

TITLE: *BACILLUS* P34 CYCLIC LIPOPEPTIDES PRODUCTION IS MODULATED BY INACTIVATED CELLS OF TARGET MICROORGANISMS

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ABSTRACT

The search for new antimicrobial molecules is one of the main objectives around the world that are concerned with combating the development of antibiotic resistance caused by many natural and anthropic factors. *Bacillus* strains are known to produce several bioactive compounds, including three main different families of cyclic lipopeptides (CLPs) called surfactins, iturins and fengycins (or plipastatins). Previous studies have shown that *Bacillus* sp. P34 isolated from a freshwater fish produces lipopeptides with antimicrobial properties, being potentially useful for different biotechnological applications. The present study aimed to investigate the production of bioactive lipopeptides by this *Bacillus* strain. Phylogenetic analysis was carried out through Multilocus Sequences Analysis of the *Bacillus* P34 housekeeping genes: *chaperone protein* (DnaK), *recombinase A* (RecA), *RNA polymerase beta subunit* (RpoB), *RNA polymerase sigma 70 factor* (RpoD), *tryptophan synthase beta chain* (TrpB). The molecular analysis indicates that P34 strain becomes to the *Bacillus velezensis* group. The gene clusters for CLPs production were found in the *Bacillus* P34 genome. Production of antimicrobial CLPs by *Bacillus* sp. P34 was performed in brain heart infusion (BHI) broth at 30 °C for 48 h at 150 rpm. CLPs induction was also investigated adding heat-inactivated cells of *Listeria monocytogenes* or spores of *Aspergillus niger* into *Bacillus* P34 culture. Lipopeptides from bacterial cultivation were extracted using *n*-butanol and analysed by high performance liquid chromatography. Antimicrobial activity was determined by disk-diffusion method, against *L. monocytogenes* and *Staphylococcus aureus*, with results expressed as activity units (AU) per milliliter. Antimicrobial activity was verified against *L. monocytogenes* and *S. aureus* with a maximum of 550 and 750 AU/mL, respectively, but a reduction ranging from 50 to 80% was observed by the *Bacillus* cultures medium with thermally inactivated cells or spores. It has been suggested that CLPs genes transcription may depend of microbial debris presence during *Bacillus* growth. Moreover an interesting aspect emerged from the experimental data, showing a direct correlation between iturin (0.245 g/L) and fengycin (2.8 g/L) production and respective antimicrobial activity against *S. aureus* and *L. monocytogenes*.

Keywords: Competition, multilocus sequences analysis, lipopeptides, antimicrobial activity.

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