Gordona hydrophobica sp. nov., Isolated from Biofilters for Waste Gas Treatment

BERND BENDINGER,^{1*} FREDERICK A. RAINEY,² REINER M. KROPPESTEDT,²
MICHAEL MOORMANN,³ AND STEFAN KLATTE²

EWE Aktiengesellschaft, Abteilung Abfallwirtschaft, Labor für Umweltanalytik, D-49661 Cloppenburg,¹
DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-38124 Braunschweig,²
and Abteilung Mikrobiologie, Universität Osnabrück, D-49069 Osnabrück,³ Germany

The taxonomic position of *Gordona* sp. strain DSM 44015^T, isolated from the packing material of a biofilter used for biological odor abatement of animal rendering emissions, has been clarified by a polyphasic study comprising chemotaxonomic, sequencing, and phenotypic results. The strain possesses a wall chemotype IV, MK-9 (H₂), as the predominant menaquinone; relatively long-chain mycolic acids (54 to 62 carbon atoms); and straight-chain, saturated, and monounsaturated fatty acids with considerable amounts of tuberculostearic acid. The polar lipids include phosphatidylethanolamine, and the G+C content of the DNA is 69 mol%. Similarity values for genes encoding 16S rRNA indicate that *Gordona* sp. strain DSM 44015^T represents a new species within the genus *Gordona* for which the name *Gordona hydrophobica* is proposed.

Actinomycetes containing mycolic acids have many common cell wall characteristics and form a suprageneric group (24, 32–34) comprising the genera *Corynebacterium*, *Rhodococcus*, *Gordona*, *Nocardia*, *Mycobacterium*, *Tsukamurella*, and *Dietzia* (28). Numerical taxonomic, chemical, and molecular systematic methods (8, 9) have been used to differentiate among the taxa.

Up to date, six species of the genus *Gordona* (33, 35) are known: *Gordona bronchialis*, *Gordona rubropertincta*, *Gordona terrae* (35), *Gordona sputi* (36, 38), *Gordona aichiensis* (18, 37), and *Gordona amarae* (18, 21). The taxonomic history of the genus *Gordona* is confusing because of renamings of original isolates (36) and recent transfers of strains from the genus *Rhodococcus* or *Nocardia* to the genus *Gordona* (18). Polyphasic taxonomic studies, including chemotaxonomic, phylogenetic, and phenotypic properties, clearly separated the species of the genus *Gordona* from those of other actinomycete genera (18).

Until now, *Gordona* species have been regarded primarily as opportunistic pathogens isolated from sputum of humans (*G. bronchialis*, *G. aichiensis*, and *G. sputi*) suffering from pulmonary lesions (35–37). Compared with the number of isolates from sputum, few *Gordona* strains have been isolated from terrestrial or aquatic habitats where mycolic acid-containing actinomycetes are generally known to be widely distributed (9). Only two *Gordona* species, *G. terrae* and *G. rubropertincta*, derive from soil (35, 36). Another strain that has recently been affiliated with the taxon *Gordona*, *G. amarae* (18), had been isolated from foam formed on the surface of aeration tanks in activated sludge-sewage-treatment plants (21). *Gordona* strains have also been isolated from the packing material (tree bark compost) of biofilters used for the treatment of malodorous animal rendering emissions (1, 2). These strains were able to use readily volatile, poorly water-soluble carbonyl compounds such as aldehydes and ketones contained in the waste gases, thus suggesting a contribution of these bacteria to the deodorization of the off-gases (1). Furthermore, these strains were found to possess extremely hydrophobic cell surfaces,

which has been related to the presence of unshielded long-chain mycolic acids (3). The aim of this study was to clarify the taxonomic position of one of these strains, *Gordona* sp. strain DSM 44015^T, on the basis of further chemotaxonomic, physiological, and genetic characteristics.

MATERIALS AND METHODS

Bacterial strains and cultivation. *Gordona hydrophobica* (DSM 44015^T) was deposited at the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen. For analysis of cellular fatty acid composition, *G. hydrophobica* was grown on TSB agar (3% [wt/vol] Trypticase soy broth [BBL], 1.5% [wt/vol] Bacto Agar [Difco]) for 4 days at 28°C. For biochemical examination, *G. hydrophobica* was harvested from GYM agar (0.4% [wt/vol] D-glucose, 0.4% [wt/vol] yeast extract, 1% [wt/vol] malt extract, 1.2% [wt/vol] agar no. 1 [OXOID]) after 3 days at 28°C. For determination of the G+C content of DNA and for cell wall analysis, cell material was produced in Trypticase soy broth (BBL) for 4 days at 28°C on a rotary shaker, harvested by centrifugation, and washed twice with distilled water.

