

JoVE: Science Education

Filamentous Fungi

--Manuscript Draft--

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Environmental Science Education Title: Filamentous Fungi

Overview:

The goal of this experiment is to isolate, observe, and quantify filamentous soil fungi using dilution and plating techniques.

Fungi are heterotrophic eukaryotic organisms, and with the exception of yeasts, are aerobic. They are abundant in surface soils and important for their role in nutrient cycling and decomposition of organic matter and organic contaminants. White rot fungi (phanerochaete chrysosporium) (Fig 1) for example are known to degrade aromatics.

Since soils generally contain millions of fungi per gram, a dilution series of the soil is often made by suspending a given amount of soil in a dispersing solution (often deionized water), and transferring aliquots of the suspensions to fresh solution, until the suspension is diluted sufficiently to allow individual discrete fungal colonies to grow on the agar plates (Fig. 4.2).

After inoculation on several replicate agar plates, the plates are incubated at 25°C and counted after they have formed macroscopic fungal colonies (Figure 4.2). Because the assumption is that one fungal colony is derived from one organism, the term **Colony Forming Units (CFUs)** is used in the final analysis, with the results expressed in terms of CFUs per gram of oven dry soil.

Normal culturable fungal counts from fertile soil are in the range of 10^6 - 10^8 fungal “propagules” (spores, hyphae, or hyphal fragments) per gram of dry soil. Culturable plate counts have been in use for enumerating organisms since the nineteenth century. They continue to be used today as they are inexpensive to perform, require little labor, are quick, and are fairly reproducible. However, they do suffer from a number of errors which must be considered when evaluating the results. The most significant of these errors is the fact that many organisms will not culture on media plates.

Procedure:

First Period

Materials:

- 25g fresh soil of each soil type
- deionized water
- 25-mL pipette
- pipette bulb
- 1 plastic vial for each soil type
- rubber bands
- plastic wrap
- benchtop balance ($\pm 0.01g$)

Steps:

1. Calculate the amount of water that needs to be added to 25g of soil to amend the soil moisture

content to a 10% on a dry weight basis. Next, add that amount of moisture (deionized water) to 25g of the soil. The equation to determine the initial moisture content of the soil is:

$$\% MC = \frac{W - D}{D}$$

where:

MC = moisture content

W = net weight

D = dry weight

The initial moisture content of the soil is determined by overnight drying of a known amount of the moist soil, and reweighing the dried soil.

The final amount of water that needs to be added to adjust the moisture content to 10% can then be calculated with the same equation using a 10% moisture content and the known dry weight of the soil.

2. Cover the containers with plastic wrap and puncture the film several times with a probe to allow aeration during incubation. Secure the film with a rubber band. Weigh the soil and wrap; you will need this information to calculate moisture loss from the soil during incubation at room temperature for one week.

Second Period

Materials:

incubated soils from Period 1

1 sterile, 95-mL water blank per soil type

3 sterile, 9-mL water blanks per soil type

150 ml Rose Bengal agar for each soil type

filter-sterilized streptomycin solution to bring the agar to 30 µg mL⁻¹

9 sterile petri dishes per soil type

6 sterile, 1-mL pipettes per soil type

deionized water

1 test tube rack with at least 3 openings

pipette bulb

pan for collecting excess agar

vinyl gloves

marking pens

benchtop balance (±0.01 g)

vortex

water bath at 45C to keep agar molten prior to pouring

1. Weigh each of the soil samples with the wrap and rubber band to record the weights. The weight loss is due to moisture loss. Thus the actual soil moisture at the time of plating can be calculated. Prepare a 1/10 dilution series of your soils as shown in Figure 4.3
2. This will give you dilutions of 10^{-1} (bottle A), 10^{-2} (tube B), 10^{-3} (tube C), 10^{-4} (tube D), and 10^{-5} (tube E) g soil mL⁻¹ suspensions.
3. Prepare two plates for each of these dilutions, for examples 10^{-2} , 10^{-3} , and 10^{-4} , (tubes B, C, and D). By adding 1.0 mL of each dilution to three separate sterile petri plates for each soil (6 plates for each soil), your final effective dilutions will be 10^{-2} , 10^{-3} , and 10^{-4} , g soil per plate. The medium is Rose Bengal-streptomycin agar. Both the Rose Bengal and streptomycin inhibit bacterial growth. For very fertile soils where soil microbial populations are high, the chosen dilutions should be higher, i.e., 10^{-3} , 10^{-4} , and 10^{-5} , g soil per plate. Your instructor will choose the dilutions for the soil.
4. Incubate plates at room temperature for one week.

