***Nocardiopsis tropica* sp. nov., *Nocardiopsis trehalosi* sp. nov., nom. rev. and *Nocardiopsis dassonvillei* subsp. *albirubida* subsp. nov., comb. nov.**

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Sixteen strains isolated from different soils and marine sediments and '*Nocardiopsis trehalosi*' VKM Ac-942^T were subjected to taxonomic analyses. The morphological and chemotaxonomic characteristics of the strains matched those described for members of the *Nocardiopsis*. Based on 16S rDNA sequence analysis, DNA–DNA hybridization values and phenotypic characteristics, including the composition of menaquinones and cell wall teichoic acids, two new species are proposed, *Nocardiopsis tropica* sp. nov. (type strain VKM Ac-1457^T) and *Nocardiopsis trehalosi* sp. nov., nom. rev. (type strain VKM Ac-942^T = JCM 3357^T = NRRL 12026^T). *Nocardiopsis dassonvillei* VKM Ac-1882^T (formerly *Nocardiopsis albirubida*) differs from other strains of this species by the presence of MK-10 as the major menaquinone, cell wall teichoic acid structure, and numerical analysis based on 115 growth and physiological characteristics. The strain is reclassified as the subspecies *Nocardiopsis dassonvillei* subsp. *albirubida*, with VKM Ac-1882^T (= DSM 43465^T = ATCC 23612^T = ISP 5465^T) designated the type.

Keywords: *Nocardiopsis dassonvillei* subsp. *albirubida*, *Nocardiopsis trehalosi*, *Nocardiopsis tropica*, 16S rDNA, DNA–DNA relatedness

INTRODUCTION

The genus *Nocardiopsis* was described by Meyer (1976) to comprise actinomycetes with fragmenting mycelium and a cell wall containing *meso*-diaminopimelic acid and no diagnostically important carbohydrates. However, strains assigned to the genus on the basis of the above characteristics were shown to be heterogeneous in composition of lipids (Goodfellow *et al.*, 1988; Grund & Kroppenstedt, 1990). Consequently, the genus *Nocardiopsis* was proposed to include only organisms with the following salient chemotaxonomic characteristics: cell wall chemotype III C (*meso* isomer of diaminopimelic acid and no characteristic sugars in whole-cell hydrolysates) (Lechevalier & Lechevalier, 1970), phospholipid type PIII (phosphatidylcholine

and phosphatidylmethylethanolamine as characteristic phospholipids) (Lechevalier *et al.*, 1977), menaquinone MK-10 with variable degrees of saturation as the major isoprenoid quinones (Collins *et al.*, 1977), fatty acids of the 3d type according to the classification of Kroppenstedt (1985) and a DNA G+C content ranging between 64 and 71 mol % (Grund & Kroppenstedt, 1990). This grouping was confirmed by 16S rDNA sequence analysis (Rainey *et al.*, 1996). The current genus *Nocardiopsis* contains seven species: *Nocardiopsis dassonvillei* (Meyer, 1976), *Nocardiopsis alba*, *Nocardiopsis listeri* (Grund & Kroppenstedt, 1990), *Nocardiopsis halophila* (Al-Tai & Ruan, 1994), *Nocardiopsis lucentensis* (Yassin *et al.*, 1993), *Nocardiopsis prasina* and *Nocardiopsis synnemataformans* (Yassin *et al.*, 1997). With the exception of *N. halophila*, species affiliation was supported by DNA–DNA reassociation data and 16S rDNA sequence analysis (Fischer *et al.*, 1983; Rainey *et al.*, 1996; Yassin *et al.*, 1997).

At the phenetic level, the differentiation of *Nocardiop-*

The GenBank/EMBL accession numbers for the 16S rDNA sequences of *Nocardiopsis tropica* VKM Ac-1457^T and *Nocardiopsis trehalosi* VKM Ac-942^T are AF105971 and AF105972, respectively.

sis species is based on the colour of the aerial and vegetative mycelium, diffusible pigments, menaquinone composition and physiological characteristics, including halotolerance and growth at different pH values (Grund & Kroppenstedt, 1990; Kroppenstedt, 1992; Al-Tai & Ruan, 1994; Miyashita *et al.*, 1984; Yassin *et al.*, 1997). Data on the cell wall teichoic acids of *Nocardiopsis* spp. show that the structure and composition of these polymers can be chemical markers of *Nocardiopsis* species (Streshinskaya *et al.*, 1998; Tul'skaya *et al.*, 1993, 1995a).