Cell wall chemistry. Isoprenoid quinones, polar lipids, cell wall diamino acids, and sugars were examined according to published methods (19). The acyl type of peptidoglycan was identified according to the method of Uchida and Aida (39) modified by passing the hydrolysate through an ion exchanger (Bond Elut column SCX, catalog no. 617303; Analytichem). Fatty acid methyl esters were prepared from wet cells (40 to 70 mg) as described previously (31) and identified by gas chromatography with a model 5898A Microbial Identification System (Microbial ID, Newark, Del.). Chain lengths of trimethylsilylated derivatives of mycolic acids (28) were determined by high-temperature gas chromatography on an HP 5790A gas chromatograph (Hewlett-Packard) equipped with a flame ionization detector on a 12-m HT5 column (part no. 051385; SGE, Ringwood, Victoria, Australia) with H₂ as carrier gas (30 ml/min). The oven temperature was ramped from 210 to 400°C at 10°C/min, holding the final temperature for 7 min. Peaks of the derivatives were identified by comparing their retention times with those of standards. Pyrolysis of mycolic acid methyl esters of *G. hydrophobica* obtained by the method of Minnikin et al. (23) and analysis of cleavage products were performed with a model 5898A Microbial Identification System (Microbial ID) under standard conditions.

Physiological tests. Qualitative enzyme tests were performed in standard microtitration plates (F-form; Greiner, Nürtingen, Germany) according to the method of Kämpfer et al. (17). The following chromogenic substrates were added to a medium (pH 7.2) consisting of 0.05 M Tris-HCl buffer, 0.05% (wt/vol) Casamino Acids (Difco), and 0.05% (wt/vol) yeast extract (Difco) at a final concentration of 2 mM: *p*-nitrophenyl (pNP)-β-D-xyloside, pNP-phosphorylcholine, and 2-deoxythymidine-5'-pNP-phosphate. The test reaction mixtures were inoculated with a standardized bacterial suspension of 0.5 to 1.0 McFarland units in 0.9% (wt/vol) NaCl and incubated for 48 h at 28°C. Growth on D-galactose, meso-inositol, L-rhamnose (all 1.0% [wt/vol]), and citric acid (Na salt) (0.1% [wt/vol]) as carbon sources was examined in yeast-nitrogen broth (Difco) (pH 7.2) at 28°C for 4 days. The results of the enzyme tests and carbon source utilization were evaluated visually against the results of duplicate controls.

Determination of G+C content of DNA. The DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion et al. (5). The G+C

* Corresponding author. Mailing address: EWE Aktiengesellschaft, Labor für Umweltanalytik, Bürgerparkstraße, D-49661 Cloppenburg, Germany. Phone: 49-4471-13381. Fax: 49-4471-13389.

TABLE 1. Chemosystematic characteristics of *Gordona hydrophobica* and related wall chemotype IV taxa containing mycolic acids^a

Taxon	Fatty acid composition ^b	Mycolate size (no. of carbon atoms)	Presence of phosphatidylethanolamine ^c	Major menaquinone(s)	G+C content of DNA (mol%)
<i>Gordona hydrophobica</i>	S, U, T	54–62	+	MK-9 (H ₂)	69
<i>Gordona</i> species	S, U, T	48–66	+	MK-9 (H ₂)	63–69
<i>Nocardia</i> species	S, U, T	44–60	+	MK-8 (H ₄ , ω-cycl)	64–72
<i>Rhodococcus</i> species	S, U, T	34–52	+	MK-8 (H ₂)	63–73
<i>Tsukamurella</i> species	S, U, T	64–68	+	MK-9	67–68
<i>Mycobacterium</i> species	S, U, T	60–90	+	MK-9 (H ₂)	62–70
<i>Corynebacterium</i> species	S, U ^d	22–36	✓ ^e	MK-8 (H ₂), MK-9 (H ₂)	51–67
<i>Dietzia</i> species	S, U, T	34–38	+	MK-8 (H ₂)	73

^a Adapted from references 8, 9, 18, 28, and 33.^b S, saturated; U, monounsaturated; T, tuberculostearic acid.^c +, present; v, variable.^d Tuberculostearic acid also present in *Corynebacterium ammoniagenes*, *Corynebacterium bovis*, *Corynebacterium cystitidis*, *Corynebacterium minutissimum*, *Corynebacterium pilosum*, *Corynebacterium urealyticum*, and *Corynebacterium variabilis* (15, 26).^e Phosphatidylethanolamine also present in *C. ammoniagenes*, *C. bovis*, *C. cystitidis*, *Corynebacterium pseudodiphtheriticum*, and *C. urealyticum* (15, 26).

content of DNA was determined by high-performance liquid chromatography (HPLC) according to the method of Mesbah et al. (22).

