INTERNATIONAL™
MILK GENOMICS
CONSORTIUM

13th International Symposium on

Milk Genomics and Human Health

"From Milk to Microbes: Omics Technologies Reveal Mechanisms of Action"

September 27–29, 2016

University of California, Davis
Conference Center
Davis, California, USA

INTERNATIONAL MILK GENOMICS CONSORTIUM

From Milk to Microbes: Omics Technologies Reveal Mechanisms of Action

Dear Participant,

On behalf of the organizing committee, I want to welcome you to the University of California, Davis for the 13th meeting of the International Symposium on Milk Genomics and Human Health. Please take this opportunity to network with your colleagues from around the world and across the scientific spectrum. This meeting is intended to be fully interactive, to build on existing collaborations, and to start new ones. In addition to the formal lectures presentations and poster sessions, we encourage you to explore the campus to see what exciting new programs UC Davis has to offer and make new friends.

As always, we are going to do our utmost to encourage scientific collaboration using every possible means. We will have various social events as part of the symposium. On Tuesday we will have a Poster Reception and on Wednesday evening, we will leave Davis and go to Mulvaney's B&L in Sacramento for the group dinner.

The organizers are also delighted to welcome you to the city of Davis and to the entire region of agriculture in Northern California. The state capitol, Sacramento, is a city with many of the opportunities to see California in 'urban' action. There are also abundant opportunities to see the Napa Valley wine region, one of the finest agricultural regions of the world. And, the San Francisco Bay Area is just an hour away!

Welcome to IMGC 2016. This is our 13th anniversary meeting and we are very excited to host this international conference to discuss the exciting discoveries of science related to milk and lactation and to plan for their applications and opportunities for renewed research. Milk and lactation have proven to be inspiring and unifying, bringing scientists together from across the physical, chemical, biological and computational sciences. We encourage you to renew acquaintances with colleagues, make new friendships and take a bold leap into the diverse fields of science around milk and lactation.

J. Bruce German
On behalf of the local Organizing Committee

Premiere Sponsors

Arla

California Dairy Research Foundation
Our Programs, Your Breakthroughs®

Dairy Australia

Dairy Farmers of Canada
Les Producteurs laitiers du Canada

nzo
nederlandse zuivel organisatie

Nutricia Research
Danone Nutricia Research

Symposium Sponsors

California Dairy Research Foundation
Our Programs, Your Breakthroughs®

Hilmar Cheese Company

NDC
National Dairy Council

Tuesday, September 27, 2016

8:15 am **Registration**

9:00 am **Welcome:** *Butch Dias, Chairman of the Board, California Dairy Research Foundation, Davis, California, USA*

9:15 am **Introduction: How lactation can decode life science's great challenges**
Bruce German, Foods for Health Institute and Dept. of Food Science and Technology, University of California, Davis, USA

9:45 am **Emerging hot topics in milk science**
Danielle Lemay, Genome Center and Foods for Health Institute, University of California, Davis, USA

10:15 am **Coffee Break**

10:45 am **Keynote: New insight on how milk "farms" the neonate gut microbiota**
David Mills, Dept. of Food Science and Technology, University of California, Davis, USA

11:30 am **Rebuilding the infant gut microbiome: Insights from ecology and evolution**
Steve Frese, Evolve Biosystems, Inc., USA

12:00 pm **Safety and tolerability following consumption of bifidobacterium longum subspecies infantis in exclusively breastfed term infants**
Jennifer Smilowitz, Foods for Health Institute, Dept. of Food Science and Technology, University of California, Davis, USA

12:30 pm **Lunch**

1:30 pm **Linking the genome of lactic acid bacteria to the metabolic response of humans having ingested fermented dairy products**
Guy Vergères, Agroscope Institute for Food Sciences, Switzerland

2:00 pm **Ruminant milk and soy solids differentially affect growth, colon gene and protein expression, and microbiota profiles in the interleukin-10 gene-deficient mouse model of inflammatory bowel disease**
Nicole Roy, AgResearch Limited, Grasslands Research Centre, New Zealand

2:30 pm **Use of the milk microbiome to predict infant fecal bacterial community composition**
Janet Williams, Dept. of Animal and Veterinary Science, University of Idaho, Moscow, USA
Student Travel Award Recipient

2:50 pm **Accurate monitoring of living and total bacterial populations in milks for improved microbial management**
Zhengyao Xue, Dept. of Food Science and Technology, University of California, Davis, USA
Student Travel Award Recipient

3:10 pm **Coffee Break**

Tuesday, September 27, 2016

3:40 pm **Identification of sialic acid-utilising bacteria in a piglet caecal community using RNA-SIP**
Wayne Young, AgResearch Limited, Grasslands Research Centre, New Zealand

4:10 pm **Sialic acid release by bacteroides thetaiotamicron from milk oligosaccharides may facilitate the growth of potentially pathogenic bacteria**
Apichaya Bunyatratchata, Dept. of Food Science and Technology, University of California, Davis, USA
Student Travel Award Recipient

4:30 pm **Award Presentation:** Chuck Ahlem, Board of Director, California Dairy Research Foundation Announces the Most Valuable Presentation of the 2015 IMGC Symposium

Update from the Most Valuable Presentation of 2015 Recipient: The role of milk lactoferrin on neurodevelopment and cognition: a dose response, randomized trial
Bing Wang, School of Medicine, Xiamen University, China, and School of Animal and Veterinary Sciences, Charles Sturt University, Sydney, Australia

5:00-6:00 pm **Joint Scientific Advisory Council/Steering Committee Meeting (Closed Session)**

5:30-7:30 pm **Poster Reception**

Wednesday, September 28, 2016

9:00 am **Keynote: Milk glycoproteomics: Preserving, enhancing, and delivering bioactivity**
Daniela Barile, Dept. of Food Science and Technology, University of California, Davis, USA

9:45 am **Milk oligosaccharides function as signalling molecules**
Bethany Henrick, Foods for Health Institute and Dept. of Food Science and Technology, University of California, Davis, USA

10:15 am **Modulation of dendritic cell differentiation and function by human milk oligosaccharides**
Michiko Shimoda, School of Medicine, University of California, Davis, USA

10:45 am **Coffee Break**

11:10 am **Enzymatic modification of bovine milk oligosaccharides and their functional properties**
Valerie Weinborn, Dept. of Food Science and Technology, University of California, Davis, USA
Student Travel Award Recipient

11:30 am **A novel method for high-throughput analysis of bovine milk oligosaccharides**
Randall Robinson, Dept. of Food Science and Technology, University of California, Davis, USA
Student Travel Award Recipient

11:50 am **Discovering natural bioactive peptides in cheese with mass spectrometry**
Randall Robinson, Dept. of Food Science and Technology, University of California, Davis, USA
Student Travel Award Recipient

12:10 pm **Pilot scale isolation of bioactive glycans from dairy co-products: capturing the whey glycome**
Joshua Cohen, Dept. of Food Science and Technology, University of California, Davis, USA
Student Travel Award Recipient

12:30 pm **Lunch**

1:30 pm **Prenatal caprine milk oligosaccharide consumption affects the development of mice offspring**
Caroline Thum, AgResearch Limited, Grasslands Research Centre, New Zealand

2:00 pm **High-throughput milk oligosaccharide analysis using a rapid cartridge-based capillary electrophoresis instrument**
Elisha Goonatileke, Dept. of Chemistry, University of California, Davis, USA

2:30 pm **Glycosylated bioactives compounds in porcine milk: identification and quantification during lactation and correlation with the fecal metagenome**
Jaime Salcedo, Dept. of Food Science and Technology, University of California, Davis, USA

3:00 pm **Coffee Break**

3:30 pm **Milk fat globules as a non-invasive source of mammary microRNA**
Christine Leroux, Institut National de la Recherche Agronomique (INRA), France

Wednesday, September 28, 2016

4:00 pm **Constitutive expression of microRNA-150 in mammary epithelium suppresses secretory activation and impairs de novo lipogenesis**
Richard Heinz, University of Colorado Anschutz Medical Campus, Aurora, USA
Student Travel Award Recipient

4:20 pm **RNA-Seq of mammary epithelial organoids from virgin and lactating glands of 15 Holstein-Friesian cows**
Hannah Lyman, Genome Center, University of California, Davis, USA
Student Travel Award Recipient

6:30-11 pm **Group Dinner**

Transportation provided to and from the Dinner venue in Sacramento.

You are invited to take group transportation. Meet with the group at the Conference Center at 6:15 pm for boarding. The bus will depart at 6:30 pm to Mulvaney's B&L in Sacramento.

The bus will begin boarding from Mulvaney's in Sacramento shortly before 11 pm and will depart at 11:15 pm, arriving in Davis at approximately 11:40 pm.

If you are driving or taking Über/Lyft, please meet us at Mulvaney's B&L, 1215 19th Street, Sacramento at 7 pm for a social hour before dinner at 8 pm. Stay for dessert, live music, networking and fun!

Thursday, September 29, 2016

9:00 am	**Keynote: Glycomic and glycoproteomic variations of human milk** *Carlito Lebrilla, Dept. of Chemistry, University of California, Davis, USA*
9:45 am	**Human milk-derived EV are a major macromolecular component in breast milk with distinct bioactive properties** *Bernd Stahl, Nutricia Research Centre for Specialized Nutrition, The Netherlands*
10:15 am	**Composition of major proteins in breast milk: High-throughput technics for quantitative analysis** *Michael Affolter, Nestlé Research Centre, Switzerland*
10:45 am	**Coffee Break**
11:15 am	**Damage to potential allergy-preventing proteins by regular heating and UVC treatment** *Kasper Hettinga, Dept. of Dairy Science and Technology, Wageningen University, The Netherlands*
11:45 am	**Comparing the sensitizing capacity of raw and processed cow's milk in a murine sensitization model for food allergy** *Ton Baars, Research Institute of Organic Agriculture, Switzerland and Betty van Esch, Utrecht Institute of Pharmaceutical Sciences, The Netherlands*
12:15 pm	**Impact of the degree of prematurity and time post partum on proteases and antiproteases in human milk and the infant stomach** *Veronique Demers-Mathieu, School of Biological and Population Health Sciences, State University, Corvallis, USA*
12:45 pm	**Closing Remarks**
1:00 pm	**Lunch**

Meet the Speakers

Michael Affolter, Ph.D.

Expert Scientist
Nestlé Research Centre, Nestec Ltd.
Vers-chez-les-Blanc
CH-1000 Lausanne, Switzerland
Phone: 0041-217-858-966
E-mail: michael.affolter@rdls.nestle.com

Dr. Michael Affolter is an expert scientist at the Nestlé Research Centre in Switzerland and is leading the protein analytics platform within the Analytical Sciences department. His team develops and integrates mass spectrometry based protein analysis technologies applied to food and nutrition research. Major application fields are milk research, immunology with a focus on food allergies and exploration of new plant protein sources for nutrition. Being educated as an analytical biochemist.

Ton Baars, Ph.D.

Senior Scientist
Research Institute of Organic Agriculture
Ackerstrasse
5070 Frick (CH), Switzerland
Phone: 0049-5545-969482
E-mail: ton.baars@fibl.org

Born in Amsterdam, Professor Ton Baars is a senior researcher for milk and animal welfare at FiBL (CH), research area for milk: fatty acids, grass-based systems, raw milk, asthma and allergies, murine models, milk production without antibiotics. Baars serves on the board of directors for the Raw Milk Institute, USA. Personal website: www.milkandhealth.com.

Daniela Barile, Ph.D.

Associate Professor
University of California, Davis
One Shields Ave.
Davis, CA 95616 USA
Phone: 530-752-9876
E-mail: dbarile@ucdavis.edu

Daniela Barile received her degrees in Pharmaceutical Chemistry and Food Science from the University of Piemonte Orientale. Her lab research aims to understand bioactive compounds formation, their recovery from food production side streams, and their specific interactions within the human body. Her lab utilizes mass spectrometry to investigate foods and a range of relatively untapped organic waste streams for valuable, healthful bioactive compounds (such as free oligosaccharides, peptides, glycoproteins and glycolipids).

Meet the Speakers

Apichaya Bunyatratchata
Student Travel Award Recipient

Graduate Student
University of California, Davis
392 Old Davis Rd.
Davis, CA 95615-21234 USA
Phone: 530-752-4137
E-mail: abunyatratchata@ucdavis.edu

Apichaya Bunyatratchata is a Ph.D. student in the Food Science Graduate Group at UC Davis. After graduating from the University of Massachusetts, Amherst with a B.S. in Food Science and a B.A. in Chemistry, she joined the Barile Lab in 2014. Her research focuses on using advanced mass spectrometry to elucidate the structures of bioactive free oligosaccharides and glycans attached to proteins, and unravel their activity on the gut microbiota.

Joshua Cohen
Student Travel Award Recipient

Graduate Student
University of California Davis
One Shields Ave.
Davis, CA 95616 USA
Phone: 267-515-3052
E-mail: jlcohen@ucdavis.edu

Joshua Cohen is a fourth-year Ph.D. student in the Food Science Graduate Group at UC Davis. After earning his B.S. in Food Science from Penn State, he began working in the Barile lab investigating technologies to isolate bioactive compounds from milk for value-addition and improved nutrition. Josh is interested in developing sustainable and scalable food processing techniques.

Veronique Demers-Mathieu, Ph.D.

Post-Doctoral Researcher
Oregon State University
School of Biological and Population Health Sciences
101 Milam Hall
Corvallis, OR 97331-6802 USA
Phone: 541-286-8366
E-mail: Veronique.Demers-Mathieu@oregon**state.edu**

Veronique Demers-Mathieu gained expertise in dairy microbiology, biochemistry, molecular medicine and enzymology during her Ph.D. studies at Laval University (Quebec, Canada). Since January of 2016, her post-doctoral research in Dr. David Dallas' lab examines the role of human milk components (enzymes, protease inhibitors and bioactive milk proteins) on preterm infant digestion and immunity. Dr. Demers-Mathieu's goal is to apply these findings to create therapeutic approaches to help premature infants digest protein appropriately to improve their digestion, immunity and development.

Meet the Speakers

Steven A. Frese, Ph.D.

Scientific Manager
Evolve Biosystems, Inc.
2121 Second St., Suite B107
Davis, CA 95618 USA
Phone: 530-747-2045
E-mail: sfrese@evolvebiosystems.com

Steven Frese is a microbial ecologist and scientific manager at Evolve Biosystems, Inc., a startup company borne out of 15 years of research at UC Davis, by the world's foremost researchers in the gut microbiome, infant nutrition, and milk. Evolve has developed the next-generation of live bio-therapeutics based on sound, evidence-based research, by understanding how nature has shaped the mother-infant-microbe triad, and how to ensure this healthy triad is supported throughout the nursing period.

J. Bruce German, Ph.D.

Professor
University of California, Davis
One Shields Ave.
Davis, CA 95616 USA
Phone: 530-752-1486
Email: jbgerman@ucdavis.edu

Bruce German received his Ph.D. from Cornell University, joined the faculty at the University of California, Davis in 1988, and is currently the director of the Foods for Health Institute and professor, at the University of California, Davis. His research interests include the structure and function of dietary lipids, the role of milk components in food and health and the application of metabolic assessment to personalizing diet and health.

Dr. German and colleagues co-founded the Milk Genomics Consortium in 2001; this year he is co-chair of the IMGC's symposium along with Dr. Danielle Lemay.

He has published more than 400 papers on milk, lipids and food, metabolism and metabolite measurements and food functions and patented various technologies and applications of bioactive agents. He is a co-founder of the biotechnology company Evolve Biosystems, which provides health solutions to infants and their mothers.

Meet the Speakers

Elisha Goonatileke

Graduate Student
University of California, Davis
One Shields Ave.
Davis, CA 95616 USA
Phone: 530-400-0222
E-mail: egoonatilleke@ucdavis.edu

Elisha Goonatileke is currently a third-year graduate student in Dr. Carlito Lebrilla's research group at the University of California, Davis. Her field of study is in Analytical Chemistry with an emphasis on mass spectrometry. She focuses on the identification, characterization, and quantification of glycoproteins and oligosaccharides in human milk.

Richard Heinz, Ph.D.
Student Travel Award Recipient

University of Colorado
Anschutz Medical Campus
2208 Ironton St.
Aurora, CO 80010 USA
Phone: 224-563-6191
E-mail: Richard.heinz@ucdenver.edu

After earning his BS in Biochemistry from the University of Illinois at Urbana-Champaign, Richard Heinz worked as a lab technologist at Northwestern University Feinberg School of Medicine prior to starting graduate school. Heinz recently completed his Ph.D. in Cancer Biology at the University of Colorado Anschutz Medical Campus, studying the roles of miR-150 in both breast cancer as well as mammary gland development.

Bethany M. Henrick, Ph.D.

Associate Director of Scientific Programs
University of California, Davis
One Shields Ave.
Davis, CA 95616 USA
Phone: 916-479-0226
E-mail: bmhenrick@ucdavis.edu

Bethany Henrick is the associate director of scientific programs at the Foods for Health Institute at the University of California, Davis. In this role, she researches the effect specific milk components play in modulating inflammation in the intestine to further our understanding of how food affects human health. Dr. Henrick's expertise in immunology and molecular biology compliments the mission of the FFHI, which engages in cutting-edge research investigating factors in human milk and food that are beneficial to overall health.

Meet the Speakers

Kasper Hettinga, Ph.D.

Associate Professor
Wageningen University
Bornse Weilanden 9
6708WG, Wageningen, The Netherlands
Phone: 31-317-482401
E-mail: kasper.hettinga@wur.nl

Kasper Hettinga received his Ph.D. at Wageningen University, for a thesis describing a new method for pathogen identification. After his Ph.D., he started research on the protein composition of human and bovine milk. Dr. Hettinga is currently working as tenured associate professor in Dairy Science and Technology at Wageningen University. His main research focus is the characterization of immunologically active proteins in bovine and human milk, focussing on the benefits of these for the newborn baby

Carlito Lebrilla, Ph.D.

Professor
University of California, Davis
2465 Chemistry Annex
Davis, CA 95616 USA
Phone: 530-752-6364
Email: cblebrilla@ucdavis.edu

Carlito Lebrilla is a Distinguished Professor at the University of California, Davis in the Department of Chemistry and Biochemistry and Molecular Medicine in the School of Medicine. He earned his B.S. degree from the University of California, Irvine and Ph.D. from the University of California, Berkeley. He was an Alexander von Humboldt Fellow and a NSF-NATO Fellow at the Technical University in Berlin. He returned to UC Irvine as a President's Fellow. He joined UC Davis in 1989. He has served as Chair of the Chemistr Department. His research is in Analytical Chemistry, primarily mass spectrometry with applications to linical glycomics and biofunctional food. He has over 320 peer-reviewed publications. He is also co-editor of Mass Spectrometry Reviews and has been on the editorial board of Molecular and Cellular Proteomics, Mass Spectrometry Reviews, Journal of American Society for Mass Spectrometry, European Mass Spectrometry, and International Journal of Mass Spectrometry.

Meet the Speakers

Danielle G. Lemay, Ph.D.

Associate Professional Researcher
Genome Center
University of California, Davis
451 Health Sciences Dr.
Davis, CA 95616 USA
Phone: 530-752-7411
E-mail: dglemay@ucdavis.edu

Danielle Lemay is an associate professional researcher at the Genome Center of the University of California, Davis where her research program encompasses the "omics" of milk, mammary biology, milk-oriented microbes, and gut health. She is also the Executive Editor of the IMGC's monthly e-newsletter, "SPLASH! milk science update." She has a Ph.D. and M.S. in Nutritional Biology from UC Davis and a B.S. in Electrical Engineering and Computer Science from MIT. Dr. Lemay is co-chair of this year's symposium.

Christine Leroux, Ph.D.

Research Director
Institut National de la Recherche Agronomique (INRA)
Herbivore Research Unit (UMRH)
F-63122 Saint Genès-Champanelle, France
Phone: 33 (0) 473624062
E-mail: christine.leroux@clermont.inra.fr

Christine Leroux is a research director at the French Institute INRA. She started her research career working on the goat alphaS1 casein polymorphism and its effects on milk component biosynthesis and secretion. For the last few years, she has been working on dairy ruminant nutrigenomics in relation with milk quality and their adaptation to nutritional restriction. She was head of the Nutrition-Genomics-Lactation team for 2 mandates (2008-2015). She is also head of the transcriptomic team of the Platform of metabolism exploration

Hannah S. Lyman
Student Travel Award Recipient

Ph.D. Student
University of California, Davis
One Shields Ave.
Davis, CA 95616 USA
Phone: 510-557-2048
E-mail: hslyman@ucdavis.edu

Hannah Lyman is a Ph.D. candidate in Integrative Genetics and Genomics at UC Davis working with Drs. Danielle Lemay and Monique Rijnkels on expression and regulation in the bovine mammary gland.

Meet the Speakers

David A. Mills, Ph.D.

Professor and Peter J. Shields Endowed Chair in Dairy Food Science
University of California
One Shields Ave.
Davis, CA 95616 USA
Phone: 530-754-7821
E-mail: damills@ucdavis.edu

David Mills is a professor in the Department of Food Science and Technology at the University of California, Davis. Dr. Mills studies the molecular biology and ecology of bacteria that play an active role in gut health or fermented foods and beverages. In the last 20 years he has mentored over 30 graduate students and postdocs and published more than 150 papers, including seminal work on lactic acid bacterial and bifidobacterial genomics. At UC Davis, Dr. Mills has worked to define, investigate and translate the beneficial aspects of human milk and its role in human health. He has previously served as a Distinguished Lecturer for the American Society for Microbiology and currently serves as an editor for the journals Frontiers in Microbiology and Systems. In 2010, Dr. Mills was awarded the Cargill Flavor Systems Specialties Award from the American Dairy Science Association. In 2012 he was named the Peter J. Shields Chair in Dairy Food Science and in 2015 he was elected a Fellow in the American Academy of Microbiology. Dr. Mills also serves on the Science/Research Advisory Boards of several food and health-focused companies and his research has helped launch two startup companies.