In this paper we present the results of a taxonomic study of 17 strains isolated from different sources and identified as members of the genus *Nocardiopsis*, including '*Nocardiopsis trehalosi*' VKM Ac-942^T (Dolak *et al.*, 1981). Based on the presenting data, we propose two new species, *Nocardiopsis tropica* sp. nov. and *Nocardiopsis trehalosi* sp. nov., nom. rev., and the new combination *Nocardiopsis dassonvillei* subsp. *albirubida* subsp. nov., comb. nov.

METHODS

Organisms and their sources. The strains studied and their sources are given in Table 1. The new strains were isolated on the QMod media used for *Frankia* sp. (Blom *et al.*, 1980), oatmeal agar (ISP medium 3) (Shirling & Gottlieb, 1966) and peptone-yeast extract agar (5 g peptone, 3 g yeast extract, 5 g glucose, 0.2 g H₂PO₄, 1 l distilled water, 15 g agar, pH 7.4) (Naumova *et al.*, 1980).

Pigmentation and morphology. Strains were grown at 28 °C on various ISP media or other diagnostic media (Shirling & Gottlieb, 1966; Gause *et al.*, 1983). Pigmentation and morphology were observed after 14 d growth. Phase-contrast microscopy and transmission electron microscopy (model JEM-7 microscope) were used to study morphological characteristics and spore surfaces.

Chemotaxonomy. Freeze-dried cells used for chemical analysis were obtained from cultures grown on liquid peptone-yeast extract broth on a rotary shaker at 28 °C for 2–3 d. The isomer of diaminopimelic acid was determined by the method of Becker *et al.* (1964). Sugars in whole-cell hydrolysates (1 M HCl, 105 °C, 30 min) were analysed by TLC as described by Hasegawa *et al.* (1983). Mycolic acids and phospholipids were examined using the methods of Minnikin *et al.* (1975, 1984). Menaquinones were extracted and purified as described by Collins *et al.* (1977), and their composition was determined using a model MAT 8430 mass spectrometer (Finnigan). For GLC analysis of fatty acids, cultures were grown in Trypticase-Soy broth (BBL Microbiology Systems) for 2 d. Cells were harvested by centrifugation and about 40 mg (w/w) of the cells was saponified, methylated, extracted and analysed using the Microbial Identification System described by Miller (1982).

Physiology. Assimilation of carbon sources at a final concentration of 1% (w/v) was tested using ISP 9 as the basal medium (Shirling & Gottlieb, 1966). The ability to grow on methanol, ethanol, propanol and butanol as sole carbon sources was examined by using the carbon-free medium of Stevenson (1967). Utilization of nitrogen sources, sulphide production and degradation of aesculin, arbutin,

pectin and Tweens was determined as described by Williams *et al.* (1983). Decomposition of other organic compounds, utilization of organic acids, production of acids from carbohydrates, urease and allantoinase, and nitrate reduction were studied as described previously (Gordon, 1967; Gordon & Mihm, 1957). The effect of inhibitors and temperature on growth was determined using peptone-yeast extract agar as the basal medium. Growth in the presence of salt at different concentrations was examined using Bennett agar as described by Al-Tai & Ruan (1994).

Numerical analysis. Phenotypic similarities were calculated using the simple matching coefficient and Jaccard's coefficient. The BIOMATRIX software was used for cluster analysis (Ozerskaya & Vasilenko, 1992).

DNA isolation and DNA–DNA hybridization study. Total DNA was isolated by the technique of Bradley *et al.* (1973). [³H]DNAs from reference strains were obtained by *in vitro* nick translation. The procedure used for hybridization experiments (membrane filter method) was described by Meyer & Schleifer (1978) and Stackebrandt *et al.* (1981). The reassociation temperature was 25 °C below the thermal denaturation midpoint of the reference DNA.

16S rDNA sequence determination. The 16S rRNA gene was amplified by using the PCR method and prokaryotic 16S rDNA universal primers fD1 and rP1 as described by Weisburg *et al.* (1991) and Zhou *et al.* (1997). The samples were subjected to an initial denaturing step consisting of 3 min at 98 °C. The PCR program used was as in Rainey *et al.* (1996). The sequences of 16S rDNA were analysed directly using the purified PCR products as the sequencing template. The sequencing reactions were performed with automated fluorescent *Taq* cycle sequencing using the ABI Catalyst 800 and a model ABI 373A automatic DNA Sequencer (Applied Biosystems), according to the manufacturer's protocol.

