Gordonia otitidis sp. nov., isolated from a patient with external otitis

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The taxonomic positions of two clinically isolated actinomycetes were established using a polyphasic approach. The two strains, IFM 10032^T, isolated from ear discharge of a 28-year-old Japanese female patient with external otitis, and IFM 10148, isolated from pleural fluid of a 60-year-old Japanese male patient with bronchitis, possessed *meso*-diaminopimelic acid as the diagnostic amino acid, MK-9(H₂) as the predominant menaquinone and mycolic acids ranging from 58 to 64 carbons. The 16S rRNA gene sequences of the two strains were most closely related to those of *Gordonia aichiensis*, *Gordonia sputi* and '*Gordonia jacobaea*'. Differences in several phenotypic characteristics together with genotypic distinctiveness distinguish strains IFM 10032^T and IFM 10148 from these three species. DNA–DNA hybridization results and the combination of genotypic and phenotypic data showed that the two strains belong to a single species, and merit recognition of a novel species within the genus *Gordonia*. The name proposed for this taxon is *Gordonia otitidis* sp. nov.; the type strain is IFM 10032^T (= DSM 44809^T = JCM 12355^T = NBRC 100426^T).

The genus *Gordonia* belongs phylogenetically to the sub-order *Corynebacterineae* (Stackebrandt *et al.*, 1997). *Gordonia* includes around 20 species (Arenskotter *et al.*, 2005), among which *Gordonia terrae* has been considered to be a cause of primary cutaneous infection, and *Gordonia aichiensis*, *Gordonia bronchialis* and *Gordonia rubropertincta* have been isolated from sputum specimens of patients with pulmonary disease. *Gordonia sputi* was also isolated from the sputa of patients with chronic pulmonary diseases (Klatte *et al.*, 1994). Therefore, most of the *Gordonia* species described hitherto have been considered as opportunistic pathogens in humans (Tsukamura, 1971, 1978, 1982). However, most of the species of *Gordonia* recently described were isolated from the environment, and have been reported to play important roles in bioremediation or the

biodegradation of pollutants (Linos *et al.*, 1999, 2002; Maldonado *et al.*, 2003; Kim *et al.*, 2000, 2003; Xue *et al.*, 2003; Yoon *et al.*, 2000).

Two hundred and thirty-five strains of pathogenic actinomycetes were isolated from clinical specimens in Japan from 2000 to 2003 and referred to our research centre for identification. Of the 235 isolates, 15 strains were found to belong to *Gordonia* species. From these 15 isolates, strains IFM 10032^T and IFM 10148 were chosen for identification. Based on 16S rRNA gene sequence analysis these two strains are most closely related to *G. aichiensis*, *G. sputi* and '*Gordonia jacobaea*' (de Miguel *et al.*, 2000). The proposed type strain of '*G. jacobaea*', MV-1 (= IFM 10484), was also obtained and used for comparative purposes. Comparative DNA–DNA relatedness data showed that strains IFM 10032^T and IFM 10148 belong to the same species, but are distinct from *G. aichiensis*, *G. sputi* and '*G. jacobaea*'. Based on our phenotypic and genotypic data, the clinical isolates represent a novel species within the genus *Gordonia*, for which the name *Gordonia otitidis* sp. nov. is proposed.

Strains IFM 10032^T and IFM 10148 were isolated in 2000 from ear discharge of a 28-year-old Japanese female patient with external otitis, and in 2002 from pleural fluid of a 60-year-old Japanese male patient with bronchitis, respectively.

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Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of IFM 10032^T and IFM 10148 are AB122026 and AB122027, respectively.

Detailed physiological properties of strains IFM 10032^T and IFM 10148 and representative *Gordonia* species are given in supplementary tables in IJSEM Online.

Both strains were isolated on blood agar plates at 37 °C for 7 days.

