Fatty Acid and Polar Lipid Composition of the Genus *Amycolatopsis*: Application of Fast Atom Bombardment-Mass Spectrometry to Structure Analysis of Underivatized Phospholipids

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Phospholipid patterns of 15 representative strains of the genus *Amycolatopsis* were recorded by two-dimensional thin-layer chromatography. The structure analysis of the isolated phospholipids was verified by fast atom bombardment-mass spectroscopy. The positive- and negative-ion spectra of the partially purified phospholipid fractions qualitatively reflect their distinctive composition. All strains contained diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. Two different types of phosphatidylethanolamine and phosphatidylmethylethanolamine were detected, viz., compounds with or without hydroxy fatty acids. These phospholipid patterns underline the integrity of the genus. Fast atom bombardment-mass spectrometry analysis of phospholipid patterns may serve as an aid for differentiation of bacterial species.

Valuable taxonomic information for actinomycete systematics has been derived from two-dimensional thin-layer chromatographic (TLC) analyses of polar lipids (16, 25–27). Phospholipids are the most common actinomycete polar

lipids, and they have been proven to be of value as taxonomic markers (10, 15, 16, 18, 21, 22, 24, 32). The phospholipids most frequently encountered in actinomycete wall envelopes include phosphatidylcholine (PC), phosphatidyl-

TABLE 1. Descriptions and sources of *Amycolatopsis* strains

Designation ^a	History
<i>A. azurea</i> DSM 43854 ^T	A. Hensen, Fachbereich Biologie der Philips-Universität Marburg/Lahn, Germany (<i>Pseudonocardia azurea</i>); NRRL 11412; S. Omura, AM 3696, soil, Japan; produces azureomycins A and B
<i>A. fastidiosa</i> DSM 43855 ^T	A. Hensen (<i>Pseudonocardia fastidiosa</i>); ATCC 31181; Chas. Pfizer Inc., FD 25028, soil, Egypt; produces macrobicyclic peptide antibiotic
<i>A. mediterranei</i> ATCC 13685 ^T	Lepetit Laboratories, ME83/973 (<i>Streptomyces mediterranei</i>), soil; produces rifamycin
DSM 40501 ^T	Lepetit Laboratories, ME83/973
<i>A. methanolica</i> NCIB 11946 ^T	J. G. Kuenen, Laboratory of Microbiology, Technical University of Delft, Delft, The Netherlands, LMD 80.32 (<i>Nocardia</i> sp.), soil, New Guinea
<i>A. orientalis</i> subsp. <i>orientalis</i> ATCC 19795 ^T	E. V. Shirling, Ohio Wesleyan University, Delaware, Ohio, ISP 5040 (<i>Streptomyces orientalis</i>); P. Pittinger, M43-05865, soil, Asia; produces vancomycin
DSM 43387 ^T	E. B. Shirling, ISP 5040
DSM 43388	M. Goodfellow, N858; R. E. Gordon, IMRU 1390; J. Scharfen, W-113/66
N875	R. E. Gordon, Rutgers University, Piscataway, N.J., IMRU 1311 (<i>Nocardia-Streptomyces</i> group); M. P. Lechevalier, FT 19; clinical specimen
N876	R. E. Gordon, IMRU 1409 (<i>Nocardia brasiliensis</i>); J. Scharfen, AK 4348; clinical specimen
N878	R. E. Gordon, IMRU 1592 (<i>Micropolyspora</i> sp.); L. K. Georg, W-2101; soil, Sudan
N879	R. E. Gordon, IMRU 1601 (<i>Micropolyspora</i> sp.); L. K. Georg, W-2156; soil, Sudan
<i>A. orientalis</i> subsp. <i>lurida</i> DSM 43134 ^T ..	L. Ettlinger, LBG A 3091 (<i>Nocardia lurida</i>); R. Hütter, ETH 24315; W. E. Grundy, NA 3-TE-19; produces ristocetins A and B
<i>A. rugosa</i> ATCC 43014 ^T	M. P. Lechevalier, Rutgers University, Piscataway, N.J., IMRU 3760; R. E. Gordon; C. di Marco; cow rumen
<i>A. sulphurea</i> ATCC 27624 ^T	Abbott Laboratories, M-319 (<i>Nocardia sulphurea</i>); garden soil; produces chelocardin

^a ^T, type strain; ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany.

