Gordonia shandongensis sp. nov., isolated from soil in China

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The taxonomic position of strain SD29^T, isolated from soil, was clarified using a polyphasic taxonomic approach. The organism produced an elementary branching mycelium which fragmented into rod/coccus-shaped elements and it possessed *meso*-diaminopimelic acid, arabinose, galactose as diagnostic diamino acid and sugars, MK-9(H₂) as predominant menaquinone, phospholipids of type PII and mycolic acid. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain SD29^T was most closely related to *Gordonia hydrophobica* DSM 44015^T and *Gordonia sihwensis* DSM 44576^T, forming a distinct but loosely related branch in the phylogenetic tree. A number of physiological properties readily separated the isolate from its nearest neighbours. It is evident from genotypic and phenotypic data that strain SD29^T represents a novel species of the genus *Gordonia*, for which the name *Gordonia shandongensis* sp. nov. is proposed. The type strain is SD29^T (=CGMCC 4.3492^T = JCM 13907^T).

The genus *Gordonia* (formerly *Gordona*), proposed by Tsukamura (1971), belongs to the mycolic acid group of the actinomycetes and the suborder *Corynebacterineae* and forms a distinct phyletic line in the 16S rRNA gene phylogenetic tree (Stackebrandt *et al.*, 1988, 1997). The genus encompasses 25 species with validly published names at the time of writing (November 2006), which can be distinguished from each other using a combination of morphological, physiological and chemical markers (Goodfellow *et al.*, 1999).

Several members of this taxon were isolated from clinical samples and have been considered as opportunistic human pathogens (Tsukamura, 1971; Klatte *et al.*, 1994; Iida *et al.*, 2005; Kageyama *et al.*, 2006). Nevertheless, most gordoniae have been isolated from other environments such as mangrove rhizosphere, soil, wastewater, oil-producing wells and activated sludge foam (Takeuchi & Hatano, 1998; Kummer *et al.*, 1999; Kim *et al.*, 2003; Maldonado *et al.*, 2003; Xue *et al.*, 2003; Shen *et al.*, 2006; Soddell *et al.*, 2006) and play important roles in pollutant biodegradation and bioremediation (Yoon *et al.*, 2000) because of their abilities to metabolize macromolecules or contaminants, for example natural rubber, benzothiophene and hydrocarbons (Linos *et al.*, 1999; Kim *et al.*, 1999; Xue *et al.*, 2003). It is therefore important for ecological reasons to unravel the species-richness of gordoniae in nature habitats. The aim

of this study is to clarify the taxonomic position of a rod/coccus-like strain isolated from farmland soil.

Strain SD29^T was isolated on ISP 2 agar (Shirling & Gottlieb, 1966), supplemented with 50 µg cycloheximide ml⁻¹, which had been incubated for 5 days at 28 °C following inoculation with a suspension of soil sample collected from farmland in Shandong Province, China. The pure cells were stored in 20 % (v/v) glycerol at –20 °C. Biomass for chemotaxonomic study was harvested by centrifugation after 3 days at 28 °C in shake flasks of ISP 2 and TSB (trypticase soy broth), washed twice with distilled water and freeze-dried. Control type strains *Gordonia hydrophobica* DSM 44015^T and *Gordonia sihwensis* DSM 44576^T were incubated as described above.

Colonial characteristics of strain SD29^T were observed on ISP 2 agar. Zeihl–Neelsen staining (Gordon, 1967) and morphological properties were recorded using a light microscope (Axioskop 20; Zeiss) and scanning electron microscope (FEI QUANTA) after 5 days on ISP 2 plates. The ability to utilize sole carbon sources for energy and growth and to decompose various substances was assessed according to the methods of Gordon & Mihm (1957). Standard chemotaxonomic analytical procedures were used as follows: Hasegawa *et al.* (1983) [isomers of diaminopimelic acid (A₂pm)], Lechevalier & Lechevalier (1980) (whole-cell sugars), Collins (1985) and Wu *et al.* (1989) (menaquinones), Minnikin *et al.* (1984) (polar lipids), Sasser (1990) and Kämpfer & Kroppenstedt (1996) (fatty acids) and Minnikin *et al.* (1975) (mycolic acids).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SD29^T is DQ420167.

