M111 Selective growth using MRS broth for raw milk microbiome of naturalized Brazilian breeds Curraleiro Pé-Duro and Pantaneiro. N. R. Soares1, M. C. Sola2, C. Gebara3, G. V. Barbacelit2, O. F. Zaccaronit2, M. C. S. Fioravantit2, E. S. Nicoliou1, and C. S. Minafra-Rezende1. Food Research Center, School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil, 2Unified Higher Education Institute, Objetivo Faculty, Goiânia, Goiás, Brazil, 3Department of Agro-Industry, Food and Nutrition, “Luiz de Queiroz” College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil, 4School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil.

The genus Enterococcus involves bacteria that are part of the natural microbiota of the gastrointestinal tract of animals and humans and is also found in water, soil, and food. In the food industry, it is used as starter or probiotic cultures, developing sensory characteristics and contributing to the balance of the intestinal microbiota. It should be noted that E. faecium can be considered probiotic or pathogenic, depending on the strain. The aim of this study was to select and identify potentially bacteriocin-producing lactic acid bacteria in raw milk from naturalized Brazilian breeds Curraleiro Pé-Duro and Pantaneiro after a selective growth on MRS medium. Raw milk samples were aseptically collected from Brazilian breeds Curraleiro and Pantaneiro (10 animals each) and aliquots were submitted to a selective growth using MRS broth at 37°C/16 h. The broth was centrifuged twice to obtain a cellular concentrate for performing molecular analysis. LAB was determined through DNA extraction from milk, the 16S rRNA gene was amplified by PCR and optimized by Illumina TrueSeq platform and analyzed with QIIME. A total of 42,899 sequences was formed. The only genus identified was Enterococcus, with higher frequency of E. faecium (86.18% of sequences) on 90% of the samples. Others species identified were E. munditii (90% of samples and 9.34% of sequences), E. faecalis (10% of samples, 3.47% of sequences), E. lactis (90% of samples, 0.84% of sequences), E. durans (45% of samples, 0.11% of sequences), and E. hirae (15% of samples, 0.05% of sequences). Our hypothesis is that lactic acid bacteria naturally present in milk of these breeds have the ability to produce inhibitory compounds against pathogens, because these animals present low mastitis incidence and produce milk with low SCC, despite the absence of management to control mastitis. These compounds could be organic acids or bacteriocins and some strains of the identified species, such as E. faecium, are bacteriocins producers. Therefore, these microorganisms have technological potential for isolation and using in dairy products and affect animal health and food safety.

Key Words: Enterococcus, molecular analysis, milk

M112 Effect of farm interventions on sporeformers and milk quality. R. A. Crespo*, B. A. Martinez, J. Stratton, and A. Bianchini, University of Nebraska-Lincoln, Lincoln, NE.

Bacillus and Paenibacillus spp. are spore-forming bacteria with the ability to survive pasteurization due to its spore structure. The powdered-milk industry may need spore counts as low as 50 spores/g to achieve the high quality standards required by foreign customers; hence, a microbial load of <5 spores/mL in raw milk would be required. Control of sporeformers below this limit is crucial to benefit the dairy industry. Interventions at the farm level are key because raw milk has been identified as a main entry point of these organisms into the milk chain. Previous research indicates that teat cleaning, changes in bedding material, and CIP procedures could be potential interventions to decrease sporeforming bacterial populations in milk. Therefore, the objective of this research was to implement these previously mentioned interventions in 2 farms and analyze their microbial effect. Samples were collected for 7 d to establish a microbiological baseline. After the interventions were applied and an acclimatization period of 15 d, samples were collected for 8 d to compare with baseline data. Environmental and raw milk samples were analyzed for mesophilic and thermophilic sporeformers. Additionally, microbial quality and prevalence of psychrotrophic sporeformers (7°C) for raw milk samples were performed. Raw milk quality analysis after interventions showed that total plate counts were around 3.0 log cfu/mL, whereas Enterobacteriaceae and coliforms were around 1.0 log cfu/mL. E. coli counts ranged from 0.25 to 0.45 log cfu/mL. Mesophilic spore counts ranged from 0.81 to 1.0 log cfu/mL, and thermophilic spore counts varied between 0.76 and 0.98 log cfu/mL in raw milk samples. Results suggest that a change in the sanitation (i.e., CIP) and bedding protocols (i.e., new bedding material) leads to a statistically significant reduction in sporeformers in raw milk. Changes in sanitizing teat dips showed different results among brands. The prevalence of psychrotrophic sporeformers did not seem to be affected by the interventions. This research showed that farm practices appear to exert an important effect on the levels of some sporeformers in raw milk.

