Classification of '*Nocardiodides fulvus*' IFO 14399 and *Nocardiodides* sp. ATCC 39419 in *Kribbella* gen. nov., as *Kribbella flavida* sp. nov. and *Kribbella sandramycini* sp. nov.

Yong-Ha Park,¹ Jung-Hoon Yoon,^{1,2} Yong Kook Shin,¹
Ken-ichiro Suzuki,³ Takuji Kudo,³ Akio Seino,¹ Hong-Joong Kim,¹
Jung-Sook Lee¹ and Sung Taik Lee²

Author for correspondence: Yong-Ha Park. Tel: +82 42 860 4620. Fax: +82 42 860 4625.
e-mail: yhpark@kribb4680.kribb.re.kr

¹ Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology (KRIBB), PO Box 115, Yusong, Taejeon, Korea

² Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejeon, Korea

³ Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01, Japan

By 16S rDNA sequence analysis, two strains identified previously as members of the genus *Nocardiodides*, namely '*Nocardiodides fulvus*' IFO 14399 and *Nocardiodides* sp. ATCC 39419, were found to form lines of descent distinct from the genus *Nocardiodides*. Therefore, the two strains were studied taxonomically in detail by phenotypic, particularly chemotaxonomic, characterization, and by phylogenetic analysis including other related taxa. The genomic DNA G+C contents of '*N. fulvus*' IFO 14399 and *Nocardiodides* sp. ATCC 39419 are 70 and 68 mol %, respectively. The two strains contain LL-diaminopimelic acid as the diamino acid in their peptidoglycan, together with L-alanine at position 1 of the peptide subunit. The predominant menaquinone found in the two strains is MK-9(H₄). The major cellular fatty acid found in the two strains is anteiso-C_{15:0}, which is a minor component of members of the genus *Nocardiodides*. The diagnostic polar lipid of the two strains is phosphatidylcholine. A phylogenetic tree was constructed which showed that the two strains form evolutionary lineages distinct from other LL-diaminopimelic-acid-containing taxa such as, for example, the genus *Nocardiodides*. On the basis of our data, a new genus, *Kribbella* gen. nov., and two new species, *Kribbella flavida* gen. nov., sp. nov. and *Kribbella sandramycini* gen. nov., sp. nov., are proposed. It is also proposed that the new genus be included in the family *Nocardioideaceae*.

Keywords: *Kribbella flavida*, *Kribbella sandramycini*, *Nocardiodides* spp., LL-diaminopimelic acid, phylogeny

INTRODUCTION

Relatively few bacterial genera containing LL-diaminopimelic acid (LL-A₂pm) in the cell wall peptidoglycan are known when compared to the number of genera containing meso-diaminopimelic acid. However, the number of such taxa has recently been considerably increased. The actinomycete genera containing LL-diaminopimelic acid in the cell wall fall in five phylogenetic lineages that represent five families

within the class *Actinobacteria* (Stackebrandt *et al.*, 1997): (i) *Streptomyces* (Waksman & Henrici, 1943) (*Streptomycetaceae*); (ii) *Sporichthya* (Lechevalier *et al.*, 1968; Rainey *et al.*, 1993) (*Sporichthyaceae*); (iii) *Nocardiodides* (Prauser, 1976) and *Aeromicrobium* (Miller *et al.*, 1991) (*Nocardioideaceae*); (iv) *Intrasporangium* (Kalakoutskii *et al.*, 1967), *Terrabacter* (Collins *et al.*, 1989) and *Terracoccus* (Prauser *et al.*, 1997) (*Intrasporangiaceae*); (v) *Propioniferax* (Yokota *et al.*, 1994), *Luteococcus* (Tamura *et al.*, 1994), *Microtholus* (Nakamura *et al.*, 1995) and *Friedmanniella* (Schumann *et al.*, 1997) (*Propionibacteriaceae*). In this study, a new genus containing LL-diaminopimelic acid in the peptidoglycan is proposed. 16S rDNA sequence analysis shows that two strains identified previously as members of the genus

Abbreviation: LL-A₂pm, LL-diaminopimelic acid.

The GenBank accession numbers for the 16S rDNA sequences of '*Nocardiodides fulvus*' IFO 14399 and *Nocardiodides* sp. ATCC 39419 determined in this work are AF005017 and AF005020, respectively.

