**W43 Microbiological quality of pasteurized milk from Minas Gerais state, Brazil.** E. H. P. Andrade, M. O. Leite*, M. R. A. Moura, T. Roza, C. F. A. M. Penna, M. M. O. P. Cerqueira, L. M. Fonseca, and M. R. Souza, *Federal University of Minas Gerais*, *Belo Horizonte, Minas Gerais, Brasil*.

The objective of this work was to evaluate the microbiological quality of pasteurized milk from different regions of Minas Gerais state, Brazil, from 2006 to 2008, to verify compliance with Brazilian standards. This state is the largest dairy producer region in Brazil, with about 7 million metric tons of milk production for the year 2008. The analyzed parameters were: aerobic mesophilic microbial counting, total and thermotolerant coliforms, *Salmonella* spp. presence and *Staphylococcus* coagulase positive counting. It was observed that 42 (70%) of the 60 samples were in disagreement with Brazilian microbial standards with at least one parameter not in compliance with the requirements. Only 18 (30%) was in accordance to the legal requirements for all parameters evaluated. Of the total of samples used in this study, 18 samples (30%) presented more than the maximum allowance for aerobic mesophilic microbial counts, 35 (58.3%) were in disagreement for total coliforms, with 23 samples (38.3%) above the limit for thermotolerant coliforms, and 2 samples (3,3%) with *Salmonella* contamination.

**Key Words:** milk quality, microbiology

**W44 The relationships between somatic cell count and bacteriology on milk quality and production in dairy goats.** K. N. Baker*, S. D. Horner, D. K. Rucker, L. C. Nuti, and G. R. Newton, *Prairie View A&M University*, *Prairie View, TX*.

Somatic cell count (SCC) in milk is an indicator of mammary infections and a barrier for production of grade A milk. The relationships between mammary infection and SSC are not clear in goats. Two experiments were conducted to evaluate the incidences of sub-clinical mastitis on mammary health and milk production. The effects of a protective teat dip and intra-mammary infusion of antibiotics on milk production, composition, SCC and bacteriology were evaluated in a 2 by 2 factorial design. At dry-off and on d 3–5 of the next lactation, goats (n = 50) were screened for the presence of intra-mammary infections. At dry-off 80% of the halves sampled were negative for bacteria. This percentage increased to 84% at the start of the next lactation. Mammary treatments did not influence the incidence of mammary infection at the start of lactation when compared with halves receiving no treatments. Content of milk fat (5.77 ± 0.5%), protein (3.92 ± 0.04%), lactose (6.06 ± 0.07%) and SCC (721 ± 302/ml) were not affected by mammary treatments. Next, milk was collected from goats (n = 65) once a month from March through November to evaluate the relationships between milk production, SCC and bacteriology. SCC in uninfected halves displayed a curvilinear increase ($P < 0.01$) with lowest values recorded in March (260 ± 11.1/ml) and highest values observed in November at the end of lactation (2075 ± 11.1/ml). SCC in infected halves did not differ during the sampling period ($P > 0.1$) and was consistently higher than samples from uninfected halves. In March SCC was 1242 ± 556/ml in infected halves. Values remained elevated through out the sampling period, reaching a peak in November (3158 ± 556/ml). Milk production by infected and uninfected halves did not differ. Production was highest in March (1.18 ± 0.03 L/half) and steadily declined to a nadir in November (0.48 ± 0.03 L/half; $P < 0.01$). Therefore, sub-clinical mastitis contributes to elevated SCC levels through out lactation. However, elevated SSC in uninfected halves late in lactation may also influence the ability to produce and market grade A milk.

**Key Words:** milk, bacteriology, quality

**W45 Biodiversity of enterococci in Egyptian dairy products.** S. Awad*[1], C. Snauwaert[2,3], P. Vandamme[3], A. El Attar[1], and M. El Soda[1], [1]*Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University*, *Alexandria, Egypt*, [2]*BCCM/LMG Bacteria Collection, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium*, [3]*Laboratory of Microbiology, Ghent University*, *K.L. Ledeganckstraat 35, 9000 Ghent, Belgium*.

Egyptian dairy products are generally produced under artisan conditions from raw milk without using industrial starter cultures. The main traditional cheeses are Ras (hard type), Domiati (soft type), and Mish; and the main fermented milk products are Zabady and Laban Rayeb. The quality of these products is strictly dependent on the microbial associations responsible for the fermentation, and the biodiversity of lactic acid bacteria. Enterococci are ubiquitous bacteria present in the environment and in the gastrointestinal tract of healthy animals and humans. To study the biodiversity of enterococci strains of the Egyptian Dairy products, 364 samples (raw & fermented milk and cheeses) were collected from farm houses, traditional cheese making factories and local markets in the Delta region, Egypt, and enterococci were isolated. All isolates were tested for their Gram reaction, catalase activity, and morphology. API and SDS-PAGE were used for identification of 784 isolates. Rep-PCR fingerprinting technique with the (GTG)5 primer and, in some cases pheS and 16S rRNA genes sequencing were also used to confirm the identification. These results confirm the importance of using molecular methods for exhaustive and precise identification of the microbial flora occurring in artisanal cheeses.

**Key Words:** Egyptian dairy products, (GTG)5-PCR, lactic acid bacteria

**W46 Identification, characterization, and differentiation of bifidobacteria obtained from Ukraine.** L. Tmanova*, A. Onyenwoke, and R. F. Roberts, *The Pennsylvania State University*, *University Park*.

