Gordonia soli sp. nov., a novel actinomycete isolated from soil

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A soil isolate, strain CC-AB07^T, was characterized using a polyphasic approach. This organism had chemotaxonomic and morphological properties consistent with its classification in the genus *Gordonia*. 16S rRNA gene sequence analysis showed that the novel strain formed a monophyletic branch at the periphery of the evolutionary radiation occupied by the genus *Gordonia*, its closest neighbours being the type strains of *Gordonia alkanivorans*, *Gordonia amicalis*, *Gordonia bronchialis*, *Gordonia desulfuricans*, *Gordonia polyisoprenivorans* and *Gordonia rhizosphaera*. The novel isolate was distinguished from all of these type strains using a range of phenotypic properties and by *gyrB* gene sequence analysis. It was evident from the genotypic and phenotypic data that strain CC-AB07^T should be classified as representing a novel species in the genus *Gordonia*, for which the name *Gordonia soli* sp. nov. is proposed. The type strain is CC-AB07^T (=BCRC 16810^T=DSM 44995^T).

The genus *Gordonia* belongs to the family *Gordoniaceae*, a member of the suborder *Corynebacterineae* proposed by Stackebrandt *et al.* (1997). The taxon is well-defined and can be distinguished readily from the nine other genera that constitute the suborder, notably by using chemotaxonomic, morphological and 16S rRNA gene sequence data (Soddell *et al.*, 2006a). At the time of writing the genus comprises 20 recognized species, most of which have been described in the last 10 years using polyphasic taxonomic approaches (Kim *et al.*, 1999; Iida *et al.*, 2005). All but one of these taxa have been isolated from natural and artificial habitats, including activated sludge foam (Soddell *et al.*, 2006b), industrial wastewater (Kim *et al.*, 2003), mangrove rhizosphere (Takeuchi & Hatano, 1998), tar-contaminated soil (Kummer *et al.*, 1999) and an oil-producing well (Xue *et al.*, 2003). The exception, *Gordonia otitidis* Iida *et al.* 2005, belongs to the growing number of *Gordonia* species associated with human and animal diseases (Goodfellow & Maldonado, 2006). However, in general, *Gordonia* strains are widely distributed in soil and aquatic habitats, including marine sediments (Goodfellow & Maldonado, 2006).

The aim of the present study was to determine the taxonomic position of an actinomycete, designated strain CC-AB07^T, that formed a pale orange colony on a trypticase soy agar (TSA) plate that had been incubated at 30 °C for

3 days following inoculation with a suspension of a soil sample collected from the campus of National Chung Hsing University, Taichung, Taiwan. The organism was subjected to a polyphasic taxonomic study, which showed that it was a novel species of the genus *Gordonia*.

Extraction of genomic DNA, PCR amplification and sequencing of 16S rRNA genes was carried out as described by Shen *et al.* (2005). Sequence analysis was performed using a DNA sequencer (ABI PRISM 310; Applied Biosystems) and sequence assembly by using the Fragment Assembly System program from the Wisconsin Package 9.1 supplied by the National Health Research Institute of Taiwan. The resultant sequence was compared with corresponding results taken from the RDP II database for representatives of the genera classified in the suborder *Corynebacterineae*. Phylogenetic trees were inferred using the maximum-likelihood, maximum-parsimony and neighbour-joining algorithms, as explained by Soddell *et al.* (2006a). The unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining dataset using the SEQBOOT and CONSENSE options from the PHYLIP package (Felsenstein, 1989). A nearly complete 16S rRNA gene sequence (1485 nt) that corresponded to *Escherichia coli* positions 26–1511 (Brosius *et al.*, 1978) was obtained for strain CC-AB07^T. A comparison of the sequence with those of representatives of the genera classified in the suborder *Corynebacterineae* showed that the organism fell within the evolutionary radiation occupied by the genus *Gordonia*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Gordonia soli* CC-AB07^T is AY995560.