Randall C. Robinson
Student Travel Award Recipient

Ph.D. Student
University of California, Davis
392 Old Davis Rd.
Davis, CA 95616 USA
Phone: 530-752-4137
E-mail: rcrobinson@ucdavis.edu

Randall Robinson is a fourth-year Ph.D. student in the department of Food Science and Technology at UC Davis. After earning a B.S. in chemistry from California State University, Stanislaus, he joined the Barile Lab and began characterizing health-promoting food compounds, including peptides and oligosaccharides, using mass spectrometry and novel quantification techniques. He hopes that by improving our understanding of milk's functional properties, we will be able to better utilize those attributes to improve human health.

Meet the Speakers

Nicole Roy, Ph.D.

Associate Professor
AgResearch Limited
Grasslands Research Centre
Tennent Drive, Private Bag 11008
Palmerston North, 4442, New Zealand
Phone: 64 6 351 8110
E-mail: nicole.roy@agresearch.co.nz

Associate Professor Nicole Roy earned her Ph.D. at Laval University, Québec and completed her post-doctoral studies at the Rowett Research Institute, Scotland. She is a principal scientist and science team leader at the Food Nutrition & Health team at AgResearch (21 staff and 10 Ph.D. students). Her research focus is food-microbe-host interactions and gastrointestinal function. Dr. Roy is also one of the science leaders for the High-Value Nutrition National Science Challenge and a principal scientist in the Riddet Institute Centre of Research Excellence.

Jaime Salcedo Dominguez, Ph.D.

Postdoctoral Researcher
University of California, Davis
One Shields Ave.
Davis, CA 95616 USA
Phone: 530-564-3032
E-mail: jsalcedodominguez@gmail.com

Jaime Salcedo Dominguez earned his B.S. and Ph.D. in Chemistry and Food Science at the University of Valencia. His research focuses on evaluating the content and biological effects of gangliosides in human milk and infant formulas. After working as a lecturer at the University Cardenal Herrera-CEU, Dr. Salcedo joined Dr. Barile's group at UC Davis as a postdoctoral researcher and has since worked on unraveling milk glycan profile, content and beneficial properties by improving current analytical techniques, with a final purpose of improving infant health.

Michiko Shimoda, Ph.D.

Assistant Professor
Department of Dermatology
University of California, Davis School of Medicine
2921 Stockton Blvd. Rm. 1630 IRC building
Sacramento, CA 95817 USA
Phone: 916-734-2156
E-mail: mshimoda@ucdavis.edu

Michiko Shimoda received her Ph.D. from the Tokyo University of Agriculture and Technology. She conducted NIH funded research projects on B cell antigen presentation as a principal investigator at the Medical College of Georgia before joining UC Davis. She is currently an assistant professor and the technical director of the Immune Monitoring Core at UC Davis. She is involved in several translational research projects to study immune modulation by glycans in autoimmune diseases and cancers.

Meet the Speakers

Jennifer Smilowitz, Ph.D.

Associate Director and Professional Researcher
University of California, Davis
One Shields Ave.
Davis, CA 95616 USA
Phone: 310-403-4483
E-mail: jensm@ucdavi.edu

Jennifer Smilowitz is the associate director of the Human Studies Research Program for the Foods for Health Institute at UC Davis. She holds a Ph.D. in Nutritional Biology with an emphasis in Endocrinology from the University of California, Davis. Dr. Smilowitz's education, training and the research program she has built over the past eight years have led her to the conclusion that improving the human condition starts with the health of babies. Her research has been widely translational and she is currently conducting several ongoing intervention trials involving dietary interventions that support the growth of beneficial gut microbes in infants and children.

Bernd Stahl, Ph.D.

R&D Director of Breastfeeding Research
Danone Nutricia Research
Uppsalalaan 12
3584 CT, Utrecht, the Netherlands
Phone: 31 611 7979 84
Email: Bernd.STAHL@Danone.com

Bernd Stahl is R&D director of breastfeeding research at Danone Nutricia Research. He earned his Ph.D. in biology at the Westfaelische Wilhelms-University Muenster, Germany. His research was focused on characterization of biologically relevant molecules obtained from human milk and other natural sources by mass spectrometry in connection with other analytical methods. He has contributed significantly to research on specific prebiotic principle for early life nutrition. He was conferred the Mattauch-Herzog Award of the German Society for Mass Spectrometry. He has been chair of ILSI Europe Task Force Prebiotics since 2008. He is the author and co-author of more than 100 scientific publications, reviews and book chapters. Dr. Stahl is a member of the American Society for Nutrition (ASN), American Society for Mass Spectrometry (ASMS), American Chemical Society (ACS), German Biochemical Society (GBM), German Society for Mass Spectrometry (DGMS), European Milk Bank Association (EMBA), International Milk Genomics Consortium (IMGC) and the International Society for Research in Human Milk and Lactation (ISRHML).

Meet the Speakers

Caroline Thum, Ph.D.

Postdoctoral Fellow
AgResearch Limited
Grasslands Research Centre
Tennent Drive, Private Bag 11008
Palmerston North, New Zealand
Phone: 64 6 351 8606

Caroline Thum is a postdoctoral fellow from AgResearch-Riddet CoRE exploring the beneficial effects of goat and sheep milk components on gut-brain development. The current research is an extension of her key research area at AgResearch on exploring the beneficial health effects of non-bovine milk components. During her Ph.D. studies at Massey University, New Zealand, she explored the beneficial effects of goat milk oligosaccharide on intestinal health using in vitro and in vivo mouse models.

Betty van Esch, Ph.D.

Senior Scientist
Utrecht Institute of pharmaceutical Sciences
Faculty of Science
Utrecht University
Universiteitsweg 99,
3584 CA, Utrecht, The Netherlands
Phone: 316 23070485
E-mail: e.c.a.m.vanesch@uu.nl

Dr. Betty van Esch is a post-doc in immunopharmacology. Her work is aimed at the elucidation of immune modulatory and immune-programming mechanisms by nutrition. Her main focus is allergen-specific tolerance induction and nutritional and pharmaceutical intervention studies in allergic diseases using experimental models of food allergy, COPD and allergic asthma. She teaches and supervises a post-doc, three Ph.D. students and two research technicians.

Guy Vergères, Ph.D.

Head of the Research Group Functional Nutritional Biology
Agroscope
Schwarzenburgstrasse 161
3006 Bern, Switzerland
Phone: 41 (0) 58 463 81 54
E-mail: guy.vergeres@agroscope.admin.ch

Guy Vergères is head of the research group Functional Nutritional Biology at Agroscope, Federal Department of Economic Affairs, Education and Research in Bern, Switzerland. He is also a lecturer on nutrigenomics at ETHZ and the University of Lausanne. The translational research of Dr. Vergères focuses on the nutritional properties of fermented dairy products, making use of the recent advances in analytical strategies in food (foodomics) and nutrition (nutrigenomics) sciences.

Meet the Speakers

Bing Wang, M.D., Ph.D.

Professor of Physiology and Nutrition
Charles Sturt University
Locked Bag 588, Boorooma Street
Wagga Wagga NSW 2678, Australia
Phone: 61 2 6399 4549
E-mail: biwang@csu.edu.au

Professor Bing Wang received her medical degree from Tianjin Medical University, China and her Ph.D. in Nutritional Science from the University of Sydney. She led nutritional neurodevelopment research programs in academia and industry before joining Charles Sturt University in 2012. Her research is focused on elucidating the molecular basis of how nutrients alter metabolic responses important in neurodevelopment from fetus to late life, and in translating basic science discoveries to application.

Valerie Weinborn DVM, MS
Student Travel Award Recipient

Food Science Ph.D. Candidate
University of California, Davis
392 Old Davis Rd.
Davis, CA 95616 USA
Phone: 530-220-2930
E-mail: vweinborn@ucdavis.edu

Valerie Weinborn earned her DVM from the University of Chile and holds an M.S. in Nutrition and Foods from the Institute of Nutrition and Food Technology, Chile. Currently, she is a Ph.D. student in Food Science at the University of California, Davis in the Barile lab. Her interests include functional ingredients, specifically prebiotics, and applying this science in the industry. Her current research is on the identification of highly complex modified carbohydrates with bioactive properties from dairy streams.

Janet Williams, Ph.D.
Student Travel Award Recipient

Postdoctoral Fellow
University of Idaho
875 Perimeter Dr. MS2330
Moscow, ID 83844-2330 USA
Phone: 208-874-3213
E-mail: janetw@uidaho.edu

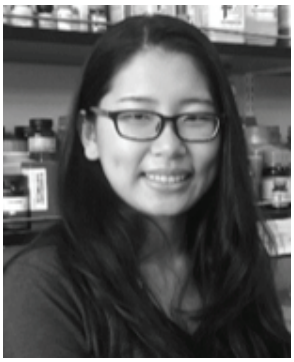
Janet Williams completed her Ph.D. in bioinformatics and computational biology from the University of Idaho in 2016. As part of her doctoral research she studied microbial ecologies of human milk and the gastrointestinal tract. Her goal is to utilize her rich background in lactation physiology combined with her newly gained knowledge of computational methods to better understand complex interactions that exist between humans and their microbes — particularly those that influence milk composition and maternal and infant health.

Meet the Speakers

Wayne Young, Ph.D.

Senior Scientist
AgResearch Limited
Grasslands Research Centre
Tennent Drive
Palmerston North, New Zealand
Phone: 64 6 351 8056
E-mail: wayne.young@agresearch.co.nz

During his Ph.D., completed in 2011, Wayne Young studied the effects of digestion-resistant carbohydrates on the gut microbiota and host physiological at the University of Otago (New Zealand), supervised by Professor Gerald Tannock. After his Ph.D., he joined AgResearch as a staff Scientist where he continues to research the complex microbe host interactions in the gastrointestinal tract, and in particular, how these interactions change in response to diet.



Zhengyao "Zeya" Xue
Student Travel Award Recipient

University of California, Davis
1139 RMI North
392 Old Davis Rd.
Davis, CA 95616 USA
Phone: 530-601-3207
E-mail: zhxue@ucdavis.edu

Zhengyao Xue is a Ph.D. student in Dr. Maria Marco's laboratory in the Department of Food Science and Technology at UC Davis. Her research project is to develop rapid and cost-effective microbial detection methods for dairy companies to implement on site. She received her B.S. in Life Sciences from Nanjing University in China and she was recently awarded a Feeding Tomorrow Graduate Scholarship from the Institute of Food Technologists.

Tuesday, September 27, 2016

9:15 am **How lactation can decode life science's great challenges**

Bruce German, Foods for Health Institute and Dept. of Food Science and Technology, University of California, Davis, USA

The life sciences are turning to the 21st century's challenges and opportunities: how to feed 10 billion people, how to produce that food sustainably, how to prevent disease and how to enhance human performance through a century-long lifespan. Lactation, the genetic secret to success of mammalia, has also wrestled with such challenges for hundreds of millions of years. Research and its translation to commercial practice can use the principles learned under this relentless selective pressure of evolution. The IMGC has been guided by lactation as the Rosetta Stone for the genetics of diet and nourishment for good reasons. Lactation is not a simple recipe, it is an encoded dictionary and encyclopedia. Both the 'words' and the 'deeds' of nourishment are intrinsic to the subset of mammalian genomes dedicated to lactation. Milk is far more than just a complete and comprehensive diet and is an inspiring two-way dialog between a mammalian mother and her infant. Decoding this remarkable system of nourishment, protection and performance will need all of the tools of modern science from genomics to molecular anthropology, chemistry to social behavior. The collaborative model of the IMGC is a vivid example of how scientists from multi-disciplines, geographies and backgrounds can unify around a common theme: how does milk nourish babies?

9:45 am **Emerging hot topics in milk science**

Danielle Lemay, Genome Center and Foods for Health Institute, University of California, Davis, USA

In April 2012, the IMGC began publishing an e-newsletter, "SPLASH! milk science update," which features four articles on emerging topics in milk science each month—that's 48 new articles on milk science each year. By the time of the IMGC conference in 2016, we will have published over 200 articles! This talk will reveal the most exciting milk science topics of the previous 12 months. It will also include a behind-the-scenes tour of "SPLASH! milk science update,": who are the current writers and editors, how topics are selected, and the basics of our publication cycle. The SPLASH! newsletter has helped to grow the IMGC with more than 50,000 visits to the website each year. Nearly all traffic to the IMGC website is the result of SPLASH! content.

The talk will also reveal milk science topics expected to emerge in the coming year and beyond. A vision for the future will be presented with suggestions solicited from the audience to further increase the impact of IMGC publications.

10:45 am **KEYNOTE: New insight on how milk "farms" the neonate gut microbiota**

David Mills, Dept. of Food Science and Technology, University of California, Davis, USA

Human milk contains numerous components that shape the microbial content of the developing infant gastrointestinal tract. A prominent feature of milk is an array of complex glycans and glycoconjugates that serve a passive immune function by sequestering and deflecting pathogens while simultaneously

enriching a protective, milk-oriented microbiota (MOM) often dominated by bifidobacteria. Recent research suggests the timing of establishment, and proper function of, a MOM is critical for infant development. An infant's MOM is initially established through environmental transfer to the gut and subsequently shaped by diet (milk) and host genetics. Once established, MOMs dominated by bifidobacteria exhibit low residual milk glycans and higher levels of short chain fatty acids in the feces, suggesting a strongly saccharolytic colonic microbiota.

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

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

11:30 am **Rebuilding the infant gut microbiome: Insights from ecology and evolution**
Steve Frese, Evolve Biosystems, Inc., Davis, CA, USA

Steven A. Frese1, Andra Hutton1, Lindsey N. Contreras1, Claire A. Shaw1, Jackelyn Moya2,3, Melissa A. Breck2,3, Annette Fineberg MD4, Mark Underwood5, Jennifer T. Smilowitz2,3
1. Evolve Biosystems, Inc., Davis, CA
2. Department of Food Science and Technology, University of California, Davis;
3. Foods for Health Institute, University of California, Davis;
4. Sutter Health, Sutter Davis Hospital, Davis, CA;
5. Department of Neonatology, UC Davis Children's Hospital, Sacramento, CA

The scientific community has been increasingly intrigued by the epidemiologic descriptions of intestinal microbiomes of individuals with metabolic, autoimmune and inflammatory diseases. However, the mechanisms behind many of these observations have not been explained, resulting in the absence of clear intervention strategies. Ongoing research at UC Davis has made a number of important discoveries linking the evolution of lactation and the mammalian gut microbiome. These breakthroughs are revealing the mechanisms for controlling this microbial ecosystem and its importance to the development, disease resistance, and long-term health outcomes of milk-fed neonates. A central paradigm in the control of this ecosystem is the explicit gut microbe-glycan interaction, driven by dietary components and shown to have a profound effect on the gut microbial ecology and metabolism. Thus, dietary glycans have the potential to encourage specific taxa that benefit the host, or to facilitate the expansion of dysbiotic taxa and damage the host.

Previous work at UC Davis and elsewhere has highlighted the importance of key complex milk glycan substrates in cross-feeding intestinal bacterial populations, resulting in gut dysbiosis, or outright infection. By applying an understanding of the ecological forces governing the gut microbiome, and how evolutionary pressures have shaped this relationship, we have developed solutions that incorporate these concepts and deployed them in a variety of clinical settings. In doing so, we found that we are able to formulate stable replacement microbial communities to reduce the abundance of taxa associated with dysbiosis in neonates. Applying this to animal models, we found that these communities were sufficient to dramatically alter health outcomes even in a real life outbreak scenario. In humans, we applied this same strategy in a 'restoration ecology' approach to restore a keystone species, in a controlled clinical trial of Bifidobacterium longum subspecies infantis in human infants and found that stable, specific associations between this subspecies and the milk-fed human infant had a profound effect on both the gut microbiome and the host. Together, these findings fundamentally alter the paradigm of gut microbiome therapeutics and offer a sound, mechanistic pathway toward gut microbiome reconstruction and alleviation of disease in both humans and animals, starting in milk-fed neonates.

12:00 pm Safety and tolerability following consumption of bifidobacterium longum subspecies infantis in exclusively breastfed term infants

Jennifer Smilowitz, Foods for Health Institute and Dept. of Food Science and Technology, University of California, Davis, USA

Jennifer T. Smilowitz1,2, Melissa A. Breck1,2, Jackeyln Moya1, Annette Fineberg MD3, Kathleen Angkustsiri4,5, Mark Underwood MD5
1. Department of Food Science and Technology, University of California, Davis;
2. Foods for Health Institute, University of California, Davis;
3. Sutter Health, Sutter Davis Hospital, Davis, CA;
4. UC Davis MIND Institute, University of California, Sacramento, CA;
5. Department of Neonatology, UC Davis Children's Hospital, Sacramento, CA

For the first six months of life, Bifidobacterium longum subspecies infantis (B. infantis) is the dominant strain of intestinal bacteria in breast-fed infants delivered vaginally. However, in developed nations, infants delivered by either cesarean section or vaginally have lower levels of intestinal B. infantis than infants born in developing nations. B. infantis has been well-tolerated when provided as a supplement to premature infants, but such data has not yet been reported. One objective of the ongoing Infant Microbiota and Probiotic Intake (IMPRINT) Study was to determine if daily consumption of B. infantis by exclusively breastfed infants for 21 days would be safe and well-tolerated. Before Day 6 postnatal, women and their infants delivered either vaginally or by section-section, were randomized into a lactation support (LS) group or lactation support plus B. infantis (BiLS) group. For women who delivered by cesarean section, the two groups were matched for the time when membranes ruptured before birth (≤ 6 hr or > 6 hr). Starting with Day 7 postnatal, and for 21 consecutive days thereafter, infants in the BiLS group were given daily doses of 1x10^10 CFU of B. infantis (sealed individual sachets provided by Evolve Biosystems Inc., Davis, CA) mixed into 5 mL of their mother's breastmilk. Mothers collected their infants' feces and filled out daily questionnaires about infant feeding and gastrointestinal events. Safety and tolerability was determined from daily logs that prompted parents to

assess their infants' feeding, stooling frequency, consistency, gut symptoms and health outcomes. Birthweight and birth length were self-reported and infants were also weighed by study personnel 1 and 2 months postnatal. Compliance was determined by counting B. infantis sachets used bi-weekly. Compared with the LS group, there were more multiparous women enrolled in the BiLS group ($P < 0.05$). There were no differences between groups for infant gestational age at birth, weight, or breastmilk intake. There was a significant time effect ($P < 0.01$), time* intervention interaction ($P < 0.0005$) and intervention effect ($P<0.0005$) for the daily number of infant stools. The number of infant stools significantly increased from baseline ($P< 0.0005$) for infants in the LS group and decreased from baseline ($P < 0.05$) for infants in the BiLS group and significantly decreased during the Post-intervention period for both groups ($P<0.0005$). The change in stool consistency from baseline to the end of the intervention period was significantly different between groups. The mean percent of watery stools decreased and the percent of soft stools increased by 36% from Baseline to the end of the intervention period for infants randomized into the BiLS group compared with 7% for infants in the LS group ($P< 0.05$). Parity was unrelated to the number of infant stools or the change in infant stool consistency. The number of adverse events reported were not different between the LS and BiLS groups and the events reported were considered normal for healthy infants. There was no difference in the number of adverse events reported between the LS and BiLS groups, including blood in infant stool or infant body temperature, and parental ratings of GI-related infant outcomes such as general irritability, upset feelings in response to spit-ups, discomfort in passing stool or gas, and frequency of flatulence. Furthermore, there were no differences in the use of antibiotics, gas-relieving medications, or parental report of infant colic, jaundice, number of illnesses, sick doctor visits and medical diagnoses of eczema. B. infantis was determined to be well-tolerated and did not result in any adverse events in normal in healthy term infants.

1:30 pm **Linking the genome of lactic acid bacteria to the metabolic response of humans having ingested fermented dairy products**

Guy Vergères, Agroscope, Institute for Food Sciences, Berne, Switzerland

Fermentation is a major transformation process in the food and beverage industry and up to one third of products consumed worldwide are fermented. Humans have been fermenting foods and beverages since at least seven millennia, first to protect them from microbial spoilage, later to provide them with attractive sensory properties. This dietary behavior is likely to have impacted on physiological functions other than olfactory processes. Only recently in human history, in particular with the pioneering work of Metchnikoff at the beginning of the 20th century, has food fermentation been considered from the point of view of health benefits. This initial research has later evolved into the field of probiotics. Probiotic research is, however, still struggling on how to translate scientifically sound data into clinically relevant information. In this context, the recently gained knowledge on the key role of the gut microbiome on human health is triggering a scientific renewal, shifting research questions from the search for specific properties of individual bacterial strains to the importance of gut microbial diversity. Taken together, the current knowledge suggests that diets rich in fermented foods may support health by delivering a diverse pool of bioactive compounds (bacteria, bacterial metabolites, products of fermentation) to the gastrointestinal tract and, eventually, to the organism.