Speciation of bifidobacterial isolates using traditional biochemical and phenotypic methods is tedious and often provides inconclusive results. DNA-based methods often yields clearer results. Ten freeze-dried bifidobacterial strains used as probiotics in Ukraine and identified by the supplier as *Bifidobacterium adolescentis* (2), *Bifidobacterium bifidum* (2), *Bifidobacterium longum* (4), *Bifidobacterium animalis* (1), and *Bifidobacterium infantis* (1) were characterized using polymerase chain reaction (PCR), pulsed field gel electrophoresis (PFGE) and allelic profiling. After anaerobic growth on MRS-cysteine (MRS) at 37°C for 72 h, single colony isolates were picked and evaluated using PCR primers specific for the genus, relevant species and for *B. animalis* ssp. *lactis*. All 10 isolates were identified as members of the genus *Bifidobacterium*. However, species-specific PCR revealed all 10 isolates were *B. animalis* ssp. *lactis*. Further evaluation using PFGE to assess strain relatedness showed all 10 isolates gave PFGE patterns identical to the type strain DSMZ 10140$^T$ when digested with *Spe*I. When digested with *Xba*I, 9 of the isolates gave patterns identical to DSMZ 10140$^T$. One strain, RT09, had one extra band when digested with *Xba*I. Allelic profiling of the Ukrainian bifidobacterial strains, revealed 4 distinct groups.

Interestingly, 6 (60%) of the isolates fell into the same cluster as that containing the common commercial probiotic strain BB-12. Our results demonstrate the conventional phenotypic methods used to characterize these isolates were sufficient to assign the correct genus, but not the correct species. These findings highlight the importance of employing molecular methods when typing bifidobacterial isolates.

**Key Words:** *Bifidobacterium animalis* ssp. *lactis*, allelic profiling, probiotics

**W47 Buffering capacity affects starter bacteria in nonfat probiotic yogurt.** M. Michael, R. K. Phebus, and K. A. Schmidt*, *Kansas State University*, *Manhattan*.

Sodium acetate has been reported to increase acid production and growth yield of some lactic acid bacteria in the growth medium predominately due to its buffering capacity. A buffering agent in yogurt mix may counteract the lethal effect of acid accumulation on starter and probiotic bacteria resulting in greater microbial counts at the end of fermentation. The objective of this study was to determine if changes in yogurt mix's buffering capacity could enhance starter and/or probiotic counts at the end of fermentation. Four nonfat yogurt mixes were prepared with 0.25% sodium acetate (SA) and 4 with no supplement (NS). Mixes were inoculated with yogurt starter alone or yogurt starter and *B. animalis*, *L. acidophilus* or both probiotics, and fermented in a bioreactor at 40°C until pH 4.50. Buffering curves of mixes were generated and pH, titratable acidity (TA) and microbial enumeration were done on an hourly basis during fermentation. Two replications were done and differences in means were determined using LSD at $\alpha = 0.05$ with SAS. Results showed that SA-supplemented mixes had greater buffering capacity at pH <6.00 than that of NS mixes. At the end of fermentation, SA supplementation resulted in greater S. thermophilus counts (~4 to 5%) in yogurts fermented with starter and *B. animalis* or *L. acidophilus*, and greater *L. bulgaricus* counts (~6%) in yogurts fermented with starter and *B. animalis* as compared with NS yogurts. *B. animalis* growth was not affected by the supplementations or fermentation bacteria. No significant differences in *L. acidophilus* counts were observed at the end of fermentation based on supplementation or fermentation bacteria. At pH 4.50, SA-supplemented yogurts had greater TA compared with NS yogurts. In general, fermentation time was longer for SA-supplemented mixes or mixes fermented with *L. acidophilus*. These results suggest that SA supplementation increased the buffering capacity of yogurt mix which improved the starter bacteria counts at the end of fermentation; however, further research should address if SA supplementation of yogurt mix could improve the viability of starter and/or probiotic bacteria in yogurt during storage.

**Key Words:** yogurt, probiotics, buffering capacity

**W48 Identification of lactic acid bacteria in Taiwanese ropy fermented milk and evaluation of their microbial ecology in different milk.** K. N. Chen[1], S. Y. Wang[2], and M. J. Chen*[2], [1]*Tungnan University*, *Taipei, Taiwan*, [2]*National Taiwan University*, *Taipei, Taiwan*.

Taiwanese ropy fermented milk (TRFM) is a domestic fermented milk. Its real original is unknown, but it has spread from family to family in northern Taiwan. TRFM has a stringy texture, a good diacetyl flavor and a pleasant taste. The purpose of this study was to identify species of lactic acid bacteria (LAB) in TRFM and to study their microbial dynamics during the fermentation process. The effects of different type of milk on the microbial ecological profiles were also investigated in this study. Ten grams of TRFM starters were homogenized in a laboratory blender. Concentrations of the viable LAB and yeasts in suspensions were obtained by serial plating dilutions. A combination of conventional microbiological cultivation, polymerase chain reaction-denaturing gradient gel electrophoresis and DNA sequencing was used to identify microorganisms and study their microbial dynamics. Identification results indicated that *Lactococcus lactis* ssp. *cremoris* and *Leuconostoc mesenteroides* ssp. *mesenteroides* were the major LAB in TRSM. Interestingly, three groups were identified as *Lactococcus lactis* ssp. *cremoris* using r16S DNA sequencing, but they showed different DGGE patterns and assimilation of carbohydrates. In addition, the microbial dynamics study in different fermentation stages demonstrated that *Lc lactis* ssp. *cremoris* was the most dominant bacterial species in the samples, followed by *Leu. mesenteroides* ssp. *mesenteroides* with no differences among the fermentation stages. Finally, the microbial distribution profiles showed that the microbial ecology was different in bovine, caprine and reconstituted milk, which might further affect the characteristics of the product. In this study, we demonstrated that *Lc lactis* ssp. *cremoris* and *Leu. mesenteroides* ssp. *mesenteroides* were the major LAB in Taiwanese ropy fermented milk. The percentages of the prevalent LAB populations present at different stages during the sample fermentation were similar. we also showed that the type of milk had a great influence on the microbial ecology.

**Key Words:** ropy fermented milk, microbial ecology, DGGE

**W49 Summary of a 2-year study involving screening, characterization, and environmental scanning of bacteria with the potential to produce ropy milk in a farm.** A. Laubscher*[1], H. Guo[1], K. White[1], B. Rossi Paneto[1], A. Cano[1], R. Cano[2], and R. Jiménez-Flores[1], [1]*Dairy Products Technology Center, California Polytechnic State University*, *San Luis Obispo,* [2]*Biological Sciences Department, California Polytechnic State University*, *San Luis Obispo*.

