W316 Preliminary study of bacteriocin production in tina bacterial biofilms. N. Fuca*[1], C. Pediliggieri[1], M.-N. Madec[2], V. Chuat[2], S. Lortal[2], F. Valence-Bertel[2], and G. Licitra[1], *[1]CoRFiLaC, Ragusa, Italy, [2]INRA UMR 1253 - Science et Technologie du Lait et de l'Oeuf, Rennes Cedex, France.*

Tina is the wooden vat used in the cheese making process of the Sicilian PDO Ragusano cheese. The existence of microorganisms (bacteria) consortia in tina samples has a profound implication both for milk colonization and its fermentation process. Tina biofilm is mainly constituted of lactic acid bacteria and it is free of pathogens. Different factors can explain this absence: nutritional competitiveness, low pH, temperature cycles during cheese making and the production of inhibitory bacteriocin-like substances by lactic acid bacteria. The aim of this work is the assessment of class II bacteriocins production by the microflora of tina biofilm. Biofilm samples were collected from 11 different farms of the Hyblean Region. The ability to produce bacteriocin was screened by microbiological, genetic, and confocal microscopy investigations. *Lactococcus lactis* ssp. *cremoris* (bacteriocin producer) and nisin were chosen as positive controls. The colonies of each biofilm, resulted positive to the microbiological test, were isolated and screened again for bacteriocin production by streaking each colony onto the surface of M17 and BHI agar plates, previously inoculated with the chosen sensitive strains. The spot-on-a-lawn method showed that nearly one-third of the 11 biofilm samples were active against *Listeria innocua* 20Li and that half of them were active against *Lactococcus lactis* CNRZ-117. PCR assay performed using class II bacteriocin specific primers revealed that amplification was obtained for 9 out of 11 biofilms. Confocal microscopy observations registered a decrease of *Lactococcus lactis* CNRZ-117 and *Listeria innocua* 20Li viability in the presence of the producer strains' supernatant, measured over time (between 3 and 48 h). Furthermore, the agar test by streaking of the isolated positive colonies confirmed their antagonistic activity against *Lactococcus lactis* CNRZ-117 and *Listeria innocua* 20Li, supporting their positive role in producing bacteriocin-like substances. This study highlights tina LAB ability to produce anti *Listeria* compounds, mantaining biofilm safety.

Key Words: bacteriocin, lactic acid bacteria, pathogen

W317 Effect of *Lactobacillus acidophilus* NS on plasma cholesterol level in diet-induced obese mice. M. Song*[1], S. Park[2], H. J. Lee[3], B. J. Min[3], S. U. Jung[3], S. H. Park[3], E. Kim[2], and S. Oh[1], *[1]Division of Animal Science, Chonnam National University, Gwangju, Republic of Korea, [2]Department of Biological Sciences, Chonnam National University, Gwangju, Republic of Korea, [3]Research & Business Development Center, Nong Shim Co., Ltd., Seoul, Republic of Korea.*

Reductions of plasma total and LDL cholesterol are major strategies to decrease the risk of cardiovascular diseases. Several studies have reported that administrations of probiotics and yogurt containing probiotics both induce resistance to diet-induced body weight gain and increase of plasma cholesterol and triglyceride levels. The objective of this study was to investigate whether *Lactobacillus acidophilus* NS effectively reduces plasma cholesterol level in high fat diet-fed mice. In addition, we also evaluated the probiotic properties of *L. acidophilus* NS such as acid resistance, bile tolerance, adherence onto HT-29 cells, and cholesterol-assimilation activity. In animal study, 7-wk-old male C57BL/6N mice were fed with a normal diet, a high-fat diet (HFD) or a HFD with *L. acidophilus* NS (~1.0×10^8 cfu) for 10 wk. Total cholesterol and LDL cholesterol levels were significantly lower in mice fed with a HFD with L. acidophilus NS than in those fed with a HFD only while HDL cholesterol level was similar in these 2 groups. To understand the mechanism of the cholesterol lowering effect of *L. acidophilus* NS on HFD-mediated increase of plasma cholesterol level, we determined mRNA levels of genes involved in cholesterol homeostasis in the liver. Expressions of SREBP-2 and LDLR in the liver were dramatically reduced in mice fed with a HFD as compared with those of mice fed with a normal diet. When *L. acidophilus* NS was administrated orally to mice fed with a HFD, a HFD-induced suppression of SREBP-2 and LDLR expression in the liver was abolished. These results suggest that the oral administration of *L. acidophilus* NS increased the expressions of SREBP and LDLR in the liver which were inhibited by high fat intake, leading to a decrease in plasma cholesterol level. *L. acidophilus* NS could be useful probiotics for cholesterol-lowering dairy products and the improvement of hyperlipidemia and hepatic lipid metabolism.