Fig. 1. Scanning electron micrographs of aerial mycelia of '*Nocardioideis fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419. (a, b) '*N. fulvus*' IFO 14399. Bars, 10 µm (a) and 1 µm (b). (c, d) *Nocardioideis* sp. ATCC 39419. Bars, 10 µm (c) and 1 µm (d).

Nocardioideis, namely '*Nocardioideis fulvus*' IFO 14399 (Ruan & Zhang, 1979) and *Nocardioideis* sp. ATCC 39419 (Matson & Bush, 1989) form phylogenetic lineages distinct from *Nocardioideis* species. Therefore, we investigated the phenotypic characters of these two strains to determine their taxonomic positions. On the basis of our data, we propose that they should be established as members of a new genus, *Kribbella* gen. nov.

Nesterenko *et al.* (1985) proposed the family *Nocardioideaceae* to accommodate the genera *Nocardioideis* (Prauser, 1976) and *Pimelobacter* (Suzuki & Komagata, 1983). However, three *Pimelobacter* species were transferred to the genus *Nocardioideis* or reclassified as the genus *Terrabacter* (Collins *et al.*, 1989). Before the proposal of Nesterenko *et al.* (1985), O'Donnell *et al.* (1982) proposed that *Arthrobacter simplex* (*Pimelobacter simplex*) should be assigned to the genus *Nocardioideis* as *N. simplex*. *Pimelobacter jensenii* was transferred to the genus *Nocardioideis* as *N. jensenii* and *Pimelobacter tumescens* was reclassified in the new genus *Terrabacter* as *T. tumescens* (Collins *et al.*, 1989). *T. tumescens* was shown to be distantly related to the genus *Nocardioideis* from phylogenetic inference based on 16S rRNA sequences (Collins *et al.*, 1989; Tamura & Yokota, 1994). Therefore, the genus *Nocardioideis* was the only member of the family

Nocardioideaceae before the genus *Aeromicrobium* was included in the family (Stackebrandt *et al.*, 1997).

The genus *Aeromicrobium*, which is phylogenetically related to the genus *Nocardioideis*, was proposed by Miller *et al.* (1991). In our study, the new genus, *Kribbella* was found to be phylogenetically related to the genera *Nocardioideis* and *Aeromicrobium* despite differences in some chemotaxonomic characters and formation of lines of descent distinct from the genera *Nocardioideis* and *Aeromicrobium*. It is thought appropriate that the genera *Nocardioideis* and *Aeromicrobium* and the new genus *Kribbella* are grouped in a single family. Therefore, we propose that the new genus is included in the family *Nocardioideaceae*.

METHODS

Bacterial strains and culture conditions. '*N. fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419 were usually cultivated at 30 °C on International *Streptomyces* Project (ISP) medium no. 2, which contains (l⁻¹) 4 g yeast extract, 10 g malt extract and 4 g glucose (pH 7.3). The two strains were also cultivated on nutrient agar (Difco), trypticase soy agar (Difco), glucose-asparagine agar (Lindenbein, 1952) and ISP agar media nos 1, 3, 4, 5 and 7 (Shirling & Gottlieb, 1966) to examine the morphological characteristics. The two strains and some reference strains were also grown for 4 d on nutrient agar (Difco) for fatty acid methyl ester (FAME)

Table 1. Utilization of various compounds as sole carbon and energy sources by *Nocardioideis albus* KCTC 9186^T, '*Nocardioideis fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419

+, Positive reaction; —, negative reaction; w, weakly positive reaction.

Compound	<i>N. albus</i> KCTC 9186 ^T	' <i>N. fulvus</i> ' IFO 14399	<i>Nocardioideis</i> sp. ATCC 39419
L-Arabinose	+	—	w
D(+)-Galactose	+	—	+
D(–)-Ribose	+	—	+
D(+)-Glucose	+	+	+
D(–)-Fructose	+	—	+
D(+)-Mannose	+	—	+
D(+)-Xylose	+	—	+
α-L(+)-Rhamnose	+	—	+
D(+)-Cellobiose	+	+	+
Lactose	+	—	+
Maltose	+	+	+
α-D(+)-Melibiose	—	+	+
Sucrose	+	+	+
D(+)-Trehalose	—	+	+
D(+)-Melezitose	—	+	+
D(+)-Raffinose	—	+	+
Adonitol	—	+	+
myo-Inositol	—	+	+
D-Mannitol	+	+	+
D-Sorbitol	—	—	—
Inulin	—	+	+
Glycerol	+	—	+
Sodium acetate	—	—	—
Disodium fumarate	+	+	+
Trisodium citrate	—	—	—
Disodium succinate	+	+	+
Sodium benzoate	—	—	—

analysis. Reference strains for FAME analysis included *Nocardioideis albus* KCTC 9186^T, *Nocardioideis jensenii* KCTC 9134^T, *Nocardioideis luteus* KCTC 9575^T, *Nocardioideis simplex* KCTC 9106^T, *Nocardioideis pyridinolyticus* KCTC 0074BP^T, '*N. fulvus*' JCM 3335^T, *Aeromicrobium erythreum* NRRL B-3381^T, *Aeromicrobium fastidiosum* KCTC 9576^T and *Terrabacter tumescens* KCTC 9133^T.