Prevention of microbial contamination in raw milk is an important objective in farms where value added is tied with quality. Recent reports of ropy milk have made us aware again of the problem. Ropy milk is characterized by its viscosity and tendency to form a slimy thread. The viscous character of the milk is produced by a complex oligosaccharide present in the capsule of different microorganisms. Over 250 raw milk samples were received from plants throughout the southern states. Of the 5 types of colonies observed, only one appears to have the "ropy" characteristic of mucoid. Isolated mucoid strains were tested for ropy milk production by inoculation in UHT milk and incubation at 25°C for 36 h. The "ropy test" proved successful with 100% reproducibility, but it was discovered that not all mucoid isolates produce a positive RM test. Using API biochemical identification tests, over 80% of the 160 positive "ropy tests" are in the *Klebsiella* species. This result gives us the belief that the presence of ropy milk can be correlated to coliform counts. The objectives were to identify high-risk areas for contamination of the responsible bacteria, the correlation with coliform and *Escherichia coli* (E/C) counts, and development of a subjective or quantitative method to evaluate the risk of finding ropy milk producing bacteria. Ten locations were examined on the Cal Poly Dairy Farm, with bedding having the highest E/C counts and the most probable source for ropy-causing bacteria contamination on the farm. The threshold for the enumeration of ropy-causing bacteria was determined to be only 2.5 CFU/10 mL in a sterile milk sample was enough to turn the milk ropy. With tests performed in triplicate, the threshold of the ropy-causing bacteria is much higher in the presence of typical raw milk microorganisms, suggesting a poor competitive nature. Our results indicate that "ropyness" is a result of a presumptive *Klebsiella oxytoca/pneumoniae* (coliform) a poor-competing bacteria and ubiquitous under poor sanitary conditions, in particular those associated with biofilm formation.

**Key Words:** milk quality, *Klebsiella*, ropy milk

**W50 Screening of *Lactobacillus casei* strains for the application of yogurt starter and probiotics.** J. K. Choi*, J. H. Im, and G. B. Kim, *Department of Animal Science & Technology, Chung-Ang University*, *Anseong 456-756, South Korea*.

*Lactobacillus casei* is one of the most important probiotics, and it is widely used in functional foods and dairy products. As a trial for the development of a new starter culture, more than 200 lactic acid bacteria strains were isolated from raw milk and healthy human feces. The strains showing excellent growth and acid production ability in the 10% skim milk media were selected and identified as *Lactobacillus casei* by the result of API carbohydrate fermentation pattern and 16S rDNA sequence analysis. Among the selected strains, *L. casei* CU2604 and CU3204 were further investigated for their physiological characteristics as a starter culture comparing them with a commercial strain, *L. casei* Shirota. Both CU3204 and CU2604 strains showed good acid production and growth characteristics in milk, which are comparable with those of *L. casei* Shirota strain. However, *L. casei* CU2604 was the only selected strain that had a similar sugar fermenting pattern and PFGE band pattern compared with *L. casei* Shirota. Furthermore, CU2604 showed better tolerance to bile and to pH than *L. casei* Shirota. In addition, to assess the effect of *L. casei* strains in irritable bowel syndrome (IBS), the inhibitory effect of the selected strains against nitric oxide (NO) production of lipopolysaccharide (LPS)-stimulated RAW 264.7 cells was measured. Among the tested *L. casei* strains, *L. casei* MCL was observed to have the greatest NO inhibitory activity. Based on these results from this study, we anticipate the possible use of *L. casei* MCL strain as a new probiotic and CU2604 as a new starter culture.

**Key Words:** *Lactobacillus casei*, yogurt starter, probiotics

**W51 Effect of yogurt consumption on the human intestinal microbiota.** H. J. Kim*[1], S. J. Eom[1], Y. T. Ahn[2], J. H. Lee[2], C. S. Huh[2], and G. B. Kim[1], [1]*Department of Animal Science and Technology, Chung-Ang University*, *Anseong 456-756, South Korea*, [2]*Research and Development Center. Korea Yakult Co. Ltd.*, *Yongin 449-901, South Korea*.

In this study we investigated the effect of consumption of yogurt on the fecal microflora of 40 healthy volunteers. The subjects were randomly divided into experimental (n = 20) and control (n = 20) groups for a double-blind placebo-controlled clinical study. The experimental group consumed yogurt 2 times a day (150mL each) for 3 weeks and the control groups consumed the same amount of milk acidified with lactic acid. Fecal samples at defined time points before, during, and after the period of yogurt ingestion were collected and analyzed. The fecal population of lactobacilli and bifidobacteria was determined by culture-based methods using Rorosa SL agar and TOS-propionate agar media, respectively, and subsequent colony PCR for the confirmation of the target Genus. The comprehensive dynamics of intestinal microbiota in response to yogurt consumption was analyzed using a bacterial barcoded pyrosequencing.

The population of bifidobacteria in the fecal sample of experimental group increased from 9.65±0.56 to 10.20±0.41 ($log_{10}$ cells/g wet feces, mean ± SD) during the ingestion of yogurt, but it tended to be normalized (9.67±0.84) when they stopped the ingestion. The similar tendency was observed in the population of lactobacilli during the experiment; however, there were no significant changes in the control group. Molecular analysis of the human fecal bacterial populations by pyrosequencing of 16S rRNA tags revealed that yogurt consumption induced significant alterations of the gut microbiota. The ratio of *Firmicutes* to *Bacteroidetes* was 1.23, 0.59, and 2.01 at the time points before, during, after the period of yogurt ingestion, respectively. This study provides evidence that the gut microbiota could be modulated by dietary intenvention such as yogurt ingestion and further studies should be done to better understand the modulation of the gut microbiota associated with health attributes in well-defined human clinical studies.

**Key Words:** yogurt, pyrosequencing, microbiota

**W52 The effect of fermented yogurt on the prevention and treatment of diarrhea in animal models.** J. H. Im*[1], J. K. Choi[1], M. H. Lee[2], J. H. Sim[2], C. S. Huh[2], and G. B. Kim[1], [1]*Department of Animal Science & Technology Chung-Ang University*, *Anseong 456-756, South Korea*, [2]*Research and Development Center. Korea Yakult Co., LTD.*, *Yongin 449-901, South Korea*.

