Gordonia araii sp. nov. and *Gordonia effusa* sp. nov., isolated from patients in Japan

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Two bacterial strains, IFM 10211^T and IFM 10200^T, were isolated from the sputum of two Japanese patients, and were subjected to a polyphasic taxonomic study. The two strains were found to have morphological, physiological and chemotaxonomic properties that were consistent with their assignment to the genus *Gordonia*, except for a few chemotaxonomic characteristics. Almost complete 16S rRNA gene sequences of the two strains were determined; the data showed that they are related distantly to *Gordonia amarae*, *Gordonia hirsuta*, *Gordonia hydrophobica* and *Gordonia sihwensis*, showing 16S rRNA gene sequence similarities to the type strains of these species of 96.2–97.9%. DNA–DNA relatedness data coupled with the combination of genotypic and phenotypic data indicated that the two strains are representatives of two novel, separate species. The names proposed to accommodate these two strains are *Gordonia araii* sp. nov. (type strain IFM 10211^T=DSM 44811^T=NBRC 100433^T=JCM 12131^T) and *Gordonia effusa* sp. nov. (type strain IFM 10200^T=DSM 44810^T=NBRC 100432^T=JCM 12130^T).

The genus *Gordonia* belongs to the suborder *Corynebacterineae* within the order *Actinomycetales* (Stackebrandt *et al.*, 1997). This suborder also contains the genera *Corynebacterium*, *Dietzia*, *Mycobacterium*, *Nocardia*, *Skermania*, *Rhodococcus*, *Tsukamurella*, *Turicella* and *Williamsia* (Goodfellow, 1998; Goodfellow *et al.*, 1999; Kämpfer *et al.*,

1999). These genera are mutually distinguishable using a combination of biochemical, chemotaxonomic and morphological characteristics. The genus *Gordonia* was erected by Stackebrandt *et al.* (1988), and at the time of writing comprises 19 recognized species. Among them, *Gordonia aichiensis*, *G. bronchialis*, *G. otitidis*, *G. sputi* and *G. terrae* have been isolated from clinical specimens (Iida *et al.*, 2005). *G. bronchialis*, *Gordonia rubropertincta* and *G. sputi* were specifically isolated from the sputum of patients with pulmonary disease (Klatte *et al.*, 1994; Tsukamura, 1978).

Two hundred and thirty-five strains of pathogenic actinomycetes isolated from clinical specimens were referred to our research centre for identification during 2000–2003. Among these clinical specimens, strains IFM 10211^T (in 2001) and IFM 10200^T (in 2002) were isolated from sputum specimens of Japanese patients with kidney dysfunction and

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Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Gordonia araii* IFM 10211^T and *Gordonia effusa* IFM 10200^T are AB162800 and AB162799, respectively.

A table giving detailed physiological characteristics of members of the genus *Gordonia* is available as supplementary material in IJSEM Online.

pulmonary disease, respectively. These organisms were found to be related to the genus *Gordonia* based on their phenotypic characteristics and TLC profile of mycolic acids. Furthermore, the two strains were found to cluster with the type strains of *Gordonia amarae*, *G. hydrophobica*, *G. sihwensis* and *G. hirsuta* based on 16S rRNA gene sequence analysis, but differences in several phenotypic characteristics and their genetic distinctiveness render them distinguishable. Comparative DNA–DNA relatedness data indicated that the strains belong to different species. Based on the phenotypic and genotypic data, we therefore suggest that the two clinical isolates represent two novel species of the genus *Gordonia*.

Strains IFM 10211^T and IFM 10200^T were isolated respectively from the sputum of a 48-year-old male Japanese patient with bacterial pneumonia, and of a 74-year-old Japanese male patient with kidney dysfunction, and referred to our research centre for identification.

Strains IFM 10211^T and IFM 10200^T and reference strains *G. amarae* IFM 0210^T, *G. hirsuta* IFM 10287^T and *G. hydrophobica* IFM 10286^T were cultured on Mueller–Hinton II (MH II; Difco Laboratories) agar slants with 1% glucose and 1% glycerol for 1 week at 30 °C. For extraction of DNA and sequencing, bacterial strains were cultured in brain heart infusion (BHI; Difco Laboratories) broth containing 1% glucose for 4 days at 30 °C. For DNA–DNA hybridization experiments, strains were cultured in BHI broth containing 2% glucose and 2% glycine for 3 days at 30 °C. Micromorphological properties were observed by using electron microscopy according to a previously described method (Kageyama *et al.*, 2004a). TLC analysis of mycolic acids for the differentiation of actinomycete genera containing these acids was performed according to the method of Miyadoh (2001). Carbon utilization tests were performed as described by Takeuchi & Hatano (1998). The modified Ziehl–Neelsen method was used for the 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 described by Lechevalier & Lechevalier (1980) and analysed by TLC (Miyadoh, 2001). Methyl esters of cellular fatty acids mycolic acid trimethylsilyl esters were prepared and analysed as described by Klatte *et al.* (1994) using the standard Microbial Identification System (Microbial ID Inc.). Isoprenoid quinones were extracted by the method of Collins *et al.* (1977) and were analysed by HPLC with a Cosmosil 5C₁₈ column (4.6 mm × 150 mm; Nacalai Tesque Inc.). A mixture of methanol and 2-propanol (2 : 1, v/v) was used as the elution solvent.

