Title Detection of 5 CFU/g of *Escherichia coli* O157:H7 on lettuce using activated charcoal and real-time PCR without enrichment

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Abstract

A sample treatment method which separates *Escherichia coli* O157:H7 from lettuce and removes PCR inhibitors allowing 5 CFU/g of target cells to be detected using real-time PCR is described. Lettuce leaves inoculated with *E. coli* O157:H7 were rinsed with 0.025% sodium dodecyl sulfate (SDS). In this study, there were two major factors that strongly affected the recovery of *E. coli* O157:H7 during sample preparation, the amount of bentonite coated activated charcoal used to remove PCR inhibitors and the agitated contact time of the samples with the coated charcoal. When 3.0 g of activated carbon coated with bentonite were mixed with target cell suspensions (30 ml) derived from 50 g of lettuce, a high recovery of *E. coli* O157:H7 (93%) was obtained. Sample agitation with bentonite coated activated charcoal for 15 min resulted in 95% recovery of *E. coli* O157:H7. When a commercial DNA purification resin was used for detection of *E. coli* O157:H7 without the use of the bentonite treated charcoal, the real-time PCR (Rti-PCR) failed to detect 1×10^2 CFU/g. In contrast, with the use of use of bentonite coated activated charcoal and a commercial DNA purifying resin together, Rti-PCR was able to detect 5 CFU of *E. coli* O157:H7/g of lettuce which was equivalent to 2.8 CFU/Rti-PCR. Such a successful detection level was the result of the bentonite coated activated charcoal's ability to absorb the PCR inhibitors released from seeded lettuce during detachment. A standard curve was generated by plotting the Ct values against the log of CFU of target bacterial cells. A linear range of DNA amplification was exhibited from 5.0×10^0 to 1.0×10^4 CFU/g by using Rti-PCR.