ABSTRACT

RESEARCH PAPER: Detection of *Cronobacter sakazakii* in Powdered Infant Milk Formula Using Real-time PCR

STUDENT: Myoung-Su Kim

DEGREE: Master of Arts

COLLEGE: Sciences and Humanities

DATE: December, 2011

PAGES: 15

Cronobacter sakazakii is a neonatal pathogen that has been found commonly in contaminated dried infant milk formula and milk powder. The fluorogenic selective marker, 4-Methylumbelliferyl-α-D-glucoside and secondary selective markers, sodium thiosulfate & ferric citrate have been used in differential media to indicate the presence of *C. sakazakii* based on α-D-glucosidase enzymes unique to this pathogen. This research will compare four enrichment broths for maximum recovery from powdered infant milk formula: *C. sakazakii – Enterobacter sakazakii* enrichment (ESE) broth, Tryptic Soy Broth (TSB), *Enterobacteriaceae* enrichment (EE) broth, and M-*Coliform* broth. Differential selective and nonselective agars including Trypticase Soy Agar (TSA), Violet red bile agar (VRBA), Violet red bile D-glucose agar (VRBDGA), and a newly developed KJ medium will be compared for the efficacy of isolation for the species and for optimal α-D-glucosidase activity with the fluorogenic selective marker, 4-Methylum-

belliferyl-α-D-glucoside and secondary selective markers. *C. sakazakii* strains ATCC 29544, ATCC 29004, ATCC 12868, and ATCC 51329 will be utilized as positive controls to run in artificially contaminated powder infant milk formula (PIMF) with each enrichment broth. DNA will be extracted from enrichments, which will be examined using real-time TaqMan PCR in order to compare to culture-based detection to determine relative sensitivities between the two approaches. The fluorogenic selective marker, secondary selective markers, and using a TaqMan probe PCR protocol will prove to be a rapid and specific powerful tool for the detection of *Cronobacter sakazakii* in powdered infant milk formula.