Description of Nocardiopsis synnemataformans sp. nov., Elevation of Nocardiopsis alba subsp. prasina to Nocardiopsis prasina comb. nov., and Designation of Nocardiopsis antarctica and Nocardiopsis alborubida as Later Subjective Synonyms of Nocardiopsis dassonvillei

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Data from chemotaxonomic and 16S ribosomal DNA sequence analyses of an isolate obtained from the sputum of a kidney transplant patient identified the isolate as a member of the genus Nocardiopsis. DNA-DNA hybridization data, as well as physiological characteristics, indicated that the isolate represents a new species of the genus Nocardiopsis, designated Nocardiopsis synnemataformans; the type strain is strain IMMD D-1215 (= DSM 44143). In addition, DNA-DNA hybridization data, as well as the results of biochemical tests, indicated that Nocardiopsis alborubida DSM 40465 T, Nocardiopsis antarctica DSM 43884T, and Nocardiopsis dassonvillei DSM 43111T represent a single species designated N. dassonvillei. We also found that Nocardiopsis alba subsp. alba DSM 43377 T and N. alba subsp. prasina DSM 43845T are genetically different and therefore propose that N. alba subsp. prasina be elevated to species status as Nocardiopsis prasina comb. nov., whose type strain is strain DSM 43845.

The genus Nocardiopsis was described by Meyer (25) for bacteria that were previously designated either “Streptothrix dassonvillei” (5), “Nocardia dassonvillei” (12), or “Actinomadura dassonvillei” (19). Currently, the genus Nocardiopsis comprises the following validly described species and subspecies: Nocardiopsis dassonvillei (25), Nocardiopsis alborubida (15), Nocardiopsis antarctica (1), Nocardiopsis listeri (15), Nocardiopsis lucentensis (38), Nocardiopsis halophila (2), Nocardiopsis alba subsp. alba (15), and N. alba subsp. prasina (15, 28). All of the species contain meso-diaminopimelic acid in their peptidoglycan and no characteristic sugars in whole-cell hydrolysates (i.e., they have cell wall chemotype III), have a type II phospholipid pattern (phosphatidylcholine is the characteristic phospholipid), contain characteristic menaquinones with 10 isoprene units having a high degree of hydrogenation [MK-10(H10, H8)], and have fatty acid profiles which include saturated, unsaturated, iso, and anteiso acids and tuberculostearic acid.

In a recent review of the phylogenetic structure of the actinomycetes, the genus Nocardiopsis was shown to represent a distinct lineage within the radiation of the order Actinomycetales (9). A combination of phylogenetic position and morphological and chemotaxonomic properties supported the creation of the family Nocardiopsiaceae, which includes the genus Nocardiopsis (30). The 16S ribosomal DNA (rDNA)-based intragenic structure of the genus Nocardiopsis was shown to include a highly related species group containing N. dassonvillei, N. alborubida, and N. antarctica and a second group of closely related species comprising N. alba subsp. alba, N. alba subsp. prasina, and N. listeri. N. lucentensis occupies a position intermediate between the two groups (30).

The actinomycete genera that are considered human pathogens include the genera Actinomadura, Cellulomonas, Corynebacterium, Dermatophilus, Mycobacterium, Nocardia, Nocardiopsis, Rhodococcus, and Streptomyces. Although Nocardiopsis species are infrequently encountered in clinical practice, N. dassonvillei is a potential cause of human infections, including conjunctivitis (22), mycetomas (33), skin infections (29, 34), and extrinsic alveolitis (4). In this report we describe the morphological, chemotaxonomical, physiological, and phylogenetic characteristics of strain IMMD D-1215 T, which was isolated from the sputum of a kidney transplant patient. We also investigated the species boundaries of members of the genus Nocardiopsis by performing a DNA-DNA hybridization study.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The Nocardiopsis strains used in this study included N. dassonvillei DSM 43111 T, N. alborubida DSM 40465 T, N. listeri DSM 40297 T, N. alba subsp. alba DSM 43377 T, N. alba subsp. prasina DSM 43845 T, N. antarctica DSM 43884 T, and N. lucentensis DSM 44045 T. N. halophila was not included in this study because a culture was not provided when it was requested from the original authors. Strain IMMD D-1215 T was isolated from the sputum of a 35-year-old Turkish patient who had received a renal transplant. All of the strains were subcultured as described previously (40) on brain heart infusion (BHI) agar (Difco), glucose-yeast extract-malt extract (GYM) agar, and the media of Shirling and Gottlieb (32).

