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CULTURING MYXOMYCETE PLASMODIA FOR CLASSROOM USE

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The Myxomycetes are organisms which exhibit both animal and plant characteristics. This dual nature is of considerable interest and worthy of consideration in planning an elementary botany course. Since the plasmodial and fruiting stages can easily be cultured, and recognized, the study of these organisms is a practical project.

In the present study bark was collected fro mwell rotted elm, cottonwood, soft maple, oak stumps and from decaying elm branches found in cool moist places. Oak, ash, aspen, box elder and elm leaves were collected from pockets in the sod where they had gathered and wintered. These collections were made at Iowa City during the latter part of February, 1946.

Collected bark was cut into suitable lengths to fit into standard petri dishes fifteen millimeters deep. It is essential that the pieces of bark be small enough to allow some space, preferably five to ten millimeters, between the pieces of bark and the top of the petri dish, otherwise the plasmodia may crawl to the top of the petri dish, from which place they are less conveniently transferred. For classroom use the standard petri dishes were found to be the most convenient, although larger containers may be used if desired. The pieces of bark were thoroughly moistened with distilled water. If any excess water collected in the bottom of the dish, it was drained off. The leaves, cut into suitable lengths to fit into the petri dishes, were soaked in distilled water for a few minutes. Several of these pieces were pressed together and placed in dishes.

The material stored at room temperature, but not in direct sunlight, developed plasmodia first. The cultures at room temperature, but in partial darkness, were somewhat slower in producing plasmodia. Some which were placed in an oven at 30°C. did not produce any.

The development of plasmodia was as follows:

TABLE I

Species	Plasmodia Color	Substrate	Time
*Physarum confertun**	white	very rotten inner portion of elm bark	10 days
Ophiotheca chrysosperma	white, then rosy	outer portion of oak bark	10 days
Arcyria denudata	white	outer portion of elm branch bark	15 days
Physarum melleum	yellow	elm leaf	19 days
Unknown	white	elm leaf	19 days
Unknown*	pink	ash leaf	19 days
Didymium squamulosum	white	ash leaf	27 days

Unknown	white	outer portion of cotton-wood bark	22 days
*Physarum didermoides**	white	outer portion of elm bark	22 days
*Physarum**	yellow	outer portion of elm bark	26 days
Physarum nucleatum	white	outer portion of elm branch bark	29 days
Unknown	white	inner portion of soft maple bark	28 days
Didymium nigripes	white	oak leaf	31 days
Didymium anellus	white	ash leaf	35 days

* Developed along yeast streaks.

The pieces of bark or leaves on which the plasmodia developed were placed on Knop's agar in petri dishes. The following formula for Knop's solution was used.

TABLE II

$Ca(NO_3)_2$	1.0 gm.
KNO_3	0.25 gm.
KH_2PO_4	0.25 gm.
$MgSO_4$	0.25 gm.
$FePO_4$	trace
Distilled H_2O	1 liter

Dilute this solution 1:4 with distilled water

Fifteen grams of agar was dissolved in one liter of diluted Knop's solution and the resulting solution was tubed, autoclaved at fifteen pounds for fifteen minutes and poured into sterile petri dishes. Plasmodia usually crawl off the bark or leaves onto Knop's agar in a few hours (Fig. 1). It was found that yeast increased the mass of some species (see Table I) and these organisms developed along traces of yeast which were streaked on the agar (Fig. I). The yeast may be made either with yeast from the inner portion of a moist yeast cake or from a yeast culture.

The yeast culture was made by adding a small amount of moist yeast cake to nutrient agar and allowed to grow several days before using. When two percen maltose was added to the agar solution and after hardening was streaked with yeast, the plasmodia increased in mass but the plates were easily contaminated and consequently were not suitable for demonstration purposes.

Plasmodia may be transferred to fresh agar plates by cutting the agar around the plasmodium, slipping a spatula under the piece of agar and placing it on a fresh plate. Plasmodia can be kept active for approximately thirty days, by frequently transferring them and feeding yeast.

These cultures may be made at any time of the year but are less satisfactory during the summer months, unless it is possible to find a cool place to grow them.

For classroom use the petri dishes containing the plasmodia grown on agar may be turned over, placed under the low power of the mi-

croscope and the naked protoplasm, gross movement and streaming of protoplasm may be studied. The protoplasmic streaming is first in one direction, it then slows down, stops and reverses its direction. This process may be timed by students and usually fascinates them.

The swarm cells may be used to illustrate motile gametes, the union of gametes and are usually very interesting. Swarm cells can be germinated from some species such as *Reticularia lycoperdon,* and the more common species *Enteridium rozeanum* by placing the spores in rain water which has not been collected in a metal container. In this study, the spores of *Reticularia lycoperdon* were placed in water boiled with a small amount of charcoal to remove metal ions. These were stored at room temperature and germinated in about forty-eight hours. It is possible for some collections of some species to germinate in ninety minutes, although the time will vary both with the species and with the particular collection. After a collection has once been found to produce swarm cells it may be kept for a number of years and will produce swarm cells when needed.

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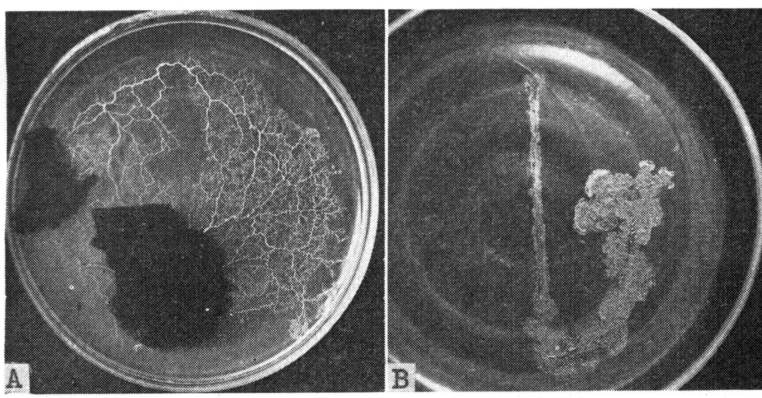

Figure 1. A Plasmodium crawling off bark of soft maple. B. Plasmodium developing along a yeast streak.