Preliminary investigations in culture of some Myxomycetes

By

P. U. Indira and *R. Kalyanasundaram*

University Botany Laboratory, Madras-5

With one plate

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Introduction

Most of the reported work pertaining to the spore to spore culture of Myxomycetes has been confined to members of the order Physarales. The first report of the laboratory cultivation of a member of the Stemonitales is perhaps that of Alexopoulos (1959). The work presented here relates to the successful spore to spore culture of three species of Myxomycetes representing the orders Physarales, Stemonitales and Trichiales respectively; the emphasis being on the morphology of the plasmodium. Detailed studies on the pre-plasmodial stages, which are in progress, will be reported later.

Materials and Methods

The following species were chosen for the present study:

1. *Physarum compressum* Alb. et Schw. (Order Physarales) collected from dead, felled branches of *Erythrina indica* at Pollibetta, Coorg, in May 1962.

2. *Stemonitis herbatica* Peck (Order Stemonitales) collected from sandy garden soil, Rajah Annamalaipuram, Madras, in September 1961.

3. *Arcyria cinerea* (Bull.) Pers. (Order Trichiales) collected from the bark of dead, felled branches of *Terminalia* sp. at Pollibetta, Coorg, in May 1962.

Two types of media were used i. e., 4% oatmeal agar and 3.5% carrot decoction agar. Petri dishes of 10 cm diameter and 250 ml Erlenmeyer flasks were used for culturing. For detailed microscopic observation of the plasmodia, the latter were grown on glass slides kept in Petri dishes under water, and fed with pulverized oats (McManus, 1961).

Spore suspensions were made in sterile tap water and seeded on the agar surface in Petri dishes and Erlenmeyer flasks under sterile conditions

by means of a nichrome wire loop. Subculturing was done at intervals of one month or less. For glass-slide cultivation, a portion of the agar bearing the plasmodium was cut out and transferred to the glass slide. The cultures were incubated in a moist chamber at 22 °C (± 2 °C), as the room temperature was found to fluctuate widely, the maximum being above 30 °C during a greater part of the year. Such high temperatures were found unsuitable for plasmodial growth.

Results

Plasmodia appeared in all the cultures: the plasmodial reticulum of *Physarum* becoming apparent on the agar surface in about a week, that of *Stemonitis* in two weeks and that of *Arcyria*, in three weeks after inoculation.

The Plasmodium

Gross Morphology

Physarum compressum has a typical physaraceous plasmodium with large, prominent veins and well-defined advancing fans (plate I, fig. 3). It is whitish in colour and forms, on glass, a network with large, angular meshes. The protoplasm is highly granular. When the plasmodium is actively feeding on oatgrains the protoplasmic granules seem to consist almost entirely of oat particles. The bounding limits of the veins in such a plasmodium are obscured by a heavy coating of extruded oat particles which cover the entire length of the veins.

In contrast to this, the plasmodium of *Stemonitis herbatica*, when growing on glass, is barely visible to the naked eye except as a few long, whitish strands. Microscopic examination, however, reveals the presence of an exquisitely fine network of delicate veinlets in between these strands (plate I, fig. 2). There are no definite fans. The veinlets bear small, finger-like processes scattered all over them and usually clustered near the axils (plate I, fig. 1). The protoplasm is transparent, and not granular as in *Physarum* except when the plasmodium is actively feeding on oats, during which time the veins are filled with granules of oat. However, there is no coating of these on the veins.

The plasmodium of *Arcyria cinerea* shows characteristics intermediate between the two types described above. The veins are not as prominent as those of *Physarum*, though larger than those of *Stemonitis*. The reticulum is also intermediate between the two types in its intricacy, and possesses advancing fans as in *Physarum*. Slender finger-like processes similar to those of *Stemonitis* occur scattered over the smaller veinlets. The proto-

plasm has greater resemblance to that of *Stemonitis* in that it is not very granular, except while feeding.

