***Gordonia nitida* sp. nov., a bacterium that degrades 3-ethylpyridine and 3-methylpyridine**

Jung-Hoon Yoon,¹ Jay Jung Lee,² Seok-Sung Kang,¹ Mariko Takeuchi,⁴
Yong Kook Shin,¹ Sung Taik Lee,² Kook Hee Kang³ and Yong-Ha Park¹

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

¹ 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 Department of Food and Life Science, Sungkyunkwan University, Chuncheon-dong 300, Jangnan-gu, Suwon, Korea

⁴ Institute for Fermentation, Osaka, 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532-8686, Japan

A bacterial strain, LE31^T, which is capable of degrading 3-ethylpyridine and 3-methylpyridine, was isolated from an industrial wastewater and was taxonomically studied by using a polyphasic approach. Strain LE31^T was identified as a member of the genus *Gordonia* on the basis of chemotaxonomic characteristics and phylogenetic inference-based 16S rDNA sequence. The cell wall contained meso-diaminopimelic acid, arabinose and galactose (wall chemotype IV). The predominant menaquinone was MK-9(H₂). The mycolic acids contained 47–55 carbon atoms. The major fatty acids were C_{16:0}, C_{18:1} ω9c, 10-methyl-C_{18:0} (TBSA). The G+C content of DNA was 67 mol%. The 16S rDNA sequence of strain LE31^T was most similar to that of the type strain of *Gordonia rubropertincta*. The differences in some phenotypic characteristics and the genetic distinctiveness distinguish strain LE31^T from the *Gordonia* species described previously. Therefore it is proposed that strain LE31^T should be placed in the genus *Gordonia* as a new species. The name *Gordonia nitida* is proposed for strain LE31^T. The type strain of the new species is strain LE31^T (= KCTC 0605BP^T = KCCM 80004^T).

Keywords: *Gordonia nitida* sp. nov., 3-ethylpyridine, 3-methylpyridine, bioremediation

INTRODUCTION

The genus *Gordonia* was proposed by Tsukamura (1971), but the three original species of the genus were subsequently reclassified in the genus *Rhodococcus* (Goodfellow & Alderson, 1977). However, *Rhodococcus* species could be divided into the two aggregate groups by serological properties and chemotaxonomic properties, such as mycolic acid composition and menaquinone profiles (Goodfellow, 1986). Stackebrandt *et al.* (1988) found that the two aggregate groups are phylogenetically distinct on the basis of 16S rRNA sequences and revived the genus *Gordonia*. Now, the name *Gordonia*, instead of *Gordona*, is being recognized because it is etymologically correct (Stackebrandt *et al.*, 1997). The members of the genus *Gordonia* are widely distributed in nature (Goodfellow, 1992; Takeuchi & Hatano, 1988). Some *Gordonia* species have also been isolated from activated sludge in

aeration tanks of biological sewage-treatment plants (Lemmer & Kroppenstedt, 1984) and from the packing material of a biofilter used for biological odour abatement (Bendinger *et al.*, 1995; Klatte *et al.*, 1996). Nevertheless, little is known about *Gordonia* species or strains that degrade toxic aromatic compounds, unlike the genus *Rhodococcus*, a phylogenetic neighbour, which is a very important taxon from the point of view of bioremediation (Briglia *et al.*, 1996; Finnerty, 1992; Grund *et al.*, 1992; Lenke *et al.*, 1992). This has encouraged both attempts to discover putative novel *Gordonia* species capable of degrading toxic environment-contaminating compounds and extensive investigation of the physiological properties of members belonging to the genus *Gordonia*. Therefore, the discovery of *Gordonia* species or strains that degrade aromatic compounds may be very meaningful, since it suggests that the genus *Gordonia* also has potential in bioremediation. This work may provide a new insight into the biological diversity of species belonging to the genus *Gordonia*. In this study, a strain (LE31^T) that can degrade 3-ethylpyridine and 3-

The GenBank accession number for 16S rDNA sequence of strain LE31^T is AF148947.

