Title: Influence of Quorum Sensing Systems on the biofilm formation and on the interaction with epithelial cells by atypical EPEC

Authors: COUTO, S.C.F; BUERIS, V.; CULLER, H. F.; HIGA, J. S.; YANG, M. J.; SIRCILI, M. P.

Institution: Laboratório de Genética - Instituto Butantan, São Paulo, SP (Avenida Vital Brasil, 1500, Prédio Novo, CEP 05503-900, São Paulo - SP, Brazil)

Abstract: The survival of human pathogens during long periods in the environment is related to their ability to form biofilm. Interactions within the bacterial communities can promote the dissemination of antimicrobial resistance genes and virulence factors. As vastly described in the literature, the formation of biofilm is regulated by Quorum Sensing (QS) in various bacterial species. Bacteria frequently employ more than one AI to regulate multiple characteristics, and various QS circuits are directly or indirectly associated. Since these systems are involved in complex signaling cascades, it is possible that in the absence of a signaling pathway that is under control of a determined QS system, other pathway might be employed in a compensatory manner. The aim of this study was to investigate the influence of QS genes on biofilm formation and on the interaction with epithelial cells by an atypical EPEC strain. Mutant strains on QS related genes were obtained by homologous recombination. Biofilm production was analyzed through the Crystal Violet binding assay and it was observed that the biofilm produced by the *luxS* mutant increased in comparison to the biofilm produced by the wild type strain. On the other hand, deletion of *qseC* on the single mutant and on the double mutant strain resulted on the same amount of biofilm produced by the wild type. The *sdiA* mutant strain had a drastic decrease in biofilm formation. In constrast, the triple mutant showed similar results to the wild type strain. Bacterial interaction with epithelial cells was analyzed after interaction with HeLa cells after 6 hours of incubation. The deletion of the *luxS* gene caused an increase of adhesion both on HeLa cells and on the cover slip. On the other hand, the deletion of *qseC* showed a remarkable decrease on the number of attached bacteria. Unexpectedly, the double deletion had no effect on the amount of attached bacteria. The *sdiA* mutant strain showed similar results when compared to the wild type strain. Interestingly, the triple mutant strain behaved similarly to the *qseC* mutant strain. The results obtained with the double mutant strain indicate an antagonistic interaction between the *qseC* and *luxS* genes, and it seems that *sdiA* plays a role in this intricate system. These data reinforce the idea that biofilm formation and host colonization are complex phenotypes tightly regulated in response to environmental signals, and that a vast number of regulation mechanisms are involved, including QS.

Keywords: Pathogenicity, Gene Regulation, Biofilm, Quorum Sensing

Technical support: Youko Miura Yamamoto

Support Agency: CAPES