
Slime Molds

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Storage:

Actively growing cultures may be maintained at room temperature (22°C) in a dark drawer or cabinet.

All slime molds are characterized by naked assimilative stages. The assimilative body is multinucleate in *Physarum polycephalum*, a true slime mold, and uninucleate in *Dictyostelium discoideum*, a cellular slime mold. In contrast to bacteria, the nuclei of slime molds are enclosed within nuclear membranes and are like the nuclei in higher plants and animals. Hundreds of species of slime molds are known and most of these are universally distributed throughout the world. They are found in nature on moist dung, wood, soil, and other vegetation. They feed on bacteria, protozoa, fungal spores, and other decaying organic material.

Physarum polycephalum



A plasmodial slime mold found under cool, humid, and dark conditions in nature. It is an excellent teaching tool: its life cycle is easy to manipulate in culture, and it allows a unique opportunity for students to work with living protoplasm.

Life History

Plasmodium

The plasmodium of *Physarum polycephalum* is a bright yellow glistening multinucleate mass that can move in an amoeboid fashion. It ingests solid food particles in the same manner as an amoeba and can also absorb dissolved nutrients. It crawls towards its food, surrounds it, and secretes enzymes to digest the food. As the food is digested, *Physarum* deposits waste particles and moves away from them. *Physarum* gains energy and nutrients it needs for further growth from its food. Migrating plasmodia of *physarum* are typically fan-shaped and are composed of a network of vein-like strands or tubules. Toward the base of the fan these tubules are large with very few branches, but they become smaller and more frequently branched toward their tips. At the advancing margin, no vein-like organization is seen; instead, a continuous layer of protoplasm is present. Each tubular strand of the plasmodium consists of a hyaline (glassy, transparent, or translucent) semisolid outer layer of protoplasm and a fluid inner portion. No cell wall is present. Within the tubules, the more fluid portion of the protoplasm undergoes rapid rhythmic streaming: it flows in one direction for a few seconds, slows to a stop, then reverses and flows in the opposite direction. The streaming portion is usually granular in appearance due to the presence of pigment granules, bacteria cells and other matter ingested by the plasmodium.

Sclerotium

If conditions remain favorable, the plasmodium continues to take in food and other material as it enlarges. Under adverse conditions where temperature and moisture are not suitable for continued growth, the plasmodium may be converted into a resting structure termed a sclerotium. Sclerotia are hardened masses of irregular form consisting of many minute cell-like components. Under favorable conditions, the sclerotium can be reactivated (converted) to actively growing plasmodial stage.

Culturing

- I. Using the dried sclerotial stage (Constructing a moisture chamber)
 - A. Open a petri-dish and place a fully moistened (not dripping wet) piece of filter paper inside.
 - B. Place a piece of the dried sclerotium (sclerotial side up) on the moistened filter paper.
 - C. Be careful not to let this preparation dry out, moisten with water droplets as necessary.
 - D. After 24 hours or so, growing, active, plasmodia should be present.
- II. Using actively growing plasmodia (can also be used with sclerotial stage)
 - A. Place a few sterile oat flakes in a sterile petri-dish. Pour physarum agar into the dish and allow to solidify.
 - B. Using a scalpel or a sterile swab, remove a dime-size piece of the plasmodia and place in center of the agar plate.
 - C. Incubate at 25°C and within a few hours, actively growing plasmodia will be present spreading across the plate.

Dictyostelium discoideum



A cellular slime mold found in soil, on dung, and other places where bacteria are abundant. A variety of interesting exercises can be done with *Dictyostelium*, from observing its life history to studying differentiation and taxis responses.

Life History

Dictyostelium

Uninucleate amoebae of *Dictyostelium* move over the substrate, while feeding upon bacterial cells and reproducing by fission. During this time, each amoeba acts independently from all others. The assimilative stage may go on indefinitely if environmental conditions are favorable and there is an adequate supply of food. Depletion of the food supply, humidity, increasing concentration of amoebae, pH, and other factors affect this stage's duration.

The next stage of development is the aggregation stage. The myxamoebae move together, forming branched stream-like groups. Shortly amoebae converge on a central collection point, forming a single heap. This movement is a chemotropic response to a substance called acrasin, a steroid, which is secreted by the amoebae. The rounded heap of amoebae soon assumes a definite elongate form called pseudoplasmodium, slug, or grex. The cartridge-shaped pseudoplasmodium of *Dictyostelium* migrates away from the place of aggregation. The slug resembles a blob, and leaves a trail of slime as it migrates. The pseudoplasmodium is composed of many myxamoebae, and if placed in a drop of water and shaken, the myxamoebae will separate and resume individual activity.

Migration of the pseudoplasmodium may last for several hours, varying with environmental conditions, especially humidity. It may last up to 10-20 days if the pseudoplasmodium is kept in very moist conditions. The length of migration directly affects stalk length, with a greater migration period resulting in greater stalk length of the fruiting structure. If cultures are exposed to drying conditions, the next stage in the life cycle is initiated.

Following migration, the pseudoplasmodium stops gliding and forms a fruiting body termed a sporocarp. As the sporocarp forms, the cells differentiate into a base, a stalk, and a globular mass. Each amoeba in the mass secretes a wall around itself, producing an encapsulated spore. On germination, each spore gives rise to a single myxamoeba.

Culturing

A. Pour lactose agar plates and allow to solidify.

B. Streak culture of *Escherichia coli* B. or *Enterobacter aerogenes* onto the plate.

C. Remove several sporocarps from a mature culture of *Dictyostelium*, and streak them across the agar in the area inoculated with bacteria.

D. Incubate in the dark at 25°C for 24-72 hours. In 24 hours individual amoebae will be present. The first aggregation will occur from 24-44 hours after inoculation. After 48 hours or so, pseudoplasmodium will be present and will begin to migrate. By the 4th-5th day, sporocarps should be present.

For Further Reading

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