**Gordonia phthalatica** sp. nov., a di-n-butyl phthalate-degrading bacterium isolated from activated sludge

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**Abstract**

A phthalate esters-degrading bacterial strain, designated QH-11T, was isolated from an activated sludge wastewater treatment plant in Beijing, PR China. The cells were aerobic, Gram-stain-positive, non-motile, catalase-positive, oxidase-negative, short rods and formed white colonies on trypticase soy agar. This isolate contained meso-diaminopimelic acid as the diagnostic diamino acid and whole-cell hydroxysates contained arabinose and ribose. Diphosphatidylglycerol and phosphatidylethanolamine were the predominant polar lipids. According to the results of full-length of 16S rRNA gene sequence analysis, QH-11T represented a member of the genus *Gordonia* and showed the highest sequence similarity to *Gordonia hydrophobica* DSM 44015T (99.2 %), but was distinguishable by a low level of DNA–DNA relatedness (37.8 %). Genome-based comparisons indicated a clear distinction from the top ten most similar type strains (16S rRNA gene sequence) with pairwise average nucleotide identities (ANI) between 74.6 and 83.4 %. The predominant respiratory quinone was MK-9(H2), the mycolic acids present had 56 to 62 carbon atoms, and the major fatty acids were C16:0 (33.3 %), C17:1ω8c (23.4 %) and C18:1ω9c (17.9 %). The DNA G+C content was 68.0 mol%. On the basis of the results of DNA–DNA hybridization, ANI and physiological and biochemical tests, it is proposed that QH-11T represents a novel species of the genus *Gordonia*, for which the name *Gordonia phthalatica* sp. nov. is proposed. The type strain is QH-11T (CICC 24107T =KCTC 39933T).

The genus *Gordonia*, a member of the family Nocardiaceae, phylum Actinobacteria, was proposed by Tsukamura [1]. Numerous members of the genus *Gordonia* have been isolated from various environments, such as human clinical specimens, animal faeces, estuary sand, mangrove rhizosphere, soil and wastewater, and described [2], and many of these species were associated with degradation of environmental pollutants. According to the List of Prokaryotic names with Standing in Nomenclature, at the time of writing, there are 39 species with validly published names the genus *Gordonia* [3].

In the course of screening of dibutyl phthalate (DBP)-degrading strains from activated sludge of a municipal wastewater treatment plant in Beijing, PR China, a Gram-stain-positive, non-motile, aerobic, non-spore-forming strain, designated QH-11T, was isolated. The degradation of dibutyl phthalate by this isolate has been subsequently studied [4]. The isolate was routinely cultured on nutrient broth or nutrient agar (NA; Difco) at 30 °C and preserved in a glycerol suspension (20 %, v/v) at −70 °C. The results of 16S rRNA gene sequence analysis indicated that QH-11T was most closely related to the members of the genus *Gordonia*. The aim of this study was to describe the exact taxonomic position of QH-11T by using a polyphasic taxonomic approach. *Gordonia hydrophobica* DSM 44015T=JCM 10086T was used as the reference strain and cultured under the same conditions.

The sequences encoding 16S ribosomal RNA (locus tag: ACH46_000885; 1528 bp) and gyrB (locus tag: ACH46_00025; 2061 bp) were extracted from the genome of QH-11T. Phylogenetic trees were reconstructed using the neighbor-joining [5], minimum-evolution [6] and maximum-parsimony [7] methods in the MEGA 7.0 program [8]. The percentages of replicate trees in which the associated
taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [9]. The evolutionary distances were computed using the maximum composite likelihood method [10] and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. For the genome sequencing of QH-11T, genomic DNA of QH-11 was isolated from an overnight cell suspension culture using the Wizard Genomic DNA Purification Kit (Promega). The genome of QH-11 was sequenced using the 454 GS FLX+ (Roche) and MiSeq (Illumina) systems (Majorbio). Remaining gaps were filled by sequencing PCR products. The complete genome sequence data of QH-11 has been deposited in the DDBJ/EMBL/GenBank database under the accession number CP011853. The DNA–DNA relatedness between QH-11T and Gordonia hydrophobica DSM 44015T was determined by a modified renaturation method described previously [11, 12], using a hybridization temperature of 82°C. The pairwise average nucleotide identities between the genome of QH-11T and the genomes of the closely related type strains, identified from the phylogenetic analysis of the 16S rRNA sequences, were determined with the algorithm using EzGenome [13] as described by Goris et al. [14]. The draft genome sequence of Gordonia humi CC-12301T has also been sequenced recently by our group. The DNA G+C content of QH-11T was determined from the complete genomic DNA sequence.