Constipation is the most prevalent health condition in the world. Common symptoms are difficult stool passage, infrequent stools, or both. Dairy products such as milk and yogurt improve intestinal function. The objective of this study was to investigate the improvement of intestinal function, the prevention of constipation, and the curative value of supplying fermented milk products to mice and rats. Constipation was induced by oral administration of loperamide and experiments were executed for 5 consecutive days. All experiments separated research subjects into 3 groups; the control, the treatment of loperamide alone, and the treatment of low, medium, and high levels fermented milk. The results showed that the effects varied significantly among different levels of doses in that: 1) The digestive tract transfer rate showed that the control 44.2%, low levels 51.7%, medium levels 67.4%, high levels 67.7%; 2) Constipation preventive effect showed that the water and food intake, and the amount and number of feces decreased significantly with loperamide alone, low, medium levels ($P < 0.001$, $P < 0.05$, $P < 0.01$). However the treatment of loperamide with fermented milk increased the number of feces significantly at high level ($P < 0.05$), amount of feces increased significantly at low, high levels ($P < 0.05$, $P < 0001$), also water contents increased. 3) Effective treatment of constipation showed that the water and food intake decreased significantly; the number of feces increased significantly at medium level ($P < 0.001$), weight of feces showed similar results. Water contents increased significantly at medium, high levels ($P < 0.05$, $P < 0.001$). These results suggest that the repetitive ingestion of fermented yogurt is effective to prevent and treat constipation in animal models.

**Key Words:** animal trials, fermented yogurt, constipation

**W53 Effect of milk fermented by *Lactobacillus rhamnosus* on an experimental infection with *Salmonella enterica* ssp. *enterica* serov. Typhimurium in gnotobiotic and conventional mice.** A. H. Mendonça[1], M. M. O. P. Cerqueira*[2], J. R. Nicoli[2], M. O. Leite[2], M. R. Souza[2], L. M. Fonseca[2], R. M. N. Drummond[2], R. M. E. Arante[2], and C. F. A. M. Penna[2], [1]*Ministry of Agriculture*, *Brasília, Distrito Federal, Brasil*, [2]*Federal University of Minas Gerais*, *Belo Horizonte, Minas Gerais, Brasil*.

An experimental infection with *Salmonella enterica* ssp. *enterica* serov. Typhimurium was evaluated in gnotobiotic (GN) and conventional (CV) mice previously treated or not with milk fermented by a *Lactobacillus rhamnosus* strain isolated from healthy human newborn. Conventional mice received 0.1 mL probiotic milk (8.0 log CFU) daily, 10 d before the oral pathogenic challenge (5.0 log CFU). Then, probiotic treatment was continued until the end of the experiment. Probiotic treatment in germ-free mice consisted of a single dose at the beginning of the experiment

and a challenge 10 d later (3.0 CFU). Protective effect was observed in both GN and CV animals in terms of histopathology and morphometric data but in different anatomical site. This protection was observed in liver and intestines, respectively for GN and CV mice. However, *Salmonella enterica* ssp. *enterica* serov. Typhimurium populations were similar in the feces of both treated and control GN mice. Concluding, a protective effect by *L. rhamnosus* against experimental *Salmonella enterica* ssp. *enterica* serov. Typhimurium was observed. This protection was not due to the reduction of the population of pathogens in the intestine.

**Key Words:** fermented milk, *Lactobacillus rhamnosus*, probiotic

**W54 Influence of bovine and caprine caseinomacropeptide on the viability of *E. coli* and *L. rhamnosus* in acidic conditions.** G. Robitaille*, C. Lapointe, D. Leclerc, and M. Britten, *Food Research and Development Centre, Agriculture and Agri-Food Canada*, *St-Hyacinthe, QC, Canada*.

Caseinomacropeptide (CMP) is a 7-kDa phosphoglycopeptide fragment released from κ-casein during chymosin-induced renneting of milk. Bovine CMP differs from caprine CMP by 19 substitutions, 2 insertions, by glycosylation extent and the level of phosphorylation. The objective of the study was to analyze the effects of pepsin-treated CMP (CMPpt) on the survival of *E. coli* and *L. rhamnosus* in acidic conditions. To induce acid shock, bacterial cells were incubated at 37°C in PBS with or without additive (CMPpt, non-glycosylated CMPpt (aCMPpt) and glycosylated CMPpt (gCMPpt) isoforms) from bovine and caprine. Viability (CFU/mL) was determined at 0, 15, 30, 60 and 90 min. As expected, *E. coli* was sensitive to low pH. The bacterial viability decreased by more than 1.5 log within 15 min at pH ≤3.0. At pH 3.5, it took about 90 min to reach a similar decrease. When added to the media, CMPpt was bactericidal in a dose dependent manner, reducing survival by more than 90% within 15 min at ≥0.25 mg/ml. This indicates that the encrypted bioactive peptides within CMP are released by pepsin proteolysis and are bactericidal against *E. coli* at low pH. Moreover, the effectiveness of CMPpt to kill *E. coli* at pH 3.5 was not significantly affected by the presence of linked oligosaccharides or by the origin of the milk. This suggests that the active peptide is located in the N-terminal portion of the polypeptide, a region of high homology between species that does not carry any phosphate or oligosaccharide. Survival of *L. rhamnosus* at pH 2.9 decreased to about 5% within 60 min. Supplementation with bovine or caprine CMPpt has an inverse effect. It increased viability to values as high as 50%, with similar efficiencies for aCMPpt and gCMPpt. These results suggest that peptic digests of bovine and caprine CMP may act as antimicrobial agents against *E. coli* in a gastric context without any deleterious effect on the resistance of a probiotic to gastric pH.

**Key Words:** caseinomacropeptide, bacterial growth, gastric pH

**W55 Screening of β-galactosidase-containing probiotic for the production of galacto-oligosaccharides and its optimal preparation conditions.** Y. Gao, X. Mi, L. Feng, R. Zhong, B. Qian, and S. Zhang*, *Department of Food Science and Technology, School of Agriculture and Biology, Shanghai Jiao Tong University*, *Shanghai, China*.

