Gordonia caeni sp. nov., isolated from sludge of a sewage disposal plant

Sathiyaraj Srinivasan,^{1,2} Giho Park,³ Hyejin Yang,¹ Supyong Hwang,¹ Yoonjung Bae,¹ Yong-An Jung,⁴ Myung Kyum Kim¹ and Myungjin Lee³

Correspondence

Myungjin Lee
mgeneli@nate.com

¹Department of Bio & Environmental Technology, Division of Environmental & Life Science, College of Natural Science, Seoul Women's University, 623 Hwarangno, Nowon-gu, Seoul 139-774, Republic of Korea

²Research and Development Division, H-Plus Eco Ltd, BVC no. 301, KRIBB, Eoeun-dong, Yuseong-gu, Daejeon, 305-333, Republic of Korea

³Daewoo Institute of Construction Technology, 60 Songjuk-dong, Jangan-gu, Suwon-si, Gyeonggi-do 440-210, Republic of Korea

⁴Korea Testing Certification, Chemical Industry Team, 587-10, Sinsa-Dong, Gangnam-Gu, Seoul, Republic of Korea

A Gram-stain-positive, strictly aerobic, short-rod-shaped, non-motile strain (designated MJ32^T) was isolated from a sludge sample of the Daejeon sewage disposal plant in South Korea. A polyphasic approach was applied to study the taxonomic position of strain MJ32^T. Strain MJ32^T showed highest 16S rRNA gene sequence similarity to *Gordonia hirsuta* DSM 44140^T (98.1 %) and *Gordonia hydrophobica* DSM 44015^T (97.0 %); levels of sequence similarity to the type strains of other recognized *Gordonia* species were less than 97.0 %. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain MJ32^T belonged to the clade formed by members of the genus *Gordonia* in the family *Gordoniaceae*. The G + C content of the genomic DNA of strain MJ32^T was 69.2 mol%. Chemotaxonomically, strain MJ32^T showed features typical of the genus *Gordonia*. The predominant respiratory quinone was MK-9(H₂), the mycolic acids present had C₅₆–C₆₀ carbon atoms, and the major fatty acids were C_{16:0} (34.6 %), tuberculostearic acid (21.8 %), C_{16:1ω7c} (19.5 %) and C_{18:1ω9c} (12.7 %). The peptidoglycan type was based on *meso*-2,6-diaminopimelic acid as the diagnostic diamino acid with glycolated sugars. On the basis of phylogenetic inference, fatty acid profile and other phenotypic properties, strain MJ32^T is considered to represent a novel species of the genus *Gordonia*, for which the name *Gordonia caeni* sp. nov. is proposed. The type strain is MJ32^T (=KCTC 19771^T=JCM 16923^T).

The genus *Gordonia* (formerly *Gordona*) was proposed by Tsukamura (1971) to accommodate a mycolic acid-containing group of actinomycetes belonging to the family *Gordoniaceae* of the suborder *Corynebacterineae* (Stackebrandt *et al.*, 1997; Goodfellow *et al.*, 1998). *Gordonia* are Gram-positive, non-motile short-rods to coccids, containing peptidoglycan of A1γ type with *meso*-diaminopimelic acid as the diamino acid and muramic acid with *N*-glycolyl residues often with arabinose and galactose or glucose as cell-wall sugars. Members of the genus *Gordonia* have been isolated from a variety of sources, such as clinical samples and various environmental sources

including xenobiotic compounds and soil (Park *et al.*, 2009; Kämpfer *et al.*, 2010).

In the course of collecting micro-organisms from sludge at the Daejeon sewage disposal plant in South Korea, a Gram-stain-positive, non-motile, aerobic, non-spore-forming strain, designated MJ32^T, was isolated. On the basis of 16S rRNA gene sequence analysis, strain MJ32^T was considered to belong to the genus *Gordonia*. It was subjected to a polyphasic taxonomic investigation and the results indicated that it represents a novel *Gordonia* species.

For isolation of strain MJ32^T, 1 g sludge sample was mixed with 50 ml saline (0.85 % NaCl), vortexed and serially diluted; 100 µl of each dilution was spread onto 0.1 × R2A agar (Difco) and incubated at 30 °C for 1 week. Single colonies of strain MJ32^T were transferred onto new plates and the purified colonies were tentatively identified by

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MJ32^T is JF806526.

