Dairy Foods: Probiotics, Bioactives, and Health

T46 Effect of milk fat globule membrane phospholipids in the adherence of probiotic lactic acid bacteria—Modelling interactions in the human gut. J. Ortega-Anaya*, D. Rocha-Mendoza, I. Garcia-Cano, and R. Jimenez-Flores, The Ohio State University, Columbus, OH.

The assortment of polar lipids that constitute the milk fat globule membrane (MFGM) affects infant gastrointestinal development and one of the possible mechanism might be via bacteria-lipid interaction that in turn affect the gut microbiome. However, there is scarce information about this topic. In our research group, we have proven that different lactic acid bacteria (LAB) bind and utilize different mixtures of MFGM phospholipids. Based on this information, we hypothesized that probiotic LAB modify their adherence properties when MFGM phospholipids are present during cell growth. To test this, we performed surface binding studies of bacterial cells grown in a defined medium (control), and with added MFGM phospholipids (0.5% wt/vol). We used a quartz crystal microbalance with dissipation (QCM-D) approach to measure binding kinetics. Our results show that the addition of MFGM during bacterial growth greatly alters the adherence properties to gold surfaces and more importantly, to intestinal cells as well. We calculated the affinity of the bacterial cells, via the total mass adsorbed, and were able to determine biochemical parameters such as the affinity constants (K_d) and found that L. casei and L. reuteri grown in MFGM increased significantly their basal binding affinity whereas the opposite effect was observed in cells of P. acidilactici, L. plantarum and L. delbrueckii. Interestingly, it was observed that regardless of the basal adherence properties, bacterial cells grown in MFGM, except for L. plantarum, displayed an unusual affinity toward intestinal cells characterized by such a strong binding affinity, that they are detached from the gold scaffold surface. Further experiments are needed to understand this interaction but based in our results, it is evident that in the presence of MFGM phospholipids, probiotic cells are subjected to metabolic changes or conditioning to surface attachment, which modify their binding properties having an impact in the gastrointestinal environment.

Key Words: adherence studies, LAB and milk fat globule membrane (MFGM), intestinal cells

T47 Effects of supplementation of citrulline and Lactobacillus helveticus ASCC 511 on the intestinal epithelial cell integrity. S. W. Ho and N. Shah*, The University of Hong Kong, Hong Kong.

Citrulline is an amino acid and a precursor of arginine that is believed to have the same beneficial effects as arginine. Lactobacillus helveticus ASCC 511 (LH511) utilizes arginine to produce extra energy for cell growth via arginine deiminase (ADI) pathway. Supplementation of citrulline and LH511 is considered to provide both benefits. The effects of LH511+Cit-2mM were examined on IPEC-J2 cell line and were used to determine anti-adhesion effects against pathogenic infection, the effect on cell integrity by measuring transepithelial electrical resistance (TEER) and that on tight junction (TJ) proteins (claudin-1, occludin and zonula occluden-1 (ZO-1)) expression by qPCR and Western blot analysis. The anti-apoptotic effect was also determined by a flow cytometric method. The adhesion level of LH511 on IPEC-J2 cells was enhanced when incubated with 2 mM citrulline. LH511+Cit-2mM exhibited the protective effect against the adhesion of enterohemorrhagic (EHEC) and enteroinvasive (EIEC) Escherichia coli (E. coli), and it also significantly improved TEER and stimulated TJ proteins expression. Treatment with LH511+Cit-2mM showed greater effects than arginine and citrulline alone. This study suggests that LH511 enriched with citrulline might be a potential supplement for enhancing the health of the intestine.

Key Words: citrulline, Lactobacillus helveticus, intestinal cells

T48 Incorporation of bigels into yogurt to improve survival of probiotics. X. Zhuang*, S. Clark, and N. Acevedo, Iowa State University, Ames, IA.

