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The Genus *Nocardiopsis* Represents a Phylogenetically Coherent Taxon and a Distinct Actinomycete Lineage: Proposal of *Nocardiopsaceae* fam. nov.

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The genus *Nocardiopsis* was shown to be phylogenetically coherent and to represent a distinct lineage within the radiation of the order *Actinomycetales*. The closest relatives of the genus *Nocardiopsis* are members of the genera *Actinomadura*, *Thermomonospora*, *Streptosporangium*, and *Microtetraspora*. The intrageneric structure of the genus *Nocardiopsis* is shown to consist of a highly related species group containing *Nocardiopsis dassonvillei*, *Nocardiopsis alborubida*, and *Nocardiopsis antarctica* and a second group of less highly related species comprising *Nocardiopsis alba* subsp. *alba*, *Nocardiopsis alba* subsp. *prasina*, and *Nocardiopsis listeri*. *Nocardiopsis lucentensis* occupies a position intermediate between the two species groups. The results of a 16S ribosomal DNA sequence analysis are generally consistent with the available chemotaxonomic, phenotypic, and DNA-DNA hybridization data. The phylogenetic position and the morpho- and chemotaxonomic properties of *Nocardiopsis* species support the creation of a family for the genus *Nocardiopsis*, *Nocardiopsaceae* fam. nov.

Meyer described the genus *Nocardiopsis* for the species *Actinomadura dassonvillei* in 1976 on the basis of the morphological characteristics and cell wall type of this organism (21). The genus *Nocardiopsis* currently comprises seven validly described species, *Nocardiopsis alborubidus*, *Nocardiopsis albus*, *Nocardiopsis antarcticus*, *Nocardiopsis dassonvillei*, *Nocardiopsis halophila*, *Nocardiopsis listeri*, and *Nocardiopsis lucentensis*. *Nocardiopsis albus* includes two subspecies, *Nocardiopsis albus* subsp. *albus* and *Nocardiopsis albus* subsp. *prasina*. Arriving at this current taxonomy has involved the exclusion of previously recognized *Nocardiopsis* species and the inclusion of new species transferred from other genera. Five species, *Nocardiopsis coeruleofusca*, *Nocardiopsis flava*, *Nocardiopsis longispora*, *Nocardiopsis mutabilis*, and *Nocardiopsis syringae*, were transferred to the genus *Saccharothrix* on the basis of their chemotaxonomic characteristics (9, 16), and Kroppenstedt et al. (14) have transferred *Nocardiopsis africana* to the genus *Microtetraspora*.

The following two species were created by Grund and Kroppenstedt (10) on the basis of chemotaxonomic and numerical taxonomic data: *Nocardiopsis alborubidus*, for the invalid species "*Actinomyces alborubidus*"; and *Nocardiopsis listeri*, for the invalid streptomycete "*Streptomyces listeri*."

The genus *Nocardiopsis* is currently defined on the basis of chemotaxonomic markers, since *Nocardiopsis* strains cannot be differentiated from members of the genus *Saccharothrix* morphologically (13, 17). The salient chemotaxonomic features, as described previously (13), include phospholipid type III (19) with the diagnostic phospholipids phosphatidylcholine and phosphatidylmethylethanolamine. The menaquinone type is type 4c2 (13), and the main menaquinones contain 10 isoprene subunits in their side chains with variable degrees of saturation. The fatty acid type is type 3d (12), and the fatty acids include iso-branched, anteiso-branched, and 10-methyl-branched fatty acids. High levels of octadecenoic acid (oleic acid) are also present. Diagnostic for all members of the genus *Nocardiopsis* is the combination of 15 to 20% anteiso-$C_{17:0}$ (14-methylhexadecanoic acid) together with 20 to 25% 10-methyl-$C_{18:0}$ (tuberculostearic acid; 10-methyl-octadecanoic acid) or its precursor, oleic acid (7, 12, 22). This combination of fatty acids is unique among bacteria and can be used to differentiate *Nocardiopsis* species from all other bacteria. The peptidoglycan contains *meso*-diaminopimelic acid, and no diagnostic sugars are present (cell wall chemotype III/C [18]). Other chemotaxonomic features include a lack of mycolic acids, the presence of muramic acid of the acetyl type, and DNA G+C contents between 64 and 69 mol% (13). The combination of these characteristics can be used to differentiate *Nocardiopsis* species from other actinomycetes.

