Polar Lipid Composition in the Classification of *Nocardia* and Related Bacteria

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Strains representing the mycolic acid-containing taxa *Nocardia*, *Mycobacterium*, *Gordona*, *Corynebacterium*, *Bacterionema*, and the "rhodochrous" complex were analysed for polar lipids by two-dimensional, thin-layer chromatography; diphosphatidylglycerol and phosphatidylinositol were found in all strains and phosphatidylethanolamine was absent only in extracts of *Corynebacterium* and *Bacterionema*. Mono- and diacyl phosphatidylinositol dimannosides were present in all *Nocardia*, *Mycobacterium*, *Corynebacterium*, and rhodochrous strains and in the single strain of *Gordona aurantiaca* examined; *Bacterionema* and strains of the other *Gordona* species had only a monoacyl phosphatidylinositol dimannoside. Phosphatidylglycerol was present in substantial amounts in extracts of two strains of *Bacterionema matruchotii* and in reduced proportions in strains of several other species. Unidentified glycolipids were detected in the lipids of a majority of the organisms investigated.

Lipids can provide good characters for the classification and identification of bacteria (10, 31, 42). In mycobacteria and coryneform and nocardioform bacteria the distribution of long-chain components, in particular the mycolic acids, has diagnostic value (1, 2, 3, 12, 25, 26, 31). Less attention has been paid to the value of polar lipids as taxonomic markers. Inconsistencies between various reports (17, 19, 21, 22, 37, 48-50) on the distribution of polar lipids in nocardioform and related taxa require clarification. The most characteristic polar lipids of actinomycetes are the phosphatidylinositol mannosides (PIMs); a few mycobacteria contain major amounts of phosphatidylinositol dimannosides (PIDMs) with three or four fatty acid residues in each molecule co-occurring with lipids having up to five mannose units (6, 35). Phosphatidylinositol monomannosides have been reported from *Nocardia leishmanii* (50), *Nocardia coeliaca* (49), *Nocardia polychromogenes* (17, 48), *Nocardia asteroides*, *Nocardia caviae*, and strains labeled *Nocardia erythropolis* and *Nocardia farcinica* (37). However, another report (19) highlighted the presence of PIDMs in strains of *N. coeliaca* and *N. polychromogenes*. In a more extensive investigation (22) of the value of phospholipids in the taxonomy of coryneform and nocardioform bacteria, the number of mannose units in the PIMs was not determined.

In the present study, the polar lipid patterns of strains of *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Gordona*, and the "rhodochrous" complex (11) were compared and the PIMs of representative nocardiae were examined in more detail. Two strains of *Bacterionema matruchotii*, recently shown to contain fatty and mycolic acids (1a) similar to those of *Corynebacterium*, were also studied.

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.

The mycobacteria, nocardiae, and gordonae were grown in shake culture at 30°C for 2 to 5 days in modified Sauton medium (33) and the corynebacteria were grown in nutrient broth (Oxoid) supplemented with 1% (wt/vol) Tween 80. *Bacterionema* strains were grown in brain heart infusion (Difco) medium, supplemented with glucose (0.5%, wt/vol), soluble starch (0.2%, wt/vol), and sodium nitrite (0.05%, wt/vol) (pH 7.4) in static culture for 7 to 10 days at 37°C. For rhodochrous strains, Sauton medium was amended with vitamin B₁ (50 mg/liter). Cultures were 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 of free lipids. Suspensions of approximately 1 g of freeze-dried bacteria in 40 ml of chloroform-methanol (2:1, vol/vol) were stirred overnight at room temperature. Extracted organisms were removed by filtration and washed with additional extraction solvent, and the combined extracts were