Morphological and physiological tests. The morphology of cells was examined by light microscopy and scanning electron microscopy. Catalase activity was determined by bubble formation in a 3% hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1% tetramethyl-*p*-phenylenediamine. Hydrolyses of gelatin, casein and starch, and production of urease were determined as described by Cowan & Steel (1965). Hydrolyses of aesculin and arbutin were determined according to the method of Kurup & Fink (1975). Hydrolysis of elastin was determined according to the method of Williams *et al.* (1983). Tests for utilization of various substrates as sole carbon and energy sources were performed as described by Shirling & Gottlieb (1966) except that CuSO₄ was not included in the trace salts solution. Most of the substrates were tested at a concentration of 1% (w/v); the exception was glycerol (0.1%,

w/v). The utilization results from the two strains were checked over 4 weeks.

Isolation of DNA. Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996).

Chemotaxonomic characterizations. The diamino acid of the peptidoglycan was determined by the method described previously (Komagata & Suzuki, 1987). The amino acid molar ratio of the peptidoglycan was determined as described previously (Sasaki *et al.*, 1998). Menaquinones were analysed as described previously (Komagata & Suzuki, 1987) using reversed-phase HPLC. Fatty acids were extracted and analysed according to the instructions of the Microbial Identification System (MIDI; Microbial ID). Polar lipids were extracted and analysed by the procedure described by Minnikin *et al.* (1984).

Determination of G+C content. The G+C content was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and dephosphorylated, and the resulting nucleosides were analysed by reversed-phase HPLC.

DNA–DNA hybridization. DNA–DNA hybridization to determine genomic relatedness was performed by the dot-blot

Table 2. Cellular fatty acid profiles of '*Nocardioideis fulvus*' IFO 14399, *Nocardioideis* sp. ATCC 39419, representatives of the genera *Nocardioideis*, *Aeromicrobium* species and *Terrabacter tumescens*

Species: 1, *Nocardioideis albus* KCTC 9186^T; 2, *Nocardioideis jensenii* KCTC 9134^T; 3, *Nocardioideis luteus* KCTC 9575^T; 4, *Nocardioideis simplex* KCTC 9106^T; 5, *Nocardioideis pyridinolyticus* KCTC 0074BP^T; 6, '*Nocardioideis fulvus*' JCM 3335^T; 7, '*Nocardioideis fulvus*' IFO 14399; 8, *Nocardioideis* sp. ATCC 39419; 9, *Aeromicrobium erythreum* NRRL B-3381^T; 10, *Aeromicrobium fastidiosum* KCTC 9576^T; 11, *Terrabacter tumescens* KCTC 9133^T.

