

# Viability and conservation of a collection of soybean-associated bacteria after preservation for more than 20 years by deep-freezing in glycerol

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Microbial genetic resources refer to taxonomically well-defined, physiologically characterized, genetically stable, authenticated, documented, quality-controlled, and long-term preserved microbial strains. The characterization of microbial biodiversity is essential not only for maintaining natural ecosystems but also for research and development purposes in the most diverse areas, promoting the bioeconomy. The Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries (CMMAABio) constantly works to add value to the Brazilian microbial biodiversity conserved in its installation. Here, we evaluate the viability and proper conservation of 481 endophytic and epiphytic soybean-associated bacteria preserved by deep-freezing in glycerol for more than 20 years. The strains had been previously isolated from leaves, stems, and roots of soybean plants [*Glycine max* (L.) Merrill], cultivars Foscarin and Cristalina, cultivated in soil with and without pre-planting application of glyphosate herbicide during three development stages (V6, R2, and R6) in the 2000/2001 and 2001/2002 harvests. The original stock was prepared with one replication in 10% Tryptic Soy Broth with a final concentration of 20% glycerol. Microbial strains were kept at the Department of Genetics (ESALQ/USP) from their isolation until 2012 when they were sent to Embrapa Agroenergia, remaining preserved until 2023 in the original form. For viability tests, the bacteria were reactivated from the stock in plates containing Tryptic Soy Agar (streak plate method), incubated at 28°C for up to 7 days, and stored in a cold chamber. When more than one morphological type grew on the same plate, subsequent purification steps were performed until pure cultures were obtained. For conservation, an isolated colony of each strain was inoculated in TSB, followed by overnight incubation (28°C; 200 rpm). The same culture was preserved by deep-freezing (20% glycerol) and cell pelleting (-20°C) for future genomic DNA extraction. The results showed that all 481 strains were viable even after more than 20 years of deep-frozen and going through different conditions of manipulation and transport. So far, 377 of the 481 strains have been evaluated. Eighty-eight strains showed some level of cross-contamination and were purified, which resulted in the addition of 117 to the original 377 strains. Thus, it has been possible to conserve 471 strains of soybean-associated bacteria. These include well-characterized and documented bacteria regarding their potential to promote plant growth and will have their taxonomic identification reconfirmed in the future.

**Key words:** Microbial Culture Collections; Bacteria; Endophytic; Epiphytic.

## Viabilidade e conservação de uma coleção de bactérias associadas à soja após preservação por mais de 20 anos por ultracongelamento em glicerol

Nós avaliamos a viabilidade da coleção de 481 bactérias associadas à soja (endofíticas e epifíticas), que se encontrava preservada por mais de 20 anos. Os resultados mostraram que as 481 linhagens estavam viáveis após este período de preservação, pelo método de ultracongelamento em glicerol. De 377 linhagens avaliadas, 88 mostraram níveis de contaminação cruzada e foram purificadas. Isso resultou no acréscimo de 117 linhagens às 377 avaliadas, e na conservação de 471 bactérias até o momento. A recuperação de bactérias de alto valor agregado da biodiversidade brasileira, com aplicação reportada para promoção de crescimento vegetal, será acompanhada da confirmação taxonômica.

**Palavras-chave:** Coleções de Culturas Microbianas; Bactérias; Endofíticas; Epifíticas.

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