Dairy Foods Symposium: Towards a Mechanistic Understanding of Probiotic Function in Man and Animals

624 Application of "omic" tools to understanding probiotic action. T. R. Klaenhammer*[1,2], [1]*North Carolina State University*, *Raleigh,* [2]*Southeast Dairy Foods Research Center*, *Raleigh, NC*.

Lactic acid bacteria are associated with various plant and animal niches and play a key role in the production of fermented dairy foods and beverages, with some species also exerting probiotic properties. As generally recognized as safe (GRAS) organisms, they have been orally ingested safely by humans over the centuries, often at levels exceeding 100 million per gram of food. Genome sequencing of ~30 LAB and probiotic species has revealed a path of evolution toward specialized habitats that are nutritionally rich. Comparative genomic analyses of lactobacilli occupying either food/dairy systems, or the intestinal tract (probiotic species), have identified both conserved and unique gene sets important for food/dairy fermentations, but recently have also identified key genes likely involved in functional roles that impact health. Functional genomic analyses using "omic" technologies have identified systems responsible for acid and bile tolerance, prebiotic utilization and revealed several cell surface proteins and structures on probiotic microbes that interact with host epithelial and immunomodulatory cells. Among these are lipoteichoic acids, surface layer proteins, and mucus binding proteins. Alteration of the cell surface display of such structures can dramatically alter dendritic cell binding and cytokine signaling and promote inflammatory or anti-inflammatory responses. Employing genomic tools for gene cloning, expression, and inactivation, the field is uniquely positioned to investigate mechanisms through which probiotic microbes interact with the intestinal mucosa, compete with pathogens and impact health.

Key Words: probiotic, lactic acid bacteria, genomics

625 The gastrointestinal microbiome and probiotics: Effects on intestinal physiology and mucosal inflammation. J. Versalovic*, *Baylor College of Medicine*, *Houston, TX*.

The human gastrointestinal microbiome is composed of many bacterial species that may affect signaling pathways in intestinal epithelial cells, stem cell compartments and mucosal immune cells. Differences in microbial composition may affect the intestinal mucosa in terms of effects on cell proliferation, apoptosis, antibody and cytokine production, and cell migration. Direct effects by microbial and probiotics-derived signals on specific mammalian cell signaling pathways may explain mechanisms of mucosal immunity. The gut microbiome may affect the biology of innate and adaptive immune responses in the gut mucosa. Specific components of the microbiome may have net anti-inflammatory or pro-inflammatory effects, and the relative balance of microbes may result in different patterns of mucosal inflammation. Differences in patterns of inflammation and immune responses to the gut microbiome and probiotics may determine, in part, differences in the risk of immune-mediated disorders and infections of the gastrointestinal tract.

626 An evolutionary link between bifidobacterial probiotics and milk. D. Mills*, *University of California*, *Davis*.

Bifidobacteria are commonly used as probiotics in dairy foods. Select bifidobacterial species are also early colonizers of the breast-fed infant colon, however the mechanism for this enrichment is unclear. We have previously shown that *Bifidobacterium longum* ssp. *infantis* is a prototypical bifidobacterial species that can readily utilize human milk oligosaccharides as a sole carbon source. Mass spectrometry-based glycoprofiling has revealed that numerous *B. infantis* strains preferentially consume small mass oligosaccharides, abundant in both human and bovine milks. Genome sequencing showed that *B. infantis* possesses a bias toward genes required to utilize mammalian-derived carbohydrates. Many of these genomic features encode enzymes that are active on milk oligosaccharides including a novel 40-kb region dedicated to oligosaccharide utilization. Biochemical and molecular characterization of the encoded glycosidases and transport proteins have further resolved the mechanism by which *B. infantis* selectively imports and catabolizes milk oligosaccharides. Expression studies indicate that many of these key functions are only induced during growth on milk oligosaccharides and not expressed during growth on other prebiotics. In addition, key cell surface oligosaccharide binding proteins in *B. infantis* bind both milk oligosaccharides and epithelial cell surface glycans. Moreover, growth on milk oligosaccharides results in significant increases in binding of *B. infantis* to intestinal cells in vitro. Additional sequencing of numerous *B. infantis* genomes has confirmed that these features are common among the *infantis* subspecies and likely constitute a competitive colonization strategy employed by these unique bifidobacteria. Through detailed characterization of the molecular mechanisms responsible for bifidobacterial enrichment in the gastrointestinal tract of breast fed infants, these studies provide a conceptual framework for enhancement of probiotic persistence and host-interaction through delivery in animal milks.

