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Calculating Colony-forming Units

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Abstract

This protocol describes how to estimate the number of colony-forming units.

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.

To estimate the initial number of colony-forming units in a mycangium, use the technique of serial dilution when plating. After colonies grow, multiply the number of colonies on the plate by the inverse of the initial dilution factor.

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 **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”.