	1	2	3	4	5	6	7	8	9	10	11
Saturated fatty acids											
C _{14:0}								0.6	0.7	0.6	7.0
C _{15:0}	0.7	0.3			0.4	0.3		2.2	1.7	1.0	4.9
C _{16:0}	0.7	1.3	0.6	1.1	1.2		0.6	1.1	20.0	10.1	12.5
C _{17:0}	1.5	2.7	0.5		1.2			1.8	2.4	2.1	5.3
C _{18:0}		2.0		1.3	1.0				6.1	9.7	7.8
C _{19:0}											1.6
C _{20:0}											
Unsaturated fatty acids											
C _{15:1} ω6c		0.3				0.8	0.8	3.8			0.9
C _{16:1} ω9c									0.8	1.4	0.3
C _{17:1} ω6c	15.6	5.2	15.9	13.1		14.9	1.7		0.6		
C _{17:1} ω8c	2.8	3.4	1.1	2.3	1.7	1.0	3.7	5.5	0.8		5.8
C _{17:1} ω9c										0.9	
C _{18:1} ω9c		3.3		7.3	2.0		1.7		25.6	25.1	13.9
C _{19:1} iso I*				1.6							
Branched fatty acids											
iso-C _{13:0}								0.7			
iso-C _{14:0}	2.2	1.1	0.7	1.6	0.5	2.5	9.1	14.8			4.3
iso-C _{14:0} 3-OH			0.6					0.9			
iso-C _{15:0}	3.1	3.7	3.7	1.5	5.1	4.1	5.4	11.2			8.3
anteiso-C _{15:0}					1.4	0.7	24.9	35.5			2.1
iso-C _{16:1} H*	1.1	14.0	2.1	5.4	4.2	4.7	13.5	2.9			1.0
iso-C _{16:0}	53.6	45.5	49.1	33.7	47.3	57.9	20.9	8.1	1.4		5.6
iso-C _{17:0}	1.3	1.7	1.0	2.2	1.7	0.4		1.9			
iso-C _{17:1} ω9c							6.7	4.4	0.4	0.7	
anteiso-C _{17:1} ω9c					2.12		1.1				
anteiso-C _{17:0}	0.7	0.7	1.4	0.5	14.2	1.1	3.2	1.3			1.1
iso-C _{18:0}	0.9	2.7		0.9	0.5				0.5		
10-Methyl-fatty acids											
C _{16:0}	1.5	2.5	5.0	4.0	5.0	3.6					2.8
C _{17:0}	12.2	5.4	15.0	5.4	5.5	6.3	3.3		0.3		1.2
C _{18:0}	2.3	2.4	2.9	9.3	3.4	1.0			24.2	32.9	1.0
Hydroxy fatty acids											
C _{15:0} 2-OH									0.6	0.6	
C _{16:0} 2-OH				0.7					11.3	8.9	
C _{17:0} 2-OH									0.9	1.5	
C _{18:0} 2-OH									1.1	3.7	
Summed features†											
Summed feature 4		1.2		5.1	0.8	0.7	3.6	2.8		0.9	8.1
Summed feature 5								0.7			
Summed feature 6				0.7							1.7
Summed feature 7				2.4							1.0
Summed feature 8					0.5						1.9

* The double bond position indicated by a capital letter is unknown.

† Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 4 contained one or more of following fatty acids: 15:0 iso 2-OH and/or 16:1ω7c. Summed feature 5 contained one or more of the following fatty acids: 17:1 anteiso B and/or 17:1 anteiso I. Summed feature 6 contained one or more of following fatty acids: 18:0 anteiso and/or 18:2ω6,9c. Summed feature 7 contained one or more of following fatty acids: 18:1 ω7c, 18:1ω9t and/or 18:1ω12t. Summed feature 8 contained one or more of following fatty acids: unknown fatty acid (equivalent chain length 18.756) and/or 19:1ω11c.

hybridization method. Labelling of probe and visualization of hybridized DNA were performed by using a non-radioactive ECL (enhanced chemiluminescence) direct nucleic acid labelling and detection system (Amersham). Resultant quantitative DNA-DNA relatedness was determined by using a densitometer (Bio-Rad).

16S rDNA sequencing. 16S rDNA sequencing from the two strains was described previously (Yoon *et al.*, 1998a).

Phylogenetic analysis. The 16S rDNA sequences of '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 were aligned with the 16S rRNA/16S rDNA sequences of representatives of the genus *Nocardioides* and some other actinomycete taxa by using CLUSTAL W software (Thompson *et al.*, 1994). The 16S rDNA sequences of representatives of the genera *Nocardioides* and *Aeromicrobium* and of *Terrabacter tumescens* were determined from our recent study (Yoon *et al.*, 1998a) and other reference sequences were obtained from the GenBank database, where the nucleotide sequences are available under the following accession numbers: X72377 (*Sporichthya polymorpha*), Z78207 (*Microtholus phosphovorius*), Z78208 (*Luteococcus japonicus*), Z78206 (*Friedmanniella antarctica*), D85486 (*Intrasporangium calvum*), X87110 (*Kineosporia aurantiaca*), Y11928 (*Terracoccus luteus*), X53217 (*Propionibacterium freudenreichii*), X53218 (*Propionibacterium acnes*), M23411 (*Arthrobacter globiformis*), X53163 (*Streptomyces albus*) and Z36934 (*Nocardia asteroides*). The 16S rRNA sequence of *Propioniferax innocua* was obtained from the study of Pitcher & Collins (1991). The 16S rDNA similarity values were calculated from the alignment and the evolutionary distances were calculated by using Kimura two-parameter correction with the CLUSTAL W software (Thompson *et al.*, 1994). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) on the basis of distance matrix data.