Milk is a strategic vector to deliver bacteria and bacterial products to the human organism, in particular because of the acceptance of fermented dairy products by consumers but also, from a technological point of view, because of the sustainability of the fermentation process. We have therefore conducted a series of four acute and short-term intervention studies investigating the response of healthy human subjects to a range of dairy products, including milk, yoghurt, cheese, and butter. The analytical strategy taken to investigate these interactions included a high-density data characterization of the ingested food products (foodomics) as well as of the human samples collected during the intervention (nutrigenomics). Dairy products were characterized both microbiologically, by sequencing the genomic content of the fermenting strains of lactic acid bacteria (LAB), and chemically by measuring their metabolomes. On the other hand, the response of the subjects to the ingestion of the dairy products was investigated by measuring the serum and urine metabolomes, the blood cell transcriptome, and the fecal microbiome. These datasets were complemented with validated metabolic and inflammatory blood parameters. Analyses of the metabolomes of both the dairy matrices and human samples allowed the identification of metabolites whose appearance in humans could directly be attributed to the fermentation of milk to dairy products. The microbial composition of the fecal microbiome was also modified when the ingested dairy matrix was technologically modified, indicating an impact on the composition of the gastrointestinal microbial community. Also, the postprandial blood cell transcriptome of the subjects responded differentially to the ingestion of the processed foods. Finally, sequencing the genome of a range of LABs suggested that genetic information can be linked to the metabolome of the corresponding fermented milk products, thus opening the door to screening strategies for LABs aimed at increasing metabolic diversity in fermented dairy products.

Using this panel of analyses, we were able to provide a proof of concept for an analytical strategy that links the genomic content of LABs to the metabolic response of subjects having ingested the corresponding fermented dairy products. This link remains, however, thin. A holistic understanding of these interactions will not only require an in depth analysis of the biological information contained in the living organisms used in our studies (LAB and humans) but should also be complemented by additional research initiatives.

2:00 pm **Ruminant milk and soy solids differentially affect growth, colon gene and protein expression, and microbiota profiles in the interleukin-10 gene-deficient mouse model of inflammatory bowel disease**

Nicole Roy, AgResearch Limited Grasslands Research Centre, Palmerston North, New Zealand

A.E. Russ1,2,3, M.P.G. Barnett1,3, W. Young1, J. Cooney3,4, R.C. Anderson1, W.C. McNabb1,3,4, N.C. Roy1,3,4
1. Food Nutrition & Health Team, AgResearch Grasslands, Palmerston North, New Zealand;
2. Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand;
3. Nutrigenomics New Zealand (www.nutrigenomics.org.nz);
4. Riddet Institute, Massey University, Palmerston North, New Zealand; Plant and Food Research, Hamilton, New Zealand

The term inflammatory bowel disease (IBD) refers to immune-mediated diseases characterized by chronic intestinal inflammation. Ruminant milks form an important part of the human diet, but people with IBD are often advised to avoid dairy products and may substitute them for soy products.

Presentation Abstracts

Milk and soy contain bioactive molecules with antibacterial, anti-inflammatory, and other immunomodulatory actions which may be of use in the prevention or treatment of IBD. The responses of people with IBD to milk may be influenced by their genotype, and by the composition and genotype of their intestinal microbiota. It is thus important to bear these factors in mind when investigating the effects of milk diets on the molecular pathways involved in the inflammatory process. A better understanding of these pathways may contribute to the use of milk components or different types of milk to prevent and/or reduce the inflammation associated with IBD.

The first aim of this study was to determine whether diets containing solids from different ruminant milks, or from soy, reduce intestinal inflammation in interleukin-10 gene-deficient (Il10–/–) mice, a model of human IBD. The second aim was to investigate changes in biochemical pathways and microbiota composition that may indicate mechanisms by which these diets altered the inflammatory process.

Male Il10-/- mice (15 per treatment) and C57BL/6J (control) mice (9 per treatment) were fed diets containing 40% (w/w) sheep, goat, or cow whole milk powder, 40% (w/w) soy solids, or one of two control diets (casein-free modified-AIN-76A or standard AIN-76A) from 4 to 11 weeks of age. For all diets except AIN-76A, total protein, fat, carbohydrate and energy were as similar as possible. Body weight and food intake were measured three times weekly throughout the experiment, and intestinal tissue was taken at 11 weeks of age for histopathological evaluation of inflammation and transcriptomic/ proteomic analyses. Caecal contents were also collected to characterize intestinal microbiota composition.

Male Il10-/- mice (but not controls) fed the cow and goat milk diets ate less and gained less weight than all other diet groups. Il10-/- mice fed the cow and goat milk diets had reduced colon histological injury scores relative to those on the other diets. Final bodyweight, average intake per day, and colon histological injury scores of Il10-/- mice were not different from control diets for soy and sheep milk diets. Il10-/- mice on the cow and goat milk diets also had decreased expression levels of many immune/inflammatory-related genes and altered expression patterns in immune-related pathways, processes and gene sets. Changes in the levels of some colon tissue proteins were also associated with the reduction in colon inflammation. A strain-diet interaction was seen in the composition of caecal microbiota, where community profiles could be distinguished not only by mouse strain, but also by the type of milk the mice received; particularly cow and goat.

Diets incorporating solids from cow and goat milks affected the growth of Il10–/– mice and also influenced colon inflammation relative to control diets. Reduced levels of colon inflammation in Il10–/– mice fed cow and goat milk were associated with alterations in gene and protein expression and microbiota composition, suggesting the presence of milk components with anti-inflammatory effects. Interactions between the microbiota and biochemical pathways in the colon tissue in response to dietary intervention with different types of milk, and the possible role of these interactions in reducing inflammation, requires further investigation, as does the mechanism of growth reduction. This research may contribute to preventative or therapeutic nutritional interventions for people with IBD.

2:30 pm **Use of the milk microbiome to predict infant fecal bacterial community composition**

Janet Williams, Dept. of Animal and Veterinary Science, University of Idaho, Moscow, USA
Student Travel Award Recipient

Janet E. Williams1,2, Benjamin J. Ridenhour4, Christopher H. Remien3, Sarah L. Brooker1,2, James T. Van Leuven6, Michelle K. McGuire5, and Mark A. McGuire1.
1. Department of Animal and Veterinary Sciences, University of Idaho, Moscow;
2. Program in Bioinformatics and Computational Biology, University of Idaho, Moscow;
3. Department of Mathematics, University of Idaho, Moscow;
4. Department of Biological Sciences, University of Idaho, Moscow;
5. School of Biological Sciences and Paul G. Allen School for Global Animal Health, Washington State University, Pullman;
6. Center for Modeling Complex Interactions, University of Idaho, Moscow

The feeding of human milk to infants reduces the risk of diarrheal diseases. Components in milk that are mitigating this effect are still not clearly defined. Milk is a complex fluid that contains myriad factors including a diverse bacterial community. The influence of the milk bacterial community on the structure of the infant's bacterial community is unknown but we hypothesize that bacterial populations in milk affect the presence and relative abundances of bacteria in the infant's gastrointestinal tract. To examine this, we collected milk samples and infant fecal samples from mother/infant dyads at 9 time points from day 2 to 6 mo pp. DNA was extracted and the bacterial DNA amplified using universal primers targeting the V1-V3 region of the 16S rRNA gene. Amplicons were sequenced using the Illumina MiSeq v3 2 x 300 bp protocol. Sequencing reads were filtered for quality, then joined and classified using a custom python pipeline (dbcAmplicons; https://github.com/msettles/dbcAmplicons). We utilized multivariate Poisson prediction models with cross-validation using the 'glmnet' package in R to capture the complex interactions of the milk bacterial populations on the infant fecal bacterial populations. These models provide an efficient framework that can handle the sparse and high dimensional input matrix of microbiome data. Several bacteria (e.g. Veillonellaceae, Bifidobacteriaceae, and Pasteurellaceae) in milk were positive predictors (effect of 0.019, 0.004, and 0.003, respectively) of the relative abundance of Bacteroides in infant feces. Staphylococcaceae in milk was a negative predictor of the relative abundances of Bifidobacteriaceae and Coriobacteriaceae (-0.051 and -0.240, respectively) and positive predictor of the relative abundances of Staphylococcus and Weissella (0.184 and 0.312, respectively) in infant feces. Individual mothers were found to have an effect on the relative abundances of several bacteria in their infant feces suggesting that the environment (e.g. diet), genetics of the mother, and/or other components unique to the milk from that mother predict the variation observed for those bacteria. These results suggest that members of the milk bacterial community impact the structure of the infant's bacterial fecal community. More research is needed to confirm this in a larger and/or different population. This project was funded by the Bill and Melinda Gates Foundation, the Idaho Agricultural Experiment Station, the Bioinformatics and Computational Program at the University of Idaho in partnership with the Institute for Bioinformatics and Evolutionary Studies (IBEST), and guided by the Center for Modeling Complex Interactions.

Presentation Abstracts

2:50 pm **Accurate monitoring of living and total bacterial populations in milks for improved microbial management**

Zhengyao Xue, Dept. of Food Science and Technology, University of California, Davis, USA

Student Travel Award Recipient

Zhengyao Xue1, Mary Kable1, Jessie Heidenreich2, Jeremy McLeod2 and Maria L. Marco1
1. Department of Food Science and Technology, University of California, Davis;
2. Hilmar Cheese Company, Hilmar, CA

Milk contains a broad diversity of bacteria that enter milks through farm and processing environments. These bacteria are important determinants of the quality and safety of fluid milk and processed dairy products. Currently employed methods for microbial analysis of milk (and other dairy products) typically target only a limited number of bacterial species, and therefore restrict our knowledge on the microbial community dynamics that lead to either high quality or defective dairy products. In order to improve the microbial management of dairy foods, we are developing and applying stream-lined methods for bacterial diversity measurements using culture-independent, high-throughput 16S rRNA gene sequencing. Firstly, we developed protocols for cell collection, DNA extraction, sequencing, and bioinformatic analysis that result in identifying bacterial communities in milk and cheese. Included in these approaches are methods to distinguish between living and total bacteria by the application of propidiummonoazide (PMA) on intact cells. To validate these protocols, we designed a bacterial standard comprising nine strains of dairy-associated bacterial species. DNA was extracted from the strains separately and combined as well as in the presence and absence of milk for sequencing of the 16S rRNA gene V4 regions on two different DNA sequencing platforms. These comparisons revealed significant biases and errors introduced by standard DNA preparation and sequencing methods. These errors could be addressed, in part, by more stringent bioinformatics approaches and sampling and DNA extraction methods that are comparable with automation. Secondly, we are employing these methods to identify raw and pasteurized milks that either result in high quality cheeses or those that are associated with a variety of defects (e.g. slits, soft texture, bitterness, acidic and buttery flavors).

We have thus far shown that high-quality and defective cheeses contain distinct microbiomes. Moreover, the bacteria present in cheeses that have a common defect are also variable. These results indicate that different bacterial species may share some conserved enzymatic functions, which can lead to defective products when these bacterial enzymatic properties are disproportionately high. This knowledge combined with the application of our methods to distinguish between living and total bacterial cells in milk pre- and post-pasteurization and during cheese ripening will enable predictive microbial models along dairy processing chains and ultimately result in consistent products with optimal sensory profiles and nutritive benefits.

3:40 pm **Identification of sialic acid-utilising bacteria in a piglet caecal community using RNA-SIP**

Wayne Young, AgResearch Limited Grasslands Research Centre, Palmerston North, New Zealand

Wayne Young1, Markus Egert2, Shalome Bassett1, Nicole Roy1,3, and Rodrigo Bibiloni1
1. AgResearch Ltd., Food Nutrition & Health Team, Food & Bio-based Products Group, Grasslands Research Centre, Palmerston North, New Zealand;

2. *Furtwangen University, Faculty of Medical and Life Sciences, Institute of Precision Medicine, Microbiology & Hygiene Group, Villingen-Schwenningen, Germany;*
3. *Riddet Institute, Massey University, Palmerston North, New Zealand; Arla Foods, Denmark*

Sialic acids are monosaccharides that can be found on the end of sugar chains expressed on cell surfaces. Milk contains high concentrations of sialic acids attached to the terminal end of oligosaccharides, glycolipids and glycoproteins. Sialic acids are also a key components of neural tissue. The highest concentrations of sialic acid in the body are found in the brain where they form an essential part of ganglioside structures and are therefore critical in neural transmission and synaptogenesis. Animal studies have shown a link between learning ability and levels of sialic acid in brain gangliosides and glycoproteins. Milk oligosaccharides are resistant to mammalian digestive enzymes and are instead degraded by the large bowel microbiota. Intestinal bacteria have also been shown to produce glycosidases capable of desialylating milk gangliosides. However, little is known about the fate of ingested sialic acid and sialic acid-containing nutrients in the large bowel.

The objective was to identify bacteria that utilise free sialic acid in a complex intestinal community of piglet origin, we used an established RNA-stable isotope probing (SIP) approach and fully labelled 13C-sialic acid as a model substrate.

Caecal contents from three male 15-day-old piglets were anaerobically cultured at 37oC for 24 hours in minimal media with or without 13C-labelled sialic acid (2 mg/mL), which was the only added carbon source. Total RNA was extracted from the cultures and separated on caesium trifluoroacetate density gradients by ultracentrifugation at 130,000 x g for 65 hours. After centrifugation, 15 fractions of decreasing densities (1.82-1.73 g/ml) were collected from each gradient for analysis. To investigate the capacity of the microbiota to utilise a sialic acid-conjugated substrate, separate caecal culture incubations were also carried out using the ganglioside GD3, a glycosphingolipid with sialic acid linked to a sugar chain.

Higher-density RNA, from bacteria incorporating heavier 13C into their nucleic acids by metabolising 13C sialic acid, was enriched in fractions with densities between 1.78 and 1.80 g/mL, while lighter unlabeled RNA was predominantly found in fractions with densities between 1.72 and 1.78 g/mL. Pyrotag sequencing of 16S rRNA amplicons showed that the bacterial composition profile of heavy fractions differed from that of the lighter fractions in 13C-sialic acid-fed cultures. In addition, corresponding profiles from caecal communities cultured without 13C-sialic acid were different to those cultured with 13C-sialic acid. Bacteria enriched in the heavier fractions, and therefore the likely users of free sialic acid, included Roseomonas, Prevotella, and Sphingomonas. However, the addition of GD3 as a substrate did not alter the microbial community in caecal content incubations, suggesting GD3 was not readily consumed.

While sialic acid was utilised by many members of the cultured microbial community, our results suggest that fewer taxa are capable of utilizing sialic acid-conjugated milk components. Given the importance of sialic acid in nutrition, further RNA-SIP based studies investing the microbial utilization and assimilation of labelled sialic acid-conjugated milk glycolipids and oligosaccharides is warranted.

Presentation Abstracts

4:10 pm **Sialic acid release by Bacteroides thetaiotamicron from milk oligosaccharides may facilitate the growth of potentially pathogenic bacteria**

Apichaya Bunyatratchata, Dept. of Food Science and Technology, University of California, Davis, USA

Student Travel Award Recipient

Apichaya Bunyatratchata1, Sercan Karav1, Steven A. Frese1, Claire A. Shaw1, Lindsey N. Contreras1, David A. Mills1,2, and Daniela Barile 1,2
1. Department of Food Science and Technology, University of California, Davis;
2. Foods for Health Institute, University of California, Davis

Human milk, as the sole nourishment food for infants, not only support infant's development but also highly influences the shaping of infant gut microbiota. Many members of the Bacteroides, common bacterial species in the gut, are able to utilize complex milk glycans by producing extracellular enzymes (neuraminidases and/or fucosidases) to cleave and release terminal monosaccharides such as sialic acid and fucose. In this study, we demonstrated that Bacteroides thetaiotaomicron, a common bacterium in the infant gut, secretes a neuraminidase that can release sialic acid from sialyated milk oligosaccharides (SMO). The experiment was performed in a minimal medium-containing SMO as a sole carbons source. SMO profiling by Nano-Liquid Chromatography-Chip-Quadrupole-Time-of-Flight mass spectrometry demonstrated a decrease of major SMO including 3-sialyllactose (3SL), 6-sialyllactose (6SL) and 3Hexose-1 N- acetylneuraminic acid after just a few hours of incubation. These results were further validated by measuring a concomitant free sialic acid increase in the medium, in concert with increased neuraminidase expression. B. thetaiotaomicron lacks the pathway to consume the cleaved terminal sialic acid monomers, it leaves the sialic acid in the environment, opening up the possibility for other bacterial species to consume it. To further investigate this hypothesis, we tested the ability of Escherichia coli to consume these free constituents (both SMO and free sialic acid) and found that while it was unable to consume complex milk oligosaccharides, it thrived on the free sugars released by B. thetaiotaomicron.

In agreement with other studies, the ability of Bacteroides to release sialic acid may facilitate the growth of pathogenic bacteria that can consume sialic acid as a carbon source, but lack of ability to produce glycosidases to hydrolyze milk oligosaccharides on their own. In turn, this may lead to promote the inflammation in neonates.

4:30 pm ***Update from 2015 Most Valuable Presentation Recipient***

The role of milk Lactoferrin on neurodevelopment and cognition: A dose response, randomized trial

Bing Wang, School of Medicine, Xiamen University, China, and School of Animal and Veterinary Sciences, Charles Sturt University, Sydney, Australia

Yue Chen1, Zhiqiang Zheng1, Xi Zhu1, Yujie Shi2, Dandan Tian1, Fengjuan Zhao1, Ni Liu1, Petra S. Hüppi3, Frederic A. Troy II14 and Bing Wang1,5
1. School of Medicine, Xiamen University, China;

2. Nestlé Research Center, Beijing, China;
3. Department of Biochemistry and Molecular Medicine, University of California School of Medicine, Davis;
4. Division of Development and Growth, Department of Paediatrics, University of Geneva School of Medicine, Switzerland;
5. School of Animal and Veterinary Sciences, Charles Sturt University, Sydney, Australia

Lactoferrin (Lf) is a sialic acid (Sia)-rich, iron-binding milk glycoprotein that has multifunctional health benefits, including improving neural development and cognition. The aim of this study was to investigate the dose-response effect of milk Lf intervention on gene expression in the hippocampus of postnatal piglets during neurodevelopment.

Fifty-one 3-day-old piglets were randomly allocated into a high (H) Lf dose group (n =18), a low (L) Lf dose group (n=17) and a control (C) group (n=16). Piglets were fed sow milk replacer supplemented with Lf at 285 mg/kg/d (H), 155 mg/kg/d (L) and 16 mg/kg/day (C). Piglets were euthanized at 38 days of age. RNA transcript profiling in the hippocampus was carried out using RNA isolated from 10 piglets/gp on Porcine Affymetrix GeneChips representing 20,201 S.scrofa genes. A TaqMan® Gene expression assay based on qPCR was used to validate the microarray findings. The results were analyzed using the Partek Genomics Suite 6.5 software and Ingenuity System (Chen et al 2014). The selected protein expression was analyzed using western blot and immunohistochemistry techniques.

Low-dose Lf activated neurotrophin signalling pathways and modulated expression of genes associated with neurodevelopment, learning and memory, including BDNF, FGFR, IRS1 and CAMKK1. Functional analysis showed network signalling impacted brain development, neuron structure and long-term potentiation. In contrast, piglets on the high dose of Lf showed no effect on neurotrophin signalling but an increase in gene expression and signalling pathways leading to cell death/apoptosis and decreased neurogenesis.

Low-dose Lf supplementation up-regulated neurotrophin signalling pathways associated with neurodevelopment and cognition, a finding in contrast to piglets on a high dose of Lf. The molecular mechanism(s) underling this paradoxical finding remains under study.

Funding source(s): Medical school of Xiamen University, China, Nestlé Research Centre-Beijing

Wednesday, September 28, 2016

9:00 am **KEYNOTE: Milk glycoproteomics: Preserving, enhancing, and delivering bioactivity**
Daniela Barile, Dept. of Food Science and Technology, University of California, Davis, USA

Daniela Barile1,2, Jaime Salcedo1,2, Annabelle Le Parc3, Adam Sun3, Sercan Karav1,2, Joshua Cohen1,2, Juliana DeMoura Bell1,2
1. Department of Food Science and Technology, University of California, Davis;
2. Foods for Health Institute, University of California, Davis;
3. Prolacta Bioscience®, City of Industry, CA

Human milk is the ideal food in terms of nutrition for newborns as it provides a myriad of bioactive functions that influence infant's healthy growth, before they are able to digest any other food. Much importance has been given to the free glycans known as human milk oligosaccharides (HMO), as they may enhance the development of the gastrointestinal and immune systems of human milk-fed infants. Remarkably, the very complex pool of oligosaccharides found in human milk are indigestible: they do not nourish the infant in any direct sense but rather they nourish a few select strains of commensal bacteria. Studies show that exclusive human milk feeding during the first few months of life decreases the risk of developing necrotizing enterocolitis (NEC) compared to commercial infant formula feeding. When mother's milk is not available, pasteurized donor milk can be used. While heat treatment is conventionally used to improve milk safety, it remains unknown whether such treatment could damage potentially heat sensitive components of human milk (oligosaccharides, glycoproteins, glycolipids). The transformative analytical, computational, and biological toolsets capable of answering this question are only now becoming available. Proteomics and glycomics are being applied to investigate the stability of human milk components to industrial processes: initial results reveal fluctuations in abundance of glycosylated milk bioactive compounds at various thermal treatments and storage conditions.

