**Gordonia humi** sp. nov., isolated from soil

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A Gram-stain-positive, non-endospore-forming actinobacterium (CC-12301T) was isolated from soil attached to a spawn used in the laboratory to grow the edible mushroom *Agaricus brasiliensis*. Based on 16S rRNA gene sequence similarities, strain CC-12301T was shown to belong to the genus *Gordonia* and was most closely related to the type strains of *Gordonia hydrophobica* (97.6% similarity), *Gordonia terrae* (97.5%), *Gordonia amarae* (97.5%) and *Gordonia malaquae* (97.4%). The quinone system was determined to consist predominantly of menaquinone MK-9(H2), minor amounts of MK-8(H2) and MK-7(H2). The polar lipid profile consisted of the major compounds diphosphatidylglycerol and phosphatidylethanolamine, moderate amounts of two phosphatidylinositol mannosides and phosphatidylinositol and minor amounts of phosphatidylglycerol, three unidentified glycolipids, two phosphoglycolipids and a phospholipid. Mycolic acids were present. These chemotaxonomic traits and the major fatty acids, which were C16:1 cis9, C16:0, C18:1 and tuberculostearic acid (10-methyl C18:0), supported the affiliation of strain CC-12301T to the genus *Gordonia*. The results of physiological and biochemical tests allowed clear phenotypic differentiation of strain CC-12301T from the most closely related *Gordonia* species. Strain CC-12301T therefore represents a novel species, for which the name *Gordonia humi* sp. nov. is proposed, with the type strain CC-12301T (=DSM 45298T =CCM 7727T).

The genus *Gordonia* (originally ‘*Gordona’*) was initially proposed by Tsukamura (1971), and strains of this genus belong to the mycolic acid-containing group of the actinomycetes and to the suborder *Corynebacterineae*, which forms a distinct phylogenetic lineage in the 16S rRNA gene tree (Goodfellow et al., 1998; Stackebrandt et al., 1997). At the time of writing, the genus *Gordonia* comprises 29 species, *Gordonia bronchialis* (the type species), *G. rubripertincta*, *G. sputi* and *G. terrae* (Stackebrandt et al., 1988), *G. aichiensis* and *G. amarae* (Klatte et al., 1994), *G. hydrophobica* (Bendinger et al., 1995), *G. hirsuta* (Klatte et al., 1996), *G. rhizosphaera* (Takeuchi & Hatano, 1998), *G. desulfuricans* (Kim et al., 1999), *G. alkaniivorans* (Kummer et al., 1999) [according to Arenskötter et al. (2003), *G. alkaniivorans* is an earlier heterotypic synonym of *Gordonia nitida* Yoon et al. 2000], *G. polyisoprenivorans* (Linos et al., 1999), *G. amicalis* (Kim et al., 2000), *G. namibiensis* (Brandão et al., 2001), *G. westfalica* (Linos et al., 2002), *G. sihwenesi* (Kim et al., 2003), *G. sinisedis* (Maldonado et al., 2003), *G. paraffinivorans* (Xue et al., 2003), *G. otitidis* (Iida et al., 2005), *G. araii* and *G. effusa* (Kageyama et al., 2006), *G. soli* (Shen et al., 2006), *G. defluvii* (Sodell et al., 2006), *G. shandongensis* (Luo et al., 2007), *G. malaquae* (Yassin et al., 2007), *G. lacunae* (Le Roes et al., 2008) and *G. cholesterolivorans* (Drzyzga et al., 2009). Members of the genus *Gordonia* were originally reported to be opportunistic pathogens that were isolated from various clinical material such as sputum of humans suffering from pulmonary diseases, e.g. *G. bronchialis*, *G. aichiensis* and *G. sputi* (Kim et al., 2003), but, more recently, many *Gordonia* isolates have been isolated from various environmental sources and some of them have been reported to be involved

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-12301T is FN561544.

A supplementary table and two supplementary figures are available with the online version of this paper.

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in the degradation of xenobiotic compounds, such as benzo[ghi]perylene and dibenzothiophene by *G. desulfuricans* and *G. amicalis* (Kim et al., 1999, 2000) and alkanes by *G. alkaniivorans* (Kummer et al., 1999).

Strain CC-12301T was isolated from soil attached to a spawn used in the laboratory for growing the edible mushroom *Agaricus brasiliensis* and was maintained on nutrient agar (NA; Oxoid) after incubation at 30 °C for 48 h. The strain was preserved at −80 °C in nutrient broth (NB; Oxoid) with 20 % (v/v) glycerol or by lyophilization.

