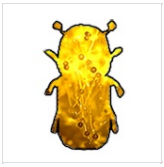

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Fungal Extraction from Beetles

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

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes how to extract fungal symbionts from beetles.

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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General notes

1. Wash the beetle with a surfactant and/or saline, to get most of the dirt off. You don't need to get every last fungal spore off the surface (we will dilute those away). You also don't need to use any toxic solutions, often used in older works. Some researchers use ethanol for surface sterilization; it probably helps in removing some contaminants, but there is also a high percentage of the mycangial symbiots that die. We have tested it!
2. Don't grind up the whole beetle – focus on the right body part. If the mycangium is in the head, use the head (most Xyleborini). If

it's in the prosternum (for example, Xyloterini, Corthylini) or pronotum (Platypodinae), use the prothorax. If it's in the mesonotum (the Xylosandrus–Anisandrus clade of Xyleborini) it gets a bit trickier, but with a little practice you will learn how to excise that general part of the body out as well. The main point is to avoid most of the surface, the alimentary canal (particularly the hind gut which is full of yeasts) and the space under elytra, which also hosts many unwanted associates (including nematodes). Yes – the space under the elytra is dirty. When you want to study the microbial associates of people's mouth, you also don't grind up the whole person.

3. Dilution plating! This is the ESSENTIAL part of the process. Plate several dilutions of your inoculum, and use the lowest one where some Colony Forming Units end up growing in the end. The goal here is to dilute away all the non-specific associates which are likely present in lower abundances, and only capture the abundant symbionts. Those are typically present in thousands of cells, so you have a good chance of getting mostly those in the lowest dilution.

Materials

- razor
- petri dishes
- ethanol in petri dish
- 1000ul and 100ul pipettes
- pipettes tips
- two pairs of fine hard forceps
- petri dishes for beetle dissection
- microscope in hood
- vials
- vial rack
- sterile 1X PBS
- alcohol-resistant marker

Procedure

Beetles: 10 female beetles

1. Remove elytra and clip wings.
2. Put each beetle in **0.5 ml 1X PBS**
3. Vortex (or sonicate) and keep/plate solution. That is the surface part of the fungal community.
4. In hood, separate beetle abdomen, head, and pronotum
5. If the beetle has mandibular pouches in the head, put the head in **0.5 ml 1X PBS** and crush it.
6. If the beetle has a pronotal/mesonotal mycnagium, remove the metanotum and transfer it in 0.5 ml 1X PBS.
7. Remove the gut, put in **0.5 ml 1X PBS** and crush it. This will be the gut symbionts, not mycangial; expect lots of yeasts.

To release fungal spores, the individual body parts can be crushed, or processed shortly in a bead beater – fill tubes with 500 µl of PBS.

Dilution plating

1. Prepare two tubes per each sample, label them "0.1" and "0.01", fill each with 500 ul of water or PBS.
2. Suspend mycangium in the tube "0.1" and vortex.
3. Plate **50 ul** of the suspension on media. Record the plate as "0.1 dilution" in [PLATES].[note] in the Isolations database.
4. Transfer **50 ul** of the initial suspension to the second tube ("0.01") and vortex.
5. Plate **50 ul** of the second suspension on the second media plate, and record that plate as "0.01 dilution".
6. Plate **5 ul** of the second suspension on a **third** plate, and record it as "0.001 dilution".

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