* [2]*Eastern Illinois University, Charleston, IL, USA,* [3]*USDA/ ARS, College Station, TX, USA.*

Dr. Milton James Allison, age 90, passed away on March 6, 2022. Dr. Allison had 100 publications and as many abstracts and presentations, so it is a difficult task to summarize the 70-year career of this great scientist. Milt was a microbiologist who greatly contributed to our current understanding of rumen microbiology and physiology. His vast knowledge of the mammalian gastrointestinal tract was incorporated into several invited review papers he wrote, including chapters in *Dukes' Physiology of Domestic Animals*, a standard textbook for veterinary students. He contributed to the Congress on Gastrointestinal Function [formerly the Rumen Function Conference (RFC)] by his regular attendance at meetings and by serving as RFC chair from 1983 to 1989. Over the years, he collaborated with many domestic and international scientists, as indicated in his extensive list of co-authored papers. His research was diverse, novel, and relevant to animal health and disease prevention. He began his work at the National Animal Disease Center (Ames, IA), on grain overload by describing the microbiology and biochemistry of acidosis and the physiology of ruminant eructation. He returned to acidosis later in his career, exploring the use of *Prevotella* as a probiotic to help control lactic acidosis in ruminants. Milt contributed new information fundamental to our understanding of anabolic and catabolic amino acid metabolism by rumen bacteria. His PhD work on the branched-chain volatile fatty acids demonstrated that these growth factors are important for cellulolytic and methane-producing microbes. His work on amino acid metabolism by ruminal bacteria would later lead to the study of oxidized nitrogen compounds such as nitrate, nitrite, and the phytotoxin 3-nitro-1-propanol. Milt determined that nitrate/ nitrite detoxification processes involved ruminal bacteria such as *Selenomonas*, *Veillonella*, and *Wolinella*. Milt and colleagues recognized that respiratory nitrate/nitrite reduction was a favorable electron sink that could outcompete methanogenesis. This is the basis of thenitrate-supplementation strategy to mitigate ruminal methane emissions. His work with plant toxins demonstrated that cattle exposed to nitrotoxin-producing *Astragalus* forages were more tolerant than naïve cattle, an effect achieved by in situ selection of toxin-metabolizing bacteria. This is a process that has been demonstrated for several other plant toxin syndromes. Milt, using one of his true talents, directed successful enrichment experiments to select and isolate toxin-degrading bacteria including *Denitrobacterium detoxificans*, *Synergistes jonesii*, and *Oxalobacter formigenes* (*Oxf*). These bacteria remain the only rumen bacteria known to express appreciable metabolizing activity against their respective toxin substrates (i.e., 3-nitropropanol, mimosine, and oxalate). Milt's work with *S. jonesii* has resulted in researchers worldwide using this microbe to relieve *Leucaena* toxicity problems in ruminants. Through the efforts of Milt and others, *Oxf* is now considered the primary intestinal oxalate degrader, and human colonization by this beneficial anaerobe is associated with a 70% reduction in kidney stone formation. These are significant discoveries given that the incidence of kidney stones in the United States is 5–5% over a lifetime, and Milt worked tirelessly to develop *Oxf* as an oral probiotic for the treatment and prevention of kidney stones. In closing, Milt was a military veteran, devoted husband and father, respected scientist, admired colleague and mentor, and avid fisherman—his life was one well lived and one to be remembered.

29 Branched-chain volatile fatty acid conversion into branched-chain amino acids and lipid synthesis in dual-flow cultures varying in forage and polyunsaturated fatty acid concentrations. K. E. Mitchell*[1], S. L. Kienzle[1], B. A. Wenner[1], C. Lee[2], D. H. Kleinschmit[3], M. T. Socha[3], and J. L. Firkins[1], [1]*Department of Animal Sciences, The Ohio State University, Columbus, OH, USA,* [2]*Department of Animal Sciences, The Ohio State University, Wooster, OH, USA,* [3]*Zinpro Corporation, Eden Prairie, MN, USA.*

Cellulolytic bacteria have limited transport or decarboxylation of branched-chain AA (BCAA), thus depending on cross-feeding for branched-chain volatile fatty acid (BCVFA) precursors for BCAA or for branched-chain fatty acids (BCFA) or their aldehyde derivatives (BCALD) to maintain membrane homeostasis. The study was an incomplete block design with 8 continuous cultures used in 4 periods with 8 treatments (n = 4) arranged as a 2 × 2 × 2 factorial: HF or LF (67 or 33% forage, 33:67 alfalfa:orchard grass), without or with 3% supplemental corn oil (CO), and without or with 2.15 mmol/d (5 mg/d ^{13}C) each of isobutyrate (IsoB), 2-methylbutyrate (2MB), and isovalerate (IsoV). We hypothesized that BCVFA would be used more in the HF diet because of the greater proportion of cellulolytic bacteria; however, linoleic acid from corn oil (CO) would inhibit cellulolytic bacteria or be incorporated into membranes, decreasing need for BCVFA. Recovery of the total ^{13}C dose in bacterial pellets, measured with IRMS, decreased (P = 0.03) from 14.4% with HF to 9.89% with LF. Of the recovered dose, 14.1, 24.3, and 23.6% were in Val, Ile, and Leu, respectively. The sum of *iso* even, *anteiso*, and *iso* odd BCFA had 2.48, 5.24, and 3.41% of the recovered dose. The respective BCALD (separated from BCFA by thin-layer chromatography) was 1.26, 1.81, and 0.80%. The *anteiso* BCALD increased (P = 0.05) from 1.51% with HF to 2.10% with LF. HF had greater (P < 0.01) abundance of bacteria such as *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* that require BCVFA and populations such as *Butyrivibrio* characterized with a high proportion of BCFA. Recovery of ^{13}C in *anteiso* lipids and Ile were the highest; therefore, 2MB was the BCVFA most incorporated into bacteria. IsoV was utilized for BCAA (Leu) and BCFA (*iso* odd) more than IsoB (Val/*iso* even), but IsoB was used for BCALD synthesis more than IsoV. The dose recovery in BCALD did not decrease as did that of BCFA when CO was supplemented. Though BCALD are a small percentage of bacterial lipids (<6%), their much greater relative dose recovery of ^{13}C supports their emerging importance for membrane function, perhaps to protect against oxygen stress or some role in extracellular fiber degradation.

