correlations among most of the type traits and final score suggest that continued selection on the latter is an effective means for improving the former. Heritabilities of the linear type traits were in most cases lower than those reported by others, ranging from zero for back and fore udder length to 0.31 for stature. Results of this study suggest that phenotypic selection on most of the type traits will yield low to moderate genetic improvement in the next generation.

Key Words: Type traits, Genetic correlations, Heritabilities

1298 Comparison of occurrence and yields of daughters of progeny-test and proven bulls in artificial insemination and natural-service bulls. H. D. Norman*, R. L. Powell, and J. R. Wright, *Animal Improvement Programs Laboratory, Agricultural Research Service, USDA, Beltsville, MD.*

Extent of artificial-insemination (**AI**) use was determined for Ayrshire, Brown Swiss, Guernsey, Holstein, and Jersey breedings since 1959. Yield deviations for milk, fat, and protein of daughters of progeny-test (**PT**) bulls were compared with those of daughters of AI-proven bulls and natural-service (**NS**) bulls available contemporaneously. Bulls were categorized as 1) PT through a major AI organization, 2) PT through a minor AI organization, 3) proven through a major AI organization, 4) proven through a minor AI organization, 5) marketed through AI based on an NS evaluation, or 6) used through NS. Only Holstein results are reported. Percentages of daughters that first calved in 1998 with lactation records used in USDA genetic evaluations were 15, 2, 63, 12, 2 and 6% for bulls in categories 1 through 6, respectively. Percentage of daughters sired by PT bulls increased from 8% in 1984 to 17% in 1998, while percentage sired by bulls brought into AI based on NS daughters decreased from 19 to 2% and percentage of NS daughters dropped from 12 to 6%. Percentage of daughters of AI-proven bulls from major AI organizations changed little (from 61 to 63%), but percentage from minor AI organizations increased from 0 to 12%. Those changes were caused by a large reduction in the number of bulls entering AI based on an NS evaluation, plus a moderate increase in percentage of AI use and herds participating in PT programs. From 1984 to 1998, Holstein daughters of AI-proven bulls annually produced 107 to 199 kg more milk and 2 to 5 kg more fat and protein than PT daughters and 366 to 443 kg more milk, 10 to 14 kg more fat, and 9 to 11 kg more protein than NS daughters based on mean yield deviations. Those mean yield differences supported PTA differences (not shown). Use of AI in place of NS would increase annual income of producers by approximately $96 per cow.

Key Words: Artificial insemination, Natural service, Progeny test

1299 Genetic correlations between semen production and economic traits of swine. S. H. Oh*[1], M. T. See[1], T. E. Long[2], and J. M. Galvin[2], [1] *North Carolina State University, Raleigh, NC,* [2] *NPD USA, Roanoke Rapids.*

Currently boars selected for commercial use as AI sires are usually evaluated on grow-finish performance and carcass characteristics. If AI sires were also evaluated and selected on semen production, it might be possible to reduce the number of boars required to service sows, thereby improving the productivity and profitability of the boar stud. The objective of this study was to estimate genetic correlations of semen production with average daily gain (ADG), backfat thickness (BF) and muscle depth (MD). The semen collection records and performance data for 599 boars and two generations of pedigree data were provided by NPD USA. Semen production was defined as the mean of repeated measurements of number of doses per ejaculate (1 dose = 3.05 billion sperm cells per 500 ml). Backfat thickness and MD were estimated by real-time ultrasound. Genetic parameters were estimated from a four-trait animal model using MTDFREML. Breed and contemporary group were included as fixed effects, and were highly significant($P<.0001$) for all four traits. Heritability estimates were .46 for semen production, .46 for ADG, .47 for BF and .33 for MD. The genetic correlations between semen production and ADG, semen production and BF, and semen production and MD were .21, -.01 and -.01, respectively. Genetic correlations between ADG and BF, ADG and MD, and BF and MD were .55, .54 and .39, respectively. Semen production showed a positive genetic correlation with ADG, but was not genetically correlated with BF and MD. Therefore, current AI boar selection practices should not have a detrimental effect on semen production.

Key Words: Genetic correlation, Semen, Pigs

1300 Relationship of body length to number of teats and litter size for four breeds of swine. Z.B. Johnson*[1] and R.A. Nugent, III[2], [1] *University of Arkansas, Fayetteville,* [2] *The Pork Group, Rogers, AR.*

The objective of this study was to estimate relationship of body length to number of teats and number of pigs born alive in first parity pigs from Landrace, Yorkshire, Duroc, and Hampshire breeds of swine. Data consisted of performance test records collected in a commercial swine operation from 1992 to 1999. Boars from 60% of the litters were culled at weaning based on a combination of maternal and performance indexes which differed by breed. Remaining boars and all females were weighed at 100 d of age (WT100) and selected for performance testing based on recalculated indexes. For three years (1992 to 1995), the number of teats (NT) was counted on both sexes at 100 d of age (n = 4,162 for Landrace, 18,986 for Yorkshire, 3,814 for Duroc, and 2,932 for Hampshire). For all years body length (LEN) was measured at the end of the 77-d performance test, and number born alive (NBA) at the first parity was recorded. Number of records for WT100 was 8,611, 38,979, 7,046, and 4,878 for Landrace, Yorkshire, Duroc, and Hampshire, respectively. Of these 825 Landrace, 3,140 Yorkshire, 4,237 Duroc, and 441 Hampshire had records for NBA. For each breed, genetic parameters were estimated using an animal model with litter effects and multiple-trait DFREML procedures. Three-trait models including WT100, LEN, and either NT or NBA were examined. Fixed effects included contemporary group and the appropriate age as a covariate for WT100 and LEN. Heritability estimates for NBA were 0.02 for Landrace, 0.15 for Yorkshire, 0.05 for Duroc, and 0.14 for Hampshire. Estimates of heritability for NT were 0.06, 0.29, 0.04, and 0.08 for Landrace, Yorkshire, Duroc and Hampshire, respectively. Genetic correlations between body length and NBA were 0.22, -0.02, -0.07, and 0.72 and between LEN and NT were -0.17, 0.04, 0.41, and -0.27 for Landrace, Yorkshire, Duroc, and Hampshire, respectively. Estimates of heritability of NBA and NT were low, and no consistent relationship with body length was observed in these data.

Key Words: Body length, Litter size, Number of teats

Dairy Foods
Micro

1301 Efficacy of spices alone or in combined with bifidobacteria to control *Escherichia coli* O157:H7. S.A. Ibrahim*, S.R.K. Dharmavaram, G. Shahbazi, and C.W. Seo, *North Carolina Agricultural and Technical State University, Greensboro, NC.*

Escherichia coli O157:H7 is one of the leading causes of bacterial foodborne disease outbreaks in the United States. An estimated 73,000 cases of infection and 61 deaths occur each year. Many of these outbreaks are associated with the consumption of meat and meat products such as ground beef and ground beef patties. Spices are usually added to meat products to improve the quality and shelf life. Our research hypothesis is that manganese (Mn^2), a common element in many spices, could stimulate the production of organic acids and antimicrobial compound by lactic acid bacteria. Therefore, combinations of starter cultures and spices would enhance the biosafety of these consumable products. The objective of this research was to determine the effectiveness of combinations of bifidobacteria and spices on inactivation of *E. coli* O157:H7 in ground beef. Ground Beef (93% lean meat) was inoculated with *E. coli* O157:H7 (380-94) to make the initial inoculum level of 2.0 log cfu/ml. Inoculated ground beef was mixed with different spices (garlic, ginger, jalapeno pepper and commercial spice, served as antioxidant) at the level of 2% (W/V). Bifidobacteria was then added to a final level of 5.00 log cfu/ml. Beef samples were held at 37 C for 48hr. Changes in the populations of *E. coli* in meat samples were followed on EMB agar plates. The results showed that ground beef treated with commercial spice had the highest inhibitory effect against *E. coli* ($P< 0.05$), followed by jalapeno pepper and garlic. Ginger had little effect on the

growth of *E. coli* in ground beef. The synergistic effect of spices and bifidobacteria on *E. coli* O157:H7 was higher than the effect of single spice alone ($P < 0.05$). Knowledge gained from this research project will be valuable in developing new strategies to eliminate *E. coli* O157:H7 in many meat products and ultimately improve the biosafety of these consumable products.

