STUDIES IN CELLULAR SLIME MOULDS FROM INDIAN SOILS

II. On the Occurrence of an Aberrant Strain of *Polysphondylium violaceum* Bref., with a Discussion on the Relevance of Mode of Branching of the Sorocarp as a Criterion for Classifying Members of Dictyosteliaceae

BY J. N. Rai and J. P. Tewari

(Microbiology Laboratory, Botany Department, Lucknow University, Lucknow, India)

Received October 23, 1962

(Communicated by Dr. S. N. Das-Gupta, F.A.Sc.)

INTRODUCTION

Agnihothrudu (1956) and Rai and Tewari (1960, 1961) reported about seven species of Acrasieae from Indian soils and rhizosphere of plants. Since the publication of the first paper of this series (Rai and Tewari, 1961), a large number of strains of cellular slime moulds have been under study and an aberrant strain of *Polysphondylium violaceum* Bref. showing irregular branching forms the subject-matter of this paper.

MATERIALS AND METHODS

The aberrant strain of *P. violaceum* was isolated from soil (pH 6.8) collected from Pallia, District Kheri, India, using Hay-infusion agar and small crumbs of soils as inocula (Raper, 1951). It was obtained in pure-mixed culture by the streak-method of Raper (1951). The organism has been studied in two-membered cultures with *Escherichia coli* on Hay-infusion agar (Raper, 1951), Non-nutrient agar (Singh, 1946, 1947), 0.1% Lactose-peptone agar and 0.1% Lactose-yeast agar (Blaskovics and Raper, 1957). The pH of media was adjusted between 6.5-7.0 and cultures were incubated at 22 and 25°C.

OBSERVATIONS

A short taxonomic description of the aberrant strain of *P. violaceum* based on the study of the culture grown on 0.1% Lactose-peptone agar in association with *E. coli* and incubated at 25°C. in dark is given below.

Sorocarps (Figs. 1-5, 8, 9) extremely variable in size, stem composed of 1-5 cells in thickness, each bearing a large terminal purple
sorus generally up to $296.0 \mu$ in diameter and large number of lateral branches up to $222.0 \mu$ in length disposed in an irregular fashion, a very small number of rather diminutive sorocarps showing regular whorled branching, each lateral branch bearing a small purple sorus up to $185.0 \mu$ in diameter. Spores (Fig. 6) suspended in a droplet of slime, guttulate, light purple oval to rod-shaped, sometimes slightly bent, $3.2-8.0 \mu \times 1.6-3.2 \mu$. Pseudoplasmodium initially large and wheel-like, in later stages showing rather prolonged stalked-migration up to a distance of even $2.0 \text{ cm.}$ or so (Figs. 10, 11) elongating up to about $5.0 \text{ mm.}$ just before culmination and then breaking into sub-sorocarps to give rise to the lateral branches. Microcysts (Fig. 7) produced in abundance in slide cultures, spherical, unicellular, thinwalled, hyaline, $4.8-8.0 \mu$ in diameter.
The sorocarps are strongly phototropic and in presence of light the period of stalked-migration is greatly enhanced resulting in the formation of sorocarps with large terminal sori and scanty lateral branches.

The aberrant strain differs from the normal strain (Figs. 12-14) of *P. violaceum* mainly in having stouter irregularly branched sorocarps and larger pseudoplasmodia in stalked-migration stage.

**DISCUSSION**

The family Dictyosteliaceae comprises two genera, *Dictyostelium* and *Polysphondylium*, the distinction being based mainly on the character of the sorocarp. *Dictyostelium* has unbranched or sparingly branched sorocarps, while *Polysphondylium* has branched sorocarps with branches oriented in regular verticils. Olive (1902) did not consider these differences "great enough to warrant the retention of the two forms (i.e., *Dictyostelium* and *Polysphondylium*) as distinct genera". Bonner (1959), rather in a roundabout way supporting the views of Olive (1902), noted that *Polysphondylium* and *Dictyostelium polycephalum* "might be closely allied". He pointed out that if a mutant of *Polysphondylium* arose that lacked the ability of stalk formation during migration, the immediate result might be *Dictyostelium polycephalum* and that it would be similar to a parallel mutant of *D. mucoroides* giving rise to *D. discoideum*. Filosa (1958) discovered a mutant of *D. mucoroides* which showed stalkless migration under certain cultural conditions. This mutant, however, lacked the basal disc characteristic of *D. discoideum*. However, to what extent such biological structures (e.g., the disc) are "genetically determined" has yet to be properly evaluated (Bonner, 1959). Raper and Thom (1941) and Bonner (1959) reported short periodic interruptions in the stalk formation process of *D. purpureum* and *D. mucoroides* and reported that under certain cultural conditions they were exaggerated.