Despite the important role of HMO for human health, a particular difficulty with research in this area is the lack of sufficient material for performing large functional and in vivo studies. In the search of HMO-mimics, bovine milk oligosaccharides (BMO) were discovered a few years ago. However, bovine milk contains a much lower concentration of these oligosaccharides than human milk. To this extent our group has explored the viability of dairy streams as sources and identified a multitude of BMO in cheese whey permeate. Considering that over 50 million pounds of cheese whey is produced daily just in the state of California (mostly deriving from whey protein extraction) the quantities of whey permeate available for massive extraction of bioactive oligosaccharides make it an attractive source. The concurrent application of mass spectrometry methods with novel purification techniques enable gaining valuable information about the structures of the bioactive milk oligosaccharides and guide their separation process from dairy streams. This work has also been generating valuable bioinformatic libraries containing the experimentally determined information (accurate masses measurements, tandem spectra and retention times) of human and bovine glycans to facilitate their identification in both donor human milk and dairy streams.

Additionally, the ability to use novel processing enzymes to harvest bioactive HMO-mimics (glycans from proteins) using an abundant and inexpensive substrate such as cheese whey, will enable development of new food products with health guiding capabilities for infants, immuno-compromised elderly, and the population at large as well as add value to an existing agricultural co-product.

9:45 am **Milk oligosaccharides function as signalling molecules**

Bethany Henrick, Foods for Health Institute and Dept. of Food Science and Technology, University of California, Davis, USA

The intestinal mucosa is the body's largest surface, and is among one of the most heavily glycosylated. It is thought that the principle function of these glycans is to mediate communication

with the extra- cellular environment. Given that it is becoming increasingly clear that the microenvironment of this mucosal environment has a marked influence on the downstream immune responses, we need to better understand the interactions between ingested glycans, their effect on the intestinal microflora and, our primary focus, how they may serve as signalling molecules that directly impact intestinal epithelial and immune cells. Human milk oligosaccharides (HMO) are a family of structurally diverse (~200 species) unconjugated glycans that are highly abundant in (5-20 mg/mL), and have beneficial effects such as prebiotic effects, antiadhesive antimicrobial roles, and possibly have anti-inflammatory functions in both epithelial and immune cell. Similar in structure, unconjugated glycans known as bovine milk oligosaccharides (BMO) are also present in high concentration in bovine colostrum and may potentially exhibit similar modulating functions in epithelial and immune cells.

Importantly, BMOs represent an unlimited resource, and thus it is imperative to determine their immunomodulating effects on intestinal epithelial and immune cells. Using oligosaccharide fractions derived from human and bovine colostrum at the Milk Processing Lab, University of California, Davis, we first investigated the direct effects of HMO and BMO on the well-established intestinal epithelial cell (IEC) line, Caco-2. Our data showed that HMO induced an immediate and significant increase in COX-2 and tight junction protein, ZO-1 expression, and, interestingly, a significant but relatively mild production of pro- inflammatory cytokine, IL-8 in a dose-dependent manner. Detection of IL-8 production in the supernatant showed a significant but mild increase in Caco-2 supernatant. Likewise, supernatant tested from IEC lines exposed to BMO showed significantly increased IL-8 production. We next tested the immunomodulating effects of both HMO and BMO, and observed that IECs treated with varying doses of HMO before LPS exposure showed a significant decrease in IL-8 production compared to medium alone. Conversely, IECs exposed to varying concentrations of BMO prior to LPS exposure produced significantly more proinflammatory cytokine compared to LPS alone. Taken together, these data suggest that milk oligosaccharides effect IEC signalling and should be investigated further for possible immunomodulatory therapies.

10:15 am **Modulation of dendritic cell differentiation and function by human milk oligosaccharides**
Michiko Shimoda, School of Medicine, University of California, Davis, USA

Michiko Shimoda1, Ace Gita Galermo2, Laura Olney1, Daniela Barile3, Bruce German3, Carlito Lebrilla2, and Emanual Maverakis1
1. Department of Dermatology, University of California, Davis, School of Medicine, Sacramento, CA;
2. Department of Chemistry, University of California, Davis;
3. Department of Food Science and Technology, University of California, Davis

Human milk oligosaccharides (HMO) are a family of structurally diverse (~200 species) unconjugated glycans that are highly abundant in (5-20 mg/mL), and unique to, human milk. In addition to multiple beneficial effects, there is evidence suggesting that HMO modulate immune cell responses by attenuating responses to surface inflammatory stimuli, while enhancing signals that support maturation of the intestinal mucosal immune system. However, how various types of glycans interact with immune cells through receptors and modulate their differentiation and functions is not fully understood.

Dendritic cells (DC) are professional antigen presenting cells that are essential in orchestrating innate

and adaptive immune responses in various stages of health and diseases. Previous studies have shown that manipulating DC functions is an effective approach to direct or re-direct the immune response for favorable outcomes. Furthermore, identifying safe and effective ways to modify DC functions could have tremendous benefits in human health. In this project, we tested the hypothesis that HMO modulate DC function and differentiation. We employed an established in vitro system to generate DC from monocytes or umbilical cord blood hematopoietic stem cells in the presence or absence of a group of HMO. We also studied the effect of HMO on acute DC response to danger- and pathogen- associated molecular patterns. The phenotypic and functional changes in DC were determined by flow cytometry, multiplex bioassay and gene expression analysis.

We found that DC differentiated from monocytes or hematopoietic stem cells in the presence of HMO showed substantial phenotypic and functional changes. For instance, monocyte-derived DC that developed in the presence of HMO, compared to those that developed in the absence of HMO, produced significantly reduced amounts of proinflammatory cytokines in response to bacterial lipopolysaccharide (LPS) or double stranded RNA. Furthermore, after exposure to HMO, blood DC from healthy donors became much less responsive to acute stimulation with LPS or double stranded RNA. The latter was evident by significantly reduced production of Type-I interferons and pro-inflammatory cytokines in association with reduced expression of NFkB downstream target genes. Importantly, this 'tolerance' induction in DC was dependent on the presence of sialyl-glycans in HMO. Thus, these results support the hypothesis that HMO play a pivotal role in modulating the immune system by targeting DC differentiation and function.

Interestingly, many unconjugated glycans are also present in bovine milk, a component of which may carry a similar modulatory function in humans. Thus, based on our findings, a project is ongoing with our experimental system to further characterize bovine glycan components with immune modulatory functions, for disease prevention and therapies.

11:10 am **Enzymatic modification of bovine milk oligosaccharides and their functional properties**

Valerie Weinborn, Dept. of Food Science and Technology, University of California, Davis, USA

Student Travel Award Recipient

Valerie Weinborn1, Yanhong Li2, Xi Chen2, Daniela Barile1
1. Department of Food Science and Technology, University of California, Davis;
2. Department of Chemistry, University of California, Davis

This multi-disciplinary collaborative project aims at developing new strategies that will allow the dairy industry to profit from oligosaccharides recovery. Our approach involves using whey permeate oligosaccharides as "backbones" for the production of improved functional compounds that would add great health value to dairy products.

Human milk is the ideal food in terms of nutrition for newborns but not every infant can access it. The main components of human milk are lactose, lipids and human milk oligosaccharides (HMO), which are sugars present in high concentration in mother's milk and have been associated with important functions related with newborn development and health. HMO acts as prebiotics on a few select bacteria because of their unique structural complexity. Despite the important role of

HMO, these compounds are not well represented in infant formula since it only contains plant derived oligosaccharides (OS). Plant derived OS are used as an additive in a wide range of food products, but despite having a prebiotic effect on the intestinal microbiota, they lack many of the other beneficial effects provided by HMO. This difference is mainly caused by the simplicity of their chemical structure. Increasing the structural complexity of the oligosaccharides present in infant formula, paired with the appropriate bacteria, could potentially lead to improved health in babies. In the search of sources of HMO-mimics, bovine milk oligosaccharides (BMO) were discovered a few years ago. Cheese whey permeate is a byproduct of whey protein production and currently has no commercial value. Because it is considered an environmental pollutant and cannot be readily discarded in the wastewater, the dairy industry currently must manage the costs of disposing of it as if it was hazardous waste. Our group was the first to extract, identify, and characterize a multitude of functional compounds (oligosaccharides) in cheese whey permeate, albeit in low concentration. Considering that over 50 million pounds of cheese whey is produced daily just in the state of California (mostly deriving from whey protein extraction which is now a very successful and growing market) the quantities of whey permeate available for further extraction of bioactive oligosaccharides make it an attractive source.

We successfully utilized whey streams as starting material for the development of an enzymatic approach to produce HMO-mimetics. Combining large-scale isolation of whey OS by membrane filtration, with pilot scale sialylation and fucosylation lead to the production of oligosaccharides much more similar to HMO and in enough quantities to test their expected improved biological activities. Sialylation of whey OS was completed by enzymatic addition of sialic acid by PmST1_M144D, an α2-3-sialyltransferase mutant from Pasteurella multocida. Fucosylation of whey OS was also successfully achieved by enzymatic addition of fucose by Hp1-3FT, an α1-3-fucosyltransferase from Helicobacter pylori, attaining eight fucosylated structures. The addition of sialic acid and fucose and the disappearance of the "donor" whey OS was monitored by nanoLC QToF MS. After further purification, all the fractions containing the new sialylated and fucosylated OS were tested for their improved ability to prevent the in vitro uptake of two common Pathogens Methicillin--resistant Staphylococcus aureus (MRSA) and Enterohaemorrhagic Escherichia coli (EHEC) by Caco--2 cells. Interestingly, both the fucosylated and sialylated OS decreased the uptake of MRSA but only sialylated BMO decreased the uptake of EHEC by Caco--2cells. Further scale-up of this approach will enable obtaining enough quantities to run further functional studies and eventually incorporate the new HMO-mimics into functional dairy foods.

11:30 am **A novel method for high-throughput analysis of bovine milk oligosaccharides**

Randall Robinson, Dept. of Food Science and Technology, University of California, Davis, USA

Student Travel Award Recipient

Randall C. Robinson1, Nina A. Poulsen2, Lotte B. Larsen2, and Daniela Barile1,3
1. Department of Food Science and Technology, University of California, Davis;
2. Department of Food Science, Aarhus University, Denmark;
3. Foods for Health Institute, University of California, Davis

Introduction: Oligosaccharides from mammalian milk are theorized to impart a variety of health benefits to consumers, as evidenced by studies demonstrating prebiotic and disease-preventing bioactivities. Beneficial oligosaccharides are highly concentrated in human milk, but are much

less abundant in bovine milk and related commercial products. Previous studies have shown that both oligosaccharide abundance and complexity can vary between dairy bovine breeds. However, factors affecting such differences in bovine milk are not fully understood. This study has established a method for high-throughput oligosaccharide analysis using isobaric tagging and tandem mass spectrometry which will be used to profile milk oligosaccharides in a large set of bovine milk samples,allowing correlations between breed and oligosaccharide content to be identified.

Oligosaccharide standards were labeled with commercially-available carbonyl-reactive isobaric tags in a known ratio. An LC-MS/MS method was developed on an Agilent 6520 Accurate Mass quadrupole time-of-flight (Q-TOF) mass spectrometer which uses collision-induced dissociation (CID) energies optimized for both precursor m/z and charge state to fragment oligosaccharides. Specific reporter ions originating from the tags are then used as a basis for both relative oligosaccharide quantification and deconvolution of multiplexed samples. In near future oligosaccharides will be analyzed with the optimized method in 850 milk samples collected from two Danish dairy breeds. Further, genotyping has been performed on all cows using the bovine HD SNP-chip so that the genomes can be correlated with oligosaccharide abundances and genetic parameters (heritability and genetic correlations) of bovine milk oligosaccharides can be estimated.

After collision energy optimization, relative oligosaccharide abundances taken from replicate injections of tagged standards showed excellent repeatability and sensitivity, with a coefficient of variation below 5% for each oligosaccharide standard. Oligosaccharide precursor ions of charge 1+ and 2+ demonstrated a predictable, linear trend in optimal collision energy (as evaluated by quantification accuracy, residual precursor abundance, and reporter ion signal), indicating that the method should be applicable to analysis of oligosaccharides for which standards are unavailable. A collision energy trendline for the largest bovine milk oligosaccharides, which ionize as 3+ ions, is under final optimization. The final method will have the capability to quantify the 19 most abundant bovine oligosaccharides, including fucosylated structures in trace abundance.

Relevance to the dairy industry: This work is important for the dairy industry because it will identify heritability and genetic correlations for oligosaccharides which can be used for optimizing breeding programs. In the absence of specialized software for automated oligosaccharide identification (similar to that already available for proteomics and genomics), novel tools need to be integrated into current investigative workflows to make such large studies possible.

11:50 am **Discovering natural bioactive peptides in cheese with mass spectrometry**

Randall Robinson, Dept. of Food Science and Technology, University of California, Davis, USA

Student Travel Award Recipient

Randall C. Robinson1, David C. Dallas2, and Daniela Barile1,3
1. Department of Food Science and Technology, University of California, Davis;
2. School of Biological and Population Health Sciences, Oregon State University, Corvallis;
3. Foods for Health Institute, University of California, Davis

Alterations in gastrointestinal microbiota are becoming increasingly common. Current anti-microbials are successful in treating overt infections, but are non-specific, cause many side effects, and can be

toxic to the host's cells. Non-selective interventions, notably broad-spectrum antibiotics, are proving highly deleterious to the healthy intestinal microbiota. There is a critical need for treatment options that will selectively eliminate pathogens, but at the same time improve or at least not impair the growth of commensal bacteria. Peptides can possess a variety of bioactivities, including antimicrobial functionality, and may be a favorable alternative to traditional antibiotics. Peptides occur naturally in dairy products as a result of microbial fermentation and proteolysis. Therefore, identification of bioactive sequences is important in understanding the physiological impact of these foods.

The objective of this study is to identify the sequences and potential functions of endogenous peptides from several cheeses by LC-Orbitrap MS/MS analysis, including Mimolette (France), Taleggio (Italy), and Stilton (UK), as well as traditional cheddar. Each of these cheeses contains a unique mix of micro-organisms that we hypothesized would contribute to proteolysis and bioactive peptide formation.

Water-soluble peptides were extracted from the rind and interior of each cheese and purified through C18 solid phase extraction. Each peptide extract was analyzed by LC-MS/MS using an Orbitrap Q Exactive Plus mass spectrometer in data-dependent mode. Peptides were then identified from the tandem spectra by an automated search, using the complete bovine proteome as a reference. The resulting peptide lists will be analyzed by an in-house homology search program that will identify peptide sequences from the cheeses which have previously been demonstrated to be bioactive.

The searches identified an outstandingly high number of peptides: 4,701 from Mimolette, 3,520 from Taleggio, 3,408 from Stilton, and 1827 from cheddar. Peptides ranged in length from 5 to 45 amino acid residues. The majority of peptides in each sample originated from caseins, although several other proteins, including β-lactoglobulin, lactoferrin, glycosylation-dependent cell adhesion molecule 1, butyrophilin subfamily 1 member A1, and xanthine dehydrogenase/oxidase, were also well represented in the peptide profiles. Mimolette was particularly notable in that it contained 117 lactoferrin-derived peptide sequences in the rind and only 1 lactoferrin peptide in its interior, demonstrating the differential in proteolytic factors between the two regions. A functional homology search will be conducted in the near future to identify bioactive sequences in each cheese.

12:10 pm **Pilot scale isolation of bioactive glycans from dairy co-products: Capturing the whey glycome**

Joshua Cohen, Dept. of Food Science and Technology, University of California, Davis, USA

Student Travel Award Recipient

Joshua L. Cohen1, Sercan Karav1, Juliana M.L.N. de Moura Bell1, David A. Mills2, and Daniela Barile2
1. Department of Food Science and Technology, University of California, Davis;
2. Foods for Health Institute, University of California, Davis

Complex glycans in human milk have been demonstrated to exhibit a variety of health effects in developing infants, including prebiotic, immunomodulatory, and anti-pathogenic roles. We are isolating milk glycans from dairy co-products in a multifaceted strategy to reclaim economic value from currently underutilized streams and improve nutrition for infants worldwide. We have developed several methods for pilot scale isolation of bovine milk oligosaccharides using fermentation, nano-filtration, and enzymatic treatment. These methods have yielded kg amounts of highly

sialylated milk oligosaccharides, and represent a promising translational opportunity for the dairy industry to reclaim value in co-products. In order to capture an oligosaccharide pool that resembles even more those found in human milk, we have utilized a recombinant enzyme Endo BI-1 cloned from the commensal gut bacterium Bifidobacterium longum subsp. infantis whose primary function is to cleave N-linked glycans from glycoproteins in the infant gut. Use of this enzyme has thus far been successful in small scale, however in order to examine the multiple biological functionalities of the glycans released from bovine whey glycoproteins using Endo BI-1, the magnitude of glycan recovery must be increased. We will use this enzyme in conjunction with a variety of immobilization resins and membrane filtration to scale glycan release and optimize overall process dynamics. Examining total glycan release with cutting-edge mass spectrometry and chromatographic methods will inform development of novel functional ingredients. This immobilization and subsequent glycan release in large scale will enable recovery of sufficient quantities of N-glycans to further investigate their in vitro and in vivo mechanisms of bioactivity and evaluate potential synergistic effects with bovine milk oligosaccharides.

1:30 pm **Prenatal caprine milk oligosaccharide consumption affects the development of mice offspring**

Caroline Thum, AgResearch Limited Grasslands Research Centre, Palmerston North, New Zealand

Caroline Thum1,2, Warren C. McNabb2,3, Wayne Young1,2, Adrian L. Cookson2,4, and Nicole C. Roy1,2,5

1. Food Nutrition & Health Team, Food and Bio-based Products Group, AgResearch Grasslands, Palmerston North, New Zealand;
2. Riddet Institute, Massey University, Palmerston North, New Zealand;
3. AgResearch Grasslands, Palmerston North, New Zealand;
4. Food Assurance & Meat Quality Team, Food and Bio-based Products Group, Hopkirk Institute, Palmerston North, New Zealand;
5. Gravida, National Centre for Growth and Development, The University of Auckland, New Zealand

The composition of the gastrointestinal (GIT) microbiota (commensal vs detrimental), particularly in early life, influences the development of metabolic diseases later in life. The maternal microbiota is the main source of bacteria colonizing the infant GIT and can be modified by dietary prebiotics, such as milk oligosaccharides. Caprine milk contains oligosaccharides structurally similar to human milk oligo-saccharides, which are known to stimulate the development, maturation and colonization of the neonate's GIT. However, important differences in the profile of goat and human milk oligo-saccharides have also been described. The impact of these different milk oligosaccharide profiles on the GIT microbiota and host physiology have been poorly explored.

Our objective was to determine the effects of prenatal consumption of prebiotic caprine milk oligosaccharides (CMO) on the large intestine of female mice, milk composition and offspring development. C57BL/6 mice were fed either a control diet, CMO diet, or galacto-oligosaccharide diet from mating to weaning. From weaning, half pups nursed by CMO, GOS and control-dams were fed the control diet for 30 days. CMO or GOS-fed dams had increased colon length and milk protein concentration compared to control-fed dams. At weaning, pups from CMO-fed dams had increased

body weight and colon length and increased proportions of colonic Bifidobacterium spp compared to the pups from control-fed dams. Thirty days after weaning, pups from CMO-fed dams had increased visceral fat weight compared to pups from control-fed dams. Metabolite profile of the blood plasma showed increased (2 fold) lysophosphatidylcholine (LPC) (20:4) in dams and pups 30 days post weaning and decreased (4.5 fold) LPC 16:0 in pups at weaning. Data from liver metabolic profile and gene expression are currently under analysis. In conclusion, the consumption of CMO by the dams during gestation and lactation improved the development of the pups, and the relative abundance of bifidobacteria and butyric acid in the colon, at weaning. The effects of maternal CMO consumption on lipid metabolism remain to be determined in-depth.

2:00 pm High-throughput milk oligosaccharide analysis using a rapid cartridge-based capillary electrophoresis instrument

Elisha Goonatileke, Dept. of Chemistry, University of California, Davis, USA

Jaime Salcedo1, Elisha Goonatileke2, Andres Guerrero3, Michael Kimzey3, Ted Haxo3, Carlito Lebrilla2, and Daniela Barile1
1. Department of Food Science and Technology, University of California, Davis;
2. Department of Chemistry, University of California, Davis;
3. ProZyme, Inc., Hayward, CA

Milk oligosaccharides (OS) have received much interest for their biological roles as prebiotics, antimicrobial agents and modulators of the immune system. Milk OS are structurally complex molecules that exhibit multiple isomeric forms, and are present with a large dynamic range of concentration in milk and dairy products. Many different analytical approaches and techniques have been used to characterize and quantify milk OS, each exhibiting different advantages and disadvantages.

In this study we employed Gly-Q, a simple, robust and low-maintenance analytical platform developed for the high-throughput analysis of carbohydrates. Sample preparation starting from milk or other dairy products is rapid and automatable, allowing the processing of up to 192 samples in one day. Labeled oligosaccharide samples are analyzed using the Gly-Q instrument in a high-throughput and rapid way: each run takes less than two minutes. In this study, human and bovine milk, as well as developmental dairy products enriched in milk OS, were prepared and analyzed using Gly-Q. Results were compared with milk OS data obtained with more conventional analytical techniques such as high-performance anion-exchange chromatography with pulsed amperometric detection or liquid chromatography coupled to mass spectrometry. The analytical speed of the Gly-Q instrument did not compromise milk OS compound resolution or sensitivity compared to the other analytical techniques tested.

Milk OS data obtained with the Gly-Q system was reproducible, sensitive, and compared favorably to existing analytical techniques. Because of the rapidity of measurement and inherent high throughput, this new platform will prove an invaluable tool in the fields of nutritional and medical sciences, as well as in the dairy industry R&D where it could be used to assist with new product development.

