Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rDNA sequences

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The 16S rDNAs from 38 strains of the genus *Nocardioides*, two *Aeromicrobium* species and *Terrabacter tumescens* were directly sequenced and then analysed. The mean nucleotide similarity value between the type strains of validly described *Nocardioides* species was $96.1 \pm 3.0\%$. The mean nucleotide similarity value between the type strains of validly described *Nocardioides* species and the two *Aeromicrobium* species was $93.7 \pm 1.4\%$. *T. tumescens* was distantly related to the genera *Nocardioides* and *Aeromicrobium*. The mean intraspecific nucleotide similarity value of 16S rDNA sequences from *Nocardioides albus* was $99.5 \pm 0.5\%$. The mean intraspecific nucleotide similarity of 16S rDNA sequences from *Nocardioides simplex* was 100%, except for *N. simplex* strains ATCC 13260, ATCC 19565 and ATCC 19566, which were shown not to be members of the genus *Nocardioides*. '*Nocardioides flavus*' strains IFO 14396^T and IFO 14397, and '*Nocardioides fulvus*' JCM 3335^T showed a 16S rDNA similarity value of 100% with *Nocardioides luteus* KCTC 9575^T and *Nocardioides albus* JCM 5854. '*N. fulvus*' IFO 14399 shared its highest 16S rDNA similarity with *Nocardioides* sp. ATCC 39419 at 99%. '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 formed a phylogenetic lineage distinct from the genera *Nocardioides* and *Aeromicrobium*. '*Nocardioides thermolilacinus*' strains IFO 14335^T and IFO 14336 displayed a close relationship to the genus *Streptomyces*. From 16S rDNA sequence analyses, it is considered that some strains that have been attributed to the genus *Nocardioides* should be taxonomically re-evaluated.

Keywords: *Nocardioides*, 16S rDNA sequence analysis, phylogeny

INTRODUCTION

The genus *Nocardioides* was introduced in 1976 by Prauser (15) for Gram-positive, non-acid-fast, aerobic and mesophilic nocardioform actinomycetes that form a mycelium that fragments into irregular rod- to coccus-like elements. *Nocardioides albus* is the type species of the genus. However, the inclusion of previously described *Pimelobacter* species within the genus *Nocardioides* has rendered the genus morphologically heterogeneous (2). *N. albus* (15) and *Nocardioides luteus* (16) produce a well-developed mycelium, whereas *Nocardioides jensenii* (2, 23), *Nocardioides plantarum* (3) and *Nocardioides simplex* (13,

23) form neither substrate mycelium nor primary mycelium.

The genus *Pimelobacter* was proposed in 1983 by Suzuki & Komagata (23) for *Arthrobacter* species with LL-diaminopimelic acid in the peptidoglycan. Three *Pimelobacter* species, *Pimelobacter jensenii*, *Pimelobacter simplex* and *Pimelobacter tumescens*, were first recognized. However, O'Donnell *et al.* (13) proposed that *Arthrobacter simplex* should be assigned to the genus *Nocardioides* as *N. simplex*. *P. jensenii* was transferred to the genus *Nocardioides* as *N. jensenii* and *P. tumescens* was reclassified in a new genus, *Terrabacter*, as *Terrabacter tumescens* by Collins *et al.* (2).

The genus *Nocardioides* possesses a cell wall peptidoglycan based on LL-2,6-diaminopimelic acid and glycine [wall chemotype I *sensu* Lechevalier & Le-

The GenBank accession numbers for the sequences reported in this paper are AF004988–AF005024, U61298, U81990, U82666 and U82667.

chevalier (9)] and lacks mycolic acids (15). The genus has complex cellular fatty acid profiles containing iso-, anteiso-, straight chain, unsaturated and 10-methyl-branched acids. The major fatty acids characterizing the genus are iso-C_{16:0} (12, 13). The predominant isoprenoid quinone found in the genus *Nocardioide*s is a tetrahydrogenated menaquinone with eight isoprene units (MK-8[H₄]) (13, 24).