Key Words: *E. coli* O157:H7, bifidobacteria, meat products

1302 Production of conjugated linoleic acid by *Lactobacillus acidophillus* and *Lactobacillus casei* of human intestinal origin. L. Alonso, P. Cuesta Alonso*, and S. Gilliland, *Oklahoma State Universtiy, Stillwater, OKlahoma, USA*.

Conjugated linoleic acids (CLA) are a mixture of positional and geometrical linoleic acid isomers, the predominant ones are (c9t11) and 18:2 (t10c12) (t9t11). At each position the double bond can be either in the cis- or the trans- configuration. However, it is the cis-9, trans-11-octadecadienoic acid (18:2 c9t11) that is considered to be the most biologically active isomer. It is a naturally occurring fatty acid, present mainly in foodstuffs from animal sources. CLA is produced from polyunsaturated fatty acids by rumen microorganisms during biohydrogenation. The objective of this study was to determine the ability of different cultures of lactobacilli isolated from human intestinal sources to produce CLA. Four different cultures, two strains each of *Lactobacillus acidophillus*and *Lactobacillus casei* were tested for their ability to produce CLA from free linoleic acid. Different concentrations of linoleic acid (0, 0.05, 0.1, 0.2, 0.5 mg/ml) were added to MRS broth and sterilized skim milk inoculated with the cultures of the lactobacilli, and incubated at 37° C. Samples were taken at 0, 24, 48 and 72h. For each sample the amounts of individual isomers of CLAs (9c9t11, t10c12, t9t11) and total CLA (conjugated linoleic acid) were quantitated by gas liquid chromatography. Growth in media containing 0.2% linoleic acid for 24h was most effective in CLA production. All the cultures were able to convert linoleic acid in conjugated linoleic acid in broth media and milk with a concentration range of (90.74-165.37 μg/ml) and (79.54-143.73 μg/ml) respectively. Conjugated linoleic acids have been reported to induce beneficial physiological effects including anticarcenogenic, inhibition of arteriosclerosis and enhancement of immunological function in different species. The use of lactic acid bacteria able to deconjugate linoleic acid might enrich dairy products with CLA contributing to these beneficial effects. Probiotic bacteria, such as the ones in this study, also might produce CLA in the intestinal tract following their ingestion.

Key Words: Conjugated linoleic acid, Lactobacillus, Probiotic

1303 Colonization Property of *Lactobacillus reuteri* and Its Antagonistic Activity in Mice Infected With *Salmonella enterica* serovar Typhimurium DT104. S. H. Kim[1], N. H. Kwon*[1], J. Y. Kim[1], J. Y. Lim[1], H. J. Kang[2], D. S. Lee[2], I. B. Kwon[2], B. W. Yoo[3], and Y. H. Park[1], [1]*College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University,* [2]*Lotte R&D Center,* [3]*Agribrands Purina Korea, Inc.*

The aim of this study was to compare the colonization properties of three probiotic strains, *Lactobacillus reuteri*, *L. bulgaricus* and *L. casei*, and their antagonistic activities against *Salmonella enterica* serovar Typhimurium DT104 infection in mice. Mice were fed with one of three probiotic strains (10^9 cfu/mouse) for 7 days and fecal samples were collected daily from day 8 to 11. Mice fed with *L. reuteri* continued to be present at high lactobacilli population in feces even 4 days after stopping feeding it, compared with the others. To investigate the antagonistic activity, mice were challenged with *S. enterica* serovar Typhimurium DT104 (3.7×10^8 cfu/mouse), after prefeeding with one of the above three probiotic strains for 7 days. The fecal shedding of *S. enterica* serovar Typhimurium DT104 and serum IgG and intestinal IgA against the organism were examined. The fecal shedding was dramatically decreased and *S. enterica* serovar Typhimurium DT104 was not detected in feces and intestines 3 days after challenge in mice fed with *L. reuteri*. Antibody responses of the intestinal IgA were significantly increased and relatively strong responses were also observed for serum IgG in mice fed with *L. reuteri*. These findings suggest that *L. reuteri* can survive better in the gastrointestinal tract and has superior antagonistic activity against *S. enterica* serovar Typhimurium DT104 compared with the others. Also, administration of *L. reuteri* might enhance the mucosal and systemic immune responses against *S. enterica* serovar Typhimurium DT104. Further study will be followed to define the mechanism of immunomodulatory effects of *L. reuteri*.

Key Words: *Lactobacillus reuteri*, antimicrobial activity, immunomodulatory effect

1304 Antimicrobial activity of *Lactobacillus reuteri* SD 2112 against bovine pathogens and *Escherichia coli* O157:H7. N. H. Kwon[1], S. H. Kim*[1], J. Y. Kim[1], J. Y. Lim[1], J. S. Ahn[2], B. W. Yoo[3], H. J. Kang[4], D. S. Lee[4], I. B. Kwon[4], and Y. H. Park[1], [1]*College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University,* [2]*National Veterinary Research and Quarantine Service,* [3]*Agribrands Purina Korea, Inc.,* [4]*Lotte R&D Center*.

The purpose of this study was to determine antimicrobial activity of *Lactobacillus reuteri* SD 2112 and to compare it with other lactic acid bacteria such as *L. acidophillus, L. bulgaricus, L. casei* and *Bifidobacterium longum*. Tested bovine pathogens were *Salmonella dublin* isolate, *S. enteritidis* ATCC 13076, *S. enteritica* serova *Typhimurium* DT104, *Staphylococcus aureus* MNEV, FRI 913, *Listeria monocytogenes* ATCC 11285 and *Bacillus anthracis* Sterene $34F_2$. *Escherichia coli* O157:H7 ATCC 43890 and ATCC 43894 were also included in as food-borne pathogens derived from cows. Five probiotics were incubated in 3 different growth conditions (MRS without glycerol, MRS with 0.5 M glycerol and 0.25 M glycerol solution) to obtain supernatants. Nine pathogens were inoculated on Mueller Hinton Agar containing supernatant of each 5 probiotics. Data were analyzed with Friedman two-way ANOVA by ranks in Statistical Analysis System Institute version 8. Though antimicrobial activity of *L. reuteri* in the first two conditions was not better than the others', the activity was significantly higher than those of the others in 0.25 M glycerol solution. This prominent effect might be attributable to reuterin, produced by *L. reuteri* using glycerol. We could detect the presence of reuterin in the supernatant of 0.25 M glycerol solution with Nuclear Magnetic Resonance at 500 MHz. The result of minimum bactericidal concentration has revealed that reuterin had pan-bactericidal effects against above pathogens. To examine any changes of antimicrobial activities of the probiotics, each supernatant was treated with different pH conditions, pepsin and trypsin digestion. Antimicrobial activity of reuterin was not entirely affected by any of these treatments, while the activities of the other probiotics were significantly decreased. This study has indicated that *L. reuteri* could be a good aid of bovine and other animal health when used as feed additives because of its antimicrobial activity and unchangeable characteristic even in gut environments. Also, *L. reuteri*, as feed additives, could be helpful to human by preventing *E. coli* O157:H7 being transferred from cows to human.

