TITLE: BIOACTIVE SMALL MOLECULES PRODUCED BY THE HUMAN GUT MICROBIOME MODULATE *VIBRIO CHOLERAE* VIRULENCE GENE EXPRESSION

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ABSTRACT

Humans live in symbiosis with a diverse community of microorganisms, which has evolved to carry out many specific tasks that benefit the host. Within the chemical diversity of the gastrointestinal tract, many of the molecules found likely constitute chemical cues for the communication between the microbiota and invading pathogens. The goal of this work was to investigate if molecules produced by the human gut microbiota show biological activity against the human pathogen *Vibrio cholerae*. In order to probe the unknown properties of the human gut metabolome, we extracted molecules from fresh feces of a healthy donor, from *Clostridium citroniae*, and *Bacteroides thetaiotaomicron*, and tested the effect of the extracts on *V. cholerae*, comparing bacterial growth in the absence and presence of the small-molecule extracts. In these experiments, we observed that molecules present in the gut metabolome as well as those produced by *C. citroniae* inhibit *V. cholerae* growth, although the exact reason for this effect is unknown. The extract from *B. thetaiotaomicron* did not affect pathogen growth. The next step was to determine the impact of the fecal extract on global gene expression profiles of *V. cholerae*. RNA sequencing results showed that small molecules present in the gut metabolome may have a significant impact on microbe-microbe interactions established in this environment and provide a framework for the study of other small molecules involved in microbiome-pathogen interactions. Among the genes downregulated in the presence of the fecal extract, and based on KEGG categories, 21% were involved in “cellular processes”, 19% in “metabolism”, and 41% encoded hypothetical proteins. Among the 58 genes classified in cellular processes, 40 are involved in *V. cholerae* chemotaxis. The ability of fecal and *C. citroniae* extracts to inhibit *V. cholerae* motility were confirmed with phenotypic tests, which also showed that *B. thetaiotaomicron* extracts have no significant inhibitory activity. We also investigated the role of the extracts on mucin layer penetration *in vitro* and observed that both fecal and *C. citroniae* extracts impaired *V. cholerae* mucin penetration, while *B. thetaiotaomicron* extracts did not. Future work will likely reveal new molecules in the intestinal environment that are involved in interspecies interactions. Many of these may have potential antivirulence activity that can be pursued for therapeutic purposes.

Keywords: Microbiome, Gut metabolome, Antivirulence, Bioactive small molecules, *Vibrio cholerae*

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