T282 Survival of *Bifidobacterium animalis* ssp. *lactis* BB-12 in yogurt drink is influenced by timing of probiotic addition. Z. Ba*, E. J. Furumoto, and R. F. Roberts, *Department of Food Science, Pennsylvania State University, University Park.*

Probiotic containing yogurts and yogurt-based drinks have become increasingly popular for their potential health benefits. Viability of the probiotic throughout the shelf life of these products is a critical quality parameter. The effect of time of bacterial addition into the product on survival has not been well studied. As part of an ongoing study designed to compare the efficacy of *Bifidobacterium animalis* ssp. *lactis* BB-12 (BB-12) delivered by yogurt-based smoothie drink and tablets on immune status, fecal transit time, and fecal microbiota, the timing of addition of the probiotic on survival of BB-12 in the yogurt drinks over 4 weeks of storage at 4°C was evaluated. The 2 yogurt drink treatments, (A) BB-12 added after yogurt fermentation and (B) BB-12 added before fermentation were formulated to have 23% total solids and <1% fat. The target concentration of BB-12 per 240-gram serving was log 10 ± 0.5 cfu, commonly considered an effective daily dose. Three bottles of each yogurt drink from 6 batches were analyzed. Population of BB-12 was determined by pour plating method on selective medium MRS-NNLP followed by anaerobic incubation at 37°C after 0, 1, 2, 3, and 4 weeks of storage at 4°C. No statistical difference in the population of BB-12 between treatments A and B was detected immediately following manufacture (wk 0) (initial counts of log 10.52 ± 0.05 cfu/serving and log 10.49 ± 0.14 cfu/serving, respectively). As expected the population of BB-12 declined throughout the shelf life of the products. However, the population decreased faster in treatment A than in treatment B resulting in a significant difference after 2 weeks of storage. This trend continued and at the end of shelf life the BB-12 concentration had decreased significantly in both treatment A (log 9.64 ± 0.06 cfu/serving) and treatment B (log 10.18 ± 0.13 cfu/serving) after 4 weeks of storage. The BB-12 survived significantly better in B than that in A ($P = 0.000$) indicating that BB-12 survives better when added before fermentation, possibly as a result of adaptation to the acidic environment.

Key Words: BB-12, survival, yogurt drink

T283 Pectin-whey protein microparticles containing probiotics: Release and survival of *Lactobacillus acidophilus* La5 in simulated gastrointestinal conditions. C. Gebara, K. S. Chaves, M. C. E. Ribeiro, F. N. Souza, C. R. F. Grosso, and M. L. Gigante*, *University of Campinas, Campinas, SP/Brazil.*

Once microparticles produced by ionotropic gelation are porous, the coating of particles with different materials has been proposed to increase the protective effect for the delivery of probiotics. The aim of this study was to evaluate the release and survival of *Lactobacillus acidophilus* La5 microencapsulated by ionotropic gelation with Ca^{2+} using pectin as wall material, and covered by electrostatic interaction with whey protein heat treated (80°C/30 min) or without heat treatment when exposed to conditions simulating the passage through the gastrointestinal tract. The simulated conditions were assayed with artificial gastric juice, at pH 3.0 with addition of mucin and pepsin at 37°C for 120 min, followed by artificial intestinal juice at pH 7.0 with addition of pancreatin for 300 min. A randomized block with 3 replications was used. The effect of time of exposure to simulated gastric juice and simulated intestinal juice on the viability of free and microencapsulated *Lactobacillus acidophilus* was assessed by ANOVA and the significant differences were evaluated by Tukey's test at 5% significance level. Microencapsulation conferred greater protective effect to *Lactobacillus acidophilus* La5 as compared with the free cells. However, the coating of pectin microparticles with whey protein did not confer additional protection to probiotics when exposed to simulated gastrointestinal conditions. The pectin microparticles remained intact when exposed at pH 1.2, 3.0 and after 300 min at pH 7.0. On the other hand, both microparticles coated with whey protein heat treated or without heat treatment have remained intact for 120 min exposure to simulated gastric juice but have disintegrated after 300 min exposure to simulated intestinal juice (pH 7.0). This occurrence suggests that the probiotics would be released in a different part of the intestinal tract whether delivered by one microparticle or another. Acknowledgments: FAPESP.