Presentation Abstracts

2:30 pm **Glycosylated bioactives compounds in porcine milk: identification and quantification during lactation and correlation with the fecal metagenome**

Jaime Salcedo, Dept. of Food Science and Technology, University of California, Davis, USA

J. Salcedo1, S.A. Frese1, A. Mudd3, R.N. Dilger3,4, M. Chichlowski5, B.M. Berg5, D.A. Mills1,2, and D. Barile1,2
1. Department of Food Science and Technology, University of California, Davis;
2. Foods for Health Institute, University of California, Davis;
3. Piglet Nutrition & Cognition Laboratory, University of Illinois, Urbana;
4. Division of Nutritional Sciences, University of Illinois, Urbana;
5. Mead Johnson Pediatric Nutrition Institute, Evansville, IN

Swine are an excellent animal model because their digestive system physiopathology and anatomical structure, as well as the developing brain structure and immune system bear a striking resemblance to the human infant.

Glycosylated milk components are known to exert beneficial effects on human health, including protection against bacterial infection, prebiotic activity, support of brain development and modulation of the immune system.

In the present work, we report the characterization and quantification of oligosaccharides and glycolipids in porcine milk during lactation and associate these with the pig fecal metagenome.

Milk samples were manually obtained from 3 adult Yorkshire/Hampshire pigs one day before farrowing (pre-colostrum), at farrowing (colostrum) and at days 7 and 14 post-farrowing, while fecal samples were obtained at days 14 (suckling) and 35 (weaned) after birth from the piglets fed with the analyzed milk. Milk oligosaccharides (OS) were identified by nano LC-Chip QToF, and selected OS were quantified by High-Performance Anionic Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD). Gangliosides GM3 and GD3 were quantified using a UHPLC-MS/MS, while fecal microbiome was sequenced using shotgun metagenomics.

Porcine milk OS composition shifts during lactation. During the first days of lactation acidic OS are the most abundant. As lactation progresses an increase in neutral and fucosylated OS is observed. The total content of gangliosides (as well as the gangliosides GM3 and GD3) vary in content across lactation. Interestingly, the fecal microbiota composition appears to be shaped by the porcine milk OS such that a change in the sialic acid and fucose consuming taxa correlated with variations the respective concentrations of these OS in sow milk.

This study presents the first comprehensive characterization of the types and abundance of glycosylated compounds in porcine milk, and highlights striking similarities with human milk. The potential role of OS in shaping microbiome composition in piglets requires further investigation but appears to represent an excellent model for investigation of interventions that may benefit the human formula fed infant.

3:30 pm Milk fat globules as a non-invasive source of mammary microRNA

Christine Leroux, INRA, Saint-Genès-Champanelle, France

D. Lago-Novais1,2, K. Pawlowski1*, J.A.A. Pires1, C. Bevilacqua3, L. Mobuchon1,3, C. Boby1, Y. Faulconnier1, S. Bes1, P. Martin3, and C. Leroux1*
1. INRA, VetAgroSup, Saint-Genès-Champanelle, France;
2. Universidade Federal da Bahia, Brazil;
3. INRA, AgroParisTech, Jouy-en-Josas, France
**: Equal contribution of the two first authors*

Genomic mechanisms involved in the regulation of milk component synthesis and their secretion are not completely understood. MicroRNA (miRNA, small noncoding RNA) have been shown to influence mammary gland (MG) development and function. Samples for MG gene expression studies are usually obtained via invasive and expensive methods (biopsy or post-mortem) that limit high throughput and dynamic genomic analyses. Recently, milk fat globules (MFG) have been used to assess the mRNA content of the secretory mammary epithelium first in humans1,2 and in ruminants3,4, thus demonstrating that MFG may be an alternative to utilization of MG tissue as a source of mRNA representative of mammary epithelial cells for subsequent gene expression studies. However, the use of the bovine MFG as the source of miRNA has not been studied. The objective of this study was to assess MFG as a source of small RNAs to profile miRNA and to ensure that MFG miRNomes are representative of MG miRNA expression, by comparing the expression of candidate miRNA in MFG and MG sampled from mid-lactation Holstein cows.

Total RNA was extracted from MFG (n=6) and MG (n=6) using TRIzol (ThermoFisher, Inc, USA). Nine miRNA (miR-29a, miR-125b, miR-126, miR-141, miR-148a, miR-204, miR-223, miR-320a, miR-494), chosen on the base of their high abundance in the MG5,6. Their expression was quantified by RT-qPCR (ThermoFisher Inc, USA). The results are expressed as fold change of MFG relative to MG data and using U6 as internal reference. Statistical analyses were performed using a t-test (DataAssitTM software, ThermoFisher Inc) and $P < 0.05$ was considered significant. Target genes of studied miRNA and corresponding putative pathways were investigated using Pathway Studio software.

Among the nine miRNA, two (miR-126 and miR-204) were not detected in MFG whereas they were abundant in MG, three were significantly more abundant in MG than in MFG: miR-29a, miR-125b, and miR-148a exhibiting a fold change value of 23.2, 13.9 and 8.7, respectively. Four studied miRNA were detected in both MFG and MG as equally expressed. Such results suggest different hypothesis. The first one is that there are selective mechanisms of miRNA transfer to milk fat. However, we cannot exclude the second hypothesis that miRNA lacking in MFG are not expressed in epithelial cells, but arising from other MG cell-types. Laser microdissection of mammary epithelial cells, currently in progress, will allow to corroborate the first or the second hypothesis. Among the biological processes targeted by the two miRNA detected exclusively in the MG tissue, there were the adherens junctions and cell-substrate adhesion which are involved in cellular structure. In addition, common biological processes were only enriched with miR-126 and miR-204. Among them, two were related to differentiation and related to regulation mechanisms. Together these biological processes related to miR-126 and miR-204 could be specific to MG cells.

In conclusion, MFG can be used as non-invasive source of miRNA but do not reflect exactly the MG miRNome. Further research is warranted on the composition of MFG miRNome and on the modulation of their secretion in milk. A comprehensive study is currently in progress to compare miRNome from MG and MFG using bovine miRNA microarrays in dairy cows submitted to a nutrient restriction and inflammation protocol. A better knowledge of MFG's miRNome is crucial before using miRNA from MFG as biomarkers of nutritional status or mammary inflammation in ruminants. Moreover, the MFG miRNome could increase the healthy quality of milk for the consumers. Indeed, the action of milk miRNA could be modulated by their packaging within MFG and/or extracellular vesicles which may play an important role for their transfer and function in consumers7.

1Maningat et al. (2007) J. of endocrinology 195: 503-511; 2Maningat et al. (2009) Physiological Genomics, 37: 12-22; 3Brenaut et al. (2012) J. of Dairy Science 95: 6130-6144; 4Canovas et al., (2014) Scientific Reports 4: 5297; 5Le Guillou et al. (2014) PloS ONE 9, e91938; 6Mobuchon et al. (2015) BMC genomics 16: 285; 7Alsaweed et al. (2015) J. of Cellular Biochemistry 116: 2397-2407

4:00 pm

Constitutive expression of microRNA-150 in mammary epithelium suppresses secretory activation and impairs de novo lipogenesis

Richard Heinz, University of Colorado Anschutz Medical Campus, Aurora, USA

Student Travel Award Recipient

Coordinated changes in protein expression govern progression from pregnancy to lactation during mammary gland (MG) postnatal development. In published profiling of miRNA expression in whole mouse MGs, many miRNAs declined precipitously between pregnancy and lactation. We postulated that the decreased expression of miRNAs in the MG during this stage of development allows translation of genes critical for milk production.

To test this hypothesis, we isolated mammary epithelial cells (MECs) and performed simultaneous expression profiling of miRNA and mRNA. We identified microRNA-150 (miR-150) as having the highest fold decrease between pregnancy day 14 (P14) and lactation day 2 (L2). This was verified by qRT-PCR and in situ hybridization (ISH). Pathway analysis of miR-150 predicted targets significantly increasing in MECs between P14 and L2 suggested that miR-150 might play a regulatory role in lipid and cholesterol biosynthesis. By crossing WAP-Cre with ROSA26-lox-STOP-lox-miR-150 transgenic mice, we forced expression of miR-150 in the mouse mammary epithelium from late pregnancy throughout lactation to override the natural decrease.

Compared to offspring nursed by litter mate controls, 3-day old pups nursed by the bi-transgenic dams had approximately 80% decrease in survival ($p<0.0001$) and surviving pups had smaller milk spots. Foster litters and surviving biological pups nursed by bi-transgenic dams showed failure to thrive. Compared to L2 MGs from normal dams, forced expression of miR-150 resulted in a decrease in alveolar density. This was due to alveoli not becoming distended with milk and not because of precocious involution. Microarray performed on L2 RNA samples from MECs of both genotypes revealed that the majority of genes downregulated after forced expression of miR-150 were involved in lipid synthesis more than any other pathway critical to lactation. Suppression (~30-70%) of four predicted targets at the protein level was confirmed by western blot: FASN ($p=0.0001$), ACACA ($p=0.01$), OLAH

(p=0.006), and STAT5B (p=0.0005). Mammary epithelial localization of FASN and ACACA suppression was confirmed by immunohistochemistry. Quantitative gas chromatography mass spectrometry revealed a significant reduction in medium-chain fatty acids, including the sum of all de novo synthesized fatty acids (p=0.003) along with 16:0 (p=0.02) and 18:0 (p=0.03), in MECs from mice with constitutive expression of miR-150 compared to control mice. These results strongly suggest that the decline in miRNAs, such as miR-150, are necessary for successful lactation by allowing translation of critical genes including Fasn, Olah, Acaca and Stat5b.

4:20 pm **RNA-Seq of mammary epithelial organoids from virgin and lactating glands of 15 Holstein-Friesian cows**

Hannah Lyman, Genome Center, University of California, Davis, USA

Student Travel Award Recipient

Hannah S. Lyman1, Keith Bradnam2, Jessica Elswood3, Adrian Molenaar4, Kuljeet Singh3, Joanne Dobson3,
Kim Oden3, Ian Korf1, Danielle G. Lemay1, and Monique Rijnkels3
1. Genome Center, University of California, Davis;
2. Institute of Cancer Research, London, England;
3. Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station;
4. AgResearch Ltd, Grassland Research Center, Palmerston North, New Zealand;
5. AgResearch Ltd, Ruakura Research Center, Hamilton, New Zealand

Although the process of lactation is vital to the commercial production of milk, normal mammary biology is incompletely understood. Next-generation sequencing studies have enabled unprecedented visibility into the genes that are expressed to produce milk. However, previous RNA sequencing (RNA-Seq) has been based on whole mammary glands or milk cells and only a few animals. In the current study, we investigated gene expression in mammary epithelial organoids isolated from the glands of 15 dairy cows in which biopsies were taken from the same cow before their first pregnancy and then again at peak lactation.

Mammary gland biopsies were obtained from 25 Holstein-Freisian cows in New Zealand at approximately 14 months of age. Of the 25 cows, 24 were mated and 19 were sampled at 40–60 days lactation. Sequencing-quality RNA was obtained from mammary epithelial organoids of 15 pairs of virgin and lactation samples. Transcripts were aligned to the UMD3.1/BosTau6 genome release with Ensembl 78 annotations. Differential expression analysis was performed with DESeq2.

RNA-Seq yielded an average of 72 million reads per sample. Over 12,000 genes were expressed in the mammary epithelial organoids of these dairy cows. Consistent with previous studies, we find transcripts corresponding to abundant milk proteins, which are highly expressed in the lactating mammary gland. Mitochondrial genes are highly expressed during lactation, reflecting the increased energy needs of the lactating state. The high number of animals in this study enabled us to detect significant fold changes as small as 1.15-fold. Principle component analysis (PCA) shows a clear segregation of virgin and lactation samples, but with greater heterogeneity among the lactation samples. PCA results suggest that there are three lactation subsets of 8, 5, and 2 animals,

respectively. Given that the animals had similar milk yield and that bovine mammary gland tissue is highly heterogeneous, we suspect that the variance in the samples is due to tissue heterogeneity. Even with this heterogeneity, over 8,000 genes were differentially expressed between virgin and lactation. A deeper understanding of these vast regulatory changes requires both knowledge of how these changes in expression arise, and the biochemical impact of the resulting quantitative differences. To this end, we are generating ATAC-seq data, which exposes active chromatin regions, and performing pathway enrichment analysis to display the functional consequences of gene expression changes.

By integrating genomic and transcriptomic data in the context of biological networks we hope to describe the regulatory logic and the functional consequences that lead to the unique and specialized biology of the lactating mammary gland. This information is needed to improve the efficient production of milk for better sustainability.

Thursday, September 29, 2016

9:00 am **KEYNOTE: Glycomic and glycoproteomic variations of human milk**

Carlito Lebrilla, Dept. of Chemistry, University of California, Davis, USA

Glycans play key roles in diseases and in maintaining health. Despite their importance, our abilities to measure and determine precise structures have been limited because of the large structural heterogeneity and the complicated structures. Our research has focused on developing advanced separation methods, accurate and structurally informative mass spectrometry techniques and automated informatics tools that now make rapid throughput profiling of glycans possible. These methods are employed to determine the role of glycans in nutrition allowing us to observe and characterize the glycan characterization during lactation. Glycan profiling of human milk oligosaccharides and glycoconjugates including glycoproteins and glycolipids are further helping us understand how these compounds establish the gut microbiome.

9:45 am **Human milk-derived EV are a major macromolecular component in breast milk with distinct bioactive properties**

Bernd Stahl, Nutricia Research Centre for Specialized Nutrition, The Netherlands

Martijn J.C. van Herwijnen1, Marijke I. Zonneveld1,2, Soenita Goerdayal3, Esther N.M. Nolte–'t Hoen1, Johan Garssen2,4, Bernd Stahl4, A.F. Maarten Altelaar3, Frank A. Redegeld2, and Marca H.M. Wauben1

1. Department of Biochemistry & Cell Biology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands;

2. Division of Pharmacology, Department of Pharmaceutical Sciences, Faculty of Science, Utrecht University, The Netherlands;

3. Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences;

4. Nutricia Research Centre for Specialized Nutrition, Utrecht, The Netherlands

Human milk is a complex biological fluid containing various macromolecular components with distinctive functions: it provides nutrition, it boosts immunity, it induces tolerance and it supports the epithelial barrier. Generally, each function can be allocated to specific macromolecular structures. The milk fat globules provide most of the lipids while casein micelles provide most of the proteins, making them the nutritional components. The bioactive components of milk are found in the whey fraction, which include antimicrobials, IgA, cytokines and growth factors. In addition, human milk is rich in oligosaccharides that feed the gut microbiota of the newborn. Recently, milk-derived extracellular vesicles (EV) have been identified adding another component to milk. However, their importance and function has not been addressed in detail. This in part is due to the difficulty to obtain human milk and to purify EV from other milk components. Consequently, the molecular composition of human milk-derived EV has not been fully elucidated.

In this study, an extensive LC-MS/MS proteomic analysis was performed on EV isolated via density-based separation from human milk of 7 individual donors. The proteome of milk-derived EV was analyzed for EV-associated markers and compared to the whole milk proteome, which was manually constructed from 38 previously published milk proteomics studies. These included the analysis of whole milk, serum, whey, casein and milk fat globules with a total of 2698 individual proteins.

A total of 1963 proteins were identified in milk-derived EV, including EV-associated proteins like CD9, Annexin A5 and Flotillin-1. Next, the milk-derived EV proteome was compared to the whole milk proteome. Remarkably, 633 proteins identified in milk-derived EV have not yet been identified in milk to date. Interestingly, these novel proteins included proteins involved in regulation of cell growth and controlling inflammatory signaling pathways, while milk proteins not associated to EV were involved in protein metabolism. Using site of expression analysis, we identified that immune cells are the likely producers of milk-derived EV rather than mammary epithelial cells.

In conclusion, these data show that milk-derived EV have an extensive proteome and harbor proteins previously unidentified in milk. These novel proteins have the capacity to support the infant's developing gastrointestinal tract and its immune system. In addition, this study provides an expansion to the whole milk proteome, illustrating that milk-derived EV are a distinct macromolecular component with a unique functional proteome.

10:15 am **Composition of major proteins in breast milk: High-throughput techniques for quantitative protein analysis**

Michael Affolter, Nestlé Research Centre, Switzerland

Michael Affolter1, Clara L. Garcia-Rodenas1, Gerard Vinyes-Pares2, Rosemarie Jenni1, Iris Rogger01, Ornella Avanti-Nigro1, Carlos Antonio De Castro1, Ai Zhao3, Yumei Zhang3, Peiyu Wang4, Sagar K. Thakkar1, and Laurent Favre1

1. Nestlé Research Center, Nestec Ltd., Switzerland;

2. Nestlé Research Center Beijing, Nestec Ltd., People's Republic of China;

3. Department of Nutrition and Food Hygiene, Peking University, People's Republic of China;

4. Department of Social Medicine and Health Education, Peking University, People's Republic of China

Human breast milk protein composition may be impacted by lactation stage or factors related to geographical location. The present study aimed at 1) implementing a high-throughput protein quantification technique to 2) assessing the temporal changes of major breast milk proteins over lactation stages in a large cohort of urban mothers in China [1].

Various approaches have been exploited to identify and quantify milk proteins. Chromatographic or electrophoretic methods have been used to profile major milk proteins whereas immuno-based techniques, i.e. ELISA or antibody arrays, were the method of choice for quantitative analysis of individual proteins. More recently, targeted LC-MS techniques have been developed to separate and quantify specific milk proteins. As the low throughput nature of these methodologies is often the limiting factor for application to larger numbers of samples, an innovative microfluidic chip-based method (LabChip) has been implemented and validated for the present analysis. 450 breast milk samples covering 8 months of lactation were analyzed for α-lactalbumin, lactoferrin, serum albumin, total caseins, immunoglobulins (IgA, IgM and IgG) and transforming growth factors (TGF) 1 and 2 content either by the microfluidic chip- or ELISA-based quantitative methods.

Results will be presented for all measured proteins and method performance criteria will be discussed for this novel approach. The combination of results from total protein measurements and total casein content determination allowed also an estimation of the whey to casein ratio present in the milk. Despite some limitations in the quantification of individual caseins with the microfluidic system, we believe that the minimal sample preparation and the 96-well sample format ideally combines speed and robustness of the analysis process and thus paves the way for a new technological standard for the measurement of proteins in future studies addressing breast milk protein composition.

[1] Michael Affolter, Clara L. Garcia-Rodenas, Gerard Vinyes-Pares, Rosemarie Jenni, Iris Roggero, Ornella Avanti-Nigro, Carlos Antonio De Castro, Ai Zhao, Yumei Zhang, Peiyu Wang, Sagar K.Thakkar and Laurent Favre, Temporal Changes of Protein Composition in Breast Milk of Chinese Urban Mothers and Impact of Caesarean Section Delivery, Nutrients 2016, 8, 504; doi:10.3390/nu8080504.

11:15 am

Damage to potential allergy-preventing proteins by regular heating and UVC treatment

Kasper Hettinga, Dept. of Dairy Science and Technology, Wageningen University, The Netherlands

Kasper Hettinga1, Bob Comstock2, Bethany Michele Henrick3, Erika von Mutius4, Bruce German3, and Markus Ege4
1. Food Quality & Design group, Wageningen University, The Netherlands;
2. Tamarack Biotics, Fresno, CA;
3. Department of Food Science and Technology, University of California, Davis;
4. Dr. von Hauner Children's Hospital, Ludwig Maximilians University Munich, Germany

At the IMGC-2014, data was presented on the difference in the breast milk proteome of allergic versus non-allergic mothers, which was subsequently published (Hettinga et al, 2015). The major outcome of that study was that protease inhibitors were increased in milk of mothers with house dust mite allergy, one of the main allergies in the Western world. Hence, protease inhibitors may play a causal role in the development or prevention of house dust mite allergy; therefore, they have been patented by pharmaceutical industry. Besides breast milk, also bovine milk contains protease inhibitors. The aim of the present study was to determine the heat damage to immune-active bovine milk proteins in general and protease inhibitors in particular.

Bovine milk was heat-treated at different temperatures (from low pasteurization to ultra-high temperature sterilization). Also a non-thermal treatment (UV-C irradiation) was tested as a comparator to thermal treatment of milk. After these milk treatments were done, non-native proteins were removed according to Zhang et al. (2016), followed by LC/MSMS-based proteomics according to Hettinga et al. (2015). Heat damage was calculated as percentage of the protein concentration / activity lost due to heat or UVC treatment, in comparison to the original raw milk. The remaining activity of immune-active proteins was assessed by bacteriostatic assays in both heat and UVC treated milk.

Around 300 proteins were identified in the thermal and UVC treated milk samples combined. Heat damage to the immune-active proteins ranged from around 10% for low pasteurization to 90% for UHT sterilization, showing decreasing proteins concentrations with increasing heating intensities. Protease inhibitors decreased more after low pasteurization (-40%) than other immune-active proteins (-10%), whereas the decrease after UHT sterilization was similar for all immune-active proteins (-85 to -90%).

UVC also damaged proteins, however with different physical-chemical characteristics, as determined by chromatography profiles. Both immune-active proteins (-10%) as well as protease inhibitors (-48%) show a similar decrease after UVC treatment compared to low pasteurization (-10% and -40%, respectively). The bacteriostatic assays corroborated the findings from the proteome analyses.

Damage to immune-active proteins and protease inhibitors depends on the treatment applied to bovine milk. The effect of heat treatment depends strongly on heating intensity, with pasteurization showing relatively high retention of immune-active proteins. UVC treatment is an interesting alternative to heat pasteurization. The physical-chemical nature of the damage by UVC to the proteome remains to be elucidated.

