

TITLE: Regulation of two-component system gene expression in *Salmonella enterica* serovar Typhimurium by microbiome-derived small molecules

AUTORES: De Pina, L.C.^{1,2}; Rêgo, A.M.²; Alves, E.S.³; Ferreira, N.V.²; Pauer, H.²; Boente, R.³; Lobo, L.A.³; Domingues, R.M.C.P.³; Ferreira, R.B.R.³ & Antunes, L.C.M.²

INSTITUTION: ¹Programa de Pós-Graduação em Biociências (PPGB-UERJ), Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro; ²Centro de Referência Prof. Hélio Fraga, Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz; ³Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro.

The interaction between members of the human gut microbiome, host cells and invading pathogens often occurs through small molecules (metabolites). The perception and effective response of a microorganism to the different conditions found in its environment is important for its adaptation, survival and dissemination. Two-component systems (TCS) allow the perception and response to environmental changes by regulating the expression of specific genes. Our group previously showed that an organic extract of human feces, as well as the specific metabolite 3,4-dimethylbenzoic acid (DMB), inhibit the ability of *Salmonella* to invade host cells. The present work proposes to investigate the impact of the human gut metabolome as well as small molecules produced by *Clostridium citroniae* (a member of the gut microbiome) on the expression and activity of *Salmonella* TCS genes. Metabolites (from feces or *C. citroniae* cultures) were extracted using ethyl acetate and added to LB broth. The pH of the medium was adjusted (~7.4), and the solution was filter-sterilized. *Salmonella* was grown in the presence or absence of the organic extracts as well as DMB under aerobic and anaerobic conditions until it reached mid-log growth. RNA was isolated using the RNeasy Mini Kit (Qiagen) and cDNA synthesis was performed using the QuantiTect Reverse Transcription Kit (Qiagen). Real-time PCR was performed using QuantiNova SYBR Green RT-PCR Kit and the Applied Biosystems 7500 system with primers targeting almost all TCS systems of *Salmonella*. Our results showed that several TCS involved in *Salmonella* virulence (*ssrAB*, *envZ-OmpR*, *qseCB*, *phoQP*) were differentially regulated by these small molecules both in aerobic and anaerobic conditions. EnvZ-OmpR and SsrAB are directly involved in the regulation of *Salmonella* Pathogenicity Islands 1 and 2, respectively. QseCB is crucial for *Salmonella* quorum sensing, sensing of host hormones, and swimming motility. Several other TCS were also regulated, including TorSR, which is involved in trimethylamine *N*-oxide respiration, and may be important for *Salmonella* survival in the anaerobic environment of the human gut. Interestingly, the effect of bioactive molecules on gene expression is distinct under aerobic and anaerobic growth conditions. Our results show that important signaling pathways of *Salmonella* virulence can be modulated by metabolites present in the human gut microbiome, and open avenues for further research into microbiome-pathogen intercellular signaling in the gut environment.

Keywords: *Salmonella*, Two-component system, metabolome, microbiome.

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