T131 *Lactobacillus plantarum* L67 suppresses allergic inflammation. Sooyeon Song*[1], Anna Jeong[1], Geun-Bae Kim[2], Dong-June Park[3], and Sejong Oh[1], *[1]Div. of Animal Science, Chonnam National University, Gwangju, South Korea, [2]Dept. Animal Science, Chung-Ang University, Anseong, South Korea, [3]Korea Food Research Institute, Seongnam, South Korea.*

Bisphenol A (BPA) is a widely used monomer of polycarbonate plastics and epoxide resin that has been implicated in allergy related disease. Allergy is an abnormal immune response to an allergen. Type I hypersensitivity is an immunoglobulin (Ig) E-mediated allergic disorder. This study investigated the inhibitory effect of the *Lactobacillus plantarum* L67 extract on BPA induced allergic inflammatory response in RBL-2H3 cells. RBL-2H3 cells were treated with bisphenol A (50 μ*M*/mL) and co-treated with the *L. plantarum* L67 extract (5–100 μg/mL) for 30 min. histamine releasing, β-hexosaminidase and intracellular Ca^{2+} level were estimated in the medium. IgE production and activities of PKC, iNOS were measured ELISA and Western blotting separately. Also IL-4 and TNF-α were measured by RTq-PCR. The histamine releasing of treatment with the *L. plantarum* L67 extract considerably decreased in a concentration dependent manner. When the cells were treated with the 100 μg/mL *L. plantarum* L67 extract, the values of histamine releasing decreased 0.48-fold compared with the BPA treatment alone. The addition of BPA caused a 1.43-fold increase in IgE levels compared with control, while the values of IgE amounts decreased by 1.31, 1.28, 1.14, 1.08 and 1.04 at addition *L. plantarum* L67 extract (5–100 μg/mL) in the presence of BPA, compared with that for the BPA treatment alone. Our results showed that *L. plantarum* L67 extract inhibited the histamine releasing, β-hexosaminidase, productions of IgE, the intracellular Ca^{2+} level, and activity of iNOS in BPA treated RBL-2H3 cells but also the results indicated that the *L. plantarum* L67 extract inhibits expression of cytokines related to allergy such as TNF-α and IL-4 on the degranulation stage of mast cell. The *L. plantarum* L67 extract can inhibit to occur allergy caused by environmental hormone such as BPA.

Key Words: bisphenol A, β-hexosaminidase, *Lactobacillus plantarum*

T132 Metal-chelating and ACE-inhibitory activity of a milk fermented with bacteria isolated from double cream cheese of Chiapas, Mexico. Claudia Y. Figueroa*, Gustavo F. Gutiérrez, and Humberto Hernández, *Escuela Nacional de Ciencias Biológicas-IPN, México City, México.*

During the fermentation of milk with lactic acid bacteria, biologically active sequences, known as bioactive peptides, can be produced. Among the most important biological activities attributed to these peptides are metal-chelating (mainly iron and calcium) and ACE-inhibitory activities. Three probiotic strains (*Lactobacillus plantarum*, *Lb. pentosus* and *Lb. acidipiscis*) were isolated from double cream cheese produced in Chiapas (Mexico). This study aims to assess the metal-chelating (iron and calcium) and ACE-inhibitory activity during the milk fermentation by each microorganism and by a mixed culture. Fermentations were performed using reconstituted skim milk powder. Milks were inoculated with 2% of each of the microorganisms or mixed culture. Fermentations were performed at 37°C for 48 h. Samples were taken every 8 h for measurement of microbial growth, protein concentration, degree of proteolysis (TNBS method), metal-chelating (calcium and iron) and ACE-inhibitory activity. Variations on free amino group concentration with respect to the initial concentration that could indicates proteolysis during fermentation were observed. The initial iron-chelating activity was high (68 ± 2%), and was attributed to the fact that some milk proteins have the ability to bind iron. However, during the fermentation the iron-chelating activity increased (99 ± 0.6% of iron-chelating activity in *Lb. plantarum* fermentation). The other fermentation batches showed a lower percentage of iron-chelating activity. In the case of the calcium-chelating activity, an initial activity of 5 μ*M* Ca^{2+}/mg protein which increased up to 18 μ*M* Ca^{2+}/mg protein in *Lb. acidipiscis*, *Lb. plantarum* and the mixed fermentations could be measured. *Lactobacillus plantarum* fermentation showed the lowest ACE inhibitory activity (its maximum value is 78 ± 2.3% of ACE-inhibition) while other fermentations reached 97 ± 3% of ACE-inhibition activity. The initial ACE-inhibition activity was 35 ± 3%. All the fermentation batches performed generated metal-chelating (calcium and iron) and ACE-inhibitory activities in these in vitro tests which could be important for the production of functional dairy products.