Raper (1951) noted that "Dictyostelia with white sori and flexuous sorophores are so variable and so commonplace as to preclude anything approaching a precise definition of this species (e.g., *D. mucoroides*)". He noted that Brefeld (1869) "either through wisdom or neglect" did not specify any range of dimensions for mature sorocarps of *D. mucoroides*. In the light of the works of Potts (1902), Olive (1901, 1902), Pinoy (1907), Singh (1947) and his own isolates approximating *D. brevicaule* and others producing irregularly branched sorocarps with yellow to yellowish sorophores bearing milk-white sori, Raper (1951) thought it "prudent to adopt a broad concept of *D. mucoroides*, and to regard this name as applicable to a series of variant types rather than attempt to limit its use to a few strains which generally
produce sorocarps in a particular size range". These forms have been grouped together in "D. mucoroides complex for want of specific criteria upon which to base specific diagnoses" (Raper, 1951; Sussman, 1956; Raper, personal communication).

Professor Raper who kindly examined the strain under study remarked (personal communication) that it "should be assigned to the species Polysphondylium violaceum Brefeld. It is quite irregular in its branching habits under most cultural conditions but we do see enough definite whorls to indicate that this is where it belongs and that it represents probably an aberrant form rather than a new species". He has also noticed irregularly branched strains with white and purple sori. Somewhat similar relatively unbranched forms of Polysphondylium with intermediate morphology have also been isolated by Prof. Cohen (personal communication). The provisional species Dictyostelium polystelium Cohen (Cohen, 1953) has been described to be branched shrubby and with some characteristics of Polysphondylium. This would also thus appear to bridge the gap between these two genera of Dictyosteliaceae.

It is well known that these simple slime molds are extremely sensitive to culture conditions. As a practical criterion Prof. Cohen accepts (personal communication) the "form which is given on agar at pH 6 with minimal nutrients, so that the accumulation of metabolites would have the least disturbing effect". He feels that practically all Acrasieae have a "normal" form as compared to the forms which may be assumed on more concentrated or alkaline media. Raper is of the view that "the patterns achieved by sorocarps in these slime molds are so extremely sensitive to the conditions under which the slime molds are cultivated" that he tends calling "anything Polysphondylium that produces a well-defined whorl under any condition" that can be achieved.

In the light of the above discussion, it is clear that members of Dictyosteliaceae deserve a thorough taxonomic re-evaluation as to mode of branching of the sorocarp and other sorophore characters as criteria for classifying genera of this family.

**Summary**

An aberrant strain of Polysphondylium violaceum Bref. showing irregular branching is described. The need for re-evaluating mode of branching of the sorocarp as a criterion for classifying genera of Dictyosteliaceae has been discussed.
ADDENDUM

Since this paper has been sent to press Huffman and Olive (1963) have described an interesting morphogenetic variant of Dictyostelium mucoroides. The strain described had aggregation centres giving rise to several sorogens and these sorogens, during culmination, often showed fusion with one another, a condition simulating to those found in D. polycephalum. Curiously enough, generally the culminating mass separated into two parts, the apical mass giving rise to the terminal sorus and the extensive basal mass, often splitting into two or more pieces, gave rise to the lateral branches. The mature fructification was thus Polysphondylium-like, except for the lateral branches which tended to be irregularly arranged. The strain showed strong compatibility with typical strains of Dictyostelium mucoroides. It is likely that the change in this strain may be similar to that speculated in transition from D. mucoroides to Polyspondylium and "that this strain represents an important phylogenetic link in the Dictyosteliaceae". Huffman and Olive (1963) also remark that P. violaceum "may have evolved from a variant of D. purpureum, with unique features" of their strain. The aberrant strain of Polysphondylium violaceum described in this paper may thus represent such a variant and lend added support to Huffman and Olive's (1963) view that "Dictyostelium mucoroides-purpureum complex represents a pivotal point in the phylogeny of Acrasiales" and may serve as a "genetic pool" providing "considerable degree of plasticity for continuing evolution and adaptation to changing environments". The changes observed in the morphogenetic variant of D. mucoroides (Huffman and Olive, 1963) and the aberrant strain of Polyspondylium violaceum, described in this paper, may both be aimed at attaining better dispersal of the spores.

ACKNOWLEDGEMENT

The authors are grateful to Prof. Kenneth B. Raper of the Wisconsin University and Prof. Arthur L. Cohen of the Oglethorpe University for their most generous help and kind criticism and to the Council of Scientific and Industrial Research, India, for financial assistance.

REFERENCES

Agnihothrudu, V. "Occurrence of Dictyosteliaceae in the Rhizosphere of plants in Southern India," Experientia, 1956, 12, 149.


**EXPLANATION OF PLATE XXVII**

Figs. 8-11. *Polysphondylium violaceum* Brefeld, aberrant strain

Fig. 8. Colony on 0·1% Lactose-yeast agar with *E. coli* at 20°C in dark.

Figs. 9. Sorocarps, ×31·5.

Figs. 10, 11. Stalked-migrating pseudoplasmodia, ×31·5.

Figs. 12-14. *Polysphondylium violaceum* Brefeld, normal strain

Fig. 12. Stalked-migrating pseudoplasmodium showing breaking up into sub-sorocarps ×31·5.

Fig. 13. Stalked-migrating pseudoplasmodium and part of the sorocarp, ×31·5.

Fig. 14. Part of the sorocarp, ×31·5.