There are currently five validly described *Nocardioide*s species, namely *N. albus* (15), *N. jensenii* (2), *N. luteus* (16), *N. plantarum* (3) and *N. simplex* (13). *Nocardioide*s *fastidiosa* was proposed in 1989 by Collins & Stackebrandt (1) but transferred to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* in 1994 [Tamura & Yokota (24)]. In addition to the five validly described *Nocardioide*s species, there are three invalidly described *Nocardioide*s species, namely '*Nocardioide*s *flavus*' (19), '*Nocardioide*s *fulvus*' (19) and '*Nocardioide*s *thermolilacinus*' (11). However, the type strain of '*N. flavus*' is assumed to be a synonym of *N. luteus* (17). Two strains of '*N. thermolilacinus*' are considered to be streptomycetes since they exhibit a *Streptomyces*-like life cycle, are susceptible to *Streptomyces* phages, and lack susceptibility to *Nocardioide*s phages (17).

The genus *Aeromicrobium* was proposed by Miller *et al.* (12) for *Arthrobacter* sp. strain NRRL B-3381 in 1991. *Arthrobacter* sp. strain B-3381 is a non-mycelial, non-sporulating actinomycete that produces the macrolide antibiotic erythromycin (4). Strain NRRL B-3381 was previously placed in the genus *Arthrobacter* solely on the basis of phenotypic traits. However, this bacterium differed in many ways from *Arthrobacter globiformis*, the type species of the genus *Arthrobacter*. Therefore, it was reclassified into a new taxon, the genus *Aeromicrobium*, as *Aeromicrobium erythreum* sp. nov. on the basis of other phenotypic traits, chemotaxonomic analysis and 16S rDNA sequence analysis (12). The genus *Aeromicrobium* is aerobic, non-motile and Gram-positive. Its cells typically form irregular rods and irregular coccoid elements (12). The major fatty acids characterizing the genus are C_{16:0}, C_{18:1} and 10-methyl-branched C_{18:0} (12, 24). The predominant isoprenoid quinone found in the genus *Aeromicrobium* is a tetrahydrogenated menaquinone with nine isoprene units (MK-9[H₄]) (12, 24). The type species of the genus is *Aeromicrobium erythreum* NRRL B-3381. The second *Aeromicrobium* species, *Aeromicrobium fastidiosum*, was transferred from the genus *Nocardioide*s on the basis of its fatty acid profile, isoprenoid quinone type and 16S rDNA sequence analysis (24).

The morphological heterogeneity found between *Nocardioide*s species may cause confusion in assigning new isolates to the genus *Nocardioide*s. Moreover, those *Nocardioide*s species which do not form a mycelium may be difficult to distinguish from *Aeromicrobium* species. Therefore, chemotaxonomic properties and 16S rRNA sequences are regarded as

necessary for their differentiation (24). In the present study, the 16S rDNA sequences of 38 strains of the genus *Nocardioide*s, two *Aeromicrobium* species and *T. tumescens* were examined. We investigated the inter- and intraspecific phylogenetic relationships of the genus *Nocardioide*s and the phylogenetic positions of invalid *Nocardioide*s species based on 16S rDNA sequences. Intraspecific phylogenetic relationships as well as interspecific relationships of the genus may be important for defining unknown strains at the species level. Intraspecific phylogenetic relationships of the species may also be necessary for reclassification of strains which are most likely to be currently misidentified. The 16S rDNA sequences determined were used to investigate the phylogenetic relationships between the genera *Nocardioide*s, *Aeromicrobium* and *Terrabacter*; the sequences were also compared with those of other actinomycete taxa.

METHODS

Bacterial strains. Table 1 summarizes the strains used in this study and the GenBank accession numbers for the 16S rDNA sequences. All strains used in this study were grown in shake flasks containing trypticase soy broth (BBL) supplemented with glucose (0.75 % w/v) at suitable temperatures. The broth cultures were checked for purity before they were harvested by centrifugation.

Chromosomal DNA isolation. The chromosomal DNAs were isolated by the method described previously (27).