Key Words: probiotics, microencapsulation, microcapsules

T284 Influence of some medicinal spices on the bile tolerance of *Streptococcus thermophilus* ST-M5. M. Sanchez-Vega*[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.*

Spices such as onion, garlic, turmeric, and ginger are known for their medicinal properties such as being antimicrobial, and their potential for the treatment of cancer and cardiovascular diseases. *Streptococcus thermophilus* is a culture bacterium having health beneficial effects. Bile tolerance is an important probiotic characteristic. Although most studies use non water soluble spice extracts, the effect of pure spice juice on bacterial performance is not known. The objective was to elucidate the effect of spices on the bile tolerance of *Streptococcus thermophilus* ST-M5. Bile tolerance of *S. thermophilus* was analyzed using MRS broth supplemented with 0.3% (wt/vol) oxgall and 1% (vol/vol) of freshly extracted spice juice. Sample without spice juice acted as a control. Samples were incubated at 37°C for 5 h, in which they were removed hourly for plating for plating. Growth was determined by plating every hour for a period of 5 h. *S. thermophilus* was incubated aerobically at 37°C for 48 h. Data were analyzed using Proc Mixed model with a Tukey adjustment of Statistical Analysis System. Experiments were conducted in triplicate. Counts at 0 and 1 h of incubation were 10 and 9 log cfu/mL respectively. None of the 4 spices showed any significant ($P > 0.05$) difference in counts when compared with control up to 1 h. After 2 h of incubation, there were significantly lower counts for garlic, with a reduction of 1.3 log cfu/mL when compared with control. At 3 h of incubation, all spices showed significant lower counts when compared with control, with an average reduction of 1.8 log cfu/mL, except for onion whose reduction was 1.4 log cfu/mL. After incubating for 4 and 5 h, all spices showed significant lower counts when compared with control, with a maximum reduction of 3.1 log cfu/mL for garlic and a minimum of 2 log cfu/mL for onion. Although these spices showed significantly lower counts, *S. thermophilus* was still viable, showing that these spices can be used alongside with this probiotic bacterium allowing health benefits from both sources.

Key Words: spice, probiotic

T285 Effect of month on the composition and quality of milk from Holstein cows in a hot-arid environment. J. Méndez*[1], M. Mellado[1], F. G. Véliz[1], M. A. de Santiago[1], J. E. García[1], and A. Zúñiga[1], [1]*Autonomous Agrarian University Antonio Narro, Saltillo, Mexico,* [2]*Autonomous Agrarian University Antonio Narro, Torreon, Mexico.*

This study was designed to evaluate the month of the year on the variation of milk composition and microbial content of intensively-managed Holstein cows in northern Mexico (26°N). The milk samples were obtained from about 3,200 cows, which were sampled 4 times per month from January to December of 2011 and 2012. Bacteriological analyses were performed daily in the bulk tank milk. The MIXED procedure of SAS with the PDIFF option was used to detect differences among mean monthly milk components, somatic cell counts and bacteriological measurements. Non-linear analyses were also performed to describe the monthly trend of milk composition and quality. Only month was accounted for in the models with year included as covariable. A quadratic (U-shaped; $r^2 = 0.86$) model best described the relationship between month of the year and total solids, with the highest ($P < 0.05$) value in November (12.27%) and the lowest in July (12.03). Also, a U-shaped trend ($Y = 3.23 + 0.22/1 + 0.08x - 0.001 x^2$; $r^2 = 0.95$) showed the best fit to the relationship between month and milk protein content, with the highest level (3.22%; $P < 0.01$) in December and the lowest (3.12%) in April. A clear decrease during the warmer months ($Y = 8.73–0.52x/1–0.058x-0.0001x^2$; $r^2 = 0.93$) was observed for non-fat solids but no differences among month for this variable was detected. Differences in the milk content of urea nitrogen (range 12.28–13.39 mg/dL), lactose (range 4.75–4.82%), fat (range 3.36–3.44), coliform bacteria counts (range 18.3–68.33 cfu/ml), standard microbial count and somatic cell counts (range 236,149–295,335 cells/mL) were not detected among months. The thermoduric strain of bacteria count showed a v-shaped tendency ($Y = 0.14–0.06 \cos(-0.02x + 0.14)$; $r^2 = 0.83$) with the highest ($P < 0.05$) values in the coldest months and the lowest values in the summer. This results show that, except for lactose, coliform bacteria counts and urea nitrogen milk content, all other milk components and microbiological variables showed a noticeable depression during the hottest month of the year.