Key Words: probiotic, cholesterol, high-fat diet

W318 Comparative genome analysis of *Lactobacillus curvatus* strains isolated from cheese and fermented sausage. C. J. Oberg*[1,2], M. D. Culumber[1], T. S. Oberg[2], J. R. Broadbent[2], D. J. McMahon[2], and J. L. Steele[3], *[1]Weber State University, Ogden, UT, [2]Western Dairy Center, Utah State University, Logan, [3]University of Wisconsin, Madison.*

Recent studies of cheese microbiology have revealed that *Lactobacillus curvatus* is an increasingly common component of the nonstarter lactic acid bacteria (NSLAB) population in aged Cheddar cheese. We recently sequenced the genome of *L. curvatus* WSU01, an NSLAB isolated from aged Cheddar cheese manufactured at Utah State University. The WSU01 genome was sequenced using a whole-genome shotgun strategy on a 454 GS Titanium pyrosequencer. The sequence was assembled into a 1.98 Mb draft genome consisting of 131 contigs, and preliminary genome annotation was performed using the RAST algorithm (rast.nmpdr.org). To learn more about the role of *L. curvatus* in cheese ripening, we compared the WSU01 genome to the 1.83 Mb genome of *L. curvatus* CRL 705, a strain isolated from dried fermented sausage. Plasmid DNA is common among *L. curvatus*, and CRL 705 is known to contain 2 plasmids (12.3 kb and 18.6 kb). WSU01 appears to contain at least one extrachromosomal replicon that includes genes for conjugative mobilization. Overall, comparative genome analysis using a 90% amino acid identity threshold revealed there were 390 proteins in WSU01 that were absent in strain CRL 705, and 196 CRL 705 proteins that were not encoded in the WSU01 genome. Notable examples of proteins unique to WSU01, which could contribute to growth and flavor production by this organism in Cheddar cheese included those associated with propanediol metabolism, arginine utilization, bacteriocin production, and fatty acid biosynthesis.

Key Words: *Lactobacillus curvatus*, NSLAB, genome

W319 Genomic analysis of *Lactobacillus* WDC04, a novel species associated with late gas production in cheese. C. J. Oberg*[1,2], M. D. Culumber[1], T. S. Oberg[2], F. Ortakci[2], J. R. Broadbent[2], and D. J. McMahon[2], *[1]Weber State University, Ogden, UT, [2]Western Dairy Center, Utah State University, Logan.*

A new heterofermentative *Lactobacillus* species (WDC04), which may be associated with late gas production in Cheddar, was isolated from aged cheese following incubation on MRS agar at 6°C for 35 d. WDC04 had 97% 16S rRNA sequence identity with *Lactobacillus suebicus* strain CECT5917 (AJ575744) and *Lactobacillus vaccinostercus* (AB218793). This bacterium only fermented 2 of the 50 substrates tested, ribose and galactose, on API CH50 fermentation panels. To learn more about the metabolic capabilities of *Lactobacillus* sp. WDC04, total genomic DNA was sequenced using a whole-genome shotgun strategy on a 454 GS Titanium pyrosequencer. The sequence was assembled into a 1.90 Mb draft genome consisting of 105 contigs, and preliminary genome annotation was performed using the RAST algorithm (rast.nmpdr.org). Genome analysis confirmed the pathway for ribose metabolism as well as a glycerol fermentation pathway that are likely related to the oligotrophic nature of this organism. This is the first report on a genome from a previously unknown nonstarter lactic acid bacterium in aged Cheddar cheese. Further study of this novel bacterium may shed new insight on the phenomenon of late gas production in Cheddar.

Key Words: *Lactobacillus*, gas production, genome

W320 Comparative analysis of blp gene clusters in bacteriocin-producing strains of *Streptococcus thermophilus*. J. Renye* and G. Somkuti, *Agricultural Research Service, USDA, Wyndmoor, PA.*

