PURIFICATION OF A MARINE ANTIMICROBIAL PROTEIN FROM BACTERIUM *Pseudoalteromonas* sp.

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Introduction. Nowadays, marine microorganisms are recognized as producers of secondary metabolites active against many biological targets, which have their own characteristics and structures of the marine environment (1). The *Pseudoalteromonas* genus has attracted attention in the field of biotechnology by the ability the produce compounds of high and low molecular weight as antimicrobial proteins (2). Today, few publications are dedicated to antibiotic proteins from marine bacteria whereas the bacteriocins produced by terrestrial bacteria are recognized to be an excellent source of antibiotic proteins and polypeptides. However, marine bacterium represents certainly and great potential reservoir not sufficiently investigated. On the other hand, protein purification is vital for the characterization of the function structure and interactions of the protein of interest. During the steps of developing new products with potential therapeutic use, it is important to isolate, purify and identify the compound. The aim of this study was to identify and purify compound associated to the cells of the marine bacterium *Pseudoalteromonas* sp, which showed antimicrobial activity again pathogenic *Staphyloccus aureus* MRSA.

Methods. *Pseudoalteromonas* sp produces a protein with inhibitory activities against *S. aureus* MRSA as control. A crude extract was isolated from the bacterial biomass by sonication before ethanol precipitation and salting out with $(NH_4)_2SO_4$. The Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protein profiles of crude extract was performed and antibacterial activity was assayed by direct bioautography in-gel. The antimicrobial protein was purified using gel-filtration and ion-exchange chromatography.The protein concentration and antibacterial activity were determined using the method of BCA with bovine serum albumin as a standard and standard disk diffusion (Kirby-Bauer) method, respectively.

Results. The SDS-PAGE protein profiles of crude extract of the sonicated biomass of *Pseudoalteromonas* sp. showed the presence of one band of approximately 80 kDa. The direct bioautography (Fig. 1), revealed an area of strong growth inhibition at the height of the band of 80 kDa when it was incubated in Petri dishes inoculated with the control strain S. *aureus* MRSA, suggesting that the compound with antimicrobial activity was a protein of high molecular weight. During HPLC purification steps, the crude extract from *Pseudoalteromonas* sp showed to display one fraction (Fig. 2) and antibacterial activity was detected in this. After the two steps of gel-filtration and anion exchange chromatography, 0.062 mg of highly purified were obtained with a specific activity of 575 U/mg.

Fig. 1. Antimicrobial activity against *S. aureus* MRSA developed by direct bioautography. E refers to crude extract; V refers to vancomycin standard

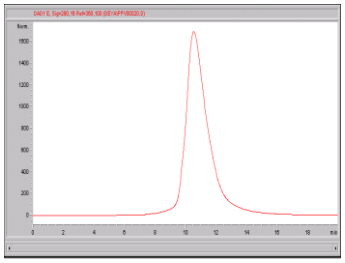

Fig. 2. Gel filtration chromatography of marine microbial proteins from *Pseudoalteromona*s sp.

Conclusions. The marine bacterium *Pseudoalteromonas* produces an antimicrobial protein of a molecular weight of approximately 80 kDa

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