Key Words: coliform, somatic cell count, milk protein

T286 Development of a pilot test system for demonstration and evaluation of CIP cleaning. Y. Yu* and R. Roberts, *Department of Food Science, The Pennsylvania State University, University Park.*

Cleaning in place (CIP) is widely used in the dairy industry. Electrolyzed oxidizing (EO) water offers an attractive alternative to traditional chemicals for cleaning and sanitation. EO water is produced via electrolysis of a dilute sodium chloride solution, which results in a sodium hydroxide solution (pH ~11.0 and ORP ~ 1168 mV) and an acidic solution (pH ~2.5, ORP ~ −850 mV and >100 ppm of chlorine). Walker et al. (2005) evaluated EO water as a cleaning agent for on-farm milking systems and found it to be effective in a cleaning/sanitizing system. The use of EO water in CIP applications for heated dairy processing systems has not been evaluated. In this project, a pilot scale system was constructed to allow evaluation and optimization of EO water as CIP agent for dairy processing equipment. The test system was composed of a 4-gallon double jacked stainless steel vessel, whose temperature could be controlled by running cooling or heating media through the jacket, a probe and microprocessor based chart recorder for monitoring temperature during fouling and cleaning, a variable frequency drive pump, and a static spray ball for CIP cleaning. A range of pump speeds were evaluated and it was determined a flow rate of 8.3 L/min provided adequate coverage for CIP cleaning of the system using a riboflavin removal method. A 4-step CIP procedure for the test system was developed. The effectiveness of cleaning was assessed using ATP bioluminescence and residual protein detection, which is comparable to a standard manual cleaning procedure. Results indicated the system would serve as a suitable test bed for optimization of CIP procedure using EO water. Preliminary experiments using EO water in a 4-step manual cleaning procedure suggested that EO water can serve as a suitable cleanser for dairy processing equipment. With the test system validated experiments are underway to optimize temperature and time required for cleaning the system soiled with cold milk and when soiled during heating of milk. Use of this test system to demonstrate principals of cleaning in a senior level dairy products processing course will also be presented.

Key Words: CIP, EO water

T287 Development of a fresh cheese model to evaluate novel antilisterials. M. L. Van Tassell*[1], L. Vazquez-Portalatin[2], S. R. Takhar[1], and M. J. Miller[1], *[1]University of Illinois at Urbana-Champaign, Urbana, [2]University of Puerto Rico at Mayaguez, Mayaguez.*

Hispanic-style fresh cheeses such as queso fresco are difficult to preserve without compromising delicate organoleptic properties. Inherently low salt content, neutral pH, and high water activity predispose such cheeses to carriage and proliferation of *Listeria monocytogenes.* Incorporation of novel antimicrobials into cheese-making processes for investigating antilisterial activity is complicated by manufacturing constraints. Consequently, few studies have focused on in situ testing of antilisterials in fresh cheeses despite evidence of discrepancies between in vitro and in situ efficacy of many antimicrobial compounds in food matrices. To allow for high-throughput testing of novel antilisterials incorporated into fresh cheeses directly, we have developed a small-scale laboratory queso fresco model that can be completed in a biosafety cabinet. Milk of variably scaled batches can be blended with desired antimicrobials and mixed with rennet before being divided into microcentrifuge tubes for incubation. Cheese production is carried out in vitro, including centrifugation to replace conventional pressing techniques. *Listeria* can be incorporated at any step to replicate diverse contamination scenarios and recovery from the final cheeses can be carried out with common selective media. We have validated this model with nisin and commercial fermentate, clearly demonstrating detection of listerial inhibition over a multi-week shelf-life. This model allows for rapid and convenient production of small-scale fresh cheeses for screening antilisterials in an appropriate food matrix.

