THE LABORATORY CULTIVATION AND DEVELOPMENT OF THE MYXOMYCETES PHYSARELLA OBLONGA AND PHYSARUM DIDERMOIDES[1]

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The literature revelant to the cultivation of myxomycete plasmodia on artificial media reveals only one record of the cultivation of *Physarella oblonga* (Berk. & Curt.) Morgan and but two records of the laboratory cultivation of *Physarum didermoides* (Ach.) Rost. Gilbert (1931) stated that in a few instances sporangia of the first species had been obtained by placing spores on slightly acidified corn meal agar; aside from this one statement the writer has found no other references concerning the culture *P. oblonga*, although the species has received some cytological and morphological study. Bisby (1914) described capillitium development, and the gross details of sporangium development of this species were described and figured by Emoto (1934); both Bisby's and Emoto's observations, however, were made on naturally occurring material. Constantineanu (1906), in an extensive paper on the development of Myxomycetes, described the cultivation of *P. didermoides* on various types of media but included no figures and made no statement concerning maintenance of the species in culture by means of plasmodial transplants. Kambly (1939) stated that he was able to obtain plasmodia of *P. didermoides* on carrot decoction agar but that on this medium the plasmodia did not flourish and never completed their life cycles. The writer has had considerable success with the culture of both species and finds them well-suited for the demonstration of sporangial development as well as the illustration of pigmented and non-pigmented plasmodial types. Both are slow-growing when compared with such a species as *Physarum polycephalum* Schw., but they grow rapidly enough that after twelve or fourteen days inocula for thirty or forty cultures may be obtained from a single petri plate culture.

MATERIALS AND METHODS

Two types of media have been employed in these studies: (1) corn decoction agar, prepared by grinding 25–50 gms. of field corn, adding 500 ml. of distilled water, autoclaving for one hour at fifteen pounds pressure, filtering, adding enough distilled water to bring the total volume to one liter, adjusting to pH 5.2–5.4, adding 15 gms. of agar and sterilizing; (2) corn decoction agar, as prepared above, with 25 gms. of filter paper (finely divided by triturating wet in a mortar) suspended in each liter. Both species grew quite well on both types of media; because of the greater ease of preparation, plain corn decoction agar was used in most of the work.

Smith (1929) has shown that spores of various species of Myxomycetes remain viable for many years, but so far the writer has had consistently better results with spores not over two or three years old. Unsterilized medium may be used, but, because these plasmodia grow slowly, it is best to use sterile medium in order to hold contamination to a minimum, although even with sterile medium the cultures are never completely free of other organisms.

CULTIVATION OF PHYSARELLA OBLONGA

Spores from a two months old collection of this species, when placed in a drop of distilled water in a corn decoction agar slant, germinated and in fifteen days

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or less small yellow plasmodia were visible. Some variation in the time required for plasmodium formation is to be expected, since age, vitality of the spores, and other factors undoubtedly influence both spore germination and plasmodium growth. After plasmodia had appeared in the test tubes they were maintained in the vegetative stage by transferring small blocks of agar, on which there were bits of plasmodia, to fresh medium in 75 mm. petri dishes. Subsequent transfers were made in the same manner. If cultures are stored in the dark, subcultures need not be made at lesser intervals than three weeks. By such methods the species was maintained in culture continuously for nine months; Fig. 2 shows a nine day old culture of *P. oblonga*; Fig. 5 shows a bit of plasmodium enlarged four diameters.