Phylogenetic analysis. The 16S rDNA sequences of *Nocardiopsis* strains were initially aligned with those of type species of the class *Actinobacteria* (Stackebrandt *et al.*, 1997) and subsequently with members of the family *Nocardiopsaceae* (Rainey *et al.*, 1996) using the ae2 editor (Maidak *et al.*, 1997). Evolutionary distances were calculated by the method of Jukes & Cantor (1969). Phylogenetic dendrograms (DeSoete, 1983) were constructed using sequences from different sets of reference strains. Bootstrap analysis was used to evaluate the tree topology of the neighbouring data by performing 1000 resamplings (Felsenstein, 1985).

Nucleotide sequence accession numbers. The GenBank accession numbers of the 16S rRNA gene sequences of strains *Nocardiopsis* sp. VKM Ac-1457^T and '*N. trehalosi*' VKM Ac-942^T determined in this study are AF105971 and AF105972, respectively. These sequences were compared with the following published sequences from EMBL: *N. alba* DSM 43377^T, X97883; *N. dassonvillei* DSM 43111^T, X97886; *N. dassonvillei* DSM 43884, X97885; *N. dassonvillei* DSM 40465, X97882; *N. listeri* DSM 40297^T, X97887; *N. lucentensis* DSM 44048^T, X97888; *N. prasina* DSM 43845^T, X97884; *N. synnemataformans* DSM 44143^T, X99593; *Actinomadura madurae* DSM 43067^T, X97889; *Microtetraspora glauca* DSM 43311^T, X97891.

Table 1. List of *Nocardiopsis* strains used in this study and their sources

Species	Strain	Other designation of strain or source of new isolate	Reference
<i>N. alba</i>	VKM Ac-1883 ^T	DSM 43377 ^T , JCM 9419 ^T , IFO 15097 ^T	Lechevalier & Lechevalier (1970); Grund & Kroppenstedt (1990)
<i>N. alba</i>	VKM Ac-1879	DSM 43120, IMRU 1572	Grund & Kroppenstedt (1990)
<i>N. alba</i>	VKM Ac-1884	DSM 43378, IMRU 714, Goodfellow A119	Gordon & Horan (1968); Grund & Kroppenstedt (1990)
<i>N. dassonvillei</i>	VKM Ac-797 ^T	DSM 43111 ^T , ATCC 23918 ^T , IMRU 509 ^T	Lechevalier & Lechevalier (1970); Meyer (1976)
<i>N. dassonvillei</i>	VKM Ac-773	DSM 43235, ATCC 23219, IMRU 1250	Lechevalier & Lechevalier (1970); Meyer (1976)
<i>N. dassonvillei</i>	VKM Ac-836	DSM 43884, ATCC 43517, JCM 6843	Abyzov <i>et al.</i> (1983); Yassin <i>et al.</i> (1997)
<i>N. dassonvillei</i>	VKM Ac-1882 ^T	DSM 40465 ^T , ATCC 23612 ^T , ISP 5465 ^T	Kudrina (1957); Yassin <i>et al.</i> (1997)
<i>N. listeri</i>	VKM Ac-1881 ^T	DSM 40297 ^T , ATCC 27442 ^T , ISP 5297 ^T	Erikson (1935); Grund & Kroppenstedt (1990)
<i>N. lucentensis</i>	VKM Ac-1962 ^T	DSM 44048 ^T , ATCC 51300 ^T , JCM 9420 ^T	Yassin <i>et al.</i> (1993)
<i>N. prasina</i>	VKM Ac-1880 ^T	DSM 43845 ^T , JCM 3336 ^T , IFO 14423 ^T	Miyashita <i>et al.</i> (1984); Yassin <i>et al.</i> (1997)
' <i>N. trehalosi</i> '	VKM Ac-942 ^T	IFO 14201 ^T , JCM 3357 ^T , NRRL 12026 ^T	Dolak <i>et al.</i> (1981)
<i>Nocardiopsis</i> spp.	VKM Ac-1176	Arctic soil, Russia	This study
	VKM Ac-1453	Intestinal tract of <i>Goloturia</i> sp.	This study
	VKM Ac-1455	Rhizosphere of <i>Casuarina</i> sp., Seychelles	This study
	VKM Ac-1456	Rhizosphere of <i>Casuarina</i> sp., Seychelles	This study
	VKM Ac-1457 ^T	Rhizosphere of <i>Casuarina</i> sp., Seychelles	This study
	VKM Ac-1458	Rhizosphere of <i>Casuarina</i> sp., Seychelles	This study
	VKM Ac-1459	Rhizosphere of <i>Casuarina</i> sp., Seychelles	This study
	VKM Ac-1460	Rhizosphere of <i>Casuarina</i> sp., Seychelles	This study
	VKM Ac-1485	Soil, Seychelles	This study
	VKM Ac-1486	Soil, Seychelles	This study
	VKM Ac-1487	Rhizosphere of <i>Casuarina</i> sp., Seychelles	This study
	VKM Ac-1490	Rhizosphere of <i>Casuarina</i> sp., Seychelles	This study
	VKM Ac-1498	Rhizosphere of <i>Casuarina</i> sp., Seychelles	This study
	VKM Ac-1751	No data	This study
	VKM Ac-1758	Polluted soil, Russia	This study
	VKM Ac-1759	No data	This study