Morphology and pigmentation. Strain IMMD D-1215 T was grown on yeast extract-malt-extract agar (ISP medium 2), oatmeal agar (ISP medium 3), and inorganic salts-starch agar (ISP medium 4), as described by Shirling and Gottlieb (32), and on GYM agar and was examined for pigmentation, aerial mycelia, and other morphological features. Cultures were grown at 37°C for 4 weeks, and observations were made at weekly intervals. Air-dried smears from GYM agar were stained by Gram’s method and the Ziehl-Neelsen method in order to determine the Gram reaction and acid fastness, respectively. For scanning electron microscopy, a culture grown at 37°C for 5 days on ISP medium 2 was prepared by cutting agar blocks from the growth medium, fixing them with glutaraldehyde, and dehydrating them by using a graded ethanol series. The

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dehydrated blocks were then critical point dried, mounted on aluminum stubs, and sputter coated with gold-palladium. Finally, they were observed with a Zeiss digital scanning electron microscope (model DSM 950).

**Chemical characteristics.** Peptone-yeast extract-iron agar (ISP medium 6) and tyrosine agar (ISP medium 7) were used to determine melanoid pigment production. Decomposition of adenine, guanine, hypoxanthine, xanthine, tyrosine, elastin, keratin, and testosterone was determined by the method of Gordon and Smith (14), esculin decomposition was determined as described by Gordon (10), and casein and gelatin hydrolysis was determined by the method of Gordon and Mihan (13). Urea decomposition was tested by using urea agar base (Oxoid code CM 53) after the addition of 2.2% urea. Carbohydrate assimilation and simultaneous utilization of a substrate as a carbon source and a nitrogen source were determined as described previously (40).

Nitrate reductase was determined as described by Gordon (11). β-Glucosidase and β-galactosidase activities were determined by the method of Tsuchakura (36). Phosphatase activity was determined by the method of Kupur and Schmitt (17). Tolerance to salt was determined by growing the organism on GYM agar plates supplemented with 0, 4, 6, 8, 10, 12, and 14% (wt/vol) NaCl. The sensitivity of the organism to various antibiotics was studied by using the agar dilution technique with microscopic reading of the results as previously described (31). Various concentrations (0.20 to 128.0 pg/ml) of mezlocillin, amoxicillin plus clavulanic acid, erythromycin, clindamycin, vancomycin, gentamicin, amikacin, tobramycin, imipenem, tetracycline, ciprofloxacin, and ofloxacin were tested.

**Cell chemistry.** For chemotaxonomic analyses, freeze-dried cells were obtained from cultures grown in BHI broth (Difco) on a rotary shaker at 37°C for 7 days. Analyses of whole-cell hydrolysates for characterization of amino acids and sugars were performed as described by Becker et al. (3) and Lechevalier (20). Respectively, cellular fatty acid and mycolic acid methyl esters were prepared from whole-cell methanolysates as described previously (27). The presence of both fatty acid and mycolic acid methyl esters was detected by thin-layer chromatography, and the fatty acid methyl ester profile was determined by gas chromatography-mass spectrometry. Menaquinones were extracted and purified as previously described (8), and the menaquinone composition was determined by using a Finnigan Mat 212 mass spectrometer. Phospholipids were extracted, purified, and characterized as previously described (39).

DNA isolation and characterization. DNA was isolated by chromatography on hydroxyapatite by using the method of Cashon et al. (7). Guanine-plus-cytosine (G+C) contents were determined by high-performance liquid chromatography (24). DNA-DNA hybridization studies were carried out by using the thermal denaturation method (38).