Streptococcus thermophilus LMD-9 contains a gene cluster for the production of a bacteriocin-like peptide (Blp); yet expression depended on the exogenous addition of a quorum sensing induction peptide. In contrast, *S. thermophilus* ST110 naturally produces a bacteriocin with unique antipediococcal activity. PCR analysis of ST110 confirmed the presence of several components of the blp gene cluster. Inactivation of blpC, known to encode the quorum sensing induction peptide, resulted in impaired antipediococcal activity. Analysis of the blp gene cluster in ST110 by chromosomal walking revealed that it was 2 kb shorter and contained 9 fewer open reading frames (ORF) when compared with LMD-9. The nucleic acid sequence encoding components of the blp quorum sensing system (blpABC and blpHR) were identical between both strains, yet real-time PCR analysis showed the expression of BlpC was over 500-fold higher in ST110. In ST110, sequence analysis revealed 2 ORF, designated blpU and blpK, which may encode the bacteriocin based on the presence of a double glycine leader peptide. In the ST110 blpC knockout strain the expression of blpU and blpK was 21 and 39-fold lower when compared with the ST110 parent strain, suggesting they are needed for optimal expression of the bacteriocin. In LMD-9 blpD was shown to be essential for the strains antimicrobial activity; and its absence from the ST110 gene cluster further supports the possibility of either blpU or blpK encoding the actual bacteriocin. When compared with LMD-9, where BlpU serves as an accessory peptide to enhance BlpD activity, blpU expression was 174-fold higher in ST110 and 8-fold higher in the ST110 blpC knockout strain. Analysis of the promoter region of blpU in ST110 detected 2 direct repeats required for binding of the response regulator which were identical to those found within the blpD promoter of LMD-9, suggesting BlpU may have an essential role in the antipediococcal activity of ST110.

Key Words: *Streptococcus thermophilus*, bacteriocin

W321 Influence of phospholipids on the viability of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* under acid stress. B. Chinnasamy*, S. Clark, and A. Mendonca, *Iowa State University, Ames.*

Improved extraction methods of phospholipids (PLs) from by-products such as buttermilk and whey and their potential health benefits have opened new avenues for dairy PLs applications in foods; one such application is fortification of yogurt. However, it is critical to evaluate the influence of PLs on the viability of common lactic acid bacteria (LAB) before application in yogurt. As post acidification is a limiting factor for viability of LAB, the objective of this study was to investigate the influence of PLs on viability of *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB) under normal and acid stressed conditions (pH of 4.5 and 4.2, respectively). Yogurt cultures, ST and LB, were grown individually in M17 and MRS broths and later inoculated in broths fortified with one of 3 PLs: phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS); the PLs were fortified at the rate of 0.01% w/v. The pH in the broths were adjusted to 4.5 and 4.2 before inoculation and stored at refrigeration temperature for 20 d to mimic yogurt storage conditions. The LAB were enumerated on d 0, 5, 10 and 20 using the pour plate technique; 3 replications were completed. ST showed varied response in the presence of PLs; improved viability with PC, a slow decline with PE and decreased viability with PS was noted in comparison to control (without PLs) at pH 4.5. While decreased viability was observed in the presence of PE and PS at pH 4.2, ST showed improved viability in the presence of PC by the end of the storage period. Generally, viability of LB declined during the study period, but the decline was slower in the presence of PE (pH 4.5 and 4.2) and PC (at pH 4.2) than without PLs. The results suggest that individual PLs do not have profound beneficial or inhibitory influences on ST and LB under acid stress conditions. For practical applications in yogurt, influence of additional PLs and combined PLs on LAB is currently being investigated.

Key Words: phospholipid, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*

W322 Influence of the tina wooden vats biofilm composition on milk microbial growth under Ragusano cheese-making conditions. S. Carpino*[1], I. Schadt[1], V. Giummarra[1], and G. Licitra[1,2], *[1]CoRFiLaC, Regione Siciliana, Ragusa, Italy, [2]DISPA, Catania University, Catania, Italy.*

Ragusano cheese is a brine-salted pasta filata raw-milk cheese. The raw milk is placed in the traditional tina for cheese making. Starters are not added, but the tina biofilm and the natural milk flora are responsible for the milk acidification. The aim of the present study was to investigate the influence of the tina biofilm on the microbial composition of milk which has undergone the usual cheese-making procedure except for the rennet addition step. Biofilm samples were obtained from the inner surface area of 100 cm^2 of 11 wooden tinas using sterile swabs which were then suspended in peptone water. Aliquots of a UHT milk sample were inoculated (InoMB) with the biofilm samples. Tina surface area per volume milk was 1.5 times the usual exposure conditions. Inoculated milk samples were then incubated (Inc.MC) under Ragusano cheese-making conditions: 65 min at 35°C, 45 min at 40°C (first cooking), 120 min at 45°C (second cooking), 24 h at 15°C (time before brining). Biofilm, inoculated and incubated samples were analyzed for TBC, for counts of mesophilic lactobacilli (ME_LB), mesophilic lactococci (ME_LC), thermophilic lactobacilli (TH_LB), thermophilic lactococci (TH_LC) and enterococci (EC). A linear model was used to test the effects of log-transformed biofilm counts on log-transformed counts of inoculated and incubated milk samples. Biofilm samples had highest variations in TH_LC and ME_LC ($1 – 5.5 \times 10^3$ and $1 – 1.3 \times 10^3$ cfu/cm^2, respectively), followed by TH_LB and ME_LB ($1 – 7 \times 10^2$ and $1 – 3.8 \times 10^3$ cfu/cm^2, respectively) and by EC ($1 – 6.4 \times 10^1$ cfu/