16S rDNA sequencing. Two primers described previously (22), 9F [5'-GAGTTTGATCCTGGCTCAG-3'; positions 9-27 (*Escherichia coli* 16S rRNA numbering)] and 1542R (5'-AGAAAGGAGGTGATCCAGCC-3'; 1542-1525), were used for amplification of the 16S rRNA gene. The 5' ends of the two primers were phosphorylated using T4 polynucleotide kinase (New England Biolabs) according to the instructions of the Strandase ssDNA preparation kit (Novagen). The 16S rRNA gene was amplified as described previously (28) using phosphorylated 9F plus non-phosphorylated 1542R and phosphorylated 1542R plus non-phosphorylated 9F, respectively. The PCR products were precipitated with 1 µl 1 % (w/v) glycogen, 9 µl 3 M sodium acetate (pH 5.2) and 70 µl 2-propanol, and were resuspended in 10 µl distilled water.

The strands containing phosphorylated primer from PCR products were selectively digested using λ exonuclease according to the Strandase ssDNA preparation kit instructions. The ssDNA templates produced were used directly for the sequencing reaction. Sequencing was performed as described previously (8) using α -³⁵S-labelled dATP and a DNA sequencing kit (US Biochemical) according to the manufacturer's instructions. The sequencing primers were derived from conserved regions of previously described 16S rRNA gene sequences of eubacteria. The forward primers used were 373F [5'-AATGGGCGC-AAGCCTGAT-3'; positions 373-390 (*E. coli* 16S rRNA numbering)], 792F (5'-ATACCCTGGTAGTCCAC-3'; 792-808), and 1392F (5'-GTACACACCGCCCGT-3'; 1392-1406). The reverse primers used were 330R (5'-GTGTCTCAGTCCCAGTGT-3'; 330-313), 536R (5'-GWATTACCGCGGCKGCTG-3'; 536-519), 926R (5'-CCGTCAATTCMTTTRAG-3'; 926-910), 1406R (5'-

Table 1. Strains used in this study and accession numbers of 16S rDNA sequences

The following abbreviations are used: KCTC, Korean Collection for Type Cultures, Taejeon, Korea; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Saitama, Japan; DSM, DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; ATCC, American Type Culture Collection, Rockville, MD, USA; IFO, Institute for Fermentation, Osaka, Japan; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA.

Strain	Source	Accession no.
<i>Nocardioides albus</i>	KCTC 9186 ^T (JCM 3185 ^T)	AF004988
<i>Nocardioides albus</i>	JCM 5851	AF004989
<i>Nocardioides albus</i>	JCM 5852	AF004990
<i>Nocardioides albus</i>	JCM 5853	AF004991
<i>Nocardioides albus</i>	JCM 5854	AF004992
<i>Nocardioides albus</i>	JCM 5855	AF004993
<i>Nocardioides albus</i>	JCM 5856	AF004994
<i>Nocardioides albus</i>	JCM 5857	AF004995
<i>Nocardioides albus</i>	JCM 5858	AF004996
<i>Nocardioides albus</i>	DSM 46107	AF004997
<i>Nocardioides albus</i>	JCM 5859	AF004998
<i>Nocardioides albus</i>	JCM 5860	AF004999
<i>Nocardioides albus</i>	JCM 5861	AF005000
<i>Nocardioides albus</i>	JCM 5862	AF005001
<i>Nocardioides albus</i>	JCM 5863	AF005002
<i>Nocardioides albus</i>	JCM 5864	AF005003
<i>Nocardioides albus</i>	DSM 43873	AF005004
<i>Nocardioides albus</i>	DSM 43874	AF005005
<i>Nocardioides jensenii</i>	KCTC 9134 ^T (DSM 20641 ^T)	AF005006
<i>Nocardioides luteus</i>	KCTC 9575 ^T (JCM 3358 ^T)	AF005007
<i>Nocardioides plantarum</i>	NCIMB 12834 ^T	AF005008
<i>Nocardioides simplex</i>	KCTC 9106 ^T (JCM 1363 ^T)	AF005009
<i>Nocardioides simplex</i>	JCM 1366	AF005010
<i>Nocardioides simplex</i>	JCM 1367	AF005011
<i>Nocardioides simplex</i>	NCIMB 12919	AF005012
<i>Nocardioides simplex</i>	ATCC 13260	U81990
<i>Nocardioides simplex</i>	ATCC 15799	AF005013
<i>Nocardioides simplex</i>	ATCC 19565	U82666
<i>Nocardioides simplex</i>	ATCC 19566	U82667
' <i>Nocardioides flavus</i> '	IFO 14396 ^T	AF005014
' <i>Nocardioides flavus</i> '	IFO 14397	AF005015
' <i>Nocardioides flavus</i> '	JCM 3335 ^T	AF005016
' <i>Nocardioides flavus</i> '	IFO 14399	AF005017
' <i>Nocardioides thermolilacinus</i> '	IFO 14335 ^T	AF005018
' <i>Nocardioides thermolilacinus</i> '	IFO 14336	AF005019
<i>Nocardioides</i> sp.	ATCC 39419	AF005020
<i>Aeromicrobium erythreum</i>	NRRL B-3381 ^T	AF005021
<i>Aeromicrobium fastidiosum</i>	KCTC 9576 ^T (JCM 8088 ^T)	AF005022
<i>Terrabacter tumescens</i>	KCTC 9133 ^T (DSM 20308 ^T)	AF005023
<i>Nocardioides</i> -like isolate (' <i>N. pyridinoliticus</i> ')	OS4 ^T , S.-K. Rhee	U61298
<i>Nocardioides</i> -like isolate	NSP41, Y. K. Cho	AF005024

