Adhaeribacter terreus sp. nov., isolated from forest soil

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A bacterial strain, designated DNG6^T, was isolated from forest soil of the Changbai mountains, Heilongjiang province, China. Cells of strain DNG6^T were Gram-negative, 0.3–0.5 μm in diameter and 1.0–2.0 μm in length, strictly aerobic and produced large amounts of extracellular fibrillar material. Growth occurred at 16–33 °C (optimum, 28 °C), at pH 6.0–9.0 (optimum, pH 7.0–7.5) and in the presence of 0–1 % NaCl (optimum, 0 %). Strain DNG6^T contained MK-7 as the major respiratory quinone and iso-$C_{15:0}$ (40.6 %) and summed feature 4 comprising iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B (26.5 %) as the major cellular fatty acids. The DNA G+C content was 48.1 mol% (T_m). Phylogenetic analysis based on 16S rRNA gene sequences indicated that the closest relative of strain DNG6^T was *Adhaeribacter aquaticus* MBRG1.5^T with 93.9 % sequence similarity. Based on these results, it is concluded that strain DNG6^T represents a novel species of the genus *Adhaeribacter*, for which the name *Adhaeribacter terreus* is proposed, with strain DNG6^T (=CGMCC 1.6961^T=NBRC 104235^T) as the type strain.

The genus *Adhaeribacter* was created by Rickard *et al.* (2005) as a new member of the family '*Flexibacteraceae*' phylum *Bacteroidetes* (formerly the *Cytophaga–Flavobacterium–Bacteroides* group). Members of the genus *Adhaeribacter* are Gram-negative, non-motile, obligately aerobic, chemo-organotrophic organisms producing copious amounts of extracellular fibrillar material. The genus currently comprises a single species, *Adhaeribacter aquaticus*, isolated from a potable water biofilm (Rickard *et al.* 2005).

In an attempt to study the microbial diversity and cultivability of forest soil of the Changbai mountains, Heilongjiang province, China, bacterial strains were isolated by using serial dilution (Janssen *et al.*, 2002) and inoculation on a medium designed for this study [containing (l^{-1}): 1.5 g Bacto tryptic soy broth (TSB), 15 g gellan]. Plates were sealed with Parafilm and incubated at 25 °C for 1–4 weeks. Strain DNG6^T was isolated after 1 week on several plates that had been inoculated with the 10^{-7} dilution. Each of these plates displayed two or three pink colonies of strain DNG6^T.

A nearly complete 16S rRNA gene of strain DNG6^T (1513 bp) was amplified and sequenced as described by Zhang *et al.* (2003). DNA BLASTN searches on NCBI (Altschul *et al.*, 1990) showed that strain DNG6^T was phylogenetically related to members of the genera *Adhaeribacter* and *Hymenobacter*. Based on 16S rRNA gene sequence similarities, the closest relatives of strain DNG6^T were *A. aquaticus* MBRG1.5^T (93.9 %), *Hymenobacter ocellatus* Myx 2105^T (90.7 %) and *Hymenobacter gelipurpurascens* Txg1^T (90.1 %); these are all members of the family '*Flexibacteraceae*'. Alignments of 16S rRNA gene sequences of members of this family were performed with the CLUSTAL_X program (version 1.64b; Thompson *et al.*, 1997) and the positions for insertions or deletions were excluded for calculations. Phylogenetic trees based on 16S rRNA gene sequence analysis were constructed by using the neighbour-joining and maximum-parsimony methods with Kimura's two-parameter calculation model in MEGA version 3.1 (Kumar *et al.*, 2004). The neighbour-joining phylogenetic tree clearly showed that strain DNG6^{T}and *A. aquaticus* MBRG1.5^T formed a distinct phylogenetic lineage that formed a cluster with the genera *Effluviibacter*, *Pontibacter*, *Adhaeribacter* and *Hymenobacter* of the family '*Flexibacteraceae*' (Fig. 1). The topology of the maximum-parsimony tree was essentially the same (data not shown).

The morphological, physiological and biochemical characteristics of strain DNG6^T were investigated using routine cultivation on R2A agar (Reasoner & Geldreich, 1985) at 30 °C. Growth was assessed in TSB broth (30 g l^{-1}, at strengths 1, 0.5, 0.2, 0.1 and 0.05) and Luria–Bertani (LB; at strengths 1 and 0.1). The Gram reaction was performed according to Gerhardt *et al.* (1994) with cells grown on R2A agar at 30 °C for 3 days. Morphological observation was performed by transmission (H-600; Hitachi) and

Abbreviation: PHB, polyhydroxybutyrate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DNG6^T is EU682684.