**16s rDNA sequence determination.** Genomic DNA extraction, PCR-mediated amplification of 16S rDNA genes, and a sequence determination were performed as described by Rainey et al. (30). A model 373A DNA sequencer (Applied Biosystems, Inc., Foster City, Calif.) was used to electrophorese the sequencing reaction mixtures. The 16S rDNA sequences determined in this study were manually aligned with previously published sequences (23) using the aez method.

**Nucleotide sequence accession number.** The 16s rDNA sequence of strain IMMIB D-1215T is available from the EMBL under accession no. Y15393.

**RESULTS**

**Colonial morphology.** Strain IMMIB D-1215T grew well on BHI agar, GYM agar, ISP medium 2, and ISP medium 3. On these media the vegetative hyphae were pimento colored and the aerial hyphae were white. On ISP medium 4 growth was weak, and the vegetative hyphae appeared to be yellowish and to bear white aerial mycelia. No diffusible pigment was produced.

**Micromorphology.** The hyphae of strain IMMIB D-1215T were gram positive and not acid fast. The vegetative hyphae were well-developed with irregular branches penetrating the agar and thus formed compact colonies on the agar surface. Synnemata were formed from spiral aerial hyphae that fragment into nonmotile spores. Synnemata were well-developed with irregular branches penetrating the agar surface, and the vegetative hyphae appeared to be yellowish and to bear white aerial mycelia. No diffusible pigment was produced.

**Physiological characteristics.** Strain IMMIB D-1215T decomposed adenine, esculin, elastin, gelatin, casein, hypoxanthine, tyrosine, xanthine, and urea but not guanine, keratin, and testosterone. It utilized xylose, glucose, galactose, rhamnose, maltose, cellobiose, mannositol, citrate, and gluconate as carbon sources but did not utilize arabinitol, lactose, raffinose, trehalose, sucrose, melezitose, adonitol, meso-erythritol, myo-inositol, sorbitol, acetyl, benzolate, lactate, α-hydroxybenzoate, p-hydroxybenzoate, adipate, isovalyl alcohol, 2,3-butanediol, 1,2-propanediol, and paraffin. Table 1). Strain IMMIB D-1215T utilized alanine, gelatin, and proline as simultaneous carbon and nitrogen sources, but did not utilize acetamide and serine. Catalase, nitrate reductase, β-glucosidase, β-galactosidase, and phosphatase activities were detected. Cultures tolerated NaCl at levels up to and including 10%. Melanine pigments were not produced on either peptone-yeast extract-iron agar (ISP medium 6) or tyrosine agar (ISP medium 7). The following Mics were measured: mezlocillin, 2.0 pg/ml; amoxicillin plus clavulanic acid, 2.0 pg/ml; imipenem, 2.0 pg/ml; erythromycin, 8.0 pg/ml; clindamycin, >128 pg/ml; tetracycline, ≤0.2 pg/ml; vancomycin, ≤0.2 pg/ml; gentamicin, ≤0.2 pg/ml; tobramycin, ≤0.2 pg/ml; amikacin, ≤0.2 pg/ml; ciprofloxacin, 2.0 mg/ml; and ofloxacin, 8.0 mg/ml.

**Cell chemistry.** The cell wall of strain IMMIB D-1215T contained meso-diaminopimelic acid and no characteristic sugars (wall chemotype III). Mycolic acids were not detected. The fatty acid profile consisted of major amounts of straight-chain saturated and unsaturated fatty acids and branched-chain fatty acids of the iso and anteiso types in addition to 10-methyl branched-chain fatty acids with 16, 17, and 18 carbon atoms. The phospholipid type was PIII sensu Lechevalier et al. (21), and phosphatidylcholine was the characteristic phospholipid. Other phospholipids, such as phosphatidylethanolamine, phosphatidylglycerol, and diphasatidylglycerol were also detected. The predominant menaquinones were MK-10 and MK-10(H9); in addition, there were detectable amounts of MK-10(H11), MK-10(H12), and MK-10(H13) and traces of MK-9.

**DNA characteristics.** The G+C content of the DNA of strain IMMIB D-1215T was 74.1 mol%. The levels of relatedness as determined by DNA-DNA hybridization between strain IMMIB D-1215T DNA and DNAs from N. dssonvillii DSM 43111T, N. alborubida DSM 40465T, N. antarctica DSM 43884T, N. lucentensis DSM 44048T, N. alba subsp. alba DSM 43377T, N. alba subsp. prasina DSM 43845T, and N. listeri DSM 40297T are given in Table 2 and ranged from 14.0 to 55.2%.

