

Isolation of Dictyostelid Cellular Slime Molds from Soil/Leaf Litter

1. Collect small samples (a few tablespoons full) of the “soil” at the decomposition zone beneath intact fallen leaves and debris. Active slime mold cells feed upon the variety of bacteria that are responsible for organic decomposition. Place samples in small, clean plastic bags (sandwich bags will do nicely).
 2. Using a standard balance and plastic weight boat, weigh out 4-6 grams of sample. The actual weight can vary. Just be sure to record the weight.
 3. Tear off the “top” of a “whirl-pac” bag provided and transfer the weighed sample to the “whirl-pac” (or fresh sandwich bag).
 4. With a graduated cylinder, measure out a quantity of distilled water in milliliters equal to 9x the gram weight of your soil sample. E.G. If you weigh out 5.5 g sample, you would want to measure 49.5 ml water (9 x 5.5).
 5. Add this water to the soil in the whirl-pac, close the bag securely and mix by shaking the soil water mixture to create a uniform suspension. (If your soil is rather sandy, larger mineral particles will quickly settle making a suspension harder to maintain).
 6. Put a blue pi pump onto an unplugged 1.0 or 2.0 ml pipets, but slip the pi pump onto the tapered tip end of the pipet (the opposite end that you would usually use). In this fashion, draw up 0.8 – 1.0 ml of your soil suspension into the pipet.
 7. Transfer 0.2 ml of suspension onto the agar surface of each of 2 or 3, 60mm culture dishes provided. The medium in these dishes is a rather undefined “hay-infusion” agar, phosphate buffered to a pH of 6 (optimum for slime molds cells, but a bit too basic for the rapid growth of many filamentous fungi)
- Note: In most studies, this original 1:10 soil/water dilution is further diluted to 1:25 by taking a 5.0 ml aliquot of the 1:10 dilution and mixing it with 7.5 ml sterile, distilled water, before dispensing 0.5 ml of the more dilute suspension onto hay infusion agar in 95-100 mm diameter culture plates.
8. Using another pipet (cotton-plugged this time), in the more usual configuration with the wider plugged end inserted into the pi pump, transfer 2-3 drops of an *Escherichia coli* suspension into each of your culture dishes.
 9. With the lids of the dishes on, swirl the soil/bacteria suspension to spread the mixture evenly over the agar surface. Prop the lids on the rims of the dishes for a few minutes to allow excess moisture to evaporate, but put the lids on securely after the “wet” look on the surface has disappeared so the plates don’t dry out completely. It usually takes only 10-15 minutes for the wet look to disappear.
 10. With a marking pen, label the SIDE of the lid of each dish with your initials and today’s date. Stack the dishes and incubate at ambient room temperature (out of direct sunlight) for several days. Within 3-4 days, noticeable aggregations, pseudoplasmodia and fruiting bodies should begin to appear on the agar surface of the plates.