Colonies and cell morphology were observed after growth on nutrient agar for 2 days at 30°C. Cell morphology was observed by transmission electron microscopy (H-7650; Hitachi). The Gram reaction, gliding motility and growth under anaerobic conditions for QH-11 were determined using the API 50CH, API Coryne and API ZYM systems (bioMérieux) (majorbio). Remaining gaps were filled by sequencing PCR products. The complete genome sequence data of QH-11 has been deposited in the DDBJ/EMBL/GenBank database under the accession number CP011853. The DNA–DNA relatedness between QH-11T and Gordonia hydrophobica DSM 44015T was determined by a modified renaturation method described previously [11, 12], using a hybridization temperature of 82°C. The pairwise average nucleotide identities between the genome of QH-11T and the genomes of the closely related type strains, identified from the phylogenetic analysis of the 16S rRNA sequences, were determined with the algorithm using EzGenome [13] as described by Goris et al. [14]. The draft genome sequence of Gordonia humi CC-12301T has also been sequenced recently by our group. The DNA G+C content of QH-11T was determined from the complete genomic DNA sequence. Colony and cell morphology were observed after growth on nutrient agar for 2 days at 30°C. Cell morphology was observed by transmission electron microscopy (H-7650; Hitachi). The Gram reaction, gliding motility and growth under anaerobic conditions for QH-11T were examined according to the methods described by Dong and Cai [15]. Motility was tested with the hanging-drop technique [16]. The temperature (4, 10, 15, 20, 25, 27, 30, 34, 37, 39 and 42°C) and pH ranges (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0, 10.0, 11.0 and 12.0) and salt tolerance [1.5, 2.5, 3.0, 3.5, 4.0, 5.0 and 6.0% (w/v) NaCl] for growth were determined by measuring the turbidity (OD600) of cultures grown in a 150 mL flask containing 100 mL nutrient broth and incubated at 30°C. Catalase activity was determined by assessing bubble production in 3% (v/v) H2O2, and oxidase activity was determined using 1% (v/v) tetramethyl-p-phenylenediamine. For DBP, mono-n-butyl phthalate (MBP) and phthalic acid (PA) utilization tests, QH-11 and Gordonia hydrophobica DSM 44015T were inoculated into liquid mineral salts medium containing 200 mg l−1 of each substrate, respectively. Substrate utilization was assessed by microbial growth by measuring the increase of the biomass (OD600) combined with visible turbidity after 72 h of incubation. Additional physiological properties and enzyme activities were determined using the API 50CII, API Coryne and API ZYM systems (bioMérieux) according to the manufacturers’ instructions.

Cells for fatty acid methyl ester analyses were harvested from nutrient agar plates incubated at 30°C for 3 days. Fatty acids were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.1). The fatty acids were analyzed by GC (model 7890; Hewlett Packard) and identified by using the RTSSBA6 database of the Microbial Identification System [17]. The isoprenoid quinones of QH-11T were extracted with chloroform/methanol (2:1), purified by TLC according to the method of Collins [18] and analyzed by HPLC using an XDB-C18 column (Agilent). Polar lipids of QH-11T and the reference strain were determined according to the method of Minnikin et al. [19]. Whole-cell hydrolysates were analyzed to determine their amino acid and sugar contents by methods described previously [20, 21]. Mycolic acids analysis was performed by the Leibniz-Institut Deutscher Sammlung von Mikroorganismen und Zellkulturen.

