

TITLE: Production, partial purification and characterization of bacteriocin-like inhibitory substances (BLIS) produced by *Pediococcus* spp.

AUTHORS: KUNIYOSHI, T.M., VIEIRA, V.B., OLIVEIRA, R.P.S

INSTITUTION: Faculdade de Ciências Farmacêuticas – Universidade de São Paulo, São Paulo, SP (Av. Prof. Lineu Prestes, 580, Bloco 16 – Semi industrial, CEP 05508-000, São Paulo-SP, Brazil).

One of today's great challenges from the scientific area is the search of new compounds in order to substitute antibiotics. Bacteriocins are peptides produced by a number of bacteria that harbor bacteriostatic or bactericide effect over pathogenic and deleterious microorganism present in industrial processes. The ones produced by lactic acid bacteria (LAB) present an interest on industry as some of them are probiotic, therefore, acknowledged as safe on this field. A vast gamma of lineages from *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* genera are known for producing classes I and II bacteriocins. In this context, the aim of this work was to study the BLIS production from different lineages *Pediococcus* genus. This group is known for producing the class II bacteriocin named pediocin. The gene that codifies pediocin from these different lineages was sequenced and a global alignment was performed based on the pediocin sequences already deposited in the databank (GenBank). Upon the amino acid sequence analysis, it was possible to detect different aminoacid residues in comparison to the commercial pediocin PA-1, being included the 42nd conserved cysteine substitution with serine. The cell free supernatant (CFS) was obtained from each lineage of *Pediococcus* spp., partially purified with ammonium sulfate precipitation (40%) and purified in a SepPak C18 column and later analyzed in tricine-SDS-PAGE. The inhibition spectrum from the partially purified BLIS was determined using different Gram-Positive and Gram-Negative as bioindicator lineages. Besides, it was verified that BLIS lost its antimicrobial activity when treated with proteolytic enzymes (1mg/mL trypsin, papain or pepsin) but not when treated with α -amylase, lipase, Triton X-100, SDS, Tween 20, Tween 80, Urea, NaCl and EDTA indicating the proteinaceous nature of this compound. It was also evaluated the effect of different parameters (pH, temperature, agitation, sugar cane vinasse supplementation) on BLIS productivity using fractional factorial designs and rotational central composite design (RCCD).

Keywords: Bacteriocin-like inhibitory substances, *Pediococcus* spp., pediocin

Development Agency: Fundação de Amparo à Pesquisa do Estado de São Paulo