Title: STUDY OF BLIS (BACTERIOCIN-LIKE INHIBITORY SUBSTANCES) PRODUCED BY *STAPHYLOCOCCUS* SPP. ISOLATED FROM DOGS

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Abstract:

Bacteriocins are antimicrobial peptides ribosomally synthesized by prokaryotes with activity against micro-organisms related to the producer species. Many bacteriocins have been suggested as alternative agents either in food biopreservation or in prevention/treatment of bacterial infections due to their antimicrobial action against human, plant and animal pathogens, showing potential biotechnological applications. Bacteriocin-like inhibitory substances (BLIS) are antimicrobial agents similar to bacteriocins but whose amino acid sequences have not been determined yet. Members of the Staphylococcus genus have been described in the literature as bacteriocin producers. In this work, three strains of *Staphylococcus* sp. (P1, P16 and I3), isolated from healthy dogs or with otitis externa, were selected in order to identify new bacteriocins with potential biotechnological applications. Some phenotypic characterization was performed with these strains. Only Staphylococcus sp. P16 produced acetoin in the Voges-Proskauer test; all strains exhibited biofilm production, cadmium resistance and negative reaction for lecithinase production. The antimicrobial susceptibility test was performed by disk diffusion using eleven antimicrobials. Staphylococcus sp. P1 was resistant to penicillin, tetracycline, ampicillin and rifampicin, and Staphylococcus sp. P16 and I3 were only resistant to penicillin and ampicillin. Bacterial identification by MALDI-TOF mass spectrometry suggests that Staphylococcus sp. P1, P16 and I3 belong to the species Staphylococcus intermedius, Staphylococcus schleiferi and Staphylococcus pseudintermedius, respectively. PCR reactions were performed to detect in the bacterial genome the presence of the ten staphylococcin structural genes described in the literature and genes encoding the five classical staphylococcal enterotoxins. None of the BLIS seems to be similar to the staphylococcins investigated and the BLIS-producer strains did not present the genes encoding the classical staphylococcal enterotoxins. Plasmid profile was determined by agarose gel electrophoresis and all BLIS-producer strains presented plasmid forms of different sizes. All BLIS were shown to inhibit Gram-positive bacteria of different genera. Antimicrobial activity was detected in the supernatant of all BLIS-producer cultures after growth of the strains in BHI broth for 24 h at 37° C with shaking, which will allow their further purification and biochemical characterization.

Keywords: BLIS, bacteriocin, Staphylococcus, staphylococcin, dog

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