The probiotic yogurt market is strong due to the potential health benefits that probiotics can provide to the host. However, many factors can cause the loss of probiotics viability, including processing conditions and the high acidity of yogurt. The objective of this study was to use bigel technology, a novel encapsulation system, to preserve viability of probiotics incorporated into yogurt. Bigels, composed of an oleogel emulsion (OGE) blended with a hydrogel (HG), were formulated. The OGE was prepared with 20% wt/wt oleogelators (5:5 soy lecithin: stearic acid), 10% wt/wt milk, and soybean oil as the continuous phase. The HG was composed of 25% wt/wt whey protein concentrate (WPC80) and 75% wt/wt deionized water. Probiotic bigels were prepared by homogenizing OGE and HG, followed by incorporation of Lactobacillus acidophilus and Bifidobacterium lactis suspended in milk. For Sundae-style yogurt, 18% wt/wt probiotic bigels were placed at the bottom of containers and covered with yogurt. For Swiss-style yogurt, 18% wt/wt probiotic bigels were mixed into containers with yogurt at a constant stir rate. Three controls were also included in the experimental design: yogurt without probiotics and bigel, yogurt with only probiotics (no bigel), and probiotic bigels from the Sundae-style yogurt. Probiotics viability at 4°C was monitored via plate counts for 6 weeks. The results showed that both the presence of phospholipids and the bigel structure enhanced L. acidophilus and B. lactis viability in yogurt. Throughout 42 d of storage, the presence of soy lecithin exhibited up to 2 log higher cfu/mL values than the control. Additionally, B. lactis and L. acidophilus survived for 7 d longer when inoculated in the structured system than control. The present study demonstrated that probiotics can be efficiently entrapped in bigel systems which extend their viability when incorporated in yogurt. This approach shows a promising future for its application to improve efficacy of probiotics in commercial yogurt production.

Key Words: bigel, probiotics, phospholipids

T49 Growth and short-chain fatty acid production by potential probiotic lactobacilli. J. Renye*, A. Hotchkiss, and A. White, Dairy and Functional Foods Research Unit, ERRC, ARS, USDA, Wyndmoor, PA.

Prebiotics are nondigestible food ingredients selectively used by beneficial bacteria within the colon to improve host health. Inulin and fructo-oligosaccharides (FOS) are well-studied prebiotics that can be metabolized by strains of lactobacilli and bifidobacteria; and are associated with improved digestive health in humans due to the production of short-chain fatty acids (SCFA). Prebiotics have also been shown to improve the growth, survival and bioactivities of probiotics, which has led to the development of synbiotics, where pre- and probiotics are delivered together to optimize their beneficial activities. In this study, we screened 87 strains of lactobacilli for their ability to grow with inulin (Synergy 1) or FOS (P95) provided as the sole source of fermentable carbohydrates. Growth in modified MRS broth (no glucose) contain-
ing 1% inulin or FOS (m/v) was monitored for 24 h in a Cytation 5 multi-mode plate reader (BioTek). Nine lactobacillus strains fermented both prebiotics, reaching an optical density (OD600) ≥ 1.2, including L. casei (strains: LC3, 441 and ATCC 4646); L. helveticus (strains: 1842 and 1929); L. lactis FARR; L. paracasei ssp. paracasei 4564; L. acidophilus 1426; and L. reuteri 1428. Bifidobacterium breve 2141 was also screened and fermented both prebiotics reaching an OD > 1.6. High-performance liquid chromatography was used to identify SCFAs in cell free supernatants (CFS) from 20 cultures that reached an OD ≥ 0.5. For the 9 lactobacillus strains above, the concentration of lactic acid was between 175 and 206 mM, and L. helveticus 1929 produced the highest concentration of acetic acid (~19 mM). In the presence of FOS, the highest concentrations of propionic (3.9–6.2 mM) and butyric acids (0.9–1.2 mM) were detected in CFS from L. reuteri 1428, L. paracasei ssp. paracasei 4564 and L. plantarum 23115. With inulin, L. acidophilus 1426 and L. delbrueckii ssp. lactis 735 produced the highest concentrations of propionic acid (~4.2 mM); and L. acidophilus 1426, L. paracasei ssp. paracasei 4564 and L. plantarum 23115 produced the most butyric acid (1.0 mM). Results from this study are essential to identify lactobacillus strains suitable for the development of synbiotics utilizing FOS or inulin as prebiotics components.

