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Cultivating Melanized Fungi from Biological Soil Crust and Rock Surfaces V.1

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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.

Keywords: Melanized fungi, black yeasts, culturing, biological soil crust, rock surfaces

MATERIALS

MATERIALS

✕ Asparagine Contributed by users

✕ Glucose P212121 Catalog #Glucose

✕ Potassium phosphate (dibasic) P212121

✕ Petri dish, 100x15mm Fisher Scientific Catalog #FB0875712

✕ Falcon® Serological Pipettes, 1 mL 1000 Pipettes Stemcell Technologies Catalog #38001

✕ 1.5mL Microtubes Contributed by users

- 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.

The diagram illustrates the serial dilution process for Biocrust inoculation. It is organized into two rows of four columns each.

Top Row (Dilution Preparation):

- Column 1:** Shows a microcentrifuge tube containing 0.1g Biocrust and 1mL Water. The dilution is labeled as "No diltn. 10^0 ".
- Column 2:** Shows 100 μ L of the solution being added to a tube containing 900 μ L of Water. The dilution is labeled as "1/10 diltn. 10^{-1} ".
- Column 3:** Shows 100 μ L of the 10^{-1} dilution being added to a tube containing 900 μ L of Water. The dilution is labeled as "1/10 diltn. 10^{-2} ".
- Column 4:** Shows 100 μ L of the 10^{-2} dilution being added to a tube containing 900 μ L of Water. The dilution is labeled as "1/10 diltn. 10^{-3} ".

Bottom Row (Inoculation):

- Column 1:** Shows 100 μ L of the 10^{-1} dilution being added to three petri dishes. The dilution is labeled as "1/10 diltn. 10^{-1} ".
- Column 2:** Shows 100 μ L of the 10^{-2} dilution being added to three petri dishes. The dilution is labeled as "1/10 diltn. 10^{-2} ".
- Column 3:** Shows 100 μ L of the 10^{-3} dilution being added to three petri dishes. The dilution is labeled as "1/10 diltn. 10^{-3} ".
- Column 4:** Shows 100 μ L of the 10^{-3} dilution being added to three petri dishes. The dilution is labeled as "1/10 diltn. 10^{-4} ".

Additional Information:

- An illustration of a petri dish being poured into shows the instruction: "Pour 50C media on top of plate Swirl slowly".

Visual protocol for clarity.

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

1m

- 3.1** Add 1000 μ L Sterile H₂O into the same tube. 30s
- 3.2** Label this 10⁰. 30s
- 4** Vortex (on highest setting) sample for 10 minutes until solution becomes a slurry. 10m
- Note**

Rock surface samples may not become a slurry.
- 5** **Preparing the Serial Dilution** 2m
- Prepare 3 other microcentrifuge tubes with 900 μ L sterile H₂O.
- 5.1** Label them 10⁻¹, 10⁻², 10⁻³. 45s
- 5.2** Take 100 μ L slurry solution from 10⁰ and add it to 10⁻¹. Vortex 10⁻¹. 30s
- 5.3** Then take 100 μ L 10⁻¹ and add it to 10⁻². Vortex 10⁻². 30s
- 5.4** Then take 100 μ L 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).

Note

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 $^{\circ}\text{C}$ *) and ready to go at this point.

Note

*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.

Note

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

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

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