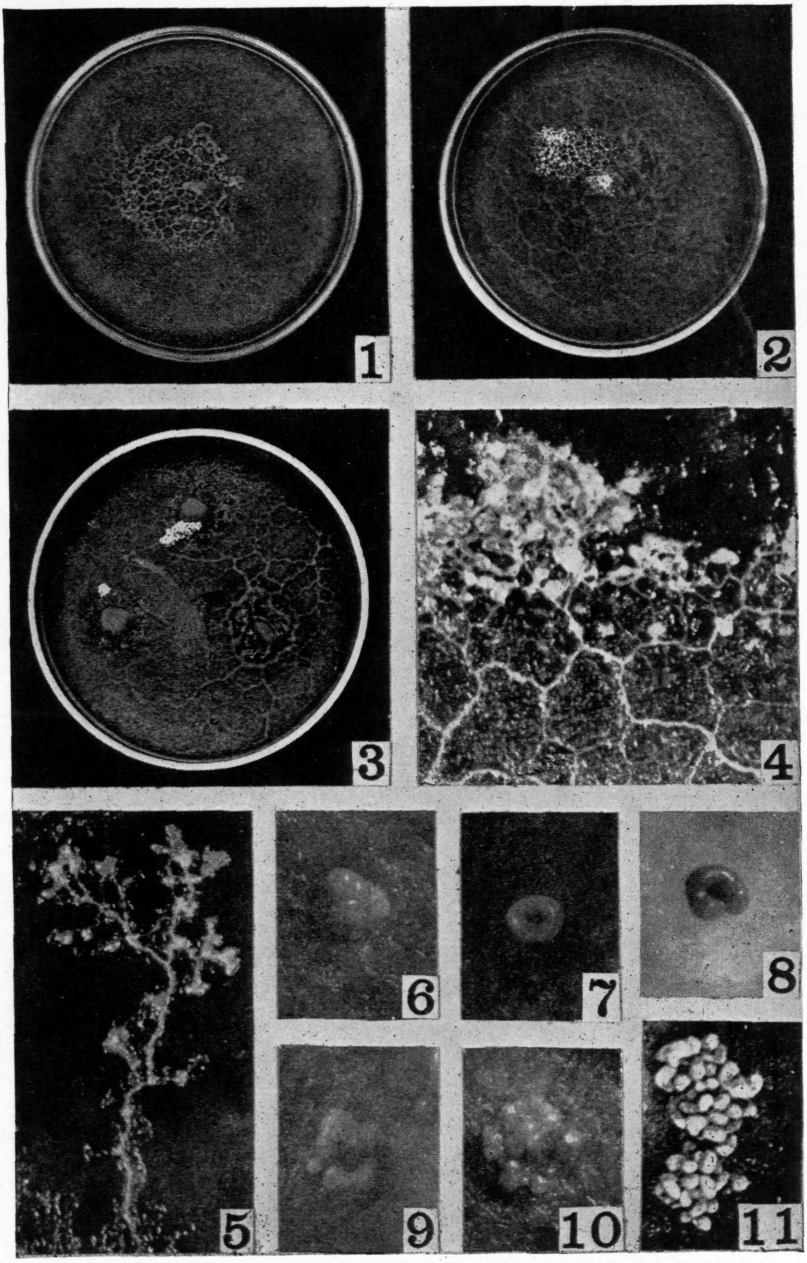
In order to determine if light has any effect upon fruiting, cultures were placed both in light and darkness. Of fifty cultures placed in the dark, fruiting bodies were formed in but two, and, of course, all of these cultures were exposed to light for short periods, since they were examined daily; sporangia appeared in forty out of sixty cultures which were placed in the light. Thus, light affects this species in the same way as previously reported for other species with yellow plasmodia (Gray, 1938). Under laboratory conditions, if plasmodia are left undisturbed, fruiting bodies formed in 15–18 days, while if they are covered with black cloth or placed in a drawer, plasmodia may remain in the active vegetative state for periods as long as seventy days provided the agar substrate does not become dry.

The time required for the development of sporangia from plasmodium is 11–12 hours, once sporangial formation has started. Fruiting is manifested when enlargements appear in the larger strands of the plasmodium (Fig. 6); protoplasm flows into these enlargements and they become still larger with the final result that the strands of plasmodium connecting them disappear. Shortly after this, an umbilicus appears in the top of each globule of protoplasm (Fig. 7) and soon definite stipes are formed. The stipes gradually elongate, and just before maximum length is attained, the stipe bends just below the developing sporangium, and thus the sporangia are typically nodding although in some instances they may be borne upright. Sporangia retain the same yellow color as the plasmodium until final form is attained, and then they gradually darken; however, as soon as the stipes become apparent, they gradually darken from the basal portion upward. Emoto (1934) has figured the various stages in the development of sporangia of this species; all such stages are not shown here.

