

TITLE: THE EFFECTS OF DIFFERENT FREEZE-DRYING PROTECTIVE AGENTS ON THE VIABILITY OF PHOSPHATE SOLUBILIZING BACTERIA FROM THE GENUS *BACILLUS*

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ABSTRACT

Phosphate solubilizing bacteria (PSB) play an important role in plant nutrition through the increase in P uptake by the plant. The preservation of these microorganisms by storage for long periods is necessary to ensure reproducible results, continuity of the research, and for commercial purposes. Freeze-drying is one of the most common methods used to store microbial culture collections. However, the freezing and drying processes must be performed in the presence of protective agents to preserve the cell viability. The aim of the present study was to compare the efficiency of different protective agents and rehydration media for preserving the viability of PSB of the genus *Bacillus* subjected to freeze-drying process. The bacteria used were BRM033111 (*Bacillus thurigiensis*), BRM033109 (*Bacillus pumilus*) and BRM034840 (*Bacillus subtilis*), stored at the Collection of Multifunctional and Phytopathogenic Microorganisms of Embrapa Milho e Sorgo. Nine different protectants and two rehydration media were tested to maintain the bacterial viability during the freeze-drying. The percentage of bacterial survival was determined by comparing the colony forming units (CFUs) counts before and after freeze-drying. Survival rates varying between 80 and 100% were obtained for *B. thurigiensis* and *B. subtilis* using 10% sucrose or 5% glucose as protective agents. The 0.85% saline solution or the mixture of 10% sucrose and 5% peptone also provided high protection to *B. thurigiensis* (80% survival). The results showed differences in the bacterial viability among the isolates depending on the rehydration media used. Among the protective agents tested, the bacterial rehydration in 0.85% saline solution gave better recovery for *B. subtilis* and rehydration in 10% non-fat skim milk gave better recovery for *B. thurigiensis*, compared with the other methods. However, for *B. pumilus*, the best rehydration media varied according to the protective agent used. Survival rates of 60% to 70% were obtained for *B. pumilus* when 5% glucose/10% non-fat skim milk or 10% sucrose + 5% peptone/0.85% saline were used as protective agent/rehydration media, respectively. The use of 5% glucose as protective agent for freeze-drying of PSB-*Bacillus* was effective for preserving the viability of all bacterial strains tested, thus this is the best method for preserving the viability of PSB-*Bacillus* by freeze-drying.

Keywords: biosolubilizers, lyophilization, microbial culture collection, preservation.

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