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TABLE 1. *Test strains*

Laboratory no.	Strain	Source ^a
B57	<i>Bacterionema matruchotii</i>	G. H. Bowden, J1247
B58	<i>B. matruchotii</i>	G. H. Bowden, J1429
C12 ^b	<i>Corynebacterium bovis</i>	NCTC 3224
C33	<i>Corynebacterium xerosis</i>	NCTC 8755
N663 ^b	<i>Gordona aurantiaca</i>	NCTC 19741
N654 ^b	<i>Gordona bronchialis</i>	NCTC 10667
N661 ^b	<i>G. bronchialis</i>	H. Mordarska, T1
N655 ^b	<i>Gordona rubra</i>	NCTC 10668
N656	<i>Gordona terrae</i>	NCTC 10669
M13	<i>Mycobacterium aurum</i>	NCTC 10438
M25 ^b	<i>Mycobacterium chitae</i>	NCTC 10485
M158 ^b	<i>Mycobacterium diernhoferi</i>	R. Bönicke, SN1418
M164	<i>Mycobacterium duvalii</i>	J. M. Grange, 51
M61	<i>Mycobacterium fortuitum</i>	I. Baess, F656
M62	<i>M. fortuitum</i>	I. Baess, F141
M79 ^b	<i>Mycobacterium gallinarum</i>	M. Tsukamura, 2506
M168	<i>Mycobacterium gilvum</i>	J. M. Grange, 35
M183 ^b	<i>Mycobacterium kansasii</i>	J. L. Stanford, 50
M60	<i>Mycobacterium phlei</i>	I. Baess, F89
N290	<i>M. phlei</i>	NCTC 8151
M176	<i>Mycobacterium rhodesiae</i>	NCTC 10779
M32 ^b	<i>Mycobacterium salmoniphilum</i>	ATCC 13756
M184	<i>Mycobacterium scrofulaceum</i>	J. L. Stanford, 15
M57	<i>Mycobacterium smegmatis</i>	I. Baess, F21
M59	<i>M. smegmatis</i>	I. Baess, F87
M63	<i>M. smegmatis</i>	I. Baess, 108
M101 ^b	<i>M. smegmatis</i>	ATCC 14468
M11 ^b	<i>Mycobacterium thermoresistibile</i>	NCTC 10409
N233	<i>Nocardia asteroides</i>	R. Olds, CN750
N317 ^b	<i>N. asteroides</i>	ATCC 19247
N14	<i>Nocardia brasiliensis</i>	NCTC 10300
N318 ^b	<i>N. brasiliensis</i>	ATCC 19296
N36 ^b	<i>Nocardia caviae</i>	NCTC 1934
N563	<i>N. caviae</i>	J. Lacey, 1912
M99 ^b	rhodochrous strain	ATCC 13808 (<i>Rhodococcus rhodochrous</i>)
N41 ^b	rhodochrous strain	NCIB 8863 (<i>Nocardia calcarea</i>)
N53 ^b	rhodochrous strain	NCIB 8147 (<i>Jensenia canicruria</i>)
N84	rhodochrous strain	S. T. Williams, E41 (<i>Nocardia corallina</i>)
N146	rhodochrous strain	M. Turner, 39
R43	rhodochrous strain	ATCC 25685

^a ATCC, American Type Culture Collection, Rockville, Md.; NCIB, National Collection of Industrial Bacteria, Aberdeen, United Kingdom; NCTC, National Collection of Type Cultures, London, United Kingdom. G. H. Bowden, Dept. of Bacteriology, Dental School, Turner Street, London; H. Mordarska, Hirsfeld's Institute, Wrocław, Poland; R. Bönicke, Institute for Experimental Biology and Medicine, Borstel, Federal Republic of Germany; J. M. Grange, School of Pathology, Middlesex Hospital, London, United Kingdom; I. Baess, Statens Seruminstitut, Copenhagen, Denmark; M. Tsukamura, Chubu Chest Hospital, Oku, Aichi-Ken 474, Japan; R. Olds, Pathology Dept., Cambridge Univ., United Kingdom; J. Lacey, Rothamsted Experimental Station, Harpenden, United Kingdom; S. T. Williams, Botany Dept., Liverpool Univ., United Kingdom; N. Turner, Botany Dept., Nottingham Univ., United Kingdom.

^b Type strain.

evaporated to dryness at low temperature (<37°C) under reduced pressure. Lipid extracts were redissolved in 1 ml of chloroform-methanol (2:1, vol/vol) and stored in stoppered containers at -18°C.

