951 Wooden vat to produce PDO Ragusano cheese is a living system. G. Licitra*[1,2], L. Tuminello[2], N. Fucà[2], P. Campo[2], S. Lortal[3], and S. Carpino[2], [1]*D.A.C.P.A. University of Catania*, *Catania, Italy*, [2]*CoRFiLaC, Regione Siciliana*, *Ragusa, Italy*, [3]*UMR Science et Technologie du Lait et de l'Oeuf*, *Rennes Cedex, France*.

Tina is the traditional wooden vat daily used for the cheese making process of the P.D.O. Ragusano cheese. A previous study has shown the biofilm microstructure and has demonstrated the safety and efficiency of the *tina* as natural inoculation system in the cheese making process. Ragusano cheese is produced from November to May, when native pastures are available; so the *tina* is not used during the summer period. The aim of the present work was to verify the presence and survival of the biofilm on the inner surface of the dried inactive *tina*. Samples were taken, in October, from 2 dried *tinas* belonging to 2 different farms; the biofilm of those *tinas* had been previously (Lortal et al., 2009) analyzed when the same *tinas* were used for the Ragusano cheese production. By using a sterile blade 2 small wood micropieces (15 × 5 × 1 mm about) were removed from 2 opposite sides of the internal surface of each *tina*. *Tina* samples were analyzed by scanning electron (SEM) and confocal laser scanning microscope (CLSM). SEM and CLSM images showed a rich biofilm constituted by big and close microbial communities immersed in an abundant exopolysaccharide matrix which provides the necessary nutrients to the bacteria and protects them from the external environmental conditions. CLSM images highlighted that biofilm is mainly made of live bacteria. Heterogeneous bacterial colonies cover uniformly the whole surface of the *tina* forming a compact multilayered biofilm with a thickness of about 20 - 30 µm. The biofilm goes through the surface in the external wood vessels which look fully stuffed by bacteria and exopolysaccharide matrix. SEM images showed also the presence of biofilm inside the *tina* sample as far as 400–600 µm from the surface. This study demonstrated that at the end of the summer period a microbial biofilm still survives and maintains an intact and well organized structure.

Key Words: biofilm, wooden vat, Ragusano cheese

952 Survival of *Lactobacillus acidophilus* in Boursin-like cheese after gastric and enteric conditions in vitro. A. M. Liserre*[1], P. B. Zacarchenco[1], K. M. O. dos Santos[2], F. C. A. Buriti[2], L. S. Gonçalves[1], and L. R. Monteiro[1], [1]*Instituto Tecnologia Alimentos. Av. Brasil*, *Campinas, SP, Brasil*, [2]*EMBRAPA, Centro Nacional de Pesquisa de Caprinos e Ovinos*, *Sobral, Ceará, Brasil*.

In this study, Boursin-like cheeses were made from goat's milk at the facilities of EMBRAPA Caprinos (Brazilian Agricultural Research Corporation) in the town of Sobral, Brazil. The objective was the evaluation of the survival of probiotics in the cheese after treatment in simulated gastric and enteric juices. The cheeses added with *Lactobacillus acidophilus* were evaluated after 14 d refrigerated storage to quantify the surviving probiotics after treatment simulating gastrointestinal conditions. For this purpose, cheese samples were added to an acid solution at pH 2.5 containing pepsin (3g/L) for 120 min. Next, the pH was changed to 5.6 for another 120 min and finally changed to pH 7.5 for the last 120 min. During the enteric fluid simulation steps the samples were additionally added with bile in a proportion adequate to obtain a final concentration of 3g/L, so as to simulate small intestine conditions. Probiotic counts in the cheese samples after 14 d storage were 7.74 log CFU/g. After the test simulating gastrointestinal conditions, the counts had been reduced to 4.02, 2.42 and <2.00 log (detection limit) CFU/g in the cheeses exposed to gastric (pH 2.5) and enteric (pH 5.6 and pH 7.5) fluids, respectively. Brazilian legislation requires a minimum of 10^8 to 10^9 viable cells per daily portion of a probiotic product. The portion size of 30g of the cheese contained around 10^8 viable cells before the simulation tests but, after action of the gastrointestinal juices the counts were lower than the detection limit of the test, yielding an unsatisfactory result.

Key Words: probiotic, goat milk, cheese

953 Addition of probiotic bacteria modifies the biodiversity of other lactic acid bacteria in Cheddar cheese. B. Ganesan*[4,3], B. C. Weimer[1], G. Rompato[2], J. Pinzon[1], P. Desai[2,3], C. Brothersen[4,3], and D. J. McMahon[4,3], [1]*University of California*, *Davis,* [2]*Center for Integrated BioSystems, Utah State University*, *Logan,* [3]*Department of Nutrition, Dietetics, and Food Sciences, Utah State University*, *Logan,* [4]*Western Dairy Center, Utah State University*, *Logan*.

