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 IMMIB 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 43884^T, and *Nocardiopsis dassonvillei* DSM 43111^T represent a single species designated *N. dassonvillei*. We also found that *Nocardiopsis alba* subsp. *alba* DSM 43377^T and *N. alba* subsp. *prasina* DSM 43845^T 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 PIH phospholipid pattern (phosphatidylcholine is the characteristic phospholipid), contain characteristic menaquinones with 10 isoprene units having a high degree of hydrogenation [MK-10(H₆, 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 morphologic and chemotaxonomic properties supported the creation of the family *Nocardiopsiaceae*, which includes the genus *Nocardiopsis* (30). The 16S ribosomal DNA (rDNA)-based intragenomic 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 less

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 IMMIB 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 44048^T. *N. halophila* was not included in this study because a culture was not provided when it was requested from the original authors. Strain IMMIB 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 IMMIB 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).

Physiological characteristics. Peptone-yeast extract-iron agar (ISP medium 6) and tyrosine agar (ISP medium 7) (32) 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 Mihm (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 Tsukamura (36). Phosphatase activity was determined by the method of Kurup 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 μ g/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 methanolsates 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 Cashion 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 renaturation 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 (30) by using the acz editor (23).

Nucleotide sequence accession number. The 16S rDNA sequence of strain IMMIB D-1215^T is available from the EMBL under accession no. Y-13593.

RESULTS

Colonial morphology. Strain IMMIB D-1215^T 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-1215^T 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 wrapped together to form long ropes (Fig. 1a). In a later stage of growth the hyphae fragmented into small rod-shaped elements (Fig. 1b). Single zigzag hyphae were also observed, which fragmented in a similar way to form rod-shaped elements. Sporangia and motile elements were not observed.

Physiological characteristics. Strain IMMIB D-1215^T 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, mannitol, citrate, and gluconate as carbon sources but did not utilize arabinose, lactose, raffinose, trehalose, sucrose, melezitose, adonitol, *meso*-erythritol, *myo*-

inositol, sorbitol, acetate, benzoate, lactate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, adipate, isoamyl alcohol, 2,3-butanediol, 1,2-propandiol, and paraffin (Table 1). Strain IMMIB D-1215^T 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 μ g/ml; amoxicillin plus clavulanic acid, 2.0 μ g/ml; imipenem, 2.0 μ g/ml; erythromycin, 8.0 μ g/ml; clindamycin, >128 μ g/ml; tetracycline, $\leq$ 0.2 μ g/ml; vancomycin, $\leq$ 0.2 μ g/ml; gentamicin, $\leq$ 0.2 μ g/ml; tobramycin, $\leq$ 0.2 μ g/ml; amikacin, $\leq$ 0.2 μ g/ml; ciprofloxacin, 2.0 μ g/ml; and ofloxacin, 8.0 μ g/ml.

Cell chemistry. The cell wall of strain IMMIB D-1215^T 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 type PIII *sensu* Lechevalier et al. (21), and phosphatidylcholine was the characteristic phospholipid. Other phospholipids, such as phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol, were also detected. The predominant menaquinones were MK-10 and MK-10(H₂); in addition, there were detectable amounts of MK-10(H₄), MK-10(H₆), and MK-10(H₈) and traces of MK-9.

DNA characteristics. The G+C content of the DNA of strain IMMIB D-1215^T was 74.1 mol%. The levels of relatedness as determined by DNA-DNA hybridization between strain IMMIB D-1215^T DNA and DNAs from *N. dassonvillei* DSM 43111^T, *N. alborubida* DSM 40465^T, *N. antarctica* DSM 43884^T, *N. lucentensis* DSM 44048^T, *N. alba* subsp. *alba* DSM 43377^T, *N. alba* subsp. *prasina* DSM 43845^T, and *N. listeri* DSM 40297^T 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-1215^T generated in this study and *Nocardiopsis* 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 shown in Table 3. The 16S rDNA sequence similarity values between strain IMMIB D-1215^T and the reference strains ranged from 97.7 to 99.8% (Table 3). These results clearly demonstrate that strain IMMIB D-1215^T is a member of the phylogenetic cluster defined previously that represents the genus *Nocardiopsis* (30). The highest 16S rDNA sequence similarity values between strain IMMIB D-1215^T and reference strains were the similarity values with *N. antarctica*, *N. alborubida*, and *N. dassonvillei* (99.8, 99.5, and 99.3%, respectively).