16S rDNA sequencing. Genomic DNA isolation and PCR-mediated amplification of the genes encoding 16S rRNA (16S rDNA) were performed as described by Rainey et al. (27). Double-stranded PCR products were sequenced with the Taq Dye-Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif.) according to the protocol of the manufacturer. Sequence reactions were electrophoresed with the Applied Biosystems 373A DNA Sequencer. The 16S rDNA sequences were aligned against the sequences currently available from the public databases. Evolutionary distances were calculated according to the method of Jukes and Cantor (16). The phylogenetic dendrogram was constructed from the distance matrices by the algorithm of De Soete (6).

Nucleotide sequence accession number. The EMBL accession number for the 16S rDNA sequence of *G. hydrophobica* DSM 44015^T is X87340.

RESULTS

Cell wall analysis. The results of this study and previous chemotaxonomic analyses of *G. hydrophobica* are shown in Tables 1 and 2 along with the data for other related taxa (2). The cell wall of *G. hydrophobica* contained arabinose and ga-

lactose as major cell wall sugars, *meso*-diaminopimelic acid as the only diamino acid (variation A1γ sensu Schleifer and Kandler [30]), and N-glycolated muramic acid moieties of peptidoglycan, thus possessing the characteristic wall chemotype IV sensu Lechevalier and Lechevalier (20) of genera containing mycolic acid. The polar lipids were composed of diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, and phosphatidylethanolamine. MK-9 (H₂) was found to be the predominant menaquinone with minor amounts of MK-8 (H₂) (less than 5%).

Cellular fatty acids. Analysis of simple fatty acids from whole cells exhibited a pattern of straight-chain, saturated, and monounsaturated fatty acids with significant amounts of tuberculostearic acid (10-methyl octadecanoic acid) (1% 14:0, 2% 15:1 *cis* 10, 4% 15:0, 13% 16:1 *cis* 10, 27% 16:0, 4% 17:1 *cis* 9, 4% 17:0, 2% 10-Me-17:0, 14% 18:1 *cis* 9, 1% 18:0, 26% 10-Me-18:0). The mycolic acids of *G. hydrophobica*, as determined from trimethylsilylated derivatives by high-temperature gas

TABLE 2. Diagnostic characteristics of the *Gordona* species^a

Characteristic	<i>G. hydrophobica</i>	<i>G. aichiensis</i>	<i>G. amarae</i>	<i>G. bronchialis</i>	<i>G. rubropertincta</i>	<i>G. sputi</i>	<i>G. terrae</i>
Colony color white to tan ^b	+	–	+	–	–	–	–
Cleavage of the following pNP substrates (2 mM) ^c							
pNP-β-D-xyloside	–	–	+	–	–	–	+
pNP-phosphorylcholine	+	–	–	–	–	–	–
2-Deoxythymidine-5'-pNP-phosphate	+	–	–	–	–	–	–
Growth on sole carbon source ^{d, e}							
D-Galactose (1.0% [wt/vol])	+	+	–	–	+	+	+
<i>meso</i> -Inositol (1.0% [wt/vol])	–	–	+	+	–	–	–
Sorbitol (0.5% [wt/vol]) ^f	ND	–	–	–	+	+	+
L-Rhamnose (1.0% [wt/vol])	–	–	+	–	–	–	+
Citric acid (Na salt) (0.1% [wt/vol])	+	+	–	–	+	–	+
Mycolic acids ^g							
Avg carbon no.	58–60	62	50–52	64	58–59	61–62	58
FAME released on pyrolysis of MAME ^h	16:0, 17:0, (18:1), 18:0	16:0	16:1, 18:1	18:0	16:0, 18:0	16:0, 18:0	16:0, 18:0
16:1 fatty acid present ^g	+ ⁱ	+ ⁱ	+	+	+	– ⁱ	+

^a Symbols: +, ≥90% of the strains are positive; –, ≥90% of the strains are negative; ND, not determined.^b Data from references 10 and 21 and this study.^c Data for *G. amarae* are from reference 17.^d Data from references 10, 11, 12, and 21, and this study.^e The concentration used to determine carbon source utilization for *G. amarae* was 0.1% (wt/vol) except for growth on citric acid, which was tested at 0.01% (wt/vol) (10).^f Data from reference 37.^g Data from references 10 and 43 and this study.^h FAME, fatty acid methyl esters; MAME, mycolic acid methyl esters.ⁱ Data from *G. hydrophobica* DSM 44015^T, *G. aichiensis* DSM 43978^T, and *G. sputi* DSM 43896^T.