Key Words: probiotics, milk oligosaccharides, bifidobacteria

627 Assessing and maintaining probiotics in food. T. Hornbaek*, *Chr. Hansen A/S*, *Hoersholm, Denmark*.

Dairy products, and specifically yogurt-like products, form the largest segment by far in the market for probiotic foods. Dairy products are excellent vehicles for delivering useful probiotic bacteria such as Bifidobacteria and introducing them into the gastrointestinal tract. Increased consumer awareness of the health benefits of probiotics has led to numerous new probiotic product launches both in the market for fermented milk products as well as other food categories. In the development of new probiotic food products, there are several factors to consider including: 1) the documentation of the probiotic strain; 2) the ability to produce the probiotic strain in large scale; and 3) successful application in the food product. This presentation will focus on the last aspect: how to obtain successful probiotic food applications. Challenges with respect to sensory impact and survival of probiotics in different food applications will be discussed. Recent work will be presented to illustrate various ways of increasing probiotic cell counts and stability in dairy as well as non-dairy applications. Ways to circumvent the lack of important nutrients and the potential negative effect of different processing conditions will be discussed, also in relation to the use of adjunct cultures.

Without having bio-markers related to probiotic effects, the best way to ensure the probiotic properties of a food product is by measuring probiotic cell counts throughout shelf life. Our newest findings will be presented on the correlation between traditional plate counting methods and novel probiotic cell count methods such as qPCR and flow cytometry. Furthermore, a dynamic, multi-compartmental model system will be presented which provides the ability to estimate survival rates of probiotics delivered in different food vehicles on their way through the stomach and small intestine.

Key Words: probiotic, food, survival

628 Translating the science into efficacy claims on probiotic or prebiotic products in the US market. M. E. Sanders*, *Dairy & Food Culture Technologies*, *Centennial, CO*.

The US marketplace is home to an increasing number of food products labeled "probiotic" or "prebiotic." Although the scientific definitions of these terms are clear, they lack legal definition in the US. Responsible companies seek scientific substantiation for claims. Translating science into product claims starts with conducting research on endpoints that are compatible with the category of product. A product claiming to cure, treat, mitigate or prevent disease is a drug, not a food. Claims stating the effect of the product on the normal structure or function of the human body (structure/function claim), or on reducing the risk of a diet-related, chronic disease (health claim) are allowed for food. However, many studies comprising the body of research on probiotics and prebiotics would be considered drug studies by the FDA, and therefore would not be suitable as primary substantiation for a structure/function claim. Endpoints such as prevention of allergy, reducing the incidence of intestinal or respiratory infections among healthy children or managing symptoms of irritable bowel syndrome are seen by the FDA as drug uses. Securing public funding to conduct such research might trigger a request to file an Investigational New Drug Application with the FDA. Such a process – especially for a study recruiting healthy subjects - seems like an unnecessary burden for researchers with no intention to market a drug. Endpoints such as modulation of immune system function and alteration of colonizing microbiota fall under the structure/function claim rubric, but the consumer benefit from such endpoints is not always obvious. The arena of crafting "truthful and not misleading" product claims that accurately reflect scientific substantiation is a challenge. In addition to compliance with regulatory statutes, claims are scrutinized by other audiences, such as: consumers, healthcare professionals, media professionals, consumer watchdog organizations, advertising watchdog organizations (e.g., the National Advertising Division of the Better Business Bureau), and litigious elements in society.

Key Words: probiotic, prebiotic, claims

629 Strategic application of direct-fed microbials to livestock for growth efficiency and production. E. Davis* and T. Rehberger, *Dansico*, *Waukesha, WI.*

The establishment of the commensal microbiota in the gastrointestinal tract of the neonate is the impetus for the development of a functional immune system, with ramifications on metabolic functions related to subsequent growth in later production stages. The administration of probiotics, termed direct-fed microbials (DFM) when delivered to livestock, affords an opportunity to dictate a portion of the microbial consortia and thereby exert a positive influence on production efficiency and health. With the use of culture-independent molecular techniques, microbial diversity in the intestinal tract of neonatal pigs has been assessed and members of the intestinal microbial population identified that were positively correlated to specific immune cell phenotypes and growth performance traits. The administration of a *Bacillus*-based DFM to sows enhanced specific *Lactobacillus* populations in piglets at 3 and 10 d of age compared with pigs born to unsupplemented sows, with differences also observed in nutrient composition and immune cell phenotypes within colostrum, piglet growth, and immune cell populations in the gastrointestinal tracts of piglets. Although early delivery of a DFM may be ideal to influence early microbial colonization, administration of microorganisms in later production stages can still elicit improvements in production efficiency. Administration of *Propionibacterium* strain P169 to dairy cows during late gestation and early lactation altered ruminal metabolism toward increased propionate, resulting in increased milk production without increasing DMI. Host-microbial interactions during early post-natal development and in later production stages indicate that strategic applications of DFM to livestock can positively impact growth efficiency and production.

Key Words: probiotics, swine, cattle