Key Words: prebiotic, Lactobacillus, probiotic

T50 Preparation of γ-aminobutyric acid-enriched fermented compound beverage by Lactobacillus plantarum 326. K. Zhuang1, H. Li1, Z. Zhang1, X. Feng1, S. Fu1, T. Li1, Y. Jiang1,3, H. Zheng2, and C. Man1, 1Key Laboratory of Dairy Science, Ministry of Education, College of Food Science, Northeast Agricultural University, Harbin, China, 2California Polytechnic State University, San Luis Obispo, CA, 3YANGDA Kangyuan Dairy Company Limited, Yangzhou, China.

Whey and corn oligopeptides are by-products from cheeses and cornstarch manufacturing processes respectively. Both of these by-products are highly nutritious. It was found in our previous research that some lactic acid bacteria (LAB) can utilize γ-glutamate in corn oligopeptides to produce γ-aminobutyric acid (GABA). Moreover, research have shown blood-pressure-lowering effect of GABA-enriched dairy foods. Therefore, the objective of this study is to develop a GABA-enriched fermented compound beverage made up of whey and corn oligopeptides. Four species of LAB, including Lactobacillus plantarum, Lactobacillus bulgaricus, Streptococcus thermophilus and Lactobacillus acidophilus, were screened as single starter or mixed as compound starter. The quantity of substrates were characterized with viable counts and yield of GABA as evaluation indicators. Results showed that the optimal starter was L. plantarum 326 and the optimal substrates were 8% whey powder and 4% corn oligopeptides. In addition, the optimization of initial pH value, fermentation temperature and time were carried out using Box-Behnken response surface methodology. The optimal conditions of fermentation were 68 h fermentation at 32°C with an initial pH 5.4. The content of GABA reached 180.4 mg L\(^{-1}\) in the beverage which significantly upregulated the synthesis of vitamin B\(_6\) and protect HUVECs function through activating PDXP and the PI3K/AKT/ERK1/2 pathway. For the first time, we revealed that lactoferrin could induce the synthesis of vitamin B\(_6\), protect HUVECs function through activating PDXP and the related pathway.

Key Words: lactoferrin, vitamin B\(_6\), human umbilical vein endothelial cells (HUVEC)

T51 Lactoferrin induces the synthesis of vitamin B6 and protects human umbilical vein endothelial cell (HUVEC) functions by activating PDXP and the PI3K/AKT/ERK1/2 pathway. Y. Wang1,2, H. Li1,2, H. Yang1,2, J. Wang*1,2, and N. Zheng1,2, 1State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

As a nutritional active protein in foods, multiple studies of the biological activities of lactoferrin had been proved, including antioxidant, anti-inflammatory, antitumor, antibiosis and antiparasitic effects, while the mechanism related to its protection of cardiovascular system remained elusive. In the present work, the effect of lactoferrin on the viability of HUVECs (human umbilical vein endothelial cells) was detected to select the proper doses, the transcriptomics detection and data analysis were performed to screen out the special genes and the related pathway. Meanwhile, the regulation of lactoferrin in the functional factors thromboxane A\(_2\) (TXA\(_2\)) and prostacyclin (PGI\(_2\)) was detected. Then, siRNA fragment of the selected gene pyridoxal phosphate synthase (PDXP) was transfected into HUVECs to validate its role in protecting HUVECs function. Results showed that lactoferrin inhibited expression of TXA2 and activated expression of PGII2, as well as activated expression of PDXP, which significantly upregulated the synthesis of vitamin B\(_6\) and protect HUVECs function through activating PDXP and the related pathway.