ACGGGCGGTGTGTRC-3'; 1406–1392), and 1512R (5'-ACGGHTACCTTGTTACGACTT-3'; 1512–1492).

Phylogenetic analysis. The 16S rDNA sequences of the test strains were aligned using CLUSTAL W software (25). The 16S rDNA similarity values were calculated from the alignment. The phylogenetic trees were constructed by the neighbour-

joining method (20) from a distance matrix calculated by CLUSTAL W (25).

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in this study have the GenBank accession numbers shown in Table 1. The accession numbers for reference 16S rDNA/rRNA sequences used in our

analysis are as follows: *Streptomyces albus*, X53163; *Streptomyces bluensis*, X79324; *Streptomyces caelestis*, X80824; *Streptomyces tendae*, D63873; *Rhodococcus rhodochrous*, X70295; *Rhodococcus erythropolis*, X79289; *Arthrobacter globiformis*, M23411; *Luteococcus japonicus*, D85487; *Micrococcus luteus*, M38242; *Sporichthya polymorpha*, X72377; *Microtholamatus phosphovorus*, Z78207; *Propionibacterium freudenreichii*, X53217; and *Friedmanniella antarctica*, Z78206. The 16S rRNA sequence of *Propioniferax innocua* was obtained from the study of Pitcher & Collins (14).

RESULTS AND DISCUSSION

The 16S rDNAs from 41 strains attributed to the genera *Nocardioides*, *Aeromicrobium* and *Terrabacter* were amplified *in vitro* by PCR, and their nucleotide sequences were determined directly using non-phosphorylated strands of PCR products whose 5'-phosphorylated strands were selectively digested by λ exonuclease. The 16S rDNA sequences determined correspond to the region between positions 28 and 1524 of *E. coli* 16S rRNA.

The mean 16S rDNA similarity value found between the type strains of validly described *Nocardioides* species was $96.1 \pm 3.0\%$ (Table 2). The mean nucleo-

tide similarity value found between the type strains of validly described *Nocardioides* species, except for the relationship between *N. albus* KCTC 9186^T and *N. luteus* KCTC 9575^T which showed a high 16S rDNA similarity (99.0%), was $94.1 \pm 1.0\%$ (Table 2). The type strains of the *Nocardioides* species formed phylogenetic lineages distinct from each other within the evolutionary radiation encompassed by the genus *Nocardioides* (Figs 1 and 2). However, some strains of the genus *Nocardioides* were distantly related to the cluster of the genus *Nocardioides* as shown by the phylogenetic tree in Fig. 1. The two *Aeromicrobium* species, which were previously identified as members of different species from a DNA-DNA relatedness test as well as by phenotypic traits (24), showed a 16S rDNA similarity of 98.2% (Table 2). *Aeromicrobium* species showed a mean nucleotide similarity value of $93.7 \pm 1.4\%$ with the type strains of the five validly described *Nocardioides* species (Table 2) and formed a phylogenetic lineage separated from the evolutionary radiation encompassed by the genus *Nocardioides* (Figs 1 and 2). *T. tumescens* showed 16S rDNA similarity values of 89.4–90.1% with the type strains of validly described *Nocardioides* species and 88.8–90.0% with *Aeromicrobium* species (Table 2). The phylo-

