**TITLE:** PURIFICATION AND PARTIAL CHARACTERIZATION OF THE BACTERIOCIN PRODUCED BY *Lactobacillus plantarum* ST16PA

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**ABSTRACT:**
Bacteriocins are antimicrobial peptides ribosomally synthesized specially by lactic acid bacteria. Their application is vast and comprises fields as food preservation, oral care, veterinary use, skin care, among others. To have deeper knowledge of these peptides, establish an efficient purification protocol is extremely necessary. This study aimed to evaluate a purification protocol and, from this, obtain the characterization of the bacteriocin produced by *L. plantarum* ST16Pa. Thus, the strain was cultured in Man-Rogosa-Sharpe (MRS) broth and the resulted cell-free supernatant (CFS) was used to carry out the following assays. The bacteriocin was precipitated by adding ammonium sulfate to 100 mL of CFS until reaching 20% (w/v) saturation and, then, stirred for 2 h at 4 °C. After centrifugation for 30 min at 4470 *g* and 4° C, the resulting pellet was resuspended in 10 mL of 25 mM ammonium acetate buffer (pH 6.5) and loaded on a previously activated $C_{18}$ solid phase extraction (SPE) cartridge, named as OASIS® HLB, which was washed with gradual concentration of isopropanol in the same buffer above mentioned. The antimicrobial activity of each step was tested against the bioindicator strain *Listeria innocua* 6a CLIST 2865 using the spot-on-the-lawn method. Regarding the characterization, the bacteriocin protein nature was evaluated by testing the effect of the proteolytic enzymes pepsin, chymiotrypsin, and proteinase XIV (1 mg/mL) and its molecular weight was estimated in a tricine–SDS–PAGE gel. Part of this gel was overlaid with the bioindicator strain, embedded in Brain Heart Infusion agar, to determine the position of the active bacteriocin. The results demonstrated that the proposed purification protocol worked well for this bacteriocin. At final step using the SPE OASIS® HLB, despite low yield (only 3%), the purification fold increased approximately 13.7 as compared with the CFS. The tricine–SDS–PAGE reveled a single antimicrobial band with molecular weight of about 2 and 5 kDa. The proteolytic enzymes assay resulted in complete inactivation of antimicrobial activity, confirming the bacteriocin protein nature. In conclusion, the proposed protocol attained satisfactory recovery results, however, were not sufficiently suitable to obtain a pure molecule, needing more purification steps. Only with an ultrapure bacteriocin it will be possible to achieve the complete characterization of this molecule (amino acid and nucleotide sequences).

**Keywords:** bacteriocin, purification, characterization, antimicrobial activity, *Lactobacillus plantarum*

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