Key Words: cheese, *Listeria*, antimicrobial

T288 Antimicrobial susceptibility profile and toxigenic genes detection in *Staphylococcus* spp. samples isolated from Brazilian artisanal cheeses. D. L. S. Oliveira[1], L. S. Carmo[2], L. B. Acurcio[1], R. D. Castro[1], F. M. Sant'Anna[1], C. F. A. M. Penna[1], M. O. Leite[1], L. M. Fonseca[1], S. H. C. Sandes[3], A. M. Silva[4], M. M. O. P. Cerqueira*[1], and M. R. Souza[1], *[1]Departmento de Tecnologia e Inspecao de Produtos de Origem Animal, Escola de Veterinaria, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [2]Fundacao Ezequiel Dias, Belo Horizonte, Minas Gerais, Brazil, [3]Departamento de Genetica, Instituto de Ciencias Biologicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [4]Universidade Federal de Sao Joao Del Rey, Sete Lagoas, Brazil.*

The objective of this study was to detect the presence of enterotoxin coding genes and analyze the antimicrobial susceptibility profile of 15 *Staphylococcus* spp. samples isolated from Serra da Canastra artisanal cheeses produced in Brazil. The bacterial identification was made through molecular techniques based on the amplification of a fragment of rDNA 16S gene. The detection of *sea*, *sec*, *sed* and *see* was carried

out by PCR-Multiplex and *seb* and *tst* by individuals PCR-Uniplex. Antimicrobial susceptibility profile was determined according to the disc diffusion method. The 15 samples of *Staphylococcus* spp. were identified as *S. aureus* ssp. *aureus* (67%), *S. saprophyticus* ssp. *bovis* (20%) and *S. warneri* (13%). No gene for synthesis of classic staphylococci toxins was identified in none of the analyzed samples. Regarding the antibiogram, the 15 tested samples presented, in general lines, low resistance to the 20 tested antimicrobials. All samples presented resistance to sulfonamide. Resistance was also observed to penicillin (80% of the samples), ceftazidime (60%) and oxacillin (40%). Although, for the other 16 tested antimicrobials, resistance rates were below 30%. A total of 26.7% of the samples were resistant to ciprofloxacin, tetracycline, ampicillin and amoxicillin, and 13.3% to chloramphenicol, sulfatrim and nitrofurantoin. The other tested drugs (vancomycin, gentamicin, clindamycin, eritromicin, imipenem, cefoxitin, amikacin, ceftriaxone and cefaclor) were efficient against 100% of the tested samples. Although *Staphylococcus* spp. tested samples were not able of producing enterotoxins and TSST-1, they can represent public health risk due to phenotypic resistance to most common antimicrobials used in animal and human therapies.

Key Words: *Staphylococcus*, PCR-multiplex, antimicrobial susceptibility

T289 Analysis of a genetically distinct strain of the monomorphic subspecies *Bifidobacterium animalis* ssp. *lactis,* the complete genome of *Bifidobacterium animalis* ssp. *lactis* ATCC 27673. J. R. Loquasto*[1], R. Barrangou[1,2], E. G. Dudley[1], B. Stahl[2], and R. F. Roberts[1], [1]*Department of Food Science, Penn State University, University Park,* [2]*DuPont Danisco USA Inc., Madison, WI.*

Bifidobacterium animalis ssp. *lactis* (BAL) is a widely consumed probiotic microorganism, commonly added to a variety of foods, including fermented dairy foods such as yogurt. Characteristics making this subspecies desirable for use as a probiotic include its perceived health benefits as well as technological advantages over organisms in the same genus. The genomes of 9 strains of this subspecies have been sequenced and are publicly available. Analysis of these genomes reveals very little genetic diversity leading to the term "monomorphic" being used to describe subspecies. In previous work, Delétoile et al. (2010) used multilocus sequence typing (MLST) to characterize several bifidobacterial species and revealed BAL ATCC 27673 had a unique MLST type. As part of an effort to assess the genetic diversity in the *B. animalis* ssp. *lactis* group, this strain was chosen for full genome sequencing. Following DNA isolation, 454 shotgun pyrosequencing was conducted, contigs were aligned and assembled using the genome of BAL DSM 10140 as a reference scaffold and the genome was closed using a combination of PCR and Sanger sequencing. The full genome of ATCC 27673 was 1,963,012 bp long, contained 1,616 genes, 4 rRNA operons and had a G+C content of 61.55%. Further analysis revealed 5 distinct genomic islands differing from other strains of the same subspecies. In 4 islands, either mobile genetic elements or phage elements are present. In island 5, the largest island, a novel CRISPR locus was identified. This locus contains 81 novel spacers different from any spacers observed in other strains of BAL. In addition BAL ATCC 27673 was found to contain a type I-E CRISPR-cas system, whereas all other strains of this subspecies contain a type I-U system. This analysis revealed ATCC 27673 represents a novel strain of *B. animalis* ssp. *lactis*, differing substantially from other stains of the subspecies. This work suggests that the 9 commercial strains sequenced to date may represent only a limited portion of the genetic potential of this subspecies.