Table 2. Percentage 16S rDNA/16S rRNA similarity for representatives of the genus *Nocardioides*, *Aeromicrobium* species, *Terrabacter tumescens*, and some other actinomycete taxa

1, *N. albus* KCTC 9186^T; 2, *N. jensenii* KCTC 9134^T; 3, *N. luteus* KCTC 9575^T; 4, *N. plantarum* NCIMB 12834^T; 5, *N. simplex* KCTC 9106^T; 6, *N. simplex* ATCC 13260; 7, *N. simplex* ATCC 19565; 8, '*N. fulvus*' IFO 14399; 9, '*N. thermophilacinus*' IFO 14335^T; 10, *Nocardioides* sp. ATCC 39419; 11, *Aeromicrobium erythreum* NRRL B-3381^T; 12, *Aeromicrobium fastidiosum* KCTC 9576^T; 13, *T. tumescens* KCTC 9133^T; 14, *Nocardioides*-like isolate OS4; 15, *Nocardioides*-like isolate NSP41; 16, *S. albus*; 17, *S. bluensis*; 18, *S. caelestis*; 19, *S. tendae*; 20, *R. rhodochrous*; 21, *R. erythropolis*; 22, *Arthrobacter globiformis*; 23, *L. japonicus*; 24, *M. luteus*; 25, *S. polymorpha*; 26, *M. phosphovorus*; 27, *P. freudenreichii*; 28, *P. innocua*; and 29, *F. antarctica*.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1																												
2	94.9																											
3	99.0	94.9																										
4	93.3	93.1	93.9																									
5	94.7	94.0	95.0	93.7																								
6	90.3	92.2	91.1	90.5	90.7																							
7	89.9	91.9	90.7	90.3	90.2	99.4																						
8	91.3	93.5	91.4	91.3	91.7	91.2	91.2																					
9	89.1	90.9	89.5	89.0	88.9	90.6	90.4	89.7																				
10	91.3	93.5	91.4	91.3	91.7	91.5	91.6	99.0	89.8																			
11	92.3	95.0	92.6	92.8	92.3	92.7	92.4	94.4	90.4	94.5																		
12	92.8	94.6	93.1	92.6	92.3	91.4	91.1	93.0	89.8	93.3	98.2																	
13	89.6	90.1	89.9	89.4	89.5	89.7	89.5	89.5	90.9	89.5	90.0	88.8																
14	95.0	95.2	95.5	95.1	95.7	91.4	91.3	92.7	89.3	92.7	94.2	93.5	89.6															
15	94.8	93.8	95.1	94.3	98.6	90.1	90.1	92.1	89.0	91.9	92.8	92.7	89.6	96.4														
16	89.9	89.6	89.4	88.3	87.9	90.4	90.3	89.2	95.8	89.2	90.3	89.5	89.5	88.3	87.2													
17	89.3	91.0	89.8	88.8	89.4	90.4	90.2	90.0	97.7	89.7	90.6	89.7	90.4	89.8	89.5	95.0												
18	89.7	91.2	89.6	89.2	89.7	90.4	90.2	89.6	98.6	89.3	90.4	89.7	91.0	89.3	89.2	95.9	97.3											
19	89.0	91.0	89.4	89.3	88.9	90.6	90.6	89.9	98.7	89.7	90.7	90.1	91.0	89.1	89.1	96.0	97.8	98.3										
20	88.1	89.4	88.6	89.0	88.8	94.4	94.5	89.7	89.8	89.6	89.8	88.8	89.8	89.4	88.5	89.9	90.0	89.7	90.0									
21	89.9	91.9	90.7	90.3	90.2	99.5	100	91.3	90.4	91.6	92.4	91.1	90.1	91.3	90.1	90.6	90.2	90.2	90.4	94.6								
22	88.8	89.2	89.1	89.3	90.2	90.6	90.2	89.8	91.6	89.9	91.0	89.7	93.1	89.4	90.2	90.9	91.4	91.3	89.7	89.8	90.2							
23	91.3	91.8	90.9	89.9	89.4	88.1	87.7	90.9	89.1	90.9	91.6	91.8	89.7	90.9	89.5	89.1	89.0	89.6	87.8	87.7	88.4							
24	88.8	89.8	88.9	89.4	89.5	90.2	89.6	89.5	90.8	89.4	90.2	89.5	92.7	89.4	89.3	91.1	91.5	90.9	90.5	89.7	89.6	95.9	88.9					
25	88.6	90.5	89.1	88.4	88.5	90.0	90.2	91.1	91.5	90.8	90.2	89.3	92.4	90.0	88.8	91.9	91.5	91.4	90.9	89.6	90.2	91.1	87.8	90.5				
26	90.5	92.5	90.9	89.4	90.8	88.8	88.9	91.0	88.6	91.1	91.8	91.6	89.9	91.6	90.9	87.4	88.8	88.5	89.4	88.2	88.9	88.8	92.0	87.8	89.9			
27	88.6	89.4	89.2	88.7	89.5	88.5	88.7	89.1	87.4	89.1	89.3	88.8	88.5	89.6	89.1	85.6	87.8	87.5	87.7	88.7	88.7	87.9	91.5	88.0	87.6	90.0		
28	90.6	91.4	90.2	89.5	89.6	88.4	88.5	91.9	89.3	91.6	91.2	90.9	88.6	90.6	89.7	88.6	89.5	90.1	89.0	86.2	88.6	88.1	92.2	88.1	87.7	91.2	90.7	
29	90.9	92.6	91.4	90.5	90.9	89.9	90.0	92.6	89.9	93.1	93.4	92.9	90.7	92.2	91.0	89.1	89.6	89.8	90.1	87.4	90.0	89.9	92.3	89.3	90.1	94.0	90.4	92.2