cm^2). Biofilm TBC ($P < 0.01$), ME_LB ($P < 0.01$), ME_LC ($P < 0.01$), TH_LB ($P < 0.01$), and TH_LC ($P < 0.05$) counts were highly associated with counts of the inoculated milk samples. Moreover, biofilm EC ($P < 0.05$), ME_LC ($P = 0.05$), and TH_LB ($P < 0.05$) were associated with counts of the incubated milk. The counts of respective Inc.MC samples were estimated to vary by 2.80×10^6 cfu/mL EC, by 1.75×10^8 cfu/mL MESO_LC and by 1.42×10^3 cfu/mL TH_LB. Considering equal levels of all biofilm bacteria, the EC growth was most abundant during incubation.

Key Words: Ragusano, cheese, biofilm

W323 Growth of specific lactic acid bacteria (PCR-DGGE) in relation to volatile compounds (SMart Nose and GC/MS) in biofilm inoculated milk under Ragusano cheese-making conditions. S. Carpino*[1], I. Schadt[1], T. Rapisarda[1], C. Randazzo[2], and G. Licitra[1,3], *[1]CoRFiLaC, Regione Siciliana, Ragusa, Italy, [2]DiGeSA, Catania University, Catania, Italy, [3]DISPA, Catania University, Catania, Italy.*

Ragusano cheese is a brine-salted pasta filata raw-milk cheese. Starters are not added, but the biofilm of the wooden vat (tina) and the natural milk flora are responsible for the milk acidification. The aim of the present study was to investigate the growth of specific LAB species in relation to the development of volatile compounds in milk which has been inoculated with biofilm from different tinas and which has undergone the usual cheese-making procedure except for the rennet addition step. Biofilm samples were obtained from the inner surface of 11 wooden tinas (A-K) with sterile swabs suspended in peptone water. Aliquots of a UHT milk sample were inoculated with the biofilm samples. Tina surface area per volume milk was 1.5 times the usual exposure conditions. Incubated samples were analyzed by PCR-DGGE to determine LAB species, with SMart Nose and by GC/MS. The following LAB species were identified: *Lb. plantarum* and *Lb. paracasei* (mesophilic lactobacilli), *Lc. lactis* and *Lc. mesenteroides* (mesophilic lactococci), *Lb. delbrueckii* and *Lb. helveticus* (thermophilic lactobacilli), *S. thermophilus* (thermophilic lactococcus) and *E. faecalis* (enterococcus). Data were evaluated with principal component analysis (PC1: 23%; PC2: 14%). Four (B, E, F, J) represented the greatest volatiles' variation. Samples E and F were similar in profile, but different from B and J. Profiles of B and J differed also. *Lb. paracasei* was associated to hexane 2,3,4-trimethyl and heptane-2,2,4,6,6-pentamethyl, *E. faecalis* and *Lb. delbrueckii* to tetradecanal, *Lb. plantarum* to nonanoic acid and benzeneacetaldehyde, *Lc. lactis* to octanal. Differences in volatile compounds were most related to *Lc. mesenteroides* and *Lb. paracasei* which separated B from E and F samples, but not to *Lb. plantarum*, *S. thermophilus* and *Lb. delbrueckii*. Sample J was distinguished from the others by 2-dodecanone. Further studies are still needed to better understand the relationship between individual LAB species and flavor production in cheese.