FIG. 1. Phylogenetic dendrogram indicating the relationship of *G. hydrophobica* to all species of the genus *Gordona* and related taxa. Bar = 5 substitutions per 100 nucleotides, as determined by measuring the length of the horizontal lines connecting two species. A bootstrap analysis yielded a 100% confidence level for the position of the branch of the *Gordona* group.

chromatography, were similar in size as described for members of the genus *Gordona* (8, 43) (Table 2), having an overall range of 54 to 62 carbon atoms (12% C₅₆, 11% C₅₇, 30% C₅₈, 16% C₅₉, 26% C₆₀, and 5% C₆₁ and traces of C₅₄, C₅₅, and C₆₂) with a mean chain length of between 58 and 60 carbon atoms. The composition of fatty acid methyl esters released by pyrolytic cleavage of methyl mycolates was mainly 18:0 (58%) and 16:0 (26%), with additional amounts of 17:0 (12%) and 18:1 *cis* 9 (4%).

Physiological test. The results of physiological testing of *G. hydrophobica* are included in Table 2. *G. hydrophobica* could enzymatically hydrolyze the chromogenic substrates pNP-

phosphorylcholine and 2-deoxythymidine-5'-pNP-phosphate, respectively, while pNP-β-D-xyloside remained uncleaved. D-Galactose and citric acid (Na salt) were utilized by *G. hydrophobica* as sole carbon sources, while *meso*-inositol and L-rhamnose were not.

Analysis of 16S rDNA. Almost the complete (>1,500 nucleotides) 16S rDNA sequence was determined for *G. hydrophobica* and compared with those of reference strains of mycolic acid-containing actinomycetes. The phylogenetic dendrogram in Fig. 1 shows the relationship of *G. hydrophobica* DSM 44015^T to all species of the genus *Gordona* and representatives of related genera for which sequence data are available. *G. hydrophobica* DSM 44015^T is part of a cluster composed of all valid species composing the genus *Gordona*, thus being clearly separated from other mycolic acid-containing genera on the basis of 16S rDNA data. Furthermore, the values for *G. hydrophobica* DSM 44015^T of similarity to members of the genus *Gordona* (Table 3) were in the range of 96.1 to 97.9% while the similarity to other genera was less than 94.1%. *G. hydrophobica* DSM 44015^T showed the highest level of similarity (97.9%) to *G. rubropertincta* DSM 43197^T.

Determination of G+C content of DNA. The G+C content of DNA of *G. hydrophobica*, as determined by HPLC, was 69 mol%.

DISCUSSION

During the last several years, a significant number of mycolic acid-containing actinomycetes have been isolated from the environment, biotechnological plants used for soil or groundwater remediation (4, 40, 41), and waste gas treatment plants (7, 25, 42) and have been shown to degrade recalcitrant or readily volatile pollutants (13, 14, 29). Such isolates need to be identified reliably. Chemotaxonomy is a powerful tool for differentiation among mycolic acid-containing bacteria and should be applied for their preliminary classification (2).

Strains of the genus *Gordona* were isolated from the packing material of biofilters used for biological odor abatement of malodorous animal rendering emissions (1, 2). Further taxonomic studies performed for strain DSM 44015^T clarified its position within the genus *Gordona*. The sequence data clearly showed that *G. hydrophobica* DSM 44015^T belongs to the genus *Gordona* and, moreover, provided evidence for the exclusion of this strain from other known species of the genus. This

