Nov 20, 2020

Mini culture slants for long term storage of fungi

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1 Works for me dx.doi.org/10.17504/protocols.io.bnu3meyn

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes how to store fungi in slants.

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.

DOI

dx.doi.org/10.17504/protocols.io.bnu3meyn

DOCUMENT CITATION

You Li, Jiri Hulcr 2020. Mini culture slants for long term storage of fungi. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bnu3meyn

CREATED

Oct 23, 2020

LAST MODIFIED

Nov 20, 2020

DOCUMENT INTEGER ID

43643

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Small culture slants have the advantage of easy storage, integration with our database system, and safe shipping to collaborators.

1. Sterilize 2mL cryotubes by autoclaving or prolonged exposure to UV light.
2. Under a biosafety hood, arrange the open tubes in a rack so that the open end of the tubes face almost horizontally (about 15°).
3. Expose the tubes to UV light again if necessary to ensure they are not contaminated.
4. Add about 1mL of autoclaved media (usually PDA) to the bottom of the tube.
5. Let the media harden and cool UNDER AN ANGLE in the hood before adding the tube caps.

6. Store in the fridge.

Following this, you may culture the fungus on the slant media, label it with a fungi vial number, and place it in the incubator, slightly open with parafilm, to grow. After the media has a culture and covers the whole slant, you may add 10-20% glycerol to fill the vial, close it completely and use the “Mr. Frosty” jar to slowly bring it down to freezing in the -80C. It can then be moved to a box in the -80 for long term storage.

For sending the tubes to collaborators:

1. Keep the culture for a couple days before shipping to make sure the fungus is growing and isn’t contaminated.
2. Wrap the lid-tube joint with parafilm, so that the lid doesn’t get loose.
3. Tape the tube to a piece of paper so that it doesn’t roll around in the envelope during shipping.
4. Make sure it is sufficiently padded and that the tube and envelope are labeled.
5. Ship IMMEDIATELY: there is very little oxygen for the fungus to breathe.

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