Key Words: bioactive peptides, metal-chelating activities, ACE-inhibitory activity

T133 Virulence and regulator gene expression in *Bacillus* spp. from ultrapasteurized organic milk. Alyssa Grutsch* and John McKillip, *Ball State University, Muncie, IN.*

We isolated a *Bacillus amyloliquefaciens* strain present in ultra-high temperature (UHT) pasteurized organic whole milk to ascertain virulence determinants present in this species, as well as the pattern of virulence gene expression over time in a model food (UHT milk) system compared with the type strain *Bacillus cereus* ATCC14579. The overall goal of this project was to genotypically and phenotypically characterize thermoduric *B. amyloliquefaciens* virulence potential and the presence of the global regulator effector PlcR. Recovery of bacteria from milk required an enrichment in brain-heart infusion broth, incubated aerobically for >24 h, after which time samples were spread-plated onto tryptic soy agar (TSA) plates to recover *Bacillus* spp. Resulting colonies were streak plated onto TSA to ensure purity of culture before catalase testing, Gram and spore-staining to presumptively identify to the genus level. Pure cultures were biochemically identified to the species level using the Microgen *Bacillus* ID system (Hardy Diagnostics), yielding an identification of *Bacillus firmus*, and validated further using fatty acid profiling and 16S rDNA sequencing, which revealed the isolate to be a strain of *B. amyloliquefaciens*. To confirm presence of the target genes in each isolate, DNA was extracted from pure cultures during late log phase. Quantified DNA template was used in real-time (SYBR Green-based) PCR with primers specific for each of the target genes: *plcR*, *codY*, *nheA* and *hblC*, and the16S rRNA gene as a control. PCR results indicated no significant difference existed between average melting temperatures (T_m) of 16s rRNA ($P = 0.435$) and *plcR* ($P = 0.341$) of *B. amyloliquefaciens* and *B. cereus*. The applications of this project will be to determine if parameters regarding shipment, storage, and shelf life of UHT organic milk should be revisited, to ensure quality before consumption of product that may harbor thermoduric toxigenic *Bacillus* spp.

T134 Sequencing and annotation of novel plasmids from *Lactobacillus curvatus*. Jordan Hendricks[1], Craig Oberg*[1], Michele Culumber[1], Taylor Oberg[2], Donald McMahon[2], and Jeff Broadbent[2], *[1]Weber State University, Ogden, UT, [2]Utah State University, Logan, UT.*

Lactobacillus curvatus, a nonstarter lactic acid bacteria (NSLAB), is rapidly becoming the most prevalent NSLAB strain isolated from aged Cheddar cheese. Plasmids often carry genes that confer advantageous traits, which provide a survival advantage to the organism. Understanding the genotype of *Lb. curvatus* plasmids could provide important information concerning genes that provide *Lb. curvatus* an advantage in the cheese environment. Plasmids were isolated from 2 different strains of *Lb. curvatus*, WSU-1 and LFC-1. These strains were isolated from distinct geographical areas. WSU-1 contained 7 plasmids while 4 plasmids were detected in LFC-1. Similarities, at least in size, exist between the largest (40kb) and smallest (1240 bp) plasmids in both *Lb. curvatus* strains. The genomes of both strains have been sequenced and several putative plasmid contigs identified. These sequences were paired with similarly sized plasmids isolated from the organisms and the identified open reading frames compared. Each strain contained a plasmid, of different sizes, with nearly identical genes that coded for an anti-toxin part of a toxin-antitoxin system. Plasmid contigs also coded for cation transport proteins that could promote survival in aging cheese. Variation in plasmid profiles between the 2 *Lb. curvatus* strains also suggests multiple strains may be circulating in cheese plants. WSU-1 and LFC-1 strains carry a greater complement of plasmids than are typically found in dairy lactobacilli. Maintaining a large set of plasmids has a high metabolic cost to the cell, indicating these plasmids contain genes of value to the organism.