RESULTS AND DISCUSSION

Pigmentation and morphological characteristics

The aerial mycelium formed by most isolates was white to off white, while the vegetative mycelium was yellow–brown or colourless. Strain VKM Ac-1457^T differed from the other strains in this study by the formation of ochra-red to orange vegetative mycelium pigments on oatmeal and Czapek's agars and formation of yellow to orange–yellow diffusible pigments on oatmeal and glycerol-nitrate agars. Distinguishable substrate mycelial pigments were reported previously in *N. synnemataformans* (pimento colour) (Yassin *et al.*, 1997), *N. halophila* (coral-red to red) (Al-Tai & Ruan, 1994) and *N. dassonvillei* DSM 40465 (brownish-red or yellow–orange) (Grund & Kroppenstedt, 1990; Kroppenstedt, 1992). All strains that exhibited aerial hyphae showed the typical zig-zag fragmentation and formation of smooth, elongated spores of irregular size. Aerial hyphae were not observed in *N. listeri*

VKM Ac-1881^T and *N. alba* VKM Ac-1879. Morphological structures such as sunnemata (Yassin *et al.*, 1997) were not discovered.

Chemotaxonomic characteristics

All strains contained the *meso*-isomer of diamino-pimelic acid in their cell walls; diagnostic sugars were not observed. No mycolic acids were detected in whole-cell methanolysates. Phospholipid pattern type PIH *sensu* Lechevalier *et al.* (1977) was determined in lipid extracts. The major menaquinone contained 10 isoprene subunits in the side chain with a variable degree of saturation; MK-10(H₄), MK-10(H₆) or MK-10(H₂) were predominant in different new isolates. In addition, different combinations of minor amounts of MK-9(H₂), MK-9(H₄), MK-9(H₆), MK-11(H₄) or MK-8(H₂) were observed in some strains. *Nocardiopsis* sp. VKM Ac-1457^T possessed MK-10(H₆, H₈), and '*N. trehalosi*' VKM Ac-942^T contained mainly MK-10(H₄,

Table 2. Fatty acids of *Nocardioopsis* strains studied

Name	Strain	Fatty acid composition (% of total fatty acids)										
		16:0	18:0	i16:0	i15:0	i17:0	ai15:0	ai17:0	18Me	16:1	17:1	18:1
<i>N. dassonvillei</i>	VKM Ac-797 ^T	2.2	5.8	26.7	0.8	2.1	3.2	19.8	6.4	1.2	5.2	18.3
<i>N. dassonvillei</i>	VKM Ac-836	1.9	5.5	30.2	0.5	2.6	2.2	20.3	13.5	1.6	3.3	12.5
<i>Nocardioopsis</i> sp.	VKM Ac-1453	5.9	6.6	21.8	1.0	1.4	6.6	14.0	5.3	3.4	8.6	9.2
<i>Nocardioopsis</i> sp.	VKM Ac-1456	4.3	3.9	23.9	1.1	2.2	5.4	16.7	3.4	1.3	5.2	14.3
<i>Nocardioopsis</i> sp.	VKM Ac-1457 ^T	4.1	5.0	23.8	1.1	1.6	6.8	15.2	4.4	1.7	6.9	18.9
<i>Nocardioopsis</i> sp.	VKM Ac-1459	6.7	7.2	24.4	1.3	1.7	6.0	16.9	5.1	3.4	3.4	13.0
<i>Nocardioopsis</i> sp.	VKM Ac-1490	3.7	7.5	28.2	0.7	1.4	3.5	15.7	4.6	1.3	5.7	7.5

Fig. 1. Dendrogram showing the relationships among strains of the genus *Nocardioopsis*. The dendrogram is based on 115 growth and physiological characteristics and was drawn by using Jaccard's coefficient values (%).

