A STUDY ON THE LIFE CYCLE OF PHYSARUM CINEREUM GROWN IN CULTURE*

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ABSTRACT

An experiment on the culture of *Physarum cinereum* from spore to spore on sterilized bark and leaves in a moist chamber has been completed. The swarm cells escape through a deep wedge-shaped rupture in the spore walls. The posterior end of the swarm cell has a contractile vacuole and also sends out pseudopodia. They may be fused at their posterior ends. The swarm cell has flagella of equal length. One of the flagella is usually directed more or less straight forward, while the other is directed posteriorly at an angle greater than 90° from the first. The plasmodium belongs to the type of phaneroplasmodium, which is almost colorless or watery-white. By using single spore cultures it has been proved that a single myxamoeba is capable of producing a plasmodium, which indicates that this organism is homothallic. Ultra-thin sections of the spore have been made, and the structures have been photographed under the electron microscope.

INTRODUCTION

Among the more than four hundred known species of the Myxomycetes, only a relatively few have been induced to complete their entire life cycle on artificial media. *Physarum cinereum* (Batsch) Pers. is a very common cosmopolitan myxomycete growing on the soil, leaves, bark or even on grassy ground. But no one has ever reported it before growing in culture on artificial media starting with spores. The present work is a study of its culture from spore to spore on artificial media and also on sterilized bark and leaves in a moist chamber. Corn meal agar and Bold's (1949) modified Bristal agar appear to be satisfactory for culturing this organism. This experiment was carried on in a crude culture with bacteria, but free from fungi.

An electron microscopy of the spore has also been made. Since no one has ever reported the making of sections for the study of the structure of the spore of myxomycetes, this may well be the first study ever made of the fine structures of the spores with ultra thin sections under an electron microscope, and this may be a starting point in helping mycologists to know more about the nature and morphology of the spores of myxomycetes.

MATERIAL AND METHODS

A specimen collected from the air by Dr. Malcon R. Brown, Jr. fruited on an agar plate. The fruitification was identified as *Physarum cinereum*.

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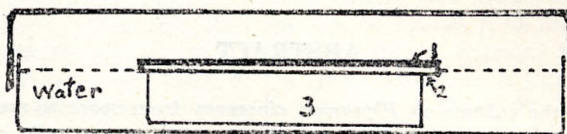
The spores of this specimen were used to start cultures on artificial media, and its life cycle and structures at different stages were examined.

In the studying of the germination of spores, a special moisture chamber has been used. The apparatus was set up as described in Figure 1.



Fig, 1

A large square coverglass (1) of 4 cm^2 was sterilized with alcohol and then a very thin layer of sterilized Bristal agar (2) was poured on one surface of this cover glass. After the agar had solidified, 3 drops of Bristal solution were added and then the liquid containing the spores of the specimen was spread on the agar surface by means of a sterilized platinium wire. The cover glass was inverted over an empty small Petri dish (3). This was then put into a 10 cm Petri dish so that the water could touch the agar surface on the cover glass. The Petri dish was covered and incubated at 25°C. This was examined with a microscope under high magnification from time to time without contamination.

Half strength Difco corn meal agar was used for cultivating the swarm cells and mixamoebae to produce plasmodia. 20 ml of the medium sterilized in autoclave for 15 minutes at 15 pounds pressure was poured into each of the standard sized sterilized Petri dishes. Spores in water were spread on the solidified agar surface. The plates were then incubated at 25°C. The plasmodium was separated from molds by repeated transplanting of a little bit of the plasmodium into a new plate of medium.

After the plasmodia were free from mold, a small amount of pulverized sterilized rolled oats was sprinkled over them. Some of the plasmodia were transferred to oats agar media.

The bark and leaves were also used as culture media, especially for the purpose of observing the slime mold growing on a more natural substratum, as the fruitification produced there is likely more nearly normal and of greater value for identifying the species. In preparing the moist chamber for bark or leaf culture, pieces of bark or leaves were placed on the filter paper at the bottom of a standard sized Petri dish, and a small amount of water was added. The Petri dishes were covered and together with the bark or leaves were sterilized in an autoclave under 15 pounds pressure for one hour. A little piece of agar with plasmodium was then transplanted on to the bark or leaf, after they were cold, and then incubated at 25°C.

The method of single spore culture is as follows: a mature fruiting body was

placed into a sterilized test tube to make a spore-suspension with 3 ml of sterilized water. Pour the spore-suspension on a plain agar plate and spread evenly by an "L"-shaped glass rod which was sterilized by dipping in alcohol and flaming in the fire. Use a fine needle to pick up a single spore together with a bit of agar under the stereoscope. The spore was then transplanted on a half strength Difco corn meal agar plate with glass rings separating the agar surface from the outside of the ring in the Petri-dish. The ring was about one centimeter in diameter and one centimeter high. The plate was then cultivated at 25°C.