Table 1. Physiological and biochemical characteristics that differentiate QH-11T from Gordonia hydrophobica DSM 44015T.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strains: 1, QH-11T; 2, Gordonia hydrophobica DSM 44015T. Data are from this study unless otherwise indicated. All strains are positive for glycerol, α-glucose, α-fructose, aesculin, sucrose, alkaline phosphatase, cystine arylamidase, β-glucosidase, esterase (C4), esterase lipase (C8), leucine arylamidase, Naphthol-AS-BI-phosphohydrolase and α-glucosidase. All strains are negative for erythritol, α-xylene, rhamnose, 5-keto-gluconate, α-arabinose, α-arabinose, β-ribose, α-arabinose, β-xylose, adonitol, methyl-β-xylose, d-galactose, d-mannose, l-sorbose, dulcitol, inositol, manitol, sorbitol, methyl-α-β-mannoside, methyl-α-β-glucoside, N-acetyl glucosamine, amyligalacturonic acid, salicin, cellobiose, maltose, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, α-l-fucose, a-leucomelitose, L-arabitol, 5-ketogluconate, 2-ketogluconate, valine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, β-fucosidase, α-chymotrypsin, α-mannosidase, pyrazinamidase, urease and gelatin hydrolysis. All strains are Gram-stain-positive non-motive bacteria that do not produce acid from glucose, ribose, xylose, mannitol, malonic acid, lactose, succrose and glycogen. —, Negative; +, positive; W, weakly positive reaction.</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony pigmentation</td>
<td>Creamy-white</td>
<td>White</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>0–8%</td>
<td>0–6%</td>
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<tr>
<td>Nitrate reduction</td>
<td>−</td>
<td>+</td>
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<td>Phthalic acid</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Hydrolysis/assimilation of:</td>
<td></td>
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<tr>
<td>Glycerol</td>
<td>+</td>
<td>W</td>
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<td>α-fructose</td>
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<td>W</td>
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<td>Sucrose</td>
<td>W</td>
<td>+</td>
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<tr>
<td>Trehalose</td>
<td>−</td>
<td>W</td>
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<tr>
<td>Enzymatic activities</td>
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</tr>
<tr>
<td>Trypsin</td>
<td>W</td>
<td>+</td>
<td></td>
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<tr>
<td>β-Glucosidase</td>
<td>W</td>
<td>+</td>
<td></td>
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<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>W</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>+</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Pyridoxyl arylamidase</td>
<td>W</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>68</td>
<td>69*</td>
<td></td>
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</tbody>
</table>

Data taken from Bendinger et al. [24].
(DSMZ) Identification Service, Germany, according to their established protocols.

After two days growth on NA at 28 °C, QH-11T formed irregular, creamy-white colonies, which were thick and 2–3 mm in diameter. Cells were Gram-stain-positive, catalase-positive, oxidase-negative, non-motile, rod-shaped and non-spore-forming. Growth was not observed below 10 °C or above 42 °C, with an optimum at around 30 °C. The pH range for growth occurred at pH 5.0–10.0. Growth at more than 10% salinity was not observed. The substrate utilization tests indicated that both QH-11 and DSM 44015T could utilize DBP and also the main intermediate product MBP. Unlike QH-11, Gordonia hydrophobica DSM 44015T could not utilize the other main intermediate product PA. Other phenotypic characteristics are summarized in Table 1 and in the species description.

The G+C content of the DNA of QH-11T was 68 mol%, a value within the range for species of the genus Gordonia.

QH-11T showed the highest level of 16S rRNA gene sequence similarity to Gordonia hydrophobica DSM 44015T (99.2%), Gordonia humi CC-12301T (98.3%) Gordonia alkanivorans NBRC 16433T (98.1%) and Gordonia

![Fig. 1. Neighbor-joining phylogenetic tree derived from 16S rRNA (a) and gyrB (b) gene sequences, showing the relationships of QH-11T with members of the genus Gordonia and other closely related genera. All the branches were also recovered by the minimum-evolution and maximum-parsimony methods. Bar, 1(a) and 1(b) nucleotide substitutions per 100 nucleotides.](image-url)
The genome sequences deposited in NCBI. The results indicated that the neighbour joining algorithm revealed that QH-11 of DNA average nucleotide identities (ANI) were determined recommended 70% value used as the main criterion for distinctiveness of QH-11 and separated from other species of the genus *Gordonia*. The topologies of the minimum-evolution and maximum-parsimony trees were essentially the same. We also compared the *gyrB* gene sequence with all available *gyrB* gene sequences for members of the genus *Gordonia*. QH-11 exhibited the highest *gyrB* gene sequence similarity value (88.5%) to *Gordonia hydrophobica* DSM 44015\(^\text{T}\) (Fig. 1b). DNA–DNA hybridization experiments revealed low levels of DNA–DNA relatedness between QH-11 and *Gordonia hydrophobica* DSM 44015\(^\text{T}\) (37.8%), much lower than the recommended 70% value used as the main criterion for definition of a bacterial species [22], which confirmed that this novel strain deserves separate species status. In addition, average nucleotide identities (ANI) were determined between QH-11 and all the type strains with available genome sequences deposited in NCBI. The results indicated that the highest ANI values of QH-11 were 83.4% with *Gordonia hydrophobica* DSM 44015\(^\text{T}\). Meanwhile, the ANI for QH-11 to *Gordonia* neofilaecia NRRL B-59395\(^\text{T}\) was 80.6%, that to *Gordonia* sihwensis NBRC 108236\(^\text{T}\) was 80.3%, that to *Gordonia* shandongensis DSM 45094\(^\text{T}\) was 78%, that to *Gordonia* humi CC-12301\(^\text{T}\) was 76.8%, that to *Gordonia* desulfuricans NBRC 100010\(^\text{T}\) was 75%, that to *Gordonia* alkanivorans DSM 44455\(^\text{T}\) was 74.9%, that to *Gordonia* amicalis DSM 44462\(^\text{T}\) was 74.6%, that to *Gordonia* rubripertincta NBRC 101908\(^\text{T}\) was 74.6% and that to *Gordonia* amicalis NBRC 100051\(^\text{T}\) was 74.6%, which are well above the threshold of 95–96% for species delineation [23].

