TITLE: *Citrobacter rodentium* chemical signaling mediated by QseC and QseE sensor kinases.

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ABSTRACT:
Enteropathogenic and enterohemorrhagic *Escherichia coli* are gastrointestinal human pathogens, and they do not have an animal model broadly accepted in the literature. They albeit in their genome the *Locus of Enterocyte Effacement (LEE)* pathogenicity island, responsible to encode important virulence factors. The histidine sensor kinases QseC and QseE regulate directly LEE in these pathogens. The norepinephrine (NE) and epinephrine (Epi) hormones regulate directly LEE thought the QseC and QseE sensors, and modulate their virulence. *Citrobacter rodentium* is a natural murine pathogen, which exhibit homology of 67% their genes with these human pathogens, including LEE, and becomes an alternative model to *in vivo* studies as bacteria carrying LEE island. Herein the chemical signaling was studied by phenotypic assays in *C. rodentium* wild type (Wt), and *ΔqseC*, *ΔqseE* and *ΔqseC*/*ΔqseE* isogenic sensor mutants, through epithelial cells adhesion and motility assays, in this case to evaluate swarming in this non-motile rodent pathogen. The bacterial strains were grown aerobically in Luria-Bertani medium (LB) broth or agar, at 37ºC. The NE was solubilized in dimethylsulfoxide (DMSO), and pH adjusted. The HeLa cells were seeded 24 wells plates during 24 hours to a 80% confluence. After that, the cells were washed, and the assay was performed the bacterial cell adhesion in semi-confluent cell monolayers in presence and absence of NE, in concentration 50nM, 1 μM and 50 μM, for 3 and 6 hours. Posteriorly, serial dilution of the samples was made, the colony forming units (CFU) were counted. In motility test like swarming, the bacterial strains were grown in LB broth overnight, and inoculated 5 μl of the strains on the surface of LB semi-solid medium (0,5% agar), with addition of 1 μM NE. The control group was added DMSO in same concentration of NE. Thereafter the surface growth halos were measured at 12, 24, 36 and 48 hours. NE does not seems to affect the adhesion in 3 and 6 hours assays in HeLa cells, since do not have statistical differences between the control group and treat group. Conversely NE affected swarm formation in this bacteria, since halo measured were larger in both Wt and *qseC* mutant treated groups, comparing to non-NE control group. NE modulate responses in this murine pathogen and seems to be QseC-independent. Other chemicals signaling tests are still necessary to better elucidate the full role of sensors QseC and QseE in C. *rodentium* pathogenesis.

Keywords: *Citrobacter rodentium*, chemical signaling, histidine kinase sensors.

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