Analytical thin-layer chromatography of polar lipids. A two-dimensional system involving silica gel plates impregnated with sodium acetate (27) was used for analysis of polar lipids. Chromatograms were developed in the first dimension with a mixture of chloroform-methanol-water (65:25:4, by volume) and in the second dimension with chloroform-

acetic acid-methanol-water (80:18:12:5, by volume). Spraying with 50% sulfuric acid followed by charring at 180°C revealed spots corresponding to all lipids. Specific spray reagents for lipid phosphate (9), α -glycols (periodate-Schiff) (41), sugars (α -naphthol) (15), and free amino groups (ninhydrin in water-saturated butanol) were also employed. Diposphatidylglycerol gave a positive reaction with the lipid phosphate spray alone; phosphatidylethanolamine also reacted positively with ninhydrin. Phosphatidylinositol gave a characteristic yellow-

brown colour (41) with periodate-Schiff reagent; PIMs and glycolipids were positive with both periodate-Schiff reagent and α -naphthol. Phosphatidylglycerol gave an immediate intense purple colour (41) with periodate-Schiff reagent.

Analysis of the proportions of inositol, mannose, and long-chain fatty acids in PIMs. Samples of the two distinct suspected PIDMs were isolated by preparative thin-layer chromatography using layers (1 mm) of Merck silica gel H prepared by using a slurry made up in 0.2% (wt/vol) aqueous sodium acetate. Bands of total polar lipids were applied to chromatograms which were developed three times in chloroform-acetic acid-methanol-water (80:18:12:5, by volume) resulting in bands containing a mixture of the least polar mannoside (I) and phosphatidylinositol separated from the one corresponding to the more polar mannoside (II) (see Fig. 1b); separated zones were located by brief exposure to iodine vapour. The least polar mannoside (I) was isolated by chromatography of the mixture of it and phosphatidylinositol by using triple developments of chloroform-methanol-water (65:25:4, by volume). The purity of the separated lipids was checked by analytical thin-layer chromatography, and further purification was made, when necessary, by using the same procedure.

Samples (5 to 10 mg) of purified PIMs were dissolved in 4% sulfuric acid in methanol (5 ml), equal amounts of molar solutions of methyl arachidate (eicosanoate) (Sigma) in hexane and mannitol (British Drug Houses) in methanol were added, and the solutions were kept in screw-capped (polytetrafluoroethylene-lined) 20-ml tubes at 50°C overnight. After cooling, excess barium carbonate was added, the mixtures were shaken, and fatty acid esters were extracted with three successive portions of hexane (5 ml). The combined hexane extracts were examined, after evaporation to dryness, by gas chromatography using a Pye 104 flame ionisation instrument fitted with a glass column (200 by 0.4 cm) containing polyethyleneglycol adipate (10%) supported on Chromosorb W and nitrogen as carrier gas at an oven temperature of 180°C. Individual fatty acid methyl esters were identified by comparison of their retention times with authentic standards using both the above polar column and one (150 by 0.4 cm) containing a nonpolar methyl silicone (SE-30) phase (3% on acid-washed Celite 545) (180°C).

The remaining methanolic mixtures were centrifuged to remove barium sulfate and carbonate, the precipitates were washed twice with methanol (5 ml), and the combined supernatants, were taken to dryness under reduced pressure. Dry samples of sugars and polyols were dissolved in dry pyridine (2 ml) in a stoppered flask, and the solutions were treated with hexamethyldisilazane (0.6 ml) and trimethylchlorosilane (0.2 ml) and then kept at room temperature for 20 min. The total mixture was evaporated to dryness at low temperature, the residue was dissolved in hexane, and, after filtration, the hexane solution was examined for trimethylsilyl ethers of polyols by gas chromatography on the SE-30 column at an operating temperature of 150°C.

Factors which allow the ratios of the constituents to be calculated (44) were determined by subjecting, in triplicate, samples of methanolic solution containing molar quantities of mannitol, mannose, and inositol to the procedure previously described.

