ensuite (c'est à ce moment que se développe le sporange)[5]; c) puis la croissance du sporangiophore reprend. Ces résultats confirment toute une série de recherches relatives à la croissance du Phycomyces[5].

Essais 2: Les sporangiophores sont placés horizontalement lorsqu'ils mesurent respectivement 2, 4, 8 ou 16 mm. La Figure 2 (*O*) qui représente les variations de l'angle de courbure (entre l'axe du sporangiophore et l'horizontale; chaque point correspond à la moyenne de 100 mesures) permet les conclusions suivantes: a) tous les sporangiophores présentent un géotropisme négatif; b) les temps de présentation et de réaction sont d'autant plus faibles que les sporangiophores sont plus courts; c) la géosensibilité est donc maximum lorsque les sporangiophores sont dans leur première phase de croissance (avant que les sporanges ne soient formés).

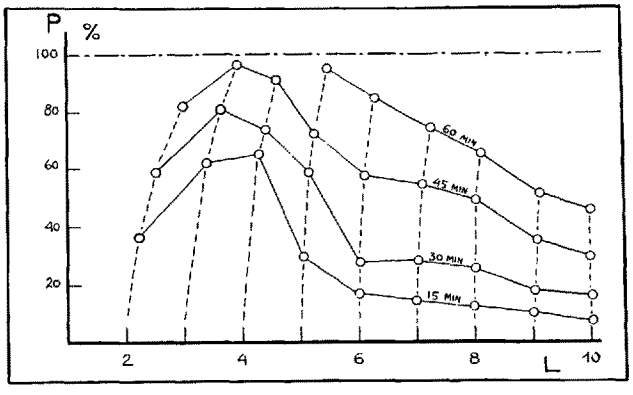

Fig. 3. Nombre de sporangiophores réagissant apogéotropiquement. *P* (%) pour-cent des sporangiophores présentant une courbure supérieure à 5°. *L* longueur des sporangiophores au moment de l'excitation.

Essais 3: Les champignons sont éclairés ½ h (lumière blanche: 60 W, 650 lm, 150 lux; distance: 25 cm; rayons parallèles à l'axe des sporangiophores), juste avant d'être placés horizontalement. Sous l'action de ce prétraitement lumineux (Fig. 2, *L*), les temps de présentation et de réaction sont considérablement accrus. Cette perte de géosensibilité pourrait être interprétée par la photoinactivation des auxines telle qu'elle fut analysée chez quelques plantes supérieures[6].

Essais 4: Des sporangiophores, longs de 2 à 10 mm, sont disposés horizontalement. Toutes les 15 min, on détermine le % des champignons qui ont répondu par une courbure (supérieure à 5°) à l'excitation gravitique. De la Figure 3, qui rend compte de ces essais (chaque point est une moyenne de 120 mesures), on peut tirer les conclusions suivantes: a) les sporangiophores mesurant de 3 à 4 mm sont ceux qui répondent le plus rapidement à l'excitation gravitique; b) pour les sporangiophores plus longs (ceux dont le sporange est déjà formé), le temps de réaction est nettement supérieur.

Ainsi l'apogéotropisme du Phycomyces paraît lié à la croissance de son sporangiophore et à la formation de son sporange. Il est probable que les auxines, qui président au développement du Phycomyces[7], soient responsables des variations de géosensibilité observées.

P. E. Pilet

Kerkhoff Department of Biology, California Institute of Technology, Pasadena et Institut de Botanique, Université de Lausanne, le 4 janvier 1956.

Summary

Under definite conditions of culture, growth of sporangiophore of Phycomyces has been investigated by Shadowgraphic method. During sporange formation, the rate of growth is the smallest (Fig. 1). The apogeotropic sensitivity of sporangiophores is the greatest before sporange formation; presentation and reaction times increase with age and strongly with light pre-treatment (Fig. 2, 3). Auxin could be responsible for apogeotropic reactions of Phycomyces.

[5] G. H. Banbury, J. exp. Bot. *3*, 86 (1952). – E. S. Castle, J. gen. Physiol. *9*, 407 (1927); Amer. J. Bot. *29*, 664 (1942). – M. Grosper, Bot. Zbl. *36*, 414 (1919). – A. N. J. Heyn, Protoplasma *25*, 372 (1936). – L. J. J. Pop, Proc. Acad. Sci., Amsterdam *41*, 661 (1938). – P. A. Roelofsen et W. L. Houwink, Acta bot. Neederland *2*, 218 (1853).

