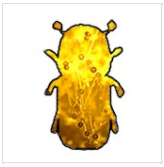

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Fungi Permanent Storage

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1 Works for me dx.doi.org/10.17504/protocols.io.bnu5mey6

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes how to store and revive ambrosia fungi.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

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Because some fungi may not survive well after freezing, also keep a duplicate, preserving in water vials, especially for Raffaelea and Geosmithia fungal species.

Freezing in -80 Celsius

- Glycerol (most fungi and bacteria do well in 10%, some need 20%).
- Put three agar plugs (or just chunks of mycelium, or one big chunk, whatever is easier) from each plate into its respective storage vial. Agar plugs (cubes with fungus) or pieces of mycelium can be cut with scalpel; make sure that scalpel is perfectly sterilized

after each culture. Take the growing edge of the fungus, or a whole growing colony, not the old crusty center.

- If you are using minislants – just pour the sterile 20% glycerol in the minislant tube
- Place in Mr. Frosty (blue/white container on top of the fridge). Fill up the bottom compartment with isopropyl alcohol (in the chemicals storage). Put in -80C freezer. It will freeze slowly, 1C per minute.
- **Record in the isolations_current Database**. Slant vial number should register in the Table "SLANTS_AND_VIALS". Each isolate will record which plate it comes from and relate information. Vial numbers should be wrote both on the top and side of vials

Water vial in room temperature

- Eppendorf tubes with 1ml sterile water.
- Put agar plugs (or just chunks of mycelium, or one big chunk, whatever is easier) from each plate into its respective storage vial. Agar plugs (cubes with fungus) or pieces of mycelium can be cut with scalpel; make sure that scalpel is perfectly sterilized after each culture. Take the growing edge of the fungus, or a whole growing colony, not the old crusty center.
- Write vial numbers on the cap of teh tube. Number should be the same as slant vials freezing in -80C.

Reviving

Prepare:

- vials with 1mL PBS. (Label them with numbers corresponding to frozen samples)
- equal number of PDA plates (label these with regular database numbers)
- sterilizer, scalpel

1. Take out the sample. 2. Cut gel disk inside the tube with carefully sterilized scalpel. 3. Use only one half (other half stays). 4. Put half-disk in vial with PBS, shake briefly to rinse off surplus glycerol or mineral oil, and put on PDA plate.

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