RESULTS

Polar lipid patterns. The results of the two-dimensional, thin-layer chromatographic analyses of the polar lipids of the organisms studied are shown in Fig. 1-9. The patterns obtained were quite complex and many components could not be positively identified. Certain lipids, however, could be clearly recognised by their characteristic chromatographic migration and staining properties with specific spray reagents. The most mobile phospholipid in all cases co-chromatographed with authentic diphosphatidylglycerol (DPG). Phosphatidylethanolamine (PE) was definitely absent only in representatives of *Corynebacterium* (Fig. 4) and *Bacterionema* (Fig. 5), but in certain other strains (Fig. 1d; 2c,d,e; 3b,c,d,f; 6f; 7a,d; 9b) the proportion of this lipid was very low indeed. Phosphatidylinositol was detected in all extracts. Two lipids having the staining properties of glycopospholipids were detected in all of the extracts studied apart from those of representatives of *Bacterionema* (Fig. 5) and most *Gordona* strains (Fig. 3a,b,c,d,e) in which only the most polar lipid was found. These characteristic glycopospholipids were identified, as will be described in detail below, as PIDMs having three or four fatty acid residues. The only other polar lipid identified with any certainty was phosphatidylglycerol (PG) which was present in substantial amounts in the lipids of *Corynebacterium* (Fig. 4) and of *Bacterionema* (Fig. 5), but its occurrence in the lipids of *Mycobacterium*, *Nocardia*, *Gordona*, and rhodochrous strains was very sporadic (Fig. 1b,c,d,e; 2a,b,d; 6a,b,c; 7c,e; 8a,b,c,d,e). No other unidentified phospholipids were detected in the lipid extracts studied, but most strains contained unidentified glycolipids (G) whereas certain strains (Fig. 1a,b; 2a,c,e,f; 4a,b; 5a,b; 6b,f; 7a,d,e; 8a,c,d; 9b) had other unidentified polar lipids. No further studies were carried out on the structures of these unidentified lipids.

Composition of the PIMs of *Nocardia*. Samples of the two PIMs from the lipids of *N. asteroides* N317 (Fig. 1b), *N. brasiliensis* N318 (Fig. 1c), and *N. caviae* N36 (Fig. 1e) were isolated by preparative thin-layer chromatography. Equimolar amounts of methyl arachidate and mannitol were added to samples of the purified lipids and the mixtures were subjected to acid methanolysis. Fatty acid methyl esters were

FIG. 1. Two-dimensional thin-layer chromatograms (2D TLC) of polar lipids from representatives of *Nocardia asteroides* (a, b), *N. brasiliensis* (c, d), and *N. caviae* (e, f). Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PDM, phosphatidylinositol dimannoside; G, glycolipid.

FIG. 2. Two-dimensional thin-layer chromatograms of polar lipids from representatives of the rhodochrous complex (Table 1). For abbreviations see Fig. 1.

extracted into hexane and their ratios were determined by gas chromatography. The fatty acids derived from the PIMs were simple mixtures of 16- and 18-carbon saturated acids and an 18-carbon unsaturated acid. Ratios of mannose, inositol, and mannitol were determined

by gas chromatography of their trimethylsilyl ether derivatives. The results of these studies are given in Table 2. If it is assumed that each molecule contains a single phosphodiester group and a single inositol unit, then these lipids are PIDM having either four (I) or three

FIG. 3. Two-dimensional thin-layer chromatograms of polar lipids from representatives of *Gordonia*. For abbreviations see Fig. 1.

FIG. 4. Two-dimensional thin-layer chromatograms of polar lipids from strains of *Corynebacterium bovis* and *C. xerosis*. Abbreviations: AG, acidic glycolipid; for others see Fig. 1.

FIG. 5. Two-dimensional thin-layer chromatograms of polar lipids from strains of *Bacterionema matruchotii*. For abbreviations see Fig. 1.

(II) fatty acid residues. It is more correct to describe these lipids as di- and monoacylated PIDMs, respectively, as the phosphatidyl unit, by definition, contains two fatty acids. The chromatographic behaviour of these lipids is consistent with that found for the di- and monoacylated PIDMs isolated from strains of *Mycobacterium* (6, 35).

DISCUSSION

The polar lipids of mycolic acid-containing genera are complicated mixtures of many different lipid types. Phospholipids and glycolipids are the main lipids found; no evidence was obtained for the presence of lipids similar to the ninhydrin-positive ornithine-amide lipids iso-

lated previously from strains of mycobacteria (38, 39). Phosphatidylinositol (PI) and DPG were the only two lipids found in all the organisms studied. This result is in accordance with studies on the lipids of authentic strains of *Nocardia* (22, 37), *Mycobacterium* (35), and *Corynebacterium* (8, 22). Less well characterised strains such as *N. polychromogenes* (17, 19, 48), *N. coeliaca* (19, 49), *N. leishmanii* (50), *N. erythropolis* (22, 37), *N. opaca*, *N. rubra*, *N. rugosa* (22), *N. farcinica* (22, 37), and *Corynebacterium alkanolyticum* (21) are also reported to contain these two lipids. The reported absence of PI in an early study (23) of the lipids of *N. brasiliensis* and a rhodochrous strain may have been a result of the difficulty in separat-

FIG. 6. Two-dimensional thin-layer chromatograms of polar lipids from representatives of *Mycobacterium fortuitum* and *M. smegmatis*. For abbreviations see Fig. 1.