As β-galactosidase carriers, whole probiotic cells were screened out for the production of galacto-oligosaccharides (GOS) and the optimal production conditions were investigated in this study. In this process, probiotics including 2 strains of *Bifidobacterium bifidum*, 2 strains of *Lactobacillus helveticus* and one strain of *Lactobacillus delbrueckii* ssp. *bulgaricus* were employed. A high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was utilized for the sugar composition analyses in the prepared products. Results showed: top 3 GOS production prebiotics were *Bifidobacterium bifidum* BB03, *Bifidobacterium bifidum* BB02 and *Lactobacillus delbrueckii* ssp. *bulgaricus* with the production of GOS at 201.8 g/L100g, 160.1 g/L and 129.4 g/L, respectively. The optimal production conditions of *Bifidobacterium bifidum* BB03 were as follows: 0.20 g freeze-dried cells were transferred into a flask with 50 mL of lactose aqueous solution (473.4 g/L), shaken at the speed of 150 rpm and cultured at 37 centigrade for 8h. The fractions of GOS in this product were proved to be with 197.7 g/L dimer GOS and 4.1 g/L tetramer GOS. In addition, the production of galactose, glucose and the residual lactose in the product were presented to be 1.9 g/L, 94.3 g/L and 1.3 g/L, respectively.

**Key Words:** galacto-oligosaccharides, probiotic, HPAEC-PAD

**W56 Characterization and partial purification of antimutagenic peptide produced by *Lactobacillus plantarum* CNU 2116.** J. W. Jeong*[1], B. H. Yoon[1], D. J. Park[3], Y.-S. Son[2], and S. Oh[1], [1]*Division of Animal Science, Chonnam National University*, *Gwangju, South Korea*, [2]*Division of Bioscience & Technology, Korea University*, *Seoul, South Korea*, [3]*Korea Food Research Institute*, *Gyeonggi-do, South Korea*.

Intestinal lactic acid bacteria (LAB) are closely associated to the host's health because the presence of LAB are an important bio-defense factor in preventing colonization and subsequent proliferation of pathogenic bacteria in the intestine. Some probiotics such as *Lactobacillus* species can intoxicate the carcinogens including chemical mutagens. The antimutagenic activity of 24 LAB strains was investigated using 3 mutagens (4-nitroquinoline-N'-oxide, 4-NQO; N-methyl-N'nitro-N-nitrosoguanidine, MNNG; and 2-amino-3- methylimidasol quinoline, IQ). In the Ames test, dose-dependent activity was exhibited significantly against 4NQO, MNNG, and IQ. *Lactobacillus casei* KCTC 13086 and *L. plantarum* strains showed the highest anti-4NQO activity (62.1%) among the tested strains of LAB. The active substance was found to be sensitive to trypsin (71500 units/mL). This indicates that antimutagenic substance is proteinaceous in nature. The molecular weight of the antimutagenic peptide was estimated as an approximately 762 Da using tricine-SDS-PAGE. N-terminal amino acid residue sequence from the purified peptide was identified as $NH_2$ -Xaa-Leu-Glu-Xaa-Lys-Lys-Ala-Glu-Xaa-Ile-Thr-Thr. Compared with other sequences in the NCBI database using Blast program, we found no significant sequence similarity to previously reported antimutagenic peptides.

**Key Words:** antimutagenic activity, lactic acid bacteria, characterization

**W57 Characterization of microorganisms isolated from biofilms formed on whey reverse osmosis membranes.** A. C. Biswas*, M. Avadhanula, S. Anand, and A. Hassan, *Midwest Dairy Foods Research Center, Dairy Science Department, South Dakota State University*, *Brookings*.

Microbial biofilm is a complex structure made up of organisms embedded in polymeric matrices of biological origin attached to a substratum. The present investigation was undertaken to characterize the multispecies microbial consortia isolated from reverse osmosis (RO) membranes drawn from active industrial whey filtration process at 2, 4, 6, 8, 10, 12, and 14 mo of age. The ability of the different bacterial isolates to produce capsule and slime, and increase whey viscosity as indications of exopolysaccharides (EPS) production was also studied. Results showed that *Bacillus* sp. was present on almost all RO membranes from 4 to 12 mo. *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Aeromonas*, *Corynebacterium*, and *Pseudomonas* species were also

encountered frequently. *Escherichia coli* and *Klebsiella oxytoca* were detected only on 8, 12 and 14 mo old membranes. Among all isolates, about 25% did not produce capsules or slime, or increased viscosity of whey, which indicated lack of EPS production. This reflects that strains not producing EPS can also be involved in biofilm formation. However, since the EPS production was only tested in planktonic cells, there is still a possibility that they produce EPS in biofilm matrices. Interestingly, the EPS production was more pronounced in isolates from older (8 to 14 mo old) as compared with newer (2 to 6 mo old) membranes. Studies also confirmed the ability of individual isolates to form biofilms under static in vitro conditions. Predominance of *Escherichia coli* and *Bacillus sp*. was observed during the reproduction of mixed species biofilms. The study provides useful information on the predominant bacterial species in biofilms formed on whey processing membranes. This will help in developing more effective cleaning and sanitation regimens.

**Key Words:** biofilm, exopolysaccharides (EPS), RO membrane

**W58 Transcriptional analysis of a very broad spectrum lantibiotic produced by *Bifidobacterium longum* DJO10A.** J. H. Lee*, X. Li, and D. J. O'Sullivan, *University of Minnesota*, *St Paul*.