Differentiation of *Nocardiopsis* species is currently based on the color of the mycelium and the results of comparative physiological tests (13). Recently, the presence of novel cell wall teichoic acids has been reported in *Nocardiopsis* species (24, 25). The authors of these studies suggested that species-specific teichoic acids are present in *Nocardiopsis dassonvillei*, *Nocardiopsis antarcticus*, and *Nocardiopsis albus* subsp. *albus*, but until this trait has been examined for all *Nocardiopsis* species, its taxonomic value will remain unknown.

In recent years the application of rRNA sequence analysis to the systematics of the actinomycetes has helped bring some order to the taxonomy of this phylum. The combination of new data from rRNA analyses with previously available phenotypic information has given us a better understanding of the true relationships among various actinomycete taxa. The recent review of Embley and Stackebrandt (4) provided a comprehensive overview of the phylogenetic structure of the actinomycetes. In this review, *Nocardiopsis dassonvillei* was considered an actinomycete of uncertain phylogenetic affiliation, but was tentatively placed between the family *Thermomonosporaceae* (2) and the family *Streptosporangiaceae* (8). Although the genus *Nocardiopsis* is phylogenetically related to these families, it can be excluded from them by its unique combination of chemotaxonomic markers.

In order to clarify the phylogenetic position of the genus *Nocardiopsis* and to investigate the phylogenetic coherence of

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TABLE 1. Strains for which 16S rDNA sequence data were obtained in this study

Strain	EMBL accession no.
Nocardiopsis alborubidus DSM 40465^T	X97882
Nocardiopsis albus subsp. *albus* DSM 43377^T	X97883
Nocardiopsis albus subsp. *prasina* DSM 43845^T	X97884
Nocardiopsis antarcticus DSM 43884^T	X97885
Nocardiopsis dassonvillei DSM 43111^T	X97886
Nocardiopsis listeri DSM 40297^T	X97887
Nocardiopsis lucentensis DSM 44048^T	X97888
Actinomadura madurae DSM 43067^T	X97889
Actinomadura kijaniata DSM 43764^T	X97890
Microtetraspora glauca DSM 43311^T	X97891
Microtetraspora salmonea DSM 43678^T	X97892
Thermomonospora curvata DSM 43183^T	X97893

this genus, 16S ribosomal DNA (rDNA) sequence data were obtained from all available type strains and analyzed.

MATERIALS AND METHODS

Strains and culture conditions. The actinomycete strains investigated in this study are listed in Table 1. Cell material for DNA extraction was grown on DSM medium 65 (3). The wet biomass used for fatty acid analysis was obtained from cultures grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) for 4 days at 28°C.

Preparation of genomic DNA and amplification of the 16S rRNA gene. A single colony was removed from an agar surface and dispersed in 400 μl of saline-EDTA buffer (150 mM NaCl, 10 mM EDTA; pH 8.0). The resulting preparation was incubated at 37°C for 30 min, after 5 μl of a lysozyme solution (10 mg/ml) was added. Then 5 μl of a proteinase K solution (15 mg/ml) and 10 μl of a sodium dodecyl sulfate solution (25%, wt/vol) were added; this was followed by incubation at 55°C for 30 min. The lysate was extracted with an equal volume of phenol, and this was followed by centrifugation. An equal volume of chloroform was added to the aqueous layer, and the preparation was mixed and centrifuged. DNA was recovered from the aqueous phase by using a Prep-A-Gene kit (Bio-Rad, Hercules, Calif.). The purified DNA was eluted from the binding matrix in 50 μl of sterile distilled H_2O.

The 16S rDNA was amplified by the PCR in a reaction mixture containing 1× PCR buffer (Boehringer, Mannheim, Germany), each deoxynucleoside triphosphate at a concentration of 200 μM, 50 to 100 ng of genomic DNA, 0.5 μg of primer 27f (5′GAGTTTGATCCTGGCTCAG3′), and 0.5 μg of primer 1525r (5′AGAAAGGAGGTGATCCAGCC3′). The final volume of the PCR mixture was adjusted to 100 μl by adding distilled H_2O, and the reaction mixture was overlaid with 80 μl of sterile mineral oil. Thermal cycling was performed with a model 480 apparatus (Perkin-Elmer, Foster City, Calif.). The samples were subjected to an initial denaturing step consisting of 3 min at 98°C, after which 2 U of *Taq* polymerase was added to each sample at 90°C. The thermal profile used was 28 cycles consisting of 1 min of primer annealing at 52°C, 2 min of extension at 72°C, and 1 min of denaturation at 94°C. A final extension step consisting of 5 min at 72°C was also included. PCR amplificants were detected by agarose gel electrophoresis and were visualized by UV fluorescence after ethidium bromide staining.

