

# Identification of *Bacillus* Strains Using The Matrix-Assisted Laser Desorption-Ionization Mass Spectrometry (MALDI-TOF MS) for Biotechnology Purposes

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*Bacillus* genus is compound for Gram-positive strains, attractives for industrial use because of its broad genetic manipulation capability, favorable growth in large-scale fermentations and its ability to produce secondary metabolites. MALDI-TOF (*Matrix Assisted Laser Desorption-Ionization*) is currently a physicochemical technique utilized for bioprospecting of bacterial species. This method has been successfully used in the investigation of proteins and peptides, therefore applied for taxonomic identification of microorganisms. The present study aimed identify *Bacillus* strains by MALDI-TOF technique, in order to be applied in industrial processes. Initially, were isolated 20 strains of *Bacillus* sp., 10 from ECOVITA™ (PR-01, PR-02, FB-01, FB-02 and EV-01 to EV-06), one (PA-01) from *Opuntia ficus-indica*, an autochthonous plant of Brazilian Northeast, two isolated from the larvicide (Vectolex™) (BS-236217, BS-15931, BT-51450), and seven from Probiotic Complex (BC-01, BC-02, BC-03, BS-148, BS-198, BS-664 and BS-666). Morphological characteristics (sporulated Gram-positive rods) of isolates and preliminary biochemical tests confirmed of *Bacillus* genus, however, were not conclusive for identification of species. For MALDI-TOF analysis, all bacterial strains were preserved in medium Agar Muller Hinton. Each colony was inactivated in absolute ethanol, followed by protein extraction with 70% formic acid and acetonitrile and addition of matrix of  $\alpha$ -cyano-4-hydroxy-cinnamic acid to obtain specific mass spectrum specific for each 16S rRNA sequence using Autoflex III equipment (Bruker Daltonics™). Two independent spectra were taken for each sample and contained 50 to 60 mass peaks in the 2–10 kDa range. The final identification of the strains was made by matching the generated spectra in the database, with classification score for species from 2.00 to 3.00. *Bacillus subtilis* (score 2.043 for BS-198), *Lysinibacillus fusiformis* (score 2.116 for BS-236317 and 2.232 for BS-666), *Bacillus cereus* (score 2.503 for BT-51450 and score 2.503 for PR-02) and *Bacillus licheniformis* (score 2.06, FB-02 strain) were the highest species identified. The MALDI-TOF is a high-sensitivity and high-throughput technique that allowed phyloproteomic identification of the *Bacillus* species in the samples studied. This bacterial genus is known to synthesize antibiotics and produce lipopeptides cyclics with industrial interest, suggesting future biotechnological applications. The next step would be to produce biosurfactants for biotechnology purposes.

**Keywords:** Phyloproteomic identification, Autoflex III, *Bacillus* sp.

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