Morphological properties, Gram-staining and cell morphology were observed by phase-contrast microscopy as described by Kämpfer & Kroppenstedt (2004).

The 16S rRNA gene sequence of strain CC-12301T was determined and analysed as described previously (Kämpfer & Kroppenstedt, 2004). The 16S rRNA gene sequence of strain CC-12301T was a continuous stretch of 1438 bp. Multiple sequence alignment and analysis of the data were performed using the software package MEGA version 4 (Tamura et al. 2007) as well as with the ARB software package (version December 2007; Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (release July 2008; Pruesse et al., 2007). Genetic distances were calculated on the basis of pairwise comparisons using the ARB program and clustering was performed with the neighbour-joining (Supplementary Fig. S1, available in IJSEM Online) and maximum-parsimony (results not shown) methods using MEGA 4 and bootstrap values based on 1000 replications.

Tree reconstruction using the maximum-likelihood method with fastDNAml (Olsen et al., 1994) was performed with the ARB software package (Fig. 1). Tree topology was further tested without filters. Several differences could be observed between these trees. Sequence similarity calculations on the basis of a pairwise comparison indicated that the closest relative of strain CC-12301T were the type strains of *G. hydrophobica* (97.6 %), *G. terrae* (97.5 %), *G. amarae* (97.5 %) and *G. malaquae* (97.4 %). Type strains of other *Gordonia* species revealed 16S rRNA gene sequence similarities <97.4 %. Almost the same values were obtained for pairwise similarity calculations based on the algorithm of the EzTaxon program (Chun et al., 2007). These similarities were not reflected in the trees. However, the close relationship of strain CC-12301T to *G. hydrophobica* is clearly shown in Fig. 1, and the cluster of *G. hydrophobica*, *G. sihwaensis*, *G. cholesterolivorans*, *G. paraffinivorans*, *G. hirsuta* and *G. shandongensis* shown in Fig. 1 was also reported by Drzyzga et al. (2009). From other studies (e.g. Soddell et al., 2006; Yassin et al., 2007), it is clear that many *Gordonia* clusters are not stable and that relationships are not supported by high bootstrap values. If the results obtained with different treeing algorithms disagree, the closest relatives of a given sequence are identified on the basis of individual sequence similarities. It has been shown previously that members of distinct *Gordonia* species can show relatively high 16S rRNA gene sequence similarity, e.g. the type strains of *G. bronchialis* and *G. terrae* share 98.3 % similarity, which corresponds to 25 nucleotide differences, while members of these species show DNA–DNA relatedness within the range 16–21 % (Zakrzewska-Czerwinska et al., 1988). A similar result was obtained for the type strains of *G.*
aichiensis and G. sputi, sharing 99.7% 16S rRNA gene nucleotide similarity and 38–40% DNA–DNA relatedness (Goodfellow et al., 1998; Klatte et al., 1994). For this reason, only the type strains of the most closely related species (G. hydrophobica, G. terrae and G. amarae) were included in DNA–DNA hybridization experiments.

For mycolic acid, quinone and polar lipid analyses, cells were grown on PYE medium (0.3% yeast extract, 0.3% peptone by vol.) and detected by charring at 140 °C after spraying with ethanolic molybdophosphoric acid (5%, w/v).

Table 1. Physiological properties of Gordonia humi sp. nov. CC-12301T and related type strains of the genus Gordonia

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**Hydrolysis of:**

| p-NP β-1,4-xylolose            | –   | –   | +   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| p-NP phosphorylcholine         | +   | +   | +   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| 2-Deoxythymidine-5′-p-NP phosphate | –   | +   | +   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |

