

Title Reduction of *Escherichia coli* O157:H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and *trans*-cinnamaldehyde

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Abstract

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 has been recognized as a major foodborne pathogen responsible for frequent gastroenteritis outbreaks. Phages and essential oils can be used as a natural antimicrobial method to reduce bacterial pathogens from the food supply. The objective of this study was to determine the effect of a bacteriophage cocktail, BEC8, alone and in combination with the essential oil *trans*-cinnamaldehyde (TC) on the viability of a mixture of EHEC O157:H7 strains applied on whole baby romaine lettuce and baby spinach leaves. The EHEC O157:H7 strains used were Nal^R mutants of EK27, ATCC 43895, and 472. Exponentially growing cells from tryptic soy (TS) broth cultures were spot inoculated on leaves and dried. EHEC cells were placed at low, medium, and high inoculum levels (10^4 , 10^5 , and 10^6 CFU/mL, respectively). Appropriate controls, BEC8 (approx. 10^6 PFU/leaf), and TC (0.5% v/v) were applied on treated leaves. The leaves were incubated at 4, 8, 23, and 37 °C in Petri dishes with moistened filter papers. EHEC survival was determined using standard plate count on nalidixic acid (50 µg/mL) Sorbitol MacConkey agar. No survivors were detected when both leaves were treated with BEC8 or TC individually at low inoculum levels after 24 h at 23 and 37 °C. When the EHEC inoculum size increased and/or incubation temperature decreased, the efficacy of BEC8 and TC decreased. However, when the two treatments were combined, no survivors were detected after 10 min at all temperatures and inoculum levels on both leafy greens. These results indicated that the BEC8/TC combination was highly effective against EHEC on both leafy greens. This combination could potentially be used as an antimicrobial to inactivate EHEC O157:H7 and reduce their incidence in the food chain.