Key Words: *Lactobacillus reuteri*, Antimicrobial activity, Probiotics

1305 Autoaggregation behavior of bifidobacteria as influence by media composition and incubation temperatures. V. Rada[1], J. Medkova[1], S. A. Ibrahim*[2], O.A. Hassan[2], G. Shahbazi[2], and Y. Murad[3], [1]*Czech University of Agriculture Prague, Prague, Czech Republic,* [2]*North Carolina Agricultural and Technical State University, Greensboro, NC.,* [3]*Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL*.

Bifidobacteria are of increasing interest to the dairy industry. The incorporation of such culture into the human diet corresponds to the emergence of a new generation of dairy products, which use the beneficial effect of bacteria to improve intestinal metabolism. However, if the use of bifidobacteria as a dairy product supplement is to be justified, these cultures must possess certain characteristics, one of which is the ability to autoaggregate in the human intestinal tract. Therefore, the objective of this research was to determine the autoaggregation behavior of bifidobacteria as influence by media composition and incubation temperatures. Eight strains obtained from commercial culture collection and six strains isolated in our laboratory were tested for their autoaggregation ability. These strains were grown in different media (MRS, TPY, and Wilkins-Chalgren) and incubated at different incubation temperatures (30, 37 and 42 C). The autoaggregation ability of these strains was determined at different time intervals during the incubation period. Results showed that Tween 20 and Tween 80 reduced autoaggregation behavior. TPY broth, increased the autoaggregation behavior of the tested strains, where as Wilkins-Chalgren and MRS reduced autoaggregation behavior. This could be due to the reduced growth in these media. The

autoaggregation behavior significantly differed among tested samples. Higher incubation temperature, (42 C) compared to lower incubation temperature (30 C), increased the ability of strains to autoaggregate. Our data suggest that autoaggregation is an important trait that contributes to the ability of bacteria to colonize in the intestinal tract. Our results also suggest that this ability should be used as a preliminary step to determine a potentially suitable culture for specific dairy food applications. Further studies are required to determine the molecular mechanism of autoaggregation.

Key Words: autoaggregation, bifidobacteria

1306 Bacteriological quality of bulk tank milk in Pennsylvania. B. M. Jayarao*, S. R. Pillai, D. R. Wolfgang, C. M. Burns, and L. J. Hutchinson, *The Pennsylvania State University, University Park, PA, USA*.

Studies conducted over the last two decades have shown that examination of bulk tank milk (BTM) is useful for diagnosing multiple problems (current and potential) that might exist in a dairy herd related to milk quality and mastitis pathogens. An extension and research program was conducted in Pennsylvania from April 2000 through March 2001 focused on BTM analysis. The research project involved surveying BTM quality in Pennsylvania. A total of 126 dairy producers from 14 counties in Pennsylvania participated in the study. Four BTM samples were collected at interval of 15 days from each of the participating dairy herds. The BTM samples were examined for somatic cell count (BTSCC) and differential bacterial counts including; 1) Standard plate count (SPC), 2) Preliminary incubation count (PIC), 3) Laboratory pasteurization count (LPC), 4) *Staphylococcus aureus* (SA) count, 5) Coagulase negative staphylococcal (CNS) count, 6) Streptococci and streptococci-like organisms (SSLO) count, 7) Coliform count (CC) and 8) Gram-negative non-coliform (NC) bacteria. The findings of the study are summarized in Table 1. BTSCC are expressed as cells/ml while bacterial counts are expressed as cfu/ml.

Table 1. Somatic cell and bacterial counts in bulk tank milk

Test	Mean	Median	Mode	Std.error
BTSCC	363,214	347,500	9520-737,500	142830.39
SPC	7672	4193	180-62,825	796.48
PIC	22,913	12,250	500-139,750	2452.82
LPC	388	133	5-6400	66.65
SA	45	34	0-275	4.07
CNS	1246	693	60-15,175	173.86
SSLO	1390	890	15-11,402	152.62
CC	159	60	5-4130	41.81
NC	838	226	0-15,475	203.43

Key Words: bulk tank milk, milk quality, mastitis

1307 Production of exopolysaccharides by *Lactobacillus rhamnosus* RW-9595M: influence of carbon source and ratio carbon / nitrogen. M. I. Cote*[1], D. Roy[1], and J. C. Vuillemard[2], [1]*Food Research and Developement Centre,* [2]*Dairy Research Centre STELA*.

Lactic acid bacteria (LAB) produce exopolysaccharides (EPS) which have a beneficial effect on organoleptic properties of fermented dairy products. EPS from LAB may enhance the immune system. Furthermore, EPS from *Lactobacillus rhamnosus* RW-9595M stimulate the production of cytokines (IL-6 and IL-12 as well as TNF and IFN-γ). It seems to be a potential in these EPS to help a immune response which would favour both anti-infection and anti-allergy reactions. The aim of this work was to study the influence of carbon source and the ratio carbon/nitrogen on growth and EPS production by *L. rhamnosus* RW-9595M. Batch fermentations were carried in BMM (basal minimum medium) at 37°C, with a pH maintained at 6.0 and a constant agitation at 100 rpm. Optical density at 600nm, cell counts (CFU mL^{-1}), carbon consumption ($g.L^{-1}$) and EPS production expressed as total sugar ($mg.L^{-1}$) were measured. It has been observed that the nature and the concentration of carbon source influenced growth and EPS production by *L. rhamnosus* RW-9595M. EPS biosynthesis increased steadily when cells entered in stationary growth phase. The BMM supplemented with glucose or fructose+glucose (1:1) at a concentration of 40 $g.L^{-1}$ reached an EPS production of respectively 1457.3 $\pm$ 386.9 $mg.L^{-1}$ and 1205.4 $\pm$ 121.3 $mg.L^{-1}$ after 72 hours of fermentation. However, after 24 hours of fermentation, the EPS production rate was higher with fructose+glucose (1:1) 901 $\pm$ 177.2 $mg.L^{-1}$ as compared to 405 $\pm$ 31.6 $mg.L^{-1}$ for glucose. The ratio carbon/nitrogen was modified to enhance the EPS production by adding different concentrations of casamino acids or tryptone to BMM medium containing either glucose, fructose+glucose (1:1) or isoglucose (high fructose syrup) at 40 $g.L^{-1}$. Values of 1747.5 $\pm$ 192.7 $mg.L^{-1}$, 1913.1 $\pm$ 309 $mg.L^{-1}$ and 2184.7 $\pm$ 172.5 $mg.L^{-1}$ were obtained in term of EPS production after 72 hours of fermentation, by combining 2.0 $g.L^{-1}$ of tryptone to fructose+glucose (1:1), isoglucose and glucose respectively. After 24 hours of fermentation an EPS production of 1816.6 $\pm$ 95.9 $mg.L^{-1}$ was reached with isogluse and tryptone, compared to 1662.1 $\pm$ 135.8 $mg.L^{-1}$ with fructose+glucose (1:1) and tryptone and 1067.9 $\pm$ 275.4 $mg.L^{-1}$ with glucose and tryptone. These results confirm that *L. rhamnosus* RW-9595M is a highly EPS-producing strain.