RESULTS

Morphological and physiological characters

'*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 exhibited mycelial structures on most media. Mycelial structures were observed best on ISP agar medium no. 3 (oatmeal agar). Vegetative mycelia showed hyphae which were extensively branched and penetrated into agar media; they often fragmented into rod- to coccoid-shaped and rod elements. Aerial mycelia consisted of hyphae that fragmented into short to elongated rod-like elements (Fig. 1). Colonies of '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 were pasty and had lichenous shapes with irregular edges. The two strains are strictly aerobic, Gram-variable and non-acid-fast. They showed catalase, oxidase and urease activities. Arbutin, casein, elastin, aesculin and Tween 80 were hydrolysed. Starch was not hydrolysed by '*N. fulvus*' IFO 14399 and was weakly hydrolysed by *Nocardioides* sp. ATCC 39419. The utilization of various compounds as sole carbon and energy source is shown in Table 1, in which results from the two strains are compared with those of *N. albus* KCTC 9186^T, the type species of the genus *Nocardioides*. '*N. fulvus*' IFO 14399 grew very slowly in this test. '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 grew at pH 5 and 9. The two strains grew

at 20 and 37 °C. However, they grew weakly at 40 °C and did not grow at 45 °C.

Chemotaxonomic characters and DNA base compositions

'*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 have LL-diaminopimelic acid as the diamino acid in their cell wall peptidoglycan, indicating that the two strains are members of wall chemotype I (Lechevalier & Lechevalier, 1970). The peptidoglycan of the two strains showed a similar molar ratio of amino acids D-glutamic acid:glycine:L-alanine:D-alanine:LL-A₂pm of 1:1:1:0.5:0.63. The presence of sufficient amounts of L-alanine in the peptidoglycan strongly suggests that the two strains have L-alanine at position 1 of the peptide subunit. The predominant menaquinone found in the two strains is a tetrahydrogenated menaquinone with nine isoprenoid units [MK-9(H₄)]. The major cellular fatty acid of the two strains is anteiso-C_{15:0}, which is a minor component of members of genus *Nocardioides*. On the other hand, '*N. fulvus*' IFO 14399 was also found to have significant amounts of iso-C_{16:0} and iso-C_{16:1} H, whereas *Nocardioides* sp. ATCC 39419 had significant amounts of iso-C_{14:0} and iso-C_{15:0} (Table 2). Two-dimensional TLC of polar lipids indicated the presence of phosphatidylcholine and the absence of phosphatidylethanolamine. The genomic DNA G+C contents of '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 are 70 and 68 mol %, respectively.

Phylogenetic analysis

The nearly complete nucleotide sequences of 16S rDNAs from the two strains were determined directly, following PCR amplification. The sequences of the 16S rDNAs determined from '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 were 1472 and 1470 bp, respectively, which corresponds to the regions between positions 28 and 1524 by comparison with the 16S rDNA of *Escherichia coli*. The 16S rDNA sequences of the two strains were compared with 16S rDNA and 16S rRNA sequences of representatives of the genus *Nocardioides* and some other actinomycete taxa, mainly those containing LL-diaminopimelic acid in the cell wall. '*N. fulvus*' IFO 14399 showed levels of 16S rDNA similarity of 91.4% to '*N. fulvus*' JCM 3335^T, 91.3–93.5% to type strains of the genus *Nocardioides*, and 93–94.4% to two *Aeromicrobium* species. *Nocardioides* sp. ATCC 39419 showed levels of 16S rDNA similarity of 91.3–93.5% to type strains of the genus *Nocardioides* and 93.3–94.5% to two *Aeromicrobium* species. The level of 16S rDNA similarity between '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 was 99%. The phylogenetic positions of '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 relative to members of the genera *Nocardioides* and *Aeromicrobium* and some other actinomycete taxa are shown in the tree constructed by the neighbour-joining method (Fig. 2).

Fig. 2. Unrooted phylogenetic tree showing the positions of '*Nocardioides fulvus*' IFO 14399, *Nocardioides* sp. ATCC 39419, LL-diaminopimelic acid-containing actinomycete taxa and some other actinomycete taxa.

DNA–DNA relatedness

The percentage DNA–DNA relatedness was 43 % for '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419. '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 exhibited levels of DNA–DNA relatedness of 38 % and 32 %, respectively, to *N. albus* KCTC 9186^T, the type species of the genus *Nocardioides*, which was used as reference strain.