methylpyridine was isolated from a wastewater from a wastewater-treatment plant and was considered to be a member of the genus *Gordonia*. 3-Ethylpyridine and 3-methylpyridine are alkylpyridines that cause serious problems for human health as environmental contaminants (Sims & O'Loughlin, 1989; Kaiser *et al.*, 1996). Although some bacterial strains that are able to degrade pyridine have been described (Shukla & Kaul, 1974; Watson & Cain, 1975; Yoon *et al.*, 1997), little is known about bacterial strains that degrade 3-ethylpyridine and 3-methylpyridine. Accordingly, the aim of this study was to determine the exact taxonomic position of strain LE31^T. We describe the morphological and physiological characteristics and also the phylogeny based on 16S rDNA sequence and DNA relatedness with some *Gordonia* species of strain LE31^T. On the basis of the data described below, we propose a new species of the genus *Gordonia*, i.e. *Gordonia nitida*, for strain LE31^T.

METHODS

Bacterial strains. Strain LE31^T was isolated by selective enrichment from an industrial wastewater in Taegu, Korea. The wastewater sample was inoculated in 50 ml minimal salts medium (Yoon *et al.*, 1997) containing (per litre) 1 g K₂HPO₄, 0.5 g NaH₂PO₄·2H₂O, 0.25 g KCl, 0.25 g MgSO₄·7H₂O, 1 ml trace-element solution (Lee *et al.*, 1991) supplemented with 0.5 mM each of some alkylpyridines including 3-ethylpyridine and 3-methylpyridine. This medium was incubated at 30 °C on a horizontal shaker at 150 r.p.m.. When it was confirmed that the 3-ethylpyridine and 3-methylpyridine in the medium had disappeared, a portion of the suspension was transferred into 50 ml fresh medium and the medium was reincubated. The disappearance of 3-ethylpyridine and 3-methylpyridine was determined by spectrophotometry (Beckman DU-68). After three successive transfers, the suspension was plated on solid media containing 0.5 mM each of 3-ethylpyridine and 3-methylpyridine to isolate pure cultures. Single colonies were tested to investigate ability to degrade 3-ethylpyridine and 3-methylpyridine in minimal salts medium (Yoon *et al.*, 1997) containing (per litre) 1 g K₂HPO₄, 0.5 g NaH₂PO₄·2H₂O, 0.25 g KCl, 0.25 g MgSO₄·7H₂O, 1 ml trace-element solution (Lee *et al.*, 1991). The degradation of 3-ethylpyridine and 3-methylpyridine from each broth culture was checked by spectrophotometry. Of the isolates, strain LE31^T was selected for further studies.

Gordonia aichiensis DSM 43978^T, *Gordonia amarae* DSM 43392^T, *Gordonia bronchialis* DSM 43247^T, *Gordonia hirsuta* DSM 44140^T, *Gordonia hydrophobica* DSM 44015^T, *Gordonia rubropertincta* DSM 43197^T, *Gordonia sputi* DSM 43896^T and *Gordonia terrae* DSM 43249^T were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen). *Gordonia rhizosphaera* IFO 16068^T was obtained from the IFO (Institute for Fermentation, Osaka, Japan).

Culture conditions. For the investigation of morphological and physiological characteristics, strain LE31^T was in most cases cultivated on trypticase soy agar (TSA; BBL) at 30 °C. Cell biomass for the analysis of cell wall, mycolic acids and menaquinone was obtained from trypticase soy broth (TSB; BBL) culture. Strain LE31^T was cultivated at 30 °C on a horizontal shaker at 150 r.p.m.. Cell biomass for DNA extraction of strain LE31^T and *Gordonia* species was pro-

duced on TSB (BBL) supplemented with glucose (0.75%, w/v). The broth cultures were checked for purity before being harvested by centrifugation. Strain LE31^T and *Gordonia* species were also cultivated at 30 °C for 7 d on TSA (BBL) for fatty acid methyl ester analysis.

Morphological characteristics. The morphology of the cells was examined by light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). For SEM, cells from the early growth phase and the stationary growth phase were prepared by the method described previously (Bozzola & Russell, 1991) and the specimens were examined by using a model 535M scanning electron microscope (Philips). The presence or absence of flagella was determined using TEM with cells from exponentially growing cultures. The cells were negatively stained with 1% (w/v) phosphotungstic acid and, after air drying, the grids were examined by using a model CM-20 transmission electron microscope (Philips).