Key Words: exopolysaccharides, Lactobacillus rhamnosus, fermentation

1308 Utilization of dot blots to screen probiotic Lactobacilli for mucin binding. J. Newman* and R. Jimenez-Flores, *California Polytechnic State University, San Luis Obispo, CA*.

Probiotics are live microbial food ingredients that benefit the host#s health, specifically, the gastrointestinal tract (GI). Mucin, a highly glycosylated protein, is a major constituent of mucus and therefore is present in the GI tract. Mucin is also present in the milk fat globule membrane. To be effective, probiotic organisms must adhere to intestinal cells to successfully colonize the intestine. Adherence to intestinal cells involves the ability to bind to mucin. The objective of this project was to develop a method to explore the mucin binding ability of probiotic *Lactobacillus* species. Bovine sub maxillary mucin, porcine stomach mucin and various milk fractions were diluted in series, in concentrations that ranged from 1-5% to 0.001-0.005%. A serial dilution was tested for each purified protein or buttermilk fraction. The proteins were immobilized on PVDF membrane using a dot blotter and then the membrane was blocked with gelatin. The different bacterial strains under study were biotinylated using EZ-Link^{TM}Sulfo-NHS-LC-Biotin. These labeled cells were used to wash the membranes containing the immobilized proteins. Streptavidin-horseradish peroxidase conjugate was used to develop the blot with o-phenylenediamine dihydrochloride substrate. Results demonstrate the ability of NCFM cells to bind to bovine mucin. Our work suggests this is an effective method to screen probiotic *Lactobacillus* species for mucin-binding ability.

Key Words: Probiotics, Mucin, Lactobacillus

1309 The use of a *Lactobacillus* cell extract in growth media designed for lactic cultures. H. Gaudreau*[1], C.P. Champagne[1], and P. Jelen[2], [1]*Food Research and Development Center,* [2]*Alberta University, Edmonton, Canada*.

Some probiotic bacteria grow poorly in milk. However, the addition of ruptured yoghurt cells to milk could potentially stimulate the growth of probiotic cultures in milk through hydrolysis of lactose into glucose and galactose, by hydrolysis of casein into peptides and amino acids and by the presence of growth factors contained in cell lysates. A crude cellular extract (CCE)of *Lactobacillus delbrueckii* subsp. *bulgaricus* was produced using a high pressure homogenizer. Lactase and proteolytic capacity of this extract and its ability to promote growth of probiotic strains was evaluated. After two passes throught the homogenizer (17 000 psi), 85% of initial cells were ruptured and lactase activity of the extract was maximum. With a 2g/L CCE supplementation of 6% sterile skim milk, 85% of initial lactose was transformed into glucose and galactose over a 12 h incubation period at 37°C. An automated spectrophotometric method was used to evaluate the growth-promoting properties of non-heated CCE and heated-CCE (121°C/5min) in MRS-type media, in comparison with yeast extracts and tryptones on the growth of 3 strains of *Lactobacillus acidophilus* and 2 *Lactobacillus rhamnosus* cultures. In heated-CCE, enzymes were inactivated. The growth of one strain of *Lactobacillus acidophilus* was non significantly different in media containing yeast extracts or heated-CCE but for all other cultures the growth-promoting properties of heated or non-heated CCE was poor. However, heated-CCE had better growth-promoting properties than non-heated CCE. Thus, thermolabile inhibitory compounds were found in non-heated CCE when MRS-type media were used. In pH-controlled fermentations of *Lactobacillus rhamnosus* R011 in 6% (w/w) sterile skim milk with or without 2 g/L non-heated CCE, biomass obtained in the two media was almost the same. However, time required

to obtain maximal biomass (fermentation time) was much shorter in the CCE-enriched milk. Moreover, 2 times more lactic acid was produced in supplemented milk than in native milk.

Key Words: Starters, Media, Lactobacillus

1310 Development and validation of immunological approachs for the evaluation of probiotic adhesion to Caco-2 cells. Gwenaelle Le Blay*, Melanie Gagnon, Christophe Lacroix, and Ismail Fliss, *Dairy research centre (STELA), Laval university.*

Probiotics are widely recognised as beneficial for human health. Amongst the different criteria for selecting them, attachment to the intestinal mucosa remains one of the most important. Indeed, this ability is regarded as essential for probiotics to exert their beneficial effects, such as the exclusion of entero-pathogenic bacteria or immunomodulation of the host. Due to the difficulties of assessing adherence of probiotics strains *in vivo*, *in vitro* adherence assays have been developed. *In vitro* adhesion to Caco-2 cells has been extensively used to select for adhesive properties. Different methods have been developed for detecting highly adherent probiotic strains. The principal ones are Gram staining and radiolabelling. These methods successfully detect probiotic adherence, however, they present several limitations. The Gram staining can not differentiate bacteria with the same Gram staining and the extensive washing steps may influence the determination of the adherence potential. As for the radiolabelling assay, which is more accurate but more time consuming, it can not either distinguish between different bacterial strains in competition studies. In this study, we propose to use an Enzyme Linked Immunosorbent Assay (ELISA) and the histo-immunostaining assay to quantitavely and qualitatively study the adhesion of different strains of probiotic bacteria and pathogens to Caco-2 cells. Different antibodies were then produced in order to specifically detect target bacterial strains. These specific antibodies were labelled with different molecules including enzymes and fluorochromes, and used for the simultaneous and specific detection of different bacterial species attached to Caco-2 cells. Results were compared to those obtained by the traditional microbiological method using specific selective media. Both immunological techniques were shown to be very effective. They were safe, economic, and have the high advantage to allow the manipulation and comparison of different bacterial species simultaneously.

Key Words: Probiotic, Adhesion, Immunological approachs

1311 Development of endospore-specific primers for the TRFP analysis of microbial populations in milk powder. M. M. Arendts*, A. J. Rife, and R. Jimenez-Flores, *California Polytechnic State University, San Luis Obispo CA.*

A comprehensive risk assessment of the microbial quality of milk powder should include information of endospores as well as viable bacteria. Current methods for detection for endospore contamination in milk and dairy products are labor intensive and time consuming. Molecular methods offer a unique and sensitive tool for rapid microbial detection over traditional methods. Previous work in our laboratory has focused on the study of viable bacterial populations using 16s rDNA primers that can be detected via Terminal Restriction Fragment Patterns (TRFP). The overall objective of this work is to study microbial populations, including a specific focus on exclusively endospore forming bacteria, by utilizing a combination of primers in the TRFP. One set of primers, specific for 16s RNA, has been successfully used in several experiments to assess microbial ecology of milk powder. The second set, designed for the exclusive detection of endospore formers, is the specific objective of this work. Two genes of endospore forming bacteria were potential targets for primer development, SpoIIA and GerC3. SpoIIA shows lower exclusivity since it does not distinguish between endospore formers other bacteria present in milk samples. The GerC3 primer set is specific for the germination gene in *Bacillus subtilis*, and was shown to amplify 70% of the endospore forming strains of the Dairy Products Technology Center (DPTC) library. However, the designed degeneracy of these primers represent a problem for positive identification of TRF patterns. Because this gene has not been sequenced in the most common endospore forming bacteria found in milk powder we undertook the task of sequencing fragments for re-design of these GerC3 primers.