Key Words: Ragusano, cheese, biofilm

W324 Influence of different concentrations of lactose on the growth of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 and *Streptococcus thermophilus* ST-M5. B. Mena*[1] and K. Aryana[1,2], *[1]School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, [2]Department of Food Science, Louisiana State University Agricultural Center, Baton Rouge.*

Lactic acid bacteria ferment lactose. The objective of this study was to evaluate the effect of various concentrations of lactose on the growth of yogurt culture bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Sterile MRS broth was inoculated separately with freshly thawed *Lactobacillus bulgaricus* LB-12 and *Streptococcus thermophilus* ST-M5 and different amounts of lactose (1, 2, 3, 4, and 5% w/v) were added. No added lactose was used as control. Growth was determined by plating in duplicate at 0, 4, 10, 12, 24, 36, 48 and 60 h of incubation period for both microorganisms. Growth of *L. bulgaricus* was determine by pour plating using MRS agar pH 5.2 and anaerobically incubation at 43°C for 72 h. Growth for S. thermophilus was determine by pour plating using *Streptococcus thermophilus* agar and aerobically incubation at 37°C for 24 h. Data were analyzed using ANOVA of SAS with Duncan adjustment. Three replications were conducted. At hours 36, 48 and 60, use of 5% lactose (w/v) resulted in significantly highest log cfu/mL of *S. thermophilus*, while only 4% lactose (w/v) resulted in significantly highest log cfu/mL of *L. bulgaricus*. It is concluded that both these lactic acid bacteria had a slightly different lactose optimum for growth.

Key Words: lactose, yogurt, bacteria

W325 Influence of whey protein isolate on growth of *Streptococcus thermophilus* ST-M5. L. Vargas*[1] and K. Aryana[1,2], *[1]School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, [2]Department of Food Science, Louisiana State University Agricultural Center, Baton Rouge.*

Whey protein is used in manufacture of some dairy products to increase protein content and as a replacement of solids. The objective was to study the effect of whey protein isolate (WPI) on the growth of *Streptococcus thermophilus* ST-M5. Sterile MRS broth was inoculated with freshly thawed *S. thermophilus* and WPI was added in different amounts namely, 0 (control), 1, 2, 3, 4, and 5% w/v. The MRS broth containing WPI was incubated at 37°C for up to 60 h. Growth was determined by pour plating using *Streptococcus thermophilus* agar followed by aerobic incubation at 37°C for 24 h. Data were analyzed using ANOVA of SAS. Three replications were conducted. At 24 and 60 h, significantly lower counts were observed for control compared with WPI use. With the use of 5% WPI the *Streptococcus thermophilus* ST-M5 counts were 2 log cfu/mL higher than the control. Use of WPI improved growth of *Streptococcus thermophilus* ST-M5.

Key Words: yogurt, whey, culture bacteria

W326 Effect of several health beneficial spices on the bile tolerance of *Lactobacillus bulgaricus* LB-12. M. Sanchez-Vega*[1] and Kayanush Aryana[1,2], *[1]School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, [2]Department of Food Science, Louisiana State University Agricultural Center, Baton Rouge.*

Functional foods have become an important and rapidly expanding market. Spices such as garlic and ginger are widely used for their antibacterial properties and as a preventative for cardiovascular diseases. Onion and turmeric have been studied for its potential use in decreasing the risk of developing diabetes and along with garlic, they have been researched for their anticancer properties. *Lactobacillus bulgaricus* is a culture bacterium with some health benefits. Bile tolerance is an important probiotic characteristic. Influence of pure spice juice on the bile tolerance of culture bacteria is not known. Objective was to elucidate the effect of garlic, ginger, onion and turmeric on bile tolerance of *Lactobacillus bulgaricus* LB-12. Bile tolerance of *L. bulgaricus* was analyzed in MRS-THIO broth supplemented with 0.3% (w/v) oxgall and 0.2% (w/v) sodium thioglycolate, and 1% (v/v) of freshly extracted spice juice. Sample without spice juice were the control. Samples were incubated at 43°C for 5 h, but removed hourly

for plating. Growth was determined by pour plating using MRS agar pH 5.2. Plates were incubated anaerobically at 43°C for 72 h. Data were analyzed using Proc Mixed model with a Tukey adjustment of SAS. Experiments were conducted in triplicate. At 0 h of incubation (8.4 log cfu/mL), ginger showed a significant higher count with a difference of 0.5 log cfu/mL when compared with control. Garlic and onion showed significantly lower counts after 2 h of incubation with a difference of 1 log cfu/mL when compared with control. After 3 h of incubation, garlic and ginger showed significantly lower counts with a difference of 1 log cfu/mL when compared with control. After 4 and 5 h, all spices showed significantly lower counts with a minimum difference of 1.5 log cfu/mL for garlic and a maximum of 3 log cfu/mL for turmeric, when compared with control. Even though these spices showed significantly lower counts, *L. bulgaricus* was still viable, indicating that these spices can be used in combination with this bacterium allowing health benefits from both bacteria and spices.

Key Words: spice, bile, probiotic