Fig. 1. Phylogenetic tree showing the relationships among *Nocardioides* strains, *Aeromicrobium* species and *Terrabacter tumescens* used in this study. Scale bar represents 2 nucleotide substitutions per 100 nucleotides.

Fig. 2. Unrooted phylogenetic tree showing the relationships among representatives of the genus *Nocardioides*, *Aeromicrobium* species, *Terrabacter tumescens*, and some other actinomycete taxa. Scale bar represents 2 nucleotide substitutions per 100 nucleotides.

genetic tree shows that *T. tumescens* is distantly related to the genera *Nocardioides* and *Aeromicrobium* (Figs 1 and 2) as shown by previous studies (2, 12, 24).

The mean intraspecific nucleotide similarity of 16S rDNA sequences from *N. albus* was $99.5 \pm 0.5\%$ (Fig. 1). The phylogenetic relationships between *N. albus* strains are shown in Fig. 1. The 16S rDNA similarity value between *N. albus* strains is within the range of 16S rDNA similarity values that have a high possibility of being recognized as the same species, according to current species definitions from a compilation of available 16S rDNA/rRNA sequences and DNA–DNA reassociation data (21). The current phylogenetic definition of a species in bacteriology states that 'strains with approximately 70% or greater DNA–DNA relatedness' are members of the same species (26). According to Stackebrandt & Goebel (21), organisms that have less than 97% sequence homology will not reassociate to more than 60%, no matter which hybridization method is applied. The strains of *N. albus* formed a distinct cluster that separated from other validly described *Nocardioides* species, except *N. luteus* KCTC 9575^T, as seen in the phylogenetic tree shown in Fig. 1. In our study, *N. luteus* KCTC 9575^T was closely related to *N. albus* strains (Figs 1 and 2). *N. luteus* KCTC 9575^T showed mean nucleotide similarity values of $99.5 \pm 0.5\%$ with *N. albus* strains and, in particular, a 16S rDNA similarity of 100% with *N. albus* JCM 5854 (Fig. 1). Although *N. luteus* KCTC 9575^T could be differentiated from *N. albus* by the difference in the colours of the primary and aerial mycelium and by the DNA–DNA reassociation value, and it has been published as a new species of the genus *Nocardioides* (16), 16S rDNA homologies between *N. albus* strains and *N. luteus* KCTC 9575^T are much too high for these to be described as different species (Fig. 1). Therefore, the taxonomic relationship between *N. albus* strains and *N. luteus* KCTC 9575^T should be examined using other taxonomic markers as well as a re-examination of DNA–DNA relatedness. *N. luteus* KCTC 9575^T and *N. albus* JCM 5854 also showed 16S rDNA similarity values of 100% with '*N. flavus*' strains IFO 14396^T and IFO 14397, and '*N. fulvus*' JCM 3335^T (Fig. 1). '*N. flavus*' IFO 14396^T was shown to be related to *N. luteus* KCTC 9575^T (17) and has been described as a strain of *N. luteus* in catalogues of some culture collections. Although '*N. fulvus*' JCM 3335^T showed DNA–DNA homology of 41% with the type strain of *N. albus* (17), its exact taxonomic position is vague. From our 16S rDNA analysis, '*N. fulvus*' JCM 3335^T was shown to be closely related to *N. luteus* KCTC 9575^T and *N. albus* strains (Fig. 1). The phylogenetic relationships among *N. albus* strains, *N. luteus* KCTC 9575^T, '*N. flavus*' strains and '*N. fulvus*' JCM 3335^T also should be re-examined by DNA–DNA relatedness tests.