Bacteria in cheese face abiotic stresses during manufacture and storage that impact their abilities to produce flavors, survive, and interact with other bacterial populations. Apart from the added starter cultures non-starter lactic acid bacteria (NSLAB) are derived from milk handling, cheese equipment, and human contact. Occasionally, flavor adjunct bacteria are added at the beginning of cheese manufacture to modify the flavor of the product. Probiotic bacteria are added to foods with the goal of reaping the microbes' human health benefits; however, addition to cheese poses new stress conditions that may change the characteristics of the microbe. In this study, probiotic bacteria (lactobacilli and bifidobacteria) were added during Cheddar cheese manufacture to determine their survival during the aging process in different milk fat levels. Starter culture and NSLAB populations were determined using real time-quantitative PCR using primers specific for the different bacterial genera or species of interest. Bifidobacteria were initially added at 2.5×10^6 CFU/g cheese and survived the aging process with a small population reduction after 280 d. Added *Lactobacillus acidophilus* populations (10^7 CFU/g cheese) and NSLAB populations (10^8 CFU/g cheese) increased significantly ($P < 0.05$) by 10 to 100-fold during the same time. Analysis of the added probiotic and starter culture using propidium monoazide for differentiating live and dead bacteria by PCR indicated that these microbes not only survived, but increased in numbers significantly ($P < 0.05$) by 10 to 100-fold over aging independent of fat level. In conclusion, probiotic bacteria are capable of surviving throughout the cheese-making and aging process, indicating that delivery via hard cheeses is possible.

Key Words: lactic acid bacteria, probiotic, survival

954 Production of microcapsules of *Lactobacillus acidophilus* to add in dairy products. A. M. Liserre*[1], P. B. Zacarchenco[1], C. R. Meneze[3], A. E. C. Antunes[2], G. M. B. Q. Cardozo[1], and I. Moreno[1], [1]*Tecnolat/ Instituto de Tecnologia de Alimentos*, *Campinas, São Paulo, Brasil*, [2]*UNICAMP; Universidade Estadual de Campinas - Limeira*, *Limeira, São Paulo, Brasil*, [3]*Universidade de Santa Maria*, *Rio Grande do Sul, Brasil*.

Probiotics have attracted attention because of their benefits to human health. However, studies indicate that probiotics may not survive well in dairy products and during their passage through the gastrointestinal tract, due to stress factors such as acidity, low storage temperature, and presence of lactic and acetic acids, bile salts and digestive enzymes.

Microencapsulation is a technology used to improve viability of probiotic. The objective of this study was the development of *Lactobacillus acidophilus* microcapsules with cellulose acetate phthalate, maltodextrin, glycerol, Hi-maize, Tween 80 and skim milk powder prepared by the spray dryer technique. In vitro release of probiotics from the microcapsules was investigated using citrate-phosphate buffer solution (pH 4.5) and in phosphate buffer solution (pH 6.0 and pH 7.5). Aliquots were removed after 60, 120 and 180 min agitation at 150 rpm at 37°C. The number of released cells was determined by pour plating in MRS LP (37°C/72h) under anaerobiosis. Changes in bead integrity with time at pH 7.5 were monitored by optical microscopy and photographed. The spray dryer technique was efficient to obtain probiotic encapsulated cellulose acetate phthalate based microcapsules. The viability of probiotic were very good showing counts of 5.70, 8.85 and 9.37 log CFU/g after 180 min of dissolution at pH 4.5, 6.0 and 7.5, respectively. Microcapsules promoted a controlled release of probiotics in different pH values, because the highest retention of cells occurred at pH 4.5. Theses microcapsules can be applied to dairy products because at the pH of milk (pH 6.0) the release of probiotics was high.

Key Words: probiotic, microencapsulation, acetate phthalate cellulose

955 Novel immunostimulatory activities of CpG oligodeoxynucleotides from *Streptococcus thermophilus*. T. Shimosato*[1], M. Fujimoto[1], M. Tohno[2], T. Sato[3], H. Otani[1], and H. Kitazawa[4], [1]*Shinshu University*, *Kamiina, Nagano, Japan*, [2]*National Institute of Livestock and Grassland Science*, *Nasushiobara, Tochigi, Japan*, [3]*Yokohama City University*, *Yokohama, Kanagawa, Japan*, [4]*Tohoku University*, *Sendai, Miyagi, Japan*.