Strains IFM 10032^T, IFM 10148, *G. aichiensis* IFMO473^T, *G. sputi* IFM 0477^T and '*G. jacobaea*' IFM 10484 were cultured on Muller–Hinton II (MH II; Difco) agar slants with 1 % glucose and 1 % glycerol for 1 week at 27 °C. For extraction of DNA and sequencing, bacterial strains were cultured in a 1 % glucose containing brain heart infusion (BHI; Difco) broth. Morphological observations under a scanning electron microscope (model S-5200; Hitachi) were made on cultures grown on BHI agar.

Carbon source utilization tests were performed as described by Takeuchi & Hatano (1998). Biochemical characterization of the isolates was performed using the Biolog GP2 MicroPlate assay (Biolog). A modified Ziehl–Nielsen method was used for acid-fast staining test. Whole-cell hydrolysates were analysed for diaminopimelic acid (DAP) isomers using TLC (Staneck & Roberts, 1974). Whole-cell sugars were prepared as detailed by Lechevalier & Lechevalier (1980) and were analysed by a TLC method (Miyadoh, 2001). Fatty acid methyl esters and mycolic acid trimethylsilyl ethers were prepared and analysed as described by Klatte *et al.* (1994) using the standard Microbial Identification System (MIDI Inc.) for automated GC analyses (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Menaquinones were extracted from freeze-dried biomass and analysed as described by Chun & Goodfellow (1995).

Preparation of genomic DNA samples for sequencing was performed using the guanidine thiocyanate method of Kageyama *et al.* (2004a, b).

16S rRNA genes were amplified and sequenced by PCR employing six prokaryotic 16S rRNA gene universal primers. PCR was performed with a DNA thermal cycler (TaKaRa) using 35 cycles of denaturation at 94 °C for 60 s, primer annealing at 60 °C for 60 s and primer extension at 72 °C for 120 s. DNA sequences were determined with an automatic sequence analyser (ABI PRISM 3100; PE Applied Biosystems) using the BigDye terminator cycle sequencing ready reaction kit (PE Applied Biosystems). The 16S rRNA gene sequences were analysed and species related to the isolates were identified by performing a BLAST sequence database search. Nucleotide 200 substitution rates (K_{nuc} values) were calculated (Kimura & Ohta, 1972). Phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei, 1987), and the topology of the trees was evaluated by a bootstrap analysis of the sequence data using CLUSTAL W software (Thompson *et al.*, 1994).

Preparation of genomic samples for DNA–DNA hybridization was performed using a modified method of that described by Saito & Miura (1983). DNA base compositions were estimated by HPLC (Tamaoka & Komagata, 1984) and levels of DNA–DNA relatedness were determined by the

method of Ezaki *et al.* (1989), using photobiotin and microplates.

Whole-cell hydrolysates of strains IFM 10032^T and IFM 10148 contained *meso*-DAP as the only diamino acid of the peptidoglycan and arabinose plus galactose as major whole-cell sugars. The fatty acid pattern of strain IFM 10032^T comprised straight-chain saturated and unsaturated fatty acids plus tuberculostearic acid (Table 1). Although this pattern is roughly the same in all gordoniae, there are qualitative and quantitative species-specific and often intra-specific differences (Klatte *et al.*, 1994) (Table 1). The predominant menaquinone is MK-9(H₂), but substantial amounts of MK-8(H₂) are also present. The G + C contents of the DNA of strains IFM 10032^T and IFM 10148 are 64.9 and 65.2 mol%, respectively.

The above results indicated that strains IFM 10032^T and IFM 10148 have properties consistent with their assignment to the genus *Gordonia* (Minnikin *et al.*, 1980; Stackebrandt *et al.*, 1988; Klatte *et al.*, 1994).

Strains IFM 10032^T and IFM 10148 produced short elementary branching hyphae that disintegrated into rod- and cocci-like elements. They showed the typical rod–coccus growth cycle that is usually found among strains of the genus *Gordonia* and related taxa (Goodfellow, 1992).

Table 1. Fatty acid profiles of strain IFM 10032^T and types strains of its closest phylogenetic relatives

Strains: 1, *G. otitidis* IFM 10032^T; 2, *G. aichiensis* IFM 0473^T; 3, *G. sputi* IFM 0477^T; 4, '*G. jacobaea*' IFM 10484. Values are percentages of total fatty acids; –, not detected. Abbreviations are exemplified by the following: *cis*-7-16:1, *cis*-7 hexadecenoic acid (palmitoleic acid); 10-methyl 18:0, 10-methyl octadecanoic acid (tuberculostearic acid).