Key Words: Endospore, Milk Powder, Terminal Restriction Fragment Patterns

1312 Survival of a five strain cocktail of E. coli O157:H7 during thermalization and the 60 days aging period of hard cheese made from unpasteurized milk. J. Schlesser*[1], J. Dunn[2], K. Madsen[2], and R. Gerdes[2], [1]*Food and Drug Administration, NCFST, Summit-Argo, IL,* [2]*Illinois Institute of Technology, NCFST, Summit-Argo, IL.*

The purpose of this study was to investigate the adequacy of the 60-day minimum aging to eliminate the foodborne pathogens in hard cheese made from unpasteurized milk. Hard cheese was made from unpasteurized milk inoculated with 10^{3} cells/ml of a five-strain cocktail of acid-tolerant *E. coli* O157:H7 (Strain Numbers: 43895, SEA 13B88, 932, C7927, and ENT 9490). Samples of unpasteurized milk, curd and whey were collected during the cheese manufacturing process. After pressing, the blocks of hard cheese were packaged into plastic bags, and sealed with a vacuum-packaging machine, and aged at 7^{o} C. After 1 week, the cheese blocks were cut into smaller uniform-sized pieces, and vacuum sealed in clear plastic pouches for ease of sampling at the various aging intervals. Samples were plated and enumerated for *E. coli* O157:H7 using BCM# for *E. coli* O157:H7 (+) Plating Medium. Populations increased to 10^{4} in the drained curd and to 10^{5} at milling and pressing. Populations of *E. coli* in cheese aged for 60 and 180 days at 7^{o} C decreased by less than 1 log and 3 logs, respectively. Low levels of *E. coli* O157:H7 could still be detected at 360 days of aging. Cheese runs conducted with unpasteurized milk inoculated with *E. coli* O157:H7 at the 10^{1} level showed similar results. These studies appear to confirm prior reports in the literature that suggest 60-day aging may be inadequate to eliminate *E. coli* O157:H7 during cheesemaking. Thermalization, a sub-pasteurization heat treatment, was evaluated as a method to improve the safety of hard cheeses made from unpasteurized milk Thermalization runs at 148^{o} F (64.4^{o} C) for 16 seconds were conducted on unpasteurized milk inoculated with *E. coli* O157:H7 at 10^{5} CFU/ml. A 5-D *E. coli* O157:H7 reduction by thermalization was shown. After pre-enrichment and enrichment of the 25-ml thermalized milk sample, growth of *E. coli* O157:H7 was observed suggesting very low levels of survivors or recovery of cells injured by the heat treatment. Thermalization appears to be a process that would improve the safety of hard cheeses made from unpasteurized milk.

Key Words: raw milk cheese, thermalization, *E. coli* O157:H7

1313 Microbiological analysis of processor obtained milk samples: Experimental determination of shelf-life. Todd Pritchard*[1] and Emmanuelle Monteith[2], [1]*Northeast Dairy Foods Research Center, Burlington, VT,* [2]*Dept. Nutrition and Food Sciences, University of Vermont, Burlington, VT.*

Grade A fluid milk is subject to the regulations put forth in the Pasteurized Milk Ordinance (PMO). The PMO includes a microbiological standard of less than 20,000 CFU/ml. This standard is based on quality, not safety, related issues. The goal of our research was to determine what percentage of milk samples maintained at ideal temperature conditions were still within the PMO code for microbiolgical quality at the carton encoded sell by date. A total of 204 milk samples were directly obtained from 12 of 13 fluid milk processors in the Maine-New Hampshire-Vermont region of New England during the period of April 2001 through December 2001. The milk was maintained below 41F at all times during collection and evaluation. Analysis of the samples included a preliminary coliform evaluation and a total plate count evaluation over the time period from collection until the carton encoded sell by date. Coliform and total plate counts were determined utilizing 3M CC petrifilm and 3M AC petrifilm respectively. Fifty-one percent (104/204) of the samples were still within PMO code at the carton encoded sell by date. The number of samples meeting PMO code varied by plant with a low of 19% to a high of 83.3% passing. An evaluation of the samples meeting PMO code also revealed that there was variation from state to state. An evaluation of the samples which failed to meet PMO code indicated that 60 of the samples were still within PMO code at least 7 days post processing and that among these samples, 14 were still within PMO code at least 10 days post processing. The overall percentage of samples within PMO code at least 7 days post production was calculated to be 80.4% and 57.8% of the samples were still within PMO at least 10 days post production. Our results indicate that some processors may wish to re-evaluate the shelf life of their products. Furthermore,

consumers may wish to utilize milk as close to the day of purchase as possible to ensure they are consuming the highest quality milk available.

Key Words: milk, quality, shelf-life

1314 Characterization of the novel lactococcal food-grade vector pRAF800 based on melibiose fermentation. I. Boucher*, C. Vadeboncoeur, and S. Moineau, *Universite Laval, Quebec, Canada.*

A food-grade plasmid vector named pRAF800 was developed for the genetic engineering of industrial *Lactococcus lactis* strains using: 1) the melibiose fermentation phenotype conferred by the *Lactococcus raffinolactis aga* gene encoding an alpha-galactosidase; 2) the minimal replicon of *L. lactis* plasmid pSRQ800 to ensure plasmid maintenance. pRAF800 is therefore constituted of two divergently oriented genes separated by non-coding regions, each of them containing a unique cloning site. The expression profile of pRAF800 was monitored by RT/PCR in *L. lactis* MG1363. Results indicated that the *aga* gene would be expressed from the putative promoter located immediately upstream (TTGACA-N17-TATAAT) and would terminate at non-specific sites located in the *rep*B gene encoding the replication initiator. Similarly, the *rep*B transcript is likely to initiate at a promoter located upstream, in the replication origin, and would encompass *aga* to end at a transcriptional terminator identified upstream of the *aga* promoter. This particular transcription profile suggests multiple avenues for the exploitation of pRAF800. One of the two proposed cloning sites offers the opportunity to express cloned genes from the plasmid promoters while the other site might be adapted for the expression of genes from their own promoter. Expression of *aga* from pRAF800 was also examined in the *L. lactis* industrial strain SMQ-741 by enzymatic activity measurement. Alpha-galactosidase activity was induced by galactose and melibiose but not by glucose or lactose, indicating that a gene regulation is present. Consequently, the introduction of pRAF800 into an industrial *L. lactis* strain should not cause a metabolic burden to the cells during the manufacture of the starter culture as well as during the milk fermentation where lactose is the principal energy source.

