

TITLE: Effect lactoferrin on *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*

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ABSTRACT:

The genus *Bacteroides* is composed by several species that are present in large numbers in the human intestinal microbiota, such as *Bacteroides fragilis*. This specie is known to manipulate the immune defenses and is the most frequent strict anaerobe isolated from infections. Others species, like *Bacteroides thetaiotaomicron* are responsible for important metabolic functions related to the host organism and has the capacity to modulate the expression of genes related to carbohydrate metabolism. The prevalence of this species in the human microbiota is regulated by several factors of the host, including proteins with antimicrobial activities such as lactoferrin. This glycoprotein is present in various secretions, and its main activity is to chelate iron ions. When lactoferrin reaches the digestive tract, gastric, pepsins digest it generating a bactericidal peptide called lactoferricin. In this context the study was to evaluate growth changes in species *B. thetaiotaomicron* and *B. fragilis*, in response to the presence of holo and apo-lactoferrin, and the peptide lactoferricin B in semi-defined medium for *Bacteroides spp* supplemented with different sources of iron. In addition, we studied the effect of this molecule on the formation of biofilm. The strains used are part of the collection of the Anaerobic Biology lab cultures (IMPPG / UFRJ) and were routinely grown in pre-reduced and anaerobically sterilized (PRAS) culture media. All experiments were performed in an anaerobic chamber (80% N₂, 10% CO₂ and 10% H₂). The strains were cultivated in semi-defined medium without iron for *Bacteroides* and incubated for 24 hours at 37 ° C. After this period the cultures were adjusted to OD₆₀₀ = 0,4. The minimal inhibitory concentration (MIC) for holo-lactoferrin, apo-lactoferrin, and lactoferricin B was determined for *B.fragilis* and *B.thetaiotaomicron* in broth microdilution assays. Both strains were resistant to physiological relevant concentrations of lactoferrin (1mg/ml). To evaluate the effect on bacterial metabolism, growth curves were measured spectrometrically (OD_{600nm}). Biofilm formation in polystyrene plates was quantified by crystal violet incorporation. No inhibition of growth by lactoferrin was observed, in fact, the effect of lactoferrin was mildly stimulatory for bacterial growth. In contrast, biofilm formation was strongly inhibited in the presence of lactoferrin.

Keywords: *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, biofilm, lactoferrin, lactoferricin

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