FIG. 7. Two-dimensional thin-layer chromatograms of polar lipids from strains of *Mycobacterium thermoresistibile*, *M. salmonophilum*, *M. phlei*, and *M. gilvum*. For abbreviations see Fig. 1.

FIG. 8. Two-dimensional thin-layer chromatograms of polar lipids from strains of *Mycobacterium aurum*, *M. chitae*, *M. gallinarum*, *M. diernhoferi*, *M. duvalii*, and *M. rhodesiae*. For abbreviations see Fig. 1 and 4.

FIG. 9. Two-dimensional thin-layer chromatography of polar lipids from strains of *Mycobacterium kansasii* and *M. scrofulaceum*. For abbreviations see Fig. 1.

ing this lipid from PIMs. DPG is commonly found in bacterial lipids but PI is restricted, so far, to actinomycetes, coryneform bacteria, and strains of *Micrococcus* (10, 42). PG is a common constituent of bacterial lipids (10, 42) but is not routinely found in the lipids of actinomycetes and coryneform bacteria. It has been shown that in certain bacteria DPG is synthesized from two molecules of PG (14); in relatively slow-growing actinomycetes it is possible that most of the cellular PG has been converted to DPG.

PE was found in this study to occur in the lipids of *Nocardia*, *Gordona*, *Mycobacterium*, and the rhodochrous strains (Fig. 1–3, 6–9) but not in those from *Corynebacterium* (Fig. 4) or *Bacterionema* (Fig. 5) strains. Komura et al. (22) found only traces of PE in certain strains of *Corynebacterium*, but two strains labeled *C. fascians* and *C. equi* did, however, contain this lipid. In numerical phenetic studies, *C. equi* and *C. fascians* strains have been recovered in the rhodochrous taxon (11, 12). The very small proportions of PE detected in certain extracts (Fig. 1–3, 6, 7, 9) make this lipid a very unreliable chemotaxonomic marker. It has been clearly shown that the proportions of PE in certain strains of *Bacillus* (30) and *Pseudomonas* (28) may be dramatically changed by variation of the growth environment and a possible interchangeability of this lipid with neutral polar glycolipids proposed (29, 30). In this connection, it is interesting to note that, in many extracts containing small amounts of PE, glycolipids are prominent (Fig. 1d; 2c,d,e; 3b,c,d,e,f; 6f; 7a; 9b), and when much PE is present, glycolipids are low in proportion (Fig. 1f; 2b,f; 7b,c; 8e). Neutral glycolipids of the glycosyl diacylglycerol type have been reported in extracts of *N. polychromogenes* (19), *Actinomyces viscosus* (51), *Corynebacterium aquaticum* (20), *Rothia dentocariosa* (34), and many coryneform taxa (31, 42). Acylated glucoses were found in *N.*

TABLE 2. Ratios of mannose and fatty acids to inositol in the phosphatidylinositol mannosides of *Nocardia* strains

<i>Nocardia</i> strain	Polarity ^a	Fatty acid ^b proportion (%)			Total fatty acids/arachidic acid = (R)	Mannose/mannitol = (S)	Mannose/inositol	Fatty acids/inositol = (2R/S)
		C _{16:0}	C _{18:0}	C _{18:1}				
<i>N. asteroides</i> N317	I	40.3	23.2	36.5	0.108	0.056	2.25	3.85
	II	53.4	17.1	29.5	0.681	0.450	2.09	3.02
<i>N. brasiliensis</i> N318	I	30.3	42.5	27.2	0.239	0.127	2.12	3.76
	II	55.7	15.2	29.1	0.495	0.328	2.26	3.02
<i>N. caviae</i> N36	I	48.1	12.3	39.6	0.681	0.343	2.26	3.96
	II	52.4	13.0	34.6	0.268	0.203	2.08	2.64

^a I, Least polar; II, most polar mannoside.