Physiological characteristics. Catalase activity was determined by bubble formation in a 3% hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1% *p*-aminodimethylaniline oxalate. Nitrate reduction was studied as described previously (Lanyi, 1987). Hydrolysis of casein, starch and Tween 80 and the production of urease and DNase were determined as described previously (Cowan & Steel, 1965). Hydrolysis of aesculin and arbutin was determined according to the method of Kurup & Fink (1975). Hydrolysis of elastin was determined according to the method of Williams *et al.* (1983). Assimilation of D-arabitol, D-galactose, glucose, *myo*-inositol, D-ribose, sucrose, D-trehalose, D-turanose, L-rhamnose, L-aspartate, benzoate, citrate, gluconate, succinate, L-alanine, L-leucine, L-proline, L-valine and acetamide was determined as described previously (Takeuchi & Hatano, 1998). Acid production from glucose, D-galactose, *myo*-inositol, D-sorbitol, sucrose, D-trehalose and L-rhamnose was determined as described previously (Takeuchi & Hatano, 1998).

Chemotaxonomic characterization. The isomer type of diamino acid in the cell wall was analysed using TLC according to the method described previously (Komagata & Suzuki, 1987). The sugars of the cell wall were analysed as described previously (Saddler *et al.*, 1991). Menaquinones were analysed as described previously (Komagata & Suzuki, 1987), using reversed-phase HPLC. Mycolic acids were extracted and purified as their methyl esters according to the procedures described by Minnikin (1988). The numbers of carbon atoms in the mycolic acids were calculated from the mass spectra of the methyl esters taken from an Autospec M mass spectrometer (Micromass) operated in electron-impact mode. Fatty acids were extracted and analysed according to the instructions of the Microbial Identification System (MIDI, Microbial ID).

Determination of G + C content. Chromosomal DNAs were isolated and purified according to the method described previously (Yoon *et al.*, 1996) with the exception that ribonuclease T1 was used together with ribonuclease A. The G + C content was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

DNA-DNA hybridization. DNA-DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. The type strains of all validly described *Gordonia* species were used as reference strains for DNA-DNA hybridization.

Table 1. Some differential characteristics of strain LE31^T and other *Gordonia* species

+, Positive reaction; –, negative reaction; + w, weakly positive reaction; v, variable reaction; ND, not determined.

Characteristic	Strain LE31 ^T	<i>G. aichiensis</i>	<i>G. amarae</i>	<i>G. bronchialis</i>	<i>G. hirsuta</i>	<i>G. hydrophobica</i>	<i>G. rhizosphaera</i>	<i>G. rubropertincta</i>	<i>G. sputi</i>	<i>G. terrae</i>
Assimilation of:*										
Galactose	–	+	–	–	ND	+	–	+	+	+
Inositol	–	–	–	+	+	–	–	ND	–	–
Rhamnose	–	–	–	v	–	–	+ w	v	–	v
Ribose	+	–	+	–	–	–	–	+	–	–
Sucrose	+	–	+	+	–	+	–	+	–	+
D-Turanose	+	–	+	+	–	+	ND	–	–	–
Aspartate	–	+	–	–	–	–	–	–	–	–
Benzoate	–	–	–	–	–	–	–	+	–	+
Citrate	+	+	+	–	–	+	+	–	+	+
Gluconate	+	–	–	+	–	–	+	+	–	–
Succinate	–	–	–	+	–	+	–	–	+	–
L-Alanine	+ w	+	–	+	–	–	+	+	–	–
Leucine	–	–	–	+	+	–	+ w	–	–	–
Proline	+	–	+	+	–	+	+ w	–	–	–
L-Valine	–	–	+	+	–	+	+ w	–	–	–
Acetamide	–	–	–	v	–	+	–	–	–	–
Degradation of:†										
3-Ethylpyridine	+	–	–	–	–	–	–	–	–	–
3-Methylpyridine	+	–	–	–	–	–	–	–	–	–
Acid production from:*										
Rhamnose	–	ND	ND	+	ND	ND	+ w	ND	ND	–
Trehalose	+	ND	ND	+	ND	ND	–	ND	ND	+
Mean no. carbon atoms in mycolic acids	47–55	62	48–54	64	60–64	58–60	60–62	58–59	61–62	58

* Data for *Gordonia* species other than strain LE31^T were obtained from the study by Takeuchi & Hatano (1998). Data for assimilation of D-turanose are from Klatte *et al.* (1996).

