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Cultivating Melanized Fungi from Biological Soil Crust and Rock Surfaces

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1 Works for me dx.doi.org/10.17504/protocols.io.3fxgjpn

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ABSTRACT

As the interest to understand melanized fungi becomes more of a focus due to pathological diseases, there needs to be a clearer method to isolate and identify the fungi from their surroundings. Culture independent analysis has helped bloom scientist understandings of the vast quantities of microorganisms around us, but culturing and identifying has always been a struggle. We must look into utilizing different microbiological techniques to help better understand, isolate, and to apply Koch's postulates to prove they are the cause of said disease. Growing and isolating fungi has always been an issue especially if the fungi in question is a slow growing fungi, where fast growing fungi or other microorganisms can grow and surpass the field of view and compete with the slow growing fungi. A series of dilutions, antibiotics and oligotrophic media can all counteract the issues to provide you with a clear window to help isolate your fungi in question.

There is still a need to verify that living strains of organisms are actually present in the environment. One approach is to use "culture dependent" method to obtain strains of organisms present in the biocrust. This is achieved by plating biocrust soils onto microbiological media in order to further isolate life strains. Serial dilution is used to isolate the fungi from the soil. This method involves making a soil slurry by diluting the soil sample with increasing higher proportion of water or media in order to get a low starting concentration of starting spores or material growing on the Petri dishes.

MATERIALS

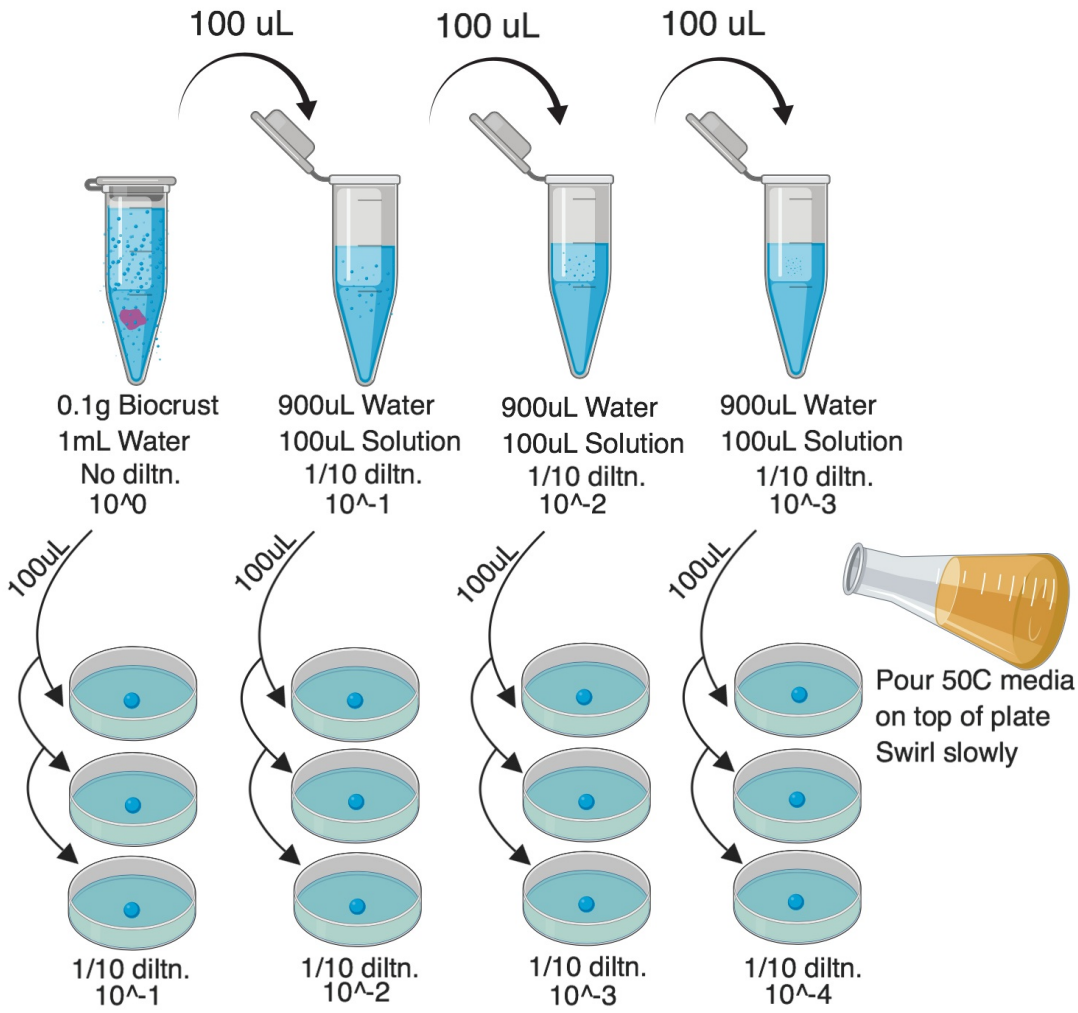
NAME	CATALOG #	VENDOR
Asparagine		
Glucose	Glucose	P212121
Potassium phosphate (dibasic)	View	P212121
Petri dish, 100x15mm	FB0875712	Fisher Scientific
Falcon® Serological Pipettes, 1 mL 1000 Pipettes	38001	Stemcell Technologies
1.5mL Microtubes		