DISCUSSION

Two '*Nocardioides fulvus*' strains were proposed by Ruan & Zhang (1979). In our recent 16S rDNA sequence analysis (Yoon *et al.*, 1998a), '*N. fulvus*' JCM 3335^T (strain 71-N86^T) showed 16S rDNA sequences identical to that of *N. luteus* KCTC 9575^T, whereas '*N. fulvus*' IFO 14399 (strain 65-N86) showed a low level of 16S rDNA similarity of 91.3–93.5 % to '*N. fulvus*' JCM 3335^T and the type strains of the genus *Nocardioides*. '*N. fulvus*' IFO 14399 showed its highest level of 16S rDNA similarity of 99 % to *Nocardioides* sp. ATCC 39419, which also showed low levels of 16S rDNA similarity of 91.3–93.5 % to the type strains of the genus *Nocardioides*. This observation led us to examine the exact taxonomic positions of '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 by using additional taxonomic traits such as phenotypic, particularly chemotaxonomic, characters.

'*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 had LL-diaminopimelic acid as the diamino acid in the cell wall peptidoglycan. The major menaquinone found in '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 was tetrahydrogenated menaquinone with nine isoprenoid units [MK-9(H₄)], whereas the genus *Nocardioides* contains a tetrahydrogenated menaquinone with eight isoprenoid units [MK-8(H₄)] (O'Donnell *et al.*, 1982; Tamura & Yokota, 1994). The actinomycete genera which contain MK-9(H₄) as the major menaquinone among LL-A₂pm-containing taxa include *Aeromicrobium*, *Friedmanniella*, *Luteococcus*, *Microthlax*, *Propioniferax* and representatives of the genus *Propionibacterium* (Schumann *et al.*, 1997).

Cellular fatty acid profiles show that '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 are clearly distinguishable from the members of the genus *Aeromicrobium* as well as the genus *Nocardioides*. The genus *Nocardioides* has significant amounts of iso-C_{16:0} and 10-methyl branched acids as its major fatty acids, and the genus *Aeromicrobium* has significant amounts of C_{16:0}, C_{18:1}, and 10-methyl-C_{18:0} as its major fatty acids. However, the major fatty acid found in '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 is anteiso-C_{15:0}, which is scarcely detected in the members of the genera *Nocardioides* and *Aeromicrobium* (Table 2). '*N. fulvus*' IFO 14399 and

Table 3. Differential characteristics of '*Nocardioideis fulvus*' IFO 14399, *Nocardioideis* sp. ATCC 39419 and LL-A₂pm-containing related taxaData for LL-A₂pm-containing related taxa are from Schumann *et al.* (1997).

Taxon	Cell morphology	Major menaquinone(s)	Amino acid at position 1 of peptide subunit	Polar lipids*	G + C content (mol %)
Strains IFO 14399 and ATCC 39419	Hyphae	MK-9(H ₄)	L-Alanine	PC	68–70
<i>Nocardioideis albus</i>	Hyphae	MK-8(H ₄)	L-Alanine	PG, DPG, PL, PG-OH	66.5–68.6
<i>Nocardioideis simplex</i>	Rods, cocci	MK-8(H ₄)	LyAlanine	PG, DPG, PL, PG-OH	71.7
<i>Aeromicrobium</i>	Rods	MK-9(H ₄)	L-Alanine	PE, PG	71–73
<i>Friedmanniella</i>	Cocci in packets	MK-9(H ₄)	Glycine	PI, PG, DPG, PL	73
<i>Luteococcus</i>	Cocci	MK-9(H ₄)	L-Alanine	PI, PG, DPG, GL	66–68
<i>Microclunatus</i>	Cocci	MK-9(H ₄)	Glycine	PI, PG, DPG, PL	67.9
<i>Propioniferax</i>	Rods	MK-9(H ₄)	L-Alanine	PE, PG, PL, GL	59–63
<i>Propionibacterium propionicum</i>	Pleomorphic	MK-9(H ₄)	Glycine	ND	63–65
<i>Terrabacter</i>	Rods, cocci	MK-8(H ₄)	L-Alanine	PE, PI, DPG, PL	69.8–73.4
<i>Terracoccus</i>	Cocci	MK-8(H ₄)	Glycine	PE, PI, PG, DPG	73
<i>Intrasporangium</i>	Hyphae	MK-8	L-Alanine	PI, PIM, PG, DPG	68.2
<i>Sporichthya</i>	Hyphae	MK-9(H ₆), MK-9(H ₈)	L-Alanine	PI, PG, DPG, PL	70.1
<i>Streptomyces</i>	Hyphae	MK-9(H ₆), MK-9(H ₈)	L-Alanine	PE, PI, PIM, DPG	69–73

* DPG, diphosphatidylglycerol; GL, unknown glycolipid(s); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PG-OH, phosphatidylglycerol containing 2-hydroxy fatty acids; PI, phosphatidylinositol; PL, unknown phospholipid(s); ND, not determined.