The complete fatty acid compositions of QH-11\(^\text{T}\) and the reference strain are given in Table 2. The fatty acid profile of QH-11\(^\text{T}\) was mainly composed of C\(_{16:0}\) (33.3%), C\(_{17:1}\) \(\omega 8c\) (23.4%), and C\(_{18:1}\) \(\omega 9c\) (17.9%). Although the predominant fatty acid (C\(_{16:0}\)) was identical to that of the reference strain and other species of the genus *Gordonia*, there were some obvious differences between QH-11\(^\text{T}\) and DSM 44015\(^\text{T}\). QH-11\(^\text{T}\) contained a higher amount of C\(_{17:1}\) \(\omega 8c\) and a lower amount of C\(_{18:1}\) \(\omega 9c\) than DSM 44015\(^\text{T}\). QH-11\(^\text{T}\) contained menaquinone MK-9(H\(_2\)) as the major respiratory quinone and minor amounts of MK-9, MK-9(H\(_6\)) and MK-8(H\(_2\)), which is very similar to those of other species of the genus *Gordonia* with validly published names. The polar lipid profile of QH-11\(^\text{T}\) consisted of diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE) as
major components, plus phosphatidylinositol (PI), a glycolipid, an unknown phosphatidyl glycolipid, an unidentified phospholipid and three unidentified lipids. The cell-wall peptidoglycan contained MK-9(H₂) and minor amounts of MK-9(H₆) and MK-9(H₈). The polar lipid profile is composed of diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE) as major components, plus phosphatidylinositol (PI), a glycolipid, an unknown phosphatidyl glycolipid, an unidentified phospholipid and three unidentified lipids. The cell-wall peptidoglycan contained meso-diaminopimelic acid as the diagnostic amino acid and arabinose and ribose. Mycolic acids with chain lengths of 56–62 carbon atoms are present. Major fatty acids are C₁₆:0, C₁₇:1ω8c and C₁₈:1ω9c.

Cells are aerobic, Gram-stain-positive, catalase-positive, oxidase-negative, non-sporulating, non-motile, rod-shaped cells that occur singly (0.3–0.5 × 0.8–1.6 μm). Colonies grown on NA are irregular in shape and creamy-white in colour. The optimum temperature for growth is 30 °C but grows at temperatures from 15 to 40 °C, at pH 5.0–10.0 (optimum pH 7.0) and in the presence of 0–8 % of NaCl (optimum growth in the absence of NaCl). Gelatin and starch are not hydrolysed. Nitrate is not reduced. In API ZYM and API Coryne strips, alkaline phosphatase, cystine arylamidase, acid phosphatase, trypsin, β-glucosidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, lipase (C14), α-galactosidase and pyrrolidonyl arylamidase are present, but valine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, β-fucosidase, α-chymotrypsin, α-mannosidase, pyrazinamidase and urease are absent. In API 50CH strips, positive for glycero1, D-glucose, D-fructose, aesculin and sucrose, but negative for erythritol, D-xylene, rhamnose, 5-ketogluconate, D-arabinose, L-arabinose, D-ribose, L-xylene, adonitol, methyl β-D-xylkoside, D-galactose, D-mannose, L-sorbose, dulcitol, inositol, manitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetyl glucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and 2-ketogluconate. The quinone system consists of MK-9(H₂) and minor amounts of MK-9, MK-9(H₆) and MK-9(H₈). The polar lipid profile is composed of diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE) as major components, plus phosphatidylinositol (PI), a glycolipid, an unknown phosphatidyl glycolipid, an unidentified phospholipid and three unidentified lipids. The cell-wall peptidoglycan contained meso-diaminopimelic acid as the diagnostic amino acid and arabinose and ribose. Mycolic acids with chain lengths of 56–62 carbon atoms are present. Major fatty acids are C₁₆:0, C₁₇:1ω8c and C₁₈:1ω9c.

The type strain, QH-11ᵀ (CICC 24107ᵀ=KCTC 39933ᵀ), was isolated from an activated sludge wastewater treatment plant in Beijing, PR China. The DNA G+C content of the type strain is 68 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This article does not contain any studies with human participants or animals performed by any of the authors.
References