[6] L. Brauner, Naturwissenschaften *40*, 23 (1953). – F. Franck, Thesis, Tübingen (1951). – A. W. Galston, Bot. Rev. *16*, 361 (1950). – P. E. Pilet, Bull. Soc. bot. suisse *60*, 6 (1950); Bull. Soc. vaud. Sci. nat. *65*, 197 (1952).

[7] F. Kögl et B. Verkaaik, Z. physiol. Chem. *280*, 162 (1944). – R. Pohl et W. Rother, Biol. Zbl. *72*, 364 (1953).

Occurrence of Dictyosteliaceae in the Rhizosphere of Plants in Southern India

The Dictyosteliaceae are generally considered to represent a group of coprophilous organisms, since frequently such material represented mainly the source of isolates investigated by many[1]. It was Krzemienewskis[2] who pointed out that *Dictyostelium mucoroides* Brefeld could be obtained from cultivated soils in Poland. Later Raper and Thom[3] isolated the same organism from soils of United States and Singh[4] from soils in England.

There is no report of the occurrence of these organisms in soils of India. In the course of investigations on the rhizosphere microflora of plants the author very frequently observed *D. mucoroides* Brefeld in bacterial plates incubated for a period well over two weeks. With a view to investigate the occurrence of these organisms in soils of South India, 35 soil samples (20 of cultivated and 15 of uncultivated) and rhizosphere soils of 48 species of crop plants and common weeds growing in above soils were analysed. Soil suspensions prepared by agitating 10 gm of soil or 5 gm of root samples in 100 ml of sterile tap water were streaked on ¼ strength of hay infusion and dung infusion agar[5]. In addition to the above media lactose-peptone agar[6] and *Aerobacter* circles[4] were also employed. Root bits of different plants were incubated on all the above media at laboratory temperature (28–34°C) and the observations extended for a period of one month. The number of soils and species of plants yielding different members of Dictyosteliaceae are presented in the Table.

[1] O. Brefeld, Abh. Senckenberg naturf. Ges. Frankfurt *7*, 85 (1869). – F. W. Olive, Proc. Boston Soc. Nat. Hist. *30*, 451 (1902).

[2] H. and S. Krzemieniewski, Acta Soc. bot. Poloniae *4*, 141 (1927).

[3] K. B. Raper and C. Thom, J. Wash. Acad. Sci. *22*, 93 (1932).

[4] B. N. Singh, J. gen. Microbiol. *1*, 11 (1947).

[5] K. B. Raper, J. agric. Res. *55*, 289 (1937).

[6] K. B. Raper, Quart. Rev. Biol. *26*, 169 (1951).

Species of Dictyosteliaceae	Cultivated soils	Uncultivated soils	Rhizosphere soils
Dictyostelium mucoroides Brefeld	18	4	36
D. discoideum Raper	2	0	6
D. minutum Raper	3	1	8
D. purpureum Olive	2	0	8
Polysphondylium pallidum Olive	6	2	18
P. violaceum Olive	2	2	7

D. mucoroides Brefeld was found to be present in many soils. Greater number of isolations were made from cultivated than uncultivated soils. In uncultivated soils, rhizosphere samples of weeds in many instances yielded species of Dictyosteliaceae.

Hay infusion, dung infusion and lactose-peptone agar were found to be best suited for the isolation of different species. In many instances 2–3 species were isolated from individual samples. Rhizosphere sample of pigeonpea (*Cajanus cajan*) in one instance yielded 4 species and that of pea-nut (*Arachis hypogea*) 5 species, which is indicative of the abundance of these forms in the cultivated soils here. *Aerobacter* circles invariably yielded 2 starins of *D. mucoroides* Brefeld one of which seemed to agree with *D. giganteum* Singh[4].

It is of interest to note that this is perhaps the first time that the Dictyosteliaceae are reported from uncultivated soils. The results of the writer emphasize that the members of this group are typically soil inhabitants and being predatory by nature, the rhizosphere of plants with divers groups of bacteria in great abundance might be one of their important ecological habitats.

Work on the growth of some members of Dictyosteliaceae in pure mixed cultures with bacteria isolated from soils and rhizosphere of different plants is underway and the details of these investigations will be reported elsewhere.

I am thankful to Professor T. S. SADASIVAN for his help and advice, to Professor KENNETH B. RAPER of The University of Wisconsin for confirming the identity of some of the isolates and to Dr. H. G. THORNTON, F. R. S. of the Rothamsted Agricultural Experiment Station for the culture of *Aerobacter* (1912) used in the above investigation.