Third Period

Materials:

incubated plates from Period 2
lactophenol mounting fluid
pressure or transparent tape
dissecting probe
forceps
microscopic slides
immersion oil
microscope
fungal identification key

1. Make colony counts at one and only one dilution of each soil. The plates that are counted should have discrete countable colonies. Overgrown plates should not be counted. Likewise, plates with <10 colonies should not be counted. Note and describe the cultural characteristics of three different colonies. ~~The number of fungal colonies per gram of soil is equal to the number of colonies counted on the plate multiplied by the reciprocal of the dilution plated. For example, if 46 colonies are counted at a dilution of 10^{-6} , then the CFU per gram of soil is $10^6 \times 46$ or 4.6×10^7 .~~
2. Prepare pressure tape (transparent tape) mounts on slides for detailed microscope study using the following procedure:
 - deposit a drop of lactophenol mounting fluid at the center of a clean glass slide
 - cut a strip of clear cellophane tape about 3 cm long from the stock roll. To avoid contaminating the adhesive surface, use forceps when handling the tape. A dissecting needle will aid in freeing the tape from the forceps.
 - the adhesive side of the tape is applied to the surface of a sporulating fungus colony. Take care to avoid excessive pressure on the tape or too dense a mass of hyphae and spores will be collected.

- remove the tape from contact with the fungus colony and apply it, adhesive side down, to the drop of mounting fluid on the glass slide. Rub the tape gently with a smooth, flat instrument to express air bubbles.
- Examine the tape mount microscopically under the oil immersion objective with oil.
- Identify ~~three~~two different fungal genera using the supplied fungal identification key (figure 4-4).

Results:

a) Colony Counts

The number of fungal colonies per gram of soil is equal to the number of colonies counted on the plate multiplied by the reciprocal of the dilution plated. For example, if 46 colonies are counted at a dilution of 10^{-5} , then the CFU per gram of soil is $10^5 \times 46$ or 4.6×10^6 .

b) Identification of Three Different Fungal Genera

Fungi can be identified by examination of the fruiting bodies and fungal spores, both of which are characteristic for a given fungal genus. The ability to discern different fungi microscopically takes practice and experience, but the fungal identification key (Fig 4.4) will get you started. Fungi often observed include *Penicillium* and *Aspergillus*.

Applications:

Dilution and plating of soil fungi can be used as an indication of the health of a soil. Normally a "healthy" fertile soil will have 10^5 to 10^6 fungi per gram of soil. It can also be utilized to isolate pure cultures of specific fungi, subsequently evaluated for specific properties, such as the ability to degrade organic compounds. These can be detrimental as in the case of white rot fungi, or beneficial when toxic organics are degraded through biodegradation. Other uses of pure cultures of fungi include the isolation of fungi for antibiotics. For example, the first antibiotic ever was penicillin, produced by the soil-borne fungus *Penicillium*. This was first discovered by Sir. Alexander Fleming in 1929.

Comment [DM1]: We'll need a results section added too. You sort of start on it in the Third Period part of the Procedure, namely:

- Note and describe the culturable characteristics of 3 different colonies
- Examine the tape mount microscopically under the oil immersion objective with oil
- ID 3 different fungal genera

If you could elaborate on the description, examination, and identification in the Results section, we should be good to go.

Comment [DM2]: Could we bulk this up a little bit? Ideally we would include 3 applications.

Figures and Legend

Figure 4.1 White rot on birch. (Photo by Sten; licensed under Creative Commons Attribution)

Figure 4.2 Soil fungi isolated from a surface soil grown in a Petri dish containing Rose Bengal Agar. Photo courtesy K.L. Josephson. From *Environmental & Pollution Science*, 2nd Ed., Academic Press, San Diego, CA.