Key Words: NSLAB, plasmids, *Lactobacillus curvatus*

T135 Comparative analysis of prebiotics on growth kinetics, fermentation, and antioxidant activity of probiotics. Evelyn Puspitasari*[1], Chi Kong Yeung[2], and Marie Yeung[1], *[1]Biological Sciences Department, California Polytechnic State University, San Luis Obispo, CA, [2]Dairy Science Department, California Polytechnic State University, San Luis Obispo, CA.*

Prebiotics are nondigestible oligosaccharides that selectively stimulate the growth of beneficial bacteria in the human intestine. Fructooligosaccharide (FOS) and inulin are among the most common prebiotics used in food products and dietary supplements. Previous studies examining the bioactivity of lactulose suggest that it also has a prebiotic potential. Lactulose is a derivative of lactose in which the glucose moiety is isomerized to fructose in the presence of heat; and hence can be found in heated milk. The goal of this study was to establish growth kinetics of common probiotics cultured in FOS, inulin or lactulose as the sole carbohydrate source. Fermentation of the prebiotics and the antioxidant activity of spent medium were also characterized. Dextrose and non-probiotic species were included for comparison. Eight commercial and ATCC strains were cultured in a semi-defined modified MRS, modified MRS plus L-cysteine-HCl, or peptone medium containing 1% carbohydrate. When incubated at 37°C in aerobic condition, except for *Lactobacillus casei*, probiotic strains cultured in prebiotics did not reach maximum growth rate or yield compared with dextrose. Multiple pairwise comparisons showed that lactulose tended to produce better growth than FOS and inulin in *L. rhamnosus* and *L. acidophilus*. In anaerobic condition, non-probiotic species were able to catabolize all carbohydrate sources, but displayed weaker lactic acid production (0.13 ± 0.06%) relative to *Bifidobacterium* spp. (0.30 ± 0.09%) or *Lactobacillus* spp. (0.56 ± 0.27%), as measured by titratable acidity. Antioxidant activity, with Trolox as the reference standard, was inversely correlated with the pH of the spent medium after fermentation ($P = 0.022$). Among the 3 prebiotic substrates, lactulose yielded the lowest pH in *L. acidophilus* and *B. infantis*, while FOS appeared to be preferred by *L. casei* and *B. bifidum*. Antioxidant activity was overall the highest from lactulose fermentation. In conclusion, lactulose is a promising prebiotic ingredient that can be incorporated in functional food products.

Key Words: prebiotic, probiotic, lactulose

T136 Heat tolerance of *Lactoccocus lactis* with prior subjection to mild heat stress. Ingrid Osorio* and Kayanush J. Aryana, *School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA.*

Lactococcus lactis has been associated with cheese manufacturing. It is important that the cultures used are able to survive to adverse heat conditions in manufacturing of probiotic process cheese. The hypothesis was whether prior exposure to mild heat would enhance heat tolerance of *L. lactis.* The objective was to evaluate the effect of prior exposure to mild heat on the growth of *Lactococcus lactis*. *L. lactis* R-604 was subjected to heat shock at 40 or 50°C for 1 h. Control was not subjected to heat shock. Cultures were subsequently incubated for 24 h at 30°C followed by subjecting them to batch pasteurization (for ice cream mix) at 71.11°C for 30 min. M17 Agar with 10% w/v lactose was used for plating. Plates were incubated aerobically at 30°C for 48 h. Each experiment was conducted 3 times. Counts observed after subjecting mildly heat treated *L. lactis* to batch pasteurization were 4.4 Log for 40°C, 4.3 Log at 50°C, and 1 Log for the control. Exposure of *L. lactis* R-604 to mild heat enhanced its tolerance to heat (batch pasteurization).

Key Words: heat tolerance, dairy culture

T137 Effect of ultraviolet light exposure and mild heat shock on the salt tolerance of *Lactococcus lactis*. Ernesto E. Gonzalez-Duran* and Kayanush J. Aryana, *School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA.*

Lactococcus lactis is a dairy culture bacterium widely used in dairy products which contain salt (NaCl) such as cheese and salted butter. Osmotic conditions generally hinder the growth of both pathogen and desirable bacteria. It has been observed that exposure to an environmental stress can develop resistance to several stresses. Some studies have shown that short UV (UV) light exposure build up resistance to acid, ethanol, hydrogen peroxide and heat induced stress. There are many proteins and low molecular weight compounds that are produced under more than one stress condition which protects the cell. If salt tolerance is enhanced in desirable bacteria they would survive better compared with pathogens in salty environments. The hypothesis was whether salt tolerance of *Lactococcus lactis* can be enhanced. The objective was to study the influence of UV light and heat shock on salt tolerance of *L. lactis*. *L. lactis* R-604 cells were exposed to mild stresses of UV light (245 nm) for 5 min or a heat shock at 50°C for 25 min. A control sample was run without any stress. Samples were transferred to M17 broth with 5 concentrations of NaCl (0, 1, 3, 5 and 7% w/v) and incubated aerobically at 30°C for 5 d. Plating was conducted immediately after inoculation and every 24 h for 5 d in M17 agar with 0.5% of lactose and incubated aerobically at 30°C for 48 h. Three replications were conducted. On d 1 and 2 no differences were observed for either UV light or heat shock treated cells when exposed to 0, 1 and 3% w/v NaCl, however growth inhibition was observed using UV light or heat shock at 7% w/v NaCl. On d 1,2 and 3, resistance to salt was improved using UV light or heat shock treated cells when exposed to 5% w/v NaCl compared with control. On d 5, mild heat shock and UV light exposure had no effect on salt tolerance. Until d 4, different amount of NaCl and days of salt exposure had a differential effect on growth of *L. lactis* R-604.