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FIG. 1. Two-dimensional TLC of polar lipids from *Amycolatopsis* strains. Chloroform-methanol-water (65:25:4 [vol/vol]) was used as the solvent in the first direction, and chloroform-acetic acid-methanol-water (80:15:12:4 [vol/vol]) was used in the second direction. PC, phosphatidylcholine; IMMIB, Institute for Medical Microbiology and Immunology of the University of Bonn.

glycerol (PG), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). Less commonly detected polar lipids include phosphatidylmethylethanolamine (PME).

Lechevalier et al. (16, 18) divided the phospholipids extracted from aerobic actinomycetes into five groups on the basis of the distribution of certain nitrogenous phospholipids. Members of the family *Pseudonocardaceae* were recovered in two of these groups. Among them, *Amycolatopsis*, *Kibdelosporangium*, and *Saccharomonospora* strains have a type II phospholipid pattern; that means that they contain PE but not phosphatidylcholine.

Characterization and identification of microorganisms constitute a major goal of diagnostic microbiology. Many systems and methods have been developed for this task, but in recent years increasing emphasis has been placed on the need to reduce the time involved and to introduce automated

systems into diagnostic microbiology. One of the methods likely to enable rapid characterization of microorganisms is fast atom bombardment (FAB)-mass spectrometry (MS). As shown by Barber et al. (4), polar, nonvolatile compounds can be analyzed. Fenwick et al. (8) showed that FAB provides abundant quasimolecular ions for phospholipids, as well as characteristic fragment ions. A comparative study of various soft ionization techniques has been reported for the structural characterization of phospholipids (2, 9, 19, 35). Münster and Budzikiewicz (28) and Münster et al. (29) showed that negative-ion FAB-MS is a suitable method for analysis of intact phospholipids. Heller et al. (11, 12) and Pramanik et al. (31) reported the use of negative-ion FAB and tandem MS in profiling mixtures of bacterial phospholipids.

In this study, partially purified classes of phospholipids

FIG. 2. Negative-ion FAB mass spectrum of the components separated by preparative TLC from *A. fastidiosa* DSM 43855^T (PI).

isolated from different species of the genus *Amycolatopsis* were subjected to negative- and positive-ion FAB as well as FAB-tandem MS to verify their structural assignments as already identified by two-dimensional TLC and to evaluate the usefulness of these methods for phospholipid analysis from bacterial sources.

MATERIALS AND METHODS

Strains and culture conditions. Stock cultures were taken from the culture collection of the Institute for Medical Microbiology and Immunology of the University of Bonn as listed in Table 1. The strains were maintained on brain heart infusion agar (Difco) at 4°C. The strains were grown in brain heart infusion broth (Difco) under agitation on a rotary shaker (150 rpm) for 7 days. At maximum growth, cultures were checked for purity, killed with formaldehyde (1% [vol/vol]), harvested by centrifugation, washed with distilled water, and freeze-dried.

Extraction of phospholipids. Lyophilized cells were extracted overnight with chloroform-methanol (2:1 [vol/vol]). The cells were then separated by filtration, and the filtrate was evaporated under vacuum with a rotary evaporator. The precipitation method of Andrews (1), which uses ice-cold acetone, was used to separate the phospholipids from other lipids. The dry residue was dissolved in 2.5 to 5 µl of chloroform per mg and cooled in a deep freeze for 10 min. Excess ice-cold acetone was added until no more milky precipitate formed. The mixture was centrifuged at 2,000 × g for 10 min at 0°C to pellet the precipitate, and the supernatant was transferred to another stoppered tube. The precipitate was further washed with 1 ml of ice-cold acetone per mg. Both tubes were left in a freezer overnight, and the precipitates were collected on the following day and dried thoroughly under nitrogen.