*Compared in this study and confirmed.
†Compared in this study but not confirmed.
‡Not reported by Linos et al. (1999); result from this study in parentheses.
Mycolic acids were detected in the extract of strain CC-12301T. The mycolic acid spot showed almost identical chromatographic behaviour to the mycolic acids of G. terrae DSM 43249T and G. hydrophobica DSM 44015T, which were extracted and analysed concurrently (results not shown). The two reference strains were reported to contain mycolic acids containing predominantly 56–60 carbon atoms; hence, strain CC-12301T can also be considered to have mycolic acids of similar size. The quinone system consisted predominantly of menaquinone MK-9(H2), with minor amounts of MK-8(H2) and MK-7(H2), in the ratio 93.0 : 6.5 : 0.4. The polar lipid profile was composed of the major compounds diphosphatidylglycerol and phosphatidylethanolamine. Furthermore, moderate amounts of two phosphatidylinositol mannosides and phosphatidylinositol and minor amounts of phosphatidylglycerol, three unidentified glycolipids, two phosphoglycolipids and a phospholipid were detected (Supplementary Fig. S2a). This profile clearly distinguished CC-12301T from G. hydrophobica DSM 44015T and G. terrae DSM 43249T, which were also examined (Supplementary Fig. S2b, c). In the two reference strains, none of the three unidentified glycolipids was detected, and the phosphatidylinositol mannoside that exhibited the same behaviour in the second chromatographic dimension as phosphatidylinositol was detected only in trace amounts in G. hydrophobica DSM 44015T and not detectable in G. terrae DSM 43249T.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain CC-12301T was similar to those of the other closely related type strains G. hydrophobica DSM 44015T and G. terrae DSM 43249T (Supplementary Table S1). It was composed mainly of the fatty acids C16:1 cis9, C16:0 cis18:1 and tuberculostearic acid (10-methyl C18:0). Results from analysis of mycolic acids, quinones, polar lipids and fatty acids are in excellent agreement with the traits listed in the genus description (Stackebrandt et al., 1988) and, hence, support the affiliation of strain CC-12301T to the genus Gordonia.

Results of the physiological characterization are given in Table 1 and the species description, with methods described previously (Kämpfer et al., 1991). The results show that strain CC-12301T was clearly different from the most closely related Gordonia species.

As indicated above, DNA–DNA hybridization experiments were performed with CC-12301T and the type strains G. hydrophobica DSM 44015T, G. terrae ATCC 25594T and G. amarae DSM 43392T using the method described by Ziemke et al. (1998), with a minor variation in the nick translation step, where 2 µg DNA was labelled over a 3 h incubation at 15 °C.

We assumed a G+C content of 67 mol% for strain CC-12301T in the DNA–DNA hybridization experiments. Strain CC-12301T showed relatively low DNA–DNA relatedness to G. hydrophobica DSM 44015T (27.7 %, reciprocal 25.5 %), G. terrae ATCC 25594T (33.4 %, reciprocal 34.7 %) and G. amarae DSM 43392T (40.2 %, reciprocal 34.8 %). The observed physiological differences between these type strains (Table 1) as well as the unique polar lipid profile clearly warrant the creation of a separate species.

**Description of Gordonia humi sp. nov.**

Gordonia humi (hu’mi. L. gen. n. humi of/from soil, the source of the type strain).

Coccoid cells, about 1.0–1.5 µm in diameter. Gram-stain-positive and oxidase- and catalase-positive, showing an aerobic respiratory metabolism. Good growth occurs after 3 days of incubation on TSA, R2A agar and nutrient agar at 25–30 °C. The quinone system consists of the predominant compound menaquinone MK-9(H2) and minor amounts of MK-8(H2) and MK-7(H2). The polar lipid profile is composed of the major compounds diphosphatidylglycerol and phosphatidylethanolamine. Moderate amounts of two phosphatidylinositol mannosides and phosphatidylinositol and minor amounts of phosphatidylglycerol, three unidentified glycolipids, two phosphoglycolipids and a phospholipid are also present. Mycolic acids are present. Major fatty acids are C16:1 cis9, C16:0 cis18:1 and tuberculostearic acid (10-methyl C18:0) (Supplementary Table S1). The type strain is able to utilize D-glucose, sucrose, N-acetyl-D-glucosamine, citrate and proline, but not D-galactose, L-rhamnose, D-ribose, turanose, D-adonitol, myo-inositol, caprate, 4-aminoobutyrate, L-alanine, asparagine or L-leucine. Further details of carbon source utilization (including differentiating characters on the basis of identical test conditions) are indicated in Table 1.

The type strain is CC-12301T (=DSM 45298T =CCM 7727T), which was isolated from a spawn used for growing the edible mushroom Agaricus brasiliensis.

**Acknowledgements**

We are grateful to Gundula Will for excellent technical assistance. This work was supported by the National Science Council, Taiwan ROC, and the Council of Agriculture, EY, Taiwan ROC.

**References**