H₆). Strain *N. dassonvillei* VKM Ac-1882^T (formerly *N. alborubida*) contained MK-10 as the major menaquinone; minor amounts of MK-10(H₂) and MK-11 or MK-9 and MK-8 were also determined in various experiments. The fatty acid pattern of the strains under study was composed of mainly iso- and anteiso-branched acids combined with tuberculostearic acid (Me18), straight chain saturated (16:0, 18:0) and unsaturated (16:1, 17:1, 18:1) acids (Table 2); no hydroxy fatty acids were detected. All these characteristics are typical of the genus *Nocardioopsis* (Grund & Kroppenstedt, 1990).

Numerical analysis

The results of numerical analysis based on 115 growth and physiological characteristics are presented by a dendrogram (Fig. 1). Seventeen strains, including novel isolates and *N. dassonvillei* VKM Ac-797^T, *N.*

dassonvillei VKM Ac-773, *N. dassonvillei* VKM Ac-836 and *N. dassonvillei* VKM Ac-1882^T, formed a common phenetic cluster at $S_j = 66\%$. While most of the strains exhibited S_j values higher than 72%, strains VKM Ac-1453 and VKM Ac-1882^T branched separately. Members of other species included in the numerical analysis, *N. alba* and *N. listeri*, isolate VKM Ac-1457^T and strain '*N. trehalosi*' VKM Ac-942^T, defined individual lineages which were separated from each other and from the cluster of *N. dassonvillei* at S_j levels of 53–63%.

16S rDNA sequence analysis

The almost complete 16S rDNA sequences of strain *Nocardioopsis* sp. VKM Ac-1457^T and '*N. trehalosi*' VKM Ac-942^T which we determined contained 1452 and 1474 nucleotides, respectively. Comparison of the sequences of these strains with reference sequences

Fig. 2. Phylogenetic tree showing the position of *Nocardiopsis* strains based on 16S rDNA analysis. Sequences of the genus *Streptosporangium* served as an outgroup. Numbers within the dendrogram indicate the percentages of occurrence of the branching order in 1000 bootstrapped trees. Bar, 1 nucleotide substitution per 100 nucleotides.

Table 3. DNA relatedness between tested *Nocardiopsis* strains

Name	Strain	DNA relatedness (%)		
		<i>N. dassonvillei</i> VKM Ac-797 ^T	<i>N. dassonvillei</i> VKM Ac-1882 ^T	<i>Nocardiopsis</i> sp. VKM Ac-1457 ^T
<i>N. dassonvillei</i>	VKM Ac-797 ^T	100	72	29
<i>N. dassonvillei</i>	VKM Ac-773	92	64	22
<i>N. dassonvillei</i>	VKM Ac-836	83	80	30
<i>Nocardiopsis</i> sp.	VKM Ac-1485	89	75	26
<i>Nocardiopsis</i> sp.	VKM Ac-1459	82	65	28
<i>Nocardiopsis</i> sp.	VKM Ac-1498	78	74	23
<i>Nocardiopsis</i> sp.	VKM Ac-1751	89	73	26
<i>Nocardiopsis</i> sp.	VKM Ac-1453	74	57	24
<i>N. dassonvillei</i>	VKM Ac-1882 ^T	71	100	39
<i>N. alba</i>	VKM Ac-1883 ^T	29	25	21
<i>N. lucentensis</i>	VKM Ac-1963 ^T	37	21	29
<i>N. listeri</i>	VKM Ac-1881 ^T	31	37	22
<i>N. prasina</i>	VKM Ac-1880 ^T	32	33	26
<i>Nocardiopsis</i> sp.	VKM Ac-1457 ^T	31	36	100
' <i>N. trehalosi</i> '	VKM Ac-942 ^T	16	17	22

indicates that both organisms are members of the *Nocardiopsis* phylogenetic cluster, supported by 100% of the bootstrapped trees (Fig. 2). Within this cluster, '*N. trehalosi*' VKM Ac-942^T was determined to be phylogenetically isolated, defining the deepest branch within the genus *Nocardiopsis*. The binary sequence similarity of this strain with other species of the genus *Nocardiopsis* was found to be from 96.2 to 97.6%, demonstrating that '*N. trehalosi*' belongs to a new species. Isolate VKM Ac-1457^T formed a distinct

lineage with an 80% bootstrap replication value attached to the cluster of *N. alba*, *N. prasina*, *N. listeri* and *N. lucentensis* with a sequence similarity between 98.0 and 98.3%. These values of 16S rDNA sequence similarity are approximately the same or less than the similarity values between other closely related species of this genus. The data obtained indicate that strain VKM Ac-1457^T most likely belongs to a new species. However, high degrees of 16S rDNA sequence similarity (97% and higher) were demonstrated to be of

Table 4. Differentiating characteristics of *Nocardiosis* species and subspecies

+, All strains are positive; d +, most of the strains are positive; –, all strains are negative; d –, most of the strains are negative; ND, no data.

