Title Use of bioluminescence to examine parameters associated with Salmonella enterica serotype Poona and Escherichia coli O157:H7 contamination of produce

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Abstract

Numerous foodborne illnesses have been associated with fresh fruits and vegetables resulting from contamination with *E. coli* O157:H7and *Salmonella spp* . Many studies have been undertaken to determine the potential sources of contamination of fresh produce however limited investigation has been conducted on factors affecting pathogen survival on harvested produce. Determination of metabolic activity in situ is problematic due to current methodology which often requires removal of the bacteria and destructive assays. The main objective of this research was to evaluate the use of bioluminescence as method to monitor metabolic activity of *E. coli* O157:H7 and *Salmonella enterica* serotype Poona. Both bioluminescent *E. coli* O157:H7 and S. Poona were constructed by transformation with a plasmid containing a *lacluxCDABE* cassette for the determination of potential carbon utilization and patterns of utilization associated with fruits and vegetables. A bioluminescent strain of S. Poona was also constructed by recombination of a *luxCDABE* gene cassette fused to the kanamycin resistance marker into the chromosome for long term studies to overcome plasmid instability. Carbon utilization patterns based on increased luminescence varied with different extracts analyzed with commercial produce extracts and lab extracts both in overall luminescence and kinetics for both plasmid based constructs. In situ radish studies showed carbon was bioavailable from the germinating seed as well as from the root suggesting that root exudates may support survival of *E. coli* O157:H7. In situ assays using wounded cantaloupes showed that S. Poona could migrate from the site of inoculation into adjacent mesocarp tissue. These results suggest bioluminescence can be used as a tool to examine metabolic activity and as a marker to monitor pathogen movement within the produce.