M113 Increasing producer profitability through farm-level interventions designed for optimization of spore counts in raw milk. R. L. Evanowski*, D. J. Kent, N. H. Martin, K. J. Boor, and M. Wiedmann, Cornell University, Ithaca, NY.

Spore-forming bacteria, such as Paenibacillus sp. and Clostridium sp., can survive pasteurization and affect the quality of dairy products (e.g., spoilage in fluid milk and late blowing in certain cheeses). With the demand for higher quality finished products that can be distributed further and to new markets, dairy processors are becoming more concerned with low spore counts in raw milk. Some processors have begun to offer premiums to producers who can supply low spore count raw milk for certain applications. The present study used results from a previous data collection to develop and test intervention strategies aimed at reducing transmission of spore-forming bacteria from environmental sources into bulk tank raw milk. These strategies involved (1) training milking staff to focus on teat end cleaning during milking preparation, and (2) implementing changes in towel treatment (i.e., use of detergent, chlorine bleach, and drying). Study design included collecting bulk tank raw milk samples for a week before and a week after initiating the intervention strategies (e.g., milker training on the importance of teat end cleaning and towel treatment). The interventions were conducted 3 times over the course of 15 mo at 5 New York farms, each of which had varying management practices. Teat end condition and udder hygiene scores were also collected. The 288 raw milk samples to date were analyzed for mesophilic and thermophilic spore counts. Results showed bulk tank milk mean spore counts of 1.5 and 1.3 cfu/mL for MSC and TSC respectively before the intervention, and bulk tank milk mean spore counts of 0.9 cfu/mL after the intervention for both MSC and TSC for a spore reduction of 41% and 42% in bulk tank raw milk for MSC and TSC respectively. This was found to be significant using a mixed effects linear regression model. The intervention strategies tested provided an easy to execute milking hygiene enhancement (e.g., focusing on teat end hygiene and towel washing procedures) that can reduce bulk tank
Sporulating behavior influences the population dynamics of sporeformers during raw milk holding. N. Awasti*, S. Anand1, and G. Djirad, Midwest Dairy Food Research Center, Dairy and Food Science Department, South Dakota State University, Brookings, SD, and Department of Mathematics and Statistics, South Dakota State University, Brookings, SD.

Thermoduric sporeformers are predominant in raw milk and form thermoduric endospores. Our previous research showed these sporeformers to cause biofouling of dairy contact surfaces and membranes, leading to cross contamination of final products. A critical factor influencing thermal inactivation is their form as vegetative cells or endospores. It would thus be of interest to understand the population dynamics of sporeformers in raw milk during storage at low temperatures. In our previous study, a low sporulating strain of Bacillus licheniformis showed an increasing trend in vegetative cell population during 72 h of storage at 10°C or higher, while maintaining spore population relatively static. In continuation, this study investigates population dynamics of a high sporulating strain of B. licheniformis (ATCC 14580). Raw milk samples were separately spiked with an average 4.0 log vegetative cells and 2.0 log spores/mL, and stored at 4°C, 6°C, 8°C, 10°C, and 12°C for 0, 24, 48, and 72 h. Standard protocols were followed for enumerating vegetative cells and spores. Three trials were conducted, in replicates of 3, and means were compared using ANOVA. Contour plots were developed using quadratic regression models to predict the population of vegetative cells and spores. In the vegetative cell spiking study, cell population remained mainly unchanged for 72 h up to 10°C, with more than 1.0 log change observed only at 12°C. As it was a sporulating strain, the spore spiking study validated a shift toward spores during storage at 4°C to 8°C, with evidence of some parallel germination at 10°C or higher. The regression models helped us to develop contour plots across the holding temperature and duration. Based on the initial cell population of the spore former, such contour plots would help predict the presence of vegetative cells and spore populations in raw milk at a given time and temperature. This information will prove useful in optimizing raw milk holding conditions to keep the sporeformer population toward vegetative cells, which can subsequently be inactivated easily with thermal treatments such as pasteurization.