Hettinga KA, Reina FM, Boeren S, Zhang L, Koppelman GH, Postma SD, Vervoort JJM Wijga AH. Difference in the Breast Milk Proteome between Allergic and Non-Allergic Mothers. PLoS One 2015; 10(3): e0122234; Zhang L, Boeren S, van Hooijdonk ACM, Vervoort JJM, Hettinga KA. 2016. Proteomic study on the stability of proteins in bovine, camel, and caprine milk sera after processing. Food Research International 82: 104–111

11:45 am Comparing the sensitizing capacity of raw and processed cow's milk in a murine sensitization model for food allergy

Ton Baars, Research Institute of Organic Agriculture, Frick, Switzerland and Betty van Esch, Utrecht Institute of Pharmaceutical Sciences, Utrecht University, The Netherlands

S. Abbring1, M.A.P. Diks1, G.M. Dingjan1, T. Baars2, J. Garssen1,3, and B.C.A.M. van Esch1,3
1. Div of Pharmacology, Utrecht Institute of Pharmaceutical Sciences, Faculty of Science, The Netherlands;
2. Research Institute of Organic Agriculture (FiBL), Frick, Switzerland;
3. Nutricia Research, Utrecht, The Netherlands

Epidemiological studies show that the consumption of raw milk early in life is protective against the development of allergies later in life. The effect was found to be limited to raw milk consumption and was not observed when this milk was boiled or when pasteurized and homogenized shop milk was consumed. So milk processing seems to abolish the allergy protective effects of raw milk. The components and mechanisms involved are however still unknown. In this study the sensitizing capacity of raw and processed cow's milk was compared in a murine sensitization model for food allergy.

C3H/HeOuJ mice were sensitized orally once a week for 5 consecutive weeks with raw milk, heated raw milk (10 minutes 80°C), shop milk (pasteurized and homogenized), an 80:20 mixture of casein/whey protein (equivalent to the amount in milk; sensitized control) or PBS (non-sensitized control) using cholera toxin as adjuvant. One week after the last sensitization mice were challenged both intradermally and orally with casein/whey. Clinical parameters, such as the acute allergic skin response, anaphylactic shock symptoms and changes in body temperature were assessed upon intradermal challenge and serum specific antibodies and mast cell degranulation were measured upon oral challenge. Activated Th2-, Th1- and regulatory T-cell populations were quantified in spleen using flow cytometry and ex vivo cytokine production was measured after re-stimulation with casein/whey.

Mice sensitized with raw milk did not show any clinical symptoms upon challenge and did hardly produce specific IgE antibodies. Sensitization with the processed milk types on the contrary increased the acute allergic skin response and anaphylactic shock symptoms and caused a drop in body temperature. IgE levels were also significantly increased in these mice. No differences were observed in mucosal mast cell degranulation between groups and also Tcell populations did not differ. The production of Th2 cytokines IL-5 and IL-13 was however significantly reduced in the raw milk group compared to the processed milk groups after ex vivo re-stimulation of splenocytes with casein/whey.

In contrast to processed milk, raw milk is hardly able to induce sensitization. Allergic symptoms and IgE levels were reduced and this coincided with reduced Th2 cytokine responses. What it is in raw milk that prevents sensitization needs to be elucidated in future studies.

12:15 pm Impact of the degree of prematurity and time post-partum on proteases and antiproteases in human milk and the infant stomach

Veronique Demers-Mathieu, Oregon State University, Corvallis, USA

Veronique Demers-Mathieu1, Søren Drud Nielsen1, and David Dallas1
1. Nutrition Program, School of Biological and Population Health Sciences, College of Public Health and Human Sciences, Oregon State University, Corvallis

Human milk contains a complex system of proteases and protease inhibitors. This proteolytic system provides an apparently controlled and protein-selective proteolytic digestion in the mammary gland and the infant stomach. This balance of proteases and antiproteases may allow for assistance with protein digestion during the early stages of the infant's digestive development while also allowing immune-related proteins like lactoferrin and the immunoglobulins to remain intact and functional. Protease activity is likely to differ across the degree of prematurity because of the physiological maturation differences; however, the degree to which specific proteases and inhibitors differ across prematurity remain unexplored.

Our research aim was to determine the degree to which the concentration and activity of milk's proteases (plasmin, kallikrein, thrombin, elastase, cathepsin D, cytosol aminopeptidase and carboxypeptidase B2) and protease inhibitors (α1-antitrypsin, α1-antichymotrypsin, α2-antiplasmin, plasma serine protease inhibitor and antithrombin III) differ between human milk and the infant stomach, as well as across the spectrum of prematurity and across time post-partum. Human milk and infant gastric samples were collected from two groups of mother-infant pairs: 10 early preterm (24–26 wks of gestational age at birth (GA) and 620–770 g birth weight) and 10 late preterm (27–32 GA wks and 900–2,210 g birth weight). The activity level of each milk protease was determined by fluorometric assays, and the concentrations of these enzymes as well as gastric pepsin and the protease inhibitors were determined by ELISA assays. Total protease activity, pH and protein concentrations were also determined in these samples. Concentrations of immune-related proteins (IgA and lactoferrin) were determined by ELISA assays. Carboxypeptidase B2 was the most abundant and active protease in human milk, kallikrein was the second and plasmin the third, followed by cathepsin D, thrombin and elastase. These milk proteases were far more active and abundant than pepsin in the preterm infant stomach (milk enzymes accounted for 83-88% of total protease activity in the stomach). The early preterm infant stomach contained lower pepsin and cytosol aminopeptidase concentrations and lower plasmin, elastase and thrombin activities than the late preterm infant stomach for at least 3 weeks postpartum. However, the late preterm infant stomach had higher α1-antitrypsin concentration than in the early infant stomach, which may affect overall protein digestive capacity (all other protease inhibitor concentrations were similar between early and late preterm infants). Lactoferrin and IgA concentrations were higher in the early than late preterm milk. Across time post-partum (1 wk vs. 3 wk), in human milk, activities of plasmin, thrombin and cytosol aminopeptidase and concentrations of α1-antitrypsin, α1-antichymotrypsin, α2-antiplasmin decreased while activities of kallikrein and elastase increased. In addition, in the stomach, activity of kallikrein and elastase increased across time post-partum. Lactoferrin concentration decreased across time; however, IgA concentration remained stable, suggesting some protection from gastric digestion. Future work will examine further how protein digestion changes across gestational age and time post-partum.

1) Sialic acid release by Bacteroides thetaiotamicron from milk oligosaccharides may facilitate the growth of potentially pathogenic bacteria

Apichaya Bunyatratchata, Dept. of Food Science, University of California, Davis, USA
Student Travel Award Recipient

Apichaya Bunyatratchata1, Sercan Karav1, Steven A. Frese1, Claire A. Shaw1, Lindsey N. Contreras1, David A. Mills1,2, and Daniela Barile1,2
1. Department of Food Science and Technology, University of California, Davis;
2. Foods for Health Institute, University of California, Davis

Human milk, as the sole nourishment food for infants, not only support infant's development but also highly influences the shaping of infant gut microbiota. Many members of the Bacteroides, common bacterial species in the gut, are able to utilize complex milk glycans by producing extracellular enzymes (neuraminidases and/or fucosidases) to cleave and release terminal monosaccharides such as sialic acid and fucose. In this study, we demonstrated that Bacteroides thetaiotaomicron, a common bacterium in the infant gut, secretes a neuraminidase that can release sialic acid from sialyated milk oligosaccharides (SMO). The experiment was performed in a minimal medium containing SMO as a sole carbons source. SMO profiling by Nano-Liquid Chromatography-Chip-Quadrupole-Time-of-Flight mass spectrometry demonstrated a decrease of major SMO including 3-sialyllactose (3SL), 6-sialyllactose (6SL) and 3Hexose-1 N- acetylneuraminic acid after just a few hours of incubation. These results were further validated by measuring a concomitant free sialic acid increase in the medium, in concert with increased neuraminidase expression. B. thetaiotaomicron lacks the pathway to consume the cleaved terminal sialic acid monomers, it leaves the sialic acid in the environment, opening up the possibility for other bacterial species to consume it. To further investigate this hypothesis, we tested the ability of Escherichia coli to consume these free constituents (both SMO and free sialic acid) and found that while it was unable to consume complex milk oligosaccharides, it thrived on the free sugars released by B. thetaiotaomicron. In agreement with other studies, the ability of Bacteroides to release sialic acid may facilitate the growth of pathogenic bacteria that can consume sialic acid as a carbon source, but lack of ability to produce glycosidases to hydrolyze milk oligosaccharides on their own. In turn, this may lead to promote the inflammation in neonates.

2) Pilot scale isolation of bioactive glycans from dairy co-products: Capturing the whey glycome

Joshua Cohen, Dept. of Food Science and Technology, University of California, Davis, USA
Student Travel Award Recipient

Joshua L. Cohen1, Sercan Karav1, Juliana M.L.N. de Moura Bell1, David A. Mills2, and Daniela Barile2
1. Department of Food Science and Technology, University of California, Davis;
2. Foods for Health Institute, University of California, Davis

Complex glycans in human milk have been demonstrated to exhibit a variety of health effects in developing infants, including prebiotic, immunomodulatory, and anti-pathogenic roles. We are isolating milk glycans from dairy co-products in a multifaceted strategy to reclaim economic value from currently underutilized streams and improve nutrition for infants worldwide. We have successfully developed several methods for pilot scale isolation of bovine milk oligosaccharides using fermentation, nanofiltration, and enzymatic treatment. These methods have yielded kg amounts of highly sialylated milk

oligosaccharides, and represent a promising translational opportunity for the dairy industry to reclaim value in co-products. In order to capture an oligosaccharide pool that resembles even more those found in human milk, we have utilized a recombinant enzyme Endo BI-1 cloned from the commensal gut bacterium Bifidobacterium longum subsp. infantis whose primary function is to cleave N-linked glycans from glycoproteins in the infant gut. Use of this enzyme has thus far been successful in small scale, however in order to examine the multiple biological functionalities of the glycans released from bovine whey glycoproteins using Endo BI-1, the magnitude of glycan recovery must be increased. We will use this enzyme in conjunction with a variety of immobilization resins and membrane filtration to scale glycan release and optimize overall process dynamics. Examining total glycan release with cutting-edge mass spectrometry and chromatographic methods will inform development of novel functional ingredients. This immobilization and subsequent glycan release in large scale will enable recovery of sufficient quantities of N-glycans to further investigate their in vitro and in vivo mechanisms of bioactivity and evaluate potential synergistic effects with bovine milk oligosaccharides.

3)

Constitutive expression of microRNA-150 in mammary epithelium suppresses secretory activation and impairs de novo lipogenesis

Richard Heinz, University of Colorado Anschutz Medical Campus, Aurora, USA

Student Travel Award Recipient

Heinz RE1, Rudolph MC2, Ramanathan P3, Spoelstra NS4, Butterfield KT4, Webb PG5, Babbs BL4, Gao H6, Chen S6, Gordon M4, Anderson SM4, Neville MC5,7, Gu H4,6 and JK Richer4

1. Cancer Biology Graduate Program

2. Division of Endocrinology, Metabolism and Diabetes, School of Medicine, Departments of: 4. Pathology, 5. Obstetrics and Gynecology, 7. Physiology and Biophysics, and at the University of Colorado Anschutz Medical Campus, Aurora, CO

3. Department of Pathology, University of Texas Medical Branch, Galveston, TX.

6. Key Laboratory of Laboratory Medicine, Ministry of Education, School of Laboratory Medicine and Life Science, Wenzhou Medical University, Wenzhou 325035, China.

Coordinated changes in protein expression govern progression from pregnancy to lactation during mammary gland (MG) postnatal development. In published profiling of miRNA expression in whole mouse MGs, many miRNAs declined precipitously between pregnancy and lactation. We postulated that the decreased expression of miRNAs in the MG during this stage of development allows translation of genes critical for milk production. To test this hypothesis, we isolated mammary epithelial cells (MECs) and performed simultaneous expression profiling of miRNA and mRNA. We identified microRNA-150 (miR-150) as having the highest fold decrease between pregnancy day 14 (P14) and lactation day 2 (L2). This was verified by qRT-PCR and in situ hybridization (ISH). Pathway analysis of miR-150 predicted targets significantly increasing in MECs between P14 and L2 suggested that miR-150 might play a regulatory role in lipid and cholesterol biosynthesis. By crossing WAP-Cre with ROSA26-lox-STOP-lox-miR-150 transgenic mice, we forced expression of miR-150 in the mouse mammary epithelium from late pregnancy throughout lactation to override the natural decrease. Compared to offspring nursed by littermate controls, 3-day old pups nursed by the bitransgenic dams had approximately 80% decrease in survival ($p<0.0001$) and surviving pups had smaller milk spots. Foster litters and surviving biological pups nursed by bi-transgenic dams showed failure to thrive. Compared to L2 MGs from normal dams, forced expression of miR-150 resulted in a decrease in alveolar density.

This was due to alveoli not becoming distended with milk and no because of precocious involution. Microarray performed on L2 RNA samples from MECs of both genotypes revealed that the majority of genes downregulated after forced expression of miR-150 were involved in lipid synthesis more than any other pathway critical to lactation. Suppression (~30-70%) of four predicted targets at the protein level was confirmed by western blot: FASN (p=0.0001), ACACA (p=0.01), OLAH (p=0.006), and STAT5B (p=0.0005). Mammary epithelial localization of FASN and ACACA suppression was confirmed by immunohistochemistry. Quantitative gas chromatography mass spectrometry revealed a significant reduction in medium-chain fatty acids, including the sum of all de novo synthesized fatty acids (p=0.003) along with 16:0 (p=0.02) and 18:0 (p=0.03), in MECs from mice with constitutive expression of miR-150 compared to control mice. These results strongly suggest that the decline in miRNAs, such as miR-150, are necessary for successful lactation by allowing translation of critical genes including Fasn, Olah, Acaca and Stat5b.

4) **RNA-Seq of mammary epithelial organoids from virgin and lactating glands of 15 Holstein-Friesian cows**

Hannah Lyman, Genome Center, University of California, Davis, CA, USA

Student Travel Award Recipient

Hannah S. Lyman1, Keith Bradnam2, Jessica Elswood3, Adrian Molenaar4, Kuljeet Singh3, Joanne Dobson3, Kim Oden3, Ian Korf1, Danielle G. Lemay1, and Monique Rijnkels3

1. Genome Center, University of California, Davis;

2. Institute of Cancer Research, London, England;

3. Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station;

4. AgResearch Ltd, Grassland Research Center, Palmerston North, New Zealand;

5. AgResearch Ltd, Ruakura Research Center, Hamilton, New Zealand

Although the process of lactation is vital to the commercial production of milk, normal mammary biology is incompletely understood. Next-generation sequencing studies have enabled unprecedented visibility into the genes that are expressed to produce milk. However, previous RNA sequencing (RNA-Seq) has been based on whole mammary glands or milk cells and only a few animals. In the current study, we investigated gene expression in mammary epithelial organoids isolated from the glands of 15 dairy cows in which biopsies were taken from the same cow before their first preg nancy and then again at peak lactation. Mammary gland biopsies were obtained from 25 Holstein-Freisian cows in New Zealand at approximately 14 months of age. Of the 25 cows, 24 were mated and 19 were sampled at 40–60 days lactation. Sequencing--quality RNA was obtained from mammary epithelial organoids of 15 pairs of virgin and lactation samples. Transcripts were aligned to the UMD3.1/BosTau6 genome release with Ensembl 78 annotations. Differential expression analysis was performed with DESeq2.

Results: RNA-Seq yielded an average of 72 million reads per sample. Over 12,000 genes were expressed in the mammary epithelial organoids of these dairy cows. Consistent with previous studies, we find transcripts corresponding to abundant milk proteins, which are highly expressed in the lactating mammary gland. Mitochondrial genes are highly expressed during lactation, reflecting the increased energy needs of the lactating state. The high number of animals in this study enabled us to detect significant fold changes as small as 1.15--fold. Principle component analysis (PCA) shows a clear segregation of virgin and lactation samples, but with greater heterogeneity among the lactation samples. PCA

results suggest that there are three lactation subsets of 8, 5, and 2 animals, respectively. Given that the animals had similar milk yield and that bovine mammary gland tissue is highly heterogeneous, we suspect that the variance in the samples is due to tissue heterogeneity. Even with this heterogeneity, over 8,000 genes were differentially expressed between virgin and lactation. A deeper understanding of these vast regulatory changes requires both knowledge of how these changes in expression arise, and the biochemical impact of the resulting quantitative differences. To this end, we are generating ATAC-seq data, which exposes active chromatin regions, and performing pathway enrichment analysis to display the functional consequences of gene expression changes. By integrating genomic and transcriptomic data in the context of biological networks we hope to describe the regulatory logic and the functional consequences that lead to the unique and specialized biology of the lactating mammary gland. This information is needed to improve the efficient production of milk for better sustainability.

5) **A novel method for high-throughput analysis of bovine milk oligosaccharides**

Randall Robinson, Dept. of Food Science, University of California, Davis, USA

Student Travel Award Recipient

Randall C. Robinson1, Nina A. Poulsen2, Lotte B. Larsen2, and Daniela Barile1,3
1. Department of Food Science and Technology, University of California, Davis;
2. Department of Food Science, Aarhus University, Denmark;
3. Foods for Health Institute, University of California, Davis

Introduction: Oligosaccharides from mammalian milk are theorized to impart a variety of health benefits to consumers, as evidenced by studies demonstrating prebiotic and disease-preventing bioactivities. Beneficial oligosaccharides are highly concentrated in human milk, but are much less abundant in bovine milk and related commercial products. Previous studies have shown that both oligosaccharide abundance and complexity can vary between dairy bovine breeds. However, factors affecting such differences in bovine milk are not fully understood. This study has established a method for high-throughput oligosaccharide analysis using isobaric tagging and tandem mass spectrometry which will be used to profile milk oligosaccharides in a large set of bovine milk samples, allowing correlations between breed and oligosaccharide content to be identified.

Methods: Oligosaccharide standards were labeled with commercially available carbonyl-reactive isobaric tags in a known ratio. An LC-MS/MS method was developed on an Agilent 6520 Accurate Mass quadrupole time-of-flight (Q-TOF) mass spectrometer which uses collision-induced dissociation (CID) energies optimized for both precursor m/z and charge state to fragment oligosaccharides. Specific reporter ions originating from the tags are then used as a basis for both relative oligosaccharide quantification and deconvolution of multiplexed samples. In near future oligosaccharides will be analyzed with the optimized method in 850 milk samples collected from two Danish dairy breeds. Further, genotyping has been performed on all cows using the bovine HD SNP-chip so that the genomes can be correlated with oligosaccharide abundances and genetic parameters (heritability and genetic correlations) of bovine milk oligosaccharides can be estimated.
Results: After collision energy optimization, relative oligosaccharide abundances taken from replicate injections of tagged standards showed excellent repeatability and sensitivity, with a coefficient of variation below 5% for each oligosaccharide standard. Oligosaccharide precursor ions

of charge 1+ and 2+ demonstrated a predictable, linear trend in optimal collision energy (as evaluated by quantification accuracy, residual precursor abundance, and reporter ion signal), indicating that the method should be applicable to analysis of oligosaccharides for which standards are unavailable. A collision energy trendline for the largest bovine milk oligosaccharides, which ionize as 3+ ions, is under final optimization. The final method will have the capability to quantify the 19 most abundant bovine oligosaccharides, including fucosylated structures in trace abundance.

Relevance to the dairy industry: This work is important for the dairy industry because it will identify heritability and genetic correlations for oligosaccharides that can be used for optimizing breeding programs. In the absence of specialized software for automated oligosaccharide identification (similar to that already available for proteomics and genomics), novel tools need to be integrated into current investigative workflows to make such large studies possible.

6) Discovering natural bioactive peptides in cheese with mass spectrometry

Randall Robinson, Dept. of Food Science, University of California, Davis, USA

Student Travel Award Recipient

Randall C. Robinson1, David C. Dallas2, and Daniela Barile1,3
1. Department of Food Science and Technology, University of California, Davis;
2. School of Biological and Population Health Sciences, Oregon State University, Corvallis;
3. Foods for Health Institute, University of California, Davis

Introduction: Alterations in gastrointestinal microbiota are becoming increasingly common. Current antimicrobials are successful in treating overt infections, but are non-specific, cause many side effects, and can be toxic to the host's cells. Non-selective interventions, notably broad-spectrum antibiotics, are proving highly deleterious to the healthy intestinal microbiota. There is a critical need for treatment options that will selectively eliminate pathogens, but at the same time improve or at least not impair the growth of commensal bacteria. Peptides can possess a variety of bioactivities, including anti-microbial functionality, and may be a favorable alternative to traditional antibiotics. Peptides occur naturally in dairy products as a result of microbial fermentation and proteolysis. Therefore, identification of bioactive sequences is important in understanding the physiological impact of these foods.

The objective of this study is to identify the sequences and potential functions of endogenous peptides from several cheeses by LC-Orbitrap MS/MS analysis, including Mimolette (France), Taleggio (Italy), and Stilton (UK), as well as traditional cheddar. Each of these cheeses contains a unique mix of micro-organisms that we hypothesized would contribute to proteolysis and bioactive peptide formation.

Methods: Water-soluble peptides were extracted from the rind and interior of each cheese and purified through C18 solid phase extraction. Each peptide extract was analyzed by LC-MS/MS using an Orbitrap Q Exactive Plus mass spectrometer in data-dependent mode. Peptides were then identified from the tandem spectra by an automated search, using the complete bovine proteome as a reference. The resulting peptide lists will be analyzed by an in-house homology search program that will identify peptide sequences from the cheeses which have previously been demonstrated to be bioactive.