DISCUSSION

Chemotaxonomic data indicate that strain IMMIB D-1215^T is similar to members of the genus *Nocardiopsis* (25, 26). All of these organisms possess wall chemotype III, phospholipid type PIII, hydrogenated 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

FIG. 1. Scanning electron micrographs of strain IMMIB D-1215^T, showing spiral hyphae wrapped together to form synnemata (a), which fragment into small rod-shaped elements (b).

demonstrate that strain IMMIB D-1215^T is a member of the genus *Nocardiopsis*. The high degrees of 16S rDNA sequence similarity (99.3 to 99.8%) to the species *N. dassonvillei*, *N. alborubida*, and *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 *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 *N. antarctica*, *N. alborubida*, and *N. dassonvillei* (99.5 to 99.7%), the results of DNA-DNA hybridization stud-

ies 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 *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 *Nocardiopsis*. Strain IMMIB D-1215^T differs from *N. dassonvillei*, *N. alborubida*, and *N. antarctica* by its ability to utilize rhamnose and maltose and its inability to

TABLE 1. Differential physiological characteristics of strain IMMIB D-1215^T and other members of the genus *Nocardiopsis*^a

Characteristic	IMMIB D-1215 ^T	<i>N. dassonvillei</i> DSM 43111 ^T	<i>N. alborubida</i> DSM 40465 ^T	<i>N. antarctica</i> DSM 43884 ^T	<i>N. listeri</i> DSM 40297 ^T	<i>N. lucentensis</i> DSM 44048 ^T	<i>N. alba</i> DSM 43377 ^T	<i>N. prasina</i> DSM 43845 ^T
Hydrolysis of:								
Adenine	+	+	+	+	+	+	+	—
Hypoxanthine	+	+	+	+	+	+	+	—
Xanthine	+	+	+	+	+	—	+	+
Utilization of the following compounds as carbon sources:								
D-Xylose	+	+	+	+	—	—	—	—
D-Glucose	+	+	+	+	—	+	+	—
D-Galactose	+	d	d	d	—	—	+	—
L-Rhamnose	+	w	w	w	—	+	—	—
Maltose	+	—	—	—	—	+	+	—
Raffinose	—	—	—	—	—	+	—	—
Trehalose	—	+	+	+	—	+	+	—
Sucrose	—	+	+	+	—	+	—	—
Cellobiose	+	+	+	+	—	+	+	—
myo-Inositol	—	—	—	—	—	+	—	—
Mannitol	+	+	+	+	—	+	—	—
Acetate	—	—	—	—	—	+	—	—
Gluconate	+	+	+	+	—	+	+	—
p-Hydroxybenzoate	—	—	—	—	—	+	—	—
Paraffin	—	—	—	—	—	+	—	—
Utilization of the following compounds as sole carbon and nitrogen sources:								
L-Alanine	+	+	+	+	—	+	—	+
Gelatin	+	+	+	+	+	—	+	+
Proline	+	w	w	w	+	+	+	—
Serine	—	w	w	w	—	+	—	—

^a +, utilized; —, not utilized; w, weakly utilized; d, variably utilized.

utilize trehalose and sucrose as carbon sources. It differs from *N. listeri* and *N. alba* subsp. *prasina* by its ability to utilize xylose, glucose, galactose, rhamnose, maltose, and cellobiose. Strain IMMIB D-1215^T differs from *N. lucentensis* by its inability to utilize raffinose, trehalose, sucrose, and inositol as carbon sources. It differs from *N. alba* subsp. *alba* by its ability to utilize rhamnose as a carbon source and its inability to utilize trehalose as a carbon source. Differences between strain IMMIB D-1215^T and *N. dassonvillei* DSM 43111^T, *N. alborubida* DSM 40465^T, *N. antarctica* DSM 43884^T, *N. listeri* DSM 40297^T, *N. lucentensis* DSM 44048^T, *N. alba* subsp. *alba* DSM 43377^T, and *N. alba* subsp. *prasina* DSM 43845^T are also demonstrated by the low levels of DNA-DNA hybridization. The levels of binding between the DNA of strain IMMIB D-1215^T and the DNAs of the type strains of the other species range from 14.0 to 55.2% (Table 2).

We propose on the basis of 16S rDNA sequence analysis and chemotaxonomic data that strain IMMIB D-1215^T 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. formans, forming; M. L. part. adj. synnemataformans, synnema forming, referring to the ability of the organism to form synnemata). 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^T and other strains of the genus *Nocardiopsis*

Organism	Binding to:						
	IMMIB D-1215 ^T	<i>N. dassonvillei</i> DSM 43111 ^T	<i>N. alborubida</i> DSM 40465 ^T	<i>N. antarctica</i> DSM 43884 ^T	<i>N. listeri</i> DSM 40297 ^T	<i>N. lucentensis</i> DSM 44048 ^T	<i>N. alba</i> subsp. <i>alba</i> DSM 43377 ^T
<i>N. dassonvillei</i> DSM 43111 ^T	45.5						
<i>N. alborubida</i> DSM 40465 ^T	52.0	77.3					
<i>N. antarctica</i> DSM 43884 ^T	55.2	94.0	73.7				
<i>N. listeri</i> DSM 40297 ^T	15.0	24.9	31.1	33.0			
<i>N. lucentensis</i> DSM 44048 ^T	22.4	39.4	25.5	40.0	18.2		
<i>N. alba</i> subsp. <i>alba</i> DSM 43377 ^T	14.0	33.5	36.0	37.6	26.0	25.5	
<i>N. alba</i> subsp. <i>prasina</i> DSM 43845 ^T	35.0	11.0	ND ^a	38.0	20.0	36.0	24.25

^a ND, not determined.