Key Words: sporulation, spore, Bacillus

Feasibility of hydrodynamic cavitation, in line with HTST pasteurization, for inactivating sporeformers and spores in skim milk. P. Chaudhary*, S. Anand, and S. M. Monteagudo, Midwest Dairy Foods Research Center, Dairy and Food Science Department, South Dakota State University, Brookings, SD.

Sporeformers and their endospores are of concern for the dairy industry due to their thermal resistance. A previous study conducted in our laboratory revealed the effectiveness of a 6- to 8-pass cavitation effect (CE), in combination with batch pasteurization, in reducing vegetative cells and endospores of some common dairy sporeformers. In continuation, the current study evaluates the feasibility of reduced pass hydrodynamic cavitation, combined with HTST pasteurization for the reduction of sporeformers. The equipment set up was assembled in Davis Dairy plant to conduct a continuous cavitation and HTST pasteurization process. The spiking studies were conducted by inoculating sterilized skim milk with either vegetative cells (at 4.76 ± 0.03 log/mL) or spores (2.65 ± 0.09 log/mL) of a thermoduric strain of Bacillus coagulans (ATCC 12245). The flow of the spiked skim milk samples varied from 50 to 200 L/h, with a cavitation frequency of 60 Hz, using an APV cavitator. A small plate heat exchanger and a holding tube set up to achieve 73°C ± 1.0°C for 15 s effected the pasteurization. The spiked milk samples were processed through the above set-up for a single pass CE, followed by pasteurization to establish a baseline. The study was expanded up to 4-pass CE, combined with pasteurization, to observe their feasibility in enhancing spore inactivation. All trials were conducted thrice, with samples drawn in triplicates, and data were analyzed statistically using one-way ANOVA Tukey’s test. The initial load of 4.76 ± 0.03 log of vegetative cells was reduced to 0.91 ± 0.04 log by a single pass CE at a flow rate of 200L/h, followed by pasteurization, as compared with 1.19 logs by pasteurization alone. In the case of spores, a 4-pass CE at 100L/h flow rate was found necessary to reduce the mean spore counts from 2.65 ± 0.09 log to 2.06 ± 0.02 log. The single pass CE combined with HTST pasteurization appeared to be feasible for reducing thermoduric sporeformers; for spore reduction, multiple CEs were necessary. Further evaluation studies are in progress.

Key Words: hydrodynamic cavitation, sporeformer, spore

Efficacy of sub-pasteurization thermal treatments to inactivate Salmonella, Shiga toxin-producing Escherichia coli (STEC), and Listeria monocytogenes in fluid milk. A. Emch*, L. Goddik, J. Kovačević, and J. Waite-Cusic, Oregon State University, Corvallis, OR.