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rRNA gene sequences showing the position of strain CC-AB07^T within the radiation occupied by the genus *Gordonia*. Asterisks indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms. F and P indicate branches that were also recovered using the least-squares and maximum-parsimony methods, respectively. Numbers at the nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50% are given. Bar, 0.02 substitutions per nucleotide position.

(data not shown). 16S rRNA gene sequence similarity between strain CC-AB07^T and the type strains of members of the genus *Gordonia* ranged from 94.0 to 98.2%.

Chemosystematic studies were carried out to establish whether strain CC-AB07^T had a chemical profile consistent with its assignment to the genus *Gordonia*. Biomass for chemical studies was grown in shake flasks of glucose-yeast extract broth (Gordon & Mihm, 1962) for 5 days at 28 °C, checked for purity, harvested by centrifugation, washed twice in distilled water and freeze-dried. Standard methods were used for analysis of the isomers of diaminopimelic acid, fatty acids, isoprenoid quinones, muramic acid type, mycolic acids, polar lipids and sugars, as described by Soddell *et al.* (2006b). The isolate contained *meso*-diaminopimelic acid, arabinose and galactose (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970); *N*-glycolated muramic acid; dihydrogenated menaquinones with nine isoprene units as the predominant isoprenologue; phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides as major polar lipids (phospholipid type II *sensu* Lechevalier *et al.*, 1977) and a fatty acid profile rich in palmitic (C_{16:0}; 33.6% of the total fatty acid composition),

palmitoleic (C_{16:1}; 10.4%), oleic (C_{18:1}; 11.7%) and tuberculostearic (29.4%) acids. It was also characterized by the presence of mycolic acids that co-migrated (*R_F* value 0.47) with those extracted from *Gordonia bronchialis* DSM 43247^T. All of these properties are typical of representatives of the genus *Gordonia* (Goodfellow & Maldonado, 2006).

It is evident from the phylogenetic tree in Fig. 1 that strain CC-AB07^T forms a monophyletic branch at the periphery of the evolutionary radiation occupied by the genus *Gordonia*. The organism was most closely related to *G. bronchialis* DSM 43247^T, sharing a 16S rRNA gene sequence similarity of 98.2%, a value that corresponds to 26 nt differences at 1468 locations. Strain CC-AB07^T also shares comparatively high 16S rRNA gene sequence similarities with the type strains of *Gordonia polyisoprenivorans* (98.1%), *Gordonia rhizosphaera* (97.7%), *Gordonia amicalis* (97.6%), *Gordonia desulfuricans* (97.6%), *Gordonia terrae* (97.4%), *Gordonia westfalica* (97.4%), *Gordonia alkanivorans* (97.3%) and *Gordonia rubripertincta* (97.3%). DNA–DNA relatedness experiments were not carried out between strain CC-AB07^T and its closest phylogenetic neighbours as the type strains of several *Gordonia* species share higher 16S rRNA gene sequence similarities, but have levels of

Fig. 2. Neighbour-joining analysis based on *gyrB* nucleotide sequences showing the position of strain CC-AB07^T within the radiation occupied by the genus *Gordonia*. Numbers at the nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50 % are given. The *gyrB* gene sequences of the type strains of members of the genus *Gordonia* were retrieved from the EMBL and ICB (<http://seasquirt.inbio.co.jp/icb/>) databases. Bar, 0.02 substitution (K_{nuc}) units.

DNA–DNA relatedness well below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987). The type strains of *G. alkanivorans* and *G. rubripertincta*, for example, have a 16S rRNA gene sequence similarity of 99.1 % (which corresponds to a 13-nt difference), but a DNA–DNA relatedness value of only 52 % (Kummer *et al.*, 1999).