TABLE 3. Levels of 16S rDNA sequence similarity between strain IMMIB D-1215^T and *Nocardiopsis* reference strains

Taxon	% 16S rDNA similarity to:						
	IMMIB D-1215 ^T	<i>N. antarctica</i>	<i>N. alborubida</i>	<i>N. dassonvillei</i>	<i>N. lucentensis</i>	<i>N. alba</i> subsp. <i>alba</i>	<i>N. subsp. prasina</i>
<i>N. antarctica</i>	99.8						
<i>N. alborubida</i>	99.5	99.7					
<i>N. dassonvillei</i>	99.3	99.5	99.5				
<i>N. lucentensis</i>	98.5	98.7	98.8	98.8			
<i>N. alba</i> subsp. <i>alba</i>	98.4	98.5	98.5	98.6	98.8		
<i>N. alba</i> subsp. <i>prasina</i>	97.9	98.2	98.2	98.1	98.5	98.9	
<i>N. listeri</i>	97.7	97.9	97.8	97.9	98.2	98.3	98.7

lium is well-developed (diameter, approximately 0.35 to 0.45 μ m) with zigzag or spiral forms and is white with a slight pimento touch, which may be reflected color from the substrate mycelium. Different spirals are wrapped together to form synnemata. The mycelia of a synnema fragment in a later stage to form rod-shaped elements. Gram positive. Catalase positive and aerobic. No soluble pigment is produced. Melanoid pigments are not produced on either ISP medium 6 or ISP medium 7. Adenine, esculin, elastin, gelatin, casein, hypoxanthine, tyrosine, xanthine, and urea are hydrolyzed; guanine, keratin, and testosterone are not hydrolyzed. Assimilates xylose, glucose, galactose, rhamnose, maltose, cellobiose, mannitol, citrate, and gluconate, but not arabinose, lactose, raffinose, trehalose, sucrose, melezitose, adonitol, erythritol, inositol, sorbitol, acetate, benzoate, lactate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, adipate, isoamyl alcohol, 2,3-butanediol, 1,2-propandiol, or paraffin. Alanine, gelatin, and proline are used as sole carbon and nitrogen sources, but acetamide and serine are not used. Nitrate reductase, β -glucosidase, β -galactosidase, and phosphatase are produced. Grows in the presence of 10% NaCl. Growth occurs at 20, 30, and 37°C. No growth takes place at 42°C. Cell wall chemotype III (*meso*-diaminopimelic acid and no characteristic sugars). Phospholipid type PIII with significant amounts of phosphatidylcholine as the characteristic phospholipid. The predominant menaquinones are MK-10 and MK-10(H₂); in addition, there are substantial amounts of MK-10(H₄), MK-10(H₆), and MK-10(H₈) and trace amounts of MK-9. The fatty acid profile includes straight-chain saturated and unsaturated fatty acids, branched-chain fatty acids of the iso and anteiso types, and 10-methyl branched-chain fatty acids with 16, 17, and 18 carbon atoms. The G+C content of the DNA of the type strain is 74.1 mol%. The type strain was isolated from the sputum of a 35-year-old male who had received a renal transplant. It was isolated only once, so it is difficult to determine the risk group to which the species belongs. The type strain of *Nocardiopsis synnemataformans* is strain IMMIB D-1215 (= DSM 44143).

In a numerical phenetic study, Grund and Kroppenstedt (15) found that *N. dassonvillei*, *N. alborubida*, and *N. antarctica* are similar to each other. Recently, a comparison of 16S rDNA sequences revealed that these three species cluster together with 99.5 to 99.7% sequence similarity (30). These findings were further investigated by performing DNA-DNA hybridization studies of these *Nocardiopsis* species. The resulting binding values, 73.7 to 94.0%, indicated that genomically the type strains of these three taxa belong to the same species, assuming

that species boundaries occur at DNA similarity values of approximately 70% (37). The type strains of *N. dassonvillei*, *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. dassonvillei* was included on the Approved Lists of Bacterial Names (35), as *N. dassonvillei*; 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 intragenetic relationship of members of the genus *Nocardiopsis* based on a 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).

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