Key Words: salt tolerance, dairy culture

T138 Influence of osmotic adaptation and lactose deprivation on the salt tolerance of *Lactococcus lactis*. Ernesto E. Gonzalez-Duran* and Kayanush J. Aryana, *School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA.*

Lactococcus lactis is often used in cheese and salted butter manufacture. Mediterranean cheeses can have up to 7% NaCl. Osmotic conditions generally slow down the growth of both pathogen and desirable bacteria. Studies have shown that vegetative cells exposed to a mild stress become more resistant to lethal doses of the same stress since the first exposure starts the defense mechanisms of the cells creating an effect of cross-protection. The hypothesis was whether salt tolerance of *Lactococcus lactis* can be enhanced. The objective was to study the influence of lactose deprivation and osmotic adaptation on salt tolerance of *L. lactis*. *L. lactis* R-604 was subjected to mild stress induced by lactose deprivation (grown with no lactose in M17 broth) or prior osmotic adaption (grown with 3% w/v NaCl in M17 broth) for 24 h aerobically at 30°C. A control was run without stress (grown in M17 broth with lactose and no NaCl and incubated aerobically for 24 h at 30°C). Lactose deprived or osmotic adapted cells were transferred to M17 broth with 5 concentrations of NaCl (0, 1, 3, 5 and 7% w/v) and incubated aerobically at 30°C for 5 d. Plating was conducted immediately after inoculation and every 24 h for 5 d in M17 agar with 0.5% of lactose and incubated aerobically at 30°C for 48 h. Three replications were conducted. After the 24 h incubation in M17 broth with no lactose or 3% NaCl an increase in 4 log cfu/mL was observed. Lactose deprived cells exposed to 0, 1,3 and 5% w/v salt had a stationary phase at 11 logs for d 1 and 2. While use of 7% w/v salt reduced cells by 2 logs by d 2. On d 5 there were no differences in counts of lactose deprived cells exposed to all salt concentrations. Osmotic adapted cells exposed to 5% salt had a stationary phase at 11 logs for d 1 and 2, while cells exposed to 0,1,3 and 7% NaCl slowly declined until d 5. On d 5 there were no differences in counts of osmotic adapted cells exposed to all salt concentrations. Different amount of NaCl and days of salt exposure had a differential effect on growth of *L. lactis*. From d 3 until d 5, prior exposure to either mild stress did not have an effect on salt tolerance of *L. lactis* R-604.

Key Words: salt tolerance, dairy culture

T139 *Lactobacillus wasatchii* WDC04 associated with late gas production in aged Cheddar cheese. Lauren Montierth[1], Craig Oberg[1], Michele Culumber*[1], Donald McMahon[2], Fatih Ortakci[2], and Jeff Broadbent[2], *[1]Weber State University, Ogden, UT, [2]Utah State University, Logan, UT.*

A new species of nonstarter lactic acid bacteria (NSLAB), called *Lactobacillus wasatchii* WDC04, was identified in aged Cheddar cheese manufactured in northern Utah. This bacterium has been linked to gas formation in the latter stages of Cheddar cheese ripening. It is an obligate heterofermentative NSLAB shown to produce gas in broth cultures under the conditions of cheese aging. WDC04 prefers growth on ribose at low pH (5.0–5.5). It grows slowly at cold temperatures, which could play a role in its ability to create gas defects during cheese ripening. In aging cheese, gas formation causes swelling of the packaging and splitting of the cheese, making it unfit for consumer use. Twenty-seven aged Cheddar cheeses from around the world were tested for WDC04 using MRS medium (pH 5.2) amended with 1.5% ribose and incubated for 1 to 4 weeks. Isolates were identified using 16S rRNA gene sequencing then compared with the GenBank database and to the 16S rRNA gene from *Lb. wasatchii* WDC04. No *Lb. wasatchii* were detected in cheeses without gas defects. WDC04 was found, however, in several distinct aged commercial Cheddar cheeses produced in facilities geographically distant from the original isolation location. These results indicate *Lb. wasatchii* is more widespread than previously thought, and appears to be a causative agent of late gas defect in aged Cheddar cheeses.