Cheeses made with milk treated at temperatures below time/temperature requirements for pasteurization are subject to the 60-d aging rule as is required by the FDA for raw milk cheeses. Recent reports from the FDA have indicated their lack of confidence in the 60-d aging rule. It is likely that the FDA will be increasing their scrutiny of a variety of dairy products produced with non-pasteurized milk. Several commercial dairy companies produce cheeses made from milk that has been treated at these “sub-pasteurization” temperatures, these treatments are commonly referred to as “heat-shocked.” Currently, there is very little research on the efficacy for heat shock treatment to reduce pertinent pathogens in fluid milk. The purpose of this study is to determine the efficacy of sub-pasteurization treatments to reduce pertinent foodborne pathogens (Salmonella, Shiga toxin-producing E. coli, and L. monocytogenes) in fluid milk. Raw milk (1 mL) samples were individually inoculated with a cocktail of Salmonella, E. coli O157:H7, or Listeria monocytogenes to reach a final concentration of approximately 8 log cfu/mL. Milk samples were heated in at 63°C in a recirculating water bath for 0–6 min. Survivors were enumerated using standard serial dilution and spread plating techniques using suitable selective-differential media: Hektoen enteric agar (Salmonella), Eosin Methylene Blue agar (E. coli), or CHROMagar Listeria spp. (Listeria). Plates were incubated at 37°C for 48 h before enumeration. A thermal treatment of 63°C for 6 min resulted in a 2.85 ± 0.95 log reduction of the Salmonella cocktail. These conditions were more effective at reducing E. coli O157:H7 and L. monocytogenes achieving 5.52 ± 0.98 log reduction and 5.12 ± 0.44 log reduction, respectively. E. coli and Listeria did not have significantly different reductions for the full treatment. The reduction of Salmonella was significantly less than both E. coli O157:H7 and L. monocytogenes (Tukey HSD P < 0.05). This study demonstrates that Salmonella is more resistant to heat-shock treatment in raw milk fluid under bench-scale conditions. Therefore, Salmonella would be an appropriate target organism for future thermal validation studies in fluid milk.

Key Words: fluid milk, heat-shock
M117  Effect of freezing and hardening on injured versus intact cells of *Listeria* in ice cream mix. N. Neha1, S. Anand1, B. Kraus2, and S. Sutariya2. 1South Dakota State University, Brookings, SD, 2Wells Enterprises Inc., Le Mars, IA.

Ice cream manufacturing relies on pasteurization to eliminate any *Listeria* contamination. However, factors such as cross-contamination levels, entrapment in polymorpholeukocytes, and product matrices may influence cellular injury. Our previous studies demonstrated a dose-dependent, random presence of heat-injured cells of *Listeria* in pasteurized ice cream mix. Such cells did not show any recovery within the mix itself under normal handling conditions. The present study evaluates the effect of freezing and hardening of ice cream mix on heat-injured and intact cells of *Listeria*. Raw ice cream mix (42% TS) samples were spiked with 4.36 ± 0.13 logs per gram of *Listeria innocua* (a surrogate) and subjected to pasteurization (69°C for 30min), which resulted in the random presence of heat-injured cells. To simulate the post-pasteurization contamination of mix with intact cells, 2.65 ± 0.07 logs per gram of *L. innocua* were spiked in the pasteurized mix samples. The mixes containing injured and intact cells were followed through aging (72h at 7°C), freezing (−4.4°C), and hardening (12 h at −40°C) steps. Direct plating on *Listeria* selective agars enumerated intact cells, while *Listeria* enrichment broth (BLEB) was used to recover heat-injured cells before plating. All trials were conducted in triplicates and data were statistically analyzed. Although no post-pasteurization survivors were observed on direct plating, the enrichment protocol revealed heat-injured cells at all of the post-pasteurization stages of processing, tested. Freezing and hardening steps thus did not appear to have any detrimental effects on heat-injured cells. Injured cells have not been associated with any outbreaks; however, it would be interesting to study their ability to recover during any handling-abuse at retail or consumer end. In case of spiked intact cells, no detrimental effect of freezing and hardening was observed. This implies that post-pasteurization contamination of mix might pose a greater risk. Results from this study emphasize a need to design stage-specific critical control points to prevent any potential *Listeria* outbreaks.