Lantibiotics are bacteriocins produced by some gram-positive bacteria with a very broad antimicrobial spectrum. A reverse genomic analysis of *B. longum* ssp. *longum* DJO10A revealed the potential to produce a lantibiotic and subsequent analysis revealed it produced a small (<5 kDa) peptide, only on agar media, with an antimicrobial spectrum that included gram-negative bacteria. To further understand the regulation for production of this lantibiotic a full-genome microarray analysis with total RNA from both agar and broth cultures was conducted. This revealed a very different expression pattern between agar and broth cultures, including the lantibiotic-producing genes which were expressed much higher in agar. Interestingly, the expression of the 2-component regulatory genes in this operon, were relatively constant in both conditions, suggesting it should be functional in broth cultures. To further investigate this, a real-time PCR procedure, with a specific TaqMan probe targeting the lanA gene, was developed. This confirmed the broth and agar differential expression of *lanA* in different media, with MRS agar showing the highest gene expression. To obtain some crude lantibiotic compound, it was extracted from MRS agar using 95% methanol and partially purified via size fractionation with filtration systems. Different concentrations of this partially purified lantibiotic were used to investigate the expression of *lanA* in broth cultures. Strikingly, *lanA* gene expression was drastically increased in a dose dependant fashion in broth cultures, confirming that increasing the external signal in broth cultures allows the expression of the lantibiotic production genes, thus facilitating lantibiotic production in broth.

**Key Words:** bifidobacteria, lantibiotic, microarray

**W59 Comparison of the Baird-Parker agar, Baird-Parker-RPF and Petrifilm Staph Express in the detection and enumeration of *Staphylococcus* coagulase positive in raw milk.** A. K. R. Santos, M. O. Leite*, L. M. Fonseca, M. O. P. Cerqueira, M. R. Souza, C. F. A. M. Penna, and M. R. A. Moura, *Federal University of Minas Gerais*, *Belo Horizonte, Minas Gerais, Brasil*.

The reference methodology for *Staphylococcus* spp. enumeration recommends the use of Baird-Parker agar (BP); however, other culture media may produce results in shorter time, as Baird-Parker - RPF agar (bioMérieux, Marcy l'Etoile, France) and Petrifilm™ Staph Express Count Plate - PSE - (3M Microbiology Products, St. Paul, USA). Thus, this work was carried out to compare the efficiency of the forecited culture media for the enumeration of *Staphylococcus* spp. in 36 samples of raw milk. The experiment was designed in random blocks and the media were compared by the *t*-test. Mean *Staphylococcus* spp. count obtained by PSE (2.50 Log10 CFU/mL) was lower ($P < 0.05$) than those by BP (4.12 Log10 CFU/mL) and RPF (3.86 Log10 CFU/mL), being the latter 2 values considered similar ($P > 0.05$). The results showed viable the use of RPF, but not PSE, replacing BP for *Staphylococcus* spp. enumeration without altering the accuracy of the analysis.

**Key Words:** *Staphylococcus*, culture media, raw milk

**W60 Influence of low pressure homogenization on growth of *Streptococcus thermophilus*.** T. Muramalla[1] and K. Aryana*[1,2], [1]*Louisiana State University*, *Baton Rouge,* [2]*Louisiana State University Agricultural Center*, *Baton Rouge*.

The objective was to study the effect of low pressure homogenization on growth of *Streptococcus thermophilus*. Fat free milk was sterilized by autoclaving, chilled to 4°C, inoculated with *Streptococcus thermophilus* and homogenized at 0, 3.45, 6.90, 10.35, 13.80 MPa for 5 continuous passes. The control (0 MPa) and homogenized (3.45, 6.90, 10.35, 13.80 MPa) samples were individually inoculated in Lactobacilli MRS broth. Samples were plated in duplicate at 0, 2, 3, 4, 5, 6, 8, and 10 h using the pour plate technique. During 10 h incubation period the broth samples were kept at 37°C and plates were incubated aerobically at 37°C for 3 d. Entire experiments were replicated 3 times. Data were analyzed using proc mixed of SAS. The interaction effect of pressure x time was not significant. Both main effects pressure and time were significant ($P < 0.0001$). The homogenized samples (6.90, 10.35, 13.80 MPa) were not significantly ($P < 0.05$) different from each other but exhibited counts significantly ($P < 0.05$) higher than control (0 MPa). The homogenized samples treated to pressures of 6.90, 10.35, 13.80 MPa exhibited average counts of 11.84, 11.85, 11.83 log CFU/ml and control (0 MPa) had an average count of 11.70 log CFU/ml. The low pressure homogenization at 6.90, 10.35, 13.80 MPa had a slight yet positive significant effect on growth of *Streptococcus thermophilus*.

**Key Words:** dairy, culture, bacteria

**W61 Influence of mild pulsed electric field conditions on the growth of *Streptococcus thermophilus*.** N. Najim[1] and K. Aryana*[1,2], [1]*Louisiana State University Agricultural Center*, *Baton Rouge,* [2]*Louisiana State University*, *Baton Rouge*.

Pulsed electric field (PEF) processing involves the application of pulses of voltage for less than one second to fluid products placed between 2 electrodes. *Streptococcus thermophilus* is an important bacterium used for the production of fermented dairy products. Objective of this study was to determine the influence of a mild PEF condition on the growth of *Streptococcus thermophilus*. A range of mild pulsed electric field conditions were earlier screened by the authors to arrive at an optimum overall mild pulsed electric field condition for various probiotic characteristics. Freshly thawed *Streptococcus thermophilus* was suspended in 0.1% w/v sterile peptone water and treated in a pilot plant PEF system. The treatment was a mild PEF condition of positive square unipolar pulse of 3 µs, pulse period of 0.5 s and voltage of 1kv/cm. Control was run through PEF system but without receiving any pulsed electric field condition. Control and treated sample flow rates were kept constant at 60 mL/min. Samples were individually inoculated in lactobacilli MRS broth. Samples were plated in duplicate. Pour plates were incubated aerobically at 37°C for 3 d. Growth was determined hourly for 20 h. Experiments were replicated 3 times. The control and mild PEF treated samples had the same counts of 10.97 (+/− 0.25) log cfu/mL at 0 h. The

mild PEF treated samples reached the log phase an hour earlier than control. Although at most time points, counts were within the same log cfu/mL for the control and treated samples, the mild PEF treated samples had significantly ($P < 0.05$) higher counts compared with control for most of the time points over the 20 h of growth. The mild PEF condition enhanced growth of *Streptococcus thermophilus*.