Figure 4.3 Dilution and pour plating technique. Here, the diluted soil suspension is incorporated directly in the agar medium rather than being surface applied as in the

case of spread plating. From *Environmental & Pollution Science*, 2nd Ed., Academic Press, San Diego, CA.

Figure 4.4

Fungal identification key

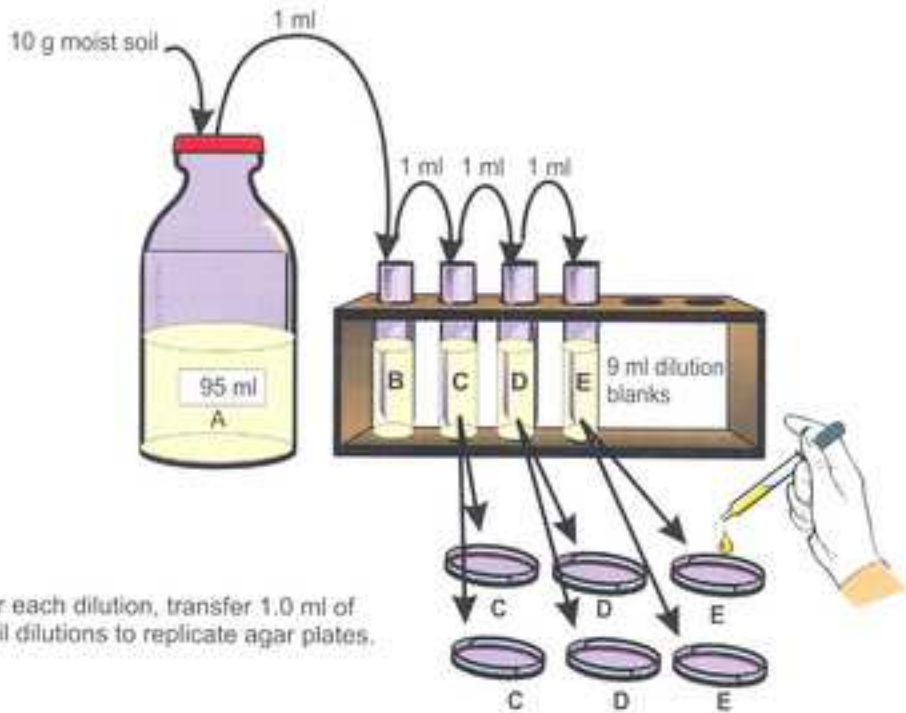
Comment [DM3]: Permission granted by Elsevier for us to use each of these.

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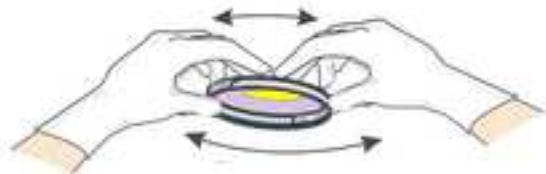
Step 1. Make a 10-fold dilution series.



Step 2. For each dilution, transfer 1.0 ml of soil dilutions to replicate agar plates.



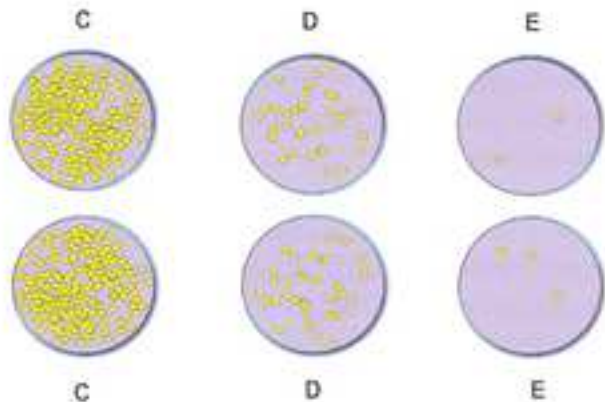
Step 3a. Add molten agar cooled to 45°C to the dish containing the soil suspension.



Step 3b. After pouring each plate, replace the lid on the dish and gently swirl the agar to mix in the inoculum and completely cover the bottom of the plate.

Step 4. Incubate plates under specified conditions.

