

TITLE: LONG-TERM PRESERVATION AND DATA MANAGEMENT OF THE EMBRAPA MILHO E SORGO *BACILLUS THURINGIENSIS* CULTURE COLLECTION

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ABSTRACT:

Bacillus thuringiensis is a rod-shaped, gram-positive, spore-forming bacterium characterized by the production of proteinaceous parasporal crystals. These parasporal inclusion bodies are toxic for certain insect larvae of the orders Coleoptera, Diptera and Lepidoptera. Therefore, *B. thuringiensis* has been used for more than five decades as biological insecticides to control agricultural insect pests. The Brazilian corporation for maize and sorghum research (Embrapa Milho e Sorgo) maintains a microbial culture collection composed of 4.860 *B. thuringiensis* strains isolated from soil samples collected in different maize production regions of Brazil. All *B. thuringiensis* strains incorporated into the collection were microscopically characterized by the formation of parasporal crystals and assayed for insecticidal activity against *Spodoptera frugiperda* larvae. In order to ensure the long-term preservation of the *B. thuringiensis* strains for continued use in the development of new biopesticides, the collection has currently been duplicated to create its backup version at -80 °C, following international quality standards. The *B. thuringiensis* strains from original stocks at -20 °C were activated by plating on LB medium agar and incubation for 24h at 30 °C. Afterwards, the cultures were replated on LB plates to obtain isolated colonies. Next, single colonies with typical *B. thuringiensis* morphology were transferred to fresh LB plates by the spreading plating method, and after 72h growth, the culture was harvested, suspended in cryoprotectant solution (25% glycerol) and stored at -80 °C. Before preservation, phase-contrast microscopy observations and the Gram staining method were used to confirm the identity of the *B. thuringiensis* isolates. A total of 2.592 *B. thuringiensis* strains were cryopreserved and maintained at the Embrapa Milho e Sorgo institutional culture collection (CMMF – Coleção de Microrganismos Multifuncionais e Fitopatogênicos). Although the collection has samples preserved for over 20 years, all isolates were reactivated, probably due to the germination of viable spores present in the original stock cultures. Currently, the data on 3.408 *B. thuringiensis* strains were transferred to the collection management software (AleloMicro), which can be partially accessed by the public through the online catalog at <http://alelomicro.cenagen.embrapa.br/>.

Keywords: AleloMicro, biological control, entomopathogenic bacteria, microorganism germoplasm bank.

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