Extraction of whole genomic DNA, PCR amplification of the 16S rRNA gene and sequencing of the PCR product were performed as described previously (Chun & Goodfellow, 1995; Huang *et al.*, 2001). The almost-complete 16S rRNA gene sequence (1398 nt) of strain SD29^T was aligned with corresponding sequences of all type strains within the genus *Gordonia* retrieved from GenBank using MEGA software version 3.1 (Kumar *et al.*, 2004). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Evolutionary distances for the neighbour-joining algorithm were calculated with the Kimura two-parameter model (Kimura, 1980) and close-neighbour-interchange (search level = 2, random additions = 100) was applied in the maximum-parsimony analysis. The topology of the neighbour-joining tree was evaluated by bootstrap analysis on the basis of 1000 replications (Felsenstein, 1985).

The morphological and chemotaxonomic characteristics of strain SD29^T were consistent with the diagnostic properties of the genus *Gordonia* (Stackebrandt *et al.*, 1988). The organism was Gram-positive, aerobic, non-motile and slightly acid-fast, formed short elementary mycelia that separated into rod/coccus-shaped elements and produced light-yellow, rough colonies, different in appearance from the control type strains, on ISP 2 agar. It contained *meso*-A₂pm, arabinose and galactose in whole-cell hydrolysates (cell-wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), MK-9(H₂) as the predominant menaquinone and phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides as diagnostic polar lipids (phospholipid type II *sensu* Lechevalier *et al.*, 1977). The mycolic acid pattern detected by TLC was identical to that of the control strain *G. sihwensis* DSM 44576^T. The fatty acid profile was composed mainly of C_{14:0} (3.5 %), C_{15:0} iso 2-OH (14.6 %), C_{16:0} (35.9 %), C_{18:1}ω9c (21.3 %) and TBSA (tuberculostearic acid, 10-methyl C_{18:0}) (14.9 %) and differed from those of related type strains in the presence of a significant amount of C_{15:0} iso 2-OH (Table 1).

Results based on BLAST searches with the 16S rRNA gene sequence against the GenBank/EMBL/DDBJ databases showed that strain SD29^T was closely related to members of the genus *Gordonia*. It lay in a monophyletic clade with *G. sihwensis* DSM 44576^T and *G. hydrophobica* DSM 44015^T in the neighbour-joining tree (Fig. 1), and shared the highest 16S rRNA gene sequence similarity (97.1 and 97.0 %, respectively) with these strains. Such low similarity values and the low bootstrap levels (37 and 40 %) supporting the clades indicated that the isolate was distinct from the type strains of *G. sihwensis* and *G. hydrophobica*. In the maximum-parsimony tree, the isolate formed an unstable clade (bootstrap level of 33 %) with *G. sihwensis*. The physiological properties listed in Table 2 also readily distinguished the isolate from the two type strains. It is apparent from the results of phenotypic and genotypic studies that strain SD29^T represents a novel taxonomic unit

Table 1. Cellular fatty acid compositions (%) of strain SD29^T and related type strains of the genus *Gordonia*

Strains: 1, SD29^T (*G. shandongensis* sp. nov.); 2, *G. alkanivorans* DSM 44369^T; 3, *G. amicalis* DSM 44461^T; 4, *G. desulfuricans* DSM 44462^T; 5, *G. hydrophobica* DSM 44015^T; 6, *G. namibiensis* DSM 44568^T; 7, *G. rubripertincta* DSM 43197^T; 8, *G. sihwensis* DSM 44576^T; 9, *G. terrae* DSM 43249^T; 10, *G. westfalica* DSM 44215^T. In addition, strain SD29^T contains 1 % C_{17:1}ω8c and 1 % C_{17:0} 3-OH, and *G. namibiensis* DSM 44568^T and *G. rubripertincta* DSM 43197^T respectively contain 1 and 3 % C_{19:1}. Data in columns 2–4, 6, 8 and 10 were taken from Linos *et al.* (1999), and data in columns 5, 7 and 9 were taken from Kim *et al.* (2003).

