

1311 Development of endospore-specific primers for the TRFP analysis of microbial populations in milk powder. M. M. Arendts*, A. J. Rife, and R. Jimenez-Flores, *California Polytechnic State University, San Luis Obispo CA.*

A comprehensive risk assessment of the microbial quality of milk powder should include information of endospores as well as viable bacteria. Current methods for detection for endospore contamination in milk and dairy products are labor intensive and time consuming. Molecular methods offer a unique and sensitive tool for rapid microbial detection over traditional methods. Previous work in our laboratory has focused on the study of viable bacterial populations using 16s rDNA primers that can be detected via Terminal Restriction Fragment Patterns (TRFP). The overall objective of this work is to study microbial populations, including a specific focus on exclusively endospore forming bacteria, by utilizing a combination of primers in the TRFP. One set of primers, specific for 16s RNA, has been successfully used in several experiments to assess microbial ecology of milk powder. The second set, designed for the exclusive detection of endospore formers, is the specific objective of this work. Two genes of endospore forming bacteria were potential targets for primer development, SpoIIA and GerC3. SpoIIA shows lower exclusivity since it does not distinguish between endospore formers other bacteria present in milk samples. The GerC3 primer set is specific for the germination gene in *Bacillus subtilis*, and was shown to amplify 70% of the endospore forming strains of the Dairy Products Technology Center (DPTC) library. However, the designed degeneracy of these primers represent a problem for positive identification of TRF patterns. Because this gene has not been sequenced in the most common endospore forming bacteria found in milk powder we undertook the task of sequencing fragments for re-design of these GerC3 primers.

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