Key Words: *B. animalis* ssp. *lactis*, probiotic

T290 Microbiological quality of nonfat dry milk and skim milk powder produced in the United States A. K. A. Ali*, K. E. Smith, K. J. Burrington, and J. A. Lucey, *Wisconsin Center for Dairy Research, University of Wisconsin–Madison, Madison.*

Microbiological quality of nonfat dry milk powder/skim milk powder (NFDM)/(SMP) is important as it can affect the quality of food products in which the powder is used as an ingredient. The objectives of this study were to determine the microbiological quality of domestically produced NFDM/SMP. In this study, 23 samples of NFDM/SMP were obtained from 4 US processors for analysis. Samples were 25 kg or 50 pound bags of powder of low, medium and high heat NFDM/SMP that have not been agglomerated or instantized and were approximately 6 to 9 mo old. Total bacterial count, aerobic thermophiles, thermoresistant bacteria, coliforms, yeasts and molds, as well as, bacterial spores including mesophilic aerobic, mesophilic anaerobic, thermophilic aerobic, and thermophilic anaerobic were determined. Powders were <10 cfu/g for coliforms, thermoresistant bacteria, yeasts and molds. Total bacterial count for powders had a range of 2.0–2.9 $\log_{10}$ cfu/g with differences due to powder manufacturer. Samples had a large variation in thermophilic aerobic bacteria numbers with a range of 2.1–4.8 $\log_{10}$ cfu/g. While the numbers of mesophilic aerobic spores and thermophilic anaerobic spores did not show notable variations, the numbers of thermophilic aerobic spores varied greatly with a range of $1 < -4.1$ $\log_{10}$ cfu/g. The high numbers of thermophilic aerobic spores observed with samples from some producers may be explained by their presence on the surfaces of the equipment and/or the formation of biofilms on stainless steel surfaces. There was no significant variation among powders based on heat classifications or between NFDM and SMP. The results of this work indicate that the microbiological quality of powders was source dependent regardless of heat classifications or whether the product was NFDM or SMP. Based on the results, powders from some manufactures may be better suited to low-spore count applications than powders from other producers.

Key Words: NFDM/SMP, bacterial spore, microbiological quality

T291 Real-time PCR and TTGE for rapid and sensitive detection of *Staphylococcus aureus* in Ras and Domiati cheese during ripening. S. Awad*, *Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.*

Microbial diversity in cheese is considered essential to the sensory richness and variety of traditional Egyptian cheeses. However, some members of these complex communities may also be responsible for cheese flavor defects or may constitute a health risk. Being able to characterize the microbial communities in raw milk and follow the dynamics of the entire populations throughout the cheese-making and ripening processes is therefore critical. *Staphylococcus aureus* is a bacterial pathogen considered a principal etiological agent of food poisoning. The aim of this study was to apply real-time PCR-based method (TaqMan RT-PCR) comparing with temporal temperature gradient electrophoresis (TTGE) technique to detect *S. aureus* in Doimiati and Ras cheeses during their ripening periods. In parallel, all cheese samples were examined to conventional methods to ensure the detection limit of 10^0 cfu per gram. In this study, *S. aureus* was detected in all Ras and Domiati cheeses after 2 mo of ripening by using TTGE. The results of real time PCR showed that the *S. aureus* could not be detected in fresh cheese samples using TTGE method but it detected in fresh cheese sample using real time PCR. The real time PCR method could be used for *S. aureus* detection as a faster, highly specific, and more sensitive alternative to microbiological method with the potential for providing of improved food-processing hygiene control.