Direct sequencing of PCR products. PCR products were purified and concentrated by using a Prep-A-Gene kit (Bio-Rad). DNA was eluted in 50 μl of distilled H_2O, and 0.5 to 1.0 μl of the resulting preparation was used in sequencing reactions. The sequencing reactions were performed with a PRISM Ready-Reaction DyeDeoxy terminator cycle sequencing kit by using Amplitaq FS (Applied Biosystems, Foster City, Calif.) and a Perkin-Elmer Cetus model 9600 thermal cycler according to the protocol and thermal profile recommended by Applied Biosystems. The sequencing primers used were primers 343r (5′CTGC TGCCTCCCGTA3′), 357f (5′TACGGGAGGCAGCAG3′), 519r (5′G[T/A]AT TACCGCGGC[T/G]GCTG3′), 536f (5′CAGC[C/A]GCCGCGGTAAT[T/A]C 3′), 803f (5′ATTAGATACCCTGGTAG3′), 907r (5′CCGTCAATTCATTTGA GTTT3′), 1114f (5′GCAACGAGCGCAACCC3′), and 1385r (5′CGGTGTGT [A/G]CAAGGCCC3′). Sequence reaction mixtures were purified as recommended by Applied Biosystems and were electrophoresed on a 6% (wt/vol) polyacrylamide sequencing gel for 12 h by using an Applied Biosystems model 373A automated DNA sequencer.

Phylogenetic analysis. The 16S rDNA sequences obtained in this study were manually aligned with actinomycete reference sequences obtained from the Ribosomal Database Project (20). Because many of the actinomycete reference sequences obtained from databases were partial sequences consisting of less than 1,300 nucleotides, two data sets were produced. The data set comprising the sequences generated in this study and the actinomycete reference sequences contained information for 1,236 unambiguous nucleotide positions present in all sequences between positions 51 and 1471 (*Escherichia coli* numbering [1]). When only the *Nocardiopsis* sequences generated in this study and the sequence of *Microtetraspora glauca* were used, a second data set, which contained information for 1,437 unambiguous nucleotide positions present in these sequences between positions 34 and 1500 (*E. coli* numbering [1]), was produced. Evolutionary distances were calculated by the method of Jukes and Cantor (11). Phylogenetic dendrograms were reconstructed by using treeing algorithms contained in the PHYLIP package (6). Tree topologies were evaluated by performing bootstrap analyses (5) of the neighbor-joining data, using 1,000 resamplings.

Saponification, methylation, extraction, and analysis of fatty acid methyl esters. Fatty acid methyl esters were obtained from wet biomass (ca. 40 mg) by saponification, methylation, and extraction (15). The fatty acid methyl ester mixtures were separated by using a model 5898A microbial identification system apparatus (Microbial ID, Newark, Del.). The fatty acid data obtained for the strains were compared qualitatively and quantitatively by using Ward's method (26) and the Microbial Identification System Library Generation software (Microbial ID, Newark, Del.).

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in this study have been deposited in the EMBL data library under the accession numbers shown in Table 1. The accession numbers of the sequences of the strains used as representatives of the main actinomycete groups are as follows: *Actinoplanes philippinensis* DSM 43019^T (T = type strain), X93187; *Arthrobacter globiformis* DSM 20124^T, M23411; *Atopobium minutum* ATCC 33267^T, M59059; *Bifidobacterium bifidum* ATCC 29521^T, M38018; *Cellulomonas flavigena* DSM 20109^T, X83799; *Dactylosporangium aurantiacum* DSM 43157^T, X93191; *Microbacterium lacticum* DSM 20427^T, X77441; *Microlunatus phosphovorus* JCM 9379^T, D26169; *Micromonospora chalcea* DSM 43026^T, X92549; *Mycobacterium tuberculosis* NCTC 7416^T, X58890; *Nocardia asteroides* DSM 43757^T, X80606; *Nocardioides albus* DSM 43109^T, X53211; *Propionibacterium freudenreichii* DSM 20271^T, X53217; *Saccharopolyspora rectivirgula* ATCC 33515^T, X53194; *Saccharothrix australiensis* ATCC 31497^T, X5192; *Sporichthya polymorpha* DSM 46113^T, X72377; *Streptomyces ambofaciens* ATCC 23877^T, M27245; *Streptomyces griseus* NCTC 9080, X61478; *Streptosporangium longisporum* DSM 43180^T, X89944; and *Streptosporangium roseum* DSM 43021^T, X89947.