Purification of phospholipids. The dry precipitate was dissolved in 5 ml of chloroform-methanol-water (60:30:4.5 [vol/vol]) and passed through a column (0.9 by 15 cm) of Sephadex G25 (coarse) at a rate of 6 drops/min with the same solvent as the eluent. The effluent was taken to dryness under a stream of nitrogen. The residue was redissolved in 5 ml of chloroform and mixed by vibration for 1 min with 3 ml of 0.5% NaCl. The emulsion was broken by centrifugation, the milky supernatant was discarded, and the washing process was repeated with fresh saline. The lower chloroform

TABLE 2. Quasimolecular ion and fatty acid composition of PI isolated from *A. fastidiosa* DSM 43855^T

Peak (m/z) of:				Fatty acids
[M-H] ⁻	[M + H] ⁺	[M + Na] ⁺	[M + 2 Na-H] ⁺	
795	797	819	841	[C _{15:0} /C _{16:0}]
809	811	833	855	[C _{16:0} /C _{16:0}], [C _{15:0} /C _{17:0}]
823	825	847	869	[C _{17:0} /C _{16:0}], [C _{15:0} /C _{18:0}]
837	839	861	883	[C _{17:0} /C _{17:0}], [C _{16:0} /C _{18:0}], [C _{15:0} /C _{19:0}]

layer was taken to dryness under a stream of nitrogen and used for TLC.

Two-dimensional TLC. The purified phospholipid extracts were dissolved in chloroform-methanol (2:1 [vol/vol]), and 10-µl samples were applied to the corners of six silica gel (Merck Kieselgel 60 F₂₅₄ no. 5554) aluminium sheets (10 by 10 cm). Chromatography was done with chloroform-methanol-water (65:25:4 [vol/vol]) in the first direction and chloroform-acetic acid-methanol-water (40:7.5:6:2 [vol/vol]) in the second direction (25). To determine the type of lipids present on the chromatograms, the following differential stains were used. Five percent ethanolic molybdophosphoric acid was used as a general detector to reveal the presence of all lipids. Upon spraying with the reagent and charring at 180°C for 5 min, blue spots appeared. Merck ninhydrin spray reagent (no. 6758) was used to detect compounds containing amino groups. After spraying and subsequent heating at 110°C for 10 min, pink spots revealed the presence of the amino compounds. α-Naphtholsulfuric acid (13) reagent was used to detect the presence of glycolipids. After spraying and charring at 120°C until maximum color developed, blue-purple spots were obtained. Periodate-Schiff reagent was adapted from the method devised by Baddiley et al. (3) and Shaw (32) and used to detect glycolipids and phosphatides which contain vicinal hydroxyl groups. The method depends on cleavage of glycols with periodate and staining of the resulting aldehydolipids with Schiff reagent. The periodate-positive lipids appear within 1 min as pink-mauve spots. The identities of PI and PG were verified by this reagent. A dried chromatogram was sprayed with the Draggendorff reagent (5, 34), whereupon phosphatidylcholine, PE, and PME appeared immediately as yellow-orange spots on a yellow

FIG. 3. CA spectrum of [M-H]⁻ (m/z 809) from *A. fastidiosa* DSM 43855^T (PI).

FIG. 4. Negative-ion FAB mass spectrum of the lowest weight ninhydrin-positive components separated by TLC from *A. rugosa* ATCC 43014^T (PE plus OH). PN, PME.

background. Dittmer and Lester reagent (7, 33) is specific for phospholipids and detects as little as 10 µg. Phospholipids appear as blue spots on a white background within a few minutes without heating.

Preparative TLC. The phospholipid mixture was fractionated into partially purified components by TLC with silica gel (Merck Kieselgel F₂₅₄ no. 5554) aluminum sheets and a developing solvent of chloroform-methanol-acetic acid-water (25:15:4:2 [vol/vol]). The developed plate was exposed to iodine vapor, and the phospholipid bands were marked with a pen and left in a fume hood until the iodine evaporated. The bands were then scraped off into a centrifuge tube, and the phospholipids were eluted from silica gel with chloroform-methanol (2:1 [vol/vol]), separated by centrifugation, and then concentrated by evaporation of the excess chloroform-methanol under a nitrogen stream. They were stored at -20°C until analyzed.

