Gordonia humi sp. nov., isolated from soil

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A Gram-stain-positive, non-endospore-forming actinobacterium (CC-12301^T) 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-12301^T 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 C_{16:1} cis9, C_{16:0}, C_{18:1} and tuberculostearic acid (10-methyl C_{18:0}), supported the affiliation of strain CC-12301^T to the genus *Gordonia*. The results of physiological and biochemical tests allowed clear phenotypic differentiation of strain CC-12301^T from the most closely related *Gordonia* species. Strain CC-12301^T therefore represents a novel species, for which the name *Gordonia humi* sp. nov. is proposed, with the type strain CC-12301^T (=DSM 45298^T =CCM 7727^T).

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. alkanivorans* (Kummer *et al.*, 1999) [according to

Arenskötter *et al.* (2005), *G. alkanivorans* 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. sihwensis* (Kim *et al.*, 2003), *G. sinesedis* (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* (Soddell *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

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

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

in the degradation of xenobiotic compounds, such as benzothiophene and dibenzothiophene by *G. desulfuricans* and *G. amicalis* (Kim *et al.*, 1999, 2000) and alkanes by *G. alkanivorans* (Kummer *et al.*, 1999).

Strain CC-12301^T 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-12301^T was determined and analysed as described previously (Kämpfer & Kroppenstedt, 2004). The 16S rRNA gene sequence of strain CC-12301^T 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 fastDNAmI (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 relatives of strain CC-12301^T 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-12301^T to *G. hydrophobica* is clearly shown in Fig. 1, and the cluster of *G. hydrophobica*, *G. sihwensis*, *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.*

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory database (accession numbers in parentheses). The phylogenetic tree was constructed using 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). Tree building was performed using the maximum-likelihood method with fastDNAmI (Olsen *et al.*, 1994). Bar, 0.10 substitutions per nucleotide position.

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

from casein, pH 7.2). Quinones and polar lipids were extracted and analysed as described by Tindall (1990a, b) and Altenburger *et al.* (1996). HPLC analysis of quinones was carried out using the equipment reported by Stolz *et al.* (2007). Mycolic acids were extracted according to Minnikin *et al.* (1975), spotted on silica gel 60 thin-layer glass sheets (Merck 105626), developed in chloroform/methanol/water (65:25:4, by vol.) and detected by charring at 140 °C after spraying with ethanolic molybdotatophosphoric acid (5 %, w/v).

