Title: Relationship between cell cycle and Pelgipeptin production in the spore forming bacteria *Paenibacillus elgii*

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Abstract:

The spore forming bacteria *Paenibacillus elgii* can produce a non-ribosomal family of antimicrobial lipopeptide named Pelgipeptin, that present a wide range of medical and veterinary applications. Media composition is crucial to the production of these antimicrobial molecules, and several studies have investigated the effect of different media composition for the production of lipopetides from *Paenibacillus* species. The medium that presented the highest yield of pelgipeptins is knows as GS and it is a chemically defined medium containing glycerol as carbon source. However it does not contain calcium ions, which are required for *Paenibacillus* sporulation. The aim of this study was to investigate how the absence of calcium influences *Paenibacillus elgii* AC13 cell cycle and its pelgipeptin production. Bacteria was cultivated on GS medium (2% glycerol, 1% (NH4)2SO4, 0.2% KH2PO4•3H2O, and 0.04% MgSO4•7H2O, at pH 7.2) and GS medium amended with 0.01% of CaCl2. Samples were collected at 24 hours interval for 141 hours. Microbial growth was evaluated by absorbance measurements at 600 nm. Cell cycle was observed using phase contrast microscopy of fresh culture slides. Pelgipeptin quantification was performed in the cell-free supernatant using UFLC and a standard curve made from known concentrations of the same lipopeptides. In the absence of calcium the bacteria could not complete sporogenesis, however, prespores were observed after 75 hours of incubation. Pelgipeptin concentration achieved 16.8 µg/mL at 48 hours of growth. Treatment with calcium reduced the period for prespores formation after 48 hours and the free spores were observed after 75 hours. Pelgipeptin concentration achieved 35.6 µg/mL after 141 hours. This result shows that *Paenibacillus elgii*, as other endospore-forming bacteria, cannot complete the sporulation process in the absence of calcium ion. However, Pelgipeptins were produced in both treatments. This suggests that the pelgipetin production is not dependent on *Paenibacillus elgii* sporulation. However, the pelgipeptin concentration on supernatant was higher on the treatment with calcium, which may be a result of passive release of the intracellular content caused by the mother cell lyses to release the bacterial endospores.

Key-words: *Paenibacillus*, pelgipeptin, calcium, sporulation

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