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

analysis of partial 16S rRNA gene sequences. The isolate was routinely cultured on nutrient agar (NA; Difco) at 30 °C and preserved as a suspension in Luria–Bertani (LB) broth (Difco) with 20 % (w/v) glycerol at –70 °C.

The genomic DNA of strain MJ32^T was extracted by using a commercial genomic DNA extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). The nearly complete sequence of the 16S rRNA gene (1433 nt) was compiled with SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank and edited via the program BioEdit (Hall, 1999). Multiple alignments were performed with the program CLUSTAL X (Thompson *et al.*, 1997). Evolutionary distances were calculated by using the Kimura two-parameter model (Kimura, 1983). A phylogenetic tree was constructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) method in the program MEGA3 (Kumar *et al.*, 2004) (Fig. 1). Bootstrap analyses, with 1000 replicates, were performed to obtain confidence levels for the branches (Felsenstein, 1985) and a maximum-likelihood

tree was constructed by using the PHYLIP program (Fig. S1 in IJSEM Online) (Choi *et al.*, 2000; Brinkman *et al.*, 2001).

On the basis of 16S rRNA gene sequence similarity, the closest relatives of strain MJ32^T were *Gordonia hirsuta* DSM 44140^T (98.1 %) and *Gordonia hydrophobica* DSM 44015^T (97.0 %). Levels of 16S rRNA gene sequence similarity to the type strains of other *Gordonia* species were less than 97 %. Consequently, the type strains of *Gordonia* species were obtained and grown under the same conditions as strain MJ32^T and used as reference strains for phenotypic, chemotaxonomic and DNA hybridization experiments.

Cell morphology and motility were observed with an Olympus light microscope (1000× magnification) by using cells grown for 24 h in nutrient broth at 30 °C. For electron microscopy, cells were gently resuspended in one drop of deionized H₂O, and samples were placed on carbon- and Formvar-coated nickel grids for 30 s. Grids were floated on one drop of 0.1 % (w/v) aqueous uranyl acetate, blotted dry and then viewed with a Carl Zeiss LEO912AB electron microscope at 100 kV under standard operating conditions. The Gram reaction was performed by

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strain MJ32^T and the type strains of recognized *Gordonia* species. Bootstrap values of >50 % (percentages of 1000 replications) are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.02 substitutions per nucleotide position. *Williamsia marianensis* MT8^T was used as an outgroup.

using the non-staining method, as described by Buck (1982). Catalase activity was assessed by bubble production in 3% (v/v) H₂O₂ and oxidase activity was assessed by using 1% (w/v) tetramethyl *p*-phenylenediamine (Cappuccino & Sherman, 2002). Anaerobic growth was examined in serum bottles with sodium thioglycolate (1 g l⁻¹) added to nutrient broth and the upper air layer was replaced with nitrogen. Physiological characteristics were determined with API 20NE, API ID 32 GN and API ZYM galleries (bioMérieux) according to the manufacturer's instructions. Growth at 4, 10, 20, 25, 28, 30, 37, 40, 42 and 45 °C and at pH 5.0–10.0 (0.5 pH unit intervals) was assessed in LB broth for 5 days. The pH of the medium was maintained via three buffers (final concentration 50 mM): acetate buffer (for pH 5.0–5.5), phosphate buffer (for pH 6.0–8.0) and Tris buffer (for pH 8.5–10.0). Salt tolerance was tested in R2A broth supplemented with 0–10% (w/v) NaCl incubated for 5 days at 30 °C. Degradation of DNA (DNase agar; Scharlau), casein, chitin, starch (Atlas, 1993), xylan and CM-cellulose (Ten *et al.*, 2004) was also investigated; reactions were read after 5 days. Growth on trypticase soy agar (TSA; Difco), R2A agar and LB agar was also evaluated at 30 °C. The phenotypic characteristics of strain MJ32^T are given in the species description and those that differentiated strain MJ32^T from the type strains of closely related *Gordonia* species are listed in Table 1. Strain MJ32^T could be differentiated from *G. hirsuta* KCTC 9816^T, *G. hydrophobica* KCTC 9814^T and *Gordonia humi* DSM 45298^T based on the following characteristics: pale yellow colonies, positive for oxidase, esterase and β -galactosidase (PNPG), and assimilation of 2-ketogluconate (α), L-fucose, maltose and melibiose; colonies of *G. hirsuta* KCTC 9816^T are light yellow and negative for oxidase.