Fatty acid	1	2	3	4
10:0	—	—	0.50	0.57
ECL 13:957*	0.61	0.76	—	0.54
14:0	2.26	1.95	2.9	1.21
<i>cis</i> -10-15:1	0.51	0.37	—	0.17
15:0	1.07	0.84	—	0.3
<i>cis</i> -7-16:1	0.56	0.46	1.02	0.23
<i>cis</i> -9-16:1	18.91	17.66	—	20.29
16:0	31.65	35.95	52.74	34.66
<i>cis</i> -9-17:1	1.45	0.43	0.75	1.16
17:0	1.1	0.78	1.45	0.4
10-methyl 17:0	0.25	—	—	0.15
<i>cis</i> -9-18:1	15.19	5.52	19.14	19.64
18:0	3.13	4.75	2.32	0.55
10-methyl 18:0	22.96	30.53	19.19	20.14
<i>cis</i> -9-19:1	0.34	—	—	—

*Unknown lipid eluting at a equivalent chain length of 13.957.

Table 2. Main mycolic acid components of *G. otitidis* sp. nov. IFM 10032^T and other type strains of *Gordonia* species

Values are percentages of total mycolic acids. Designations indicate the chain lengths of the mycolic acids, i.e. number of carbon atoms.

Strain	Ref.*	C ₄₈	C ₄₉	C ₅₀	C ₅₁	C ₅₂	C ₅₃	C ₅₄	C ₅₅	C ₅₆	C ₅₇	C ₅₈	C ₅₉	C ₆₀	C ₆₁	C ₆₂	C ₆₃	C ₆₄
<i>G. otitidis</i> IFM 10032 ^T	1											7	1	13	5	54	6	14
<i>G. aichiensis</i> IFM 0473 ^T	2													14	11	47	11	17
<i>G. sputi</i> IFM 0477 ^T	2													8	12	48	14	8
' <i>G. jacobaea</i> ' IFM 10484	1											2	—	15	6	53	6	18
<i>G. alkanivorans</i> DSM 44369 ^T	1					11	2	29	8	32	6	12						
<i>G. amarae</i> DSM 43392 ^T	2	5		30	3	40	4	18										
<i>G. amicalis</i> DSM 44461 ^T	1	1	2	7	8	24	17	19	8	10	3	1						
<i>G. bronchialis</i> DSM 43247 ^T	2													8	21	25	25	13
<i>G. desulfuricans</i> DSM 44462 ^T	1									1		5		19	2	50	3	20
<i>G. hirsuta</i> DSM 44140 ^T	2													19	10	31	9	31
<i>G. hydrophobica</i> DSM 44015 ^T	2									12	11	30	16	26	5			
<i>G. namibiensis</i> DSM 44568 ^T	1							3	3	13	11	24	14	20	7	5		
<i>G. nitida</i> DSM 44499 ^T	1					12	4	26	8	34	6	10						
<i>G. polyisoprenivorans</i> DSM 44302 ^T	3											8	3	30	6	47	1	5
<i>G. rhizosphaera</i> IFO 16068 ^T	4											7	5	26	10	34		
<i>G. rubripertincta</i> DSM 43197 ^T	2							4	3	14	11	23	17	18	5			
<i>G. sihwensis</i> SPR2 ^T	5	7	4	20	4	28	6	22	2	7								
<i>G. terrae</i> DSM 43249 ^T	2							5	1	27	4	36	3	19	1	4		
<i>G. westfalica</i> DSM 44215 ^T	1							5		20	7	34	9	18	5	2		

*Data were taken from: 1, this study; 2, Klatté *et al.* (1994); 3, Linos *et al.* (1999); 4, Takeuchi & Hatano (1998); 5, Kim *et al.* (2003).