RESULTS AND DISCUSSION

Correction of names. In the descriptions of some *Nocardiopsis* species and subspecies, the names were incorrectly derived. The names are therefore corrected as follows: *Nocardiopsis alborubida* corrig. (for *Nocardiopsis alborubidus* [sic]), *Nocardiopsis antarctica* corrig. (for *Nocardiopsis antarcticus* [sic]), *Nocardiopsis alba* subsp. *alba* corrig. (for *Nocardiopsis albus* subsp. *albus* [sic]), and *Nocardiopsis alba* subsp. *prasina* corrig. (for *Nocardiopsis albus* subsp. *prasina* [sic]). The correct names are used below.

Phylogenetic analyses. Almost complete 16S rDNA sequence data (>95% of the *E. coli* sequence [1]) were obtained for the type strains of six species of *Nocardiopsis*, including the type strains of the two subspecies of *Nocardiopsis alba*. Sequence data could not be obtained for *Nocardiopsis halophila* as a culture was not provided when it was requested from the original authors. Five new reference sequences were determined for the type strains of *Actinomadura madurae*, *Actinomadura kijaniata*, *Thermomonospora curvata*, *Microtetraspora glauca*, and *Microtetraspora salmonea* in order to include in the comparison the type species of three genera that are considered related to the genus *Nocardiopsis* (4).

The phylogenetic dendrogram shown in Fig. 1 was reconstructed from evolutionary distances by the neighbor-joining method. A total of 1,236 nucleotides present in all strains between position 51 and position 1471 (*E. coli* numbering [1]) were used for this analysis. Phylogenetic analyses in which the maximum-likelihood and unrooted parsimony methods were used produced very similar results. A phylogenetic analysis based on a comparison of the 16S rDNA sequence data showed that the genus *Nocardiopsis* is phylogenetically homogeneous, with all six species forming a distinct lineage within the radiation of the actinomycetes (Fig. 1).

Phylogenetic relationship of the genus *Nocardiopsis* with other actinomycetes. The distinct position of the genus *Nocardiopsis* and the association of this taxon with the *Actinoma-*

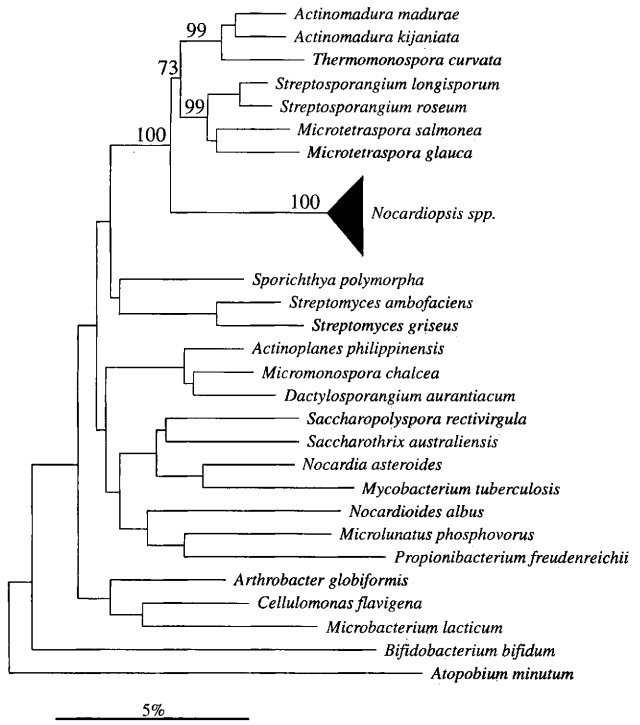

FIG. 1. Phylogenetic dendrogram reconstructed from evolutionary distances (11) by the neighbor-joining method (23), indicating the position of *Nocardiopsis* species within the radiation of representatives of the main lineages of the order *Actinomycetales*. Scale bar = 5 inferred nucleotide substitutions per 100 nucleotides. The numbers at the nodes are bootstrap values.