For quinone, mycolic acid, polar lipid and peptidoglycan analysis, cells were grown aerobically on LB medium at 30 °C. Isoprenoid quinones were extracted from bacterial cells of strain MJ32^T with chloroform/methanol (2:1, v/v), evaporated under vacuum and re-extracted in *n*-hexane/water (1:1, v/v). The crude *n*-hexane quinone solution was then purified by using silica Sep-Pak Vac cartridges (Waters) and subsequently analysed by HPLC, as described by Hiraishi *et al.* (1996). Strain MJ32^T contained menaquinone MK-9(H₂) as the major respiratory quinone and minor amounts of MK-8(H₂). The peptidoglycan was analysed as described by Schleifer & Kandler (1972) by using TLC on cellulose sheets instead of paper chromatography. The murein acyl type was determined according to the colorimetric method of Uchida *et al.* (1999). Strain MJ32^T contained *meso*-2,6-diaminopimelic acid as the diamino acid. Whole-cell sugars contained arabinose and glucose, and sugars in the peptidoglycan were glycolated as is common for *Gordonia* species. Mycolic acids were extracted according to Minnikin *et al.* (1975), spotted on silica gel TLC plates, developed in petroleum ether (boiling point 60–80 °C)/acetone (95:5, v/v) and detected by charring at 140 °C after spraying with ethanolic molybdophosphoric

acid (5%, w/v). The mycolic acids in strain MJ32^T were identical to those in the reference strains of *G. humi* and *G. terrae*, which had also been extracted and analysed along with those of strain MJ32^T. Based on TLC (Fig. S2), strain MJ32^T possessed mycolic acids comprising C₅₆–C₆₀ carbon atoms, as with the reference strains (Kämpfer *et al.*, 2010).

For analysis of fatty acid methyl esters, strain MJ32^T and the type strains of closely related species were grown on TSA and two loopfuls of bacterial cells were harvested from the third quadrant streak (according to recommendations for the MIDI Sherlock system) and subjected to saponification, methylation and extraction according to Kuykendall *et al.* (1988). The fatty acid methyl ester mixtures were separated by using the Sherlock Microbial Identification System (TSBA6; Sherlock Version 6.0; MIDI), analysed by GC (Hewlett Packard 6890) and identified according to the Microbial Identification software package (Sasser, 1990). The overall fatty acid composition of strain MJ32^T (Table 2) was very similar to those of recognized *Gordonia* species, and supported the affiliation of strain MJ32^T to the genus *Gordonia*. The major components were C_{16:0} (34.6%), tuberculostearic acid (21.8%), C_{16:1 ω 7c} (19.5%) and C_{18:1 ω 9c} (12.7%). Minor amounts of C_{14:0} (3.4%), C_{15:0} (2.5%), C_{17:1 ω 9c} (1.7%), C_{16:1 ω 9c} (1.6%) and C_{18:0} (1.2%) were also present. Strain MJ32^T contained a greater amount of C_{16:1 ω 7c} than the type strain of *G. hirsuta* and this component was absent in *G. hydrophobica* and *G. humi*. To determine the polar lipid profile of strain MJ31^T, total cellular polar lipids were extracted and examined by two-dimensional TLC (Minnikin *et al.*, 1977).

Total lipids were observed after staining with 5% ethanolic molybdophosphoric acid and spots were detected by using different reagents (Fig. S3). The results showed that strain MJ32^T contained a major amount of diphosphatidylglycerol, moderate amounts of phosphatidylethanolamine and phosphatidylglycerol, and minor amounts of phosphatidylinositol and eight unknown lipids. The polar lipid profile of strain MJ32^T was similar to those of recognized *Gordonia* species (Stackebrandt *et al.*, 1988; Kämpfer *et al.*, 2010).

For determination of the DNA G+C content, genomic DNA was extracted as described above and enzymically degraded into nucleosides. The nucleosides were analysed by HPLC as described by Tamaoka & Komagata (1984) and Mesbah *et al.* (1989). The DNA G+C content of strain MJ32^T was 69.2 mol%.