^b Abbreviations for fatty acids: C_{16:0} and C_{18:0} = hexa- and octadecanoic; C_{18:1} = octadecenoic.

polychromogenes (19), *N. coeliaca* (19), and *Corynebacterium xerosis* (7). Polar acylated trehaloses occurred in *Mycobacterium fortuitum* (46) and a strain of *Micromonospora* (45), and propionibacteria contained an acylated inositol mannoside (40, 43). It is impossible to relate any of the glycolipids detected in the present study to the types mentioned above.

The extracts of the two *Corynebacterium* strains were found to contain components (Fig. 4) whose rapid chromatographic mobility in acidic solvents was typical of acidic glycolipids (47). *M. aurum* and *M. diernhoferi* (Fig. 8a,d) produced similar lipids. It is possible that the proportion of acidic glycolipids depends on the growth environment since an interrelation of these lipids and acidic phospholipids has been proposed (29, 47). Acidic glycolipids have been detected in the lipids of *N. caviae* (36) and a streptomycete strain (4, 5).

PIMs were found in all the strains studied. The two PIMs occurring in representatives of *N. asteroides*, *N. brasiliensis*, and *N. caviae* were shown to be mono- and diacyl PIDMs. This result supports the study of Khuller and Brennan (19) who reported the presence of PIDMs in *N. coeliaca* and *N. polychromogenes* and is in contrast to the survey of Pommier and Michel (37) in which several well-authenticated species of *Nocardia* were said to contain monomannosides. Yano and co-workers (48) and Kataoka and Nojima (17) also reported the presence of monomannosides in *N. polychromogenes*, and the former authors (49, 50) found similar lipids in *N. coeliaca* and *N. leishmanii*.

The *Nocardia* (Fig. 1), *Corynebacterium* (Fig. 4), *Mycobacterium* (Fig. 6-9), and rhodochrous (Fig. 2) strains all contained both mono- and diacylated PIDMs, but the *Bacterionema* strains (Fig. 5) lacked the diacylated lipids, a result which may reflect the different growth conditions. The diacylated lipid also could not be detected in any *Gordona* strains (Fig. 3a-e) except *G. aurantiaca* (Fig. 3f), but the large proportions of PI in extracts of *G. rubra* (Fig. 3c,d) made it difficult to observe this lipid precisely. Mono- and diacylated PIDMs have previously been characterised in detail from the lipids of strains of *Mycobacterium* (6, 35). Mycobacteria also contain more polar higher oligomers having up to five mannose units (6, 35); the possible presence of such lipids in the strains presently studied has not been investigated.

The phospholipid composition of other actinomycete genera has been the subject of several studies. Streptomycetes (17, 18, 24, 37) and a strain of *Microbispora* (17) apparently contain DPG, PE, and PIMs but lack PI; *Actinomyces*

viscosus contains DPG, PE, and PI but no PIMs (51). A strain of *Micromonospora* (45) contains all four of these lipid types, but *Oerskovia turbata* (22, 37) lacks PE. The absence of PE in *oerskoviae* adds weight to the finding (16) that they are more closely related to the coryneform bacteria than to actinomycetes. Strains of *Actinomadura madurae* were reported (22) to contain DPG and PG alone, but it is shown in the accompanying paper (32) that representatives of *A. madurae* and *Actinomadura pelletieri* contain DPG, PI, and monoacylated PIDM as major phospholipids. In this latter study (32), it is also demonstrated that the polar lipids of *Actinomadura dassonvillei* are different in that PG is a major lipid and PI and PIMs are minor components, other unidentified phospholipids being also present.

The phospholipids of strains labeled *N. coeliaca* (19, 49) and *N. leishmanii* (51) are distinct in that the former contains phosphatidylcholine (PC) and the latter has PG and two PEs, one of which incorporates hydroxy fatty acids. The classification of these two species should be studied in more detail.

It appears, therefore, that the polar lipids of mycolic acid-containing genera are all rather similar in pattern but certain small differences, such as the absence of a diacylated PIDM in some *Gordona* and *Bacterionema* strains, may be of value in classifying these bacteria. Differences exist among the polar lipid patterns of some other actinomycete taxa which may distinguish them from one another and from the mycolic acid-containing genera. The true potential of polar lipids as chemotaxonomic characters for the classification of actinomycetes may only become apparent after extensive systematic studies on the effect of growth environment have been performed.

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REPRINT REQUESTS

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