Key Words: NSLAB, gas production, cheese

T140 The effect of xenon pulsed-light technology on biofilm adhered to stainless steel surfaces. Stephanie Jacquez* and Rafael Jimenez-Flores, *California Polytechnic State University, San Luis Obispo, CA.*

In food processing, inadequate sanitation procedures lead to the formation of biofilms, in which bacteria attach to surfaces and aggregate in a hydrated polymeric matrix of their own synthesis. Formation of these sessile communities and their inherent resistance to existing sanitation agents are at the root of the risk of bacterial infections for consumers. Based on this evidence, an effective method for reducing biofilm formation in dairy processing equipment is necessary. UV pulsed light technology has proven effective in eliminating microorganism populations on food products. The objective of this work is to evaluate the effect of pulsed light technology on a biofilm consisting of different dairy media (e.g., whey protein concentrate (WPC) and pure lactose) as well as 3 strains of spore forming *Bacillus* species (*B. subtilis*, *B. coagulans*, and *B. licheniformis*) adhered to square, 2.5 × 2.5, ASI 304, stainless steel coupons . Four treatment levels (no treatment, 5 bursts, 10 s, and 20 s) were applied to the coupon surfaces using the Xenon model RC847 machine. Each coupon was placed at a distance of 10.5 cm away from the UV lamp. The pulsed light effect was evaluated using the pour plate technique with 0.2% (w/v) starch TSA and incubated for 48h at 55 C. The adhesion of Bacillus to stainless steel in water as matrix was 1000 to 3000/cm^2 as measured in our laboratory. When compared with the No treatment group, there was a maximum of a 3.96 delta log kill rate in the biofilm created with whey protein concentrate when exposed to 20 s of pulsed UV light. Results indicate a difference between kill rates at 20 s with biofilms created with 5% whey protein concentrate and 5% lactose.

T141 Slime production by *Bacillus* strains affects biofilm formation on dairy separation membranes. Nuria Garcia-Fernandez*[1,2], Ashraf Hassan[1], and Sanjeev Anand[1,2], *[1]Dairy Science Department, South Dakota State University, Brookings, SD, [2]Midwest Dairy Foods Research Center, South Dakota State University, Brookings, SD.*

Our previous research showed that the hydrophobicity of the extracellular polysaccharides produced by lactic acid bacteria plays an important role in biofilm formation on dairy separation membranes. Thermoduric bacilli possess a major challenge for the dairy industry, due to their resistance to heat and cleaning agents. The objective of this study was to evaluate the effect of slime production by *Bacillus* spp. on biofilm formation on separation membranes. Two slime-producing strains; *Bacillus mojavensis* (Bc) and *Bacillus licheniformis* (K1), isolated from dairy powder and one non-slime producing variant from each of the 2 strains (K1–1 from K1 and Bc-1 from Bc) produced by spontaneous mutation were used to study attachment (in the absence of growth) and biofilm formation on polyamide RO membrane pieces. Parameters related to bacterial adhesion (cell charge, capsule production, and hydrophobicity) were evaluated to determine their contribution to differences in biofilm formation among strains. The number of viable cells on biofilm formed by the hydrophobic Bc was more than 1 log cfu/cm^2 greater that its less hydrophobic slime-negative mutant (Bc-1) ($P < 0.05$). However, counts lower by about 0.7 log cfu/cm^2 were found in biofilm formed by the hydrophilic K1 than its slime-negative mutant ($P > 0.05$). Bc and K1 slime precipitated by ethanol contained only 3.6

and 6.5% of total carbohydrates. Bacterial cell surface hydrophobicity was the only parameter that strongly related to both attachment and biofilm formation on RO membranes. To confirm its role, cell surface hydrophobicity was modified by surfactants with different hydrophilic lipophilic balance (HLB) values and attachment of the altered cells was then studied. Tween 20 (high HLB) resulted in lower attachment of Bc while Span 80 (low HLB) improved biofilm formation by K1 ($P < 0.05$). In conclusion, hydrophobic slime produced by *Bacillus* enhanced attachment and biofilm formation on RO membranes. Cleaning strategies that decrease cell hydrophobicity or increase membrane hydrophilicity would reduce risk of biofilm formation by the potential spoilage and disease-causing *Bacillus* strains.