Protoplasmic Streaming

Streaming is vigorous in *Physarum*, and involves only a small central portion of the protoplasm of the veins, the thick outer layer remaining static. This outer layer and the flowing stream (which, for the sake of convenience, will henceforth be referred to as the endoplasm) appear equally granular. In a single vein, the time taken for streaming in one direction is usually longer than in the other, and the pause before change of direction of flow is very brief. The longest pause observed did not exceed 15 seconds.

In *Stemonitis*, streaming involves almost the entire protoplasm of the vein, the bounding layer being exceedingly thin. While streaming goes on fairly regularly in the larger veins, the smaller veinlets appear static most of the time; when streaming does occur in these, it is so slow as to be hardly discernible. In the larger veins, streaming is characterised by the long pauses made by the protoplasm before it changes its direction, the long pauses usually being confined to one side. The longest pause observed extended to 125 seconds, and this is in strong contrast to the brief pauses observed in *Physarum*.

In the plasmodium of *Arcyria*, almost the entire protoplasm in the veins is involved in the streaming movements, and in this respect it resembles *Stemonitis*. The pause before the change of direction is brief as in *Physarum*, except in a few cases when it might extend up to 40 seconds.

Formation of the Reticulum

The plasmodial reticulum develops in two ways in *Physarum*. In the first, clear transparent areas appear in the continuous sheet of protoplasm of an advancing fan (plate I, fig. 3, h). These apparently are formed by the pressing down of the transparent upper limiting layer of protoplasm over the lower, and the consequent movement of the granular endoplasm away from these regions. The margins of these hyaline areas, bounded by the endoplasmic granules of the surrounding regions, are irregular and continuously change their shape as the granules move about during protoplasmic streaming. Eventually, perforations appear in the centre of these areas and enlarge gradually, forming a definite margin (plate I, fig. 3, p). The margin of the perforation is apparently formed by the coalescence of the upper and lower bounding layers of protoplasm. Considerable time, up to an hour or more, may elapse between the formation of a hyaline area and the appearance of the perforation. With the continued enlargement of

two adjacent perforations, the protoplasmic strand between them becomes organised into a regular vein. The second method i. e., branching and anastomosing, is not very common to this plasmodium and occurs when the advancing fan is very small. The fan gives rise to one or two stout processes, which elongate, branch and anastomose with already existing veins. Like the previous one, this process also is extremely slow.

In *Stemonitis*, the plasmodial reticulum forms by the second method described for *Physarum*, though differing from it in certain details. There are no fans, and the growing points are confined to the dilated, vesiculose apices of long veins (plate I, fig. 1). These give rise to numerous long, slender finger-like processes (pseudopods) which elongate, branch and anastomose. The apices of these pseudopods are very hyaline and transparent, and the protoplasm at their base appears granular in contrast.

Explanation to plate I

Figure 1

Apical region of a marginal vein of the living plasmodium of *Stemonitis herbatica* in glass slide culture, showing the finger-like processes (p). Ca. x 800

Figure 2

Portion of the live plasmodial reticulum of *Stemonitis herbatica* in glass slide culture. Ca. x 240

Figure 3

Portion of an advancing fan of the living plasmodium of *Physarum compressum* in glass slide culture; note the perforation (p) which has not yet extended up to the limits of the hyaline area (h). Ca. x 800

Figure 4

Agar culture of *Arcyria cinerea* showing the sporangia; note the plasmodial reticulum spreading on the glass surface of the culture flask in the foreground. Ca. x 4

Figure 5

Pre-fructification stage of *Stemonitis herbatica* on agar in a test tube; note the coralloid formation. Ca. x 4

Figure 6

Two sporangial clusters of *Stemonitis herbatica* on the glass surface of a test tube, away from the agar (a). Ca. x 4