Key Words: *Lactococcus lactis*, Food-grade cloning vector, Alpha-galactosidase

1315 Bifidobacteria protection study using whey protein matrice. Viel Louise-Marie*[1−2], Fliss Ismail[1], and Subirade Muriel[1−2], [1]*Centre de recherche STELA (Université Laval)Québec, Canada,* [2]*Functional Food and Nutraceutical Institute (INAF) (Université Laval) Québec, Canada.*

Bifidobacterias are probiotics which, when ingested in sufficient dose, are likely to provide many beneficial effects to the health. Numerous studies were performed on these bacterias and showed that at least 10E6 to 10E8 bacterias/g must reach the colon alive to have a significant effect. However, bifidobacterias are particularly vulnerable to acidic pH of the stomach which inhibits their biological effect. Different strategies were developed to encounter this problem and maximise cell viability. A simple one is to higher the amount of ingested bacterias for a maximum resistance to the acidic pH of the stomach to reach the colon alive. A better solution is to protect the cells by means of encapsulation. Many matrices were elaborated based on natural polymers. Despite being non-toxic and low cost, many of those matrices are reversible in phosphate buffers and most of them use organic solvents and high temperatures when they are made. Factors very deadfull for the incorporated cells. Recently, a new method based on emulsification and cold gelation process of whey proteins was elaborated in our research group (patent in press). The objective of the present project is to determine the impact of process parameters on the viability of bacterial cells incorporated in this matrice. *B*ifidobacterium *l*ongum ATCC 15707 cells were grown in modified MRS broth (0,05% L-cycteine HCl) and added in the matrice at the beginning of the matrice formation process. The pour plate method was used to follow cells viability and compared to non-encapsulated cells. Optimal process conditions were then determined with the lowest death rate. Data were analyzed using a 4 x 4 x 3 replications completely randomized factorial design. An analysis of variance was conducted. The predetermined acceptable level of probability was 5% ($P \leq 0{,}05$)for all comparisons. In the following presentation, results of the impact of process parameters on viability of probiotic bacterias (*B*ifidobacterium *l*ongum) will be exposed.

Key Words: bifidobacteria, protection, whey protein

1316 Factors influencing cell count of a probiotic *Lactobacillus crispatus* strain. Kevin Bourzac*, Ann Bernard, Dr. M. E. Sanders, and Dr. Rafael Jimenez-Flores, *California Polytechnic State University, San Luis Obispo, CA.*

The probiotic bacteria *Lactobacillus crispatus* strain HP101 was derived from a fermented dairy product in eastern Europe. Commercialization of this strain has been a challenge due to its poor growth characteristics in standard *Lactobacillus* media. The objective of this work was to analyze factors influencing growth and cell count in different media to improve the commercialization potential of this strain. Compared to the successful industrial *Lactobacillus acidophilus* strain NCFM, HP101 exhibits 2-3 log cycle fewer CFU/ml in MRS media although optical density measurements are equivalent. HP101 cells were also observed to have different morphology than NCFM when grown in MRS. NCFM cells were short, compact rods where HP101 were long and spindly (often associated with unhealthy cells). A live/dead staining procedure also indicated that a high percentage of HP101 cells were damaged or dead when grown on MRS for 24hrs. Growth in milk completely reversed the negative HP101 growth parameters. Cell morphology, cell health (as determined by the live/dead stain), and final cell count became equivalent to that of NCFM. However, since cells are not easily recovered from milk media, it is unsuitable for industry use. Therefore, applicable media adjustments Which mimicked results from growth in milk were determined. Our experimental methods included growth curve analysis, colony counts, live/dead stain analysis, peptide analysis of media and 2-D gel electrophoresis. Milk permeate supplemented with $\geq$0.3 % casein resulted in CFU and cell morphology that mimicked milk-grown cells.

Key Words: Probiotics, Permeate, Lactobacillus

1317 Production of lactic acid and antimicrobial compounds from cheese whey. A. Shahbazi* and S.A. Ibrahim, *North Carolina Agricultural and Technical State University, Greensboro, NC.*

Whey is an important by-product from the cheese manufacturing industry. Typically, 100 pounds of milk yield 10 pounds of cheese and 90 pounds of liquid whey. Disposal of liquid whey is costly due to its high BOD content. Whey is a rich source of nutrients. Many different value added products such as lactic acid, vitamins, proteins and functional ingredients can be produced from cheese whey. Developing these processes will help to reduce the waste treatment cost and add to the profitability of the dairy industry. The purpose of this project was to determine the ability of spiral-sheet membrane to immobilize bifidobacteria and to determine the performance of a spiral-sheet immobilized bacterial reactor for the production of organic acids (lactic and acetic acids) and antimicrobial compounds using cheese whey. *Bifidobacterium bifidum* (NCFB 1454) obtained in freeze-dried form was propagated by weekly transfers (2% vol/vol) in trypticase-peptone-yeast extract (TPY). A 10-liter culture was prepared and immobilized into a spiral sheet membrane in a 10-liter cylindrical bioreactor. Immobilization was achieved at room temperature (23 C) within 24 hrs. Fermentation experiments were conducted with 4.8% lactose and 4% TPY broth. When no pH control was used, the pH dropped from 6.5 to 3.8, which inhibited the bacterial activity in the bioreactor. As a result, during the subsequent experiments the pH was controlled, and it was adjusted to 6.5 by neutralizing the acid with 5N ammonium hydroxide. Samples were collected every 6 hours and were analyzed for lactic acid using HPLC, and for the production of antimicrobial compounds using the bioassay. Under the controlled temperature and pH conditions, the bioreactor effectiveness was measured as 37% conversion of lactose to lactic acid within the first 24 hours and 67% conversion rate within 48 hrs. We expect a higher conversion rate under a longer fermentation time. Our results indicate that immobilization of bifidobacteria on a spiral-sheet reactor could be used for the continuous production of lactic acid and antimicrobial compounds.

Key Words: Bifidobacteria, Immobilization, Spiral-sheet reactor

1318 Inhibition of Lactococcus lactis ssp lactis ml3 and c2 bacteriophage proliferation by chelation of Ca^{2+} with monosodium glutamate. C. L. Hicks* and I Surjawan, *University of Kentucky, Lexington, KY 40546-0215.*

Calcium (Ca^{2+}) is required for the replication of many lactococcal phages and for efficient adsorption of the phage to the host cells. Monosodium glutamate (MSG)(2.0%) chelated Ca^{2+} in the medium (M17) to inhibited c2 phage (10^5 pfu/ml) attachment and proliferation on host *Lactococcus lactis* ssp. *lactis* C2 (10^8 cfu/ml). The effect of MSG on ml3 phage attachment was similar. Phage inhibition tests were conducted by growing *L. lactis* ssp *lactis* C2 in M17 medium (10mM Ca^{2+}) with or without 2% MSG, and infecting the culture with various ml3 phage titers (10^4, 10^6, and 10^8 pfu/ml) after 15 min incubation. Culture growths was monitored by recording the absorbance (λ_{600nm}) every 10 min. At the highest ml3 phage infection level (10^8 pfu/ml), lysis of host cells occurred quickly after 2 h of incubation. When MSG (2.0 %) was added to M17 medium, cell lysis was delayed for 30 min. MSG, had a greater inhibition on cell lysis when phage titers were reduced. Cell lysis was delayed for 50 min at a phage titer of 10^6 pfu/ml and no lysis occurred when the phage titer was reduced to 10^4 pfu/ml. Inhibition of ml3 and c2 phage proliferation were compared with and without MSG present in the medium. When *L. lactis* ssp *lactis* C2 was grown in M17 (10mM Ca^{2+}) with and without 2% MSG and infected (after 15 min) with either ml3 or c2 phage (10^5 pfu/ml) both ml3 and c2 phage proliferation were inhibited equally. Lysis of host cells grown in the medium without MSG occurred after 190 min incubation. When C2 was grown in the M17 containing 2% MSG and infected (either ml3 or c2) with 10^5 pfu/ml the culture was able to reach the stationary phase and no lysis was observed. Addition of additional calcium (40 mM) restored c2 ability to lysis the host culture. These experiments suggested that 2% MSG bound most of the Ca^{2+} in M17 medium and was responsible for the inhibition of ml3 and c2 phage proliferation.