Characteristic	<i>N. tropica</i> sp. nov.	<i>N. trehalosi</i> sp. nov.	<i>N. dassonvillei</i> subsp. <i>dassonvillei</i>	<i>N. dassonvillei</i> subsp. <i>albirubida</i> subsp. nov.	<i>N. alba</i>	<i>N. listeri</i>	<i>N. prasina</i>	<i>N. lucentensis</i>	<i>N. halophila</i> *	<i>N. synnemataformans</i> †
Aerial mycelium	+	+	+	+	d +‡	–	+	+	+	+
Synnemata	–	–	–	–	–	–	–	–	–	+
Utilization of:										
L-Arabinose	+	+	+	+	–	+	+	–	+	–
Inositol	–	–	–	–	–	+	–	+	+	–
Melibiose	+	+	–	–	–	+	–	–	ND	ND
L-Rhamnose	+	+	+	+	–	+	–	+	+	+
D-Xylose	+	+	+	+	–	+	+	–	+	+
Acid from:										
Adonitol	–	–	d –	–	–	+	–	–	ND	ND
L-Arabinose	+	+	d +‡	+	–	+	+	–	+	ND
Galactose	+	–	d +‡	+	–	+	–	–	–	ND
Glycerol	+	+	d +‡	+	+	–	+	+	ND	ND
Inositol	–	–	d –	–	–	–	–	+	+	ND
D-Lactose	–	+	–	–	–	+	–	–	–	ND
Mannitol	+	+	+	+	+	–	+	+	+	ND
D-Mannose	+	+	d –	+	–	–	–	+	–	ND
Melibiose	+	–	–	–	–	+	–	–	ND	ND
L-Rhamnose	+	–	+	+	–	+	–	+	+	ND
Sucrose	+	–	d +	–	+	–	+	+	–	ND
D-Xylose	–	+	d +	+	–	+	–	–	+	ND
Decarboxylation of:										
Lactate	+	+	+	+	+	+	+	–	ND	–
Malonate	+	+	+	+	+	–	–	+	ND	ND
Oxalate	+	–	d +	+	–	–	+	–	ND	ND
Propionate	+	+	+	–	+	+	+	+	ND	ND
Decomposition of:										
Casein	–	+	+	+	+	+	+	+	+	+
Tyrosine	–	–	d +‡	+	+	+	+	+	–	+
Tween 80	+	+	d –	–	+	–	–	–	+	ND
Tween 85	+	–	d +	+	+	–	–	–	ND	ND
Nitrate reductase	–	–	d –	+	d –	+	–	+	ND	+
Urease	+	+	d –	–	d +‡	–	–	–	+	+
Growth at:										
10 % NaCl	+	–	d +	+	d –	–	+	+	+	+
20 % NaCl	–	–	–	–	–	–	–	–	+	ND
10 °C	–	–	d –	–	–	+	–	+	ND	–
42 °C	–	+	d +‡	+	d +‡	–	+	–	–	–
45 °C	–	+	–	–	–	–	–	–	–	–
Major menaquinones	10/6, 10/8	10/4, 10/6	10/4, 10/6§	10/0	10/4, 10/6	10/0, 10/2	10/4, 10/6	10/6, 10/8¶	10/6, 10/8	10/0, 10/2

* Data from Al-Tai & Ruan (1994).

† Data from Yassin *et al.* (1997).

‡ Type strain is positive.

§ The following predominant menaquinones were found in some strains of this species: 10/4, 10/2; 10/4, 9/4; 10/2, 9/2; 10/6, 9/6.

|| Data from Grund & Kroppenstedt (1990).

¶ Data from Yassin *et al.* (1993).

limited usefulness for differentiating species, and DNA pairing studies need to be performed to recognize species affiliation (Stackebrandt & Goebel, 1994).

DNA–DNA reassociation study

Fifteen strains, listed in Table 3, were hybridized against reference [³H]DNA of *N. dassonvillei* VKM Ac-797^T, *N. dassonvillei* VKM Ac-1882^T and VKM Ac-1457^T. High DNA–DNA homologies (71–92 %) were obtained between *N. dassonvillei* VKM Ac-797^T, other strains of this species and the new isolates, except for strain VKM Ac-1457^T, indicating that these strains belong to the same species (Wayne *et al.*, 1987). Strain

VKM Ac-1457^T, which differed significantly from the *N. dassonvillei* cluster at the phenotypical level, exhibited only 21–39 % DNA–DNA homology with all other strains tested. These values are in agreement with the DNA–DNA similarity values between different species of *Nocardiosis* obtained in this study and by Fischer *et al.* (1983) and Yassin *et al.* (1997).