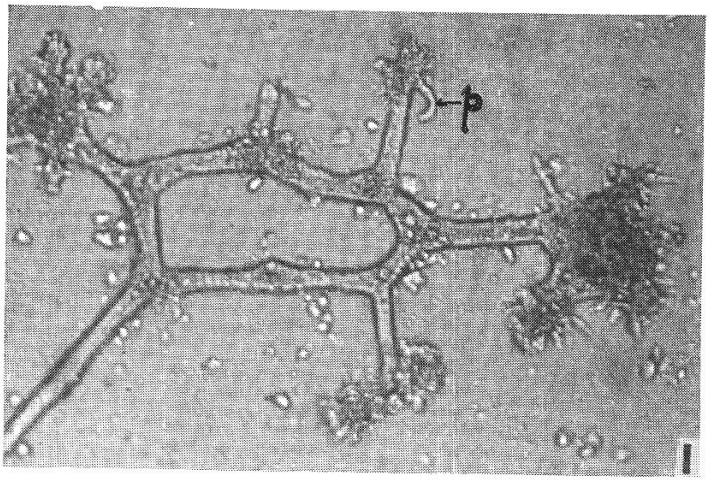
Figure 7

Typical sporangia of *Physarum compressum* formed on the cover of a Petri dish, along with remnants of the plasmodial reticulum. Natural size

Figure 8

Atypical, peltate sporangia of *Physarum compressum* on agar surface in a Petri dish. Ca.x 3

Plate I



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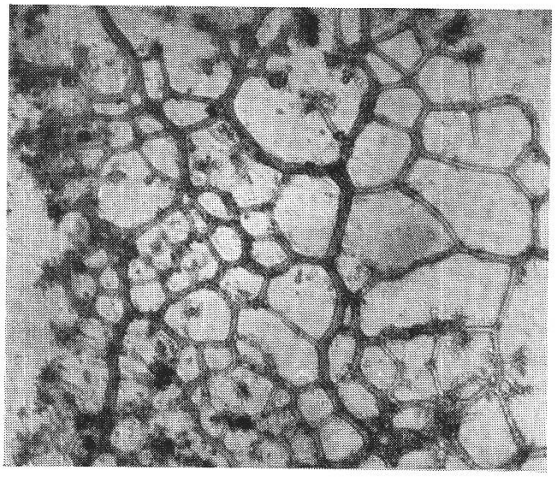

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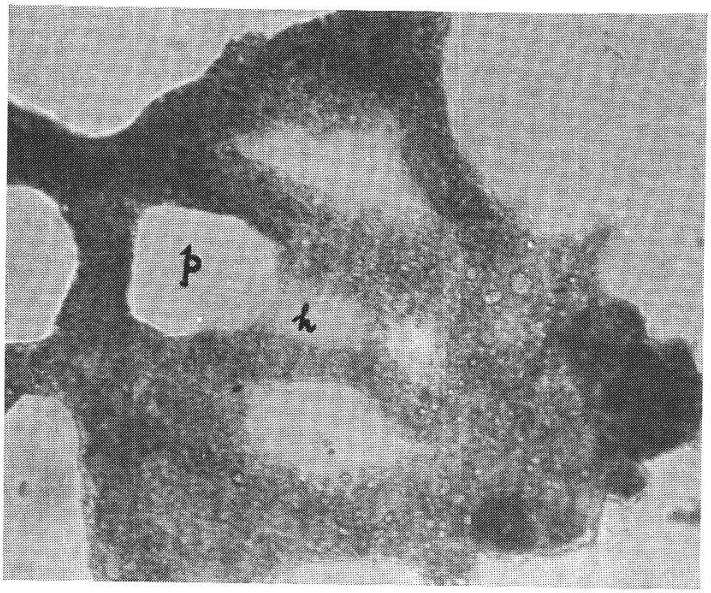