Scanning and transmission electron micrographs of cells of strain DNG6^T are available as supplementary material with the online version of this paper.

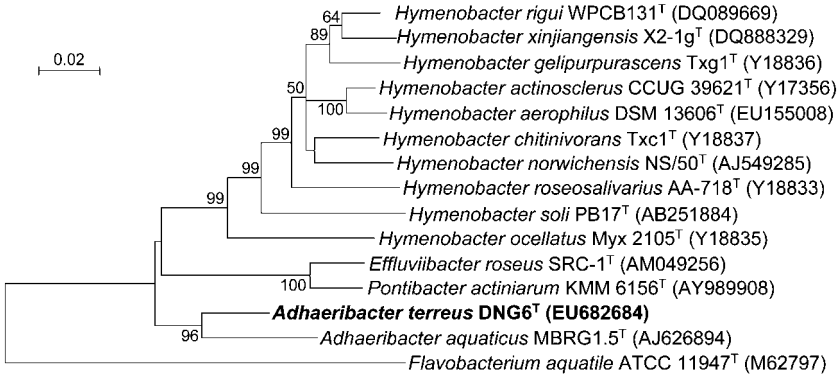

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain DNG6^T and closely related members of the family '*Flexibacteraceae*'. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. *Flavobacterium aquatile* ATCC 11947^T (M62797) was used as an outgroup. Bar, evolutionary distance (K_{nuc}) of 0.02.

scanning (FEI Quanta 2000) electron microscopy. The presence of flagella, gliding motility and growth under anaerobic conditions were examined according to Dong & Cai (2001). The temperature range for growth was determined with a TN3F temperature-gradient incubator (Advantec) from 4 to 61 °C (at 4, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17.5, 19, 21, 22, 24, 27, 28, 29, 31, 34, 36, 38, 41, 45, 49, 51, 56, 61 °C). The pH range for growth from pH 2.2 to 11 was determined by adjusting the pH of R2A broth with 5 M NaOH or HCl and verifying after autoclaving (at pH 2.2, 3, 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10 and 11). Tolerance to NaCl was examined in R2A broth supplemented with 0, 0.5, 1, 2 and 5 % (w/v) NaCl. The tests for catalase and oxidase activities and hydrolysis of casein, starch and Tweens 20 and 80 were carried out according to Dong & Cai (2001). Granules of a polyhydroxybutyrate (PHB)-like substance were identified by Sudan black staining (Dong & Cai, 2001). Carbon-source utilization tests were performed by supplementing 50 ml aliquots of minimal medium (Cohen-Bazire *et al.*, 1957) with 0.2 % of various carbon sources (see the species description). Assimilation tests for amino acids as sole nitrogen sources were determined according to Suresh *et al.* (2006). Susceptibility to antibiotics was determined using the disc diffusion method (Beijing Pharmaceutical Company) on R2A plates incubated at 30 °C for 3 days. In addition, strain DNG6^T and *A. aquaticus* MBRG1.5^T were characterized by using API 20NE and API ZYM identification systems (bioMérieux) at 30 °C.

Cells of strain DNG6^T produced large amounts of extracellular fibrillar material (Supplementary Fig. S1a, available in IJSEM Online) and accumulated granules of a PHB-like substance (Supplementary Fig. S1b). Additional physiological and biochemical characteristics of strain DNG6^T are provided in the species description. Properties that differentiate the isolate from its closest relative, *A. aquaticus* MBRG1.5^T, are detailed in Table 1.

Biomass of strain DNG6^T and *A. aquaticus* MBRG1.5^T for chemotaxonomic analyses were produced on R2A agar at 30 °C for 3 days. Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System following the manufacturer's instructions. Isoprenoid quinones were extracted and purified according to the method of Collins (1985) and analysed by HPLC (Wu *et al.*, 1989; Hu *et al.*, 2004). The predominant isoprenoid quinone of the two strains was menaquinone 7 (MK-7), which is in accordance with members of the genera *Hymenobacter*, *Effluviibacter* and *Pontibacter* (Buczolits *et al.*, 2006; Suresh *et al.*, 2006; Nedashkovskaya *et al.*, 2005). The most abundant cellular fatty acids of strain DNG6^T were iso-$C_{15:0}$ (40.6 %) and summed feature 4 comprising iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B (26.5 %). These fatty acids were also predominant in *A. aquaticus* MBRG1.5^T, although in different proportions. The detailed fatty acid compositions of the two strains are given in Table 2.

