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Polar Lipid Composition in the Classification of Some *Actinomadura* Species

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The polar lipids of *Actinomadura dassonvillei*, *Actinomadura madurae*, and *Actinomadura pelletieri* were analysed by two-dimensional thin-layer chromatography. *A. madurae* and *A. pelletieri* had a simple pattern consisting essentially of diphosphatidylglycerol, phosphatidylinositol, and monoacyl phosphatidylinositol dimannoside, but *A. dassonvillei* strains contained diphosphatidylglycerol, phosphatidylglycerol, a lipid which co-chromatographed with phosphatidylcholine, and a chromatographically mobile unknown phospholipid. Two *A. dassonvillei* strains had substantial proportions of uncharacterised glycolipids and phosphoglycolipids. Low proportions of lipids co-chromatographing with, and having the same staining reactions as, phosphatidylethanolamine, phosphatidylinositol, and diacyl phosphatidylinositol dimannoside were detected in several strains of *A. dassonvillei*.

The genus *Actinomadura* was proposed (13) for strains previously classified as *Nocardia dassonvillei* (9), *Nocardia madurae*, and *Nocardia pelletieri* (8). The genus was defined primarily on chemical and morphological criteria, although its separation from *Nocardia* was subsequently supported by numerical phenetic (6, 7) and phage sensitivity studies (26). In the more comprehensive numerical survey (6), the *Actinomadura madurae* and *Actinomadura pelletieri* clusters formed an aggregate group joined at a much lower level of similarity by the *Actinomadura dassonvillei* cluster.

Actinomadura strains have a type III amino acid and sugar pattern (2, 13) and do not contain mycolic acids (20). However, only whole organism hydrolysates of *A. madurae* and *A. pelletieri* strains contain the novel sugar madurose (3-*O*-methyl-D-galactose) (12) and prodiginine pigments (5, 14), whereas *A. dassonvillei* strains are characterised by phenazine pigments (14). Finally, the long-chain fatty acids of *A. madurae* and *A. pelletieri* have been found to be predominantly straight chain, but those of *A. dassonvillei* include a high proportion of branched-chain acids (1).

In the present study the polar lipid composition of representative strains of *A. dassonvillei*, *A. madurae*, and *A. pelletieri* was examined.

MATERIALS AND METHODS

Strains and growth conditions. Details of the strains and their sources are given in Table 1. All cultures were maintained routinely on yeast extract agar at room temperature.

Strains were grown in shake culture at 30°C for 7 to 14 days in modified Sauton medium (23), checked for purity at maximum growth, killed by shaking with formalin (1%, vol/vol), separated by centrifuging, washed with distilled water, and freeze-dried.

Extraction and analysis of polar lipids. Freeze-dried bacteria (50 to 100 mg) were initially extracted by stirring with chloroform-methanol (2:1, vol/vol) (10 ml) overnight at room temperature as described in the accompanying paper (22). A modification (4) of the method of Bligh and Dyer (3) was preferred in later studies since simple chloroform-methanol extraction gave nonlipid material which appeared on thin-layer chromatograms (see Results). Polar lipids were analysed by two-dimensional thin-layer chromatography on silica gel plates impregnated with sodium acetate (17); developing solvents and spray reagents were identical to those employed in the accompanying paper (22).

RESULTS AND DISCUSSION

The polar lipid patterns of the test strains are shown in Fig. 1. The patterns given by *A. dassonvillei* strains are different and more complex than those from *A. madurae* and *A. pelletieri*, and provide further chemical data for separating *A. dassonvillei* from the other two species (1, 5, 12, 14).

The polar lipids of *A. madurae* and *A. pelletieri* are remarkably simple in composition; diphosphatidylglycerol (DPG), phosphatidylinositol (PI), and monoacyl phosphatidylinositol dimannoside, co-chromatographing with a corresponding lipid isolated from *Nocardia* (22), were the major components. All of these polar lipids are acidic, whereas in many bacteria acidic lipids co-occur with neutral lipids such as

glycolipids or phosphatidylethanolamine (18, 19, 27). *A. madurae* strains A11, A12, and A17 contain small amounts of a lipid having the properties of PG, whereas strains A16 and A22 contain an unidentified phospholipid.

