

**Title: EXPLORATORY INVESTIGATION OF *Bacteroides fragilis* TRANSCRIPTOME SELECTED AFTER *IN VITRO* EXPOSURE TO SUBINHIBITORY CONCENTRATION OF METRONIDAZOLE.**

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**Leite**

**Abstract:**

*Bacteroides fragilis*, a common member of the gut microbiota, is widely recognized as an important pathogen associated to anaerobic infections. Elucidation of bacterial genome expression would lead to a better understanding of microbial pathogenesis. In addition, it is accepted that antimicrobial drugs may have implications in host-bacteria interactions. The aim of this study was qualitative evaluation of *B. fragilis* transcriptome after *in vitro* exposure to metronidazole at sub-inhibitory concentrations. Bacteria strains were selected after *in vitro* subcultures with metronidazole (1µg/mL). From a parent *B. fragilis* ATCC 43859 (wild-type) 4 derivative strains were considered: 1st (BfMTZ1+) and 4th (BfMTZ4+) subcultures during drug exposure, and 1st (BfMTZ1-) and 4th (BfMTZ4-) subcultures after drug exposure. Subcultures were performed in 48h intervals, in Brain Heart Infusion broth under anaerobic environment. Total RNA was extracted from 1 ml of 24h cultures using the RNeasy mini kit (Qiagen). Ribosomal RNA was depleted using Ribominus Kit and cDNA library was obtained after emulsion PCR, prior to pirossequencing (Platform 454 Roche). Out of 4312 protein coding genes, 3597 ORFs were identified (83.41%), of these, 992 were assigned to Kyoto Encyclopedia of Genes and Genomes (KEGG), and 27.57% retrieved no function associated. Among 3597 identified genes, 1544 were found in all strains, which suggest that genes are critical for *B. fragilis*. In presence of metronidazole, expression of 188 exclusive genes was identified, and after drug removal 934 genes were expressed. Overall under metronidazole selective pressure gene expression related to carbohydrate metabolism, nucleotide metabolism, amino acids, lipids, micronutrients, xenobiotics metabolism and degradation, replication mechanisms and repair and transport membrane were up regulated. Although no function was attributed to all bacteria expressed genes, elucidation of *B. fragilis* transcriptome during drug exposure, mainly at subinhibitory concentrations, would contribute with information about bacteria survival strategies under stress conditions in their residency environment.

**Keywords:** *Bacteroides fragilis*, Metronidazole, Transcriptome

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