Cell Walls of Nocardiae and Related Actinomycetes: Identification of the Genus *Nocardia* by Cell Wall Analysis

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The cell wall compositions of two strains of "true" nocardiae, *Nocardia asteroides* R 399 and *N. caviae* IM 1381, and two strains of "so-called nocardiae," *N. piracicabensis* and *N. mediterranei*, were determined. Two different methods were used for preparing the cell walls. In the one, the bacteria were sonically treated, and the cell walls were obtained by differential centrifugation; in the other, the bacteria were delipidated before sonic treatment. The cell walls of true nocardiae contain nocardic acids, identified by comparison with nocardic acids isolated from the whole cell. The peptidoglycan structures of the "true" and "so-called" nocardiae studied showed some important differences. In the true nocardiae, the glycan strand is constituted of β -*N*-acetylglucosaminyl (1 $\rightarrow$ 4) *N*-glycolylmuramic acid, and muramic acid is *N*-acetylated in the strains of "so-called nocardiae," as it is in the majority of bacteria. In addition, the peptide monomers of true nocardiae are diamidated on both the α -carboxyl group of D-glutamic acid and the (L) carboxyl group of *meso*-diaminopimelic acid, whereas the peptide monomers of the "so-called nocardiae" have only one amide substituent on the carboxyl group of *meso*-diaminopimelic acid. It is difficult to differentiate without ambiguity members of the genus *Nocardia* from the mass of the other actinomycetes which contain major amounts of *meso*-diaminopimelic acid. Our results suggest that true nocardiae may be those actinomycetes whose cell walls contain, in addition to arabinose and galactose, *N*-glycolylmuramic acid in the glycan part of the peptidoglycan and diamidated peptides in the peptide part of the peptidoglycan. In addition, true nocardiae contain nocardic acids.

The differentiation between nocardiae and related organisms is often quite difficult. Morphologically the genus *Nocardia* contains filamentous, branching bacteria (19). The validity of this characteristic as a differential criterion has been criticized in past years, and biochemical or chemical tests have been proposed to establish a more precise classification (20). Since the initial work of Cummins (7, 8), cell wall composition has proven to be most useful in classifying organisms belonging to the order *Actinomycetales*. For example, LL-diaminopimelic acid (Dpm) is found in the cell walls of streptomycetes and *meso*-Dpm in the cell walls of nocardiae (2). Moreover, some sugars, such as arabinose and galactose, are found in the cell walls of nocardiae but not in those of streptomycetes. Recently it has been proposed that the specific lipids present in whole cells of nocardiae be used to differentiate nocardiae from streptomycetes (27) and from mycobacteria (17, 21). Since the first isolation of nocardic acids from *Nocardia aster-*

oides (25) and the determination of their structure (3, 4), nocardic acids with related structures have been found in all the species of *Nocardia* so far examined. Nocardic acids are α -branched, β -hydroxylated acids of the mycolic type; they have recently been found in the cell walls of *Nocardia kirovani* in both the free and the bound form. Likewise, mycolic acids have been found in the walls of mycobacteria where they form a covalently linked lipid-polysaccharide-peptidoglycan complex (22). Another analogy between mycobacterial and nocardial cell walls is the presence of *N*-glycolylmuramic acid (MurNGly) in the glycan part of the peptidoglycan (1, 14) instead of *N*-acetylmuramic acid (MurNAc) found in the cell walls of all other bacteria. In consideration of these features, it was thought that the nocardiae could be classified on the basis of their cell wall composition, including both the lipid composition and the structure of the peptidoglycan.

We have examined the cell walls of two strains

of the "true nocardiae," *N. asteroides* and *N. caviae*, to check for the structural characteristics of the nocardic acids as previously reported for *N. kirovani* and the cell walls of two strains of the "so-called nocardiae," *N. piracicabensis* and *N. mediterranei*. *N. piracicabensis*, described by Joly (15) as a soil bacterium with great parafinolytic activity, gave a lipid profile quite different from that of other *Nocardia* strains (unpublished data). *Nocardia mediterranei* was previously placed in the genus *Streptomyces* and was then transferred to the genus *Nocardia* by Thiemann et al. (34) because it contains the *meso* isomer of Dpm in its cell wall as do true members of the genus *Nocardia*.