**16s rDNA sequence analysis.** A data set comprising the 16s rDNA sequence of strain IMMIB D-1215T generated in this study and Nocardiosis reference sequences contained information on 1460 unambiguous nucleotide positions present in all sequences between positions 34 and 1503 (Escherichia coli numbering [7]). Pairwise similarity values for sequences in this data set are listed in Table 3. The 16S rDNA sequence similarity values between strain IMMIB D-1215T and the reference strains ranged from 97.7 to 99.8% (Table 3). These results clearly demonstrate that strain IMMIB D-1215T is a member of the phylogenetic cluster defined previously that represents the genus Nocardiosis (30). The highest 16s rDNA sequence similarity values between strain IMMIB D-1215T and reference strains were the similarity values with N. antarctica, N. alborubida, and N. dssonvillii (99.8, 99.5, and 99.5%, respectively).

**DISCUSSION**

Chemotaxonomic data indicate that strain IMMIB D-1215T is similar to members of the genus Nocardiosis (25, 26). All of these organisms possess wall chemotype III, phospholipid type PIII, hydrogynated menaquinones with 10 isoprene units, and a fatty acid profile that includes saturated, unsaturated, iso, anteiso, and 10-methyl branched-chain fatty acids. They are also morphologically similar in that they produce zigzag aerial hyphae that fragment into nonmotile spores.

The results of 16s rDNA sequence comparisons clearly
demonstrate that strain IMMIB D-1215^T is a member of the genus \textit{Nocardiopsis}. The high degrees of 16S rDNA sequence similarity (99.3 to 99.8\%) to the species \textit{N. dassonvillei}, \textit{N. alborubida}, and \textit{N. antarctica} place strain IMMIB D-1215^T in this cluster (30) but do not define species affiliation. The results of DNA-DNA hybridization studies confirm that strain IMMIB D-1215^T is a member of a novel species. Although the degree of 16S rDNA sequence similarity between strain IMMIB D-1215^T 16S rDNA and \textit{N. antarctica} 16S rDNA (99.8\%) is higher than the degree of 16S rDNA similarity between strain IMMIB D-1215^T 16S rDNA and the 16S rDNAs of \textit{N. antarctica}, \textit{N. alborubida}, and \textit{N. dassonvillei} (99.5 to 99.7\%), the results of DNA-DNA hybridization studies show that the latter three species are synonyms and hybridize at levels of 73.7 to 94.0\%, while strain IMMIB D-1215^T is a distinct species whose highest level of hybridization is 55.2\%. These results demonstrate the limited usefulness of 16S rDNA sequence data for differentiating organisms at the species level in cases involving highly related species and/or strains.

In contrast to the chemotaxonomic similarities between isolate IMMIB D-1215^T and members of the genus \textit{Nocardiopsis}, the results of our physiological tests (Table 1) show that there are clear differences between strain IMMIB D-1215^T and other species of the genus \textit{Nocardiopsis}. Strain IMMIB D-1215^T differs from \textit{N. dassonvillei}, \textit{N. alborubida}, and \textit{N. antarctica} by its ability to utilize rhamnose and maltose and its inability to
TABLE 1. Differential physiological characteristics of strain IMMIB D-1215<sup>T</sup> and other members of the genus *Nocardiopsis*<sup>4</sup>

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<th>N. alborubida DSM 40465&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. antarctica DSM 43884&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. listeri DSM 40297&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. lucenensis DSM 44048&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. alba DSM 43377&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. prasina DSM 43845&lt;sup&gt;T&lt;/sup&gt;</th>
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* +, utilized; -, not utilized; w, weakly utilized; d, variably utilized.

We propose on the basis of 16S rDNA sequence analysis and chemotaxonomic data that strain IMMIB D-1215<sup>T</sup> belongs to the genus *Nocardiopsis* and represents, on the basis of DNA-DNA hybridization data and biochemical tests, a new species designated *Nocardiopsis synnemataformans* sp. nov.; this strain has been deposited in the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen as strain DSM 44143. A description of the new species is given below.