'*N. fulvus*' IFO 14399 showed low 16S rDNA similarity (91.4%) with '*N. fulvus*' JCM 3335^T and had highest 16S rDNA similarity (99%) with *Nocardioides*

sp. ATCC 39419 (Table 2). '*N. fulvus*' IFO 14399 had 16S rDNA similarities of 91.3–93.5% with the type strains of the genus *Nocardioides* and 93.0–94.4% with the two *Aeromicrobium* species (Table 2). *Nocardioides* sp. ATCC 39419 showed 16S rDNA similarities of 91.3–93.5% with the type strains of the genus *Nocardioides* and 93.3–94.5% with the two *Aeromicrobium* species (Table 2). From this 16S rDNA sequence analysis, '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 are thought to be more closely related to the genus *Aeromicrobium* than to the genus *Nocardioides*. '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 also form a phylogenetic lineage distinct from the genera *Nocardioides* and *Aeromicrobium* on 16S rDNA-based trees (Fig. 2). Therefore, the two strains are thought to be members of a new genus which is related to the genera *Nocardioides* and *Aeromicrobium*. However, chemotaxonomic, biochemical and physiological characterizations are necessary for the description of the two strains as a new genus and, in addition, DNA–DNA relatedness testing is required to investigate the relationship between '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419. These taxonomic studies are in progress in our laboratory.

N. simplex strains KCTC 9106^T, JCM 1366, JCM 1367, NCIMB 12919 and ATCC 15799 shared an identical 16S rDNA sequence (Fig. 1). *N. simplex* strains ATCC 19565 and ATCC 19566 also shared identical 16S rDNA sequences (Fig. 1). However, *N. simplex* strains ATCC 13260, ATCC 19565 and ATCC 19566 showed low 16S rDNA similarities to *N. simplex* strains KCTC 9106^T, JCM 1366, JCM 1367, NCIMB 12919 and ATCC 15799, and to other validly described *Nocardioides* species in this study (Table 2). *N. simplex* ATCC 13260 showed a 16S rDNA similarity value of 99.4% with *N. simplex* strains ATCC 19565 and ATCC 19566 (Table 2). *N. simplex* ATCC 13260 showed 16S rDNA similarities of 90.7% with *N. simplex* KCTC 9106^T and 90.3–92.2% with the other type strains of the genus *Nocardioides* (Table 2). *N. simplex* strains ATCC 19565 and ATCC 19566 showed 16S rDNA similarities of 90.2% with *N. simplex* KCTC 9106^T and 89.9–91.9% with the other type strains of the genus *Nocardioides* (Table 2). Therefore, *N. simplex* strains ATCC 13260, ATCC 19565 and ATCC 19566 were not thought to be members of the genus *Nocardioides*. *N. simplex* ATCC 13260, ATCC 19565 and ATCC 19566 were more closely related to the genus *Rhodococcus*, especially *R. erythropolis* DSM 43066^T, than to *Nocardioides* species from 16S rDNA sequence analysis (Fig. 2). *N. simplex* ATCC 13260 showed a 16S rDNA similarity value of 99.5% with *R. erythropolis* DSM 43066^T, and *N. simplex* strains ATCC 19565 and ATCC 19566 showed a 16S rDNA similarity value of 100% with *R. erythropolis* DSM 43066^T in the region corresponding to positions 28–1524 (*E. coli* 16S rRNA numbering) (Table 2). From chemotaxonomic characteristics, which are consistent with those found in the genus *Rhodococcus* (5,

