65 The detection of *Bacillus* endospores during low heat skim milk powder processing using nucleic acid technology. Amy Rife*, Dr. Rafael Jimenez-Flores, Dr. Chris Kitts, and Dr. Mark Kubinski, *California Polytechnic State University*.

Detection of endospores in milk powder could be obtained by direct PCR tests plus terminal restriction fragment patterns (TRFP) based on amplification of the 16s rDNA gene for bacterial community analysis. In the DPTC endospore library, we have detected five specific endospores that contribute to the lipolysis, casein hydrolysis, starch hydrolysis, and acid production in milk. Optimal quality powder has to be free of specific detrimental endospores. The objectives of this work were to evaluate TRFPs for the efficient detection of the five detrimental Bacillus endospores during a low heat skim milk powder processing run using a pilot plant model, to perform an ecological study of the microorganisms during the processing run, and to evaluate endospore specific gene amplification using PCR as a detection method. In addition to being used as a detection system, TRFP allows knowledge of the composition of communities and the dynamics of individual populations within that community. By using standard peaks and the Ribosomal Database Project (RDP), we are able to identify the specific microorganisms present during the powder processing stages. Our results indicate that TRFPs have proven to be a sensitive endospore detection method during low heat milk powder processing when endospores are present in the range of 103 to 105 CFU/g of milk. TRFPs also prove to be effective in classifying specific microorganisms present throughout each processing step as well as observing community transformations occurring during normal milk powder production in a pilot plant. This can lead to microbial ecology studies dealing with contamination parameters during powder production at an industrial level. TRFP analysis allowed to observe how adding a high concentration of endospores altered the microbial community and the interactions that took place within each sample. Using this technique in place of current ecological study methods, culturing biases such as temperature, nutrients and oxygen concentration are not encountered. In addition, using PCR with a selected gene found only in endospores, we are able to specifically detect those endospore-formers associated with milk powder production.

Key Words: Endospore, Milk Powder, Terminal Restriction Fragment Pattern