DNA–DNA hybridization was performed by using DNA reassociation kinetics as described by De Ley *et al.* (1970). Purified, sonicated genomic DNA (65 mg) from each strain was used according to the method of Gonzalez & Saiz-Jimenez (2005). DNA–DNA hybridization between strain MJ32^T and the type strains of closely related *Gordonia* species (selected on the basis of 16S rRNA gene sequence similarity) was performed. Mean ΔT_m and

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
D-Glucose	+	-	+	w	w	+	w	+	w	+	w	+	w	+	+	+	+
Maltose	+	-	-	+	w	w	-	-	w	+	w	+	-	+	-	+	+
D-Mannose	+	-	+	w	w	-	-	-	-	+	w	+	+	+	+	w	+
Melibiose	+	-	-	-	w	-	-	-	-	+	w	+	+	+	-	w	-
D-Ribose	+	+	-	-	+	+	-	+	+	+	+	-	+	w	-	+	w
Sucrose	+	+	-	-	+	+	-	+	+	+	+	-	+	w	-	+	w
myo-Inositol	+	+	-	-	+	+	-	+	+	+	+	-	+	w	-	+	w
D-Mannitol	+	+	-	-	+	+	-	+	+	+	+	-	+	w	-	+	w
D-Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N-Acetyl-D-glucosamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycogen	w	w	+	-	w	+	w	+	w	+	+	+	+	w	+	w	w

DNA–DNA relatedness values between strain MJ32^T and *G. hirsuta* KCTC 9816^T were 8.1 °C and 47 % ($n=4$) and between strain MJ32^T and *G. hydrophobica* KCTC 9814^T were 10.8 °C and 36 % ($n=4$). Levels of DNA–DNA relatedness between strain MJ32^T and the type strains of other closely related *Gordonia* species are given in Table S1. At the time of writing, ΔT_m values of 5 °C and DNA–DNA relatedness values of less than 70 %, considered with phenotypic and chemotaxonomic differences, are acknowledged standards for the differentiation of bacterial species (Wayne *et al.*, 1987; Stackebrandt *et al.*, 2002).

The phenotypic and chemotaxonomic characteristics of strain MJ32^T were compared with those of closely related *Gordonia* species and the results are sufficient to differentiate it from its closest phylogenetic relatives. The results of the present polyphasic study indicate that strain MJ32^T represents a novel species of the genus *Gordonia*, for which the name *Gordonia caeni* sp. nov. is proposed.

Description of *Gordonia caeni* sp. nov.

Gordonia caeni (cae'ni. L. gen. n. *caeni* of sludge).

Cells are Gram-stain-positive, strictly aerobic, non-motile, non-spore-forming short rods, 0.2–0.3 µm in diameter and 0.3–1.0 µm in length (Fig. S4). After 3 days of incubation at 30 °C on R2A agar, colonies are 0.5–0.7 mm in diameter, pale yellow, smooth, convex and circular with regular edges. Growth occurs at 20–42 °C (optimum, 30 °C; weak growth at 15 °C). The pH range for growth is pH 6.0–9.5 (optimum, pH 7.0–7.5). Growth occurs with 0–4 % (w/v) NaCl, but not with 11 %. Growth occurs on trypticase soy, LB, nutrient and R2A agars. Catalase- and oxidase-positive. Nitrate is reduced to nitrogen gas. Hydrolyses starch and casein but not chitin, CM-cellulose, DNA or xylan. In API ZYM and API 20E strips, positive for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase, esterase lipase, lipase, leucine arylamidase, α -glucosidase and β -glucosidase activities; weak gelatinase and trypsin activities are also observed. Negative for arginine dihydrolase, *N*-acetyl- β -glucosaminidase, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, valine arylamidase, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -mannosidase and urease activities. In API ID32GN strips, assimilates *N*-acetylglucosamine, L-arabinose, L-fucose, gluconate, D-glucose, L-malate, maltose, D-mannitol, melibiose, *myo*-inositol, potassium 2-ketogluconate, D-ribose, salicin, D-sorbitol and sucrose, and weakly assimilates adipate, L-alanine, citrate, glycogen, L-rhamnose and sodium acetate. Caprate, L-histidine, 3-hydroxybenzoic acid, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, itaconate, DL-lactate, D-mannose, malonate, potassium 5-ketogluconate, phenylacetate, L-proline, propionate, n-valeric acid, L-serine, suberate and sucrose are not assimilated. The predominant respiratory quinone is MK-9(H₂). The polar lipid profile comprises diphosphatidylglycerol as the major component, with moderate amounts of phosphatidylethanolamine and phosphatidylglycerol. Mycolic acids are present. The