DPG and phosphatidyglycerol (PG) are major components of the lipids of *A. dassonvillei*, but PI and a lipid having the chromatographic mobility and staining properties of a diacylated phosphatidylinositol dimannoside (22) occurred in small amounts in three strains (Fig. 1; A14, A15, and A118). *A. dassonvillei* strains contain a lipid which co-chromatographed with phosphatidylcholine (PC) and a mobile phospholipid which gave negative reactions to all the specific spray reagents other than that for lipid phosphate. Two strains (A15 and A119) had substantial proportions of a glycolipid and a phosphoglycolipid giving positive reactions with α-naphthol and periodate-Schiff reagent. Small amounts of a ninhydrin-positive phospholipid, possibly phosphatidylethanolamine (PE), were found in three strains (A114, A118, A119) (Fig. 1). Lipids extracted from *A. dassonvillei* by chloroform-methanol (2:1, vol/vol), on thin-layer chromatography, gave a large spot near the origin (Fig. 1; A114, A119). This component, which gave a positive reaction for carbohydrates with α-naphthol, was not present in extracts prepared using the modified procedure of Bligh and Dyer (3, 4) (Fig. 1; A14, A15, A118).

TABLE 1. *Test strains*

Laboratory no.	Strains	Source[a]
A14[b]	*Actinomadura dassonvillei*	NCTC 10488
A15	*A. dassonvillei*	NCTC 10489
A114	*A. dassonvillei*	Laboratory strain
A118	*A. dassonvillei*	H. Prauser, RG 509
A119	*A. dassonvillei*	H. Prauser, RG 714
A11	*Actinomadura madurae*	C. Philpot, 393
A12	*A. madurae*	C. Philpot, 373
A16[b]	*A. madurae*	NCTC 5654
A17	*A. madurae*	NCTC 1070
A22	*A. madurae*	M. Mariat, 725
A7	*Actinomadura pelletieri*	C. Philpot, 377
A8	*A. pelletieri*	C. Philpot, 388S
A10	*A. pelletieri*	C. Philpot, 1065
A18	*A. pelletieri*	NCTC 3026
A19	*A. pelletieri*	NCTC 4162

[a] NCTC, National Collection of Type Cultures, London, United Kingdom. H. Prauser, Institut für Mikrobiologie und Experimentelle Therapie, Jena, DDR; C. Philpot, London School of Hygiene and Tropical Medicine, Keppel St., London, United Kingdom; M. Mariat, Institut Pasteur, Paris, France.

[b] Type strain.

However, the two methods of extraction gave practically identical patterns of polar lipids when compared.

Only three strains of *Actinomadura* had previously been examined for polar lipid composition. Komura et al. (11) found that *A. madurae* strains contained only DPG and PG, a result at variance with our data, in which all of the *A. madurae* strains contained high proportions of PI and monoacyl phosphatidylinositol dimannoside. The present data provide further evidence of the chemical similarity found between *A. madurae* and *A. pelletieri* in analyses for madurose (12), prodiginine pigments (5, 14), and simple fatty acids (1). It would be interesting to extend these studies to include recently described species of *Actinomadura* (24).

The patterns of polar lipids found for *A. madurae* and *A. pelletieri* strains are distinct from those of *Nocardia* and related organisms (22). *Bacterionema* strains (22) resemble the patterns from *A. madurae* and *A. pelletieri* but differ significantly in the presence of substantial proportions of PG and traces of glycolipids. The polar lipids of *Mycobacterium*, *Nocardia*, and the "rhodochrous" complex contain two phosphatidylinositol mannosides, PE, and numerous unidentified glycolipids (22), the patterns being very different from those given by *A. madurae* and *A. pelletieri* (Fig. 1).

The polar lipids of *A. dassonvillei* (Fig. 1), containing two unidentified phospholipids, are unlike those of any other bacteria presently described (11, 21, 22, 27). One of the unidentified lipids is probably PC, which has been found only in one actinomycete, labeled *Nocardia coeliaca* (10, 29). The other unidentified lipid (Fig. 1), from its chromatographic behaviour, probably contains a relatively high proportion of fatty acid residues and could possibly be an acylated PG or DPG. Bisphosphatidic acid, which is a fully acylated PG, has been found in lipids of a marine bacterium (15), and monoacylated PG was found in extracts of *Salmonella typhimurium* (25); fully acylated DPGs have been detected in the lipids of *Acholeplasma modicum* (16). The glycolipids and glycophospholipids of *A. dassonvillei* strains A15 and A119 must be examined further and compared with similar lipids from other bacteria (21, 22, 28).

These preliminary data suggest that polar lipid analyses may provide good characters for the classification of *Actinomadura* species. However, further studies on additional *Actinomadura* species are required to determine the range of variation at present accommodated in this taxon.