Results: The searches identified an outstandingly high number of peptides: 4,701 from Mimolette, 3,520

from Taleggio, 3,408 from Stilton, and 1827 from cheddar. Peptides ranged in length from 5 to 45 amino acid residues. The majority of peptides in each sample originated from caseins, although several other proteins, including β-lactoglobulin, lactoferrin, glycosylation-dependent cell adhesion molecule 1, butyrophilin subfamily 1 member A1, and xanthine dehydrogenase/oxidase, were also well represented in the peptide profiles. Mimolette was particularly notable in that it contained 117 lactoferrin-derived peptide sequences in the rind and only 1 lactoferrin peptide in its interior, demonstrating the differential in proteolytic factors between the two regions. A functional homology search will be conducted in the near future to identify bioactive sequences in each cheese.

7) **Enzymatic modification of bovine milk oligosaccharides and their functional properties**

Valerie Weinborn, Dept. of Food Science and Technology, University of California, Davis, USA

Student Travel Award Recipient

Valerie Weinborn1, Yanhong Li2, Xi Chen2, and Daniela Barile1
1. Department of Food Science and Technology, University of California, Davis;
2. Department of Chemistry, University of California, Davis

Project goal: This multi-disciplinary collaborative project aims at developing new strategies that will allow the dairy industry to profit from oligosaccharides recovery. Our approach involves using whey permeate oligosaccharides as "backbones" for the production of improved functional compounds that would add great health value to dairy products.

Background: Human milk is the ideal food in terms of nutrition for newborns but not every infant can access it. The main components of human milk are lactose, lipids and human milk oligosaccharides (HMO), which are sugars present in high concentration in mother's milk and have been associated with important functions related with newborn development and health. HMO acts as prebiotics on a few select bacteria because of their unique structural complexity. Despite the important role of HMO, these compounds are not well represented in infant formula since it only contains plant-derived oligosaccharides (OS). Plant derived OS are used as an additive in a wide range of food products, but despite having a prebiotic effect on the intestinal microbiota, they lack many of the other beneficial effects provided by HMO. This difference is mainly caused by the simplicity of their chemical structure. Increasing the structural complexity of the oligosaccharides present in infant formula, paired with the appropriate bacteria, could potentially lead to improved health in babies. In the search of sources of HMO-mimics, bovine milk oligosaccharides (BMO) were discovered a few years ago. Cheese whey permeate is a byproduct of whey protein production and currently has no commercial value. Because it is considered an environmental pollutant and cannot be readily discarded in the wastewater, the dairy industry currently must manage the costs of disposing of it as if it was hazardous waste. Our group was the first to extract, identify, and characterize a multitude of functional compounds (oligosaccharides) in cheese whey permeate, albeit in low concentration. Considering that over 50 million pounds of cheese whey is produced daily just in the state of California (mostly deriving from whey protein extraction which is now a very successful and growing market) the available quantities of whey permeate available for further extraction of bioactive oligosaccharides make it an attractive source.

Results: We successfully utilized whey streams as starting material for the development of an

enzymatic approach to produce HMO-mimetics. Combining large-scale isolation of whey OS by membrane filtration, with pilot scale sialylation and fucosylation lead to the production of oligosaccharides much more similar to HMO and in enough quantities to test their expected improved biological activities. Sialylation of whey OS was completed by enzymatic addition of sialic acid by PmST1_M144D, an α2-3-sialyltransferase mutant from Pasteurella multocida. Fucosylation of whey OS was also successfully achieved by enzymatic addition of fucose by Hp1-3FT, an α1-3-fucosyltransferase from Helicobacter pylori, attaining eight fucosylated structures. The addition of sialic acid and fucose and the disappearance of the "donor" whey OS was monitored by nanoLC QToF MS. After further purification, all the fractions containing the new sialylated and fucosylated OS were tested for their improved ability to prevent the in vitro uptake of two common Pathogens Methicillin--resistant Staphylococcus aureus (MRSA) and Enterohaemorrhagic Escherichia coli (EHEC) by Caco--2 cells. Interestingly, both the fucosylated and sialylated OS decreased the uptake of MRSA but only sialylated BMO decreased the uptake of EHEC by Caco--2cells. Further scale--up of this approach will enable obtaining enough quantities to run further functional studies and eventually incorporate the new HMO--mimics into functional dairy foods.

8) **Use of the milk microbiome to predict infant fecal bacterial community composition**

Janet Williams, Dept. of Animal and Veterinary Sciences, University of Idaho, Moscow, USA
Student Travel Award Recipient

Janet E. Williams1,2, Benjamin J. Ridenhour4, Christopher H. Remien3, Sarah L. Brooker1,2, James T. Van Leuven6, Michelle K. McGuire5, and Mark A. McGuire1
1. Department of Animal and Veterinary Sciences, University of Idaho, Moscow;
2. Program in Bioinformatics and Computational Biology, University of Idaho, Moscow;
3. Department of Mathematics, University of Idaho, Moscow;
4. Department of Biological Sciences, University of Idaho, Moscow;
5. School of Biological Sciences and Paul G. Allen School for Global Animal Health, Washington State University, Pullman;
6. Center for Modeling Complex Interactions, University of Idaho, Moscow

The feeding of human milk to infants reduces the risk of diarrheal diseases. Components in milk that are mitigating this effect are still not clearly defined. Milk is a complex fluid that contains myriad factors including a diverse bacterial community. The influence of the milk bacterial community on the structure of the infant's bacterial community is unknown but we hypothesize that bacterial populations in milk affect the presence and relative abundances of bacteria in the infant's gastrointestinal tract.

To examine this, we collected milk samples and infant fecal samples from mother/infant dyads at 9 time points from day 2 to 6 mo pp. DNA was extracted and the bacterial DNA amplified using universal primers targeting the V1-V3 region of the 16S rRNA gene. Amplicons were sequenced using the Illumina MiSeq v3 2 x 300 bp protocol. Sequencing reads were filtered for quality, then joined and classified using a custom python pipeline (dbcAmplicons; https://github.com/msettles/dbcAmplicons). We utilized multivariate poisson prediction models with cross-validation using the 'glmnet' package in R to capture the complex interactions of the milk bacterial populations on the infant fecal bacterial populations. These models provide an efficient framework that can handle the sparse and high dimensional input matrix of microbiome data. Several bacteria (e.g. Veillonellaceae, Bifidobacteriaceae, and

Pasteurellaceae) in milk were positive predictors (effect of 0.019, 0.004, and 0.003, respectively) of the relative abundance of Bacteroides in infant feces. Staphylococcaceae in milk was a negative predictor of the relative abundances of Bifidobacteriaceae and Coriobacteriaceae (-0.051 and -0.240, respectively) and positive predictor of the relative abundances of Staphylococcus and Weissella (0.184 and 0.312, respectively) in infant feces. Individual mothers were found to have an effect on the relative abundances of several bacteria in their infant feces suggesting that the environment (e.g. diet), genetics of the mother, and/or other components unique to the milk from that mother predict the variation observed for those bacteria. These results suggest that members of the milk bacterial community impact the structure of the infant's bacterial fecal community. More research is needed to confirm this in a larger and/or different population. This project was funded by the Bill and Melinda Gates Foundation, the Idaho Agricultural Experiment Station, the Bioinformatics and Computational Program at the University of Idaho in partnership with the Institute for Bioinformatics and Evolutionary Studies (IBEST), and guided by the Center for Modeling Complex Interactions.

9) **Accurate monitoring of living and total bacterial populations in milks for improved microbial management**

Zhengyao Xue, Dept. of Food Science and Technology, University of California, Davis, USA
Student Travel Award Recipient

Zhengyao Xue1, Mary Kable1, Jessie Heidenreich2, Jeremy McLeod2 and Maria L. Marco1
1. Department of Food Science and Technology, University of California Davis, Davis;
2. Hilmar Cheese Company, Hilmar, California

Milk contains a broad diversity of bacteria that enter milks through farm and processing environments. These bacteria are important determinants of the quality and safety of fluid milk and processed dairy products. Currently employed methods for microbial analysis of milk (and other dairy products) typically target only a limited number of bacterial species, and therefore restrict our knowledge on the microbial community dynamics that lead to either high quality or defective dairy products. In order to improve the microbial management of dairy foods, we are developing and applying stream-lined methods for bacterial diversity measurements using culture-independent, high-throughput 16S rRNA gene sequencing. Firstly, we developed protocols for cell collection, DNA extraction, sequencing, and bioinformatic analysis that result in identifying bacterial communities in milk and cheese. Included in these approaches are methods to distinguish between living and total bacteria by the application of propidiummonoazide (PMA) on intact cells. To validate these protocols, we designed a bacterial standard comprising nine strains of dairy-associated bacterial species. DNA was extracted from the strains separately and combined as well as in the presence and absence of milk for sequencing of the 16S rRNA gene V4 regions on two different DNA sequencing platforms. These comparisons revealed significant biases and errors introduced by standard DNA preparation and sequencing methods. These errors could be addressed, in part, by more stringent bioinformatics approaches and sampling and DNA extraction methods that are comparable with automation. Secondly, we are employing these methods to identify raw and pasteurized milks that either result in high quality cheeses or those that are associated with a variety of defects (e.g. slits, soft texture, bitterness, acidic and buttery flavors). We have thus far shown that high quality and defective cheeses contain distinct microbiomes. Moreover, the bacteria present in cheeses that have a common defect are also variable. These results indicate that different bacterial species may share some conserved

enzymatic functions, which can lead to defective products when these bacterial enzymatic properties are disproportionately high. This knowledge combined with the application of our methods to distinguish between living and total bacterial cells in milk pre- and post-pasteurization and during cheese ripening will enable predictive microbial models along dairy processing chains and ultimately result in consistent products with optimal sensory profiles and nutritive benefits.

10) Anaerobic rumen fungi: Probiotic for dairy cows?

Isabel Gigli, Facultad de Agronomía, Universidad Nacional de La Pampa, Argentina

Mario Calafat1, Aníbal Pordomingo2, and Isabel Gigli1
1. Facultad de Agronomía, Universidad Nacional de La Pampa, Argentina;
2. Instituto Nacional de Investigación Agropecuaria, EEA Anguil, Argentina

Among volatile fatty acids (propionate, butyrate and acetate) produced by ruminal microflora, propionate is the one that directly impacts total milk production. Once absorbed by ruminal papilla, propionate is transformed into glucose in the liver, and then used in the mammary gland to synthesize lactose. Changes in the cows' diet and subsequent changes in the ruminal microflora could affect volatile fatty acids production. Ruminal microflora consists of bacterial protozoa, methanogenic archaea and anaerobic fungi. These anaerobic fungi penetrate the cellulose material and therefore collaborate with the bacterial degradation. As a first step in evaluating the possible use of ruminal fungi as a probiotic, we studied the kinetics of dry matter and volatile fatty acids production of Panicum Vigatum in two development stages (senescence and green). In previous studies, we determined that the optimal antibiotic combination and concentration to obtained an enriched fungi culture was 0.2 mg/ml of Streptomycin plus 1.25 mg/ml of Penicillin. Briefly, whole rumen content, kindly provided by INTA (National Institute of Agricultural Technology), from eight different cows adapted to natural pasture was collected. At the laboratory, these samples were strained through a double-layered cloth, pooled, and kept under CO_2 flushing. Previously, Panicum Vigatum—senescence and green leaves—were cut in 5mm diameter discs by a cork borer punched and cultured in the presence of antibiotic, as previously described, in anaerobic condition. Control samples were cultured in similar condition but without antibiotics. Neocallimastix fungi were identified by light microscopy of preparations stained with lactophenol blue solution. Dry fiber matter disappearances and volatile fatty acid concentrations were determined at 48 and 160 hours. In the senescence leaves samples, there was no statistical significance in the percentages of dry fiber matter disappearances or on the volatile acid concentrations between the two groups (control and enriched fungi culture). On the contrary, when green leaves were inoculated, the dry fiber disappearance and the concentration of propionate were higher ($p \leq 0.05$) in the enriched fungi group at 48 hours (control 3.3 nmol/l ± 0.07 vs enriched fungi 4.1 nmol/l ± 0.45) and at 160 hours (2.5 nmol/l ± 0.07 vs 3.0 nmol/l ± 0.45). We conclude that ruminal anaerobic fungi favored propionate production when green leaves are inoculated. These preliminary results encourage us to continue our studies to determine if anaerobic ruminal fungi could function as probiotic to increase propionate concentration in the rumen and consequently to produce a positive impact on milk production.

The authors acknowledge the financial support from POIRe (Proyectos Orientados de Investigación Regional) 29014-05-UNLPam and Weizur SRL for funding this project.

11) Differences in the pre-weaning gut microbiomes of dairy and beef calves

Daisy Johnson, University of California, Davis, USA

Daisy Johnson1, Steve A. Frese1, and David A. Mills1
1. University of California, Davis

Milk is known to both seed and feed the microbes that colonize mammalian gastrointestinal tract. In particular, milk contains complex glycans that serve a prebiotic function enriching beneficial microbes in the mammalian colon. We hypothesize that the development of the gut microbiota in bovine mammals represents an interesting model to understand the co-association of milk glycans with specific milk enriched microbial populations. Pre-weaning management of dairy and beef calves occurs differently.

Beef calves are often nursed from their mother and fed hay ad libitum while dairy calves are typically given milk replacer and grain after an initial feeding of colostrum. In order to understand these pre-weaning gut microbiome changes, fecal swabs were collected from 9 beef calves and 22 dairy calves daily for the first two weeks and then weekly until they were fully weaned. Initial microbiota analysis of dairy calves indicates a pre-weaning predominance of Bacteroides species during the milk replacer feeding which decreases during weaning with an increase in Ruminococcaceae and Prevotella at weaning. Comparative analysis between the pre-weaned beef and dairy calf gut microbiomes will reveal both population and functional differences that arise from these two modes of pre-weaning feeding and aid in understanding how potential pathobiont populations (such as Escherichia coli) become established early on in these commercially important livestock.

12) The bacterial composition of milk is seasonally dependent and undergoes dramatic shifts in dairy processing facilities

Mary Kable, Department of Food Science and Technology, University of California Davis, USA

Mary E. Kable1, Yanin Srisengfa, Miles Laird, Jose Zaragoza, Jeremy McLeod2, Jessie Heidenreich2 and Maria L. Marco1
1. Department of Food Science and Technology, University of California Davis;
2. Hilmar Cheese Company, Hilmar, CA

Background: Raw milk harbors diverse bacteria that can have significant impacts on the quality and safety of fluid milk and dairy products. Currently, the bacterial composition in raw milk after transport in tanker trucks and within commercial processing facilities is not well understood. Therefore, we set out to identify the bacterial composition of raw milks entering and moving through large-scale, dairy product-manufacturing plants in California.

Methods: Raw bovine milks were collected from tanker trucks arriving at two dairy processors in the California San Joaquin Valley during three seasons (spring, summer and fall) and from five large volume silos during two days in the summer season. Additionally, a total of 142 milks were collected in duplicate once every 1.5 to 4.5 hours during spring (71) and late summer (71) from 9 and 11 types of equipment, respectively, within the early milk processing steps including storage, pasteurization, separation and ultrafiltration. Half of each of the milks collected from within the processing facilities

were treated with propidium monoazide (PMA) to prevent DNA from dead bacterial cells from being PCR amplified and sequenced. Illumina MiSeq sequencing of the 16S rRNA V4 region was performed to characterize the bacterial diversity in these milks.

Results: Examination of milk from 899 tankers revealed highly diverse bacterial populations. However, even with this complexity, a core microbiome was present consisting of 29 taxonomic groups and high proportions of Streptococcus, Staphylococcus and unidentified members of Clostridiales. Comparisons between the raw tanker milks also showed seasonal differences. Raw milk collected in the spring contained the most diverse bacterial communities with the highest total cell numbers but were also the most homogenous, with the lowest variation between the different tankers tested. Transfer of the milk to storage silos resulted in distinct shifts in the bacteria present, with some of the silos containing significantly higher proportions of Acinetobacter than the tankers used to fill them. Bacterial communities within the facility were also susceptible to seasonal variation that mirrored changes in the raw tanker milks. In addition to natural variability, the equipment used for short-term storage tended to have the greatest impact on determining the bacterial community structure. The feed tanks before pasteurization, concentrate silos after ultra filtration and standardized silos formed the most distinct clusters out of all equipment compared by PCoA of weighted UniFrac distances. PMA treatment of pasteurized samples resulted in a trend in reduced Streptococcus, Staphylococcus and Corynebacterium suggesting that the majority of these organisms do not survive pasteurization, although damaged cells still linger in the product. Pseudomonas was among the bacterial taxa that tended to be present in greater relative abundance after pasteurization, suggesting post-pasteurization contamination.

Conclusion: The bacterial community in raw milk is highly variable although a core set of organisms is always present. This variability in combination with the tendency for standing milk to develop outgrowths of particular taxa that are not completely controlled by pasteurization presents a challenge for dairy producers in controlling the quality of their product. This work helps to illustrate the exact nature of that challenge and these results can be applied to modify processing methods to improve the consistency of high quality dairy products.

13) **Molecular mechanisms of human milk oligosaccharide utilization by Bifidobacterium longum subsp. longum SC596 and Bifidobacterium breve SC95**

Nina Kirmiz, University of California, Davis, USA

Nina Kirmiz1, Daniel Garrido1, Daniele Lemay1, Santiago Ruiz-Moyano1, Jasmine Davis1, Sarah Totten1, Jennifer Smilowitz1, J. Bruce German1, Carlito B. Lebrilla1, and David A. Mills1
1. University of California, Davis

Human milk oligosaccharides (HMO) are an abundant component of human breast milk. Bifidobacterium is often reported as a predominant genus in the microbiota of breast-fed infants and it is believed that bifidobacteria provide positive health benefits. Previous work has shown that strains of Bifidobacterium longum subsp. infantis (B. infantis) and Bifidobacterium bifidum have the ability to grow to high cell densities on HMO as a sole carbon source and the molecular mechanisms of HMO consumption by B. infantis and B. bifidum have been previously described. Until recently, HMO consumption by Bifidobacterium longum subsp. longum and Bifidobacterium breve has been

described to a lesser extent. We have isolated two strains, B. longum subsp. longum SC596 and B. breve SC95, which can grow well on pooled HMO and individual HMO sugars as a sole carbon source. Genome analysis, transcriptomics, and functional studies were used to examine HMO consumption by B. longum subsp. longum SC596 and B. breve SC95. B. longum subsp. longum SC596 and B. breve SC95 possess a range of glycosyl hydrolases and oligosaccharide transporters induced during growth on HMO. Various glycosyl hydrolases purified from these strains have activity on HMO linkages and the family 1 solute binding proteins bind to HMO. Furthermore, we characterize a gene cluster B. longum subsp. longum SC596 possesses that allows for utilization of fucosylated HMO. This work provides insight into classes of B. longum subsp. longum and B. breve that are HMO responders. This work provides a mechanism for how select strains of bifidobacteria vigorously consume HMO, which may enhance their ability
to colonize breastfed infants.

14) **The transmission of milk-oriented microbes via the built environment: Infants and mothers as sources**

Zachery Lewis, Dept. of Food Science and Technology and Foods for Health Institute, University of California, Davis, USA

Zachery T. Lewis1,2, The Baby-Associated Built Environment (BABE) Microbiome Project Team1, and David A. Mills1,2,3
1. Department of Food Science and Technology, University of California, Davis;
2. Foods for Health Institute, University of California, Davis;
3. Department of Viticulture and Enology, University of California Davis

Bifidobacteria are a major component of the gut microbiome of breast-fed infants, where they consume human milk oligosaccharides. Bifidobacterial colonization of the infant gastrointestinal tract is associated with several positive health outcomes. It is unknown how infants acquire bifidobacteria, though it has been hypothesized that these bacteria may be passed from mother to child or between infants, possibly via the surrounding human-built environment. This study investigates potential built environment-mediated transmission of bifidobacteria to infants. To determine if bifidobacteria were present in locations frequented by infants and mothers, swab samples were collected from surfaces with which they come into contact, including surfaces in lactation rooms, baby-changing tables, and daycare centers. In order to account for the possible influence of temperature, relative humidity, and room usage on bifidobacteria deposition and survival, custom sensors were installed in rooms to monitor these factors. To test the influence of temperature on extracorporeal bifidobacterial survival, a model species of bifidobacteria (Bifidobacterium longum subsp. infantis) was incubated aerobically under an array of temperatures and assayed for viabilit at regular time intervals. Bifidobacteria were found in areas frequented by breast-feeding mothers, suggesting that mothers are potential bifidobacterial vectors. Bifidobacteria survived longer at colder temperatures and were found to survive extracorporeally for up to two weeks. Usage tracking via in-room sign-in sheets confirmed the accuracy of the infrared beam-break approach
to tracking room usage used here. Future experiments will investigate whether bifidobacteria may be transmitted between infants and how the microbiota of these surfaces differs between sites, environmental conditions, and over time.

15) Proteomic and RNA profiling of milk-derived exosomes from as1-casein deficient goats reveal that CSN1S1 genotype is capable to modulate the repertoire of molecules they conveyed

Patrice Martin, GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France

Z. Krupova1,2, C. Pechoux1, C. Henry3, M. Dumarest1, P. Defrenaix2, and P. Martin1
1. GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;
2. EXCILONE, Elancourt, France ;
3. INRA, UMR MICALIS, Plateforme d'Analyse Protéomique Paris Sud Ouest (PAPPSO), Université Paris-Saclay, Jouy-en-Josas, France

Introduction: The biosynthesis pathways responsible for the production of milk are known in general outlines but the release and function of extracellular vesicles (EVs) from mammary epithelial cells (MEC) in milk is by far less known. Studying a goat model with a "naturally occurring KO" of the gene (CSN1S1) encoding αs1-casein, we have shown that in goats homozygous for the null allele (O/O), there is a chronic endoplasmic reticulum (ER) stress due to the accumulation of the other caseins in this compartment, slowing down their secretion. Here we report on consequences of the absence of αs1-casein on MEC dysfunction and EVs secretion.