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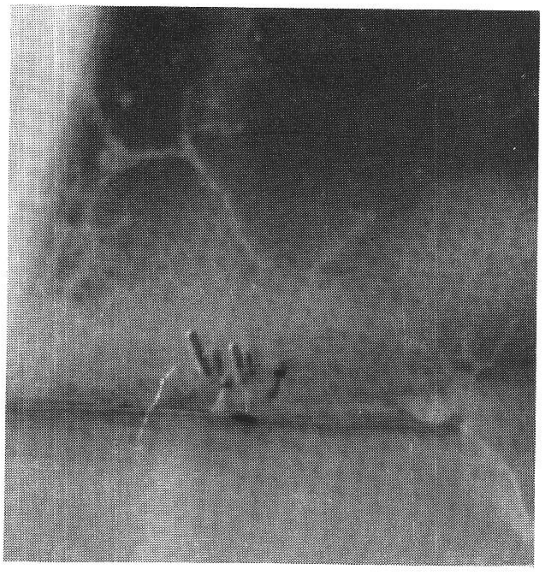

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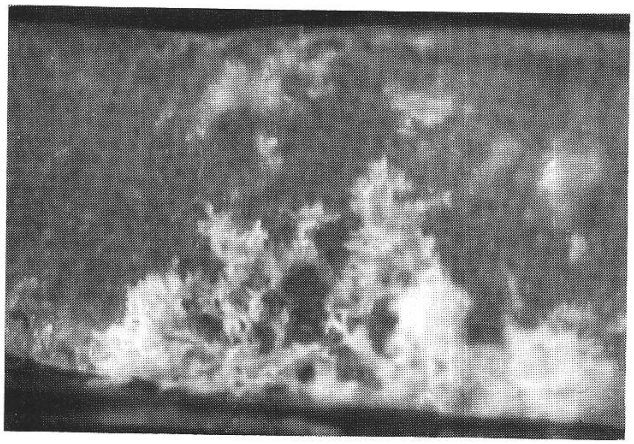

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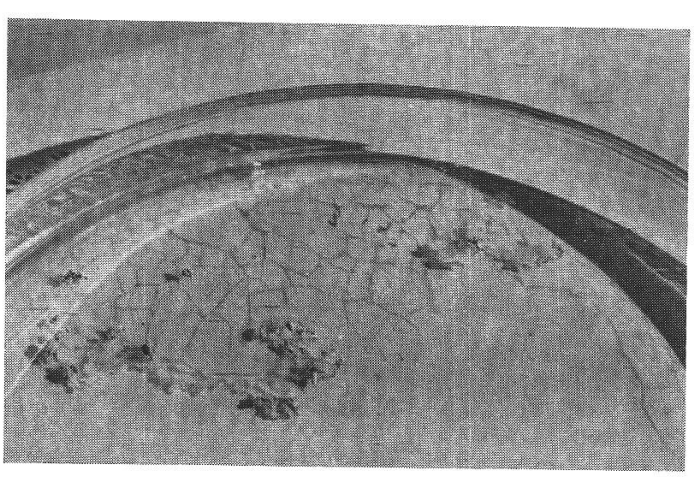

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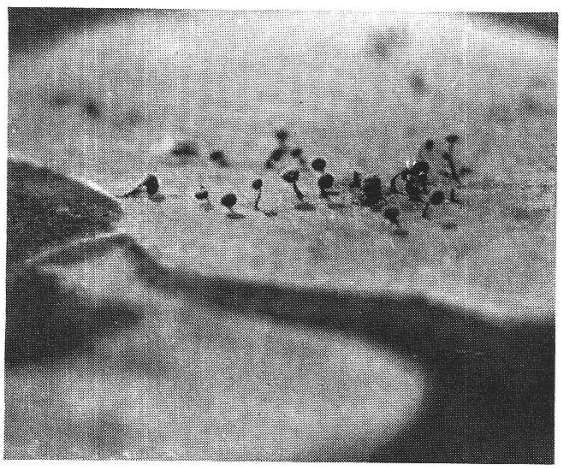

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Anastomosis occurs in two ways: a pseudopod may either grow directly towards another and fuse with it, or it may keep swinging to and fro until the tip contacts another, and then fuse with it. The entire process is accomplished within a few minutes.