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

The 16S rRNA gene was amplified and sequenced by PCR by employing six universal primers for the prokaryotic 16S rRNA gene. We performed PCR with a DNA thermal cycler (TaKaRa) using 35 cycles consisting 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. The PCR products were purified using a Centri-Sep column (Princeton Separations). DNA sequences were analysed with an automatic sequence analyser (ABI PRISM 3100; PE Applied Biosystems) by using a dye terminator cycle sequencing kit (PE Applied Biosystems).

A BLAST comparison of the 16S rRNA gene sequences of the two new isolates indicated that their closest neighbours were *G. amarae*, *G. hydrophobica*, *G. sihwensis* and *G. hirsuta*. Sequence data of related species were retrieved from GenBank. Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura & Ohta, 1972) and phylogenetic trees were constructed based on the neighbour-joining method (Saitou & Nei, 1987). Tree topology was evaluated using a bootstrap analysis of sequence data with CLUSTAL W software (Thompson *et al.*, 1994).

DNA was isolated using a modified method of that described by Saito & Miura (1983). Levels of DNA–DNA relatedness were determined using the method of Ezaki *et al.* (1989) using photobiotin and microplates.

Whole-cell hydrolysates of strains IFM 10211^T and IFM 10200^T contained *meso*-DAP as the only diamino acid of the peptidoglycan and arabinose plus galactose as major whole-cell sugars (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1980). The fatty acid profiles of strains IFM 10200^T and IFM 10211^T consisted of straight-chain saturated and unsaturated fatty acids plus tuberculostearic acid, patterns similar to those of all genera in the suborder *Corynebacterineae*. The predominant menaquinone was MK-9(H₂) for strain IFM 10211^T; strain IFM 10200^T had MK-9(H₈) and MK-9(H₆), but also a small amount of MK-9(H₄). These results were consistent with strain IFM 10211^T belonging to the genus *Gordonia*, but no such conclusion could be made for strain IFM 10200^T on this basis.

Strains IFM 10200^T and IFM 10211^T had mycolic acids with chain lengths of 62–70 and 58–64 carbons, respectively. The mycolic acid pattern of strain IFM 10211^T thus differs from those of recognized *Gordonia* species, more closely resembling that of the genus *Tsukamurella* in the suborder *Corynebacterineae*. The pattern for strain IFM 10200^T matched that of other members of the genus *Gordonia*.

Strains IFM 10211^T and IFM 10200^T produced short elementary branching hyphae that disintegrated into rod-like and cocci-like elements. Growth of strain IFM 10200^T was faster than that of IFM 10211^T; no soluble pigment was produced in either strain. Almost complete 16S rRNA gene sequences of strains IFM 10211^T (1499 bp) and IFM 10200^T (1509 bp) were determined. Phylogenetic analyses demonstrated that the two strains formed a monophyletic clade associated with *G. amarae*, *G. hirsuta* and *G. hydrophobica* (Fig. 1). On the basis of phylogenetic analysis, strains IFM 10211^T and IFM 10200^T were considered to belong to the genus *Gordonia*, but differences in the mycolic acid pattern of strain IFM 10211^T and menaquinone composition of

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences, created using the neighbour-joining method (Saitou & Nei, 1987) and K_{nuc} values (Kimura & Ohta, 1972). Numbers on the tree indicate bootstrap percentages (from 1000 replicates) for branch points. Only values >50% are indicated. The tree was unrooted, using *Williamsia muralis* MA140-96^T as an outgroup.

strain IFM 10200^T distinguished them from recognized species of the genus *Gordonia*. 16S rRNA gene sequence similarity between strains IFM 10211^T and IFM 10200^T was 97.9%; similarity values between strains IFM 10211^T and IFM 10200^T and their closest neighbour, *G. amarae* DSM 43392^T, were 97.4 and 97.7%, respectively. Lower levels of sequence similarity (<97.6%) were observed with the type strains of other neighbouring species, including *G. hydrophobica* and *G. hirsuta* (Table 1). The genomic delineation among strains IFM 10211^T and IFM 10200^T and the type strains of *G. amarae*, *G. hydrophobica* and *G. hirsuta* was supported by the DNA–DNA relatedness data (Table 1). The two new isolates showed levels of DNA–DNA relatedness of 6.2–6.9% to each other, and values in the range 3.2–10.9% to *G. amarae*, *G. hydrophobica*, *G. hirsuta* and *G. sihwensis*. These values are well below the

Table 2. Differential phenotypic properties among *Gordonia* species

Strains: 1, IFM 10211^T; 2, IFM 10200^T; 3, *G. amarae* DSM 44392^T; 4, *G. hirsuta* DSM 44140^T; 5, *G. hydrophobica* DSM 44015^T. Data were taken from Linos *et al.* (2002) and this study.

