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).

Results of Nitrogen Determinations and Serum Adsorptions (50 mg lipid)

	Lipid Extract (μg N)	Lipid with Normal Serum (μg N)	Lipid with Anti-Rh Serum (μg N)	Unadsorbed Anti-Rh Serum (titer units)	Adsorbed Anti-Rh Serum (titer units)
Rh + Lot #					
41	370	370	680	2560	80
42	340	360	460	2560	160
43	200	300	430	2560	160
44	300	480	970	2560	80
45	330	620	1120	2560	40
Rh — Lot #					
6	420	440	430	2560	2560
7	280	600	580	2560	2560
8	250	400	440	2560	1280
9	220	340	300	2560	2560
10	220	300	280	2560	2560

to determine the accuracy of the method: first, by comparison of two series of known nitrogen values run independently with this same method and, second, by using a single series of known values run with two different methods, the one described above and a distillation technique. The standard difference of the means in these series is not significant.

Results with 30 different preparations of lipid from Rh positive cells indicate that, although there is some non-specific adsorption of both anti-Rh and normal serums on the exposed lipid particle, the anti-Rh serum was without a single exception adsorbed to a greater extent than was the normal serum. A mean of 400 μg was found for the difference in N adsorption of normal serum and anti-Rh serum in the 30 preparations. 12 lipid fractions prepared from Rh negative (cde) red cells adsorbed a mean of only 30 μg more anti-Rh serum than normal serum; several lots adsorbed slightly less of the anti-Rh serum than of the normal serum. The difference between adsorption of anti-Rh on the Rh positive lipid particle and on the Rh negative lipid particle is highly significant (t of 3·27 at the 1% level of confidence). Titrations of anti-Rh serums before and after exposure to the lipid particles with hemagglutinating techniques, using calibrated pipettes, show several fold drops in titer with Rh positive lipid exposure and slight fall, if any, with the Rh negative lipid. Subjective error was avoided through one of us providing the other with unknown lipid samples for analysis, the origin of each determinable only by reference to a key.

It seems reasonable to conclude that the greater degree of adsorption of antibody nitrogen on the lipid derived from the Rh positive red cells is a specific effect. The Table shows the total nitrogen determinations in relation to 5 different lots of Rh positive lipid in sequence and 5 different Rh negative lots, together with the serum titrations.

BETTINA B. CARTER[6] and J. C. HARRIS

Department of Microbiology, State University of New York, College of Medicine at Syracuse and Veterans Administration Hospital, Syracuse, N.Y., January 18, 1956.

Zusammenfassung

Eine Reihe der Lipidfraktionen von Rh-positiv und Rh-negativ (D-positiv und D-negativ) roten Zellen menschlichen Blutes wurde der Wirkung von anti-Rho Serum (anti-D) ausgesetzt. Die dabei adsorbierte Menge von Stickstoff wurde quantitativ bestimmt. In jedem Fall adsorbierten die Lipide von Rh-positiv roten Zellen mehr Antikörperstickstoff als die aus Rh-negativ roten Zellen extrahierten Lipide. Man darf wohl daraus schliessen, dass der höhere Grad der Adsorption von Antikörperstickstoff auf Lipiden von Rh positiv roten Zellen ein spezifischer Effekt ist.

[6] Present address: Department of Biology, Western Michigan College, Kalamazoo, Michigan.

Is Lysine the Source of the Pyridine Ring in Nicotine?[1]

The suggestion of ROBINSON[2] that a genetic relationship exists between ornithine and pyrrolidine rings occurring in many alkaloids has recently received confirmation in the case of nicotine by the work of several groups[3]. ROBINSON[4] had previously called attention to a similar relationship between lysine and the pyridine ring, and noted the presence of both ornithine and lysine skeletons in nicotine.

LEETE[5] has proposed a tentative biogenetic scheme for nicotine, based on modifications of the suggestions of ROBINSON, which again suggests the possibility of lysine as a basic source of the pyridine ring. However, we have now obtained data, using (+)-lysine-ϵ-^{15}N-dihydrochloride and L(+)-lysine-^{14}C, which indicate that lysine is not incorporated as such into the nicotine molecule by root cultures of *Nicotiana tabacum* var. Turkish, grown under sterile conditions as outlined elsewhere[6]. In one experiment, 66·2 mg of the ^{15}N-lysine was fed to 100 root cultures. These were harvested[6] after one week and gave 31 mg of nicotine. A portion of this nicotine was converted to gaseous nitrogen and determined mass spectrometrically. The remainder was oxidized to nicotinic acid by means of nitric acid and analyzed similarly. The analytical results for these compounds appear in

[1] Research performed under the auspices of the United States Atomic Energy Commission.

[2] R. ROBINSON, J. chem Soc. 876 (1917).

[3] E. LEETE, L. MARION, and I. D. SPENCER, Can. J. Chem. *32*, 116 (1954). – E. LEETE, Chem. and Ind. *19*, 537 (1955). – L. J. DEWEY, R. V. BYERRUM, and C. D. BALL, Biochim. et Biophys. Acta *18*, 141 (1955).

[4] R. ROBINSON, Proc. Univ. Durham Phil. Soc. *8*, 1, 14 (1927).

[5] E. LEETE, Chem. and Ind. *19*, 537 (1955).

[6] R. F. DAWSON, D. R. CHRISTMAN, and R. C. ANDERSON, J. Amer. chem. Soc. *75*, 5114 (1953).