Fatty acid	1	2	3	4	5	6	7	8	9	10
C _{14:0}	4	4	2	2	1	2	2	1	2	2
C _{15:0}	1	1	3		4	4	2	1	1	1
C _{16:1} ω7c	1		1	1				1		12
C _{16:1} ω9c		16	14	15	13	12	15	19	16	
C _{15:0} iso 2-OH	15									
C _{16:0}	36	28	29	26	27	24	25	39	32	32
C _{17:1} ω9c		2	6		4	8	5	2	1	
C _{17:0}	1	1	5	1	4	7	3	1	1	2
10-Methyl C _{17:0}			2		4	2	1			
C _{18:1}	21	25	23	18	14	25	24	12	26	25
C _{18:0}	1	2	2	7	1	2		1	2	1
TBSA (10-methyl C _{18:0})	15	20	13	30	26	12	17	22	19	17

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences created using the neighbour-joining method in MEGA version 3.1, showing the phylogenetic positions of strain SD29^T and type strains within the genus *Gordonia*. Numbers at branching points refer to percentages of bootstrap values (from 1000 replications). Asterisks represent clades that were also recovered using the maximum-parsimony algorithm. Bar, 0.005 K_{nuc} value.

Table 2. Physiological properties that distinguish strain SD29^T from the type strains of *G. hydrophobica* and *G. sihwensis*

Strains: 1, strain SD29^T (*G. shandongensis* sp. nov.); 2, *G. hydrophobica* DSM 44015^T; 3, *G. sihwensis* DSM 44576^T. +, Positive; –, negative; d, doubtful.

Characteristic	1	2	3
Utilization of sole carbon sources:			
D-Cellobiose	+	–	–
Sucrose	–	+	+
D-Galactose	d	+	+
D-Arabinose	+	–	–
D-Ribose	–	+	–
L-Alanine	–	+	–
L-Valine	–	+	+
L-Hydroxyproline	–	–	+
L-Arginine	–	+	–
Benzoate	–	–	d
Decomposition of guanine	+	–	–
Hydrolysis of urea	+	–	+

in the genus *Gordonia*. It is therefore proposed that the strain should be recognized as the type strain of a novel species with the name *Gordonia shandongensis* sp. nov.

Description of *Gordonia shandongensis* sp. nov.

Gordonia shandongensis (shan.dong.en'sis. N.L. fem. adj. *shandongensis* referring to Shandong Province, China, from where the type strain was isolated).

Gram-positive, aerobic, non-motile, slightly acid-fast actinomycete that fragments into rod/coccus-like elements. Colonies are light yellow and rough with irregular margins on ISP 2 agar. L-Proline is used as a sole carbon source for energy and growth but L-aspartate, L-rhamnose, sodium succinate, citric acid and sebacic acid are not. Aesculin is degraded but casein, starch, tyrosine and xanthine are not. Nitrate is reduced to nitrite. The organism grows well at 20–37 °C and at pH 5.5–9.0. Other physiological properties are shown in Table 2. The cell wall chemotype is IV, and phospholipid type is PII. The principal menaquinone is MK-9(H₂). The major fatty acids are C_{16:0} (35.9 %), C_{18:1ω9c} (21.3 %), 10-methyl C_{18:0} (TBSA) (14.9 %) and C_{15:0} iso 2-OH (14.6 %). Mycolic acids are present.

The type strain, SD29^T (= CGMCC 4.3492^T = JCM 13907^T), was isolated from farmland soil collected in Shandong Province, China.

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