Nocardioideis sp. ATCC 39419 are also distinguished from the genera *Luteococcus*, *Friedmanniella*, *Microclunatus* and *Propioniferax*, which have only single species, by cellular fatty acid profiles. The genus *Luteococcus* has significant amounts of C_{17:1} and C_{16:1} as its major fatty acids (Schumann *et al.*, 1997). The genera *Friedmanniella*, *Microclunatus* and *Propioniferax* have anteiso-C_{15:0} as the major fatty acid (Schumann *et al.*, 1997), which is the same as that of '*N. fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419. However, apart from the major fatty acids, the rest of the fatty acid profiles of the three genera are different from those of '*N. fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419, although standardized conditions between them were not used. The three genera have iso-C_{15:0} as the second most predominant fatty acid and other fatty acids exist only in minor amounts (Schumann *et al.*, 1997). However, '*N. fulvus*' IFO 14399 had significant amounts (more than 10%) of iso-C_{16:0} and iso-C_{16:1} H, and *Nocardioideis* sp. ATCC 39419 had significant amounts (more than 10%) of iso-C_{14:0} and iso-C_{15:0} (Table 2). Diagnostic polar lipid profiles also show that '*N. fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419 differ from other LL-A₂pm-containing taxa in the presence of phosphatidylcholine (Table 3). Considering that phosphatidylcholine has been not found in the other LL-A₂pm-containing taxa, '*N. fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419 must be highly interesting taxa.

'*N. fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419 are also different from other taxa containing LL-A₂pm and MK-9(H₄) in showing hyphal morphology. The genera *Aeromicrobium*, *Friedmanniella*, *Luteococcus*, *Microclunatus* and *Propioniferax* do not form mycelial structures and their cells are cocci or rods (Schumann *et al.*, 1997). Among existing taxa containing LL-A₂pm and MK-9(H₄) only *Propionibacterium propionicum* shows a cellular structure of branching filaments (Charfreitag *et al.*, 1988), but this can be differentiated from '*N. fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419 by the different amino acid at position 1 of the peptide subunit (Table 3).

Despite the similarity of several chemotaxonomic characters between '*N. fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419 and some genera containing LL-diaminopimelic acid, in particular, the genera *Friedmanniella*, *Microclunatus* and *Propioniferax*, they are phylogenetically distinct from each other. The phylogenetic tree shows that '*N. fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419 form a phylogenetic lineage distinct from other LL-diaminopimelic-acid-containing taxa and some other actinomycete taxa (Fig. 2). Nevertheless, '*N. fulvus*' IFO 14399 and *Nocardioideis* sp. ATCC 39419 are thought to form phylogenetic lineages that are related to the genera *Nocardioideis* and *Aeromicrobium*, which are members of the family *Nocardiodaceae* (Stackebrandt *et al.*, 1997). The amino acid at position 1 of the

peptide subunit of the peptidoglycan supports the contention that '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 are more closely related to the genera *Nocardioides* and *Aeromicrobium* than to the members of the family *Propionibacteriaceae* (Table 3). '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 and the genera *Nocardioides* and *Aeromicrobium* have L-alanine at position 1 of the peptide subunit, whereas among members of the family *Propionibacteriaceae*, the genera *Friedmanniella* and *Micro-lunatus* and *P. propionicum* have glycine at this position.

'*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 are considered as different species by DNA-DNA relatedness and some phenotypic properties. The value of DNA-DNA relatedness for the two strains is 43 %. Strains with DNA-DNA relatedness less than 70% are considered as different species according to the current phylogenetic definition for a species (Wayne *et al.*, 1987). In addition, recent studies have shown that the two strains have distinct differences in size and nucleotide similarity of the 16S-23S internally transcribed spacer (ITS) (Yoon *et al.*, 1998b). The size of the 16S-23S ITS of '*N. fulvus*' IFO 14399 is 473 bp long, whereas *Nocardioides* sp. ATCC 39419 has two types of 16S-23S ITSs on the genome, which are 429 and 439 bp long. '*N. fulvus*' IFO 14399 exhibited levels of 16S-23S ITS nucleotide similarity of 73.6 and 75.4% to *Nocardioides* sp. ATCC 39419. The two strains also showed a difference in the composition of predominant fatty acids except for the major fatty acid, and some differences in physiological characters (Table 2).