**Description of Nocardiopsis synnemataformans** sp. nov. *Nocardiopsis synnemataformans* (syn. ne. ma. ta. for'mans. Gr. adv. syn, together; Gr. n. nema, thread; Gr. n. synnema, threads wrapping together; L.v. formare, to form; L. pres. part. for-mans, forming; M. L. part. adj. synnemataformans, synnema forming, referring to the ability of the organism to form synnema). The substrate mycelium is deep pimento colored, penetrates the agar, and bears aerial mycelia; the aerial myce-

TABLE 2. Degrees of DNA binding between strain IMMIB D-1215<sup>T</sup> and other strains of the genus *Nocardiopsis*

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<thead>
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<th>Organism</th>
<th>Binding to:</th>
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<td></td>
<td>IMMIB D-1215&lt;sup&gt;T&lt;/sup&gt;</td>
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<td>N. dassonvillei DSM 43111&lt;sup&gt;T&lt;/sup&gt;</td>
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<td>N. alba subsp. prasina DSM 43845&lt;sup&gt;T&lt;/sup&gt;</td>
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* ND, not determined.
TABLE 3. Levels of 16S rDNA sequence similarity between strain IMMIB D-1215\textsuperscript{T} and *Nocardiopsis* reference strains

<table>
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<tr>
<th>Taxon</th>
<th>IMMIB D-1215\textsuperscript{T}</th>
<th>N. antarctica</th>
<th>N. alborubida</th>
<th>N. dassonvillie</th>
<th>N. lucenensis</th>
<th>N. alba subsp. alba</th>
<th>N. alba subsp. prasina</th>
<th>N. listeni</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. antarctica</td>
<td>99.8</td>
<td>99.5</td>
<td>99.7</td>
<td>99.5</td>
<td>99.5</td>
<td>98.5</td>
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<td>98.2</td>
</tr>
<tr>
<td>N. alborubida</td>
<td>99.5</td>
<td>99.7</td>
<td>99.7</td>
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<td>99.5</td>
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</tr>
<tr>
<td>N. dassonvillie</td>
<td>99.3</td>
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<td>99.5</td>
<td>99.5</td>
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</tr>
<tr>
<td>N. lucenensis</td>
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<td>98.7</td>
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<tr>
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<tr>
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<td>98.2</td>
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</tr>
<tr>
<td>N. listeni</td>
<td>97.7</td>
<td>97.9</td>
<td>97.8</td>
<td>97.9</td>
<td>97.9</td>
<td>98.2</td>
<td>98.3</td>
<td>98.7</td>
</tr>
</tbody>
</table>

that species boundaries occur at DNA similarity values of approximately 70% (37). The type strains of *N. dassonvillie*, *N. antarctica*, and *N. alborubida* should be considered members of a single species. If taxa of equal rank are unified, the oldest legitimate name or epithet should be retained for the new combination according to Rule 42 of the *International Code of Nomenclature of Bacteria* (18). In this case, only the specific epithet of *N. dassonvillie* was included on the Approved Lists of Bacterial Names (35), as *N. dassonvillie*; therefore, this epithet has nomenclatural priority over the epithets of *N. alborubida* and *N. antarctica*, which were validly published in 1990 (15) and 1983 (1, 16), respectively.

A study of the intrageneric relationship of members of the genus *Nocardiopsis* based on 16S rDNA sequence comparison showed that *N. alba* subsp. *alba* and *N. alba* subsp. *prasina* cluster loosely (30). The close relatedness of these two subspecies based on 16S rDNA sequence analysis data was further investigated by DNA-DNA hybridization. The resulting binding value of 24.25% indicated clearly that genomically the two subspecies belong to different species, assuming that species boundaries occur at DNA similarity values of approximately 70% (37).

Based on DNA-DNA hybridization data reported here, we propose that *N. alba* subsp. *prasina* should be elevated to species rank as *Nocardiopsis prasina* (type strain, DSM 43845). The description of this taxon is the description presented by Grund and Kroppenstedt (15).

REFERENCES


