TITLE: REGULATION OF *CLOSTRIDIOIDES DIFFICILE* GENE EXPRESSION BY MICROBIOME-DERIVED SMALL MOLECULES

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ABSTRACT:

Clostridioides difficile is an important nosocomial pathogen, being considered one of the major etiological agents of antibiotic-associated diarrhea (AAD). Treatment of *C. difficile* infection consists of metronidazole for moderate diarrhea and vancomycin for severe cases, which further increases the impact on the gut microbiota. Therefore, alternative treatments to eradicate *C. difficile* while preserving the gut microbiota are necessary. Secondary metabolites from the intestinal microbiota emerge as a promising source of bioactive compounds. The aim of this study was to analyze the effect of small molecules extracted from human feces on global gene expression by *C. difficile*. Fecal samples were collected from healthy donors without prior use of antibiotics for the last month. Small molecules were extracted from fecal samples using ethyl acetate. Dried extracts were resuspended directly in culture medium, filtered, and the pH was adjusted to match that of culture medium alone. Dried residues of ethyl acetate were used as controls. Initially, we assessed the effect of fecal extracts on bacterial growth by performing growth curves and found that the fecal extract did not inhibit growth of *C. difficile* R20291. After approximately 6 hours of growth, the cultures had reached the mid-logarithmic growth phase and RNA was isolated using the Roche High Pure RNA isolation kit. The effect of the fecal extract on global gene expression by *C. difficile* R20291 was then studied through RNA sequencing using an Illumina platform. RNAseq data showed that 97 genes were upregulated in the presence of the fecal extract, including phage-related genes, genes involved in the degradation of amino acids and purine ribonucleotide biosynthesis, among others. On the other hand, growth in the presence of the fecal extract resulted in downregulation of 198 genes, such as genes associated with chemotaxis and motility, transport of carbohydrates, organic acids and alcohols, and cell membrane composition. Additionally, phenotypic motility tests were performed, and results showed that growth in the presence of the fecal extract significantly reduces *C. difficile* swimming motility. Other phenotypic tests, such as the cytotoxicity assay using Vero and HT29 cells, are currently underway to investigate the role of gut small molecules on various aspects of *C. difficile* virulence.

Keywords: *Clostridium difficile*, microbiota, small molecules, virulence factors

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