Key Words: lactoferrin, vitamin B\(_6\), human umbilical vein endothelial cells (HUVEC)

T52 Investigation and comparison of the anti-tumor effects of lactoferrin, α-lactalbumin, and β-lactoglobulin in A549, HT29, HepG2, and MDA231-LM2 models. H. Li1,2, P. Li1,2, H. Yang1,2, Y. Wang1,2, G. Huang1,2, J. Wang*1,2, and N. Zheng1,2, 1State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China, 2Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.

To investigate the anti-tumor activities of lactoferrin, α-lactalbumin, and β-lactoglobulin, 4 kinds of human tumor cells (lung tumor cell A549, intestinal epithelial tumor cell HT29, hepatocellular cell HepG2 and breast cancer cell MDA231-LM2) were exposed to 3 proteins, respectively. The effects on cell proliferation, migration, and apoptosis were detected in vitro, and nude mice bearing tumors were administered with 3 proteins in vivo. Results showed that 3 proteins (20 g/L) inhibited viability and migration, and apoptosis were detected in vitro, and nude mice bearing tumors were administered with 3 proteins in vivo. Results showed that 3 proteins (20 g/L) inhibited viability and migration, as well as induced apoptosis, in 4 tumor cells with different degrees (compare with the control, P < 0.05). In vivo, tumor weights in HT29 group (0.84 ± 0.22 g v.s. control 2.05 ± 0.49 g) and MDA231-LM2 group (1.11 ± 0.25 g v.s. control 2.49 ± 0.57 g) were significantly reduced by lactoferrin (P < 0.05); tumor weights in A549 group (1.07 ± 0.19 g v.s. control 3.11 ± 0.73 g).
g) and HepG2 group (2.32 ± 0.46 g vs. control 3.50 ± 0.74 g) were significantly reduced by α-lactalbumin (P < 0.05). Moreover, the roles of lactoferrin, α-lactalbumin, and β-lactoglobulin in regulating apoptotic proteins were validated. In summary, lactoferrin, α-lactalbumin, and β-lactoglobulin were proved to inhibit growth and development of A549, HT29, HepG2, MDA231-LM2 tumors in different degrees, via induction of cell apoptosis.

**Key Words:** lactoprotein, antitumor activity, tumor-bearing model

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**T53**  
**Modulation of intestinal epithelial permeability and mucin mRNA (MUC2, MUC5AC, and MUC5B) expression and protein secretion in Caco-2/HT29-MTX co-cultures exposed to aflatoxin M1, ochratoxin A, and zearalenone individually or collectively.** C. Wu1,2, N. Zheng1,2, Y. Gao1,2, and J. Wang*1,2, 1State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China, 2Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.

Aflatoxin M1 (AFM1), ochratoxin A (OTA), and zearalenone (ZEA) are mycotoxins commonly found in milk. The combined effects of these mycotoxins on intestinal epithelial cells have not been reported. Herein, we investigated the combined effects of AFM1, OTA, and ZEA on intestinal integrity and define the underlying mechanisms(s) of their effects in Caco-2/HT29-MTX cocultures. Our results showed that the mixtures of AFM1+OTA, AFM1+ZEA, and AFM1+ZEA+OTA significantly decreased transepithelial resistance values and increased the paracellular flux of lucifer yellow and FITC-dextrans on Caco-2/HT29-MTX cells, which indicated an increased epithelial permeability. Although the expression levels of tight junction (TJ) proteins did not change significantly, immunofluorescence analysis and transmission electron microscopy revealed that mycotoxins altered TJ proteins morphology and disrupted their structures, namely, claudin-3, claudin-4, occludin, and zonula occludens-1. Also, the present study showed that mixtures of mycotoxins significantly modulated MUC5AC and MUC5B mRNA levels and protein secretion. This study demonstrated that the effects of mixtures of mycotoxins on intestinal barrier function were more significant than AFM1 alone. More importantly, the damage of intestinal integrity caused by mycotoxins was correlated with the change of the TJ proteins location and the decrease of mucin secretion. Mixtures of AFM1, OTA, and ZEA in food might pose a health risk to consumers, particularly in children, and toxin risks should be considered.

**Key Words:** Caco-2/HT29-MTX co-culture, tight junction, mucin