Growth of Green Algae with Myxomycete Plasmodia

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Growth of Green Algae with Myxomycete Plasmodia[1]

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ABSTRACT: Myxomycete plasmodia of several species, previously freed from bacterial contaminants, were grown on oat agar. To them were added pure cultures of 10 spp. of green algae. Three species of *Chlorella* were able to enter into full associations with *Physarum didermoides* and *Fuligo cinerea,* forming green plasmodia in which the algae multiplied in light.

Although many attempts have been made to grow myxomycete plasmodia when freed from bacteria, only a few have been successful (Cohen, 1939 and 1941; Hok, 1954; Sobels, 1950). Since 1958 I have successfully grown plasmodia under such conditions (Lazo, 1960). It has been demonstrated that most plasmodia grow slowly and with difficulty in this condition, although the plasmodium of *Physarum polycephalum* is a notable exception to this general rule. Since many plasmodia occur in nature in close association with algae, it was thought that algae might possibly serve the same purpose as do bacteria in 2-membered cultures.

MATERIAL AND METHODS

The plasmodia were grown on sterile oat agar consisting of oat flakes covered with non-nutrient agar to hold the flakes in place. They were freed from bacteria by various methods (antibiotics, low pH in the culture medium, migration). The sterility was thoroughly checked by seeding pieces of plasmodia in A-C Difco broth, brain-heart infusion, peptone-glucose broth, A-C agar, brain-heart agar. The plasmodia samples that were tested were taken, not from the advancing fronts of the plasmodia, which are almost always free from bacteria, but from immediately above the oats because this gives the best conditions for the growth of possible contaminants. The samples were seeded in the broth tubes and kept in the incubator at 25° C for ten days. Then, a sample of this broth was seeded in agar plates and again in broth tubes and kept in the incubator for an additional ten days.

The bacterium-free plasmodia were grown in pure 1.5 per cent agar and oats at a temperature of 25° C.

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The plasmodia used were those of *Physarum didermoides, P. polycephalum, P. gyrosum, Fuligo septica, F. cinerea.* Later some plasmodia were used that were contaminated with bacteria: *P. pusillum, P. compressum, Didymium iridis, D. squamulosum, D. minus.*

The algae used were *Euglena gracilis, Chlorella paramoecium, C. protothecoides, C. xanthella, C. saccharophyla, C. vulgaris 259, C. pyrenoidosa, C. luteo-viridis, C. zopfingiensis, C. ellipsoidea,* all in pure culture.

The plasmodia were seeded in the oat-agar plates and allowed to grow for three to five days. Then a small portion of each plasmodium was inoculated with one drop of the algal cells on the surface of the plasmodium. After inoculation the culture plates were placed in the incubator under fluorescent lamps at a constant temperature of 25° C.

RESULTS

The algae were incorporated in the bacterium-free plasmodia of *P. didermoides* and of *F. cinerea* in several trials, but were not successfully incorporated in those of any other species. All but one of the plasmodia which had not been freed from bacteria failed to incorporate the algae; the exception was *P. didermoides.* The bacterium-free plasmodia of *P. polycephalum, P. gyrosum* and *F. septica* appear to take the algal cells and digest them, but the plasmodia do not become green. On the other hand, after three or four days the white plasmodia of *P. didermoides* and *F. cinerea* appeared green in color and under a microscope the algal cells could be seen moving with the protoplasm within the plasmodial veins. Later, the algae were deposited in the walls of the veins and, with the walls, formed a green crust which, when the plasmodium became old and dried, usually cracked. The veins then appeared dry and white.

Summarizing these results, of the species of algae tested only three, *C. protothecoides, C. xanthella,* and *C. ellipsoidea,* were able to enter into full association with two species of myxomycetes tested, *P. didermoides* and *F. cinerea.* There was, in addition, some slight evidence of temporary association between these species and *C. saccharophyla* and *P. didermoides,* but it failed to persist.

Results were negative in all trials with *P. polycephalum, P. gyrosum, P. pusillum, P. compressum, F. septica, D. iridis, D. minus* and *D. squamulosum.*

If transfers are made from a green section of the plasmodium the new plasmodia will be green also. A green plasmodium may even be obtained from a white one, provided the white one has a few algal cells but an insufficient number to make it appear green at the time of the transfer. On the other hand, if a green plasmodium is kept in darkness, one part of it may become white if in the migration process that part loses all the algae.

The association plasmodium-algae grows much better than the plasmodium alone and is much more resistant to acidity than is the plasmodium in pure culture. *P. didermoides* and *F. cinerea* associated

with *C. protothecoides* or *C. xanthella* were able to grow in a medium buffered at pH 4.5 (McIlraine, 1921). In both cases the plasmodia without algae grew only slightly or not at all at that pH.

Myxomycetes which have been purified from bacteria are rarely able to fruit. *Physarum polycephalum* is exceptional in this respect, as in many others. However, by the use of streptomycin I obtained a strain of *P. polycephalum* that never fruited unless it became contaminated. In the case of *F. cinerea* and *Chlorella,* fruiting can be obtained in about two or three weeks. The control plates do not fruit.

The bacterium-free plasmodia of *P. didermoides* do not fruit either associated with algae or alone.

The spores of *F. cinerea,* even when many appear abnormal, are viable; I have obtained plasmodia from them on a few occasions in bacterium-free condition. Algal cells were present.

DISCUSSION

It is premature to say that a symbiosis has been obtained. It does seem that some algae may function in the same way as do bacteria with some of the Myxomycetes, and in the case of *F. cinerea* they may help the fruiting process. It is not yet clear why the algae can grow on the membranes of some plasmodia and not on those of others.

Pinoy (1907) and Skupienski (1928) postulated that Myxomycetes could be considered as symbiotic organisms with bacteria. It is now clear that bacteria, at least, not only make possible the vegetative growth of the plasmodia, but even contribute to completion of the life cycle. The results here reported seem to demonstrate that some algae may replace bacteria, functioning in the same way.

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