

Title: CHARACTERIZATION OF ANTAGONISTIC SUBSTANCE(S) PRODUCED BY *Acinetobacter baumannii*

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Abstract:

Since the discovery of the first antibiotic in 1928, many other antimicrobial drugs have been developed. However, this discovery was followed by the advent of microbial resistance, hindering the therapeutic approach of infectious diseases. Thus, the search for new antimicrobials, specially naturally occurring substances, is critical because they may contribute to the control of drug resistant pathogenic microorganisms and consequently of the diseases associated with them. This study aimed the characterization and purification of antagonistic substance(s) produced by an *Acinetobacter baumannii* isolate recovered from blood culture. The strain was submitted to acid precipitation with HCl 2 N and the crude extract obtained was employed in the following steps of this investigation. The antagonistic activity was confirmed by the spread plate technique against a clinical isolate of *A. baumannii*. The extract was exposed to different pH, organic solvents, and proteolytic enzymes. The molecular mass(es) of the substance(s) present in the crude extract was(were) estimated by SDS-PAGE/in situ antagonistic activity test. The extract was also submitted to reverse-phase chromatography employing C-8 and C-18 columns. The partially purified substance(s) was analyzed by mass spectrometry. Antagonistic activity of the crude extract was found to be 320 AU/mL. The extract was inactivated by proteases and remained active after exposure to organic solvents and a broad pH range. Reverse-phase chromatography employing C-8 column generated 30 fractions among them three active fractions, 31 to 33. The pool of these fractions was chromatographed on C-18 column and eluted with 90% acetonitrile. A total of 43 fractions were recovered. The fraction 37 kept active and was submitted to mass spectrometry. Two molecular masses of 6.2 kDa and 8.3 kDa were detected showing the partial purification of the antagonistic substance(s). The results suggest the synthesis of hydrophobic antimicrobial proteinaceous substance(s) by the *A. baumannii* strain under study. A possible role as a virulence trait of the bacterium as well as a potential biotechnological applicability to the antagonistic substance(s) may be predicted.

Keywords: *Acinetobacter baumannii*, antagonistic substance, chromatography, protein purification

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