TITLE: RELATIONSHIP BETWEEN THE INTERRUPTION OF *PAENIBACILLUS ELGII* AC13 SPORULATION AND THE PRODUCTION OF PELGIPEPTIN.

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ABSTRACT:

*S*oil bacteria from the genus *Paenibacillus* produce many bioactive molecules such as antimicrobials and enzymes. In a previous study, a strain of *Paenibacillus elgii* was obtained from the Cerrado and it presented antimicrobial activity. This strain was named *P. elgii* AC13, the antimicrobial activity observed in the culture supernatant was a result of the production of lipopeptides from the Pelgipeptin family. Although most of Pelgipeptins are secreted to the supernatant, we observed their presence in the cell fraction of the culture. *Paenibacillus* is a spore-forming bacterium, therefore the purification of Pelgipeptins from the pellet was challenging due to the spore resistance to cell lysis. The objective of this study was to observe the release of pelgipeptins to the culture supernatant of spore-free cultures, by interrupting the conclusion of the cell cycle. The removal of calcium from a chemically defined medium was used to impair spore formation in *P. elgii* AC13. Samples from cultures with and without the amendment of calcium were collected at 24, 48, and 72 hours. Aliquots from supernatant and pellet were collected for qualitative analysis by mass spectrometry (MALDI-ToF) and morphological analysis by phase contrast microscopy. Lipopeptides were extracted from the pellet by sonication. MALDI-ToF analysis showed the presence of pelgipeptin in the pellet and supernatant of both treatments. Cultures on medium amended with calcium showed an increase of pelgipeptins in the supernatant, whereas the concentration of pelgipeptin remained constant in the pellet. Conversely, cultures without the amendment of calcium showed an increase in the concentration of lipopeptides in the pellet, while in the supernatant we observed a decrease of pelgipeptin concentration. Usually, peptides are secreted immediately after their synthesis, but during sporulation they can be trapped in the membrane layers, and later released with cell lysis. The calcium-free cultures presented complete cell lysis and only a few formed spores so; pelgipeptins were likely associated with cell debris due to the molecular affinity between lipopeptides and cell membranes. Alternatively, it is possible that calcium plays an indirect role in lipopeptides secretion, and the interruption of the cell cycle by calcium depletion may have caused a chemical imbalance leading to the aggregation of peptides, making it difficult to disassociate from the cell debris.

Keywords: *Paenibacillus*, sporulation, lipopetides, pelgipeptin, calcium.

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