Table 2. Fatty acid composition (%) of strain MJ32^T and the type strains of closely related *Gordonia* species

Strains: 1, MJ32^T; 2, *G. hirsuta* KCTC 9816^T; 3, *G. hydrophobica* KCTC 9814^T; 4, *G. humi* DSM 45298^T; 5, *G. alkanivorans* KCTC 9951^T; 6, *G. amarae* KCTC 9803^T; 7, *G. amicalis* KCTC 9904^T; 8, *G. bronchialis* KCTC 3076^T; 9, *G. cholesterolivorans* DSM 45229^T; 10, *G. desulfuricans* KCTC 9941^T; 11, *G. effusa* DSM 44810^T; 12, *G. malaquae* DSM 45064^T; 13, *G. rubripertincta* KCTC 9802^T; 14, *G. shandongensis* DSM 45094^T; 15, *G. sihwensis* DSM 44576^T; 16, *G. terrae* KCTC 9807^T; 17, *G. westfalica* KCTC 9954^T. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. The *cis* isomers are indicated by the suffix *c*. Fatty acids amounting to <1% of the total fatty acids are not shown. Tr, trace (<1%); ND, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
C _{14:0}	3.4	1.9	1.2	2.1	2.8	1.2	2.1	1.5	1.8	2.1	1.6	1.6	2.1	1.6	1.1	2.1	2.3
C _{15:0}	2.5	1.1	5.1	2.1	2.8	1.3	3.1	2.4	Tr	ND	1.2	1.2	2.1	1.4	1.2	1.6	1.2
C _{15:1}	ND	ND	1.6	ND	ND	ND	ND	1.5	ND	ND	ND	ND	ND	ND	1.6	ND	ND
C _{16:1ω7c}	19.5	1.2	ND	ND	ND	3.5	1.5	12.6	12.5	1.5	1.2	5.3	ND	3.2	1.2	1.3	1.2
C _{16:1ω9c}	1.6	21.3	14.6	15.9	19.8	14.3	19.1	14.6	3.6	16.2	21.5	6.1	14.6	15.3	21.3	16.5	18.3
C _{16:0}	34.6	28.3	31.2	35.1	31.8	36.5	32.1	36.1	41.1	25.6	20.3	35.5	26.1	21.6	32.5	28.3	28.3
C _{17:1ω9c}	1.7	1.2	3.2	1.5	3.1	3.1	6.8	3.6	ND	ND	1.1	ND	5.3	1.1	2.6	2.1	ND
C _{17:0}	ND	1.3	3.5	ND	1.5	1.5	4.2	1.6	1.3	1.3	1.6	3.5	4.1	6.1	1.6	2.3	3.1
10-methyl C _{17:0}	ND	ND	5.1	2.3	ND	1.2	1.8	ND	ND	ND	1.3	ND	2.1	1.1	ND	ND	ND
C _{18:1ω9c}	12.7	26.4	12.3	19.6	24.6	20.1	20.6	21.1	23.6	18.6	25.6	12.3	16.1	26.1	6.2	21.6	15.3
C _{18:0}	1.2	1.3	1.6	1.6	1.2	ND	1.6	1.6	2.1	2.1	3.1	17.6	1.1	1.0	3.5	6.1	1.5
TBSA*	21.8	15.6	20.1	18.7	12.2	16.1	6.5	1.7	13.4	32.5	21.2	16.5	26.1	20.3	26.9	17.2	28.1

*TBSA, tuberculostearic acid.

peptidoglycan type is based on *meso*-2,6-diaminopimelic acid as the diagnostic diamino acid. Glycolated cell-wall sugars such as arabinose and glucose are present in the cell-wall peptidoglycan. The major fatty acids (>12%) are C_{16:0}, tuberculostearic acid, C_{16:1 ω 7c} and C_{18:1 ω 9c}; minor amounts of C_{14:0}, C_{15:0}, C_{17:1 ω 9c}, C_{16:1 ω 9c} and C_{18:0} are also present.

The type strain, MJ32^T (=KCTC 19771^T=JCM 16923^T), was isolated from sludge at the Daejeon sewage disposal plant in South Korea. The DNA G+C content of the type strain is 69.2 mol% (HPLC).

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