Material and Methods: An improved method based on the combination of successive steps associated to biochemical characteristics of milk components was first developed for purification of milk-derived exosomes. The quality of exosomes was validated morphologically by transmission electron microscopy (TEM) with negative staining (uranyl acetate). The specific exosome protein markers were detected by Western blot and the size distribution was measured by DLS Zetasizer (Malvern) and qNANO (TRPS, Izon). Once validated, their proteome was acquired by LC-MS/MS and nucleic acid content (total RNA and miRNA) by NGS.

Results and Discussion: Our purification method gives milk-derived exosome populations free of contamination by other EVs and milk components, at sufficient concentrations to perform a full characterization while preserving their morphology. Nearly 280 proteins involved in the biogenesis of exosomes and Multi Vesicular Bodies formation, their adhesion and internalization as well as proteins associated with the membrane transport, were identified, among which 18 and 23 proteins differed between αs1-casein "KO" (O/O) and wild-type (A/A) genotypes, respectively. Ongoing profiling of RNA from milk-derived exosomes has already identified over 230 miRNA among which some (miR-148a-3p, miR30a-5p, miR-200a and miR-200b) are highly represented whatever the genotype, whereas miR-21-5p seems to be in less amount in AA goats. The presence of mRNA encoding major milk proteins (α-lactalbumin and αs2-casein) in these vesicles confirm their MEC origin.

Conclusions: Our results demonstrate the effectiveness and the potential of our method to purify milk-derived exosomes and confirm the impact of a deficit in αs1-casein on the MEC secretion phenotype, thus providing evidences that αs1-casein genotype is capable to modulate the repertoire of molecules conveyed by exosomes which is of crucial interest since miRNA stable in extracellular vesicles may play an important regulatory role in the young and may account for the health benefits of milk for the consumer.

Acknowledgements: This study was funded by the National Agency for Research (MilkChEST, ANR-12-BSV6-0013-04) and by the GIS APIS-GENE. We thank Claudia Bevilacqua (Microgenomics platform from the @BRIDGe core facility) for her invaluable assistance regarding qPCR.

16) SAHARA: Suppression before Amplification of Highly Abundant mRNA to optimize RNAseq for gene expression profiling

Patrice Martin, GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France

C. Bevilacqua1, N. Crapart1,2, C. Hue-Beauvais1, B. Brandao1, S. Lemoine3, F. Coulpier3, P. Martin1
1. GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France ;
2. EXCILONE, Elancourt, France ;
3. Plateforme de Génomique, Institut de Biologie de l'ENS, IBENS, Paris, France
Corresponding author: Claudia.bevilacqua @jouy.inra.fr

Introduction: Amplification of RNA obtained from microdissected cells remains a critical step that may impact RNAseq profiles, mainly regarding weakly expressed genes. The quality (RIN value) and quantity of RNA play an important role in the choice of amplification kit to use. Moreover, RNAseq approaches on mammary epithelial cells (MEC) isolated by laser capture microdissection (LCM) showed that, at the peak of lactation, 2/3 of the transcripts correspond to 6 genes encoding the main milk proteins: 4 caseins, □-lactalbumin and □-lactoglobulin (Canovas et al., 2014). The objectives of our study were: To amplify a few amount of RNA (less than 1 ng) extracted from cells isolated by LCM with a RIN value ranging between 6.5 and 7 without loss of transcripts. To eliminate transcripts coding for the 6 main milk proteins prior amplification of cDNA for a RNAseq analysis and thereby increase the depth of analysis to reach less expressed genes

Materials and Methods: We compared 2 kits: Ovation Human FFPE RNAseq Library Systems (Nugen) based on the Inda-C technology to deplete the 6 most abundant mRNA and ribosomal RNA, and Smarter V4 kit (Clontech) which is designed to generate high-quality, full-length cDNA directly from few hundred of cells (10 pg to10 ng of total RNA), based on the SMART system and unbiased amplification of cDNA transcripts.

The first step was to design 12-15 primers for each of the 6 targeted genes to add to the Nugen kit. MECs were microdissected from the mammary tissue of 6 lactating goats using the Arcturus system (Life technologies). Total RNA extracted from each microdissected MEC sample was used to prepare 3 libraries: 1 library with Smarter V4 kit (Clontech), 1 library with depletion of the 6 targeted genes and ribosomal RNA (Nugen C) and 1 library with depletion of Ribosomal RNA (Nugen R) to validate the impact of milk protein cDNA transcripts depletion on the transcripts profile. Sequencing of libraries thus prepared was carried out on a NextSeq system (Illumina) and differential analysis performed between the 3 protocols.

Results and Discussion: Both kits (Nugen and Clontech) show good quality sequencing however unexpected differences were observed between protocols (high level of intronic reads in Nugen libraries). The abundance of transcripts encoding the 6 major milk proteins in Nugen libraries depleted for both ribosomal RNA and milk protein transcripts was lowered to 3-4% of reads aligned on exons whereas it reached 75-80% in the Smarter library. The Smarter Kit V4 provides a larger

number of reads exploitable for expression analyses while a lot of Nugen reads are intronic. In addition, depletion does not seem to impact the transcriptomic profile of samples.

Conclusions: The strategy implemented to deplete over represented transcripts proved to be very effective, but generates a significant number of intronic reads. Regarding the amount of initial material required to build a library for RNAseq, the Smarter kit from Clontech seems best suited for this type of approach, starting from microdissected material. One ng is enough for the Smarter kit whereas 25 ng are required for Nugen libraries.

References: Cánovas A, Rincón G, Bevilacqua C, Islas-Trejo A, Brenaut P, Hovey RC, Boutinaud M, Morgenthaler C, VanKlompenberg MK, Medrano JF, Martin P (2014) Comparison of five different RNA sources to examine the lactating bovine mammary gland transcriptome using RNA-Sequencing. Sci Rep. 4:5297

Acknowledgements: This study was funded by the National Agency for Research (MilkChEST, ANR-12-BSV6-0013-04) and by the incentive funds of the INRA Animal Genetics Division.

17) **MiR-acle milk: Profiling milk-derived microRNAs using molecular barcoding technology for understanding their potential role in gastrointestinal function**

Xuejing (Ivy) Men, Grasslands Research Centre, Tennent Drive, Palmerston North, New Zealand

X. Men1,2,4, M.J. McCann1,3, A. Thompson2,4, and N. Roy1,2,3,4
1. Food Nutrition & Health Team, Food & Bio-based Products, AgResearch Limited, Palmerston North, New Zealand;
2. Riddet Institute, Massey University, Palmerston North, New Zealand;
3. Gravida: National Centre for Growth and Development, Auckland, New Zealand;
4. Massey Institute of Food Science and Technology, Massey University, Palmerston North, New Zealand.

Milk is a complex fluid that provides nutrition and aids postnatal development for infants, while also contributing to our overall health as we age. Recent evidence has identified a class of small non-coding RNAs stably expressed in milk, called microRNA (miRNA). These miRNAs function at the post-transcriptional level to influence protein translation by binding to the complementary region of mRNA and repressing translation or promoting degradation of the mRNA.

MiRNAs have been shown to be key regulators of gene expression networks and are implicated in several biological processes associated with our health. Therefore, milk-derived miRNAs may represent another mechanism by which milk influences key biological processes. There is evidence suggesting that milk-derived miRNA encapsulated in exosomes can survive digestion and be absorbed, so we hypothesise that: (1) miRNA within milk exosomes are quantifiable by the novel digital molecular barcoding technology (NanoString technologies nCounter® system), and (2) direct quantification of miRNA enables a more appropriate understanding of the role of miRNA in gastrointestinal function.

A preliminary study with crude lysate and exosomal RNA was completed to develop a method for direct quantification of miRNA in breast milk using the NanoString technologies nCounter® system. The

results showed that the column-based exosomal miRNA extraction is the most suitable method for future miRNA profiling studies, especially with low sample volume. Amongst the top 10 most abundant exosomal miRNAs identified, 5 of them were also reported as the most highly expressed miRNAs in another study using small RNA sequencing, and 3 were described as the most highly expressed miRNAs in lipid fraction of the breast milk.

Human milk miRNA profiles differ considerably between individuals, suggesting that they may be influenced by maternal health, and there may also be differences in miRNA composition and abundance. Therefore, future studies will require accurate and direct counting of miRNA in order to appropriately account for the inter-individual differences when exploring the miRNA-mediated health benefits of milk.

18) **Cross-feeding of pathobionts by Bacteroides thetaiotaomicron as a possible mechanism for Necrotizing Enterocolitis**

Ishita Shah, Dept. of Food Science and Technology, University of California, Davis, USA

Ishita M. Shah1, Steven A. Frese1,3, Gege Xu2, Carlito B. Lebrilla2, Juliana Maria Leite Nobrega De Moura Bell1, Daniela Barile1, and David A. Mills1
1. Department of Food Science and Technology, University of California, Davis;
2. Department of Chemistry, University of California, Davis;
3. Evolve Biosystems, Inc., Davis, CA

Infants born preterm and hospitalized in the NICU are at high risk of infections. A major threat is the development of an intestinal disease termed as Necrotizing Enterocolitis (NEC), which many times requires surgical intervention. With about 600,000 infant deaths occurring due to NEC globally, it clearly requires better therapeutics compared to current standard of care. NEC develops in infants harboring various pathobionts, often proteobacterial species, and clinical trials suggest intestinal dysbiosis precedes disease development. Here we show that microorganisms isolated from infants developing NEC showcase a complex cross-feeding mechanism, whereby, a clear donor-recipient relationship revolving around breastmilk sugar metabolism appears to play a pivotal role in the development of proteobacterial blooms, as predicted based on 16S rRNA marker gene sequencing, metagenomics, and mass spectrometry. Bacteroides thetaiotaomicron isolated from feces of infants that did develop disease, employs extracellular glycosyl hydroases to deconstruct human milk oligosaccharides leaving behind monomers like L-fucose in the extracellular milieu. L-fucose subsequently serves as the carbon source for pathogens like Escherichia coli and Klebsiella pneumoniae, isolated from infants who developed disease. We demonstrate the involvement of the fucose operon of E. coli in this "cross-feeding" mechanism using a chromosomal mutant, which gets rapidly outcompeted by the wild-type when tested in medium supplemented with human milk oligosaccharides and conditioned by growth of B. thetaiotaomicron. These studies reveal the complex network of mechanisms by which human milk oligosaccharides are consumed in situ and identify a cross-feeding phenotype between Bacteroides species and the pathogens that cause NEC. Understanding this cross-feeding enables the rational design of probiotic treatments to eliminate cross-feeding and reduce enrichment of pathobionts associated with NEC.

19) **Diversity and Milk Oligosaccharide Consumption of Bifidobacterium pseudocatenulatum**

Guy Shani, Dept. of Food Science and Technology and Foods for Health Institute, University of California, Davis, USA

Guy Shani1,2, Jasmine Davis2,3, Josh Cohen1,2, Sadaf Nagshbandi4, Steve Ho1 Santiago Ruiz-Moyano2, Zac Lewis1,2, Mina Popovic2,5, Steven Frese2, Juliana DeMoura Bell1,2, Daniela Barile1,2, Carlito Lebrilla2,3, David Mills1,2
1:Department of food Science and Technology, University of California, Davis
2:Foods for Health Institute, University of California, Davis
3:Department of Chemistry, University of California, Davis
4: Department of Biochemistry and Molecular Biology, University of California, Davis
5:Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

Human milk has been shown to enrich certain members of the genus Bifidobacterium in the infant gut. One species of this genus, Bifidobacterium pseudocatenulatum, has been found in multiple environments, including the gastrointestinal tracts of both human infants and adults. While this species has been frequently observed in breast fed infants, little work has been done to analyze the diversity of this organism with regard to possible genetic or physiological adaptations to the infant gut environment. To better understand this diversity, 62 members of the B. pseudocatenulatum species were isolated from various sources and analyzed for their phylogenetic relatedness by multilocus sequence analysis. Eleven unique isolates were then compared in their ability to utilize individual and pooled human milk oligosaccharides in an attempt to determine whether members isolated from breast-fed infants were capable of metabolizing these sugars. Four strains grew strongly on these human milk oligosaccharides and the specific sugar, 2-fucosyllactose and genomic sequencing indicated they possessed genes coding for fucosidases not present in the poor HMO consumers. Only one isolate possessed two distinct fucosidases, members of different glycosyl hydrolase families. These fucosidases were arranged as part of a cluster of genes with functions associated with consumption of fucosylated HMOs. The two fucosidases were expressed at higher levels during growth on HMOs and showed complementary in-vitro activity on different fucosylated HMO structures. The same strain also exhibited an ability to consume both lacto-N-tetraose and lacto-N-neotetraose, though at different rates, suggesting a preference for the former isomer. The unique ability to grow well on human milk oligosaccharides may partly explain some differences between infant- and adult-associated isolates, and will inform future synbiotic (prebiotic + probiotic) strategies for beneficially manipulating the infant gut microbiome.

20) **The fatty acid composition of human milk is associated with infant microbial community profile**

Diana Taft, Cincinnati Children's Hospital, Cincinnati, Ohio, USA

Diana H. Taft1, Kelly A. Dingess1, Christina J. Valentine1,2, Nicholas J. Ollberding1, Doyle V. Ward3, David S. Newburg4, J. Thomas Brenna5, Robert J. McMahon2, and Ardythe L. Morrow1
1. Cincinnati Children's Hospital, Cincinnati, Ohio;
2. Mead Johnson and Company, Glenview, IL;
3. University of Massachusetts Medical School, Worcester;
4. Boston College, MA;
5. Cornell University, Ithaca, NY

Background: The fatty acid (FA) composition of human milk varies between mothers and is influenced by maternal diet and body stores. Evidence suggests that dietary FA may influence the gut microbiota. The impact of dietary FAs on gut microbiota are not well characterized, particularly in breastfed infants.

Objective: We conducted a pilot study to assess the association of variation in human milk FA composition with the infant gut microbiota.

Methods: Human milk and infant stool samples were collected at 4 and 13 weeks postpartum respectively, from 32 mother-infant pairs. Infants were healthy, term singletons. Human milk FAs were measured by a modified Bligh-Dyer technique and analyzed by gas chromatography. Stool DNA was extracted and the 16s rRNA gene sequenced. Using weighted UniFrac, non-metric multi-dimensional scaling visualization, and ordisurf in QIIME and R, we tested for associations between the infant gut microbiome and 25 different FAs of human milk.

Results: Human milk octadecanoic acid (18:0 - common name, stearic acid) concentration was associated with the composition of the infant stool microbial community after adjusting for multiple comparisons (p=0.003). Streptococcus anginosus more frequently colonized infants fed milk with lower levels of stearic acid, while Bacillales, Staphylococcaceae, and Staphylococcus levels were higher in infants fed milk with higher levels of stearic acid. The percent oleic acid (18:1 cis-9) of total FAs in human milk was associated with the composition of infant stool microbial communities. Bifidobacteriales, Bifidobacteriaceae, and Bifidobacterium levels were higher in infants fed milk with lower percentage oleic acid, while Actinomycetaceae and Actinomyces more frequently colonized infants fed milk with higher percentage oleic acid.

Conclusions: This project provides initial evidence that variation in the FA composition of human milk is associated with differences in infant gut microbiota. The relevance of these microbiota signatures to infant health has yet to be determined.

21) Comparison of two in vitro models to study cheese disintegration during gastric digestion

Sylvie Turgeon, STELA Dairy Research Centre, Institute of Nutrition and Functional foods, Université Laval, Quebec City, Canada

Laurie-Eve Rioux1, Xixi Fang1, Alice Gattison1, Steve Labrie1, and Sylvie L. Turgeon1
1. STELA Dairy Research Centre, Institute of Nutrition and Functional foods, Université Laval, Quebec City, Canada, G1V 0A6

The breakdown of solid food in the stomach plays an important role in the release and hydrolysis of macronutrients and their postprandial metabolic responses. In the stomach, the forces acting in vivo on food particles during the disintegration are difficult to reproduce. Two in vitro models were developed to study the gastric disintegration of solid food: 1) anchor geometry designed to maintain food particles in suspension with a steady shear force and 2) spherical probe attached to the load cell of a texture analyzer with controlled shear/elongation force. Mozzarella and light cheddar cheeses were studied since both have similar fat content and textural properties (hardness, cohesion) but different

structures. For both cheeses, the shear/elongation model has significantly increased disintegration but much higher values were found for light cheddar compared to mozzarella (86 v.s. 63%). Protein releases during gastric digestion was correlated to the disintegration values. These results were attributed to different texture softening kinetics during gastric digestion. Mozzarella needed 60 min of soaking in gastric juice to fracture while light Cheddar fractured at G0. Understanding the disintegration kinetics may allow to tailor food structure for their optimal macronutrients bioaccessibility.

Notes:

Notes:

Notes:

Notes:

Notes:

2016 Symposium presentations will be available at:
http://milkgenomics.org/2016-symposium

username: 2016symposium

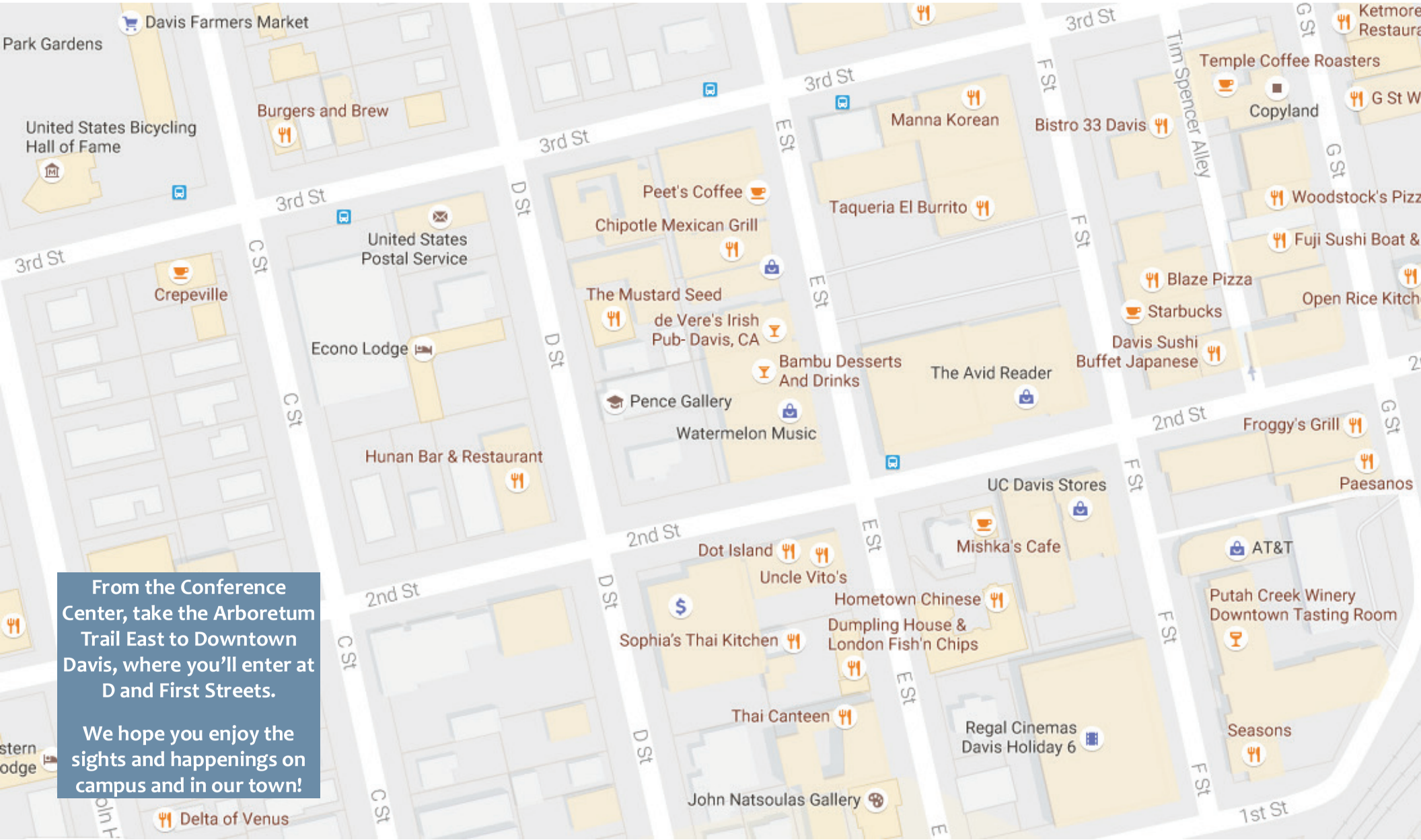
password: Davis2016

You must be logged in to view

13th International Symposium on Milk Genomics and Human Health
September 27-29, 2016
UC Davis Conference Center, Davis, California, USA

INTERNATIONAL™ MILK GENOMICS CONSORTIUM

From Milk to Microbes: Omics Technologies Reveal Mechanisms of Action



From the Conference Center, take the Arboretum Trail East to Downtown Davis, where you'll enter at D and First Streets.

We hope you enjoy the sights and happenings on campus and in our town!