Jan 06, 2021

Isolation of fungi associated with ambrosia beetles with pre-oral mycangia

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1 Works for me dx.doi.org/10.17504/protocols.io.bq7ymzpw

Protocols Bark Beetle Mycobiome

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes how to extract fungi from ambrosia beetles that have pre-oral mycangia.

Note: This protocol is specific for ambrosia beetles with pre-oral mycangia and may not be useful for beetles with different mycangia types.

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.bq7ymzpw

DOCUMENT CITATION

Kyra Lynn, Irene Barnes 2021. Isolation of fungi associated with ambrosia beetles with pre-oral mycangia. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bq7ymzpw

CREATED

Jan 06, 2021

LAST MODIFIED

Jan 06, 2021

DOCUMENT INTEGER ID

46040

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Equipment for beetle DNA extraction:

- Ethanol spray bottle
- Paper towel for drying
- Insect handling tweezers
- 1.5 ml Eppendorf tubes
- 70% ethanol
- Vortex shaker
- Distilled water
- Filter paper
- Empty Petri dishes for dissection (preferably 90 cm)
- Ethanol bath for sterilizing equipment
- Bunsen burner (any available flame) to sterilize tweezers/blades/loops
- Stereo microscope
- Biological lab tweezers
- Scalpel and scalpel blade
- Permanent lab marker pens
- Lab pipettes and pipette tips
- Inoculation loop
- Petri dishes containing specific media for different fungi (see dx.doi.org/10.17504/protocols.io.bnuwmexe for more detail).
- 2 mL screw cap tube
- Cello tape

Direct isolation from heads of female ambrosia beetles (where the pre-oral mycangium is located)

1. Clean and wipe down workspace using ethanol spray bottle and paper towel.
2. Surface disinfect recently collected (preferably living) beetle specimen by placing it in an Eppendorf tube containing 70% (v/v) ethanol, ensuring beetle is fully submerged. Use an insect handling tweezer to minimize damaging the beetle specimen.
3. Agitate tube with avortex mixer for 20 s.
4. Remove the beetle from the ethanol and place it in a 1.5 Eppendorf tube containing distilled water. Rinse twice using clean distilled water.
5. Air-dry the beetle on sterile filter paper in a laminar flow chamber.
6. Once dry, place the beetle into an empty petri dish.
7. Under the Stereo microscope, carefully separate the beetle headfrom the thoracic and abdominal segmentsusing the sterilized tweezers and scalpel.
8. Place beetle head (where the pre-oral mycangium is located) in a sterile 1.5 mL Eppendorf tubecontaining 200 uL sterile distilled water and macerate with sterile biological tweezers.
9. Store the remaining thoracic and abdominal segments of the dissected beetle in 200 µL of 70% (v/v) ethanol in 2 mL screw cap tubes for downstream beetle identification.Tubes can be sealed with cello tape and be stored at 4˚C to minimize ethanol evaporation. Care must be taken to store/label the beetles accurately to link the fungi to the beetles from which they were isolated.
10. Agitate the tube containing the macerated head with avortex mixer for 20 s.
11. Pipette 50 µL onto the selected agar (dx.doi.org/10.17504/protocols.io.bnuwmexe) and spread evenly over the agar surface using a sterile inoculation loop. The agar type will depend on the fungi you are trying to isolate. For example, to select for*Fusarium*spp. you can use*Fusarium*selective media (FSM).
12. Incubate Petri dishes at 25˚C to allow for fungal growth. a- Keep checking these cultures on a daily basis as faster growing fungi (or contaminants) will outcompete slower growers.
13. Sub-culturefungal colonies with unique morphologies onto 2% malt extract agar (MEA: 20 g/L malt extract, 20 g/L agar), amended with 100 µg L^{-1} streptomycin.
14. Perform single hyphal tip isolations to obtain pure colonies for further downstream identification.

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