Both plasmodiocarpous and sporangial types of fruiting bodies are formed by *P. oblonga* in its natural habitats; however, plasmodiocarps have appeared in only a few cultures. It has been found that sporangia are more like naturally occurring fruitings when they are formed under drier conditions; therefore, when the fruiting process is once initiated, it is advisable to remove the petri dish covers. Atypical sporangia and plasmodiocarps are generally formed when the plasmodial enlargements are irregularly shaped (Figs. 9 and 10). The type of formation in Fig. 8 may give rise to a typical sporangium but more frequently develops into a fruiting body with a saddle-shaped sporangium which is suggestive of a minute *Helvella*

EXPLANATION OF PLATE I

FIG. 1. Nine-day old culture of *P. didermoides*. × ⅔. FIG. 2. Nine-day old culture of *P. oblonga*. × ⅔. FIG. 3. Early stage in the fruiting process of *P. didermoides*, shortly after sporangia have been delimited. × ⅔. FIG. 4. Enlarged portion of plasmodium of *P. didermoides*. × 4. FIG. 5. Enlarged portion of plasmodium of *P. oblonga*. × 4. FIG. 6. Enlargement in plasmodium of *P. oblonga* which is indicative of initiation of the fruiting process. × 10. FIG. 7. Typical umbilicate immature sporangium of *P. oblonga*. × 10. FIGS. 8, 9, 10. Plasmodial enlargements which give rise to atypical sporangia. × 10. FIG. 11. Mature sporangia of *P. didermoides*. × 4.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ascocarp. Spores from fruiting bodies developed in culture are viable and will germinate and produce swarm cells and plasmodia when placed upon fresh medium; however, no viable sclerotia have been obtained thus far.

CULTIVATION OF PHYSARUM DIDERMOIDES

White plasmodia of this species were obtained by the same method as that employed for the preceding species. Plasmodia were obtained in about fifteen days after spores from a five months' old culture were sown. Of the various non-pigmented plasmodia that have been cultured by the writer, this form is probably the most suitable for laboratory cultivation. The plasmodia are quite tolerant of mechanical injury, and hence subcultures may be made employing very small amounts of plasmodium. Sporangia are formed in either light or darkness, the average time of fruiting for forty cultures being twenty-one days after the cultures were made. In order to maintain the species in its vegetative state, subcultures should be made every 18–20 days. In this manner, *P. didermoides* has been maintained in culture continuously for over six months. Once a culture is established, little care need be observed so far as the maintenance of sterile conditions is concerned, since this species is strongly mycophagous and bacteriophagous, and contaminants are rarely able to become established. Plasmodia of this species are shown in Figs. 1 and 4.

The fruiting process in this species requires 8–10 hours from initiation to completion and differs somewhat from this process as seen in *P. oblonga*; this should be expected since sporangia of *P. oblonga* are not crowded together as in *P. didermoides*. In the latter species instead of the appearance of enlargements in the plasmodial strands, initiation of the fruiting process is manifested when all of the protoplasm of the plasmodium flows into a solid lump. A few hours after this, pearly-white, discrete sporangia may be observed (Fig. 3). After the fruiting bodies have attained their final shape and size they become light, brownish-lavender in color; from this they gradually change until the blue-gray color of mature sporangia is attained. In most fruitings the white hypothallus typical of the species is present. Mature sporangia are shown in Fig. 11. In a few cultures plasmodia became pale lavender three or four days before fruiting bodies were formed, but in most cultures there was no color change until after sporangial delimitation was apparent.

Viable sclerotia of *P. didermoides* were obtained by removing the covers from petri dish cultures of actively growing plasmodia and allowing the plasmodia and agar substrate to dry slowly. Upon being moistened such sclerotia revived and streaming could be observed in about twenty-four hours. As in the preceding species, spores from sporangia which developed in culture were found to be viable.

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