1 Prepare Glucose Asparagine Agar (GAA)/1L
0.500g Dibasic Potassium Phosphate
0.500g Asparagine
10.00g Glucose
15.00g Agar

Autoclave

Prepare filtered antibiotics for 1 liter agar solution. Either Gentamycin, Tetracycline, Kanamycin, Streptomycin or Chloramphenicol will work.

2



Visual protocol for clarity.

3 1m

0.1 g of sample (either biocrust, soil or rock surface can be used) placed in microcentrifuge tube.

3.1 30s

Add **1000 µl Sterile H2O** into the same tube.

3.2 30s

Label this 10^0.

4 10m

Vortex (on highest setting) sample for 10 minutes until solution becomes a slurry.

Rock surface samples may not become a slurry.

2m

Preparing the Serial Dilution

5 Prepare 3 other microcentrifuge tubes with **900 µl sterile H2O** .

5.1 Label them 10^-1, 10^-2, 10^-3. 45s

5.2 Take **100 µl slurry solution** from 10^0 and add it to 10^-1. Vortex 10^-1. 30s

5.3 Then take **100 µl 10^-1** and add it to 10^-2. Vortex 10^-2. 30s

5.4 Then take **100 µl 10^-2** and add it to 10^-3. Vortex 10^-3. 30s

6 Set up 12 empty Petri Plates and label them with appropriate labels. I.e. Date, Media (list antibiotic used), Crust type, Dilution, Iteration (Plate 1, Plate 2 or Plate 3).

For more statistical power we must set them up in triplicate.

6.1 Pipette out **100 µl 10^0** into first three plates that were properly labeled for 10^0.

6.2 Pipette out **100 µl 10^-1** into next three plates labeled for 10^-1.

6.3 Pipette out **100 µl 10^-2** into next three plates labeled for 10^-2.

6.4 Pipette out **100 µl 10^-3** into next three plates labeled for 10^-3.

7 Make sure to have media cooled (to **50 °C***) and ready to go at this point.

*which means you can touch it with your hands without burning yourself.

7.1

Add the media slowly to avoid bubbles. Fill until agar closes all gaps on plate. Fill up to three plates at a time.

7.2

When three plates have been filled start to slowly mix the plate and slurry solution. Do a very careful "Figure 8" motion. Make sure to mix slowly and evenly without touching the lid of the plate.

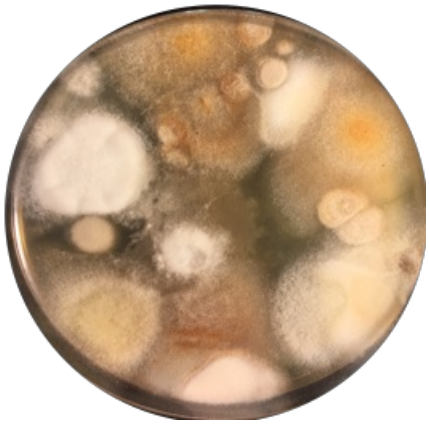
If it does touch, just replace with a new lid from an unused empty plate.

7.3 Continue repeating 5.1 and 5.2 until all plates have been filled and are cooling.

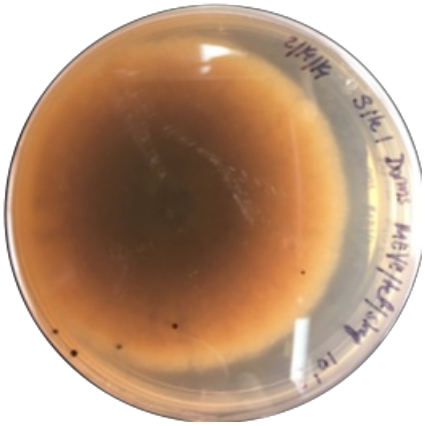
8 Let plates air dry for 24 hours, then parafilm to allow for long-term storage.

9 New fungi should start popping up on undiluted and next dilution plates in the next couple of days. Most dilute plates will also have sparser filamentous fungi growing.

9.1 Melanized fungi will take about 1-2 weeks to show up on plates.
Make sure to check on the underside of plates for black dots. Those are the fungi you are looking for!



This is an example of the least diluted plate fungal growth.



This is the back of a plate. Notice the little black dots. These are the melanized fungi/yeasts to collect.