Key Words: Lactococcal phage, MSG, Inhibition

1319 Study of the attachment of Hepatitis A virus (HAV) to stainless steel, copper, polyethylene and PVC surfaces. Irena Kukavica-Ibrulj*[1], Andre Darveau[2], and Ismail Fliss[1], [1]*Dairy Research Centre STELA, Laval University,* [2]*Biochemistry department, Laval University.*

Hepatitis A virus (HAV) is a frequent cause of food-borne infections world-wide. Many outbreaks have been commonly associated HAV with waste water or food which are served raw or only lightly cooked, such as shellfish, fruits and vegetables. A great number of reports have suggested that infected human food handlers and/or use of contaminated water may play an important role in food and surface contamination. There is no clear evidence in the literature that enteric viruses are capable to attach on solid surfaces. This study was designed to investigate the ability of HAV to attach to various food contact surfaces. An immunofluorescent method using confocal microscopy was developed for detection of HAV attached. The attachment ability of HAV was studied as a function of the type of solid surfaces (stainless steel, polyethylene, PVC and copper), temperature (4 and 20C) and contact time (2 and 4 hours). HAV was shown to attach to all four surfaces tested. This attachment level depends on the initial viral concentration and the incubation temperature. The highest attachment was obtained at 4C after a short contact time. Mechanisms implicated in this attachment phenomenon was studied by determining the surface energy values of the studied materials. The total surface energy, the Lifshitz-Van der Waals (LW) and the short range (SR) hydrogen bonding components of surface energy have been derived from contact angle determinations with help of an extended Young equation. The calculation of these parameters indicated an attractive nature of the interaction potential of the HAV attachment. In conclusion, this study confirms the attachment capabilities of HAV to different surfaces currently used in food industry. The comprehension of the HAV attachment mechanisms will permit to establish the solid base necessary to put in place better disinfection programme and consequently, reduce viral food intoxication incidence.

Key Words: Hepatitis A Virus, Attachment, Surface Energy

1320 Molecular characterisation of lactic acid bacteria of Ragusano cheese. L. Corallo[1], R. Gelsomino[1], P.S. Cocconcelli[2], P. Campo[1], S. Carpino*[1], and G. Licitra[3], [1]*Consorzio Ricerca Filiera Lattiero-Casearia, Ragusa, Italy,* [2]*Ist. di Microbiologia e Centro Ricerche Biot., Università Cattolica, Piacenza e Cremona, Italy,* [3]*D.A.C.P.A., Catania University, 95100 Catania, Italy.*

The purpose of this work was to study the natural bacterial community involved in Ragusano cheese production and the evaluation of thermal treatment as selective steps which allow selection from environmental microbiota of lactic acid bacteria responsible for Ragusano fermentation. To perform this study molecular techniques were applied to analyse bacterial population dynamics during the fermentation process of Ragusano. The analysed cheese was produced at farm level in three cheese factories from raw milk during the spring pasture of the Hyblean region of Sicily. Samples of curd, curd after first and second scalding steps, curd after 24 h of ripening and curd after stretching were immediately analysed by serially diluting in peptonate water and plating on agar media. In order understand the effect of technology on bacterial population dynamics, 1257 selected colonies of Gram positive were isolated from M17 and MRS plates, 400 from curd samples, 224 isolates from the second cooking step, 345 from 24 h ripened curd and 288 after the stretching process. The application of RAPD methodology made it possible to follow the growth kinetics of dominant strains composing the bacterial community of Ragusano and to allow the identification of 40 different bacterial populations in the three studied production processes. The taxonomical position of all the 40 biotypes was achieved by means of sequence analysis of at least 400 bp of the 5# region of the 16S rDNA gene. In curd after first scalding, Lactococcus lactis subsp. lactis and strains from Enterococcus faecium and Enterococcus durans were the isolated species. The community of curd after the second cooking step was modified and Streptococcus suis was found to be the most represented species. After the 24 hours of ripening, when the pH of curd dropped to 5.5, a modification in the bacterial community was observed and E. faecium and heterofermentative lactobacilli belonging to the L. reuteri species were identified by means of 16 S rDNA sequencing. After the stretching, variations in the natural bacterial association occurred and the most strains present were identified as Strep. macedonicus, E. faecium, E. durans, L. paracasei and L. Reuteri.

Key Words: Molecular characterisation, lactic bacteria, Ragusano cheese

1321 Cloning of heterologous pedA1 in different microbial systems. L. Beaulieu*[1,2], J-F. Jette[2], L. Laramee[2], C. Miguez[2], D. Groleau[2], and M. Subirade[1], [1]*STELA Dairy Research Centre,* [2]*Biotechnology Research Institute.*

Antimicrobial cationic peptides are important components of the innate defense mechanism of all life species. Many organisms, including fungi, insects, amphibians and humans, produce hydrophobic and amphipathic peptides which exhibit antibiotic, fungicidal, hemolytic, virucidal and tumoricidal activities by interaction with the membranes of living cells and, on this basis, many are being developed for use as a novel class of antimicrobial agents. For example, bacteriocins produced by lactic acid bacteria are presently being investigated for their potential utilisation as food preservatives. Although the bacteriocins produced by many lactic acid bacteria have an important role as future food biopreservatives, there are limitations to their practical use, such as a narrow antimicrobial spectrum, low level/unstable production, or the inability of some producing strains to grow in certain foods. Because natural producing strains tend to produce low bacteriocin quantities and because chemical peptide synthesis is expensive, industrial or therapeutical applications of bacteriocins are limited. Nevertheless, the rapid growth in our understanding of the genetics of many of these bacteriocins has provided us with the tools for considering over expression of natural bacteriocin genes, or that of engineered variants of these genes, to obtain improved bacteriocins, using unnatural producing strains capable of high protein production. Such heterologous bacteriocin production might, therefore, be used to greatly increase the usefulness of these antibacterial compounds and at the same time provide us with an interesting tool for better understanding the mechanism of bacteriocin activity and structure-function relationships. The pediocin PA-1 (*pedA1*) has been inserted in expression vectors (pCM110, pPICZαA) and cloned in *Escherichia coli*, *Methylobacterium extorquens* and *Pichia pastoris*. Expression levels are being tested using anti pedA1 polyclonal antibodies.

Key Words: Bacteriocin, Cloning, Protein expression

1322 ***In vitro* and *in vivo* inhibition of vaginal Group B *Streptococcus* (GBS) by bifidobacterial strain of human origin.** Josee Beaulieu*[1], Naceur Naimi[2], Denis Richard[2], Yvan Boutin[3], and Ismail Fliss[1], [1]*Dairy Research Centre STELA, Universite Laval, Quebec, Canada,* [2]*Centre for Research on Energy Metabolism, School of Medicine, Universite Laval, Quebec, Canada,* [3]*Transbiotech, Cegep de Levis-Lauzon, Levis, Canada.*

Group B *Streptococcus* (GBS), *Streptococcus agalactiae*, is considered to be problematic in countries all over the world. In fact, 2 or 3 out of every thousand newborns are infected with this bacterium and in 15 to 20% the infection leads to death due to severe meningitis. GBS is mostly found in the genital tracts of pregnant women and can be transmitted to newborns during labour.
In this work, the inhibition of GBS by strains of bifidobacteria was investigated *in vitro* as well as *in vivo* in an animal model. Different strains of bifidobacteria of human origin were screened for their potential to inhibit GBS. An exopolysaccharide-producing isolate (UL-2), exhibiting a high inhibitory effect against GBS and showing a good adherence to HeLa cells was selected. At pH 5.0, this isolate adhered to HeLa cells 25-fold higher than at pH 7.4. This adherence capacity was 1,6 and 3,8 times higher than that of GBS at pH 7.4 and 5.0 respectively. When UL-2 was added at a concentration of 1 X 10^7 CFU/mL, GBS adhesion to HeLa cells was inhibited by 61% and 69,5% at pH 5.0 and pH 7.4 respectively. However, a complete inhibition was obtained when UL-2 was added at a concentration of 1 X 10^9 CFU/mL. Finally, the *in vitro* inhibition of GBS by UL-2 was validated *in vivo* by using an animal model simulating GBS vaginal infection. The efficiency of UL-2 was evaluated by microbiological, immunological, histological and metabolic analysis.