Key Words: biofilm, *Bacillus*, slime

T142 Identification of gram-negative bacteria in cooling tanks of dairy farms. Magali Soares Santos Pozza*[1], Gilberto Henrique Simões[2], Maximiliane Alarvase Zambom[2], Maichel Lange[2], and Grasiele Scaramal Madrona[1], *[1]Universidade Estadual de Maringá, Maringá, Brazil, [2]Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, Brazil.*

The main parameter to verify the quality of milk is the microbiological profile and microbial contamination index. The aim of this study was to isolate and identify gram-negative proteolytic bacteria in different dairy production systems in western Paraná, Brazil. Thirty-five milk samples collected directly from milk tanks evaluated for flow cytometry by somatic cells count (SCC) and total bacteria count (TBC). Samples were diluted in peptone water and seeded in caseinate agar. The colonies were inoculated into *Escherichia coli* broth for 48 h at 35°C. Subsequently were inoculated Eosin Methylene Blue Agar (EMB) and inoculated in Bactray I and II Kit, for the purpose of identification of gram-negative oxidase negative bacteria. Twenty 7 samples showed isolation and identification of some kind of agent, with 77.1% of bacteria isolated. The mean values obtained for TBC and SCC were 1.867.000 cfu/mL and 944.000 cels/mL respectively, values considered above the current legislation. The frequencies of isolated species were *Escherichia coli* (8.6%), *Escherichia fergusoni* (8.6%), *Klebsiella oxytoca* (8.6%), *Yersinia enterocolitica* (8.6%), *Hafnia alvei* (5.7%), *Serratia liquefaciens* (5.7%) *Serratia odorifera* (5.7%), *Citrobacter freundi* (2.85%), *Klebsiella ornithinolytica* (2.85%), *Klebsiella pneumoniae* (2.85%), *Proteus mirabilis* (2.85%), *Providencia rustigiani* (2.85%), *Providencia Stuart* II (2.8 5%), *Salmonella* (2.85%), *Serratia rubidaea* (2.85%) and *Shigella flexneri* serogroup B (2.85%). Of isolates identified 42.85% of them belonged to the family *Enterobacteriaceae*, poor hygiene indicators in manufacturing processes. Proteinases produced by this microbiota are thermostable, remains intact and active after heat treatment, and the considerable economic losses, and one should trace their sources of contamination as hazards to public health.

Key Words: isolating, microorganisms, milk

T143 Can probiotic bacteria survive in a beverage made from "acid whey" from Greek yogurt? Alexis Duferene*, Dasom Park, Douglas Olson, and Kayanush J. Aryana, *School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA.*

Over the last 2 years, Greek yogurt sales and consumption have grown very rapidly leading to a $2 billion per year industry. The by-product of Greek yogurt manufacture is "acid whey," in which the industry needs to decide its utilization. For every 3 parts of milk, 1 part of Greek yogurt and 2 parts of acid whey are formed. The probiotics industry is rapidly growing since the health benefits of probiotics are widely known. Typically, probiotic bacteria do not thrive in acidic conditions. In a product such as acid whey, which naturally contains proteins, amino acids and sugars (lactose) that are needed for lactic acid bacterial growth, it will be beneficial to determine if the lactic acid probiotic *Lactobacillus acidophilus* can survive. The objectives were 1) to manufacture a probiotic acid whey beverage, 2) to determine the growth of the probiotic *Lactobacillus acidophilus* in the acid whey beverage and 3) to study any changes in pH, viscosity and titratable acidity over 5 wk of refrigerated storage. Plain yogurt was manufactured, and whey was separated from the plain yogurt to yield Greek yogurt and acid whey. Acid whey was batch pasteurized, cooled, sweetened, flavored with pineapple flavoring, inoculated with *Lactobacillus acidophilus* to 10^7 cfu/mL and stored at 4°C for 5 wk. The *L. acidophilus* counts declined from 3.2×10^7 immediately after manufacture to 1.4×10^3 at wk 3 and 90 cfu/mL at wk 5. There were no changes in pH, TA and viscosity of the flavored probiotic acid whey over storage for 5 wk which is desirable as it indicates product stability over shelf life. *L. acidophilus* survived in the flavored acid whey, although counts rapidly declined over 5 weeks. This suggests future research on methods to enhance acid tolerance of probiotic *L. acidophilus*.