† Tests for degradation of 3-ethylpyridine and 3-methylpyridine with strain LE31^T and the type strains of *Gordonia* species were performed in this study.

16S rDNA sequencing. The sequencing of 16S rDNA of strain LE31^T was performed as described previously (Yoon *et al.*, 1998).

Phylogenetic analysis and nucleotide sequence accession numbers. The 16S rDNA sequence of strain LE31^T was aligned with 16S rRNA gene sequences of *Gordonia* species and representatives of mycolic-acid-containing actinomycete taxa by using CLUSTAL W software (Thompson *et al.*, 1994). The 16S rDNA similarity values were calculated from the alignment. Gaps at the 5' and 3' ends of the alignment were omitted from further analyses. Evolutionary distance matrices were calculated by using the algorithm of Jukes & Cantor (1969) with the DNADIST program within the PHYLIP package (Felsenstein, 1993). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) as implemented within the NEIGHBOR program of the same package. The stability of relationships was assessed by bootstrap analysis of 1000 data sets with the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

The GenBank, EMBL and DDBJ accession numbers for reference 16S rDNA sequences used in this analysis are as follows: X80633 (*G. aichiensis* DSM 43978^T), X80635 (*G. amarae* DSM 43392^T), X79287 (*G. bronchialis* DSM 43247^T), X93485 (*G. hirsuta* DSM 44140^T), X87340 (*G. hydrophobica* DSM 44015^T), AB004729 (*G. rhizosphaera* IFO 16068^T), X80632 (*G. rubropertincta* DSM 43197^T), X80634 (*G. sputi* DSM 43896^T), X79286 (*G. terrae* DSM 43249^T), X84248 (*Corynebacterium diphtheriae* NCTC 11397^T), X79290 (*Dietzia maris* DSM 43672^T), X52917 [*Mycobacterium tuberculosis* (Rogall *et al.*, 1990)], X80606 (*Nocardia asteroides* DSM 43757^T), X80608 (*Nocardia brasiliensis* DSM 43758^T),

X79289 (*Rhodococcus erythropolis* DSM 43066^T), X79288 (*Rhodococcus rhodochrous* DSM 43241^T), Z35435 (*Skermania piniformis* IFO 15059^T) and X80628 (*Tsukamurella paurometabola* DSM 20162^T).

RESULTS

Morphology

Strain LE31^T is a Gram-positive and non-motile bacterium. Cells are rods in the early growth phase and cells in the exponential and stationary growth phases are cocci, which occur mainly in pairs or often singly and in short chains. Colonies of strain LE31^T are orange-coloured, circular and convex on TSA and are umbonate with slightly irregular edges as the culture ages. Colonies of strain LE31^T are glossy relative to colonies of other *Gordonia* species on TSA and nutrient agar and are slightly convex with slightly irregular edges on TSA. Neither substrate mycelia nor primary mycelia were found.

Cultural and physiological characteristics

Strain LE31^T grew well over a broad range of pH values (pH 6.0–9.0) but growth was slow or inhibited at pH values below 5.0. Strain LE31^T grew optimally at 30–37 °C and growth was very slow or inhibited at 7 °C and did not occur at 45 °C. Growth also occurred under anaerobic conditions. Strain LE31^T had catalase, urease and DNase activities but no oxidase

Table 2. Cellular fatty acid profiles of strain LE31^T and other *Gordonia* species

Taxa: 1, *G. aichiensis* DSM 43978^T; 2, *G. amarae* DSM 43392^T; 3, *G. bronchialis* DSM 43247^T; 4, *G. hirsuta* DSM 44140^T; 5, *G. hydrophobica* DSM 44015^T; 6, *G. rhizosphaera* IFO 16068^T; 7, *G. rubropertincta* DSM 43197^T; 8, *G. sputi* DSM 43896^T; 9, *G. terrae* DSM 43249^T; 10, strain LE31^T.

