IN VITRO CULTIVATION OF *DIACHEA SPLENDENS* PECK

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THE difficulties of culturing Myxomycetous species in the laboratory are well known. One of the reasons for repeated failures to obtain cultures from spores is perhaps the rapid loss of viability of spores in storage. Up to the year 1963,[1] only about 30 species are listed as having been grown from spore to spore even in crude cultures, and subsequent additions to this list are relatively few.[2]

In an attempt to obtain plasmodia for study in culture, spores of several species of Myxomycetes were separately seeded on agar surface (3% carrot decoction agar and 3% oatmeal agar) in Petri dishes and test-tubes. Of these, the following species developed plasmodia and eventually fruited in culture :

Physarum cinereum (Batsch.) Pers.,
P. vernum Fries,
P. gyrosum Rost.,
P. serpula Morgan,
Diachea splendens Peck.

Except for *P. gyrosum*[1] this is, as far as the author is aware, the first report of *in vitro* cultivation of these species. Of these, *P. serpula* and *D. splendens* could not be maintained for long in the plasmodial state as they fruited too rapidly. The remaining species are being studied in detail. An account of the culturing of *D. splendens* alone is presented here in some detail because no one seems so far to have succeeded in culturing any species of the interesting genus *Diachea*.[3]

The spores of *D. splendens* used here were from sporangia collected in nature on dead twigs and on stems and leaves of some living herbaceous plants, beneath a hedge in the University Botany Laboratory Campus, Madras. in December 1963 (Herbarium MUBL/PUI-62). Sporangia showed the typical structure of the species but had very short stipes.

When seeded on agar, plasmodia of this species appeared after about 3 weeks, and on subsequent transfer to oat agar and carrot decoction agar in 250 ml. Erlenmeyer flasks, they fruited regularly in about 2 to 3 weeks after transfer. Unlike in the other species, fruiting occurred before the plasmodium attained considerable size, and the entire plasmodium seemed to be used up in the process.

From the limited observations made, it appeared that the plasmodium of *D. splendens* was delicate, being composed of slender veins and small advancing fans. While it grew well on oat agar, often piercing into the agar and growing beneath the surface, fruiting was observed more regularly on carrot decoction agar. The fructifications usually appeared on the sides of the culture flask (Fig. 1).

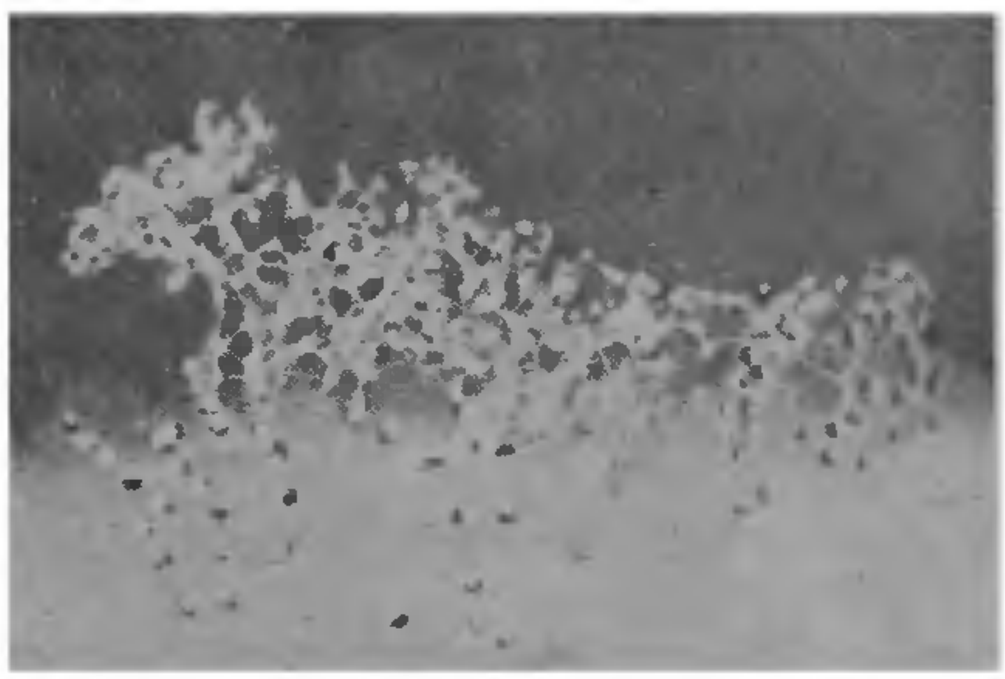

FIG. 1. Colony of fructifications of *Diachea splendens* on the sides of the culture flask, away from the agar surface (white region in the background) *c.* × 3·5.