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

Strains: 1, CC-12301^T; 2, *G. hydrophobica* DSM 44015^T; 3, *G. terrae* DSM 43249^T; 4, *G. amarae* DSM 43392^T; 5, *G. malaquae* DSM 45064^T; 6, *G. sihwensis* SPR2^T; 7, *G. aichiensis* DSM 43978^T; 8, *G. alkanivorans* DSM 44369^T; 9, *G. amicalis* DSM 44461^T; 10, *G. bronchialis* DSM 43247^T; 11, *G. desulfuricans* DSM 44462^T; 12, *G. hirsuta* DSM 44140^T; 13, *G. namibiensis* DSM 44568^T; 14, *G. nitida* DSM 44499^T; 15, *G. polyisoprenivorans* DSM 44302^T; 16, *G. rhizosphaera* NBRC 16068^T; 17, *G. rubripertincta* DSM 43197^T; 18, *G. sputi* DSM 43896^T; 19, *G. westfalica* DSM 44215^T. Data for reference strains were taken from Linos *et al.* (1999) (columns 2–4, 7, 16, 18 and 19), Yassin *et al.* (2007) (column 5) and Kim *et al.* (2003) (columns 6, 8–15 and 17); with the exception of the data for *G. malaquae* DSM 45064^T, the table is based on the compilation of Kim *et al.* (2003), using the same methods used for characterization of strain CC-12301^T. ND, No data available; *p*-NP, *p*-nitrophenyl.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Utilization of:																			
D-Galactose	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–
L-Rhamnose	–	+	+	–	–	–	–	+	+	–	–	–	–	+	+	+	+	–	–
D-Ribose	–	+	–	+	ND	–	–	+	+	–	+	–	+	+	–	–	+	–	+
Sucrose	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	–	+
Turanose	–	–	–	+	ND	+	–	+	+	+	+	–	–	+	+	+	–	–	–
D-Arabitol	–	–	+	+	ND	+	–	+	–	–	–	–	+	–	+	–	+	+	+
<i>myo</i> -Inositol	–	+	–	–	–	–	–	+	–	+	–	+	–	+	+	+	–	–	–
<i>N</i> -Acetyl-D-glucosamine	+	–	–	–	ND	+	–	+	–	+	+	–	–	–	+	+	–	–	–
Glucarate	–	+	+	–	ND	–	–	+	+	–	–	–	–	+	–	–	+	–	–
Gluconate	–	–	–	–	ND	+	–	+	+	+	+	–	+	+	+	+	+	–	–
Caprate	–	–	–	ND	ND	+	+	+	+	+	+	ND	–	–	ND	–	–	+	+
Citrate	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	+	–	+	+
4-Aminobutyrate	–	–	–	ND (–)‡	ND	+	+	–	–	–	+	ND	–	–	ND	–	–	–	–
2-Hydroxyvalerate	–	+	–	–	ND	+	–	+	+	–	+	+	–	+	–	–	–	–	–
2-Oxoglutarate	–	+	+	+	ND	+	+	+	+	–	+	+	–	+	–	+	–	+	+
Succinate	–	+	–	–	ND	–	–	+	+	+	–	–	+	+	–	+	–	+	–
3-Hydroxybenzoate	+	–	–	–	–	–	–	+	–	–	+	–	–	+	–	+	–	–	–
4-Hydroxybenzoate	–	–	–	+	–	–	–	+	+	+	+	–	+	+	+	+	–	–	–
Phenylacetate	–	–	–	–	ND	+	–	+	+	+	+	–	–	+	–	–	+	–	–
Quinate	–	–	–	+	ND	–	+	–	–	–	–	–	+	–	+	+	–	–	–
L-Alanine	–	+	–	–	+	–	+	+	+	+	+	–	+	+	+	–	+	–	–
L-Aspartate	–	–	–	–	ND	–	+	+	+	–	–	–	–	+	+	–	–	–	+
L-Leucine	–	+	–	–	ND	+	–	+	+	+	+	–	–	–	–	–	–	–	–
L-Proline	+	+	–	+	+	+	–	+	–	–	+	–	–	–	+	–	–	–	–
L-Serine	–	+	–	–	+	+	–	–	+	–	–	–	+	+	–	–	–	–	–
L-Valine	–	+	–	+	ND	+	–	–	–	+	–	–	–	+	+	–	–	–	–
Putrescine	–	–	–	+	ND	–	–	+	+	–	+	–	–	–	+	–	–	–	–
Hydrolysis of:																			
<i>p</i> -NP β-D-xyloside	–	–	+	–	ND	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>p</i> -NP phosphorylcholine	+	+	–	–	ND	+	–	+	+	–	+	+	–	+	–	–	–	–	–
2-Deoxythymidine-5'- <i>p</i> -NP phosphate	–	+	–	–	ND	+	–	–	–	–	+	–	–	–	–	–	–	–	–

*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-12301^T. The mycolic acid spot showed almost identical chromatographic behaviour to the mycolic acids of *G. terrae* DSM 43249^T and *G. hydrophobica* DSM 44015^T, 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-12301^T 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-12301^T from *G. hydrophobica* DSM 44015^T and *G. terrae* DSM 43249^T, 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 44015^T and was not detectable in *G. terrae* DSM 43249^T.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain CC-12301^T was similar to those of the other closely related type strains *G. hydrophobica* DSM 44015^T and *G. terrae* DSM 43249^T (Supplementary Table S1). It was composed mainly of the fatty acids C_{16:1} *cis*9, C_{16:0}, C_{18:1} and tuberculostearic acid (10-methyl C_{18:0}). 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-12301^T 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-12301^T was clearly different from the most closely related *Gordonia* species.

As indicated above, DNA–DNA hybridization experiments were performed with CC-12301^T and the type strains *G. hydrophobica* DSM 44015^T, *G. terrae* ATCC 25594^T and *G. amarae* DSM 43392^T 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-12301^T in the DNA–DNA hybridization experiments. Strain CC-12301^T showed relatively low DNA–DNA relatedness to *G. hydrophobica* DSM 44015^T (27.7 %, reciprocal 25.5 %), *G. terrae* ATCC 25594^T (33.4 %, reciprocal 34.7 %) and *G.*

amarae DSM 43392^T (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 C_{16:1} *cis*9, C_{16:0}, C_{18:1} and tuberculostearic acid (10-methyl C_{18:0}) (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-aminobutyrate, 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-12301^T (=DSM 45298^T =CCM 7727^T), which was isolated from a spawn used for growing the edible mushroom *Agaricus brasiliensis*.

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