The DNA base composition was determined by using thermal denaturation (Marmur & Doty, 1962) and *Escherichia coli* K-12 was used as the reference strain. The DNA G+C content of strain DNG6^T was 48.1 % (T_m), a value which is significantly higher than that reported for *A. aquaticus* MBRG1.5^T (40.0 %).

On the basis of phenotypic data and phylogenetic inference, strain DNG6^T is a member of the genus *Adhaeribacter*. However, a range of phenotypic characteristics differentiated the strain from *A. aquaticus* MBRG1.5^T (Table 1). Hence, strain DNG6^T represents a novel species of the genus *Adhaeribacter*, for which the name *Adhaeribacter terreus* sp. nov. is proposed.

Description of *Adhaeribacter terreus* sp. nov.

Adhaeribacter terreus (ter′re.us. L. masc. adj. *terreus* earthly).

Strictly aerobic, Gram-negative, heterotrophic, oxidase-positive and catalase-negative. Cells are rods, 0.3–0.5 μm in diameter and 1.0–2.0 μm in length, devoid of flagella and gliding motility, producing copious amounts of extracellular fibrillar material and intracellular granules of a PHB-like substance. Colonies on R2A agar are circular, entire and pink. Their mucous consistency makes the cells difficult to suspend in liquids or to pellet by centrifugation. No growth occurs in TSB broth (30 g l^{-1}) at strengths 1 and 0.5 or in LB at strengths 1 and 0.1, but growth occurs

Table 1. Differential properties of strain DNG6^T (*A. terreus* sp. nov.) and *A. aquaticus* MBRG1.5^T

Data are from Rickard *et al.* (2005) and this study. The two strains were tested with API 20NE and API ZYM systems in parallel. Both strains are positive for oxidase, acid phosphatase, alkaline phosphatase, leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase activities, hydrolysis of aesculin and assimilation of L-arabinose, trehalose and methionine. Both strains are negative for esterase (C4), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-mannosidase, β-glucuronidase and α-fucosidase activities, nitrate reduction, indole production, hydrolysis of starch, production of acid from glucose and assimilation of D-mannose, D-mannitol, maltose, D-lactate, adipate, malate, citrate, phenylacetate, fumarate, galactose, salicin, sorbitol, lactose and xylose. +, Positive; (+), weakly positive; −, negative.

Characteristic	Strain DNG6^T	*A. aquaticus* MBRG1.5^T
Range for growth		
Temperature (°C)	16–33	4–37
Salinity (NaCl %, w/v)	0–1	0–4
Catalase activity	−	(+)
Assimilation of:		
Adonitol	−	+
D-Fructose	−	+
myo-Inositol	−	+
Pyruvate	−	+
D-Ribose	−	+
Sucrose	−	+
L-Arginine	(+)	−
L-Alanine	−	+
L-Glutamic acid	+	−
L-Histidine	−	+
L-Isoleucine	−	+
L-Leucine	−	+
L-Lysine	−	+
L-Ornithine	−	+
L-Phenylalanine	+	−
L-Proline	(+)	+
Glycine	(+)	+
API 20NE		
Urease activity	+	−
Gelatin hydrolysis	+	−
Assimilation of:		
D-Glucose	−	+
N-acetyl-D-glucosamine	−	+
Gluconate	−	+
Caprate	−	+
API ZYM		
Esterase lipase (C8)	(+)	−
α-Galactosidase	−	+
β-Galactosidase	−	(+)
α-Glucosidase	−	+
β-Glucosidase	−	+
N-acetyl-β-glucosaminidase	−	+
DNA G+C content (mol%)	48	40

Table 2. Cellular fatty acid compositions (%) of strain DNG6^T (*A. terreus* sp. nov.) and *A. aquaticus* MBRG1.5^T

Data for strain DNG6^T and *A. aquaticus* MBRG1.5^T column A are from this study after growth on R2A agar at 30 °C for 3 days. Data for *A. aquaticus* MBRG1.5^T column B are from Rickard *et al.* (2005) after growth on R2A agar at 30 °C for 4 days. Fatty acids <0.2 % in both strains have been omitted. tr, Trace amounts (<0.2 %); −, not detected.