dura-Thermomonospora-Streptosporangium-Microtetraspora (ATSM) group were found with all of the phylogenetic analysis methods used. The relationship of the genus *Nocardiopsis* to the ATSM cluster was supported by a bootstrap value of 100%. The levels of 16S rDNA sequence similarity between the members of the *Nocardiopsis* cluster and the members of the ATSM group and between the members of the *Nocardiopsis* cluster and the members of other actinomycete lineages were 91.4 to 93.6 and 82.2 to 91.9%, respectively. These data clearly indicate that the phylogenetic position of the *Nocardiopsis* lineage is isolated and that this taxon does not fall within the radiation of the families *Thermomonosporaceae* and *Streptosporangiaceae* or the other families currently placed in the order *Actinomycetales*.

The phylogenetic distances between the *Nocardiopsis* species cluster and the ATSM group (levels of sequence dissimilarity, 6.7 to 9.2%) and between the *Nocardiopsis* species cluster and representatives of other actinomycete lineages (levels of sequence dissimilarity, 8.6 to 20.3%) can be recognized at the level of the primary structure of the 16S rDNA. There are signature nucleotides which distinguish the genus *Nocardiopsis* from members of the ATSM group (Table 2). Although the majority of the *Nocardiopsis*-specific nucleotides are single nonpairing bases that are widely dispersed in the primary structure and thus of little value as targets for specific oligonucleotide probes or primers, the region from position 1435 to position 1466, containing eight signature nucleotides, could be used as a target site. In addition to these unique nucleotides, a long helix similar to that found in the slowly growing *Mycobacterium* species was found between *E. coli* positions 455 and 479 in all six *Nocardiopsis* species investigated. This extended loop can be considered an additional genus characteristic; the nucleotide composition of this region may be useful for species differentiation (see below).

TABLE 2. Signature nucleotides that can be used to distinguish members of the genus *Nocardiopsis* from *Actinomadura*, *Thermomonospora*, *Streptosporangium*, and *Microtetraspora* species

Position(s)[a]	Nucleotide(s) in:		
	Nocardiopsis	*Actinomadura* and *Thermomonospora*	*Streptosporangium* and *Microtetraspora*
156:165	G:C	C:G/U:G	C:G/U:G
187	U	G	G
280	U	C	C
281	U	G	G
344	G	A	A
447	A	G	G
451	C	A	A
611	A	C	C
629	U	G	G
1004	G	A	A
1005	G	A	A/C
1028	A	C	C
1034	U	G	A
1034′	U	—[b]	—
1034″	G	—	—
1256′	G	—	—
1435:1466	A:U	G:C	G:C
1436:1465	C:G	C · U	C · U
1437:1464	U:A	C:G	C:G
1438:1463	U:G	C:G	C:G

[a] *E. coli* numbering (1).
[b] —, nucleotides are not present at this position.

Intrageneric structure of the genus *Nocardiopsis*. The intrageneric relationships based on comparisons of the 16S rDNA sequences of the six *Nocardiopsis* species investigated in this study are shown in Fig. 2, which was derived from a comparison of the nucleotides at 1,437 base positions, as indicated above. The corresponding 16S rDNA sequence similarity values are shown in Table 3. These data clearly demonstrate that the genus *Nocardiopsis* is a phylogenetically shallow taxon; the levels of sequence similarity for the *Nocardiopsis* strains are between 97.8 and 99.7% (Table 3). *Nocardiopsis dassonvillei*, *Nocardiopsis alborubida*, and *Nocardiopsis antarctica* form one cluster (levels of 16S rDNA sequence similarity, 99.5 to 99.7%), which is supported by a bootstrap value of 99%. *Nocardiopsis alba* subsp. *alba*, *Nocardiopsis alba* subsp. *prasina*, and *Nocardiopsis listeri* cluster together loosely, and the branching pat-

