

TITLE: INTERACTIONS OF NON-O157 SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* TO LEAFS

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ABSTRACT:

Shiga toxin-producing *Escherichia coli* are important foodborne pathogens associated with diarrhea, bloody diarrhea and hemolytic uremic syndrome (HUS) in humans. Transmission of STEC to humans can occur by ingestion of contaminated foods, direct contact with animals, or by person to person transmission. Maintenance and persistence of STEC in the environment and foods can be related to its ability to form biofilm. In a previous study one O105:H18 STEC strain isolated from bovine feces was distinguished by its great ability to form biofilm in abiotic surfaces. Considering the number of outbreaks and sporadic HUS cases associated with ingestion of ready-to-eat products, as fresh produce, we aimed to investigate the adherence ability of this non-O157 STEC strain to vegetable surfaces, and to search for the structures involved in this process. Adherence assays to rocket leaves (*Eruca sativa*) were performed for 3hs at 28°C. Preparations were then washed in PBS, fixed, and prepared for scanning electron microscopy analysis. Number of adherent bacteria in distinct areas of the analyzed leaf fragment was counted. Deletion of genes related to Ag 43 and the STEC autotransporter protein contributing to biofilm (Sab) was carried out by using the lambda red system for recombination. The O105:H18 STEC adhered to leaf surface, and bacterial aggregates were observed. The number of adherent bacteria per square millimeter of leaf was 8-folds higher compared to an O157 STEC strain. Loss of target genes in the isogenic mutants were confirmed by PCR, and all mutants were confirmed to grow at the same rate as the wild type (WT) strain under the conditions tested. Deletion of Sab reduced in almost 50% the O105:H18 adherence ability and deletion of Ag 43 almost abolished the interaction; with the number of adherent bacteria being reduced in more than 50-folds compared to WT strain. Very few bacteria were seen on the leaf surface, and these differences were statistically significant. In conclusion, the importance of both proteins and especially the Ag 43 in the interaction of O105:H18 STEC strain with leaf surface can be suggested.

Keywords: non-O157 STEC, adherence, vegetables

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