V. AGNIHOTHRUDU

University Botany Laboratory, Madras-5, December 9, 1955.

Zusammenfassung

Sechs Arten von Dictycosteliaceae sind in Indien zum ersten Mal aus bebautem und unbebautem Boden isoliert worden. Ihr Vorkommen in nichtkultivierten Böden war bis jetzt unbekannt. Die Rhizosphäre der Pflanzen scheint ein wichtiges Habitat dieser Organismen zu sein.

Specificity of Anti-RH Serum Inhibition by Lipid Extracts as Determined by Quantitative Nitrogen Assays

Recovery of a lipid fraction of human red cells which would inhibit anti-Rh serums was reported by CARTER[1]. This fraction, originally extracted with ether, now is obtained by precipitation of one volume of pooled Rh positive red cells with 3 volumes alcohol, separation of the precipitate by filtration after an overnight period, followed by extraction of the precipitate by 3 volumes of dichloromethane with periodic shakings during at least 6 h at room temperature. The separation of the dichloromethane extract by filtration is followed by evaporation of the liquid under a hood before an electric fan. The lipid residue shows a comparable yield and properties similar to extracts described previously[2].

Work was undertaken to learn the specificity of this extract obtained from Rho (D) positive red cells in relation to anti-Rh (anti-D) serum. Aliquots of the lipid, 50 mg each, were weighed in Pyrex 125 × 15 mm tubes. To one tube was added 1 ml high titered anti-Rh serum; to a second tube 1 ml pooled normal serum. These serums must be free of bacterial contamination. A third tube held lipid plus 1 ml 0·85% saline. These three tubes were placed in a 50°C water bath for 10 min, shaken vigorously and put in a 37°C water bath for 30 min. Then the tubes were kept in an ice bath for 10 min and centrifuged at 4000 rpm for 10 min. The serums were removed with capillary pipettes and saved. Then 5 ml saline was added to each tube, mixed and again the tubes were placed in ice water and centrifuged. This washing was repeated 5 times. Washings from analyses of several lots were tested for nitrogen. The fifth wash was invariably negative. Also the procedure was repeated using magnesium chloride (5%) for washing to insure against loss of nitrogen. Results were closely corroborative.

Total nitrogen determinations were run on the contents of each tube after the fifth washing. Various methods were tried: KABAT and MAYER[3] and LANNI, DILLON, and BEARD[4]. The modified method used is: (1) Remove all water from the lipid with a capillary pipette without disturbing the layer. (2) To each tube add 1 ml digestion mixture (4·46 ml saturated copper sulfate plus 1 lb. reagent grade sulfuric acid), 0·5 g reagent grade, low N content potassium sulfate and one glass bead. (3) All tubes are placed over micro burners to digest until clear. After the vapor boils off, glass marbles are put on top of the tubes. Low boiling is continued for 30 min after complete clearing. (4) Cool the tubes; place in ice bath. (5) Add 5 ml distilled water; mix for complete solution. (6) Quantitatively transfer the contents of each tube to its 100 ml flask containing 25 ml distilled water. Add 20 ml NESSLER's reagent[5] slowly, with swirling. (7) Bring to 100 ml with distilled water, mix by inversion, allow to stand for 10 min, read transmittance with a Leitz photelometer at 460 mμ wavelength. A reagent blank is run simultaneously with the lipid aliquots. A curve is constructed from results obtained with tests run as above on serial dilutions in 100 ml volumetric flasks from a stock solution of ammonium sulfate (30 μg per ml, made by dissolving 0·142 g dry reagent grade ammonium sulfate in 1 l distilled water).

Use of this method to determine nitrogen in known amounts of choline hydrochloride shows that the process completely digests choline. Two ways were used

[1] B. B. CARTER, Amer. J. clin. Path. *17*, 646 (1947).

[2] B. B. CARTER, J. Immunol. *61*, 79 (1949).

[3] E. A. KABAT and M. M. MAYER, *Experimental Immunochemistry* (Charles C. Thomas, Springfield, Ill., 1948), p. 282.

[4] F. LANNI, M. L. DILLON, and J. W. BEARD, Proc. Soc. exper. Biol. Med. *74*, 4 (1950).

[5] F. C. KOCH and T. L. MCMEEKIN, J. Amer. chem. Soc. *46*, 2066 (1924).