Taxonomic affiliation of the strains

Nocardioform morphology, chemotaxonomic characteristics and 16S rDNA sequence analysis show that all of the isolates listed in Table 1 and '*N. trehalosi*' VKM Ac-942^T are members of the genus *Nocardiosis*. As

seen from the distinct phylogenetic positions of *Nocardiopsis* sp. VKM Ac-1457^T and '*N. trehalosi*' VKM Ac-942^T within the genus *Nocardiopsis* (Fig. 2), and low DNA–DNA similarity values to the type strains of the genus (Table 3), both strains differ from each other and from other species of *Nocardiopsis*. The distinctness of these organisms is also seen by numerical analysis based on 115 growth and physiological characteristics and by cell wall teichoic acid composition. The polymers 1,5-poly(ribitol phosphate) with side chains glycerol phosphate oligomers (Streshinskaya *et al.*, 1996) and 1,3-poly(glycerol phosphate) with β -glucosyl residues (Streshinskaya *et al.*, 1998) determined in strains *Nocardiopsis* sp. VKM Ac-1457^T and '*N. trehalosi*' VKM Ac-942^T, respectively, have not been found in other species of this genus (Shashkov *et al.*, 1997; Tul'skaya *et al.*, 1993, 1995a, b). All above data demonstrate that the strains under discussion are representatives of two new species of the genus *Nocardiopsis*. We propose the name *Nocardiopsis tropica* for strain VKM Ac-1457^T, isolated from the rhizosphere of *Casuarina* sp. in a tropical region (Seychelles), and the original name *Nocardiopsis trehalosi* (Dolak *et al.*, 1981) for strain VKM Ac-942^T. These species can be distinguished from other *Nocardiopsis* spp. by the characteristics set forth in Table 4 and some structural characteristics of their cell wall teichoic acids (Streshinskaya *et al.*, 1996, 1998). The descriptions of the new species are given below.

The majority of the strains investigated were identified as *N. dassonvillei*, based on numerical analysis (Fig. 1) and the high levels of DNA–DNA hybridization between the isolates and the reference strains. Strain VKM Ac-1882^T was separated from other *N. dassonvillei* strains by the major menaquinone (MK-10), growth and physiological characteristics (Fig. 1; Table 4), and cell wall teichoic acid composition (Shashkov *et al.*, 1997). In contrast to other strains of *N. dassonvillei* with the structurally identical polymers [a core represented by alternating units of poly(glycerol phosphate) and poly(glycosylglycerol phosphate) chains and the glycosyl substituents] (Tul'skaya *et al.*, 1993, 1995b; Akimov *et al.*, 1994), strain VKM Ac-1882^T has an additional *O*-succinyl substituent in most glycosyl residues. However, the DNA–DNA similarity values between VKM Ac-1882^T and strains of *N. dassonvillei* are only slightly lower than those obtained with the type strain VKM Ac-797^T (Table 3). In our opinion, the genomic and phenetic differences do not lend sufficient support to describe a new species, but they are significant enough to consider strain VKM Ac-1882^T as a separate subspecies. We propose the name *Nocardiopsis dassonvillei* subsp. *albirubida*, taking into account the taxonomic history of this strain (Kudrina, 1957; Grund & Kroppenstedt, 1990).

Description of *Nocardiopsis tropica* sp. nov.

Nocardiopsis tropica (tro'pi.ca. M.L. adj. *tropica* referring to the tropical region where the type strain was isolated).

