744 Isolation and identification of proteolytic psychrotrophic bacteria from raw milk. Ahmed S. Zahran*[1] and Bruce F. Ward[2], [1]*Minia University* , [2]*University of Edinburgh*.

A large number of bacteria secrete extracellualr proteases, lipases and phospholipases into the external medium. The presence of these enzymes cause problems for the dairy industry. Proteases for example produce bitterness, gelation of UHT milk and decrease in the keeping quality of milk during the manufacture of cheese and other dairy products. Eight genera of protease producing psychtrophic bacteria were isolated from raw milk. Four genera were found to be Gram-positive and four were Gram-negative. The Gram-positive genera were Bacillus, Micrococcus, Corynebacterium and Staphylococcus. The Gram-negative bacteria were Flavobacterium, Pseudomonas, Acinetobacter and Cytophaga. Flavobacterium spp and Pseudomonas spp were the two main genera, they represented 48.8 and 34% respectively. Pseudomonas spp were more active in terms of protease production than Flavobacterium spp. Five different Pseudomanoas species were identified, they were P.fluorescens, P. putida, P. aeruginosa, P. diminuta and P.vesicularis. P.fluorescens was the dominant species,it represented 42.9% of the Pseudomonas species isolated. All the isolated Pseudomonas showed polar flagella when examined by transmission electron microscopy. Strain 8 synthesized the enzyme in basal and complex media. The amount of protease secreted(activity/growth)in complex medium was higher than that produced in basal medium.

Key Words: Proteolyitc, Psychrotrophic, raw milk

745 Survival of a five strain cocktail of *E. coli* O157:H7 during the 60 Days Aging Period of Hard Cheese Made from Unpasteurized Milk. Joseph Schlesser *[1], Kevin Madsen [2], and Robert Gerdes[2], [1]*Food and Drug Administration, NCFST, Summit-Argo, IL,* [2]*Illinois Institute of Technology, NCFST, Summit-Argo, IL*.

Hard cheese was made from unpasteurized milk inoculated with 10^5 cells/ml of a five-strain cocktail of *E. coli* O157:H7 (*E. coli* O157:H7 43895, *E. coli* O157:H7 SEA 13B88, *E. coli* O157:H7 932, *E. coli* O157:H7 C7927, and *E. coli* O157:H7 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; and plate count agar for total plate count enumeration. Populations of *E. coli* O157:H7 increased to 10^6 cells/gm in the drained curd and to 10^7 cells/gm at milling and pressing. Populations of *E. coli* O157:H7 in cheese aged for 60 and 90 days at 7 ^{o} C were reduced by 1-log and 1 to 2-logs, respectively. Populations of *E. coli* O157:H7 in cheese aged for 180 and 240 days at 7 ^{o} C were reduced by 2-log and 3-logs, respectively. Cheese was also made from unpasteurized milk inoculated with 10^3 cells/ml of a five-strain cocktail of *E. coli* O157:H7. Populations of *E. coli* O157:H7 increased to 10^4cells/gm in the drained curd and at milling and to 10^5 cells/gm at pressing. Preliminary results shows no change in population at 60 days of aging at 7 ^{o} C.

Key Words: aging , hard cheese , survival of pathogens

746 Production of an exopolysaccharide-containing whey protein concentrate by fermentation of whey with *L. delbrueckii* ssp. *bulgaricus* RR. E. M. Panko* and R. F. Roberts, *Pennsylvania State University*.

About 22 million metric tons of whey is produced annually in the U.S. Approximately half is manufactured into whey protein concentrate (WPC); the remainder is considered a waste stream. Using whey as a fermentation media presents the opportunity to create value-added products. Fermentation of whey with exopolysaccharide-producing (EPS^+) bacteria may result in WPC with unique functional attributes. However, exopolysaccharide-producing lactic acid bacteria are weakly proteolytic, and the amount of low molecular weight nitrogen in whey is limited. Conditions were developed to partially hydrolyze the whey proteins and then ferment the product with EPS^+ bacteria. In preliminary experiments, pasteurized Cheddar cheese whey was treated with Flavourzyme^{TM} to partially hydrolyze the protein (%DH 2-12). Fermentation (38°C, pH 5.0) with *L. delbrueckii* ssp. *bulgaricus* RR revealed that hydrolysis increased the amount of EPS produced from <5 to ca. 400 mg/L. Furthermore, low levels of hydrolysis resulted in EPS levels similar to high levels of hydrolysis. In scale up experiments, whey was separated and pasteurized, then hydrolyzed to 2% DH with Flavourzyme^{TM}. Following protease inactivation, 60 L of partially hydrolyzed whey was fermented at 38°C, pH 5.0. After fermentation the broth was pasteurized, bacterial cells were removed using a Sharples centrifuge, then the whey was ultrafiltered and diafiltered (10,000 mwco) to remove lactose and salts, freeze-dried and milled to a powder. Unfermented hydrolyzed and unhydrolyzed whey controls were processed in the same manner. The EPS-WPC ingredients contained approximately 72% protein and 6% EPS. The whey-EPS blends exhibited low protein solubility (70%, pH 7.0) and extensive protein denaturation. The ingredient (2% protein) formed stable gels (G' increased from 0 to 10^4 Pa) upon heating to 85°C. Fermented whey ingredients exhibited gelation onset at lower temperatures than the control whey (65 vs. 80°C). Partial hydrolysis of whey, followed by fermentation by an EPS-producing bacteria and downstream processing, produced functional whey-based ingredients.

Key Words: exopolysaccharide, whey protein concentrate, Lactobacillus delbrueckii ssp. bulgaricus

747 Continuous production of antimicrobial compound(s) and organic acids by bifidobacteria cells entrapped in sodium alginate beads. Z. Morrison, S.A. Ibrahim, M.M. Salameh, A. Shahbazi, and C.W. Seo, *North Carolina Agricultural and Technical State University*.

Whey contains high quantity of lactose and other nutrients, which can be easily utilized by lactic acid bacteria and probiotics to produce organic acids and value added products. Therefore, the objective of this research was to evaluate the technical feasibility of producing antimicrobial compound(s) and organic acids (lactic and acetic acids) from whey and high lactose media using a continuous fermenter inoculated with *Bifidobacterium* sp. (B-1-2) cells entrapped in sodium alginate beads. Immobilized beads were packed in a column for continuous fermentation using 1-liter Lactose MRS broth (4% lactose, 1.5% MRS, 0.005% $MnSO_4$ and 0.01% Tween 20) or whey-based medium. The broth medium was placed inside a round flask sitting in a water bath (37.5 C), and fed to the bottom of the bead column using a peristaltic pump. Samples collected at different time intervals during the fermentation period were analyzed for antimicrobial compound(s) using the disk bioassay and for organic acids using high-performance liquid chromatography (HPLC). All experiments were performed in duplicate. Under these fermentation conditions, the lactose conversion rate was 25%, and maximum organic acid produced was 2.3%. Maximum organic acids and antimicrobial compound(s) were achieved after a 24h incubation period. Results of this work showed that immobilization could offer the possibility of a more stable means of producing antimicrobial compound(s) and organic acids using laboratory media, as well as whey-based medium.

Key Words: bifidobacteria, immobilization, antimicrobial compound