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

Authors [1]SANTOS, I.N.S., [2]LILENBAUM, W., [1]ALVIANO, D.S., [1]BASTOS, M.C.F.

Institution [1] Instituto de Microbiologia Paulo de Góes – Universidade Federal do Rio de Janeiro (Avenida Carlos Chagas Filho, 373 - CCS - Bloco I - sala I-1-059 - Cidade Universitária - 21.941-902 - Rio de Janeiro - RJ - Brazil)

[2] Instituto Biomédico – Universidade Federal Fluminense (Rua Prof. Hernani Melo, 101 - 3º andar - São Domingos - 24.210-130 - Niterói - RJ - Brazil)

Abstract

Bacteriocins are antimicrobial peptides or proteins ribosomally synthesized by prokaryotes and with activity against micro-organisms. Many bacteriocins have shown potential biotechnological applications either in food biopreservation or in prevention/treatment of bacterial infections due to their antimicrobial action against human, animal and plant pathogens. In this work, the strains *Staphylococcus* sp. P1, P16 and I3, isolated from either healthy dogs or dogs with otitis externa and producers of the bacteriocin-like inhibitory substances (BLIS) P1, P16 and I3, respectively, were selected in order to identify new bacteriocins with potential biotechnological applications. Sequencing of *16S rDNA* identified these strains as *Staphylococcus pseudintermedius* P1, *Staphylococcus schleiferi* P16 and *S. pseudintermedius* I3, confirming the results obtained by MALDI-TOF mass spectrometry. All strains produced biofilm and DNase, exhibited cadmium, penicillin and ampicillin resistances and did not produce lecithinase. Only *S. schleiferi* P16 produced acetoin. *S. pseudintermedius* P1 was shown to be multidrug-resistant (to beta-lactams, tetracycline and rifampicin). The BLIS-producer strains did not present the genes encoding nine staphylococcal enterotoxins which were tested and none of the BLIS seems to be similar to the eleven staphylococcins investigated by PCR reactions using the bacterial genome as template. According to plasmid profiles, the BLIS-producer strains carry plasmid forms that may encode the genetic determinants involved in BLIS production whose location remains unknown. The best spectrum of activity was shown by BLIS P16. It inhibited bacteria of different genera including Gram-positive and Gram-negative species. This BLIS was chosen for further experiments and it was partially purified and dialysed. It revealed no hemolytic activity against sheep erythrocytes but its semipurified preparation was toxic to *Raw* and L-929 cell lines, although this toxicity could not be totally attributed only to BLIS P16 action. Additionally, this BLIS exhibited a bacteriolytic mode of action against *Micrococcus luteus* ATCC 4698, sensitivity to proteolytic degradation, partial resistance to high temperatures, and retained full activity at pH 3.0-11.0. Thus, the potential biotechnological application of BLIS P16 was highlighted and this report is the first in the literature on the production of a BLIS by a strain of *S. schleiferi*.

Key-words: bacteriocin, BLIS, *Staphylococcus schleiferi*, *Staphylococcus pseudintermedius*, biotechnological application

Funding Agencies: CNPq, FAPERJ and CAPES