Key Words: Human bifidobacterial strain, Group B *Streptococcus* inhibition, Animal model

1323 Effectiveness of cleaning and sanitizing agents against a biofilm of lactobacilli isolated from slit-defected Cheddar cheese. Cecilia Golnazarian* and Catherine Donnelly, *University of Vermont, Burlington, VT.*

Slit-defect in long-hold Cheddar cheese results in loss of salable product. Strains of lactobacilli with the ability to slit cheese have been identified and characterized for the purpose of developing a procedure for the elimination of this problem. The objectives of this research were to study the ability of the strains to form biofilm on stainless steel, and determine if commonly used cleaning and sanitizing agents would eliminate the biofilm.
Selected lactobacillus strains were inoculated, as a cocktail and individually, into containers of sterile milk with stainless steel coupons in each, then held at 31oC for 20 days. The containers were drained, and sterile milk was added on day 5, 10 and 15. On day 20, the coupons were rinsed, cleaned and/or sanitized as follows: 1) rinsed with vortexing five times in sterile phosphate buffered saline (PBS); 2) rinsed then washed in cleaning agent using agitation; 3) rinsed, cleaned, then soaked in 25C sanitizer; 4) rinsed, cleaned, then soaked in 72C sanitizer. Treatments were followed by three rinses in buffer, and a fourth rinse with agitation in Lactobacillus MRS broth. The final rinse was plated for bacterial counts and the tubes incubated for 24 hours. One coupon from each treatment was examined by scanning electron microscopy.
Growth plates for treatment one were too numerous to count (TNTC). Bacterial counts for treatments two averaged between 1.50 # 2.25 log CFU/ml, depending on agents applied. Bacterial counts for treatment 3 and 4 were <100 CFU est/ml. However, the final rinses in MRS (used to inoculate the growth plates) were turbid with growth within 24 hours. The scanning electron micrographs illustrated the presence of biofilm, as well as an apparent injury effect of cleaning and sanitizing agents on the formation of the films. Although none of the tested agents were able to eliminate the presence of the biofilm, there were noticeable differences in their effect.

Key Words: lactobacilli, Cheddar cheese, biofilm

1324 Incidence of *B. cereus* spore in raw milk by membrane filtration. Yoosoung Shin* and Heidi Schraft, [1]*University of Guelph.*

In order to determine the incidence of *B. cereus* spore in raw milk, a total of 78 raw milk samples from various sampling sites, 12 environmental swabs and finished pasteurized milk were collected.
Spores of *B. cereus* in bulk tank milk of dairy farms were enumerated to be 1-37/60 ml. The incidence of *B. cereus* spores was varied with 1-222 spores and 6-263 spores/60 ml for raw milk of delivery truck and silo tank, respectively. By enrichment of heat-treated milk, *B. cereus* spores were enumerated to be 1.0×10^1-7.4×10^7 ml^{-1} for bulk tank milk samples, 1.1×10^2-2.1×10^7 ml^{-1} for truck samples, and 1.0×10^1-4.6×10^7 ml^{-1} for silo samples, respectively. Finished pasteurized milks were contaminated with *B. cereus* by numbers of 0.5×10^1-6.3×10^4, 1.0×10^4-3.2×10^7, and 1.9×10^6 - 3.4×10^7 cfu ml^{-1} for enrichment at 7C for 14 days, 10C for 7 days, and 40C for 2 days, respectively. Environmental swab samples contained *B. cereus* with range 0.5×10^1-8.2×10^8 cfu ml^{-1} by up to 24 hours enrichment at 30C.
Overall, the incidence of *B. cereus* spore was found to be higher in silo and delivery truck than bulk tank in dairy farms. There is a tendency to find higher numbers of psychrotrophic *B. cereus* in raw milks of delivery truck and silo than in bulk tank on the farms. The result suggests the possibility of*B. cereus* residue on the surface of raw milk handling equipment after C.I.P.

Key Words: *B. cereus*, Raw milk, Membrane filtration

Forages and Pastures
Grasses and Legumes

1325 Effects of camphene, myrcene, caryophyllene oxide, and β-pinene on consumption of alfalfa pellets by sheep. R. E. Estell*[1], E. L. Fredrickson[1], D. M. Anderson[1], K. M. Havstad[1], and M. D. Remmenga[2], [1]*USDA/ARS Jornada Experimental Range,* [2]*New Mexico State University Statistics Center.*

Four experiments were conducted to examine effects of individual terpenes on alfalfa pellet intake by lambs. Forty-five lambs (9 lambs/treatment) were individually fed alfalfa pellets sprayed with either camphene, myrcene, caryophyllene oxide, or β-pinene at one of five concentrations in an ethanol carrier. Treatments (0, .5, 1, 2, and 10X) were multiples of the concentration (X) of a specific terpene on the leaf surface of *Flourensia cernua*. Terpenes were applied to alfalfa pellets (.64 $kg.lamb^{-1}.d^{-1}$, DM basis), and consumption was measured during a 20-min interval for 5 d. Lambs were adapted to handling and individual pen feeding for 10 d and were maintained and fed alfalfa pellets in one group (except during 20-min tests) at a mean total daily intake of 4.7% of BW (DM basis). Camphene and caryophyllene oxide tended to decrease intake (linear contrasts were $P = .0651$ and $P = .0504$, respectively), while myrcene and β-pinene exerted no effect on consumption of alfalfa pellets by lambs during the 20-min interval. Camphene and caryophyllene oxide may be involved in the differential herbivory of individual tarbush plants by livestock.

Key Words: Herbivory, Intake, Terpenes

1326 Influence of sward height, daily timing of concentrate supplementation and grazing time management on intake, digestibility and grazing efficiency of lactating beef cows. O.J. Gekara*, E. C. Prigge, W. B. Bryan, E. L. Nestor, and W. V. Thayne, *West Virginia University, Morgantown, WV.*

Thirty-two crossbred lactating beef cows were randomly assigned to two Kentucky bluegrass/white clover sward height (SH) treatments, either 4 to 8 cm or 10 to 12 cm, replicated four times. They were fed a concentrate supplement (T) (4.1 kg DM $animal^{-1}d^{-1}$) at 0700 (AM) or 1800 (PM), and either restricted (R) to 12 hrd^{-1} (0700 to 1900) grazing or allowed (U) to graze 24 hrd^{-1} (MGT). The experiment was repeated over three 15-d periods in May, July and August 2000. Mean SH of continuously stocked pasture throughout the experiment was 6.0 and 9.9 cm for low and high SH, respectively. The high SH treatment herbage was higher ($P < 0.05$) in fiber components and lower in CP compared to low SH pasture. An interaction, T x MGT ($P < 0.05$), on forage DM