MS. Positive- and negative-ion FAB spectra were recorded with a 731 mass spectrometer (Finnigan MAT, Bremen, Germany) equipped with an 11 NF FAB gun (Ion Tech Ltd., Teddington, United Kingdom). Measurements were made with a xenon gas pressure of 10⁻⁵ torr, the FAB potential was 7 kV, and the source temperature was ca. 30°C. The spectrometer was operated at an accelerating voltage of 8 kV, the postacceleration for positive-ion FAB was 5 kV, and that for negative-ion FAB was 7.5 kV. Collision activation (CA) measurements were performed with an HSQ 30 (Finnigan MAT) magneto-electrostatic quadrupole-quadrupole configuration (BEQQ) instrument. Argon was used as the collision gas, the collision energy was 70 eV, and the parent ion intensity was reduced to 70 to 50%. The resolution in the magneto-electrostatic (BE) part was 1,000, and the resolution in the quadrupole-quadrupole (QQ)

FIG. 5. CA spectrum of [M-H]⁻ (*m/z* 706) from *A. rugosa* ATCC 43014^T (PE plus OH).

part was unit mass resolution. Scan time was 100 mass units/5 s, and the results of five consecutive scans were averaged.

Samples were dissolved in a mixture of CHCl₃-CH₃OH-H₂O (86:14:1 [vol/vol]) and deposited in a matrix on a copper target. *m*-Nitrobenzyl alcohol was used as a matrix for positive-ion FAB, and 2 µl of this substance was mixed with 2 µl of solution. Diethanolamine was used as a matrix for negative-ion FAB, and 5 µl was mixed with 5 µl of solution; 2 µl of this mixture was applied to the target.

RESULTS

TLC. The results of two-dimensional TLC analyses of the polar lipids of the organisms studied are shown in Fig. 1. The patterns obtained were quite complex, and some components could not be positively identified. The remaining lipids, however, were clearly recognized by their characteristic chromatographic migration and staining properties with specific spray reagents. The most mobile phospholipid in all cases cochromatographed with authentic diphosphatidylglycerol (DPG) and was invariably present in all strains as a main component. However, the DPG chromatographic spots of *Amycolatopsis mediterranei* DSM 40501^T, *A. orientalis* subsp. *lurida* DSM 43134^T (Fig. 1), *A. rugosa* ATCC 43014^T, and *A. sulphurea* ATCC 27624^T were not homogeneous. They showed overlapping spots with two heads, which may be explained by the presence of another accompanying phospholipid having the same migration behavior and designated P. Other spots which react with phosphomolybdic acid and Dittmer reagent were located to the left of the DPG spot and were characterized as phospholipids P₂ and P₃. In addition, a third spot, designated P₁, reacted with both α-naphthol and phosphomolybdic acid but did not react with Dittmer reagent, indicating that it was a glycolipid.

TABLE 3. Quasimolecular ion and fatty acid composition of PE-OH isolated from *A. fastidiosa* DSM 43855^T

[M-H] ⁻	Peak (<i>m/z</i>) of:			Fatty acids
	[M + H] ⁺	[M + Na] ⁺	[M + 2 Na-H] ⁺	
690	692	714	736	[C _{15:1} /C _{16:0} + OH], [C _{16:0} /C _{16:0}], [C _{15:0} /C _{17:0}]
692	694	716	738	[C _{16:0} /C _{15:0} + OH]
706	708	730	752	[C _{16:0} /C _{16:0} + OH]
720	722	744	766	[C _{16:0} /C _{17:0} + OH]
748	750	772	794	[C _{16:0} /C _{19:0} + OH]

TABLE 4. Quasimolecular ion and fatty acid composition of PME-OH isolated from *A. rugosa* ATCC 43014^T

Peak (<i>m/z</i>) of:				Fatty acids
[M-H] ⁻	[M + H] ⁺	[M + Na] ⁺	[M + 2 Na-H] ⁺	
692	694	716	738	[C _{14:0} /C _{16:0} + OH]
720	722	744	766	[C _{16:0} /C _{16:0} + OH]
734	736	758	780	[C _{17:0} /C _{16:0} + OH], [C _{16:0} /C _{17:0} + OH]
748	750	772	794	[C _{18:0} /C _{16:0} + OH]
762	764	786	808	[C _{16:0} /C _{19:0} + OH], [C _{19:0} /C _{16:0} + OH], [C _{18:0} /C _{17:0} + OH]

