

Title Alterations of the phylloepiphytic bacterial community associated with interactions of *Escherichia coli* O157:H7 during storage of packaged spinach at refrigeration temperatures

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Abstract

This study investigated the effects of packaging and storage temperature on the spinach phylloepiphytic bacterial community and fate of *Escherichia coli* O157:H7. Freshly harvested spinach was rinsed and/or disinfected, packaged and stored under typical retail conditions (4 °C) or under temperature abuse conditions (10 °C) for a period of 15 days. The final population size of culturable epiphytic bacteria after 15 days of storage was not affected by the temperature of storage or the presence of *E. coli* O157:H7. However, analysis of the bacterial community using denaturing gradient gel electrophoresis of 16s rDNA revealed changes with time of storage and the presence of *E. coli* O157:H7. Excision and sequencing of prominent DGGE bands identified that the majority of sequences belonged to the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Alphaproteobacteria*. After 10 days of storage at 4 °C or 10 °C the population became more dominated by psychrotrophic bacteria. Removal of the epiphytic bacteria resulted in significant increases in numbers of *E. coli* O157:H7 at 10 °C and was associated with decreased expression of *E. coli* O157:H7 virulence (*stxA*, *curl*, *eaeA*) and stress response (*rpoS*, *sodB*) genes. In conclusion, storage temperature and time of storage of packaged spinach affected the diversity of the epiphytic spinach microbiota which influenced the growth, establishment, physiology and potentially virulence of *E. coli* O157:H7.