The *gyrB* nucleotide sequence of strain CC-AB07^T was determined following the procedures described by Shen *et al.* (2006). Degenerate PCR primers GYRB1 and GYRB2 were designed and used to amplify a *gyrB* fragment of approximately 1.2 kb from the genomic DNA extracted from the type strains of recognized *Gordonia* species. Sequencing primers were designed from the flanking regions of *gyrB* conserved sequences with two forward primers, GYRBF1 and GYRBF2, and the reverse primer GYRBR1. The resultant sequence was compared with corresponding *gyrB* sequences of available *Gordonia* type strains drawn from the EMBL and ICB database (<http://seasquirt.mbio.co.jp/icb/>) using the CLUSTAL_X program (Thompson *et al.*, 1997). A phylogenetic tree was inferred with the neighbour-joining algorithm (Saitou & Nei, 1987) using MEGA version 2.1 software (Kumar *et al.*, 2001). An evolutionary distance matrix was generated as described by Kimura (1980). The resultant unrooted tree was evaluated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings.

It can be seen from Fig. 2 that strain CC-AB07^T forms a distinct phyletic line in the *Gordonia gyrB* gene sequence tree. The strain is most closely related to *G. amicalis* DSM 44461^T (84.5 % *gyrB* gene sequence similarity), *G. desulfuricans* (81.3 %), *G. polyisoprenivorans* (79.8 %), *G. rhizosphaera* DSM 44383^T (78.8 %), *Gordonia sputi* DSM 43896^T (78.0 %), *G. bronchialis* DSM 43247^T (77.9 %) and *Gordonia aichiensis* DSM 43978^T (77.5 %). These data provide further evidence that isolate CC-AB07^T is not particularly closely related to representatives of recognized *Gordonia* species, especially as classifications based on *gyrB* gene nucleotide sequence data correlate well with those based on corresponding DNA–DNA relatedness data (Shen *et al.*, 2006). These workers determined *gyrB* gene sequence similarities between the type strains of 12 recognized *Gordonia* species and reported similarity values in the range 79.3–97.2 %

(41–270 nt difference); the corresponding 16S rRNA gene sequences differed within the range 0.3–3.8 %.

Strain CC-AB07^T was examined for a broad range of phenotypic properties, notably for features known to be of value in *Gordonia* systematics (Kim *et al.*, 1999; Soddell *et al.*, 2006b). Cultural characteristics were examined on a TSA plate that had been incubated for 2 days at 30 °C. Smears taken from the plate were Gram-stained (Shen *et al.*, 2005) and examined for micromorphological properties. Additional smears were stained using a modification of the Ziehl–Neelsen method (Gordon, 1967) to determine whether the organism was acid–alcohol-fast. Standard biochemical and degradation tests were carried out using well-established procedures (Kim *et al.*, 1999). The enzymic profile of strain CC-AB07^T was determined using API Coryne (bioMérieux) and API ZYM (bioMérieux) kits according to the manufacturer's instructions. Similarly, sole carbon source tests were performed using a Biolog GP-II kit. The ability of the organism to grow at a range of temperatures was determined on TSA plates and motility was tested as described by Shen *et al.* (2005). Oxidase activity was determined using oxidase reagent (bioMérieux). It is apparent from the results (Table 1) that strain CC-AB07^T can be distinguished from representatives of its close phylogenetic relatives using a combination of phenotypic attributes.

It is clear from the genotypic and phenotypic data that strain CC-AB07^T represents a novel species in the genus *Gordonia*. The name *Gordonia soli* sp. nov. is proposed for this taxon.

Description of *Gordonia soli* sp. nov.

Gordonia soli (so.li. L. gen. n. *soli* of/from the soil).

Aerobic, Gram-positive, partially acid–alcohol-fast, non-motile actinomycete that forms elementary branching hyphae that fragment into rods and cocci. Pale orange-coloured, circular colonies (about 2 mm in diameter) with filamentous margins are formed on TSA after incubation for 2 days at 30 °C. Neither aerial hyphae nor diffusible pigments are produced. Grows at pH 5.5–10.0 and at 28–35 °C, but not at 4 or 40 °C. Degrades starch, produces catalase and oxidase, but does not hydrolyse allantoin or arbutin. Acid and alkaline phosphatase, butyrate esterase,

Table 1. Phenotypic characteristics that differentiate strain CC-AB07^T from representatives of closely related *Gordonia* species