Fatty acid*	Content (%) in:									
	1	2	3	4	5	6	7	8	9	10
Saturated fatty acid										
C _{10:0}								1.1		
C _{13:0}						0.8				
C _{14:0}	2.1	1.2	5.0	1.7	1.7	1.0	1.2	2.1	2.3	2.7
C _{15:0}	1.0	0.8	2.8		4.5	10.3	2.4	2.1	1.2	1.2
C _{16:0}	43.5	28.6	19.6	29.4	32.0	12.3	20.5	48.1	27.3	30.3
C _{17:0}	1.0	1.9	5.1	1.6	3.7	26.0	5.2		2.2	2.4
C _{17:0} 3-OH	0.6									
C _{18:0}	5.2	1.5	1.4	1.6	1.1	2.6	0.7	4.5	2.8	2.0
Unsaturated fatty acid										
C _{15:1} ω5c			1.3		2.1	0.9	1.4			
C _{15:1} ω8c			1.1				0.8			
C _{16:1} ω9c	1.0	0.7	2.8	0.7			1.1			
C _{17:1} ω7c					1.3		2.5			
C _{17:1} ω8c		2.4	4.7		2.3	20.5	4.1			2.5
C _{17:1} ω9c										
C _{18:1} ω9c	12.6	19.3	13.3	24.2	12.6	13.6	16.9	27.7	21.4	20.0
C _{19:1} ω12t		0.9								
C _{20:2} ω6,9c							1.3		3.3	2.2
C _{20:3} ω6,9,12c	4.9									
Branched-chain fatty acid										
iso-C _{17:1} ω9c										1.5
10-Methyl fatty acid										
C _{17:0}		1.1	2.9		1.3	2.9	1.8			0.8
C _{18:0}	28.2	21.8	25.4	21.9	18.8	3.0	21.4	14.3	21.4	21.2
C _{19:0}							1.2			
Summed feature†										
4		17.8	10.0	17.0	18.6	3.0	13.1		16.2	10.6
8		1.1	1.5			1.7	4.0		1.5	2.2
Unknown fatty acid										
		0.9	2.6	1.1			0.6			

* Fatty acids representing less than 0.5% were omitted.

† 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 16:1 *trans*9. Summed feature 8 contained one or more of following fatty acids: unknown 18:756 and/or 19:1 *cis*10.

activity. Nitrate was not reduced to nitrite. Aesculin and Tween 80 were hydrolysed but arbutin, elastin, casein and starch were not hydrolysed by strain LE31^T. Some other phenotypic properties of strain LE31^T are represented in Table 1, together with those of *Gordonia* species.

Chemotaxonomic characteristics and DNA base composition

The cell wall of strain LE31^T contained *meso*-diaminopimelic acid as the only diamino acid and

arabinose and galactose as the major sugars, indicating that the wall chemotype is type IV (Lechevalier & Lechevalier, 1970). The major isoprenoid quinone found in strain LE31^T was dihydrogenated menaquinone with nine isoprenoid units [MK-9(H₂)]. Strain LE31^T contained major amounts of the mycolic acids with 47–55 carbon atoms. Strain LE31^T had a cellular fatty acid profile containing straight-chain saturated, unsaturated and 10-methyl-branched fatty acids and had C_{16:0}, C_{18:1} ω9c, 10-methyl-C_{18:0} (TBSA) as the major fatty acids (Table 2). Strain LE31^T is characterized by having minor amounts of one branched-

Table 3. Levels of DNA–DNA relatedness between strain LE31^T and other *Gordonia* species

Strain	Reassociation (%) with:	
	Strain LE31 ^T	<i>G. rubropertincta</i> DSM 43197 ^T
Strain LE31 ^T	100	39.8
<i>G. aichiensis</i> DSM 43978 ^T	7.7	7.4
<i>G. amarae</i> DSM 43392 ^T	3.7	4.3
<i>G. bronchialis</i> DSM 43247 ^T	16.0	15.7
<i>G. hirsuta</i> DSM 44140 ^T	8.2	6.8
<i>G. hydrophobica</i> DSM 44015 ^T	4.5	5.0
<i>G. rhizosphaera</i> IFO 16068 ^T	12.5	11.7
<i>G. rubropertincta</i> DSM 43197 ^T	44.5	100
<i>G. sputi</i> DSM 43896 ^T	4.9	3.8
<i>G. terrae</i> DSM 43249 ^T	9.5	10.0

Fig. 1. Phylogenetic tree based on 16S rDNA sequences showing the position of strain LE31^T, *Gordonia* species and representatives of mycolic-acid-containing actinomycete taxa. Bar, 0.01 substitutions per nucleotide position. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points.

chain fatty acid (iso-C_{17:1} ω9c) that was not detected in other *Gordonia* species.