MATERIALS AND METHODS

Bacterial strains. *Nocardia asteroides* R 399 (=ATCC 23824) was received from M. Tsukamura, The National Sanatorium, Obu, Chita gun, Aichi-ken, Japan. This strain was cited by Tsukamura (35) as the "proposed neotype strain" of *N. asteroides*. *N. caviae* IM 1381 was obtained from the collection of the Institut Mérieux, 69-Marcy l'Etoile, France. *N. piracicabensis* was received from S. Joly, Instituto Zimotecnico, Universidade de San Paolo, Piracicaba, Brazil, and *N. mediterranei* ME/R 6468 was received from J. E. Thiemann, Research Laboratories, Lepetit Spa, Milan, Italy.

N. asteroides and *N. caviae* were grown in Sauton medium (30) for 2 weeks at 37°C. *N. piracicabensis* was grown at 37°C for 2 weeks on a potato medium containing an extract of potatoes (200 g/liter) and glucose (20 g/liter). *N. mediterranei* was grown at 30°C for 48 hr on the following medium: glycerol, 7.5 g; sucrose, 7.5 g; yeast extract (Difco), 5 g; NaCl, 4 g; distilled water to 1,000 ml. All cells were harvested in the stationary phase.

Preparation of cell walls. Two methods were used for preparation of cell walls. For the study of the peptidoglycan, the cell walls were prepared by the method of Braun and Sieglin (5). All operations were carried out at 4°C. A 2.5-g amount of dry and defatted cells was suspended in 50 ml of distilled water and disrupted for 15 min with an MSE 500-w ultrasonic apparatus. After centrifugation for 10 min at $10,000 \times g$, the same operations were repeated twice on the residue. The supernatant fluids were treated by the method described by Braun and Sieglin (5). The cell walls thus prepared (about 250 mg) were then freeze dried.

For the study of lipids, the cell walls were prepared by the method described by Guinand et al. (14).

Preparation of nocardic acids from the cell walls. The prepared cell walls were defatted by several extractions with ethanol-ether (1:1), CHCl_3 , and CHCl_3 -methanol (1:1). The lipidic extract was saponified by a 5% solution of KOH in methanol for 2 hr. After acidification of the mixture, the unsaponifiable moiety was extracted with ether, and the acidic part was dissolved in an aqueous solution of NaOH. The aqueous solution was then neutralized, and the acid constituents were extracted with ether and esteri-

fied with diazomethane (31). Nocardic esters and non-hydroxylated esters were separated by thin-layer chromatography on silica gel G plates with hexane-ether (80:20) and detected by spraying with a solution of rhodamine B and 2',7'-dichlorofluorescein.

Enzymes. Myxobacter AL₁ enzyme was kindly provided by J. F. Petit, Institut de Biochimie, Orsay, France. This enzyme is known to attack *N*-acylmuramyl-L-alanine linkages (10). A 1-ml amount of a preparation containing 130,000 lytic units/ml was shaken with 80 mg of cell walls in 0.1 M Veronal buffer, (pH 9) for 24 hr at 37°C. Lytic units were determined by the procedure of Ensign et al. (9) using cells of *Micrococcus lysodeikticus* instead of *Arthrobacter crystallopoietes*. The cell walls of *N. piracicabensis*, *N. asteroides*, and *N. caviae* had previously been hydrolyzed with 0.1 N HCl for 12 hr at 60°C to remove the neutral sugars (galactose and arabinose).

Egg-white lysozyme (Worthington) was used to hydrolyze β -*N*-acetylglucosaminyl (1 $\rightarrow$ 4) *N*-acetylmuramyl linkages in the glycan moiety of the peptidoglycan. Incubations were at 37°C for 24 hr in 0.01 M acetate buffer (pH 5.4).

