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 diphosphatidyglycerol, 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 prodigine 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 phosphatidylglycerol (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 A119). A. dassonvillei strains contain a lipid which co-chromatographed with phosphatidylycholine (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 carbohydrate with α-naphthol, was not present in extracts prepared using the modified procedure of Bligh and Dyer (3, 4) (Fig. 1; A14, A15, A119).

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), prodigine 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 for 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 Nocardiola 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 glycephospholipids 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.

### Table 1. Test strains

<table>
<thead>
<tr>
<th>Laboratory no.</th>
<th>Strains</th>
<th>Source</th>
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<tbody>
<tr>
<td>A14*</td>
<td>Actinomadura dassonvillei</td>
<td>NCTC 10488</td>
</tr>
<tr>
<td>A15</td>
<td>A. dassonvillei</td>
<td>NCTC 10489</td>
</tr>
<tr>
<td>A114</td>
<td>A. dassonvillei</td>
<td>Laboratory strain</td>
</tr>
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<td>A118</td>
<td>A. dassonvillei</td>
<td>H. Prauser, RG 509</td>
</tr>
<tr>
<td>A119</td>
<td>A. dassonvillei</td>
<td>H. Prauser, RG 714</td>
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<td>A11</td>
<td>Actinomadura madurae</td>
<td>C. Philpot, 393</td>
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<tr>
<td>A12</td>
<td>A. madurae</td>
<td>C. Philpot, 373</td>
</tr>
<tr>
<td>A16*</td>
<td>A. madurae</td>
<td>NCTC 5664</td>
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<td>A. pelletieri</td>
<td>C. Philpot, 388S</td>
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<td>A18</td>
<td>A. pelletieri</td>
<td>NCTC 3028</td>
</tr>
<tr>
<td>A19</td>
<td>A. pelletieri</td>
<td>NCTC 4162</td>
</tr>
</tbody>
</table>

* 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.

* Type strain.
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.
ACKNOWLEDGMENTS

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REPRINT REQUESTS

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LITERATURE CITED


