

Title: INHIBITORY EFFECT OF LACTOFERRIN ON *BACTEROIDES FRAGILIS* AND *BACTEROIDES THETAIOAOMICRON*.

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Abstract:

The harmonic relation between the host and the gut microbiota is maintained by several factors, including the host immune response, which must tolerate the high counts of bacteria in the gut and simultaneously keep this population in check. Antimicrobial peptides are employed by the innate immune system to attain this purpose, and several molecules including defensins and cathelicidins are secreted in the gut. The glycopeptide lactoferrin is an important antimicrobial molecule, it binds free iron and limit the availability of this element, but it also has antimicrobial activity by disrupting bacterial membranes. Trypsin degradation of lactoferrin generates an antimicrobial peptide, lactoferricin. The activity of these two molecules on species of the genus *Bacteroides* has not been investigated. These bacteria are strict anaerobes that inhabit the human gastrointestinal tract. Two species, *B. fragilis* and *B. thetaiotaomicron*, stand out, the first is considered an opportunistic pathogen and is the anaerobe most commonly isolated from human infections. The former exert important functions in human physiology, such as digestion of complex polysaccharides. In this study, microdilution bioassays in normal or iron-free media were used to evaluate the inhibitory effect of lactoferrin and lactoferricin in *B. fragilis* and *B. thetaiotaomicron*. The dependency of *Bacteroides* for an external iron source used in the biosynthesis of heme is well documented, thus physiological concentrations of lactoferrin were tested. We also investigated the effect of these two molecules on *in vitro* biofilme formation using a standard polystyrene microplate assay. Lactoferrin was tested in its iron-saturated state as well as deprived of iron. We found that neither lactoferrin nor lactoferricin inhibited bacterial growth in the concentrations tested, despite the iron load or the culture media used. Surprisingly, lactoferrin had a mild stimulating effect on bacterial growth. This effect has been reported with transferrin, another iron chelating agent, and it is due to the ability of the bacteria to use glycans associated with transferrin. Biofilm formation, on the other hand, was highly inhibited by minute concentrations of lactoferrin. This work contributes to understanding the factors involved in the selection and prevalence of species in the gastrointestinal tract and could be used to devise new strategies for selective colonization, such as probiotics, of the gut.

Keywords: Lactoferrin, lactoferricin, biofilm, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*.

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