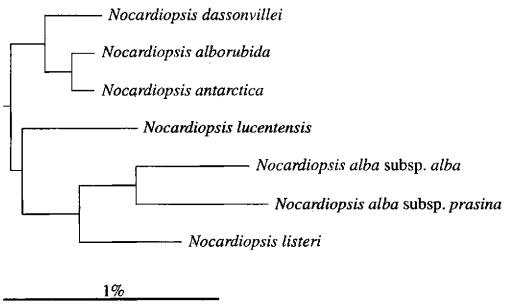

FIG. 2. Phylogenetic dendrogram reconstructed from evolutionary distances (11) by the neighbor-joining method (23), indicating the intrageneric relationships of members of the genus *Nocardiopsis*. The position of the root was determined by including the sequence of *Microtetraspora glauca*. Scale bar = 1 inferred nucleotide substitution per 100 nucleotides.

TABLE 3. Levels of 16S rDNA sequence similarity between species and subspecies belonging to the genus *Nocardiopsis*

Species or subspecies	% Similarity					
	Nocardiopsis dassonvillei	*Nocardiopsis alborubida*	*Nocardiopsis antarctica*	*Nocardiopsis lucentensis*	*Nocardiopsis alba* subsp. *alba*	*Nocardiopsis alba* subsp. *prasina*
Nocardiopsis alborubida	99.5					
Nocardiopsis antarctica	99.5	99.7				
Nocardiopsis lucentensis	98.8	98.8	98.7			
Nocardiopsis alba subsp. *alba*	98.6	98.5	98.5	98.8		
Nocardiopsis alba subsp. *prasina*	98.1	98.2	98.2	98.5	98.9	
Nocardiopsis listeri	97.9	97.8	97.9	98.2	98.3	98.7

tern of these organisms is not supported by bootstrap analysis data. Although *Nocardiopsis lucentensis* branches between these two clusters, the levels of sequence similarity between the species belonging to the second cluster are no greater than the levels of similarity between these species and *Nocardiopsis lucentensis*. The long helix found at *E. coli* positions 455 to 479 in all of the *Nocardiopsis* species investigated has a unique sequence in each of the following taxa: *Nocardiopsis dassonvillei*, *Nocardiopsis alborubida*, *Nocardiopsis antarctica*, *Nocardiopsis lucentensis*, and *Nocardiopsis alba* subsp. *alba*. Another unique sequence is found at these positions in the *Nocardiopsis alba* subsp. *prasina* and *Nocardiopsis listeri* strains. Although the nucleotide sequence of this stretch may prove to be useful for species identification, it is clear that this characteristic has little phylogenetic significance since two unrelated taxa, *Nocardiopsis alba* subsp. *prasina* and *Nocardiopsis listeri*, have the same sequence in this region.

Comparison of phenotype, chemotype, and phylotype. The results of the phylogenetic analysis of the six species of the genus *Nocardiopsis*, which demonstrated that this actinomycete lineage is distinct, are consistent with the uniqueness of this genus shown by phenotypic and chemotaxonomic data. At the species level, the close relationship of the six species based on 16S rDNA sequence analysis data indicates that the species boundary should be investigated fully by DNA-DNA hybridization analysis. To date, a comprehensive DNA-DNA hybridization study of all *Nocardiopsis* species has not been carried out. However, the limited DNA-DNA hybridization data presented in the description of *Nocardiopsis lucentensis* (27) could be compared with the 16S rDNA sequence analysis data. These DNA-DNA hybridization data demonstrated that *Nocardiopsis lucentensis* is a true species when this organism was compared with *Nocardiopsis dassonvillei*, *Nocardiopsis alborubida*, *Nocardiopsis listeri*, *Nocardiopsis alba* subsp. *alba*, and *Nocardiopsis alba* subsp. *prasina* (levels of DNA-DNA binding, 40 to 46%). An additional *Nocardiopsis* strain, designated strain A_{4-2} (which was not investigated in this study), was found to be phenotypically identical to *Nocardiopsis lucentensis*, but on the basis of a DNA-DNA binding value of 39% was clearly not related. This strain exhibited 65 and 63% DNA-DNA binding to *Nocardiopsis dassonvillei* and *Nocardiopsis alborubida*, respectively, which indicated that the latter two taxa are closely related. This relationship was reflected in the phylogeny derived from the 16S rDNA sequence analysis performed in this study.

