Proteomic analysis of growing- and sporulating-*Bacillus thuringiensis* (S811) and activity towards *Anthonumus grandis* (Coleoptera: Curculionidae) larvae

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Gram-positive spore-forming entomopathogenic bacteria use a wide range of proteins to invade, infect, and kill their hosts. Many of these proteins are synthesized during sporulation and are commonly founded in parasporal crystals. *Bacillus thuringiensis* (*B.t*.) is a well characterized group of bacteria known by their specific activity to different insect orders, as Lepidoptera, Diptera, Coleoptera, and Hymenoptera. Since years, *B.t.* entomopathogenic proteins have been used to control insect pests as bioinsecticide formulations, and more recently, their genes have been introduced in crop plants aiming to control insects. Beside of the Cry proteins, other *B.t.* proteins, such as the "VIPs" (Vegetative Insecticidal Proteins), which are soluble and synthesized during vegetative growth, have also potential of controlling insects. However, these proteins were not well explored as the Cry proteins for biotechnology purpose. The aim of this study is to characterize the proteome of the *B. thuringiensis* strain S811, from which Cry proteins show a high activity towards Coleoptera, Lepidoptera, and Diptera. Four stages of bacterial growth, including vegetative and sporulation stages, secreted and intracellular proteins, as well as bacterium activity, were analyzed against Coleoptera larvae. The different stages were defined at different time intervals after bacteria inoculation: 8 hours after inoculation (HAI)- vegetative stage, where exponential growth begins; 16 HAI- beginning of spore formation; 24 HAI- when is possible to visualize spore and crystal into the cell; and 32 HAI- beginning of sporulation process, period that is possible to observe spores and crystals in the medium. The cell features, in each stage, were evaluated by contrast-phase microscopy. Intracellular and secreted proteins from different stages were used to the bioassay with *Anthonomus grandis* larvae and to establish two-dimensional reference maps. Specific proteins from stages exhibiting high activity towards this insect were excised from the gel and identified by MALDI-TOF MS/MS.