Key Words: acid whey, probiotic

T144 Evaluation of microbial quality of raw goat and ewe's milk produced in Sabrata, Libya. Yahiah Abojnah[1], Nahed Khatabi[2], Said Gnan[2], and Marvin Moncada*[3], *[1]University of Tripoli, Tripoli Libya, [2]School of Science, Academy of Graduate Studies, Tripoli, Libya, [3]School of Animal Sciences, Louisiana State University, Baton Rouge, LA.*

The objective of this project was to evaluate the microbial quality of the raw goat and ewe's milk in the region of Sabrata, Libya. One hundred random samples of bulk tank raw ewe's and goat's milk (50 samples each) were collected from different farms in Sabrata. All samples were subjected to microbiological tests which included total plate counts (TPC), total coliform counts, yeast and mold counts, *Staphylococcus aureus*, psychotrophic, thermophilic, proteolytic and lipolytic bacteria counts as well as pH measuring. There was a significant ($P < 0.05$) differences between all samples analyzed on total plate counts, thermophilic, coliform and lipolytic bacteria counts. The obtained results showed that the mean TPC log cfu/mL were 6.36 and 4.62 respectively. Psychrotrophic and thermophilic counts were detected in 90 and 88%; 88 and 76% of the samples with mean values in log cfu/mL of 3.67 and 3.18; 2.79 and 3.56 respectively. Results also indicated that *Staphylococcus aureus* and coliforms were present in 100 and 100%; 98 and 98% of the samples with mean values in log cfu/mL 2.82 and 3.28; 3.63 and 3.18 respectively. On the other hand proteolytic and lipolytic bacteria were present in 100 and 100%; 98 and 94% of the samples with mean values in log cfu/mL 2.88 and 3.17; 2.61 and 2.61 respectively. Yeast and mold were detected in 88 and 88% of the samples with mean values in log cfu/mL < 1.17 and < 1.17. The mean values of pH in all samples analyzed were 6.46 and 6.5. In conclusion, the relatively high microbial count reflecting the poor sanitation and hygienic practices in the region evaluated.

Key Words: microbial, goat-ewe, milk

T145 Quantification of spoilage and contaminants bacteria in samples of raw milk. Magali Pozza*[1], Gilberto Simões[2], Maximiliane Zambom[2], Marcelo Neumann[2], and Paulo Pozza[1], *[1]Universidade*

Estadual de Maringá, Maringá, Brazil, [2]Universidade Estadual do Oeste do Paraná, Paraná, Brazil.

The majority of the mesophilic bacteria found in raw milk, has a big involvement in the refrigerated milk degradation and its dairy products producing enzymes with proteolytic and lipolytic action. This research was conducted in the state of west region of Parana- Brazil. The milk samples from 35 properties were collected directly from the cooling tanks and analyzed by flow cytometry for TBC and SCC. The proteolytic and lipolytic microorganisms were plated on caseinate agar and tributyrin agar both incubated at 35°C for 48 h. For all milk samples was also evaluated in the presence of Staphylococcus spp. at 3 critical points of production: from milking, hands-of-milkers, milk cooling tank and plated on Baird Parker agar being held coagulase test. It was possible to stratify the milk samples into 4 distinct treatments according to the values of somatic cells count and total bacteria count: Treatment 1 (>600.000 cells/mL), Treatment 2 (>600.000 cfu/mL), Treatment 3 (>600.000 cells/mL and cfu/mL) and Treatment 4 (<600.000 cells/mL and cfu/mL). The treatments showed mean values of proteolytic microorganisms: 2.7×10^3 cfu/mL, 1.8×10^4 cfu/mL, 1.9×10^4 cfu/mL, 1.4×10^4 cfu/mL and lipolytic microorganisms 1.0×10^5 cfu/mL, 2.1×10^5 cfu/mL, 2.0×10^5 cfu/mL, 6.2×10^4 cfu/mL, with no significant difference by Tukey test ($P > 0.05$). The dairy production systems had unsatisfactory hygienic conditions. Moderate contamination were obtained from *Staphylococcus* 9.6×10^3 cfu/mL, 2.2×10^3 cfu/mL, 1.4×10^4 cfu/mL and 3.8×10^3 cfu/mL cfu/mL; for milking, hands-of-milker, cooling tank and milk respectively. The results show the presence of *Staphylococcus* agent in dairy production systems.

Key Words: microbiology, milking, cooling tank

T146 The identification of lactic acid bacteria in the traditional Carpathian ewe's cheese. Orysysa Tsisaryk*[1], Iryna Slyvka[1], and Tomasz Bocer[2], *[1]Lviv National University of Veterinary Medicine and Biotechnology, Ukraine, [2]Rzeszow University, Poland.*