There is some correlation between the results of the phylogenetic analysis based on 16S rDNA sequence data and the results of the cluster analysis of fatty acid composition data (Fig. 3). On the basis of quantitative differences in their fatty acid patterns, the *Nocardiopsis* species can be separated into three clusters. As demonstrated in the 16S rDNA sequence analysis, *Nocardiopsis dassonvillei*, *Nocardiopsis antarctica*, and *Nocardiopsis alborubida* are similar, while *Nocardiopsis listeri*, *Nocardiopsis alba* subsp. *alba*, and *Nocardiopsis alba* subsp. *prasina* also cluster together. *Nocardiopsis lucentensis* has a distinct fatty acid type, which is not similar to the fatty acid types of the other *Nocardiopsis* species. The close relationship between *Nocardiopsis dassonvillei* and *Nocardiopsis antarctica* is also supported by the presence of a unique cell wall teichoic acid which has been found in these two species (24), while a different teichoic acid has been found in *Nocardiopsis alba* subsp. *alba* (25).

In contrast, there is little correlation between the phylogenetic data and the menaquinone composition data available. Grund and Kroppenstedt (10) found that *Nocardiopsis dassonvillei* and the two subspecies of *Nocardiopsis alba* have the same menaquinone composition. A different menaquinone composition was found for *Nocardiopsis alborubida* and *Nocardiopsis listeri* (10), while a third type was reported for *Nocardiopsis lucentensis* (27). This apparent lack of congruence provides another example of the difficulties involved in comparing the evolution of menaquinones (9).

A comparison of the 16S rDNA sequence data obtained in this study and the results of the numerical taxonomic analysis of Grund and Kroppenstedt (10) is of little value, as the inclusion of chemotaxonomic characters (i.e., menaquinone composition) in the latter study led to the placement of *Nocardiopsis alborubida* and *Nocardiopsis listeri* in an isolated position. The former species was placed outside the *Nocardiopsis* species cluster, while the latter species appeared to be no more similar to the *Nocardiopsis alba* subspecies than to *Nocardiopsis dassonvillei*.

In conclusion, a phylogenetic analysis based on the results of a 16S rDNA sequence comparison revealed that the genus *Nocardiopsis* represents a distinct lineage within the actinomycetes and hence is a taxonomically valid genus which exhibits some relatedness to the ATSM group. On the basis of the isolated phylogenetic position and the unique morpho- and

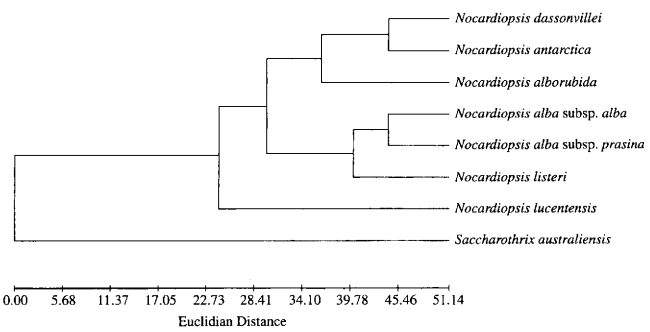

FIG. 3. Dendrogram showing relationships among *Nocardiopsis* species. The dendrogram is based on differences in the fatty acid patterns of the organisms.

chemotaxonomic properties of the genus *Nocardiopsis*, we propose that a new family should be created for this genus. The genus *Nocardiopsis* contains very closely related species. Whether the six *Nocardiopsis* species investigated in this study represent genomically well-separated species cannot be decided on the basis of 16S rDNA sequence analysis data; an answer to this question will require a comprehensive DNA-DNA hybridization study involving all available species of the genus *Nocardiopsis*.

Description of *Nocardiopsaceae* fam. nov. Rainey, Ward-Rainey, Kroppenstedt, and Stackebrandt. *Nocardiopsaceae* (No.car.di.op.sa'ce.ae. M. L. n. *Nocardiopsis*, type genus of the family; L. ending *-aceae*, ending denoting a family; M. L. n. *Nocardiopsaceae*, the *Nocardiopsis* family). The description is the same as the description given for the type genus, the genus *Nocardiopsis* Meyer 1976, 487^{AL} (21).

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