**Key Words:** injured, *Listeria*, ice cream

M118  Enhanced efficacy of nisin loaded zein microcapsules against *Listeria monocytogenes* in Queso Fresco. L. A. Ibarra-Sanchez*, Y. Feng, Y. Lee, and M. J. Miller, University of Illinois at Urbana-Champaign, Champaign, IL.

Queso Fresco (QF), a widely produced Hispanic-style fresh cheese, is known to support the growth of *Listeria monocytogenes*. Nisin is a biopreservative with antimicrobial activity against *L. monocytogenes*; however, it has limited stability in near neutral pH foods, such as QF. The objective of this study was to evaluate the efficacy of nisin microencapsulated in zein against *L. monocytogenes* in QF. Zein microcapsules with low (LNM) and high (HNM) nisin loading (3–5 and 12–13 µg nisin/mg microcapsules, respectively) were prepared with a microfluidic device and nisin concentration was quantified via HPLC. Microencapsulated and free nisin were added to QF at a final concentration of 250 µg of nisin/g of cheese, and untreated cheeses were included as a control. The antilisterial activity of microencapsulated and free nisin was tested by inoculating cheese curds with approximately 4 Log cfu/g of *L. monocytogenes* cocktail of 5 different foodborne outbreak-associated strains, and *L. monocytogenes* cells were enumerated by spread plating on PALCAM agar supplemented with ceftazidime, across 7 d of storage at 4°C. All experiments were repeated 3 times with samples prepared in duplicate. Free nisin reduced the viable cell count of *L. monocytogenes* by approximately 0.8 Log cfu/g after 3 d, but subsequent regrowth led to a final population comparable to untreated QF. All treatments with nisin-loaded zein microcapsules achieved overall lower viable cell counts relative to free nisin, notably at early cheese storage. HNM reduced the initial viable population of *L. monocytogenes* by up to 1.5 Log cfu/g after 3 d. LNM showed higher viable pathogen reduction relative to HNM, reducing up to approximately 2 Log cfu/g from the initial inoculum after 7 d of cold storage. In conclusion, our results support the use of encapsapsulation technology to improve nisin’s effectiveness to control *Listeria* in QF.

**Key Words:** Queso Fresco, *Listeria monocytogenes*, microencapsulation

M119  High-voltage atmospheric cold plasma on inactivation of *Listeria innocua* on Queso Fresco cheese. Z. Wan*, S. K. Pankaj, G. Li, and K. Keener, Iowa State Universit, Ames, IA.

Queso Fresco cheese (QFC), a type of Hispanic-style soft and fresh cheese, is a popular food in Latin-American diet. Due to its high moisture content, near neutral pH and moderate salt content, QFC provides an optimal environment for the growth of spoilage and pathogenic microorganisms. Currently, there are no effective commercial methods or technologies to reduce microorganisms in soft cheeses such as QFC. High-voltage atmospheric cold plasma (HVACP) is a novel, non-thermal technology that can be used to treat packaged food products and achieve significant reduction of pathogenic and spoilage microorganisms without compromising products’ qualities. In this study, 2 types of gases, dry air and MA50 (50% CO2, 50% N2), were evaluated for inactivation of *Listeria innocua* (LI), a non-pathogenic surrogate for *Listeria monocytogenes*, in QFC by HVACP treatment. Survival of LI after HVACP treatments was evaluated by enumeration on *Listeria* selective agar. Quality effects after HVACP treatments were analyzed for lipid oxidation, pH, and moisture content. Plasma characterization was done using optical emission spectrometry. ANOVA was used for statistical analysis of microbial and quality data. The results have shown that HVACP treatment was able to achieve a maximal of 4.9 Log10 cfu/g LI reduction after dry air treatment and 1.7 Log10 cfu/g reduction after MA50 treatment. Higher lipid oxidation was found in samples after dry air treatment than MA50 treatment due to the presence of O2 in dry air. HVACP treatments have resulted an average of 2.14 mg/kg malondialdehyde (MDA) formed in dry air treated samples, and 1.07 mg/kg MDA in MA50 treated samples. Negligible changes were observed in moisture content and pH after HVACP treatments. HVACP treated samples had an average pH and moisture content of 5.5 and 48.8%, respectively, versus 5.7 and 49.4% in control samples. LI reductions were found to be dependent on the gas composition and treatment time. The results demonstrate the efficacy of HVACP treatment for LI inactivation in QFC. This study shows the potential of HVACP technology for non-thermal processing of delicate dairy products.