Taxa: 1, CC-AB07^T; 2, *G. alkanivorans* DSM 44369^T; 3, *G. amicalis* DSM 44461^T; 4, *G. bronchialis* DSM 43247^T; 5, *G. desulfuricans* DSM 43247^T; 6, *G. polyisoprenivorans* DSM 44302^T; 7, *G. rhizosphaera* NBRC 16068^T; 8, *G. rubripertincta* DSM 43197^T; 9, *G. terrae* DSM 43249^T; 10, *G. westfalica* DSM 44215. +, Positive; –, negative. Most of the data for the *Gordonia* type strains were taken from Maldonado *et al.* (2003); all of the results for the *G. westfalica* strain were determined in the present study, as were the API ZYM results for the type strains of *G. polyisoprenivorans* and *G. rhizosphaera*. In the API ZYM tests, all of the strains produced acid phosphatase and α -glucosidase.

Characteristic	1	2	3	4	5	6	7	8	9	10
Colony colour	Pale orange	Pale orange	Red	Brown	Pink	Orange	Orange/red	Pink/orange	Pink/orange	Pastel orange
API ZYM tests:										
Alkaline phosphatase	+	–	–	+	+	+	–	–	–	–
Butyrate esterase	+	+	+	+	+	+	–	+	+	+
Caprylate esterase	+	+	+	–	+	+	+	+	+	+
Cystine arylamidase	–	+	–	–	–	+	–	–	–	–
β -Glucosidase	+	–	–	–	–	–	+	–	+	–
Leucine arylamidase	–	+	+	+	+	+	+	+	+	+
Myristate lipase	–	+	+	–	–	–	–	–	–	–
Naphthol-AS-BI-phosphohydrolase	+	+	–	+	+	+	+	+	+	+
Valine arylamidase	–	+	–	–	–	–	–	+	+	–
Biochemical tests:										
Aesculin	–	–	–	–	–	–	–	–	+	–
Arbutin	–	–	–	–	–	–	–	+	+	–
Nitrate reductase	–	+	–	+	+	–	–	–	+	+
Urea hydrolysis	+	+	–	+	+	–	–	+	+	+
Degradation of (% w/v):										
Hypoxanthine (0.4)	–	–	–	–	+	–	–	+	+	–
Tributyrin (0.1)	+	+	–	–	–	+	–	+	–	–
Tween 80 (0.1)	+	+	–	–	+	+	–	–	–	+
Tyrosine (0.5)	–	–	–	–	–	–	–	–	–	–
Uric acid (0.5)	–	–	–	+	+	+	+	+	+	–
Xanthine (0.4)	–	–	–	–	–	–	–	–	+	–

caprylate esterase, α -glucosidase, β -glucosidase, naphthol-AS-BI-phosphohydrolase and pyrazinamidase are produced, but myristate lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -mannosidase, α -fucosidase, *N*-acetyl- β -glucosaminidase, pyrrolidonylarylamidase and trypsin are not. Does not ferment D-glucose, glycogen, D-lactose, D-maltose, D-mannitol, D-ribose, sucrose or D-xylose. Utilizes *N*-acetyl-L-glutamic acid, D-alanine (weak), L-alanine, L-alanyl glycine (weak), L-alaninamide, α -cyclodextrin, β -cyclodextrin, D-fructose 6-phosphate (weak), α -D-glucose, L-glutamic acid, glycogen (weak), α -hydroxybutyric acid, β -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α -ketoglutaric acid, DL-lactic acid (weak), D-malic acid (weak), mannan, D-mannose, 3-methyl D-glucose (weak), D-psicose, L-pyrroglutamic acid, pyruvic acid (weak), putrescine, D-sorbitol (weak), succinic acid (weak), sucrose (weak), Tweens 20 and 40 and uridine 5-monophosphate as sole carbon sources. Additional phenotypic properties are given in Table 1. Chemotaxonomic properties are typical of the genus *Gordonia*.

The type strain, CC-AB07^T (=BCRC 16810^T=DSM 44995^T), was isolated from a soil sample collected from the campus of National Chung Hsing University, Taichung, Taiwan.

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