We previously reported the strong immunostimulatory effects of a CpG oligodeoxynucleotide (ODN), designated MsST, from the lacZ gene of *Streptococcus (S.) thermophilus* ATCC19258. However, there is no evidence of an anti-inflammatory response after IL-33 increase following treatment with CpG ODNs, which act via TLR9. Therefore, in this study, we focused on induction of IL-33 by CpG ODNs and examined the effects of MsST stimulation on mouse splenocytes and peritoneal macrophages.Here we show that 24 h of stimulation with MsST in mouse splenocytes and peritoneal macrophages strongly induces expression of interleukin (IL)-33, a cytokine in the IL-1 superfamily. Other IL-1 superfamily members, including IL-1α, IL-1β and IL-18, are downregulated after 24 h of stimulation of MsST. We also found that MsST-induced IL-33 mRNA expression is inhibited by the suppressive ODN A151, which can inhibit Toll-like receptor 9 (TLR9)-mediated responses. We speculate that upregulation of IL-33 in response to exposure to an external stimulus such as MsST may serve as an endogenous danger signal that alerts cells in the innate immune system to tissue damage during bacterial challenge. Our findings suggest that IL-33 is an important regulator that acts on macrophages via TLR9. Although our understanding of IL-33 is currently limited, it seems reasonable to suggest that IL-33 might counterbalance the activities of proinflammatory cytokines . In conclusion, we found a novel immunoregulatory mechanism mediated by CpG ODNs that induces IL-33. Understanding how IL-33 mediates immunoregulation via MsST activation should help in the development of therapeutic ODNs for treatment of inflammatory disease by the strong induction of IL-33. Exploiting this property may also prove useful in the design and production of new physiologically functional foods.

Key Words: CpG ODN, IL-33, *Streptococcus thermophilus*

956 Toll-like receptor 2 participates in the intestinal epithelial regulating activity of *Lactobacillus kefiranofaciens* M1 isolated from fermented milk product kefir. Y. P. Chen*, W. S. Hong, T. Y. Dai, I. N. Huang, and M. J. Chen, *National Taiwan University*, *Taipei, Taiwan, R.O.C.*

Toll-like receptors (TLRs) are pattern recognition receptors (PRRs) that recognize microbial components and endogenous ligands. Among different TLRs, TLR2 recognizes the broadest range of microbial component, including lipoteichic acid, peptidoglycan, lipopeptide and so on. TLR2 has been known to manipulate important immune function in many kinds of immune cells. However, the physiological significance of TLR2 expressed in intestinal epithelial cells is unclear. In this report, we found that TLR2 was participated in important regulatory roles of *Lactobacillus kefiranofaciens* M1, which was originally isolated from fermented milk product kefir and showed intestinal protective activity in vitro and in vivo. We cultured intestinal epithelial cell (IEC) line Caco-2 onto permeable transwell insert for 28 d until fully polarization and monolayer formation. We found that apical adding of *Lb. kefiranofaciens* M1 increased both the apical and basolateral production of intestinal restitution chemokine CCL-20 in IEC monolayer in a dose dependent and time course manner. The CCL-20 production was further blocked by using TLR2 specific neutralizing antibody. In the experimental colitis model, we used dextran sodium sulfate (DSS) to damage intestinal epithelium and to induce colitis in both wild type and TLR2 knockout mice. *Lactobacillus kefiranofaciens* M1 could ameliorate DSS-induced colitis in wild type mice group by assessing stool consistency, bleeding score, colon length shortening, histological scoring and *ex-vivo* cytokine production pattern of colon segment, while it had no such effect in TLR2 knockout mice group. In summary, *Lb. kefiranofaciens* M1 can regulate IEC restitution chemokine CCL-20 production in vitro and ameliorate DSS-induced colitis in vivo through TLR2. The data indicates that TLR2 plays an important role of *Lb. kefiranofaciens* M1 in regulating intestinal homeostasis.

Key Words: toll-like receptor, probiotics, intestinal epithelial cell

957 Inhibitory effect of Taiwanese ropy fermented milk in an ovalbumin-induced allergy mouse model. I. N. Huang*[1], T. Y. Dai[1], S. Y. Wang[2], and M. J. Chen[1], [1]*Department of Animal Science and Technology, National Taiwan University*, *Taipei, Taiwan*, [2]*Experimental Farm, National Taiwan University*, *Taipei, Taiwan*.

Taiwanese ropy fermented milk (TRFM) has a sticky consistency, which is made with the microbial action of mesophilic lactic acid bacteria (LAB). In our previous studies, we isolated and identified microorganisms from TRFM using a combination of polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and 16S rDNA sequencing. In this study, we assayed the anti-allergic abilities of TRFM and its dominant microorganisms (*Lactococcus lactis* ssp. *cremoris* and *Kluyveromyces marxianus*). The in vitro results depicted that both *L. lactis* ssp. *cremoris* and *K. marxianus* could induce Th1 (TNF-α, IL-6) and Treg (IL-10) cytokines in RAW 264.7 macrophages and murine splenocytes, which might inhibit the Th2 response and IgE production. The OVA-sensitized animal test demonstrated that oral administration of both strains generally tended to reduce serum total IgE in OVA-sensitized BALB/c mice compared with the control groups. In conclusion, the data presented clearly indicate the anti-allergic activities of TRFM microorganisms. Suppression of IgE production by oral feeding of *L. lactis* ssp. *cremoris* and *K. marxianus* probably occurs because of elevation of Th1 and Treg cytokines leading the skewness of Th1/Th2 balance toward Th1 dominance.

Key Words: allergy, lactic acid bacteria, yeast