7), and sequence comparison with the type strain of *N. simplex*, the 16S rRNA sequence of which is already known, *N. simplex* strains ATCC 13260, ATCC 19565 and ATCC 19566 were recently reclassified as the genus *Rhodococcus* (29) and, in particular, *R. erythropolis* [Gray & Thornton, 1928 (6)].

'*N. thermolilacinus*' strains IFO 14335^T and IFO 14336, which share identical nucleotide sequences, showed low 16S rDNA similarity values of 88.9–90.9 % with the type strains of the genus *Nocardioides* (Table 2) and are shown to be distantly related to the phylogenetic radiation enclosed by the type strains of the genus *Nocardioides* (Figs 1 and 2). Therefore, these two strains should be reclassified into another genus on the basis of this 16S rDNA sequence analysis. As the two strains were regarded from their morphological traits, *Streptomyces*-like life cycle, and susceptibility to phages to be streptomycete in character (17), they correspondingly showed their closest relationship with the genus *Streptomyces* from sequence analysis of their 16S rDNAs (Fig. 2). In particular, they had highest 16S rDNA similarity with *Streptomyces tendae* and *Streptomyces caelestis*, (98.7 and 98.6 %, respectively) (Table 2). Therefore, '*N. thermolilacinus*' strains IFO 14335^T and IFO 14336 may be recognized as members of the genus *Streptomyces* until their exact taxonomic position is shown by DNA–DNA relatedness and by phenotypic characteristics.

The *Nocardioides*-like isolate OS4 is able to degrade pyridine and was previously published as *Pimelobacter* sp. (10, 18). Isolate OS4 shows a mean nucleotide similarity value of 95.4 ± 0.4 % with the type strains of the genus *Nocardioides* (Table 2) and forms a distinct phylogenetic position within the genus *Nocardioides* as shown in the tree based on 16S rDNA sequences (Figs 1 and 2). Strain OS4 has been accepted for publication as a new species of the genus *Nocardioides*, '*Nocardioides pyridinolyticus*', on the basis of phenotypic characteristics and phylogenetic inference (30). However, DNA–DNA relatedness testing between strain OS4 and the five validly described *Nocardioides* species was not performed. Another *Nocardioides*-like isolate, strain NSP41, was most closely related to all *N. simplex* strains except three (strains ATCC 13260, ATCC 19565 and ATCC 19566) (Figs 1 and 2), which were previously identified as strains of *N. simplex*. The 16S rDNA similarity value between isolate NSP41 and *N. simplex* KCTC 9106^T was 98.6 %. The intraspecific 16S rDNA similarity value of *N. simplex* was 100 % in our study since *N. simplex* strains ATCC 13260, ATCC 19565 and ATCC 19566 were identified as not being members of the genus *Nocardioides*. Therefore, DNA–DNA relatedness testing between strain NSP41 and *N. simplex* strains is necessary to examine the exact taxonomic position of strain NSP41. DNA–DNA relatedness testing is important to elucidate the relationships between *N. simplex* strains and to investigate the possibility of strain NSP41 being a new species of the genus *Nocardioides*.

In conclusion, analysis of the 16S rDNA sequences was a useful method for elucidation of the phylogenetic relationship among LL-diaminopimelic acid-containing actinobacteria including the genera *Nocardioides* and *Aeromicrobium* and *T. tumescens*, and also to show the inter- and intraspecific phylogenetic relationships within the genera *Nocardioides* and *Aeromicrobium* (Figs 1 and 2). Our results led to the conclusion that most *Nocardioides* strains belong to the phylogenetic radiation encompassed by the genus *Nocardioides* despite morphological heterogeneity. However, it is concluded that some strains of the genus *Nocardioides* should be transferred to another genus and be described as a new genus (Fig. 2).

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