Four ninhydrin-positive spots were detected on TLC plates of the strains studied: two spots cochromatographed with authentic PME and PE, and two more polar spots corresponded to PME and PE acylated with hydroxy fatty acids. The structures of these four compounds were verified with FAB-MS as described in detail below. Other unidentified ninhydrin-positive phospholipids were also present in *A. orientalis* DSM 43387^T and DSM 43388 and *A. mediterranei* DSM 40501^T and were designated NP.

PG, PI, and a lipid which had the chromatographic mobility and staining properties of a diacylated PI dimannoside isolated from nocardiae were easily detected by their rapid reaction with periodate-Schiff reagent. The last two compounds were also characterized by their reaction with α -naphthol. Other unidentified phospholipids possessing vicinal OH groups and reacting with the periodate-Schiff reagent were also present in *A. orientalis* ATCC 19795^T, *A. rugosa* ATCC 43014^T, *A. sulphurea* ATCC 27624^T, *A. mediterranei* ATCC 13685^T, and all of the Institute for Medical Microbiology and Immunology of the University of Bonn strains, *A. orientalis* DSM 43388, *A. mediterranei* DSM 40501^T, and *A. orientalis* subsp. *lurida* DSM 43134^T, and designated P-OH.

FAB-MS and the identities of the different components. To verify the identities of the different components as tentatively identified by both chromatographic behavior and color reactions, partially purified fractions isolated by preparative TLC were subjected to FAB-MS. The positive- and negative-ion FAB spectra show a number of ions in the molecular ion region. In addition to the quasimolecular ion [M-H]⁻ in the negative-ion FAB spectra and [M + H]⁺ in the positive-ion FAB spectra, there were accompanying ions due to [M + Na]⁺ and [M + 2Na-H]⁺. The fragmentation pattern of intact phospholipids in both positive- and negative-ion FAB spectra shows three main regions: the molecular ion region

(*m/z* 650 to 900), the fatty acid region (*m/z* 200 to 340), and the phosphate ester region (*m/z* 140 to 260).

Figure 2 shows the positive- and negative-ion FAB spectra of a fraction isolated from *A. fastidiosa* DSM 43855^T which was tentatively identified as PI by its color reaction. The quasimolecular ions at *m/z* 795, 809, 823, and 837 were interpreted as corresponding to a series of PI with fatty acids with increasing chain lengths. The interpretation of these quasimolecular ions is shown in Table 2.

The negative-ion FAB spectrum shows peaks at *m/z* 299, 259, 281, 241, and 297 which characterize the inositol phosphate part of the molecule. Further support for the above-described structural assignment was supplied by the FAB-tandem mass spectrum of the quasimolecular ion at *m/z* 809. The daughter ion spectrum generated from *m/z* 809 displayed ions at *m/z* 553, 391, 255, 241, and 269 (Fig. 3).

TLC revealed the presence of four ninhydrin-positive components. FAB-MS analysis of the lowest fractions showed quasimolecular ions at *m/z* 690, 692, 706, 720, and 748 (Fig. 4). These were interpreted as corresponding mainly to a series of PE that contained hydroxylated fatty acids (Table 3). In the lower-molecular-weight region, intense peaks at *m/z* 180 and 140 characterized the phosphate ethanolamine part of the molecules.

These structural assignments of PE were supported by tandem MS of the daughter ion at *m/z* 706 (Fig. 5). The spectrum generated from *m/z* 706 displayed a peak with high intensity at *m/z* 255 and less intensive peaks at *m/z* 239, 241, 269, and 271 which characterize the fatty acids. The peak at *m/z* 271 corresponds to a hydroxylated fatty acid.