The plasmodium of *Arcyria* has large fans, which develop the reticulum as they advance. The pattern of formation of the reticulum conforms to the first method described for *Physarum*, but differs from it in two ways: firstly, the appearance of the perforations is spontaneous and sudden, and is not preceded by the formation of a hyaline area; secondly, the appearance, enlargement and coalescence of the perforations is rapidly completed in a few minutes' time. As the reticulum develops, withdrawal of smaller veinlets into adjacent veins on either side occurs continuously, with the result that the meshes of the network increase progressively in size from the anterior to the posterior region of an advancing fan.

Colour of the Plasmodium

It was found that in all the species the plasmodium directly obtained from spores was yellow in colour on oat agar, and white or transparent on carrot agar. After repeated subculturing, however, it appeared white or transparent on both the media in all species except *Physarum*, in which the plasmodium was often brownish on oat agar. Bacterial and fungal contaminants could not be altogether eliminated from the cultures.

Fructification

Fruiting occurred in some of the cultures of all the three species within a month after sowing the spores. The first sporangia of *Physarum* appeared in 27 days, those of *Stemonitis* in 31 days and those of *Arcyria*, in 25 days after seeding of the spores. In subsequent cultures obtained by transferring plasmodial fragments, the time interval between inoculation and fruiting varied from 18 to 30 days in *Physarum*, and 25 to 30 days in *Stemonitis*. In *Arcyria*, fruiting was observed only once, in a culture directly obtained from spores (plate I, fig. 4). Cultures obtained by transferring the plasmodium have not yielded sporangia till now.

Fruiting was preceded in *Physarum* by the condensation of the plasmodium into dense, white strands which later formed knots and eventually gave rise to scattered groups of laterally compressed sporangia (plate I, fig. 7). In *Stemonitis*, delicate ivory-white or light yellow coralloid masses appeared (plate I, fig. 5) which, after migrating all over the agar surface for two to four days, settled down, usually on plain glass surface, and produced clusters of sporangia (plate I, fig. 6). The sporangia and spores of all

the three species obtained in culture were similar to those occurring in nature, except for a few variations in *Physarum:* here, the sporangia grown in culture were sometimes discoid and peltate (plate I, fig. 8), instead of being laterally compressed and reniform. The stipe was sometimes extremely short or almost lacking. The spores obtained in culture were slightly larger ([7.5–8.0] 8.0 μ) than those found in nature ([7.0–7.5] 7.0 μ), and less spinulose. Details of the morphogenesis of the sporangium are yet to be investigated.

Discussion

Alexopoulos (1960) has classified the plasmodia of Myxomycetes into three distinct morphological types: 1. the "phaneroplasmodium", characterised by a three-dimensional structure, a reticulum with large veins, fan-shaped margins and granular protoplasm, and the confinement of streaming movement to an inner core of protoplasm in the veins; 2. the "aphanoplasmodium", characterised by its flat structure, a uniform reticulum with slender hypha-like strands, absence of advancing fans, transparent homogenous protoplasm, and the involvement of almost the entire protoplasm in the veins in the streaming movement; 3. the "protoplasmodium", which appears to represent a permanent retention of juvenile plasmodial characteristics. He points out that all the physaraceous species studied possess phaneroplasmodia and all the stemonitoid species, aphanoplasmodia while species producing the protoplasmodium form a heterogenous assemblage. In the present study, *Physarum compressum* has been found to have a phaneroplasmodium and *Stemonitis herbatica*, an aphanoplasmodium, conforming to the arrangement of these two species in their respective groups by Alexopoulos (1960). The plasmodium of *Arcyria cinerea* is intermediate between these two types, confirming a similar view expressed by Alexopoulos (1960).

