A rapid chemotaxonomic method for distinguishing mycobacterial strains

One can diagnose accurately various strains of mycobacteria by thin-layer chromatography of their respective lipids. In contrast to time consuming methods presently used, the technique outlined in this report is simple; its successful performance is well within the grasp of the thin-layer chromatographer with average skill. The method is complementary to pyrolysis–gas–liquid chromatography as a means of differentiating mycobacteria[1,2].

TABLE I

EFFECT OF THE AGE OF CULTURE AND TYPE OF MEDIA ON THE QUANTITY AND DISTRIBUTION OF LIPIDS

Each of the following pairs of observations were made on the same strain: 2 and 7; 6 and 9; 3 and 4; and 5 and 8.

No.	*Name*	*Age of culture (weeks)*	*Type of media*
1	Smooth fortuitum	6	L-J[a]
2	Rough photochromogen	4	L-J
3	Drug sensitive human	2	7H-9[b]
4	Drug sensitive human	2	P and B[c]
5	Group III	4	7H-9
6	Virulent avian	2	L-J
7	Rough photochromogen	6	P and B
8	Group III	4	P and B
9	Virulent avian	4	7H-9

[a] Lowenstein-Jensen.
[b] Middlebrook 7H-9, liquid media.
[c] Proskauer-Beck, liquid media.

Method

The bacterial lipids were extracted from heat-killed organisms with a 1:1 mixture of ethanol–diethyl ether. The lipid extract was washed with an aqueous salt solution, dried, and concentrated, using methods described earlier[3].

Approximately 50 mg of the pigmented lipid product was dissolved in 1.0 ml of toluene. Chromatography was carried out on 250 μ thick Silica Gel G plates in a solvent system consisting of hexane–diethyl ether–glacial acetic acid (9:1:0.15, v/v). All solvents were purified by appropriate treatment and distillation. Spots were applied to the origin, usually in 1 μl volumes. The period of development was of the order of 15 to 20 min, *i.e.*, the time required for the solvent front to migrate approximately 10 cm. Plates were air-dried and visualization of spots was achieved by charring with 50 % sulfuric acid.

Results

Some 17 strains covering five species of mycobacteria were analyzed by this technique. Each of the 900 or more samples analyzed gave lipid patterns which were the same for replicates of a particular species, but differed from the patterns exhibited by other species of mycobacteria (see Fig. 1).

It should be noted (Fig. 1) that the age of culture and type of media had a profound effect upon the quantity and distribution of lipids[4].

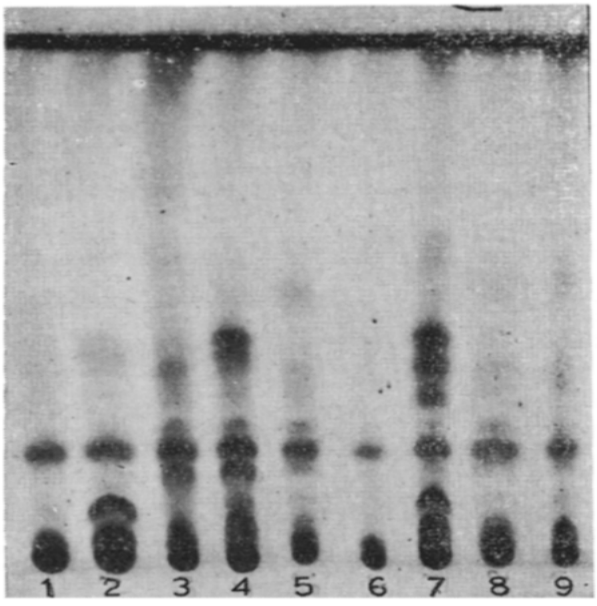

Fig. 1. Effect of the age of culture and type of media on the quantity and distribution of lipids. See also Table I.

Acknowledgement

Thanks are due to JUDITH JAMES HICKS for her diligent, skillful assistance in much of the work reported here.

National Communicable Disease Center, Public Health Service,
U.S. Department of Health, Education and Welfare,
Atlanta, Ga. (U.S.A.)

E. REINER
R. E. BEAM
G. P. KUBICA

1 E. REINER, *Nature*, 206 (1965) 1272.
2 E. REINER, *J. Gas Chromatog.*, 5 (1967) 65.
3 G. B. FREGNAN AND D. W. SMITH, *J. Bacteriol.*, 83 (1962) 828.
4 Detailed papers to be published.

Received December 16th, 1966