Chromatographic solvents. The following solvents were used: (I) butanol-pyridine-acetic acid-water (30:20:6:24); (II) butanol-acetone-water (4:5:1); (III) methanol-pyridine-10 N hydrochloric acid-water (80:10:2.5:17.5); (IV) tertiary amyl alcohol saturated with 0.1 M phthalate buffer, (pH 6); (V) butanol-acetic acid-water (5:1:2); (VI) benzene-pyridine-acetic acid (80:20:2); (VII) hexane-ether (80:20).

Gel filtrations. A column of Sephadex G 25 (3 by 150 cm) was used. The gel-filtration properties of each compound were expressed in terms of distribution coefficient $K_D = (V_e - V_o)/(V_i - V_o)$, where V_e = elution volume, V_o = elution volume of totally excluded material, and V_i = elution volume of NaCl.

Analytical procedures. Terminal amino groups (fluorodinitrobenzene method), reducing groups (Park and Johnson procedure), and hexosamines (Morgan-Elson reaction) were determined as described by Ghuysen et al. (11).

Quantitative analyses of amino acids and hexosamines (after hydrolysis with 4 N HCl for 12 hr at 95°C) were carried out by using a Technicon amino acid analyzer on a column of resin-type Chromobeads C₂ with an elution gradient from pH 2.75 to 6.10.

The amino acid sequence in the peptide moiety of the peptidoglycan was determined by Edman degradation of the peptide monomers according to the method of Gray and Smith (12).

The tetrapeptide and tripeptide monomers were separated on a column of resin Aminex Q 15 S and eluted with a linear gradient of pyridine-acetic acid buffer, 0.2 M (pH 3.1) to 2 M (pH 5.1). Amino acids were determined on a sample of each fraction after alkaline hydrolysis as described by Moore and Stein (26).

Thin-layer and paper chromatography. Qualitative analysis of amino acids was carried out by thin-layer chromatography on cellulose plates with solvent I.

The dimethylaminonaphthalene sulfonyl (=DNS= dansyl) amino acids were separated and identified by thin-layer chromatography on silica gel G plates with solvent VI.

The optical configuration of Dpm was determined as follows: *meso* and DD isomers were separated from the LL isomer by chromatography on Whatman no. 1 paper with solvent III (29). The DD or *meso* configuration was determined after dinitrophenylation by chromatography of the dinitrophenyl (DNP) derivatives on Whatman 3MM paper impregnated with 0.1 M phthalate buffer, pH 6. Solvent IV was used for developing (16).

The sugars were tested after hydrolysis with 1 N HCl for 15 hr at 105 C by chromatography on plates of silica gel G impregnated with NaH₂PO₄ and developed with solvent II. The sugars were detected by spraying with an ethanol-sulfuric acid solution of naphthoresorcinol (18).

The disaccharide units obtained by hydrolysis of the glycan moiety of the peptidoglycan with egg-white lysozyme were identified by chromatography on Whatman no. 1 paper with solvent V and detected with Sharon reagent (32).

Paper electrophoresis. Electrophoreses were carried out on Whatman no. 1 paper at pH 4.1 in pyridine-acetic acid-water (2.5:9:1,000) with a Pherograph apparatus (30 v/cm).

Reference compounds. The following compounds were used as standards for the determination of the structure of the disaccharides and peptides isolated: β -N-acetylglucosaminyl (1 $\rightarrow$ 4)-MurNAc from *Butyrivacterium rettgeri* (13), β -N-acetylglucosaminyl (1 $\rightarrow$ 4)-MurNGly from *N. kirovani* (14), the peptide monomers L-Ala- γ -D-Glu-(L)-*meso*-Dpm-(L)-D-Ala from *Bacillus megaterium* (6, 36), L-Ala-D-isoGln (isoGln: α glutamine)-(L)-*meso*-Dpm and L-Ala-D-isoGln-(L)-*meso*-Dpm-(L)-D-Ala from *Clostridium perfringens* (23), and L-Ala-D-isoGln-(L)-*meso*-Dpm-(D)-NH₂-(L)-D-Ala from *N. kirovani*. (The isolation and structural determination of this last peptide was carried out in our laboratory by M. J. Vacheron.)

