Isolation of Social Amoebae From Soil Samples

Modified by Geoff Zahn

Dictyostelium discoideum is a species of soil amoeba that serves a model organism for the study of many biological phenomena. However, *D. discoideum* is only one type of soil amoeba, or slime mold, in the world. There are countless other species of soil amoeba that have been identified and likely many more that have yet to be discovered. To compare and contrast lab quality *Dictyostelium discoideum* with true wild-type amoebae, soil samples were collected from various locations in the woods of Storrs, Connecticut. These strains were studied for their morphology, patterns in motility, chemotaxis, and development. It was found that these isolates displayed vastly different behavior from other strains in all aspects of their behavior and morphology. Based on these findings, it has been concluded that each of these samples represents a separate, distinct species.

Methods

1. Isolation of wild Dictyostelium

Eight soil samples were collected from locations nearby the Fenton River in Storrs, Connecticut. These locations included the following, mapped in figure 1.

1. Under a large pine by the river
2. Under a dry dead log
3. Material in the base of a fallen tree
4. The insides of a moist, decomposing log
5. Soil beneath very young trees
6. Just beside the river
7. Decomposing leaves beside the river
8. Near a small stream runoff

A 1:10 dilution was initially made with each sample by adding 1.0 gram of the sample to 9.0 mL of distilled water, and vortexing for 5-10 seconds. Immediately following the suspension of soil in water, a second tenfold dilution was made by pipetting 1.0 mL of the 1:10 dilution into 9.0 mL of distilled water, creating a 1:100 dilution. Debris was not pipetted.

Each dilution sample was vortexed and 200 µL of the suspension was added in drops to a hay-infusion agar plate. To these plates, one streak of an *E. coli* (Ec) culture was added in the center. The plates were incubated for 1 week at 22° C.

Following the incubation period, plates were inspected by eye and with a stereomicroscope for evidence of amoebae. Look for clearing zones in the agar or fruiting bodies. Various filamentous fungi will spread over the plate with time, so you have to check the plates frequently to find colonies before they are overgrown. When fruiting bodies or plaques were discovered, a pipette tip was used to carefully harvest them. They were then suspended in 200 µL of the EC culture before plating the EC + isolate on a hay-infusion agar plate. The fruiting bodies from these plates were harvested and re-plated in this same manner until there was no more evidence of other organisms competing or coexisting with the Dictyostelium.

2. Keeping cultures of the isolates, collection of single-celled amoebae

Because the strains being studied in this investigation were presumed to be incapable of macropinocytosis for the acquisition of nutrients, they were grown on a plate with a solid, bacterial food source. After isolating a strain, a working culture was established. For all strains, a hay-infusion plate was inoculated on the surface with 100 µl Ec. Approximately 5-10 fruiting bodies were carefully acquired from a given Dictyostelium sample using a pipette tip. The sample-laden pipette tip was applied to a small spot approximately 2 cm from the side of the

plate. The plate was then incubated at 22° C. When development began to occur at the center of the area where the sample had been applied, and a leading edge could be seen, the non-developing Dictyostelium could be collected for experiments.

New working-line plates were created every 5-7 days.

Hay-Infusion Agar:

500 mL ddH2O
10-20 g old hay
7.5 g bacto agar

Add hay to water in large wide-mouth beaker
bring to boil
simmer for 10 minutes, let cool and strain
add agar to warm liquid
Autoclave
pour into small plastic Petri plates and store sealed at 4-10 deg C