

Title: DIFFERENTIAL PROTEOMIC ANALYSIS OF OUTER MEMBRANE ENRICHED EXTRACTS OF *BACTEROIDES FRAGILIS* GROWN UNDER BILE SALTS STRESS

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Abstract:

Bacteroides fragilis is the anaerobic bacteria most commonly isolated from infectious processes. Several virulence factors contribute to the pathogenic nature of this bacterium, among them the ability to tolerate high concentrations of bile. Modulation of outer membrane proteins (OMPs) is considered a crucial event to bile salts resistance. The primary objective of this work was to isolate and to identify proteins in *B. fragilis* associated to the stress induced by high concentration of bile salts. The outer membrane of *B. fragilis* strain 638R was isolated after growth either in the presence and absence of 2% conjugated bile salts. Membrane fractions were separated on SDS-PAGE and analyzed by ESI-Q-TOF tandem mass spectrometry. Thirty-seven proteins were identified through MASCOT in addition of two others identified by BLAST alignment. Nine proteins were found to be expressed exclusively in the absence of bile salts whereas eight proteins were expressed only in the presence of bile salts. These proteins are related to cellular functions such as transport through membranes, nutrients uptake and protein-protein interactions. The protein gjl375357086 was structurally similar to secretin and was selected for disruption of gene expression. The resulting mutant strains were tested for resistance to increasing concentrations of bile salts (1%, 2%, 5% and 8%). The assays presented significant differences ($p < 0,05$) between the mutant and parental strains under the conditions where bile salts are significantly higher than physiological (5% e 8%). Minimal inhibitory concentration by microdilution method using BHI broth supplemented with dilutions of up to 20% bile salt solutions were performed. The parental strain was inhibited by 8% bile salt whereas mutant strains were inhibited by 10% bile salts. Then, minimal bactericide concentration showed that 10% and 14% bile salts are lethal to parental and mutants strains, respectively. In view of these results, the protein selected for mutant construction may be involved in bile salts efflux and influx or its deletion can affect metabolism and energy generation by the deconjugation of bile salts. This study demonstrates the role of OMPs in bile salts resistance and adaptation to environmental changes. Proteomics of OMPs also showed to be a useful approach in identification of new targets for functional analysis. In this study, it was possible to identify a protein directly involved on bile salts susceptibility.

Keywords: *Bacteroides fragilis*, bile salts resistance, ESI-Q-TOF, outer membrane proteins, secretins.

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