RESULTS

Lipid composition. Hydroxylated esters (R_F 0.49) and non-hydroxylated esters (R_F 0.89) were separated by thin-layer chromatography. Hydroxylated esters were identified with the nocardic esters isolated from whole bacteria (24). They were present only in the strains of *N. asteroides* and *N. caviae*.

Sugar composition. Arabinose and galactose were present in the cell walls of the strains of *N. asteroides*, *N. caviae*, and *N. piracicabensis*;

the strain of *N. mediterranei* contained no neutral sugar in the cell wall.

Peptidoglycan composition. Peptidoglycans prepared by the method of Braun and Sieglin (5) were hydrolyzed with 4 N HCl for 12 hr at 95 C. The results of the quantitative analysis of amino acids and hexosamines are given in Table 1.

The optical configuration of Dpm was determined by paper chromatography as described by Rhuland et al. (29) followed by paper chromatography of the DNP derivatives according to Jusic et al. (16). *Meso*- α , ϵ -Dpm was found in all of the strains examined.

Enzymatic degradation of the peptidoglycan. Myxobacter AL₁ enzyme (10) lysed the cell walls of *N. mediterranei* ME/R 6468 but solubilized the walls of the three other strains only after treatment with HCl, which eliminates the polysaccharide fraction of the walls (see Material and Methods). N-acylmuramyl-L-alanine linkages were split, and the degradation products—glycan and peptide fragments—were solubilized. The digest was filtered in 0.1 M CH₃COOH on a column of Sephadex G25. Elution profiles of all the strains were identical (Fig. 1). Three main fractions were obtained: P I, peptide monomers, K_D = 0.7; P II, peptide dimers, K_D = 0.48; P III, peptide polymers, K_D = 0. This last material was not separated from the glycan part of the peptidoglycan moiety.

Lysozyme was used to hydrolyze fraction P III (K_D = 0) at 37 C for 24 hr. After filtration in 0.1 M CH₃COOH on a column of Sephadex G25, disaccharide fragments, K_D = 0.35 (Fig. 2, peak I), were separated from the peptide polymers, K_D = 0 (Fig. 2, peak II).

Identification of the disaccharide fragments. The disaccharide fragments (Fig. 2, peak I) were chromatographed on Whatman no. 1 paper with solvent V in the presence of the following standards; β -N-acetylglucosaminyl (1 $\rightarrow$ 4)-MurNAc of *Butyrivacterium rettgeri* and β -N-acetylglucosaminyl (1 $\rightarrow$ 4)-MurNGly of *N. kirovani*. Muramic acid was found glycolylated in *N. asteroides* R 399 and *N. caviae* IM 1381 and acetylated in *N. mediterranei* ME/R 6468 and in the strain of *N. piracicabensis*.

TABLE 1. Quantitative analysis of amino acids and hexosamines^a

Organism	Ala	Glu	Dpm	MurNAc ^b	MurNGly ^b	GlcNAc ^b
<i>N. caviae</i>	1.86	1.17	1		0.81	0.91
<i>N. asteroides</i>	1.98	1.03	1		0.8	1.2
<i>N. piracicabensis</i>	1.62	1.02	1	0.94		3.95
<i>N. mediterranei</i>	1.65	1.24	1	1.26		2.05

^a Expressed as molar ratio with diaminopimelic acid (Dpm) as unit.

^b Abbreviations: MurNAc, N-acetylmuramic acid; MurNGly, N-glycolylmuramic acid; GlcNAc, N-acetylglucosamine.

FIG. 1. Filtration of *Mycobacter AL₁*-degraded walls (80 mg) on Sephadex G25 in 0.1 M acetic acid (*Nocardia mediterranei*). Hatched zone contains hexosamines (positive Morgan-Elson reaction).

FIG. 2. Filtration of lysozyme-degraded fraction P III of Fig. 1 on Sephadex G25 in 0.1 M acetic acid (*Nocardia mediterranei*). Solid lines, terminal amino groups in nanomoles; dashed line, reducing groups in nanomoles.