Property	1	2	3	4	5
Utilization of:					
D-Galactose	–	–	+	+	+
D-Ribose	+	–	+	–	–
Sucrose	+	+	+	–	+
D-Turanose	–	+	+	–	+
myo-Inositol	–	–	–	+	–
N-Acetyl-D-glucosamine	–	+	–	–	–
Glucarate	–	–	–	–	+
Citrate	–	+	+	–	+
2-Oxoglutarate	–	–	+	+	–
Succinate	–	+	–	–	+
4-Hydroxybenzoate	–	–	+	–	–
Quinate	–	–	+	–	–
L-Alanine	–	+	–	–	–
L-Aspartate	–	+	–	–	–
L-Leucine	–	–	–	–	+
L-Valine	–	+	+	–	+
Putrescine	+	+	+	–	–
Acetamide	–	–	–	–	+

70% cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987).

Strains IFM 10211^T and IFM 10200^T were also examined for a set of biochemical and physiological characteristics for comparison with those of other *Gordonia* species (Table 2). Both strains were readily distinguishable from these species based on a combination of physiological, biochemical and chemotaxonomic characteristics (see also Supplementary Table S1 available in IJSEM Online). They also differed phylogenetically from other *Gordonia* species (Fig. 1). Both phenotypic and genotypic data indicated that strains IFM 10211^T and IFM 10200^T represent two novel species of the genus *Gordonia*, for which we propose the names *Gordonia araii* sp. nov. and *Gordonia effusa* sp. nov., respectively.

Table 1. 16S rRNA gene sequence similarity and DNA–DNA relatedness between the isolated strains and related type strains

Strain	16S rRNA gene sequence similarity (%) with:		DNA–DNA hybridization (%) with DNA from:				
	1	2	1	2	3	4	5
1. Strain IFM 10211 ^T	(100)		100	6	5	5	5
2. Strain IFM 10200 ^T	97.9	(100)	7	100	3	5	5
3. <i>G. amarae</i> IFM 0210 ^T	97.4	97.7	4	3	100	2	3
4. <i>G. hirsuta</i> IFM 10287 ^T	96.2	96.2	4	4	1	100	4
5. <i>G. hydrophobica</i> IFM 10286 ^T	97.6	97.5	5	5	3	3	100

Description of *Gordonia araii* sp. nov.

Gordonia araii (a.ra'i'i. N.L. gen. n. *araii* of Arai, to honour Tadashi Arai, a Japanese microbiologist, for his valuable contributions to microbial taxonomy and secondary metabolites).

Aerobic, Gram-positive, slightly acid-fast, non-motile rods (0.3–0.5 × 0.5–1.1 µm). Colonies are white, becoming beige. Colonies are rough with irregular margins. No soluble pigment is produced. Colonies are 1.5–3.5 mm in diameter after 7 days at 30 °C on MH II medium. Utilizes glucose, but not adonitol, arabinose, erythritol, galactose, *myo*-inositol, maltose, mannose or sorbitol. Adenine, hypoxanthine, tyrosine, urea and xanthine are not hydrolysed. Contains *meso*-DAP, arabinose and galactose (cell wall chemotype IV *sensu* Lechevalier & Lechevalier, 1980). Predominant menaquinone is MK-9(H₂). Major fatty acids are C_{14:0}, C_{16:1}*cis*-9, C_{16:0}, C_{18:1}*cis*-9, C_{18:0} and 10-methyl C_{18:0}. Mycolic acids are present with a range of total carbon number from 64 to 70.

The type strain, IFM 10211^T (=DSM 44811^T=NBRC 100433^T=JCM 12131^T), was isolated from a clinical specimen.

Description of *Gordonia effusa* sp. nov.

Gordonia effusa (ef.fu'sa. L. fem. adj. *effusa* poured out, extensive, vast, broad, wide, referring to the spreading colonial growth).

Aerobic, Gram-positive, slightly acid-fast, non-motile rods (0.3–0.6 × 0.5–2.1 µm). Colonies are white, becoming beige. Colonies are rough with irregular margins. No soluble pigment is produced. Colonial growth is fast; colony diameter is 3.0–5.0 mm after 7 days at 30 °C on MH II medium. Utilizes sucrose, D-turanose, *N*-acetyl-D-glucosamine, citrate, 4-aminobutyrate, succinate, L-alanine, L-aspartate, L-valine and putrescine, but not D-galactose, L-rhamnose, D-ribose, D-arabitol or *myo*-inositol. Contains *meso*-DAP, arabinose and galactose (cell wall chemotype IV *sensu* Lechevalier & Lechevalier, 1980). Predominant menaquinones are MK-9(H₈) and MK-9(H₆); a small amount of MK-9(H₄) is also present. Major fatty acids are C_{14:0}, C_{15:0}, C_{16:0}, C_{17:0}, C_{18:0} and 10-methyl C_{18:0}. Mycolic acids are present with a range of total carbon number from 60 to 64.

The type strain, IFM 10200^T (=DSM 44810^T=NBRC 100432^T=JCM 12130^T), was isolated from a clinical specimen.

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