Our purpose was to isolate and identify lactic acid bacteria (LAB) from the traditional ewe's cheese from the Carpathian region (Ukraine), which were unknown. The objective of the study was to determine LAB from ewe's cheese by molecular genetic methods. 95 pure cultures of LAB were isolation from 3 samples of cheese. Culture media MRS and M17 were used for following isolation of pure cultures of LAB. Genomic DNA was prepared by using a set of Genomic Mini according to the instructions. Primer 1254 was used for RAPD-PCR. Primers EGE1 and 1492R were used for RFLP-PCR (Sigma-Aldrich). Two endonucleases were used for digestion amplification product: *Rsa*I and *Hinf*I (Roche). Taxonomic position of LAB strains was established under the complex cultural, morphological and biochemical properties. Seven strains of LAB of genera *Enterococcus*, 9 strains of genera *Lactobacillus*, 6 strains of genera *Lactococcus* and 6 strains of genera Leuconostoc were isolated from cheese N1. Twenty-eight strains of genera *Lactococcus* and 8 strains of genera *Enterococcus* were isolated from sample N2. Twenty-six strains of genera *Lactococcus* and 5 strains of genera *Enterococcus* were isolated from sample N3. Sixteen groups of LAB with heterogeneous properties were obtained by the method RAPD-PCR. Thirteen groups of LAB were obtained using endonuclease *Rsa*I and 11 groups of LAB using endonuclease *Hinf*I. Thirty-five cultures of LAB for heterogeneous differences were selected to determine their nucleotide sequences of the 16S rRNA gene. Taxonomic position of 20 isolates of LAB of 98-99% homology, 4 isolates of LAB with 96-94% homology, 1 isolates with 93% and 1 isolate with 90% were established by the results of the 16S rRNA gene sequencing. Taxon belonged to 7 species of *Lactobacillus plantarum* ssp., *Leuconostoc mesenteroides* ssp., *Lactococcus lactis* ssp. *lactis*, *Lactococcus garvieae* ssp., *Enterococcus faecium* ssp., *Enterococcus faecalis* ssp., *Enterococcus durans* ssp. The results of the LAB identification demonstrated a complex LAB population of Carpathian ewe's cheeses. Our examinations of LAB populations in raw ewe's milk products from Carpathian cheeses revealed 7 distinct species, some of which could be used in the dairy industry.

Key Words: Carpathian cheese, LAB, PCR-RFLP

T147 Studies of microbiological parameters of cultured butter during storage. Orysya Tsisaryk* and Lubov Musiy, *Lviv National University of Veterinary Medicine and Biotechnology, Ukraine.*

The aim of this work was to study the stability and growth ability of probiotic strains *Lactobacillus acidophilus* (*La-5*) with *Lac. lactis* ssp. *cremoris*, *Lac. lactis* ssp. *lactis*, *Lac. lactis* ssp. *diacetylactis* and *Leu. mesenteroides* spp. *cremoris*, as Flora Danica (FD) Chr. Hansen commercial starters. Four groups of cultured butter (CB) were produced: 1 – the cream fermented at 30°C by *FD* (CB1), 1:1 *FD* and *La-5* (CB2) and *La-5* (CB3); 2 – the cream fermented at 37°C by *FD* (CB4), 1:1 *FD* and *La-5* (CB5) and *La-5* (CB6); 3 (Alnarps Winter method) – 8 °→ 20 °→ 12 ° and added starter *FD* (CB7), 1:1 *FD* and La-5 (CB8) and *La-5* (CB9); 4 – added starter *FD* (CB10), 1:1 *FD* and *La-5* (CB11) and *La-5* (CB12) to the butter grain. The initial concentration of starter cultures in cream was 6 log cfu/mL. The experiment was replicated 3 times. Cultured butter was packed in polystyrene cups and stored in a refrigerator at the temperature of 4–6°C for 42 d. The cell viability of Flora Danica and *Lactobacillus acidophilus La-5* was analyzed during storage on the d 1, 7, 14, 21, 28, 35, and 42. Viable cell counts were performed by preparing serial decimal dilution in 0.1% peptone water. The *Flora Danica* strains were subsequently counted by plating (in duplicate) into M17 agar (Merck). The plates were incubated aerobically for 72 h, at 30°C. The *La-5* strains were subsequently counted by plating (in duplicate) into MRS agar (Merck). The plates were incubated aerobically for 72 h, at 37°C. The results were recorded as colony forming units (cfu) per g of product. During 14 d of storage at 4–6°C the number of viable cells *Flora Danica* and *La-5* has increased. After 14 d all samples showed a drastic decrease of the number of viable cells. The samples CB1 (7.1 log cfu/g) and CB2 (7,3 log cfu/g) possessed the best viable cells *Flora Danica* properties after 35 d of storage. The sample CB2 (7.7 log cfu/g) possessed the best viable cells *La-5* properties after 35 d of storage. The best combination with a significant growth of probiotic strains *Lactobacillus acidophilus La-5* was observed in CB2. We recommend the storage period of cultured butter less than 35 d at 4–6°C for ensure the survival the probiotic strains.

Key Words: cultured butter, Flora Danica, La-5