TABLE 3. 16S rDNA similarity values for organisms used in this study

Control no.	Sp.	% 16S rDNA similarity with control no.:															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	<i>G. hydrophobica</i>																
2	<i>G. amarae</i>	97.4															
3	<i>G. terrae</i>	97.4	97.2														
4	<i>G. rubropertincta</i>	97.9	97.0	98.4													
5	<i>G. bronchialis</i>	97.1	97.1	98.3	98.5												
6	<i>G. aichiensis</i>	96.2	96.6	96.8	97.0	97.4											
7	<i>G. sputi</i>	96.1	96.4	96.7	96.7	97.3	99.7										
8	<i>Rhodococcus equi</i>	93.9	94.0	94.5	94.2	94.2	93.1	93.0									
9	<i>Rhodococcus fascians</i>	93.0	93.0	93.3	93.0	93.0	92.1	92.0	95.7								
10	<i>Rhodococcus erythropolis</i>	93.4	93.7	93.3	93.0	93.3	92.9	92.9	96.1	97.1							
11	<i>Nocardia asteroides</i>	93.2	93.8	93.0	93.1	93.3	92.6	92.6	95.9	95.0	95.7						
12	<i>Nocardia otitidiscaviarum</i>	94.1	94.3	94.1	94.6	94.7	93.3	93.3	97.2	95.0	96.0	97.8					
13	<i>Tsukamurella paurometabola</i>	93.4	94.5	93.3	93.2	93.3	93.4	93.4	94.7	94.0	94.6	94.3	94.4				
14	<i>Mycobacterium chlorophenolicum</i>	93.7	94.0	93.4	93.1	93.7	92.8	92.8	93.5	93.4	94.0	94.2	93.9	94.5			
15	<i>Mycobacterium terrae</i>	92.8	93.2	93.0	92.6	93.0	92.8	92.5	92.9	93.0	93.2	93.3	93.0	94.0	97.4		
16	<i>Mycobacterium tuberculosis</i>	91.8	91.8	92.3	91.6	92.4	91.2	91.0	92.0	92.0	92.0	92.5	91.7	92.1	96.4	96.7	

assignment was supported by the chemotaxonomic data obtained for the strain. Among the *Gordona* species, only *G. hydrophobica* DSM 44015^T released the 17:0 fatty acid by pyrolytic cleavage of mycolic acids. In addition, phenotypic properties differentiated *G. hydrophobica* DSM 44015^T from the other described *Gordona* species. The new strain was the only one that hydrolyzed the chromogenic substrates pNP-phosphorylcholine and 2-deoxythymidine-5'-pNP-phosphate. On the basis of the data presented here, it is proposed that *G. hydrophobica* DSM 44015^T be assigned as the type strain of a new *Gordona* species, *G. hydrophobica*.

Description of *Gordona hydrophobica* sp. nov. *Gordona hydrophobica* (hy.dro.pho'bi.ca. Gr. n. *hydro*, water; Gr. adj. *phobos*, fear, dread; M.L. adj. *hydrophobica*, water avoiding, water repellent, related to hydrophobic). The species description below is based on our own data and additional information obtained from references 1, 2, and 3.

Gram-positive, non-acid-fast, regular, long rods (2 to 3 μ m) that are nonmotile, grow amycelially, and undergo a rod-coccus cycle. Light microscopy of colony or liquid culture growth shows cells mostly in aggregates. Growth in liquid culture is clear with a pellicle on the surface that rises up at the glass wall of a culture tube.

Tannish to white colonies are formed on various media. The colony morphology of the type strain is rough and flat with irregular margins. Upon primary isolation, colonies may also be convex with entire or undulate margins and a smooth surface. Subcultivation of a smooth colony on complex medium agar plates yields smooth as well as rough colonies, whereas a rough variant yields only rough colonies. Rough variants of *G. hydrophobica* have an extremely high cell surface hydrophobicity which is reduced significantly in variants forming smooth colonies.

Cleaves the pNP substrates pNP-phosphorylcholine and 2-deoxythymidine-5'-pNP-phosphate but not pNP- β -D-xyloside. Uses D-galactose, citric acid (Na salt), succinic acid (Na salt), propionic acid, and leucine as sole carbon source but does not use *meso*-inositol, L-rhamnose, gluconic acid, adipic acid, 5-aminovaleric acid, aspartic acid, asparagine, ribose, and arabinose. Does not decompose tyrosine, adenine, and xanthine and does not produce acid from sucrose aerobically or anaerobically.

Growth occurs on the readily volatile carbonyl compounds methylpropanal, 3-methylbutanal, 2-methylbutanal, butanone, 2-pentanone, and 4-methyl-2-pentanone when added as sole carbon sources into sealed agar slope cultures.

The cell wall contains *meso*-diaminopimelic acid as the only diamino acid, arabinose and galactose as major cell wall sugars, and *N*-glycolyl muramic acid moieties. The predominant isoprenolog is MK-9 (H₂); minor amounts of MK-8 (H₂) are also present. The polar lipids include diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. Contains major amounts of straight-chain, saturated, and monounsaturated fatty acids and tuberculostearic acid. Mycolic acids with 54 to 62 carbon atoms are present. Upon pyrolysis gas chromatography of mycolates, the fatty acids 16:0, 17:0, 18:0, and 18:1 are released.

The G+C content of the DNA is 69 mol%.

Isolated from packing material (tree bark compost) of biofilters for deodorization of animal rendering emissions.

The type strain is 1610/1b (=DSM 44015^T).

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