

Genus *Bacillus* Culture Collection and Related Genus - CCGB, Procedures for a Backup in Liquid N₂

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Traditionally, *Bacillus* and related sporulated genera can be preserved and stored by using drying as well as cryopreservation. In this field, lyophilization and liquid N₂ are broadly employed. The latter can be used for the purpose of backup of collections, especially for aerobic sporulated bacteria. The preservation at -196°C is a condition practiced by important collections worldwide microbial cultures, paying for bacteria, fungi and some protozoa. This work reports the methodology for backup practiced and approved for aerobic spore-forming Gram-positive bacteria, with full recovery of the lineages after 3 years of preservation and conservation in CCGB, IOC/FIOCRUZ. Preservation and conservation trials for longer under these conditions are being held. The methodology used has the following sequence:

Prepare cell biomass with ≥80% of free spores (mature spores) in a Petri dish having a solid culture media that stimulates sporogenesis → scrape off growth, transfer a full bacteriological loop to a 12 x 100mm screw capped assay tube containing 3 mL of sterile skim milk reconstituted in distilled water and containing 30% glycerol "PA" → homogenize → standardize spore suspension (100 spores per microscopic field) → keep assay tube in the refrigerator (3°C ± 1°C) for up to 1 day → moisten 6 thin strips (0,5cm x 2cm) of Whatman paper N° 4 in a sterile Petri dish, containing such heavy spore suspension → transfer to cryotube (3 per strain), identified for the preservation and storage → screw lids loosely → dry the paper strips in an incubator at 33-35°C/72h → remove from the incubator → adjust the screw lids → cool down (3°C ± 1°C) for 1hr. → freeze for 1hr. → store in liquid N₂ (-196°C). For activation (germination) of the spores, transfer the cryotube to the freezer (1hr.) followed by cooling (3°C ± 1°C) 1hr. → and finally at room temperature (1hr.). Open cryotube aseptically → select one strip of filter paper → inoculate the desired liquid culture medium → incubate 33-35°C. Perform the procedure backwards to return the cryotube to the liquid N₂. The CCGB preserves and conserves the collection by lyophilization and the backup as described.

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