On the basis of phenotypic and phylogenetic data, '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 should be differentiated from other genera containing LL-diaminopimelic acid in the cell wall. Also, '*N. fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 are considered as different species by data based on DNA-DNA relatedness and differences in some phenotypic properties. Consequently, a new genus, *Kribbella* gen. nov., and two new species, *Kribbella flavida* gen. nov., sp. nov. and *Kribbella sandramycini* gen. nov., sp. nov., are proposed. Descriptions of these taxa are given below.

Description of *Kribbella* gen. nov.

Kribbella (Kribb.el'la. M.L. dim. fem. ending -ella; M.L. fem. n. *Kribbella* arbitrary name formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, KRIBB, where taxonomic studies of this taxon were performed).

Vegetative mycelium shows hyphae which are extensively branched and penetrate into agar media; they often fragment into rod- to coccoid-shaped and rod elements. Aerial mycelium consists of hyphae that fragment into short to elongated rod-like elements. Colonies are pasty and have lichenous shapes and irregular edges. Strictly aerobic. Gram-variable.

Non-acid-fast. Catalase-, oxidase- and urease-positive. Contains LL-diaminopimelic acid as the diamino acid in the cell wall peptidoglycan (wall chemotype I). The predominant menaquinone is MK-9(H₄). The fatty acid profile consists of large amounts of branched fatty acids and the major cellular fatty acid is anteiso-C_{15:0}. The polar lipid is phosphatidylcholine. The genomic DNA G+C content is 68-70 mol%. The type species is *Kribbella flavida*.

Description of *Kribbella flavida* sp. nov.

Kribbella flavida (fla'vi.da. L. fem. adj. *flavida* pale yellow).

Vegetative mycelium shows hyphae which are extensively branched and penetrate into agar media; they often fragment into rod- to coccoid-shaped and rod elements. Aerial mycelium consists of hyphae that fragment into short to elongated rod-like elements. Colonies are pasty and have lichenous shapes with irregular edges. Strictly aerobic. Gram-variable. Non-acid-fast. Catalase-, oxidase- and urease-positive. Arbutin, casein, elastin, aesculin and Tween 80 are hydrolysed. Starch is not hydrolysed. Substrates utilized as a sole carbon and energy sources are listed in Table 1. Growth occurs at pH 5 and 9. Growth occurs at 20 and 37 °C, occurs weakly at 40 °C and does not occur at 45 °C. The major cellular fatty acid is anteiso-C_{15:0} and significant amounts (more than 10%) of iso-C_{16:0} and iso-C_{16:1} H are also found. The genomic DNA G+C content is 70 mol% (as determined by HPLC). Isolated from soil. The type strain is strain IFO 14399^T (= KCTC 9580^T).

Description of *Kribbella sandramycini* sp. nov.

Kribbella sandramycini (san.dra.my.ci'ni. M.L. n. *sandramycini* referring to an antibiotic, sandramycin).

Vegetative mycelium shows hyphae which are extensively branched and penetrate into agar media; they often fragment into rod- to coccoid-shaped and rod elements. Aerial mycelium consists of hyphae that fragment into short to elongated rod-like elements. Colonies are pasty and have lichenous shapes with irregular edges. Strictly aerobic. Gram-variable. Non-acid-fast. Catalase-, oxidase- and urease-positive. Arbutin, casein, elastin, aesculin and Tween 80 are hydrolysed. Starch is weakly hydrolysed. Substrates utilized as a sole carbon and energy sources are listed in Table 1. Growth occurs at pH 5 and 9. Growth occurs at 20 and 37 °C, occurs weakly at 40 °C and does not occur at 45 °C. The major cellular fatty acid is anteiso-C_{15:0} and significant amounts (more than 10%) of iso-C_{14:0} and iso-C_{15:0} are also found. The genomic DNA G+C content is 68.3 mol% (as determined by HPLC). Isolated from a soil in Mexico. Produces an antibiotic, sandramycin. The type strain is strain ATCC 39419^T (= KCTC 9609^T).

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