FAB-MS analysis of the lower-middle ninhydrin-positive fractions showed quasimolecular ions at *m/z* 692, 720, 734, 748, and 762 (Table 4). Negative-ion FAB showed intensive peaks at *m/z* 194 and 154 which are characteristic for a phosphate methylethanolamine moiety in the molecule. Therefore, these quasimolecular ions were interpreted as corresponding to PME with different fatty acid combinations (Table 4). The tandem mass spectrum of the quasimolecular ion at *m/z* 734 (Fig. 6) supports the above interpretation. Intensive ions at *m/z* 269, 271, and 285 correspond to the fatty acid components of the phospholipids.

FIG. 6. CA spectrum of [M-H]⁻ (*m/z* 734) from *A. fastidiosa* DSM 43855^T (PME plus OH). PN, PME.

TABLE 5. Quasimolecular ion and fatty acid composition of PME isolated from *A. rugosa* ATCC 43014^T

Peak (<i>m/z</i>) of:				Fatty acids
[M-H] ⁻	[M + H] ⁺	[M + Na] ⁺	[M + 2 Na-H] ⁺	
704	706	728	750	[C _{16:0} /C _{16:0}]
718	720	742	764	[C _{16:0} /C _{17:0}], [C _{15:0} /C _{18:0}]
732	734	756	778	[C _{17:0} /C _{17:0}], [C _{16:0} /C _{18:0}], [C _{15:0} /C _{19:0}]
746	748	770	792	[C _{18:0} /C _{16:0}]

TABLE 6. Quasimolecular ion and fatty acid composition of PE isolated from *A. fastidiosa* DSM 43855^T

Peak (m/z) of:				Fatty acids
[M-H] ⁻	[M + H] ⁺	[M + Na] ⁺	[M + 2 Na-H] ⁺	
662	664	686	708	[C _{15:0} /C _{15:0}], [C _{14:0} /C _{16:0}]
676	678	700	722	[C _{15:0} /C _{16:0}], [C _{14:0} /C _{17:0}]
690	692	714	736	[C _{16:0} /C _{16:0}], [C _{15:0} /C _{17:0}]
704	702	728	750	[C _{17:0} /C _{16:0}], [C _{15:0} /C _{18:0}]
718	720	742	764	[C _{17:0} /C _{17:0}], [C _{16:0} /C _{18:0}], [C _{15:0} /C _{19:0}]

FAB-MS analysis of the highest ninhydrin-positive fractions and the lower one showed quasimolecular ions at *m/z* 704, 718, 732, and 746 for the highest one and at *m/z* 662, 676, 690, 704, and 718 for the lower components. The negative-ion FAB spectra showed intensive peaks at *m/z* 194 and 154 (characterizing PME) for the highest components and at *m/z* 180 and 140 (characterizing PE) for the lower ones. The absence of the ions at *m/z* 257, 271, 285, and 313 atomic mass units (characterizing the hydroxylated fatty acids) suggests that both components are acylated with nonhydroxylated fatty acids (Tables 5 and 6).

The identities of the components which give rise to purpleness directly upon addition of the periodate-Schiff reagent and were tentatively identified as PG were also supported by FAB-MS. FAB analysis of these components showed quasimolecular ions in the high-mass region at *m/z* 707, 721, and 735 (Table 7). In the lower-mass region of the negative-ion FAB spectrum, intensive ions at *m/z* 211, 171, 193, and 153 are characteristic of the phosphoglycerol portion of this phospholipid class. The above interpretation was also supported by tandem MS analysis of the daughter ion at *m/z* 721 (Fig. 7).

TLC revealed the presence of a very heavy spot in all of the strains which was tentatively identified as DPG. FAB-MS analysis of the partially purified components isolated from *A. mediterranei* ATCC 13685^T showed some peaks in the higher-mass region (*m/z* 1,000 to 1,300). However, because of the very low intensity of these peaks we were not able to interpret them; they may arise from quasimolecular ions and may correspond to a mixture of DPG with a different fatty acid composition. Peaks at *m/z* 231, 312, and 319 were comparable to those obtained from a reference DPG. These ions were not found in the spectra of the other phospholipid classes, and therefore they may be regarded as characteristic for DPG.