The fructifications obtained in culture differed in many ways from those collected in nature. They were smaller in size, usually measuring 0·25 or 0·3 mm. in diameter against those in nature which measured about 0·5 mm. Often they were sessile, being grouped on the thick calcareous hypothallus. In most cases, capillitium was poorly developed.

Spores that developed in culture were highly variable in size, being 8 to 18 μ unlike those in nature which had a fairly constant diameter of 10 μ. However, most of the spores were within a narrow range of 9·5 to 11·0 μ, and the average was close to that in nature, being 10·5 μ. The most striking difference was in spore-marking. The spores in nature were marked with coarse ridges arranged in a crude reticulum. But the reticulate arrangement was not evident in the spores developed in culture which bore crude, sparsely distributed warts or spines which were occasionally united into irregular ridges.

Discussion

In view of Alexopoulos' finding[1] that members of the Physarales have a 'phaneroplasmo-

dium' and those of the Stemonitales, an 'aphanoplasmodium', it was of interest to find out the nature of the plasmodium in *Diachea* which forms a connecting link between these two orders. In so far as the rapid fruiting of the cultures permitted only a limited observation of the plasmodia, no definite statement can be made regarding the type of plasmodium in this genus. From chance observations of delicate fans and slender veins it may be suggested that the plasmodium of *D. splendens* is perhaps an intermediate type between the phanero- and aphanoplasmodia, such as that found in *Arcyria cinerea*.[4,5]

Morphological variations produced in culture, such as formation of sessile sporangia, imperfect development of the capillitium and formation of monstrous spores can perhaps be attributed to excessive moisture in the culture flasks, as it has been frequently observed that excessive moisture at the time of fruiting usually produces such irregularities.

The stipitate or sessile nature of the sporangia is sometimes used as a criterion for roughly separating the species *D. splendens* and *D. subsessilis*, the latter species tending to produce nearly sessile sporangia whereas the former is said to be prominently stipitate.[6,7] It is borne out from the present account that stipe length is a variable feature and *D. splendens* produces stipitate as well as sessile sporangia. The distinguishing feature is the ornamentation of the spore wall, as has also been pointed out by Hagelstein.[8] This feature has evolved into a well-developed, delicate reticulation in *D. subsessilis*, whereas in *D. splendens* it shows a primitive reticulation crudely formed by ridges which in turn have developed from a union of coarse warts and spines that were originally perhaps scattered, and to which state the species seems to revert under certain conditions. Such a state is perhaps common in nature, along with an intergrading series towards the reticulate form, as the spore ornamentation in this species is variously described as very coarsely warted,[6] marked with raised bands and tubercles,[9] marked with stout scattered protuberances often confluent to form ridges,[8] etc.

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CATALASE IN ACTIVATED SLUDGE

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INTRODUCTION

SINCE the early years of the scientific study of sewage and sludges, attention has been devoted mostly to the practical aspects of their treatment and utilization. Comparatively very little work has been done on the fundamental aspects of sewage purification. One of the aspects of this biological process, on which the evidence is very meagre, is the enzymes in sewage and sludges. The available information on this aspect relates mostly to the occurrence of some hydrolytic and oxidative enzymes in the slime on the material in the sewage filter,[1,2] in sewage and effluents,[3-7] in the iron bacterium M7[8] and other bacteria,[9-11] in activated sludge,[12-16] in anaerobic sludge[17] and in the sludge from synthetic media.[18] No attempt has, however, been made to extract and isolate the enzymes from even the rapidly purifying system of activated sludge and to study their relation to the aerobic process of purification.