The method of studying the fine structure of the spores was as follows:—Spores were placed in a centrifuge tube, fixed in 2% potassium permanganate solution for two hours under room temperature, washed several times with water, dehydrated gradually with ethyl alcohol, cleared in propylene oxide, and imbedded in plastic. The centrifuge was used at each step of washing and dehydration. The sections were cut 1/20 μ in thickness, examined under an electron microscope and photographic pictures were taken.

RESULT AND DISCUSSION

An experiment from spore to spore cultivated in the moist chamber has thus been completed. The spore structure was studied under the electron microscope.

Spore Germination

The method of spore germination in the myxomycetes described by Gilbert (1928), is an outstanding work. He found that there were two distinct methods of spore germination: (1) the swarm cell escapes through a deep wedge-shaped rupture in the spore wall, as in *Fuligo septica:* or (2) the swarm cell emerges through a small jagged aperture which appears in the spore wall as in *Dictydiaethalium plumbeum*. He recognized, however, that numerous species showed transitional conditions. Our material, *Physarum cinereum;* exhibits the first type of spore germination. Before germination the spore swells and increases its size (about 5/3 the original size), then the spore wall cracks and the protoplast escapes through the deep wedge-shaped rupture in the spore wall (Plate 1. fig. 2); flagella appeared after the protoplast emerged from the spore (Plate 1, fig. 1 & 3).

The length of time necessary for spore germination in this material is quite short. Germination was first observed after three hours of incubation at 25°C

Swarm cells and Myxamoebae

The swarm cells of this organism are elongate and highly metabolic, with the anterial end tapering and bearing two flagella of equal length. One of the flagella is usually directed more or less straight forward, while the other is directed posteriorly at an angle greater than 90° from the first. (Plate I, fig. 3.)

Elliot (1949) described three types of swarm cells in the myxomycetes according to their flagellation. (1) Swarm cells with one flagellum quite long and the other very short, (2) Swarm cells with one long flagellum and the other approximately half the length of the first, and (3) swarm cells with flagella of equal length. The flagella of *Physarum cinereum* corresponds to Elliot's third type.

The posterior end of the swarm cell has a contractile vacuole and also sends out pseudopodia. In a few cases I have seen swarm cells fused at their posterior ends. Most of them swim around but never fuse together (Plate I, fig. 3).

The swarm cells or two fused swarm cells lose their flagella to form myxamoebae. Myxamoebae are amoeboid structures occurring between the swarm cells and plasmodial stages.

Swarm cells or myxamoebae may encyst even though the moist condition is still quite suitable for the movement of the swarm cells.

In my spore germination chamber after ten days of incubation, active moving swarm cells were still observed.

The Plasmodium

Small plasmodia are first observed three days after spore inoculation at 25°C. The plasmodium grows and increases its size by engulfing bacteria and other organic materials. They are almost colorless or watery-white. Their structure and shape belong to the type of phaneroplasmodium. They are granular, fan shaped, with a definite margin and have veins with reversible protoplasmic streaming. This type of plasmodium is well-known in Physarum. When a little bit of plasmodium was transferred to a plate of very rich medium composed of oats agar, the plasmodium would grow rapidly. When it reaches full size before fruiting, it may sometimes become yellow in color.

Myxamoeba gives rise to a plasmodium. It frequently takes place in areas where numerous myxamoebae are aggregated. A single myxamoeba, however, is capable of producing a plasmodium. For this has been proved by the single spore culture. In our experiment single spores can produce plasmodia. Two hundred spores were plated; fifty percent of them produced plasmodium. This also indicates that this organism is homothallic.

Fruiting

When the food supply is enough for the development of large and vigorous growing plasmodium, it initiates fruitification. When a plasmodium is about to fruit, protoplasmic clump formations appear on certain areas on the agar, and they do the same on the bark or on the leaves. The clump first appears as a very dense area of protoplasm and later changes into the characteristic shape of the fruiting body. (Plate II, fig. 1). The newly formed fruiting bodies are white in color and remain

that color for as long as 12 hours. (Plate II, fig. 2). Then they become pale brown, and eventually turn to black. (Plate II, fig. 3).

Fruiting in most of the agar cultures takes place at the rim of the Petri dish.

Sporangia are sessile, closely gregarious, crowded or heaped, subglobose or elongate, merging into short plasmodiocarp, 0.3–0.5 mm. broad, calcareous, white or cinereous, or nearly limeless and iridescent to drab; peridium single, thin, more or less densely coated or flecked with lime; capillitium abundant, the nodes often angular and with the calcareous deposits extending into the internodes.

Spore and its fine structure

The color of spores in mass of this specimen is purplish-brown, and violaceous by transmitted light, minutely warty, and 3–13 μ in diameter.