The observations of Alexopoulos have been supported by McManus and Taylor (1961). These authors state that, while the blunt extensions of a migrating phaneroplasmodium are strongly demarcated from the main mass by their hyaline appearance, no such demarcation is seen in the filiform extensions of the aphanoplasmodium where the entire protoplasm is nearly hyaline. In the present study it has been observed that the filiform extensions (pseudopods) of the plasmodium of *Stemonitis herbatica* are always hyaline from the apex down to half their length. The basal half is filled with slightly granular endoplasm which is strongly demarcated, as can be readily seen by cutting out the light. Only during the forward streaming of protoplasm, or when the tip of the pseudopod contacts another and proceeds to fuse with it, does the endoplasm flow into and fill up the hyaline regions.

Alexopoulos (1960) indicates a physiological difference between the different types of plasmodia, stating that the phaneroplasmodium favours drier conditions while the aphanoplasmodium and the plasmodium of *Arcyria cinerea* develop only under water. In the present work, however, all the three plasmodia have been successfully grown under identical conditions both on agar surface and on glass under water.

It has been observed that the reversible protoplasmic streaming is rapid, vigorous and rhythmic in the physaraceous plasmodium whereas in the stemonitoid plasmodium it may at times be very slow (Alexopoulos, 1960). The present observation indicates that in *Stemonitis herbatica* the streaming movement is at times not only slow, but stops completely for long periods before it changes its direction. After this change the protoplasm pauses at the other end only for a brief period.

The mode of formation of the plasmodial reticulum in *Physarum compressum* is essentially similar in detail to the process described by Camp (1937) for *P. polycephalum*, while the process in *Arcyria cinerea* shows slight variations. In *Stemonitis herbatica* the reticulum develops by branching at the growing point followed by anastomosis of branches. That such a formation could occur in the aphanoplasmodium has already been suggested by Alexopoulos (1960).

Fruiting occurs after a varying developmental rhythm. The conditions leading to fruiting are yet to be investigated. The coralloid structures preceding fructification in *Stemonitis herbatica* are similar to those described by Alexopoulos (1959) for *S. flavogenita* and by McManus (1961) for *S. fusca*.

Summary

Physarum compressum Alb. et Schw., *Stemonitis herbatica* Peck, and *Arcyria cinerea* (Bull.) Pers. have been cultured from spore to spore on agar media.

All the three species have non-pigmented plasmodia. The plasmodium of *Physarum compressum* conforms to the "phaneroplasmodium" type of Alexopoulos (1960) and that of *Stemonitis herbatica* to the "aphanoplasmodium" type, while the plasmodium of *Arcyria cinerea* shows intermediate characteristics between these two types.

The plasmodial network is formed by the appearance and enlargement of perforations in *Arcyria cinerea*, by branching and anastomosis in *Stemonitis herbatica*, and by both ways in *Physarum compressum*.

Fruiting occurs in all the three species after a developmental rhythm varying from 20 to 35 days.

Zusammenfassung

Die Schleimpilze *Physarum compressum* Alb. et Schw., *Stemonitis herbatica* Peck und *Arcyria cinerea* (Bull.) Pers. konnten kultiviert und zum Sporulieren gebracht werden.

Alle drei Arten haben nichtpigmentierte Plasmodien. Das Plasmodium von *Physarum compressum* stimmt mit dem «Phaneroplasmodium»-Typ (Alexopoulos, 1960) überein, dasjenige von *Stemonitis herbatica* mit dem «Aphanoplasmodium»-Typ, während das Plasmodium von *Arcyria cinerea* eine Zwischenstellung einnimmt.

Das plasmodiale Netz wird bei *Arcyria cinerea* durch sich vergrössernde Löcher in der Plasmagrundmasse gebildet, bei *Stemonitis herbatica* durch Verzweigung und Anastomisierung, während bei *Physarum compressum* beide Möglichkeiten beobachtet werden können.

Alle drei Arten fruktifizieren nach einem bestimmten Entwicklungsrhythmus von 20 bis 35 Tagen Dauer.

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