**Key Words:** pulsed electric field, nonthermal, culture

**W62 Effect of mild sonication on the growth of *Streptococcus thermophilus*.** M. Moncada*[1,2] and K. Aryana[1,2], [1]*Louisiana State University Agricultural Center*, *Baton Rouge,* [2]*Louisiana State University*, *Baton Rouge*.

Mild sonication (20–40% amplitude) conditions have been reported to increase the transport of small molecules in solution enabling bacterial cell growth. *Streptococcus thermophilus* is an important lactic acid bacterium used for the production of some fermented dairy products. The objective was to study the influence of mild sonication on the growth characteristics of *Streptococcus thermophilus*. Freshly thawed culture was suspended in 0.1% peptone water and 18 mL of sample was sonicated using horn (diameter 13 mm) set at a maximum acoustic power output of 750 W, frequency 24 kHz. The treatments were amplitudes of 21, 27, 33 and 39%. In all treatments energy input into the system was kept constant at 1500 J. The control was sample not treated with mild sonication. Samples were inoculated in lactobacilli MRS broth. Samples were pour plated in duplicate. During 12 h incubation period the samples were kept at 37°C. Pour plates were incubated aerobically at 37°C for 3 d. Growth of control and treated samples were determined hourly over 12 h. Three replications were conducted. Data were analyzed using the PROC GLM of the Statistical Analysis Systems (SAS). The control and samples treated with 21% amplitude exhibited average counts of 14.55 and 15.01 log CFU/mL respectively at the end of the 12-h incubation. Similar increases in counts were observed in samples treated with 33% amplitude compared with control in which average counts at the end of the 12 h were 14.58 and 14.26 log CFU/mL respectively. With an increase in amplitude of mild sonication the counts declined but maintained higher than the control. It is concluded that the lowest sonication intensity studied (amplitude of 21%) had the best effect on increasing *Streptococcus thermophilus* counts.

**Key Words:** sonication, culture, growth

**W63 Low pressure homogenization effects on bile tolerance of *Streptococcus thermophilus*.** T. Muramalla*[1] and K. Aryana[1,2], [1]*Louisiana State University*, *Baton Rouge,* [2]*Louisiana State University Agricultural Center*, *Baton Rouge*.

The goal was to determine the influence of low pressure homogenization on bile tolerance of *Streptococcus thermophilus*. Fat free milk was autoclaved, chilled to 4°C, inoculated with *Streptococcus thermophilus* and homogenized at 0, 3.45, 6.90, 10.35, 13.80 MPa for 5 continuous passes. The control (0 MPa) and homogenized (3.45, 6.90, 10.35, 13.80 MPa) samples were individually inoculated in Lactobacilli MRS broth with oxgall bile. Samples were plated in duplicate at 0, 2, 3, 4, 5, 6, 8, and 10 h. During 10 h incubation period the broth samples were kept at 37°C. Pour plates were incubated aerobically at 37°C for 3 d. Entire experiments were replicated 3 times. Data were analyzed using proc mixed of SAS. The interaction effect of pressure × time was not significant while both the main effects pressure and time were significant ($P < 0.0001$). The homogenized samples (3.45, 6.90, 13.80 MPa) were not significantly ($P < 0.05$) different from each other but exhibited counts that were significantly ($P < 0.05$) higher than control (0 MPa). The homogenized samples treated to pressures of 3.45, 6.90, 13.80 MPa exhibited average counts of 10.72, 10.79, 10.81 log CFU/mL compared with control (0 MPa) which had an average count of 10.60 log CFU/mL. The low pressure homogenization at 3.45, 6.90, 13.80 MPa had a slight yet positive significant effect on bile tolerance of *Streptococcus thermophilus*.

**Key Words:** culture, homogenization, bacteria

**W64 Acoustical emissions generated by bacteriophages sk1 and ml3 using *Lactococcus lactis* ssp. *lactis* C2 host.** A. K. Wardani[1], C. L. Hicks*[2], and J. M. Stencel[3], [1]*University of Brawijaya*, *Malang, Indonesia*, [2]*University of Kentucky*, *Lexington,* [3]*Tribo Flo Separations*, *Lexington, KY*.

*Lactococcus lactis* ssp. *lactis* C2 bacteria in M17 medium, at 26°C for 8 h were infected with phages sk1 or ml3, and monitored using contact piezoelectric sensors attached to the sides of the growth vessel. The 2 sensors (5 to 50 kHz range) had individual characteristic and internal amplification mechanisms that were calibrated and adjusted to minimize background noise. After the sensors had been calibrated, the M17 medium was inoculated with *L. lactis* ssp. *lactis* C2 culture ($1 \times 10^9$ cfu/mL), stirred for 1 min, and allowed to grow for approx. 90 min before infection (stirred for 1 min) with phages sk1 or ml3. Infection time was set to correspond with the start of the log growth phase. Infection level was $10^5$ pfu/mL for both phages sk1 and ml3. Sound intensity from the growth chambers was measured in attojoules (aJ = $10^{-18}$ Joules) and plotted as the energy rate-per-detected acoustic wave. Acoustic peaks considered significant and beyond internal or external generated noise were those having greater than ± 3 times the sigma value of the general variation in acoustic intensity over the entire data set of each test. Energy rate data from control tests in which *L. lactis* ssp. *lactis* C2 was grown without phage sk1 or phage ml3 infections contained no acoustic peaks with intensities that exceeded the ± 3 sigma standard whereas phage sk1 or ml3 infected *L. lactis* ssp. *lactis* C2 culture contained multiple acoustic peaks with intensities that exceeded ± 3 sigma. The first peak for phage sk1 appeared at 33.2 ± 4.4 min whereas the first peak for phage ml3 appeared 40 min. Thus, the acoustic data from phage sk1 or phage ml3 infected *L. lactis* ssp. *lactis* C2 were considered to be the result of phage infection. The timings of the acoustic peaks from phage sk1 were sufficiently different from phage ml3, that these 2 phage could probably be distinguished by acoustic emission monitoring during phage infection of the bacteria.