**Key Words:** atmospheric cold plasma, *Listeria innocua*, Queso Fresco

M120  Screening of lipolytic, proteolytic, and antibacterial activities of lactic acid bacteria with biotechnological significance isolated from dairy products. I. García-Cano*, D. Rocha-Mendoza, J. Ortega-Anaya, and R. Jiménez-Flores, The Ohio State University, Columbus, OH.

Fermented dairy products such as yogurt and cheese are an important source of high quality nutrients for consumers. The wide variety of fermented products, in terms of chemical composition, are the result
of the metabolism of lactic acid bacteria (LAB). The predominant enzymes that contribute to the diversity of fermented dairy products include lipases, proteases, and antibacterial proteins such as bacteriocins and peptidoglycan hydrolases, which are synthesized directly by LAB having a high biotechnological importance. Microbiome studies based in metagenomics indicate that there are specific enzymes contributing to the fermentation process and bioavailability. The objective of this work was to identify and characterize strains of LAB that possess high enzymatic expression of lipolytic, proteolytic, or antibacterial activity. Additionally, we aimed to identify and localize the proteins involved in these processes; weather it takes place in the intra or extra cellular milieu to assess the potential application in the dairy industry. The LAB (131 strains) were identified by 16S DNA sequencing. The bacteria were grown in CGB medium and harvested by centrifugation at the end of the log phase. Both the pellet and the supernatant of each strain were tested for lipolytic, proteolytic, and antibacterial activity by agar diffusion and zymograms. Five genera of LAB were identified as most active; 2 of them showed lipolytic activity in the cell fraction, hydrolyzing tributyrin agar and α-naphthyl-acetate in zymography. The active enzyme was identified in the supernatant fraction. P. acidilactici was unique in possessing 1 bacteriocin and 2 peptidoglycan hydrolases with putative N-acetylmuramidase and N-acetylmuramoyl-1-alanine amidase activities. So far, 40, 56, and 65% of total strains showed proteolytic, lipolytic, and antibacterial activity, respectively. A significant value of this report is the characterization of these enzymatic activities and their relevance in their potential biological activity in dairy foods.

Key Words: dairy product, bioactive compound, lactic acid bacteria

M121 Addition of Lactobacillus paracasei and Lactobacillus rhamnosus bacteria to yogurts for inhibition of yeast growth and improvement of their quality. C.-H. Kim1, M. S. Nam2, and Y. W. Park3, 1Binggrae Company, Kyuunki-Do, South Korea, 2Chungnam National University, Deajeon, South Korea, 3Fort Valley State University, Ogden, UT.