Structure of the peptide monomers. Dansylation of the P I peptide monomers of each strain (Fig. 1), followed by hydrolysis, gave DNS-Ala and a mono-DNS-Dpm. Through an Edman degradation (12), *N*-terminal Ala and mono-*N*-terminal *meso*-Dpm disappeared. Dansylation and hydrolysis liberated DNS-Glu. After a second cycle of degradation, no free *N*-terminal group appeared for dansylation, demonstrating that Glu was linked to *meso*-Dpm through its γ carboxyl group. Thus the peptide sequence is Ala- γ -Glu-Dpm and the peptidoglycans belong to chemotype I (11) like those of *Escherichia coli*, *Mycobacterium smegmatis*, and *N. kirovani*. In this type of peptidoglycan, peptide monomers are composed of a mixture of tripeptides, L-Ala- γ -D-Glu-*meso*-Dpm, and tetrapeptides, L-Ala- γ -D-Glu-*meso*-Dpm-D-Ala.

The α -carboxyl group of D-glutamic acid and the carboxyl group of *meso*-Dpm not engaged in a peptide bond can be either free or amidated. The presence of amide groups in P I peptides was determined by electrophoresis at pH 4.1 with the following peptides as controls: nonamidated peptide from *Bacillus megaterium*, monoamidated peptide from *Clostridium perfringens*, and diamidated peptide from *N. kirovani* (Fig. 3). The peptides from the strains of *N. asteroides* and diamidated peptide from *N. kirovani* (Fig. 3) showed phoretic mobility as the peptide from the strain of *N. kirovani*, are diamidated while the peptides from the strains of *N. mediterranei* and *N. piracicabensis* are monoamidated like that of *C. perfringens*.

The presence of a monoamidated sequence on *N. mediterranei* and *N. piracicabensis* peptide monomers was confirmed by chromatography on resin Aminex Q 15 S. The curve obtained by the amino acid determination of a sample of each fraction after alkaline hydrolysis shows two peaks (Fig. 4): peak I (elution volume 120 ml) is a monoamidated tetrapeptide, and peak II (elution volume 134 ml), a monoamidated tripeptide. The most important eluted materials were the tetrapeptide for *N. mediterranei* (Fig. 4c) and the tripeptide for *N. piracicabensis* (Fig. 4b). As a comparison, the curve obtained from the peptide monomer of *N. caviae* (Fig. 4a) shows a main peak of diamidated tetrapeptide (III: elution volume 168 ml) and three smaller peaks: I, monoamidated tetrapeptide; II, monoamidated tripeptide; and IV, diamidated tripeptide (elution volumes: 120, 134, and 188 ml, respectively).

Location of amide substituents. The location of amide substituents on the monoamidated peptides from *N. mediterranei* and *N. piracicabensis* was determined as follows (Fig. 5).

A part of peptide monomer A (A_1 or A_2) was

FIG. 3. Electrophoresis of the peptide monomers P I. S_1 , nonamidated peptide standard from *Bacillus megaterium*; S_2 , monoamidated peptide standard from *Clostridium perfringens*; S_3 , diamidated peptide standard from *Nocardia kirovani*; NA, peptide P I from *N. asteroides*; NC, peptide P I from *N. caviae*; NM, peptide P I from *N. mediterranei*; NP, peptide P I from *N. piracicabensis*. Electrophoresis was carried out at pH 4.1 for 2 hr (30 v/cm).

submitted to an Edman degradation leading to peptide B (B_1 or B_2), and another part was deamidated by hydrochloric acid treatment (10 N HCl, 25 C, 30 hr), leading to peptide A_3 , and then submitted to an Edman degradation, leading to peptide B_3 .

Peptide A_1 is amidated on the α -carboxyl group of D-glutamic acid. Through an Edman degradation, A_1 gives a peptide, B_1 , with one amine group and one carboxyl group. Its ionic net charge, and thus its electrophoretic mobility, should be identical with that of monoamidated peptide A (2 NH_2 and 2 $COOH$). Peptide B_1 is also obtained through an Edman degradation of a diamidated peptide ($R = R' = NH_2$).