The mycolic acid pattern of strain IFM 10032^T is similar to those of *G. aichiensis*, *G. sputi*, *Gordonia desulfuricans*, '*G. jacobaea*' and *Gordonia polyisoprenivorans*, which possess similar sized mycolic acids of chain length of about C₅₈–C₆₄ and with the principal mycolic acid C₆₂ comprising about 50 % (54 % for IFM 10032^T) of the total (Table 2).

Almost complete 16S rRNA gene sequences of strains IFM 10032^T and IFM 10148 were determined and compared with those of other *Gordonia* species. Highest sequence similarity of strain IFM 10032^T was 99.5 % to *G. aichiensis* IFM 0473^T (1454/1561 nt), followed by 99.4 % (1452/1461) and 99.3 % (1453/1463 nt) to *G. sputi* IFM 0477^T and '*G. jacobaea*' IFM 10484, respectively.

The phylogenetic tree shown in Fig. 1 based on the 16S rRNA gene sequences shows that strains IFM 10032^T and IFM 10148 cluster with *G. aichiensis* IFM 0473^T, *G. sputi* IFM 0477^T and '*G. jacobaea*' IFM 10484.

Levels of DNA–DNA relatedness between IFM 10032^T and IFM 10148 were 94.7 and 95.3 %, respectively, confirming that the two strains belong to the same species. Relatedness levels of strain IFM 10032^T to *G. aichiensis* IFM 0473^T, *G. sputi* IFM 0477^T and '*G. jacobaea*' IFM 10484 were 31.1, 3.8 and 19.7 %, respectively. All these values are well below the 70 % cut-off recommended by Wayne *et al.* (1987) for the delineation of separate species.

Results of physiological tests obtained from growth on agar plates revealed that strains IFM 10032^T and IFM 10148

were able to utilize 18 out of 32 carbon sources. Strains IFM 10032^T and IFM 10148 gave identical responses in

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic positions of strains IFM 10032^T and IFM 10148 and type strains of *Gordonia* species. The tree was created using the neighbour-joining method and K_{nuc} values. Bootstrap values (from 1000 replications) are shown at branch points. Bar, 0.005 K_{nuc} value.

Table 3. Compound utilization patterns that distinguish strains of *G. otitidis* sp. nov. from *G. aichiensis*, *G. sputi* and '*G. jacobaea*'

Taxa: 1, *G. otitidis* strains IFM 10032^T and IFM 10148; 2, *G. aichiensis* IFM 0473^T; 3, *G. sputi* IFM 0477^T; 4, '*G. jacobaea*' IFM 10484. +, Positive; −, negative; ND, not determined. All four taxa are negative for utilization of 3-hydroxybenzoate.

Compound	1	2	3	4
D-Ribose	−	−	−	+
Sucrose	+	−	−	+
D-Turanose	+	−	−	+
D-Arabitol	+	−	+	+
N-Acetyl-D-glucosamine	+	−	−	−
Caprate	−	+	+	ND
4-Aminobutyrate	+	+	−	ND
Succinate	−	−	+	+
4-Hydroxybenzoate	+	−	−	−
Quinate	+	+	−	ND
L-Alanine	+	+	−	+
L-Aspartate	−	+	−	−
L-Leucine	+	−	−	+
L-Proline	+	−	−	+
L-Serine	+	−	−	+
L-Valine	+	−	−	+
Putrescine	+	−	−	+
Tyramine	−	−	−	+
Acetamide	+	−	−	ND

carbon utilization tests (Table 3). Some carbon utilization patterns useful for identifying and differentiating strains IFM 10032^T and type strains of other species of the genus *Gordonia* are given in Table 3 and under the species description below. The utilization pattern clearly showed that IFM 10032^T could be differentiated from *G. aichiensis* IFM 0473^T, *G. sputi* IFM 0477^T and '*G. jacobaea*' IFM 10484.

Representative biochemical characterization using the Biolog GP2 assay system for IFM 10032^T and IFM 10148 is shown in Table 4 (see also Supplementary Table S2), and also confirmed that IFM 10032^T could be differentiated from *G. sputi* IFM 0477^T, *G. aichiensis* IFM 0473^T and '*G. jacobaea*' IFM 10484.