Mycologists have considered the structure of the spore to be a major character in differentiation of species of Myxomycetes for a long time. The gross morphology of the spores of most species of Myxomycetes is fairly well known. But no one has ever studied their fine structures.

Yanagita (1951) and Cohen (1960) observed Myxomycetes under the electron microscope based on the observation of whole mounts, but added little to our knowledge of the structure of the spore.

In the present work ultra-thin sections (1/20 μ) have been made, and the structures have been photographed under the electron microscope.

The spore wall is composed of two layers: an outer deeply stained ektexine and an inner non-stainable endexine. The ektexine has minute protuberances on the outer surface. The endexine has a thickness of about 0.2 μ and the thickness of ektexine is about 0.1 μ. The spore has a single large nucleus and a few large mitochondria. The lipid droplets are numerous and are scattered in the cytoplasm. (Plate III.)

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Explanation of Plates

Plate I. Germination of spores. ×500.

Fig. 1. Swarm cells and empty spore walls.

Fig. 2. Protoplast escapes through the deep wedge-shaped rupture in the spore wall.

Fig. 3. Free living swarm cells, (F) showing flagella on the anterior end and (C) contractile vacuole on the posterior end.

Plate II. Fruiting. ×50.

Fig. 1. The early stage of forming the fruiting body, note the connecting protoplasmic vein; on agar medium.

Fig. 2. Newly formed fruiting bodies with white color; on bark.

Fig. 3. Older fruiting bodies turning darker color.

Plate III. Sections of spores under electron microscope. ×10,000.

Fig. 1. Section of spores fixed with potassium permanganate.
(E) endoplasmic reticulum; (En) endexine; (Ex) ektexine; (G) golgi; (L) lipoid droplets; (M) mitochondria; (N) nucleus.

Fig. 2. Section of spore fixed with osmic tetra-oxide.

Plate I

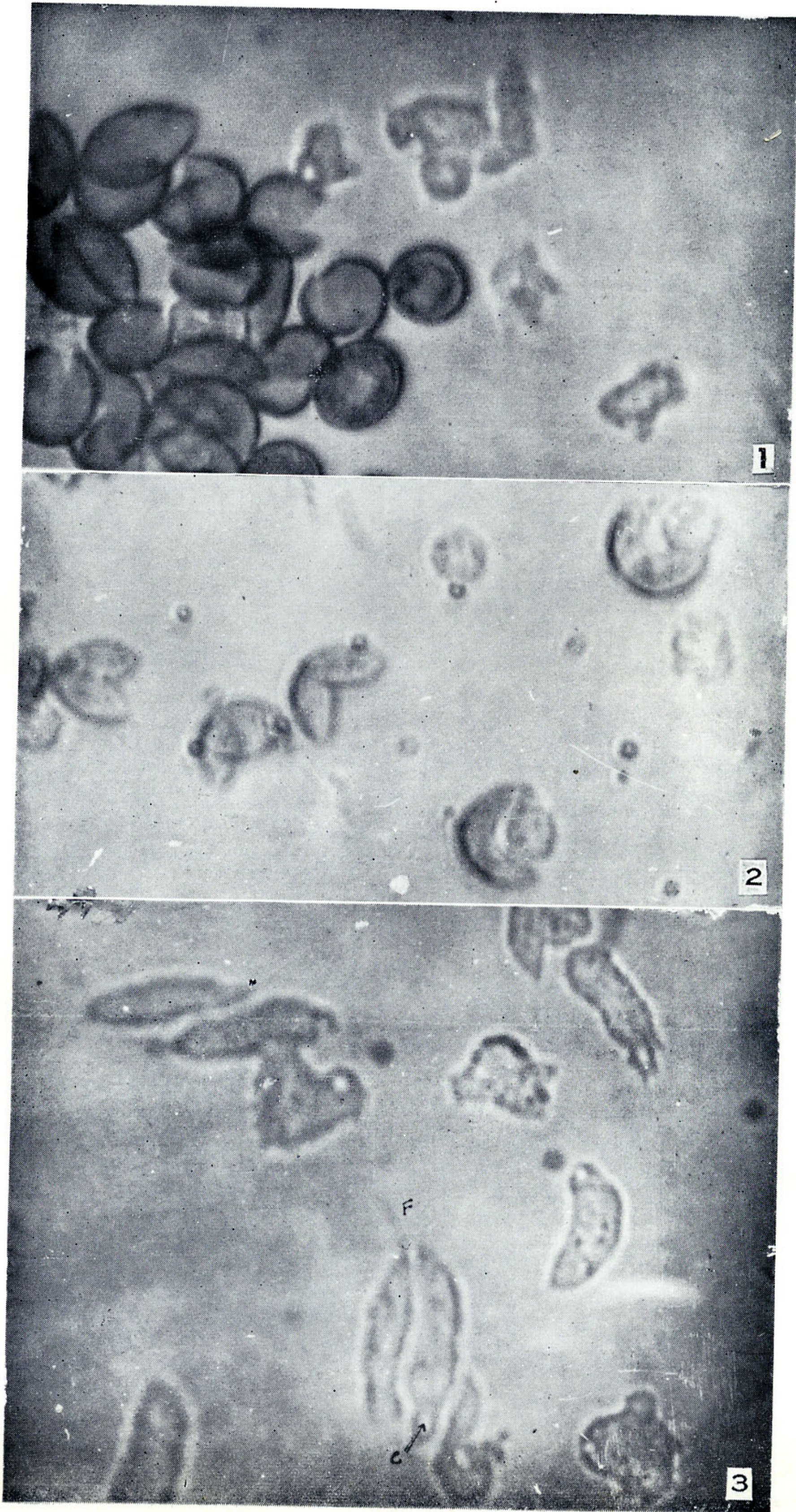
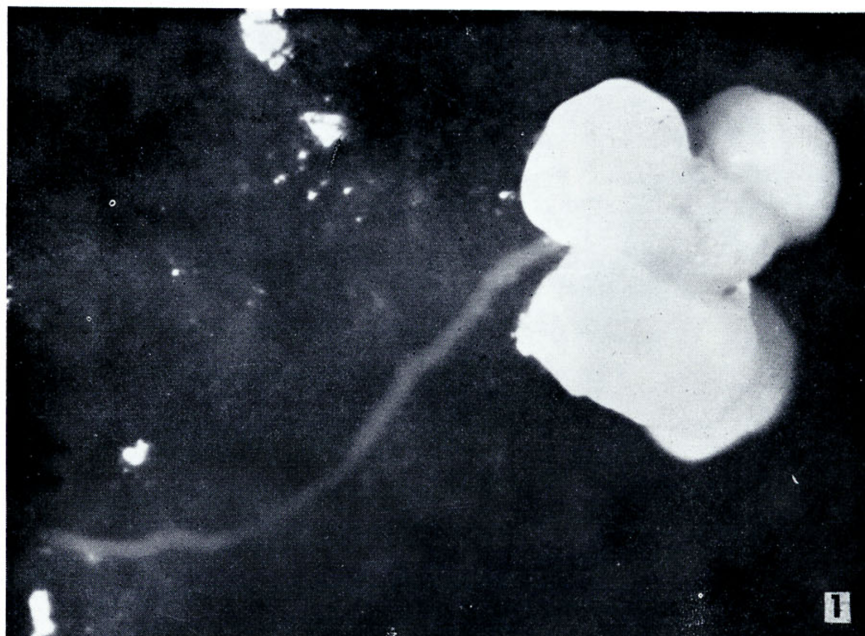