Peptide A_2 is amidated on the (D) carboxyl group of meso-Dpm. An Edman degradation gives a peptide, B_2 , with one amine group and two carboxyl groups. At pH 4.1 it is negatively charged like a nonamidated peptide monomer (2 NH_2 , 3 $COOH$), and its ionic charge is quite different from that of a monoamidated peptide monomer (2 NH_2 , 2 $COOH$).

A_3 peptides obtained from either peptide A_1 or peptide A_2 by deamination are identical ($R = R' = OH$). B_3 peptides obtained through an Edman degradation of peptides A_3 are identical with B_2 peptides ($R = OH$).

The foregoing experiment was performed upon the peptide monomers of the strains of *N. piracicabensis* and *N. mediterranei*. The diamidated peptide monomer of *N. kirovani* was used as a control. The results are shown in Fig. 6. The electrophoretic mobilities of B peptides from *N. piracicabensis* (+7.7) and *N. mediterranei* (+7.8) were similar. These peptides were slightly more anionic than the non-

FIG. 4. Purification of peptide monomers by chromatography on a column of resin Aminex Q 15 S. I. Monoamidated tetrapeptides; II, monoamidated tripeptides; III, diamidated tetrapeptides; IV, diamidated tripeptides. (a) *N. caviae*, (b) *N. piracicabensis*, (c) *N. mediterranei*.

amidated peptide of *Bacillus megaterium* (+7.5), and their electrophoretic behavior was quite different from that of the B_1 peptide from *N. kirovani* (-3.7). Thus the peptides from *N. piracicabensis* and *N. mediterranei* were in fact B_2 peptides which originated from A_2 peptides with $R = OH$ and $R' = NH_2$, i.e., they are amidated on the (D) carboxyl group of meso-Dpm.

These results have been confirmed by mass spectrometry of the tetrapeptide after acetylation and permethylation (unpublished data).

DISCUSSION

As claimed previously, cell wall composition is a useful criterion for differentiating between the various actinomycetes, for which six cell wall groups were initially described (19). Later on, two additional groups were proposed for organisms which are intermediate between the actinomycetes and true bacteria (33). In the case

FIG. 5. Location of the amide group.

FIG. 6. Electrophoresis of the peptides from Edman degradation. S_1 , nonamidated peptide standard from *Bacillus megaterium*; S_2 , monoamidated peptide standard from *Clostridium perfringens*; S_3 , diamidated peptide standard from *Nocardia kirovani*. BM, peptide B from *N. mediterranei*; BP, peptide B from *N. piracicabensis*; BK, peptide B from *N. kirovani*. B_3M , B_3P , and B_3K are deamidated peptides BM, BP, and BK. (For the significance of peptides B and B_3 , see Fig. 5). Electrophoresis was carried out at pH 4.1 for 2 hr (30 v/cm).

of the genera *Nocardia* and *Streptomyces*, a rather simple method based upon the amino acid composition of the cell walls was proposed (2) for their differentiation: Dpm is present as the LL isomer in *Streptomyces* and as the meso isomer in *Nocardia*. The results described in this

work show that the situation is more complex than originally thought and that the stereochemistry of Dpm is not a sufficient criterion for differentiating between *Nocardia* and *Streptomyces*. Table 2 summarizes the results from the four strains which were studied here and from a strain

TABLE 2. Constituents of the cell walls of various strains of *Nocardia* and "so-called *Nocardia*"^a

Organisms	Origin	Nocardic acids	Sugars		meso-Dpm	Muramic acid		Amide group	
			Gal	Ara		NAc	NGly	Dpm	Glu
<i>N. kirovani</i> IM 1374 ^b	I. Mérieux	+	+	+	+		+	+	+
<i>N. caviae</i> IM 1381	I. Mérieux	+	+	+	+		+	+	+
<i>N. asteroides</i> R 399	Japan	+	+	+	+		+	+	+
<i>N. piracicabensis</i>	Brazil		+	+	+	+		+	
<i>N. mediterranei</i> ME/R 6468	Italy				+	+		+	