These results indicate that strain IFM 10032^T merits recognition as representing a novel species of *Gordonia* (Stackebrandt & Goebel, 1994). Based on the source of isolation, from a patient with external otitis, the name *Gordonia otitidis* sp. nov. is proposed.

Because the name '*G. jacobaea*' has not been validly published in IJSEM in accordance with Rule 27 of the Bacteriological Code, the present studies included characterization of the proposed type strain of '*G. jacobaea*', MV-1 (= IFM 10484 = DSM 44880 = JCM 12643).

Table 4. Selected results of Biolog GP2 assays

Taxa: 1, *G. otitidis* strains IFM 10032^T and IFM 10148; 2, *G. aichiensis* IFM 0473^T; 3, *G. sputi* IFM 0477^T; 4, '*G. jacobaea*' IFM 10484. +, Positive; −, negative. Only those compounds that are most useful for discriminating *G. otitidis* from related species are shown; more complete results are available as Supplementary Table S2 in IJSEM Online.

Compound	1	2	3	4
α-Cyclodextrin	−	+	−	−
β-Cyclodextrin	−	+	−	−
Dextrin	+	+	+	−
Glycogen	+	+	−	−
Inulin	+	−	−	−
N-Acetyl-D-mannosamine	−	+	−	−
Amygdalin	−	+	−	−
Maltose	+	+	+	−
Maltotriose	+	+	+	−
D-Mannitol	−	−	+	−
D-Melibiose	−	+	+	−
3-Methyl glucose	−	+	−	−
D-Trehalose	+	+	+	−
β-Hydroxybutyric acid	−	+	−	+
Methyl pyruvate	+	−	+	+
Monomethyl succinate	−	+	−	−
Pyruvic acid	+	−	−	+
Succinamic acid	−	+	−	−
Succinic acid	−	+	−	−
D-Alanine	+	−	+	−
L-Alanyl glycine	+	+	+	−
L-Asparagine	−	−	+	−
L-Glutamic acid	−	+	+	−
Adenosine 5'-monophosphate	−	+	+	−
Thymidine 5'-monophosphate	−	+	+	−
Uridine 5'-monophosphate	−	+	+	−
Fructose 6-phosphate	−	+	+	−
Glucose 1-phosphate	−	+	+	−
Glucose 6-phosphate	−	+	+	−

Description of *Gordonia otitidis* sp. nov.

Gordonia otitidis (o.ti'ti.dis. N.L. gen. n. *otitidis* of otitis, inflammation of the ear).

Aerobic, Gram-positive, partially acid-fast, nitrate-reducing, non-motile actinomycetes that form rod/coccus-like elements (0.6–0.8 × 2.5–3.1 μm). Colonies are white, changing with time to apricot to pale orange. Colonies of the type strain are rough with irregular margins. Utilizes sucrose, D-turanose, D-arabitol, N-acetyl-D-glucosamine, 4-aminobutyrate, 2-hydroxyvalerate, 2-oxoglutarate, pimelate, benzoate, 4-hydroxybenzoate, phenylacetate, quinate, L-alanine, L-leucine, L-proline, L-serine, L-valine, putrescine and acetamide. Does not utilize D-galactose, L-rhamnose, D-ribose, *i*-inositol, glucarate, gluconate, caprate, succinate, 3-hydroxybenzoate, citrate, L-aspartate or tyramine. Whole-cell hydrolysates contain *meso*-DAP, arabinose and galactose

(cell-wall chemotype IV *sensu* Lechevalier & Lechevalier, 1980). Predominant menaquinone is MK-9(H₂); a small amount of MK-8(H₂) is also present. Principal mycolic acids have a chain length (58–64 carbons) equivalent to those of other *Gordonia* species. Physiological properties are given in Supplementary Table S1 available in IJSEM Online. The type strain is a clinical isolate. The G + C content of the DNA is 64.9–65.2 %.

The type strain, IFM 10032^T (=DSM 44809^T=JCM 12355^T=NBRC 100426^T), was isolated from ear discharge of a patient with external otitis, Japan.

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