DNA characteristics

The G+C content of the DNA of strain LE31^T was 67 mol%. A DNA–DNA relatedness test was per-

formed between strain LE31^T and the type strains of all validly described *Gordonia* species.

The DNA of *G. rubropertincta* DSM 43197^T was used as probe DNA as well as reference DNA for the positive control test. Strain LE31^T exhibited two independent levels of DNA–DNA relatedness of

39.8% and 44.5% with *G. rubropertincta* DSM 43197^T, the closest phylogenetic relative (Table 3). Levels of DNA relatedness between strain LE31^T and the type strains of other *Gordonia* species were 3.7–16.0% (Table 3).

Phylogenetic analysis based on 16S rDNA sequences

The 16S rDNA of strain LE31^T was amplified by the PCR and an almost complete nucleotide sequence was determined by directly sequencing single strands of 16S rDNA obtained from treatment with λ exonuclease. The 16S rDNA sequence determined for strain LE31^T was 1478 nucleotides long (> 96% of the *Escherichia coli* sequence). This sequence contains all the signature nucleotides for the family *Gordoniaceae* as described by Stackebrandt *et al.* (1997). The phylogenetic tree showed that strain LE31^T forms its phylogenetic lineage within the evolutionary radiation enclosed by the genus *Gordonia* and a coherent cluster with *G. rubropertincta* and *G. terrae* (Fig. 1). In particular, the relationship between strain LE31^T and *G. rubropertincta* DSM 43197^T is supported by a high bootstrap resampling value of 98.7%. Strain LE31^T showed a high degree of relatedness to the type strain of *G. rubropertincta*, sharing 99.2% 16S rDNA similarity. The 16S rDNA sequence similarity values for strain LE31^T and the type strains of other validly described *Gordonia* species are in the range 96.3–97.8%. These values and the results shown in the phylogenetic tree indicate that strain LE31^T is a member of the genus *Gordonia*.

DISCUSSION

Strain LE31^T exhibited the closest phylogenetic affinity to *Gordonia* species from 16S rDNA sequence comparison. Phylogenetic inference based on 16S rDNA sequences places strain LE31^T within a phyletic cluster comprising *Gordonia* species (Fig. 1). The results obtained in the chemotaxonomic analysis are consistent with the results of sequence similarity and phylogenetic inference. The chemotaxonomic data obtained from strain LE31^T are found to be most similar to chemotaxonomic properties characteristic of the genus *Gordonia* (Chun *et al.*, 1997; Takeuchi & Hatano, 1998). Therefore, both types of results (phylogenetic and chemotaxonomic) clearly indicate that strain LE31^T belongs to the genus *Gordonia*.

Morphological and phenotypic characteristics indicate that strain LE31^T differs from the *Gordonia* species described previously. When strain LE31^T was cultivated on TSA and nutrient agar together with the type strains of all validly described *Gordonia* species, it formed glossy or shining colonies unlike the other *Gordonia* species. Strain LE31^T showed physiological properties that were different from those of other *Gordonia* species (Table 1). In particular, the ability of strain LE31^T to degrade 3-ethylpyridine and 3-methylpyridine was not found in the type strains of other *Gordonia* species in our degradation test. The cellular

fatty acid pattern of strain LE31^T is similar to those of *Gordonia* species (Table 2). However, one branched-chain fatty acid (iso-C_{17:1} ω 9c), which was not detected in other *Gordonia* species, was characteristically found only in strain LE31^T, although in a minor proportion. The chain lengths of the mycolic acids of strain LE31^T were found to be relatively short when compared with those of other *Gordonia* species. DNA–DNA relatedness provides decisive evidence that strain LE31^T is genetically distinct from the *Gordonia* species described previously, since it has been recognized as an important criterion for defining species in current bacteriology (Wayne *et al.*, 1987). Strains with a level of DNA–DNA relatedness less than 70% are considered to be different species.

