

Viability and conservation of a collection of filamentous fungi associated with sugarcane plants (*Saccharum* complex)

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The Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries (CMMAABio) have been widely used in partnership with public and private institutions, seeking to obtain inputs and bioprocesses with application in the most diverse areas. Currently, 3,250 bacteria, fungi, and microalgae strains are conserved at CMMAABio and registered in the Alelo Genetic Resources Platform, but only 900 are identified at the species level. Embrapa Agroenergia maintains more than 10,000 microorganisms from all Brazilian biomes that have not yet been appropriately preserved and registered in the Alelo Platform because these steps depend on viability tests, purification, taxonomic identification, and conservation (strains in the backlog). This work aimed to evaluate the viability and properly conserve a subcollection of 223 lignocellulolytic filamentous fungi associated with sugarcane (*Saccharum* complex), preserved in sterilized distilled water at CMMAABio since 2015. That year, after receiving the material from a partner institution in a research project, each fungus was subcultured and submitted to purification to obtain a monosporic culture (if sporulating) or a purified culture, being preserved by the Castellani's method with two to four replicates. The fungi belong to 60 different genera and remained at room temperature for eight years. For viability analysis in 2023, each strain was reactivated from the original stock on PDA medium (one plate for each replica) and incubated for up to 10 days at 28°C with photoperiod. After cultivation, viable strains were preserved by deep-freezing of mycelial discs in a 20% glycerol solution in an ultra freezer (-80°C) and by Castellani's method, with several replicas. We found that four of the 223 assessed strains could not be recovered even after evaluating all the original biological material conserved (replicas). The lost strains belong to the genera *Beauveria* and *Cladosporium*, and two are unidentified *Ascomycota*. From 219 viable strains recovered, 32 were found contaminated with bacteria or other fungi, while 18 exhibited genetic instability (formation of sectors with different morphology and growth). Castellani's method was demonstrated to be cost-effective and convenient for the viability conservation of most fungi in the present study. Notwithstanding, it is recommended to be associated with long-term preservation protocols to avoid future loss of strains with high-added value from Brazilian biodiversity.

Key words: Microbial Culture Collections; Filamentous Fungi; Preservation Methods; Sugarcane.

Viabilidade e conservação de uma coleção de fungos filamentosos associados à plantas de cana-de-açúcar (complexo *Saccharum*)

O objetivo deste trabalho foi avaliar a viabilidade e conservar adequadamente uma subcoleção de 223 fungos filamentosos lignocelulolíticos associados à cana-de-açúcar (complexo *Saccharum*), pertencentes à 60 gêneros diferentes. Estes fungos estavam preservados em água destilada esterilizada desde 2015 na Coleção de Microrganismos e Microalgas Aplicadas à Agroenergia e Biorrefinarias (CMMAABio). Os resultados mostraram que 91,08% das linhagens estavam viáveis mesmo após oito anos conservadas pelo método Castellani (água destilada esterilizada). As linhagens perdidas pertencem aos gêneros *Beauveria* e *Cladosporium*, e duas são *Ascomycota* não identificadas. Não obstante, recomenda-se a associação a protocolos de preservação de longo prazo para evitar futuras perdas de linhagens de alto valor agregado da biodiversidade brasileira.

Palavras-chave: Coleções de Culturas Microbianas; Fungos Filamentosos; Métodos de Preservação; Cana-de-açúcar.

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