Plate II



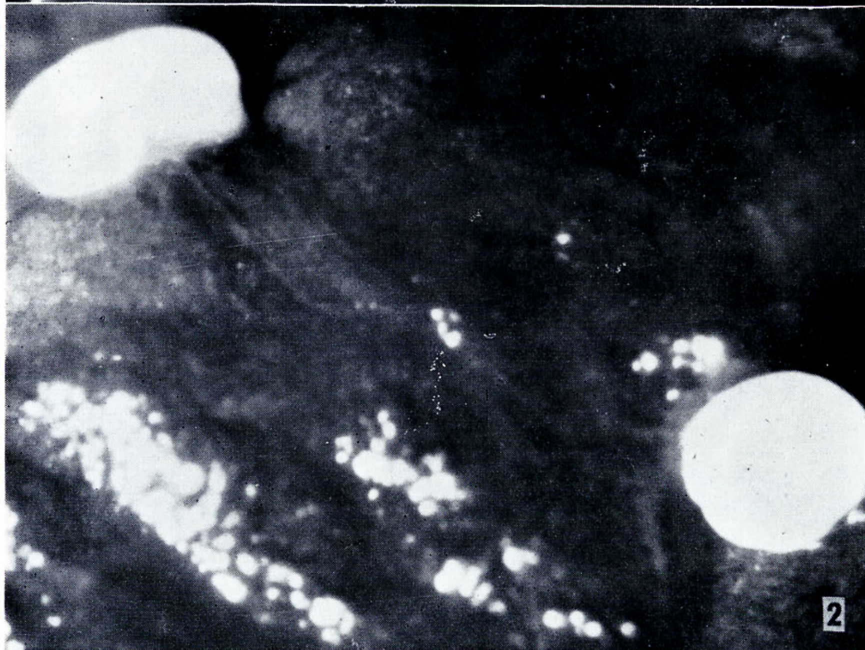


Plate III