W268 Development of endospore-specific primers for the analysis of microbial populations in milk powder. M. Arendts*[1], C. Kitts[2], and R. Jimenez-Flores[1], [1]*Cal Poly DPTC,* [2]*Cal Poly Biological Sciences.*

A comprehensive risk assessment of the microbial quality of milk powder should include information about endospores as well as viable bacteria. *Bacillus* endospores are present in raw milk, used in milk powder production, in numbers ranging from less than 10 to greater than 100 per g of solid. However, in the finished product they range from less than 1000 to over $5x10^5$ per gram, meaning that endospore-forming bacteria will have the most significant effect on the microbial quality of the powder. Molecular methods offer a unique and sensitive tool for rapid microbial detection. Our focus is to apply polymerase chain reaction (PCR) methods to detect early germination of endospores in milk products. We have studied the germination gene, GerC3, from endospore-forming members of the family *Bacillus*. This led to the development of specific primers for PCR detection. In the Dairy Products Technology Center (DPTC) endospore library, we have been able to detect five specific strains that contribute to the lipolysis, casein hydrolysis, starch hydrolysis, and acid production of milk products using our primers. The primers designed in this work identified either a 100bp or a 500bp in a conserved region of the GerC3 gene found in the five DPTC target strains. These bands have been detected during germination activity in all five of these *Bacillus* strains. Spore germination has been difficult to study because it involves extremely rapid physiological responses in a spore whose structure is biochemically intractable. We have evaluated the developed primers in Reverse Transcriptase- PCR (RT-PCR) in the early detection of specific endospores present in skim milk powder resulting in the ability to document the presence or absence of endospore forming bacteria. Results indicate that the rapid growth of endospore forming bacteria can be monitored using RT-PCR.

Key Words: Endospore detection, PCR, Milk powder