Aerobic, oxidase- and catalase-positive nocardioform actinomycete. Usually the aerial mycelium is white; however, it has a cream colour on Czapek's agar. Substrate mycelium is colourless to olive–yellow or red–orange on the different media tested. Soluble pigments are yellow–orange to yellow on oatmeal agar or glycerol-nitrate agar. Melanin is not produced on peptone-yeast extract-iron agar (ISP 6). Aerial hyphae are long and zig-zag shaped at the beginning of sporulation, fragmenting into elongated spore-like structures which subsequently subdivide into short rod-shaped spores of irregular size. Spore surface is smooth. Long branched substrate hyphae fragment into non-motile elements. L-Arabinose, D-fructose, D-glucose, D-galactose, glycerol, melibiose, maltose, mannitol, D-mannose, L-rhamnose, sucrose and D-xylose are used as sole carbon sources for growth; adonitol, dulcitol, i-inositol, lactose, D-ribose and D-sorbose are not. Acids are produced from L-arabinose, D-fructose, D-galactose, D-glucose, glycerol, maltose, D-mannitol, melibiose, L-rhamnose and sucrose, but not from adonitol, dulcitol, i-inositol, lactose, D-ribose, sorbose or D-xylose. An alkaline reaction is observed with citrate, formate, fumarate, lactate, malate, malonate, oxalate, propionate, pyruvate, sebacate, succinate and tartrate, but no alkaline reaction occurs with acetate, aconitate, benzoate or salicylate. Xanthine, hypoxanthine, calcium oxalate crystals, urea, Tween 40, Tween 60, Tween 80 and Tween 85 are decomposed; casein and tyrosine are not. Nitrates are not reduced to nitrites. Growth occurs on media supplemented with 10% (w/v) NaCl, 1.5% (v/v) butanol, 2.5% (v/v) methanol and 3% (v/v) ethanol, but no growth occurs at 12% (w/v) NaCl and 50 mg lysozyme ml⁻¹. Growth occurs at 37 °C but not at 10 °C and 42 °C; optimum growth is at 28–30 °C. The major menaquinones are MK-10(H₆, H₈). Cell wall teichoic acid contains heterogeneous 1,5-poly(ribitol phosphate) chains with lateral glycerol phosphate oligomers. The type strain is VKM Ac-1457^T. Isolated from soil in the rhizosphere of *Casuarina* sp. (Seychelles).

Description of *Nocardiopsis trehalosi* sp. nov., nom. rev.

Nocardiopsis trehalosi (tre.ha.lo'si. M.L. adj. *trehalosi* referring to 3-trehalosamine, an aminoglycoside antibiotic that is produced by the type strain of this species).

Aerobic, oxidase- and catalase-positive nocardioform actinomycete. The aerial mycelium is white to cream or yellowish grey. The colour of the substrate mycelium ranges from pale olive-brownish to pale orange–yellow. A light yellow-brownish or light orange–yellow soluble pigment is produced on some media. No melanin is observed on peptone-yeast extract-iron agar (ISP 6). Aerial hyphae are zig-zag or twisted-ribbon-like at the beginning of sporulation; spores are irregularly sized (mostly elongated) and have a smooth surface. Long branched substrate hyphae fragment

into non-motile elements. L-Arabinose, D-fructose, D-galactose, D-glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, L-rhamnose and D-xylose are used for growth as sole carbon sources, but not adonitol, i-inositol, melibiose, D-sorbitol or sucrose. Acids are produced from L-arabinose, D-fructose, glycerol, lactose, maltose, D-mannitol, D-mannose and D-xylose, but not from adonitol, D-galactose, i-inositol, melibiose, L-rhamnose, D-sorbose or sucrose. Acetate, citrate, formate, fumarate, lactate, malate, malonate, pyruvate, propionate, sebacate, succinate and tartrate are utilized; no alkaline reaction occurs with aconitate, benzoate, salicylate and oxalate. Casein, calcium oxalate crystals, hypoxanthine, xanthine, Tween 80 and urea are decomposed, but not tyrosine, Tween 40, Tween 60 and Tween 85. Decomposition of aesculin is variable. Nitrates are not reduced to nitrites. The strain is resistant to 50 mg lysozyme ml⁻¹. Growth occurs on media supplemented with 5% (w/v) NaCl, 2.5% (v/v) methanol and 3% (v/v) ethanol, but no growth occurs at 10% (w/v) NaCl and 0.5% (v/v) butanol. Growth temperature ranges between 18 and 45 °C; no growth occurs at 10 °C. Optimum growth is at 28–37 °C. The major menaquinones are MK-10(H₄, H₆). Cell wall teichoic acid of poly(glycerol phosphate) with β -glucosyl residues. Produces 3-trehalosamine. The type strain is VKM Ac-942^T (NRRL 12026^T = JCM 3357^T = IFO 14201^T). The description above is based on the data of Dolak *et al.* (1980, 1981) and our study.

Description of *Nocardiopsis dassonvillei* subsp. *albirubida* subsp. nov., comb. nov.

The description of this subspecies is as presented by Grund & Kroppenstedt (1990). The characteristics which differentiate this subspecies from other strains of *N. dassonvillei* are the following: major menaquinone MK-10; cell wall glycerol teichoic acid of mixed structure containing an additional *O*-succinyl residue; production of nitrite from nitrate; growth in 1.5% (v/v) butanol; use of lactose and melezitose as a sole carbon source; utilization of quinate; production of acid from D-mannose. The type strain is VKM Ac-1882^T (= DSM 40465^T = ATCC 23612^T = ISP 5465^T = JCM 4717^T = IFO 13392^T).

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