**Key Words:** acoustic emission, bacteriophage, *Lactococcus*

**W65 Viability of bifidobacteria and lactobacilli in skim milk with shiitake mushroom extract during refrigerated storage.** O. Hassan*[1], O. S. Isikhuemhen[1], S. A. Ibrahim[1], A. AbuGhazaleh[2], and D. Song[1], [1]*North Carolina A &T State University*, *Greensboro,* [2]*Southern Illinois University*, *Carbondale*.

Probiotics, such as bifidobacteria and lactobacilli, have been demonstrated to help establish and support strong immune systems. A key growth source for these bacteria are certain carbohydrates, so food rich in these food components would presumably help probiotics to thrive. Shiitake mushroom (*Lentinus edodes*) contains antitumor oligosaccharides and polysaccharides which could enhance probiotics growth. The objective of this study was to exam the viability of selected probiotic cultures in skim milk in the presence of different levels of Shiitake mushroom extract during refrigerated storage. *Lactobacillus reuteri* CF2–7F, *L. reuteri* DMS 20016, *Bifidobacterium breve* and *B. adolescentis* were individually inoculated into skim milk supplemented with

different concentrations of mushroom extract (0%, 1%, 2%, and 4%) and stored immediately at 4°C for 4 weeks. Aliquots were withdrawn at one-week interval to determine bacterial population, pH and titratable acidity of the milk samples. Results showed that the viability of tested strains was significantly higher in milk supplemented with Shiitake mushroom extract ($P < 0.05$) compared with the control sample. All tested strains demonstrated culture stability upon refrigerated storage and exhibited no significant loss of viability during storage conditions for 2 weeks. After 4 weeks of storage, 2 log reduction of viable cells from an initial mean of 109/ml was observed. Samples had a mean initial pH of 6.5 and titratable acidity of 0.16. Both pH and titratable acidity showed negligible change at 4°C during 2-week storage. Our results suggest that shiitake mushroom extract can be used as a natural additive in dairy products to improve the viability of probiotics during refrigerated storage and to improve consumer health.

**Key Words:** bifidobacteria, lactobacilli, mushroom

**W66 Microbiological quality of dairy protein supplements sold in Saudi Arabia markets.** S. O. Aljaloud*[1], D. Song[2], A. M. Fraser[1], and S. A. Ibrahim[2], [1]*Clemson University*, *Clemson, SC*, [2]*North Carolina Agricultural and Technical State University*, *Greensboro*.

Whey proteins are becoming popular dietary supplements here in the US and around the world. However, these ingredients are typically not sterile. There is need to investigate the microbiological safety of these products. The objective of this study was to determine the microbiology quality of whey protein supplements sold in Saudi Arabia. Twenty different dairy protein supplements were purchased from local stores in Riyadh, the capital of Saudi Arabia. These products ranged from whey protein concentrate (5), whey protein isolate (4), why protein hydrolyzed (2), whey protein concentrate lactose free (3), whey protein concentrate mineral free (2) and casein isolates (4). Samples were analyzed for several microbial quality attributes including aerobic total plate count (ATPC), psychrotrophs (PC), *Enterobacteriaceae*, total coliforms, and fecal coliforms. The presence of selected pathogens such as *Staphylococcus aureus* and *Salmonella* were investigated. Our results showed that the average bacterial population for ATPC, PC and *Enterobacteriaceae*, were 4.1, 2.1, and 1.2 log cfu/mL, respectively. Coliform groups were found in 29% of samples while 10% were fecal coliform positive as revealed by the MPN method. *S. aureus* was located in at least 25% of samples, with a mean count of 2.1 log cfu/mL. Our results confirmed that there is potential health risk with the consumption of dairy protein supplements sold in Saudi Arabia. There is a need to develop a monitoring system to check the microbiological quality of dairy protein supplements on market to assure them safe to use.

**Key Words:** microbiological quality, dairy protein supplements

**W67 Antimicrobial activity and composition of oregano essential oils from different climate zones of Colombia.** L. Betancourt*[1,3], R. Patiño[2], V. Phandanauvong[2], C. Ariza-Nieto[2], and G. Afanador-Téllez[3], [1]*Universidad de La Salle*, *Bogotá, Colombia*, [2]*CORPOICA*, *Bogotá, Colombia*, [3]*Universidad Nacional de Colombia*, *Bogotá, Colombia*.

The antimicrobial activity of oregano essential oils has been showed in the literature; however, composition and antibacterial activity of oregano essential oils (OEO) from different climatic zones of Colombia have not been studied. The aim of this study was to characterize 4 chemotypes of OEO, *Origanum majorana* (OM), *Origanum vulgare* L (OVL), *Lippia origanoides* (LO) and *Origanum vulgare* H. (OVH, from Greece). Composition was analyzed by GC/Ms and its antibacterial activity through minimum inhibitory concentration (MIC) against *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Salmonella enteritidis*, *Salmonella typhimurium* and *Escherichia coli* by broth dilution method. OVH from Greece presented the highest carvacrol content (90%), while the highest thymol was found in LO from Colombia (79%). OM was rich in Sabinyl compound (24%). OVL presented high thymol content (21%) and low carvacrol (4%). The best carvarol: thymol ratio was for OVH (25) and LO present the most very low (0.01). The amount of lowest precursors (gamma terpinene+para-cymene) was for LO (9%), but varieties of oregano produced in greenhouse conditions at high altitude (Savannah of Bogotá, 2650 AMSL)) had highest precursors content, 17% for OM and 41% for OVL. Carvacrol, OVH and LO showed the same MIC values against *S. enteritidis* (0.098 mg/ml). Carvacrol had better MIC against both *S. typhimurium* (0.098 mg/mL) and *E. coli* (0.0061 mg/mL), followed by OVH and LO. The lowest MIC against beneficial bacteria was for OM (6.25 mg/mL) against *L. acidophilus* and LO (50 mg/mL) against *B. breve*. These results clearly showed that OEO rich in thymol could have a desirable antibacterial effect on gastrointestinal tract.

**Key Words:** *Lactobacillus*, *Bifidobacterium*, *Salmonella*