

**W296 Monitoring spores and spore-forming bacteria populations in commercial skim milk powder production plants using conventional and molecular methods.** C. Murillo\*<sup>1</sup>, C. Kitts<sup>2</sup>, and R. Jimenez-Flores<sup>1</sup>, <sup>1</sup>Cal Poly Dairy Products Technology Center, <sup>2</sup>Cal Poly Biological Sciences Department.

The microflora of milk powder consists of a wide array of microorganisms of which special attention is given to *Bacillus spp.* spores and spore formers. *Bacillus spp.* spores survive well in all processing stages and inhabit the milk powder in the dormant state indefinitely. Upon reconstitution, spores may germinate, and through their enzymatic activity become detrimental to quality. The objectives of this study are to 1) enumerate total aerobes, mesophilic, and thermophilic spore populations in commercial, low-heat skim milk powder production plants; 2) characterize the microbial ecology of this process using Terminal Restriction Fragment Patterns (TRFPs); and 3) compare the changes in the ecology during this process. Fluid and powder skim milk was collected from 3 commercial facilities during spring, summer, and fall '01-'02. Sampling points included the raw milk silo, separator, evaporator, and spray dryer. Samples were normalized based on total solids. Every sample was evaluated for total aerobes, mesophilic, and thermophilic spores. For TRFPs community DNA was extracted, amplified by 16S PCR, and digested with *HaeIII* and *DpnII*. Spore formers are predominant in condensed and powdered milk, and tend to increase in the powder with increasing processing time. In raw milk mesophilic and thermophilic spores ranged from  $10^2$  CFU/g to  $7 \times 10^2$  CFU/g and  $10^2$  CFU/g to  $10^3$  CFU/g, respectively. In powder they ranged from  $10^2$  CFU/g to  $10^3$  CFU/g and  $10^2$  CFU/g to  $10^6$  CFU/g, respectively. Both spore counts from skim milk showed an increasing trend with run time and rendered the powder out of the  $10^3$  CFU/g limit. In the ecology TRF patterns successfully described microbial populations, and an overall decrease in microbial diversity between raw and powdered milk was observed. Overall, *Bacillus spp.* were found in 92 important organisms included *Clostridium spp.* (57 *Staphylococcus spp.* (29 *Streptococcus spp.* (9 *Bacillus spp.* were present in 100 from all 3 plants.

**Key Words:** Milk powder, Terminal restriction fragment patterns, Quality