DISCUSSION

The polar lipids of the genus *Amycolatopsis* are a complex mixture of many different lipid types. All of the strains contained mixtures of DPG, PG, and PI, which are acidic lipids, co-occurring with PE and PME, which are neutral lipids. The co-occurrence of acidic phospholipids with neutral ones is a phenomenon found in many bacteria (17, 23, 32).

This characteristic polar lipid pattern readily distinguishes *Amycolatopsis* spp. from *Nocardia* spp. and other mycolic acid-containing taxa that contain DPG and characteristic PI mannosides (26), as well as from other mycolateless actinomycetes, such as *Streptomyces* spp. and related wall chemotype I actinomycetes which possess DPG, PE, PI, and PI mannosides (16, 20, 23). This pattern also distinguishes

TABLE 7. Quasimolecular ion and fatty acid composition of PG isolated from *A. azurea* DSM 43854^T

Peak (m/z) of:				Fatty acids
[M-H] ⁻	[M + H] ⁺	[M + Na] ⁺	[M + 2 Na-H] ⁺	
707	709	731	735	[C _{15:0} /C _{16:0}], [C _{14:0} /C _{17:0}]
721	723	745	767	[C _{15:0} /C _{17:0}], [C _{16:0} /C _{16:0}]
735	737	759	781	[C _{16:0} /C _{17:0}], [C _{15:0} /C _{18:0}]

Amycolatopsis strains from *Actinomadura* strains which contain DPG, PI, and monoacylated PI mannosides as major phospholipids.

The presence of two forms of both PE and PME, one acylated with hydroxylated fatty acids and the other acylated with nonhydroxylated fatty acids, is to be regarded as a new chemotaxonomic characteristic for the differentiation of *Amycolatopsis* spp. from other actinomycetes. Hydroxy fatty acids, previously reported to be acylated to PE in certain streptomycetes (14, 16, 30), were identified in some strains of the following genera: *Actinoplanes*, "streptomycetes of the chainia type," *Microbispora*, *Nocardia*, *Pseudonocardia*, "streptomycetes of the streptovorticillum type," and *Streptosporangium*. The phospholipids of such strains showed two spots containing PE on TLC plates: one acylated with nonhydroxylated fatty acids and a more polar compound acylated, at least in part, with hydroxy fatty acids (16). Collins et al. (6) reported the presence of DPG, PG, and two unidentified lipids in strains of *Arthrobacter simplex* and concluded that the latter might correspond to the hydroxy fatty acid-containing PG found by Yano et al. (37, 38). Pramanik et al. (31) reported the presence of a PE series with increasing chain lengths which are acylated with hydroxy fatty acids in the lipid isolated from *Microbispora parva* ATCC 33326, *Saccharothrix mutabilis* ATCC 31520, and *Saccharothrix* sp. strain SCC 1789. Yano et al. (36) reported that "*N. leishmanmii*" has PG and two PEs, one of which incorporates hydroxy fatty acids. The increasing number of reports on the detection of phospholipids acylated with hydroxylated fatty acids in members of the order *Actinomycetales* should be regarded as an important chemical marker that could be used to differentiate members of this order. In addition, this character will extend the number of the phospholipid types sensu Lechevalier.

MS is a very efficient tool for structural elucidation of

FIG. 7. CA spectrum of [M-H]⁻ from *A. azurea* DSM 43854^T (PG).

organic compounds. FAB-MS is not applied frequently in diagnostic laboratories. The positive- and negative-ion spectra of the bacterial extracts were found to reflect their distinctive phospholipid contents qualitatively (11, 12). Intensive peaks in the negative-ion FAB spectra at m/z 299, 259, 281, 241, and 297 are characteristic for the inositol phosphate portion of PI, while those at m/z 211, 171, 193, and 153 are characteristic for the phosphoglycerol portion of PG. The ethanolamine phosphate portion of PE is characterized by intensive peaks at m/z 180 and 140, and the methyl-ethanolamine phosphate portion of PME is characterized by peaks at m/z 194 and 154.

It seems that the applicability, speed, and power of FAB-MS for identification of phospholipids and elucidation of their structures are much better than those of traditional methods, such as hydrolysis and chromatographic separation. This method may be used for differentiation of bacterial isolates at the genus level.

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