TITLE: STAPHYLOCOCCIN PEP5: PURIFICATION, STRUCTURAL CHARACTERIZATION AND ANTISTAPHYLOCOCCAL ACTIVITY

AUTHORS: FARIAS, F.M.[1,2]; ARAÚJO, T.S.[2]; AMARAL, M.J.[2,3]; ANDRADE, R.A.[2]; MIGUEL, M.A.L.[1]; ALMEIDA, M.S.[2]; BONELLI, R.R.[1]

INSTITUTION: INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES, UFRJ (AVENIDA CARLOS CHAGAS FILHO, 373, CIDADE UNIVERSITÁRIA, ILHA DO FUNDÃO, RIO DE JANEIRO)[1]; PLATAFORMA AVANÇADA DE BIOMOLÉCULAS, UFRJ (AVENIDA CARLOS CHAGAS FILHO, 373, CIDADE UNIVERSITÁRIA, ILHA DO FUNDÃO, RIO DE JANEIRO)[2]; FACULDADE DE FARMÁCIA, UFRJ (AVENIDA CARLOS CHAGAS FILHO, 373, CIDADE UNIVERSITÁRIA, ILHA DO FUNDÃO, RIO DE JANEIRO)[3]

ABSTRACT:

Bacteriocins are peptides or proteins ribosomally synthesized by prokaryotes, which have inhibitory activity against other prokaryotes. Some bacteriocins produced by *Staphylococcus* spp., known as staphylococcins, present antimicrobial activity against deteriorating and pathogenic microorganisms, with potential biotechnological applications as biopreservatives in the food industry, or as therapeutic agents against bacterial infections. The bacteriocin Pep5 is chemically distinct, since it has three thioether bridges, compared to four that the other group members normally possess. This atypical lantibiotic, produced by *Staphylococcus epidermidis* 5, still have its biotechnological applications under-explored. Thus, this project aims to structurally characterize the peptide, and to evaluate its biotechnological properties and antimicrobial potential against skin-isolated *Staphylococcus* spp. strains, due to its importance in cross-contamination by poor food manipulation. The optimal production of the bacteriocin was obtained with 12 hours incubation at 35 °C in TSB medium. High-purified preparations of Pep5 were obtained by gel filtration followed by cation exchange chromatography. The expression yield per liter of broth was approximately 4.0 mg. Mass spectrometric analyzes of this product confirmed the chemical identity of purified Pep5. The conformation of the lantibiotic was characterized by circular dichroism and multidimensional nuclear magnetic resonance spectroscopy. The bacteriocin is conformationally restricted by the thioether bridge and presents α-helical conformation in the presence of TFE and SDS. The helicoidal conformation is absent in the water-solubilized peptide. Pep5 showed an inhibitory activity against most *Staphyloccus* spp. strains (25/31) tested with minimal inhibitory concentration ranging 1.6-0.2 µM. The kinetic action of the bacteriocin was bacteriolytic for all sensitive strains tested. The peptide was susceptible to bile acid mixture, but not to gastric pepsin. Also the bacteriocin did not presented hemolytic activity. Lastly, we showed that the peptide undergoes liquid-liquid phase separation triggered by SDS, as assessed by phase contrast microscopy and turbidimetry. As far as we know, this is the first report of an antimicrobial peptide with liquid phase behavior modulated by a membrane-mimicking detergent. The antimicrobial activity accessed suggests that this feature may be related to the mechanism of action of Pep5.

Keywords: Bacteriocin, purification, structural characterization, staphylococcin Pep5, antistaphylococcal activity

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