

Title: BACTERIOCIN-LIKE ACTIVITY OF *Bacteroides* and *Parabacteroides* ISOLATES RECOVERED FROM INTRA-ABDOMINAL INFECTION PATIENTS

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Abstract:

Bacteroides and *Parabacteroides* are Gram negative obligate anaerobes associated with several endogenous diseases, among them intra-abdominal infections. The organisms synthesize bacteriocins, antibacterial substances that seem to confer competitive advantage to producing strains. The aim of this study was to evaluate the production of bacteriocin-like substances by *Bacteroides* and *Parabacteroides* isolates and subsequently to characterize one of these substances. A total of 40 bacterial isolates recovered from patients with intra-abdominal infection were included in the investigation as test strains. As indicators the test strains and 37 Gram negative and Gram positive bacteria were employed. Antagonism expression was evaluated by the overlay method employing different culture media (composition and pH). Iso- and heteroantagonism phenomena were observed for 5/12.5% and 29/72.5% test strains, respectively. Heteroantagonism was detected only against phylogenetically related bacteria. The best results were observed in Brain Heart Infusion Agar added with hemin, menadione, and yeast extract, pH 6.5. After screening one of the producer strains was selected for subsequent steps of the study aiming the characterization of the antagonistic substance. Protein extraction was performed by precipitation with ammonium sulfate. Intracellular extracts precipitated at 30% (C30) and 50% (C50) salt concentration showed antagonistic activity. Both fractions were inactivated by proteases and high temperatures, and remained active after exposure to organic solvents and a wide pH range. Minimum inhibitory and minimum bactericidal concentrations demonstrated that C30 and C50 presented bacteriostatic activity. Sequential chromatographic steps were employed in order to purify C50. The extract was subjected to ion exchange chromatography generating 50 fractions. Among them, fractions 1 to 4 which were not able to bind to the column refer to a single peak and showed antagonist activity. They were submitted to gel filtration chromatography, which generated 35 fractions. Among them fractions 2 and 3 kept active and should be subjected to reverse-phase chromatography. The results indicate that production of bacteriocin-like substances is highly disseminated among our study group. It seems reasonable to hypothesize that such substances play a relevant role in interbacterial relationships specially if we consider the complex background underlying intra-abdominal infections.

Keywords: *Bacteroides*, *Parabacteroides*, antagonistic substance, bacteriocin, intra-abdominal infection

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