SIMULTANEOUS QUANTITATION OF ESCHERICHIA COLI O157:H7, SALMONELLA AND SHIGELLA IN GROUND BEEF BY MULTIPLEX REAL-TIME PCR AND IMMUNOMAGNETIC SEPARATION

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ABSTRACT

The objectives of this study were to establish a real-time multiplex polymerase chain reaction (PCR) for simultaneous quantitation of Escherichia coli O157:H7, Salmonella and Shigella that have been implicated in a number of foodborne disease outbreaks. Genomic DNA for the real-time PCR was extracted by the boiling method. Three sets of primers and corresponding TaqMan® probes were designed to target these three pathogens. Multiplex real-time PCR was carried out with TaqMan® Universal PCR Master Mix in an ABI Prism 7700 Sequence Detection System. Final standard curves were calculated by plotting the threshold cycle ($C_t$) value against $\log_{10}$ CFU/ml by linear regression to analyze the results for each pathogen. With optimized conditions, the quantitative detection ranges of the real-time multiplex PCR for pure cultures were $10^2$ to $10^9$ CFU/ml for E. coli O157:H7, $10^3$ to $10^9$ CFU/ml for Salmonella and $10^1$ to $10^8$ CFU/ml for Shigella. When this established multiplex real-time PCR system was applied to ground beef samples, the lowest detection concentration of three pathogens were increased to $10^5$ CFU/g for E. coli O157:H7, $10^3$ CFU/g for Salmonella and $10^4$ CFU/g for Shigella. Immunomagnetic separation was then used to isolate E. coli O157:H7 and Salmonella from the beef samples. The lowest detection concentrations of three
pathogens were reduced to $10^3$ CFU/g. TaqMan® real-time PCR, combined with IMS has the potential to be a faster and more reliable method for rapid quantitation of *E. coli* O157:H7, *Salmonella* and *Shigella* in food, which will take 3 h for the whole process.