^a Abbreviations: NAc, *N*-acetyl-muramic acid; NGly, *N*-glycolyl-muramic acid.^b M. J. Vacheron et al., Eur. J. Biochem. 29: 156-166.

of *N. kirovani* which had previously been studied in our laboratory by M. J. Vacheron et al. (Eur. J. Biochem. 29: 156-166). Two strains, *N. asteroides* R 399 and *N. caviae* IM 1381, showed the same cell wall characteristics as the strain of *N. kirovani*. However, the strain of *N. piracicabensis* and *N. mediterranei* ME/R 6468 did not exhibit these same features, although their cell walls did contain meso-Dpm.

Neutral sugars (arabinose and galactose) were found in the strain of *N. piracicabensis*. The cell walls of *N. mediterranei* ME/R 6468 prepared by sodium dodecyl sulfate treatment (5) contained no sugar, whereas Thiemann (34) found arabinose and galactose in this organism by another preparative procedure. Thus, with this strain the detection of sugar in the cell wall seems to be dependent upon the method of preparation. True nocardiae always contain neutral sugars (arabinose, galactose, and sometimes glucose) in their cell walls. These sugars are the constituents of polysaccharides probably linked to the peptidoglycan and cannot be removed by sodium dodecyl sulfate treatment. An analogous constitution had been shown in the cell walls of mycobacteria (22).

The cell walls of true nocardiae contain nocardic acids, which were identified by comparison with nocardic acids isolated from the whole bacteria. This is analogous to the presence of mycolic acids in the cell walls of mycobacteria (22).

The peptidoglycans of true nocardiae have two main characteristics: (i) the glycan strand is constituted of disaccharide units of β -*N*-acetylglucosaminyl (1 $\rightarrow$ 4)-MurNGly like the glycan of mycobacteria, whereas in all other bacteria, muramic acid is *N*-acetylated; (ii) the peptide moiety is diamidated, whereas in the strains of organisms which do not exhibit the other criteria of true nocardiae, i.e., *N. piracicabensis* and *N. mediterranei*, the peptide moiety is monoamidated on Dpm.

As shown in Table 2, the cell walls of the strains of *N. piracicabensis* and *N. mediterranei* studied here are quite different from the cell walls of true nocardiae, and these two strains should be discarded from the genus *Nocardia* and placed in a different genus, perhaps in a new genus intermediate between *Streptomyces* and *Nocardia*. This action would have two consequences: (i) one of the general criteria for organisms in the genus *Nocardia*, viz., the presence of meso-Dpm and neutral sugars in the cell wall, would be inappropriate because organisms which do not belong to *Nocardia* would possess these characteristics; and (ii) the presence of nocardic acids in the lipid portion of

the cell walls of nocardiae would be a valuable taxonomic and diagnostic characteristic.

In addition, we found two other distinctive features in the cell walls of the true nocardiae studied here: (i) the presence of MurNGly in the glycan part of the peptidoglycan (the glycan part of the peptidoglycan is a polymer of disaccharide fragments— β -N-acetylglucosaminy (1 $\rightarrow$ 4)-MurNGly—linked together through β 1 $\rightarrow$ 4 linkages); (ii) the presence of diamidated peptides in the peptide part of the peptidoglycan (constituted of diamidated tripeptide and tetrapeptide units—L-Ala-D-isoGln-(L)-*meso*-Dpm-(D)NH₂ and L-Ala-D-isoGln-(L)-*meso*-Dpm-(D)NH₂-(L)-D-Ala—crosslinked through a peptide linkage between the C-terminal D-Ala of one peptide unit and the amino group located on the D carbon of *meso*-Dpm of another peptide unit).

If found in other true nocardiae, these two characters may be useful in the identification of those nocardiae not easily identified by the criteria currently used. However, the determination of these characteristics involves a considerable amount of time.

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