Yogurt is manufactured with 1.25% of active Lactobacillus delbrueckii ssp. bulgaricus and 1.25% Streptococcus thermophilus in milk by weight. Yeast contamination can occur during and after manufacture of yogurt products through unsanitary ingredients, equipment and processing personnel. Lactobacillus paracasei (Lp) and Lactobacillus rhamnosus (Lr) have been known to possess antimicrobial and heat resistant activities, respectively, against the growth of yeasts in cultured dairy products. The objectives of this study were to determine effects of the addition of Lp and Lr on inhibition of yeast cell growth in yogurts, and to evaluate the improvement of organoleptic quality and storage stability of the fermented products. Two separate experiments were conducted by additions of Lp and Lr strains at 3 different inoculation rates of 3 × 10^3, 3 × 10^4 and 3 × 10^5 cfu yeast/2L yogurt and control group without bacterial addition. All control and experimental yogurt samples were stored at 2 temperature treatments (10°C and 25°C). The number of yeast cells in all experimental yogurt samples were counted weekly, and the swellings occurred due to gas production by contaminated yeast cells in all yogurt samples were evaluated. The results showed that the Lp and Lr bacteria added yogurt groups showed much slower rates of yeast cell growth and lower frequency of swelling in comparison with those of the control yogurts, suggesting that the growth of yeast was inhibited by the additions of Lp and Lr strains to the experimental yogurts. With respect to the effect of storage temperature, the yogurts stored at 10°C had little post-acidification in the Lp and Lr added groups compared with control samples, and also showed lower post-acidification than samples stored at 25°C. These outcomes suggest that the addition of Lp and Lr bacteria to the yogurt products could improve their organoleptic quality. It was concluded that the fortification of L. paracasei and L. rhamnosus bacteria improved storage stability and organoleptic properties of the yogurts, attributable to the delayed or inhibited yeast cell growth and post-acidification in the products.

Key Words: Lactobacillus paracasei, Lactobacillus rhamnosus, yeast inhibition

M122 Growth of lactic acid bacteria in milk phospholipids enhances lipolysis and increases the possible absorption in Caco-2 cell line. D. Rocha-Mendoza*, I. García-Cano, J. Ortega-Anaya, and R. Jiménez-Flores, The Ohio State University, Columbus, OH.

Fermented milk products like yogurt have been recognized for their beneficial effects on health. This is largely due to the presence of lactic acid bacteria (LAB) who are responsible for the synthesis of bioactive compounds and other molecules that positively affect human health and also modify the physicochemical and sensory characteristics. Owing to the complexity in nutrients in milk, LAB are known to “turn on” a standard set of genes that generate enzymatic activities well identified in fermented products so far. However, little is known about the metabolic potential of LAB when grown in minimal medium or the effect on lipolytic activity specifically toward milk phospholipids (PL). In this work, we aimed to evaluate the growth of different LAB strains from our culture collection (OSU library) in a minimal medium (glucose andrypton modified) added with different concentrations of PL (0, 0.5 and 1% wt/vol) to determine lipolysis and its sub-products. The biological effect of the resulting metabolites on intestinal cells was further evaluated measuring absorption to Caco-2 cell. After screening 95 different strains of LAB we have found that L. casei, L. pentosus, L. plantarum, P. acidilactici and P. loli are able to grow in a medium enriched with an optimized concentration of milk PL (0.5% wt/vol) producing an interesting mixture of metabolites (not identified peaks by HPLC-MS). Even though the growth rate decreased with the higher concentration of milk PL, as well as biomass production, the lipolytic activity was greatly increased as determined in vitro using 4-nitrophenyl acetate and 4-nitrophenyl octanate, and α-naphthyl-acetate as substrate in zymogram experiments. We also isolated a protein with an approximate mass of 40 kDa, evaluated by SDS-PAGE, which is responsible for the predominant lipolysis observed by zymography. This enzyme product on PL modification or fragmentation represents a key factor that affects cell culture response as shown by our preliminary Caco-2 cell trials.

Key Words: milk phospholipid, lipolysis, lactic acid bacteria

M123 Rapid method for measuring the effect of prebiotics on probiotic bacterial growth. D. Hoffman*, C. Oberg, and M. Domek, Weber State University, Ogden, UT.