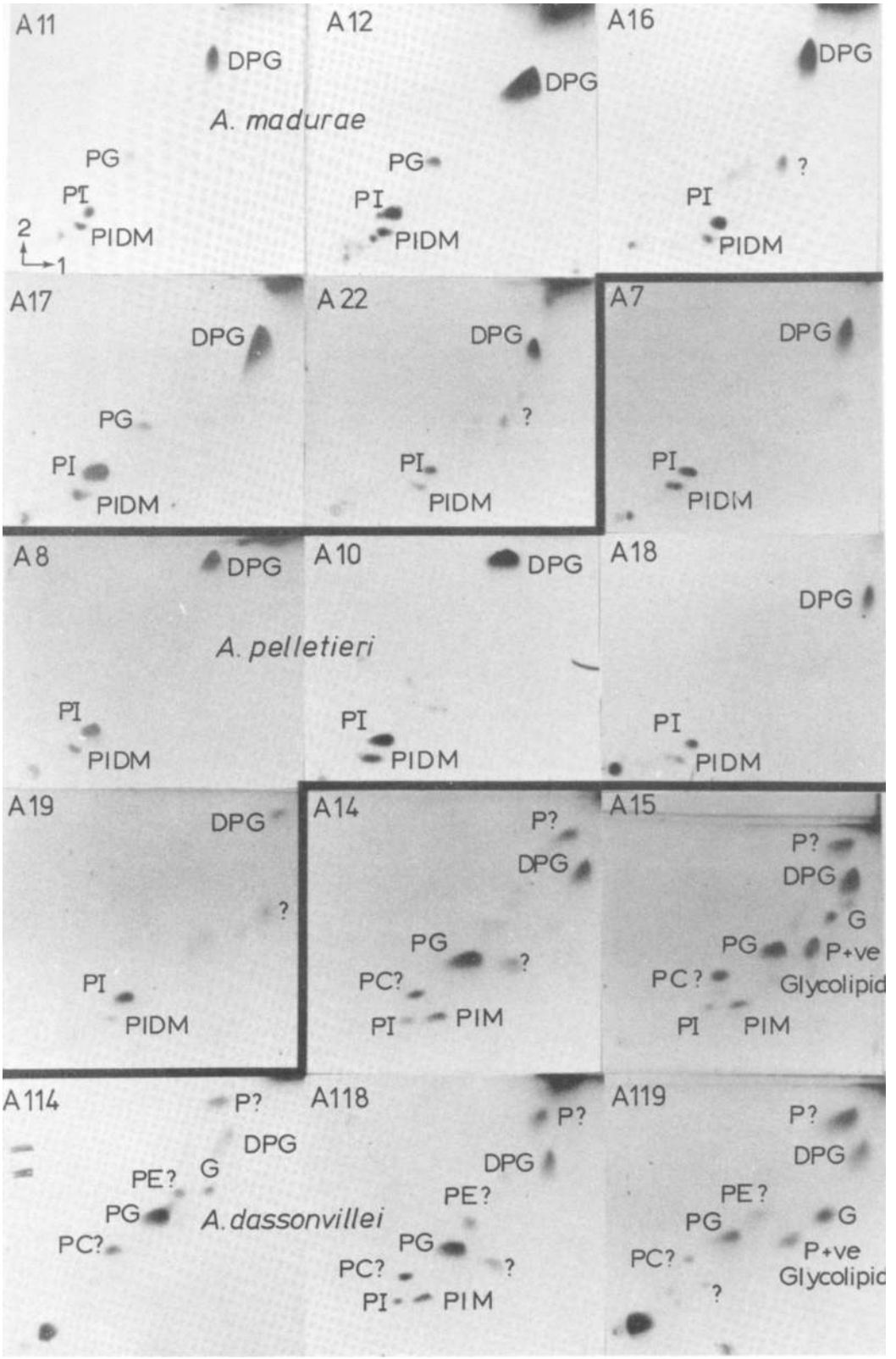

FIG. 1. *Two-dimensional thin-layer chromatograms of polar lipids from strains of Actinomadura. Chloroform-methanol-water (65:25:4, by volume) was used in the first direction, and chloroform-acetic acid-methanol-water (80:18:12:5, by volume) was used in the second direction. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIDM, phosphatidylinositol dimannoside; PIM, phosphatidylinositol mannoside; PC, phosphatidylcholine; P?, unknown phospholipid; G, glycolipid.*

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REPRINT REQUESTS

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