On the basis of the data described above, strain LE31^T represents a new species of the genus *Gordonia*, for which we propose the name *Gordonia nitida* sp. nov. The properties of the new species are summarized below.

Description of *Gordonia nitida* sp. nov.

Gordonia nitida (ni.ti'da. L. adj. *nitida* bright, shining, glossy, pertaining to the appearance of the colonies).

Cells are Gram-positive and non-motile. Cells in the early growth phase are rods and cells in the exponential and stationary growth phases are cocci, which occur mainly in pairs or often singly and in short chains. Colonies are orange-coloured, glossy and slightly convex with slightly irregular edges on TSA. Neither substrate mycelia nor primary mycelia are found. Grows well over a broad range of pH values (pH 6.0–9.0) but grow better in alkaline conditions (pH 8.0–9.0); growth is slow or inhibited at pH values below 5.0 and above 12.0. Grows optimally at 30–37 °C; growth is very slow or inhibited at 7 °C and does not occur at 45 °C. Catalase-, urease- and DNase-positive. Oxidase-negative. Nitrate is not reduced to nitrite. Aesculin and Tween 80 are hydrolysed but arbutin, elastin, casein and starch are not hydrolysed. D-Ribose, sucrose, glucose, D-turanose, D-trehalose, citrate, gluconate, L-alanine and L-proline are utilized. D-galactose, D-arabitol, *myo*-inositol, L-rhamnose, L-aspartate, benzoate, succinate, L-leucine, L-valine and acetamide are not utilized. Acids are produced from glucose, sucrose and D-trehalose. Acids are not produced from D-galactose, L-rhamnose, *myo*-inositol or D-sorbitol. Cell wall contains *meso*-diaminopimelic acid, arabinose and galactose (wall chemotype IV). The predominant menaquinone is MK-9(H₂). The major fatty acids are C_{16:0}, C_{18:1} ω 9c and 10-methyl-C_{18:0} (TBSA). Mycolic acids contain 47–55 carbon atoms. The G + C content of the DNA is 67 mol% (as determined by HPLC). Isolated from an industrial wastewater in Taegu, Korea. The type strain is strain LE31^T, which has been deposited in the Korean Collection for Type Cultures as KCTC 0605BP^T and the Korean Culture Center of Microorganisms as KCCM 80004^T.

ACKNOWLEDGEMENTS

This work was supported by grants HS2321 and HS2701 from the Ministry of Science and Technology (MOST) of the Republic of Korea.