Step 5. Count dilutions yielding 30-300 colonies per plate. Express counts as CFUs per g dry soil.



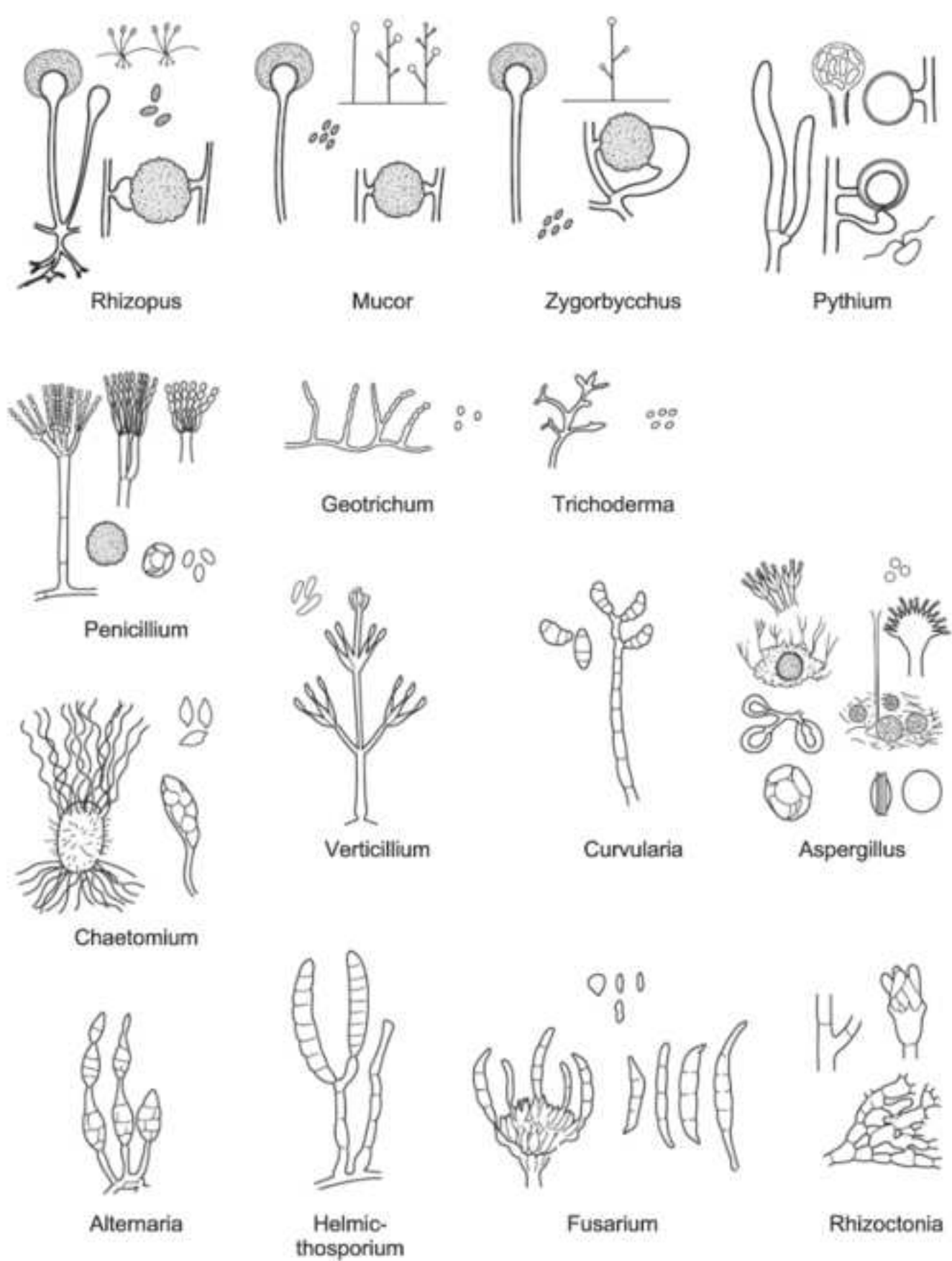


Figure 4-4 Illustrations of fruiting bodies of fungi often isolated from soil.