TITLE: Modulation of c-di-GMP signalling in atypical EPEC virulence

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ABSTRACT: Enteropathogenic *Escherichia coli* (EPEC) is one of the etiologic agents of diarrhea in children under 5 years of age, especially in developing countries. The transcription of factors that participate in the virulence, motility and biofilm formation of this pathotype is regulated by chemical signaling. An important signal molecule is the cyclic secondary messenger guanosine bis- (3'-5') -monophosphate (c-di-GMP), which regulates a number of cellular processes in bacteria. The regulation by c-di-GMP involves its synthesis from two GTP molecules by diguanylate cyclase (DGC) proteins, which contain a conserved GGDEF (Gly-Gly-Asp-Glu-Phe) domain, and its degradation by phosphodiesterases (PDE), such as YhjH, whose activity is associated with the EAL (Glu-Ala-Leu) or HD-GYP domains. To exercise its function, c-di-GMP needs to bind and allosterically alter the structure of an effector protein. One of the known c-di-GMP receptors is YcgR that is involved in the regulation of the flagella. There is a lack of studies relating the role of c-di-GMP and the pathogenesis of E. coli. The aim of this work was to analyze the influence of c-di-GMP on the regulation of virulence mechanisms in atypical EPEC. For this purpose, the O55: H7 and ONT: H25 strains were selected for the deletion by homologous recombination of the yhjH gene, which encodes a phosphodiesterase, and *ycgR*, which encodes a c-di- GMP receptor. The O55: H7Δ*ycgR* and ONT: H25Δ*ycgR* mutants showed a slight reduction of motility when compared to the wild type strains. On the other hand, the O55: H7ΔyhjH and ONT: H25Δ*yhjH* strains presented a drastic reduction of motility. The production of cellulose by ONT: H25Δ*ycgR* and ONT: H25*Δ*yhjH* mutants decreased when compared to their respective wild type strains. The curli fimbriae production assay demonstrated that O55: H7Δ*yhjH* and ONT: H25Δ*yhjH* mutants showed a change in the rdar (Red, Dry and Rough) phenotype at 26 °C. Biofilm formation was altered in the mutant strains as observed by the crystal violet colorimetric assay. These results demonstrate that c-di-GMP participates in the regulation of these phenotypes in atypical EPEC.

Keywords: atypical EPEC, biofilm, c-di-GMP, *ycgR*, *yhjH*

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