REFERENCES

- Bendinger, B., Rainey, F. A., Moormann, M., Kroppenstedt, R. M. & Klatte, S. (1995). *Gordonia hydrophobica* sp. nov., isolated from biofilters for waste gas treatment. *Int J Syst Bacteriol* **45**, 544–548.
- Bozzola, J. J. & Russell, L. D. (1991). Electron microscopy: principles and techniques for biologists. Boston: Jones & Bartlett.
- Briglia, M., Rainey, F. A., Stackebrandt, E., Schraa, G. & Salkinoja-Salonen, M. S. (1996). *Rhodococcus percolatus* sp. nov., a bacterium degrading 2,4,6-trichlorophenol. *Int J Syst Bacteriol* **46**, 23–30.
- Chun, J., Blackall, L. L., Kang, S.-O., Hah, Y. C. & Goodfellow, M. (1997). A proposal to reclassify *Nocardia pinensis* Blackall et al. as *Skermania piniformis* gen. nov., comb. nov. *Int J Syst Bacteriol* **47**, 127–131.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package) version 3.5. Department of Genetics, University of Washington, Seattle, WA, USA.
- Finnerty, W. R. (1992). The biology and genetics of the genus *Rhodococcus*. *Annu Rev Microbiol* **46**, 193–218.
- Goodfellow, M. (1986). Genus *Rhodococcus* Zopf 1891, 28^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1472–1481. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Goodfellow, M. (1992). The family *Nocardiaceae*. In *The Prokaryotes*, 2nd edn, pp. 1188–1213. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- Goodfellow, M. & Alderson, G. (1977). The actinomycete genus *Rhodococcus*: a home for the 'rhodococcus' complex. *J Gen Microbiol* **100**, 99–122.
- Grund, E., Denecke, B. & Eichenlaub, R. (1992). Naphthalene degradation via salicylate and gentisate by *Rhodococcus* sp. strain B4. *Appl Environ Microbiol* **58**, 1874–1877.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kaiser, J.-P., Feng, Y. & Bollag, J.-M. (1996). Microbial metabolism of pyridine, quinoline, acridine, and their derivatives under aerobic and anaerobic conditions. *Microbiol Rev* **60**, 483–498.
- Klatte, S., Kroppenstedt, R. M., Schumann, P., Altendorf, K. & Rainey, F. A. (1996). *Gordonia hirsuta* sp. nov. *Int J Syst Bacteriol* **46**, 876–880.
- Komagata, K. & Suzuki, K.-I. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–203.
- Kurup, V. P. & Fink, J. N. (1975). A scheme for the identification of thermophilic actinomycetes associated with hypersensitivity pneumonitis. *J Clin Microbiol* **2**, 55–61.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetales*, pp. 393–405. Edited by H. Prauser. Jena: Gustav Fischer.
- Lee, S. T., Lee, S. B. & Park, Y. H. (1991). Characterization of a pyridine-degrading branched Gram-positive bacterium isolated from the anoxic zone of an oil shale column. *Appl Microbiol Biotechnol* **35**, 824–829.
- Lemmer, H. & Kroppenstedt, R. M. (1984). Chemotaxonomy and physiology of some actinomycetes isolated from scumming activated sludge. *Syst Appl Microbiol* **5**, 124–135.
- Lenke, H., Pieper, D. H., Bruhn, C. & Knackmuss, H.-J. (1992). Degradation of 2,4-dinitrophenol by two *Rhodococcus erythropolis* strains, HL 24-1 and HL 24-2. *Appl Environ Microbiol* **58**, 2928–2932.
- Minnikin, D. E. (1988). Isolation and purification of mycobacterial wall lipids. In *Bacterial Cell Surface Techniques*, pp. 125–135. Edited by I. C. Hancock & I. C. Poxton. Chichester: Wiley.
- Rogall, T., Wolters, J., Flohr, T. & Böttger, E. C. (1990). Towards a phylogeny and definition of species at the molecular level within the genus *Mycobacterium*. *Int J Syst Bacteriol* **40**, 323–330.
- Saddler, G. S., Tavecchia, P., Lociuolo, S., Zanol, M., Colombo, L. & Selva, E. (1991). Analysis of madurose and other actinomycete whole cell sugars by gas chromatography. *J Microbiol Methods* **14**, 185–191.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shukla, O. P. & Kaul, S. M. (1974). A constitutive pyridine degrading system in *Corynebacterium* sp. *Indian J Biochem Biophys* **11**, 201–207.
- Sims, G. K. & O'Loughlin, E. J. (1989). Degradation of pyridine in the environment. *CRC Crit Rev Environ Control* **19**, 309–340.
- Stackebrandt, E., Smida, J. & Collins, M. D. (1988). Evidence of phylogenetic heterogeneity within the genus *Rhodococcus*: revival of the genus *Gordonia* (Tsukamura). *J Gen Appl Microbiol* **34**, 341–348.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Takeuchi, M. & Hatano, K. (1998). *Gordonia rhizosphaera* sp. nov. isolated from the mangrove rhizosphere. *Int J Syst Bacteriol* **48**, 907–912.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Tsukamura, M. (1971). Proposal of a new genus, *Gordonia*, for slightly acid-fast organisms occurring in sputa of patients with pulmonary disease and in soil. *J Gen Microbiol* **68**, 15–26.

- Watson, G. K. & Cain, R. B. (1975).** Microbial metabolism of the pyridine ring. *Biochem J* **146**, 157–172.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987).** International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983).** Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996).** Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.
- Yoon, J.-H., Rhee, S.-K., Lee, J.-S., Park, Y.-H. & Lee, S. T. (1997).** *Nocardioides pyridinolyticus* sp. nov., a pyridine-degrading bacterium isolated from the oxic zone of an oil shale column. *Int J Syst Bacteriol* **47**, 933–938.
- Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998).** Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* **48**, 187–194.