TITLE: TYPE THREE SECRETION SYSTEM 2 (ETT2) OF ENTEROAGGREGATIVE *E. coli* 042: EXPRESSION AND COMPARATIVE SECRETOME ANALYSIS

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ABSTRACT:

The capacity to secrete proteins to the outside environment is one of the most important virulence traits of pathogenic bacteria. Type three secretion systems (T3SS) are known to allow gram-negative bacteria deliver effector proteins from the bacterial cell core directly to the eukaryotic cytoplasm. The most studied *E. coli* T3SS, known as ETT1, has been found among EPEC and EHEC strains and is related to the delivery of effectors involved in cell-shape modification, such as the formation of A/E lesion by those bacteria. However, the analysis of *E. coli* O157:H7 genome revealed the existence of a second group of T3SS in *E.coli* named ETT2, that are related to the system found in *Salmonella sp.* in which, effectors present immunomodulator properties. ETT2 is present in numerous *E. coli* serotypes, but usually with truncated or missing genes. Enteroaggregative E. coli (EAEC)prototype strain 042 has been described as a carrier of the complete set of ETT2 encoding genes. Therefore, the aim of this work was to evaluate the secretome profile of EAEC 042 and its derivative mutant in which the ATPase coding gene (*eivC*) was deleted by site-direct mutagenesis employing a suicide delivered-based-method with pJP5603 plasmid. Bacteria cells were grown in six different conditions: DMEM, *E. coli* broth (EC), pre-conditioned DMEM, LB pH 7.0, LB supplemented with 50mM Mg (LB-Mg), and 6h growth in LB pH 6.0 followed by 3h growth in LB pH 8.0 (pH shift). Presence of the ETT2 in these preparations was then analyzed by immunofluorescence with anti-EprI antibody that recognizes the needle of the T3SS. Positive signal was observed among the cells grown in LB-Mg and on pH shift, and the last condition was chosen to perform the secretome analyzes, since it mimics the passage of the bacteria through the gastro-intestinal tract. 2-DE-gel analysis indicated differences on the profile of the mutant compared to the wild type strain. A total of 9 spots present on the secretome of 042 and absent on the mutant's secretome were selected for analysis. Our results represent evidence that ETT2 is expressed by EAEC and might be involved on secretion of proteins by these diarrheagenic bacteria. However, the range of secreted proteins by that system is yet to be determined. Further proteomic studies are in progress in order to address this issue.