Prebiotics are used to stimulate probiotic bacterial growth in the gut to optimize their health benefits. A rapid method was developed to evaluate growth enhancement by prebiotics on probiotic bacteria using a programmable spectrophotometer, microtiter plates, and commercial media, with results ready in 12 h. Lactobacillus strains were grown in MRS broth while Bifidobacterium strains were grown in MRS broth with L-cysteine. Cultures were back diluted to an OD600 of 0.1 and then inoculated into wells (48-well plate) containing individual prebiotics. Plates were placed in a Tecan Infinite M200 spectrophotometer and
incubated at 37°C with A_{600} readings taken for 12 h. Growth curves were done in triplicate with results compared with controls to determine extent of prebiotic growth enhancement. To optimize the method, MRS concentrations of 20, 35, 50, and 100% were tested at selected pH values (7.0, 5.5, 5.0, 4.5, and 4.0) using 5 probiotic cultures. Addition of the bio-catalytic oxygen-reducing reagent, oxyrase, to the test wells significantly enhanced *Bifidobacterium* species and *Lb. acidophilus* growth. Results indicated a 25% MRS broth at pH 5.0 with 2% oxyrase optimized prebiotic growth enhancement comparisons. Using this method, the stimulatory effect of prebiotics (2% vol/vol) FOS, GOS, and XOS was determined for *B. infantis* M-63, *B. longum* BB536, and *B. lactis* BL-04, *Lb. rhamnosus* LR-32, and *Lb. acidophilus* NCFM. A one-sided *t*-test was used to determine significance (*P* < 0.05) between treatments and the control (no added prebiotic). All 3 significantly improved growth of M-63 (12 h OD_{600} for GOS-.85, FOS-.68, XOS-.64, and control-.60), but only FOS significantly increased growth of BL-04 (12 h OD_{600} for GOS-.67, GOS-.60, XOS-.57, and control-.60). For BB536, just GOS (12 h OD_{600} for GOS-.75, FOS-.70, XOS-.68, and control-.70) significantly enhanced growth. GOS and FOS slightly improved growth of NCFM whereas no oligosaccharides enhanced growth of LR-32. This method allows rapid testing of inoculum levels, prebiotic concentrations, media pHs, and prebiotic combinations for any probiotic strain.  

**Key Words:** prebiotic, probiotic bacteria

**M124**  
Flax seed enhances acid tolerance of *Streptococcus thermophilus* ST-M5. I. Moppert*1 and K. Aryana2,1, 1Louisiana State University, Baton Rouge, LA, 2Louisiana State University Agricultural Center, Baton Rouge, LA.

Flax seed (*Linum usitatissimum* L.) has been reported to provide several health benefits which include, reduction of cholesterol, protection against several cancers and improvement in blood sugar. For this benefit, the consumption of 62 g of flax seed per day is recommended. Acid tolerance is an important probiotic characteristic. The objective was to determine the effect of various amounts of flax seed powder on the acid tolerance of *Streptococcus thermophilus*. M17 broth of several pH values (1, 1.5, and 2) were prepared without and with flax seed powder incorporated at 62 g/L. Flax seed powder was dispersed using a magnetic stirrer. After sterilizing and cooling the M17 broths, freshly thawed *Streptococcus thermophilus* ST-M5 was incorporated and incubated at 42°C for 4 h. Samples were drawn at 2, 2.5, 3, 3.5, and 4 h, serially diluted and poured plated on M17 agar. Pour plates were aerobically incubated at 42°C for 48 h and colonies were counted. Samples were plated in duplicate. Entire experiment was replicated 3 times. Data were analyzed using PROC ANOVA of SAS, with means being separated using Fisher Least Significant Difference test. Mean counts of control pH 2, 1.5 and 1.00 and flax seed pH 2, 1.5 and 1.00 were 9.23 ± 0.25; 8.50 ± 0.43; 4.01 ± 0.54; 9.28 ± 0.26; 8.82 ± 0.37 and 4.39 ± 0.41 log cfu/mL. Flax seed counts at pH 1.5 and 1 were significantly (*P* < 0.05) higher than control. Flax seed enhanced the acid tolerance of *Streptococcus thermophilus* ST-M5.  

**Key Words:** flax seed, yogurt, *Streptococcus thermophilus*