Key Words: real-time PCR, TTGE, *Staphylococcus aureus*

T292 Development of a rapid SNP-typing assay to differentiate *Bifidobacterium animalis* ssp. *lactis* strains used in probiotic-supplemented dairy products. S. Lomonaco*[2], E. J. Furumoto[1], J. R. Loquasto[1], P. Morra[2], and R. F. Roberts[1], *[1]Department of Food Science, Penn State University, University Park, [2]Dipartimento di Scienze Veterinarie Università degli Studi di Torino, Grugliasco TO, Italy.*

Identification at the genus, species, and strain level is required when a probiotic microorganism is added to foods. Strains of *Bifidobacterium animalis* ssp. *lactis* (BAL) have been commonly used worldwide in dairy products supplemented with probiotic. However, strain discrimination is difficult, given the high degree of genome identity (99.975%) between different genomes of this subspecies. Typing of monomorphic species can be efficiently carried out by targeting informative single nucleotide polymorphisms (SNPs). Findings from a previous study analyzing both reference and commercial strains of BAL detected SNPs that could be used to discriminate 14 groups/strains. This abstract describes the development of a primer extension reaction (PER) assay targeting multiple SNPs that can allow strain differentiation of *B. animalis* ssp. *lactis*. Based on previous data, 7 informative SNPs were selected for further testing and a multiplex preliminary PCR was optimized to amplify the 7 DNA regions comprising the selected SNPs. Extension primers (EPs), annealing immediately adjacent to the selected SNPs, were tested in simplex PER to evaluate their performance. Twenty strains belonging to 13 groups were selected and PERs were carried out on according to manufacturer's instructions (SNaPshot Multiplex System from Applied Biosystems), with minor modifications. Fragment analysis was subsequently carried out in duplicate at the Penn State University Genomics Core Facility. Simplex PER mostly gave rise to a peak of the expected color, specific to the targeted SNP and 8 specific profiles could be observed, separating the most commonly used commercial strains. Further tests will be necessary to fully assess the discriminating capacity of each EPs and to optimize a multiplex PER assay able to simultaneously interrogate the selected SNPs. Such a novel multiplex PER approach can represent a simple, rapid, flexible SNP-based subtyping method for proper characterization and identification of commercial probiotic strains of BAL from fermented dairy products.

Key Words: *Bifidobacterium animalis* ssp. *lactis*, strain identification, SNaPshot

T237 Studying the microbiological safety criteria and quality related problems of the traditional Kishk Sa'eedi. S. Awad*[1], M. El Soda[1], A. Ahmed[1], I. Nagady[1], C. Mestres[2], and D. Pallet[2], *[1]Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt, [2]CIRAD (Agricultural Research for Development), Montpellier Cedex 5, France.*

Kishk Sa'eedi is an indigenous food that is part of the rich food heritage of Egypt. The name "Kishk" refers to a group of popular fermented dairy cereal mix products common to Egypt and the Middle East within the framework of the European funded "AFTER" Project (African Food Tradition rEvisited by Research). The natural microflora in Kishk Sa'eedi were studied and characterized. An inventory of both the technological flora (lactic acid bacteria) and pathogenic germs (*Salmonella* sp., *Listeria* sp., *Clostridium* sp., *Staphylococcus aureus* coagulase positive, *Brucella* spp., yeasts, and molds) were analyzed in the final product. During the microbiological analysis of raw materials and final product, lactic acid bacteria that could potentially act as starter cultures were isolated and identified. The isolates were identified using rep-PCR as *Lactobacillus acidophilus, Lb. helveticus, Lb. del. bulgaricus, Lb. del. lactis, Lb. casei, Lb. paracasei, Lb. plantarum, Lb. rhamnosus, Lb. brevis, Lb. fermentum.* The technological criteria (stability of lyophilized, acidification, activity flavor development, antagonistic activities, slimy production, peptidase activity, antibiotic resistance and amines production) were determined for all isolates. Mixing cultures were selected to produce the second generation Kishk Sa'eedi.

Key Words: Kishk Sa'eedi, lactic acid bacteria, pathogenic bacteria