

Public Abstract

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Title:DETECTION OF VIABLE ESCHERICHIA COLI O157:H7 IN FOOD BY
PROPIDIUM MONOAZIDE REAL-TIME POLYMERASE CHAIN REACTION

The objective of this study was to develop and optimize a method that combines propidium monoazide (PMA) staining with real-time PCR to detect only viable cells of *E. coli* O157:H7. PMA is a dye that can penetrate dead cells and bind to cellular DNA, preventing its amplification via a subsequent PCR. PMA has been reported to exert less influence on DNA amplification from viable cells. Three strains of *E. coli* O157:H7 (505B, G5310 and C7927) was prepared separately and serially diluted to generate cell suspensions. Dead cells were obtained by heating. Suspensions were then treated with PMA. The optimized assay was then applied to artificially contaminated apple juice and ground beef. DNA was extracted and amplified by TaqMan® real-time PCR targeting the *uidA* gene to detect only viable *E. coli* O157:H7 cells. Plasmid pUC19 was included in each reaction as an internal amplification control (IAC) to monitor the efficiency of real-time PCR. Results showed that a treatment of 25 µM PMA with a 10-min light exposure on ice was sufficient to eliminate DNA from 10⁸ CFU/mL dead *E. coli* O157:H7 cells. The optimized assay could detect viable *E. coli* O157:H7 at as low as 10² CFU/mL in pure culture, 10⁴ CFU/mL in apple juice and 10⁵ CFU/g in ground beef, in the presence of 10⁶ CFU/mL or g dead cells. With 8 h enrichment, viable *E. coli* O157:H7 of 1 CFU/mL or g in apple juice or ground beef was detectable without interference from 10⁶ CFU/mL or g dead cells.