Inactivation of the type three secretion system ETT2 does not affect the physiology or adherence patterns of enteroaggregative *Escherichia coli* 042

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Type three secretion systems (T3SS) are a conglomerate of proteins employed by gram-negative bacteria to deliver effector proteins directly into the cytoplasm of eukaryotic cells, leading to modification on the host cell physiology and/or structure. In diarrheagenic *E. coli*, the most studied system is named ETT1, which is responsible for the establishment of the attaching and effacing lesions by enteropathogenic (EPEC) and enterohaemorrhagic (EHEC) *E. coli*. The genome sequence of *E. coli* O157:H7 revealed the existence of a gene cluster encoding components of a second type III secretion system, named ETT2, present in a numerous *E. coli* serotypes, but usually with truncated or missing genes. The 042 prototype EAEC strain carries the complete set of ETT2 encoding genes. The aim of this work was to evaluate the role of ETT2 in the physiology and adherence patterns of EAEC 042. The ATPase coding gene (*eivC*) was deleted by site-direct mutagenesis employing a suicide delivered-based-method with pJP5603 plasmid. The mutant selected did not show any modification on growth in culture media compared to wild type. Production of biofilm on polystyrene plates demonstrated that the ability to produce a thick layer of polysaccharide was preserved in the mutant strain. Adhesion to HeLa cells, after three hours of incubation, presented the same pattern as observed for the wild type cell. Finally, a brief analysis of the mutant secreted proteins profile by SDS-PAGE did not indicate a major difference on the number and intensity of the proteins precipitated from bacterial culture in Luria Bertani broth, or pre-conditioned DMEM media. Altogether, the present results indicate that the inactivation of the ETT2 system does not modify the physiology of EAEC 042 considering the analyzed traits. Our results represent an evidence that ETT2 inactivation do not interfere with the EAEC 042 physiology and adherence capacity *in vitro*. However, the range of secreted proteins by that system is yet to be determined. Proteomic studies are in progress in order to address that.

Key words: Enteroaggregative *Escherichia coli*; Type three secretion system; ETT2.

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