Key Words: cellulolytic bacteria, fatty acids, fatty aldehyde

30 Effects of dosing nontoxigenic Clostridia on bacterial populations and immunological responses in the intestinal tract of lactating dairy cows. H. W. Kim*[1], A. H. Smith[2], J. S. Thompson[2], T. G. Rehberger[2], M. N. Griffin[2], F. F. Cardoso[1], F. Cardoso[1], and R. I. Mackie[1], [1]*University of Illinois at Urbana-Champaign, Urbana, IL, USA,* [2]*Church & Dwight Co. Inc., Waukesha, WI, USA.*

Responses to toxigenic *Clostridia* in cattle have been well documented, but the effects of dosing nontoxigenic commensal *Clostridia* using a systematic cultivation approach are rare. In the present study, a total of 8 lactating dairy cows in 2 groups: untreated control (no added *Clostridia*, n = 4) or with *Clostridia* treatment (*C. bifermentans*, average 7,179 cfu/g, n = 4). Cows were fed according to milk production for 10 weeks and then euthanized for tissue and microbiology sampling. Bacterial communities were analyzed using high-throughput quantitative PCR in the buccal mucosa in lumenal and mucosal samples of the gastrointestinal tract (rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, colon, and rectum, n = 10 compartments). Transcriptomic analysis of barrier and immune-related gene (total of 18 genes) expression was also performed on rumen, jejunum, and liver samples. In the buccal mucosa, the levels of total bacteria, *Firmicutes*, *Bacteroidetes*, and *Clostridia* was higher in the *Clostridia* treated group than in the control (treated: 10.86 to

12.32 log copy number/g; control: 7.95 to 11.03 copy number/g). This finding was consistent with the bacterial load in the digesta of rumen, which indicates that the buccal microbiota can act as a proxy for the rumen microbiota. Along the gastrointestinal tract from rumen to rectum, the total bacterial level in the mucosa generally decreased to the jejunum and then increased through ileum, cecum, and colon in both groups. Similarly, levels of *Firmicutes* in the lumen tended to decrease to the jejunum and then increased to rectum. However, there were no significant differences ($P > 0.05$). Higher *Bacteroidetes* levels were observed in the digesta of ileum to rectum of the treated groups compared with the control; otherwise, *Clostridia* and toxigenic *C. perfringens* did not show any differences. In the rumen mucosa of the treated group, the junction adhesion molecule encoding gene (*JAM2*) was downregulated (−1.44 of $\log_2$ fold-change) and a gene related to the development of rumen epithelial cells (*IGFBP3*) was upregulated (1.68). However, the cow immune system did not respond to the addition of commensal *Clostridia*, and treatment did not affect the barrier function or immune responses of cows in general.

Key Words: commensal *Clostridia*, lactating cows, dosing, intestinal tract, immune function

31 The equine microbiome's subtle responses to dietary challenges can be seen over time. A. C. B. Johnson* and A. S. Biddle, *University of Delaware, Newark, DE, USA.*

This study reports the differential response of the equine gut microbiome to protein or carbohydrate based on keeper status [easy keeper (EK), medium keeper (MK), hard keeper (HK)]. Anaerobic microcosms were inoculated with equine fecal samples (n = 12 total, n = 3/EK, MK, HK of 4 breeds) with 3 dietary conditions [C = carbohydrates (cornmeal), P = protein (soybean meal), M = mix (50% C, 50% P), and F = fecal (no additive)]. Over 48 h (at hours 0, 4, 8, 16, 24, and 48), fermentation products were measured using colorimetric assays and high-performance liquid chromatography. Microbial populations were surveyed using 16S rRNA gene sequencing analyzed by QIIME2. Linear mixed models were fit with fixed effects of Treatment and Keeper status and their interactions, with random effects of HorseID. In terms of function, MK had higher pH and greater gas production ($P < 0.05$) and EK produced higher hydrogen sulfide ($P = 0.05$). Total short-chain fatty acids were not different between keeper status but the acetate:propionate ratio and isobutyrate production were highest for HK and lowest for EK ($P = 0.05$). P-treated trials had increased fermentation outputs due to lower acidity effects. P-treated samples had more α-diversity than C and M ($P < 0.001$). Starting at hour 36, P-treated samples had an increase in α-diversity, demonstrating a community resiliency to recover following fermentation. β-diversities did not differ by community ($P > 0.05$) but the Bray-Curtis PCoA plots showed that EK had a delayed response to dietary perturbation compared with MK and HK. Differential abundance testing identified microbes that responded to treatments over time uniquely to keeper status. For example, *Lactobacillales*, a prominent lactate producer, was differentially abundant for EK and MK across treatments but not a biologically significant marker in HK. Opportunistic *Gammaproteobacteria* bloomed in MK and HK by hour 48, but were only identified in EK at hour 8, indicating that EK communities can resist this growth. Although the gut microbiome compositions of keeper groups were similar, they were functionally different in processing key nutrients. MK were more adaptive to dietary challenges and HK were less efficient at nutrient utilization.

Key Words: equine, in vitro, keeper status

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