Fatty acid	Strain DNG6^T	*A. aquaticus* MBRG1.5^T	
		A	B
Straight-chain			
$C_{14:0}$	tr	0.3	−
$C_{16:0}$	0.5	0.8	−
$C_{17:0}$	0.3	−	−
Unsaturated			
$C_{15:1}\omega6c$	0.2	4.4	−
$C_{16:1}\omega5c$	4.8	13.8	16.9
$C_{17:1}\omega6c$	3.3	3.5	5.1
$C_{17:1}\omega8c$	tr	0.4	−
Branched			
iso-$C_{13:0}$	tr	0.2	−
iso-$C_{14:0}$	0.4	−	−
iso-$C_{15:0}$	40.6	32.6	22.5
iso-$C_{16:0}$	2.8	0.5	−
iso-$C_{17:0}$	1.8	1.6	−
iso-$C_{15:1}$ G	6.6	−	−
anteiso-$C_{15:0}$	0.7	3.3	4.4
anteiso-$C_{17:0}$	−	0.8	−
Hydroxy			
iso-$C_{15:0}$ 2-OH	−	−	16.5
iso-$C_{15:0}$ 3-OH	2.3	3.5	3.1
iso-$C_{16:0}$ 2-OH	0.3	−	−
iso-$C_{16:0}$ 3-OH	−	0.9	−
iso-$C_{16:1}$ OH	−	1.0	−
iso-$C_{17:0}$ 3-OH	4.4	4.8	12.1
Summed feature 1*	0.5	0.8	−
Summed feature 3*	2.3	9.6	−
Summed feature 4*	26.5	15.6	−
Summed feature 5*	0.5	−	11.2
Summed feature 9*	0.2	1.5	−

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contained iso-$C_{15:1}$ H and/or $C_{13:0}$ 2-OH. Summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{15:0}$ 2-OH. Summed feature 4 contained iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B. Summed feature 5 contained $C_{18:2}\omega6,9c$ and/or anteiso-$C_{18:0}$. Summed feature 9 contained iso-$C_{17:1}\omega7c$ and/or $C_{16:0}$ 10-methyl.

in TSB at strengths 0.2, 0.1 and 0.05. Growth is significantly increased by addition of 0.1 % gellan to the diluted TSB. Growth occurs at 16–33 °C (optimum, 28 °C), pH 6.0–9.0 (optimum, pH 7.0–7.5) and in the presence of 0–1 % NaCl (w/v) (optimum, 0 %). Nitrate is not reduced. Casein, aesculin and gelatin are hydrolysed,

but starch and Tweens 20 and 80 are not. Urease activity is present, but arginine dihydrolase activity is absent. Indole is not produced. Acid is not produced from D-glucose. L-Arabinose, trehalose, L-glutamic acid, L-methionine and L-phenylalanine are assimilated. L-Arginine, L-proline and glycine are weakly assimilated. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate, phenylacetate, adonitol, fructose, fumarate, galactose, inositol, D-lactate, lactose, pyruvate, D-rhamnose, D-ribose, salicin, D-sorbitol, sucrose, D-xylose, L-alanine, L-histidine, L-isoleucine, L-leucine, L-lysine and ornithine are not assimilated. Alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present. Weak esterase lipase (C8), cystine arylamidase, α-galactosidase and α-glucosidase activities are present. Esterase (C4), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Susceptible to (μg per disc unless otherwise stated) amikacin (30), carbenicillin (100), cefoperazone (75), erythromycin (15), gentamicin (10), penicillin G (10 U), tobramycin (10) and vancomycin (30). The predominant cellular fatty acids are iso-$C_{15:0}$ and summed feature 4 (comprising iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B). The major isoprenoid quinone is MK-7. The DNA G+C content of the type strain is 48.1 mol%.

The type strain, DNG6^T (=CGMCC 1.6961^T=NBRC 104235^T), was isolated from forest soil.

Acknowledgements

This work was supported by grants from the National Nature Science Foundation of China (30725001) and the Chinese Academy of Sciences (KSCX2-YW-G-009).

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