Adhaeribacter terrae sp. nov., a novel bacterium isolated from soil

Nabil Elderiny,1 Jae-Jin Lee,1 Yeon-Hee Lee,1 Su-Jin Park,1 Seung-Yeol Lee,1 Sangkyu Park,1 Young-Je Cho,2 Leonid N. Ten1 and Hee-Young Jung1,3,*

Abstract

Strain HY02T was isolated from a soil sample collected at Namyangju-si, Gyeonggi-do, Republic of Korea. Cells of this strain were observed to be Gram-stain-negative, short and rod-shaped. Colonies were red in colour. A 16S rRNA gene sequence analysis identified this strain as a member of the genus Adhaeribacter in the family Cytophagaceae, with the highest level of 16S rRNA gene sequence similarity to Adhaeribacter terreus DNG6T (98.08 %). This strain was positive for oxidase but negative for catalase activity and acid production from glucose. Growth of strain HY02T was observed at 15–30 °C, pH 7–8 and in the presence of 0–1 % NaCl. The isolate contained MK-7 as the predominant respiratory quinone, and C18:0, iso-C15:0 summed feature 4 (anteiso-C17:1 B/iso-C17:1 I) and C16:0 were the major fatty acids. The major polar lipid was phosphatidylethanolamine. The genomic DNA G+C content of strain HY02T was 44.0 mol%. Phenotypic and chemotaxonomic data supported the affiliation of strain HY02T with the genus Adhaeribacter. However, strain HY02T exhibited a relatively low level of DNA–DNA relatedness with A. terreus (16.3±3.5 %). Based on its phenotypic and genotypic properties, together with its phylogenetic distinctiveness, strain HY02T should be considered a representative of a novel species in the genus Adhaeribacter, for which the name Adhaeribacter terrae sp. nov. is proposed. The type strain is HY02T (=KCTC 52512T=JCM 31652T).

The genus Adhaeribacter was first described by Rickard et al. [1], with Adhaeribacter aquaticus as the type species. At the time of writing, three other species with validly published names had been assigned to this genus (www.bacterio.net/adhaeribacter.html). Cells of Adhaeribacter species are Gram-stain-negative, with a DNA G+C content of 40–48 mol% and menaquinone-7 (MK-7) as the predominant quinone system. Species of Adhaeribacter have been found in ecological niches such as potable water biofilms, forest soil and air [1–3]. In the course of screening for novel bacteria, strain HY02T was isolated from a soil sample collected in Namyangju-si, Gyeonggi-do, Republic of Korea. On the basis of 16S rRNA gene sequence analysis, this isolate was considered to be an Adhaeribacter-like strain. Strain HY02T was subjected to a detailed investigation using a polyphasic taxonomic approach that included genotypic, chemotaxonomic and phenotypic analyses. These results indicated that HY02T should be placed in the genus Adhaeribacter as a representative of a novel species.

Strain HY02T was isolated from a soil sample collected at Namyangju-si, Gyeonggi-do (N 37°36′ 25.89″ E127°10′ 07.28″), Republic of Korea. The sample was serially diluted in distilled water. One hundred microlitres of each dilution was spread onto R2A agar plates (Difco, USA). After incubation at 25 °C for 1 week, one red-coloured colony was purified by subculturing under the same conditions and was maintained as a glycerol suspension (20 %, w/v) at −70 °C. Adhaeribacter terreus KACC 14257T was obtained from the Korean Agricultural Culture Collection and used as refer- ence strain. Unless otherwise noted, the morphological, physiological and biochemical characteristics of strain HY02T and A. terreus KACC 14257T were investigated by using routine cultivation on R2A agar at 25 °C for 3 days.

For the phylogenetic analysis, the 16S rRNA gene was amplified from chromosomal DNA using the universal bacterial primers, 9F and 1512R, as described previously [4]. Purified PCR products were sequenced by Genotech (Dea-jeon, Republic of Korea). A nearly complete 16S rRNA sequence was compiled using SeqMan software (DNASTAR) and compared with those of closely related species using BLAST from NCBI (www.ncbi.nlm.nih.gov). The 16S rRNA sequences of related taxa were obtained from GenBank and

Author affiliations: 1School of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea; 2School of Food Science and Biotechnology/Food and Bio-Industry Research Institute, Kyungpook National University, Daegu 41566, Republic of Korea; 3Institute of Plant Medicine, Kyungpook National University, Daegu 41566, Republic of Korea.

*Correspondence: Hee-Young Jung, heeyoung@knu.ac.kr

Keywords: Adhaeribacter; Bacteroidetes; soil bacteria.

The NCBI GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain HY02T is LC177335.1. Two supplementary figures are available with the online Supplementary Material.
assembled in BioEdit [5]. Multiple sequence alignments were performed with CLUSTALX [6]. Tree topologies were inferred by using the neighbour-joining (NJ) [7], maximum-likelihood (ML) [8] and maximum-parsimony (MP) [9] methods in the program MEGA7 [10]. NJ and ML phylogenetic trees were constructed using the Tamura three-parameter model. A bootstrap analysis with 1000 replicates was used to indicate the confidence levels of branches [11].

The nearly complete 16S rRNA gene sequence of strain HY02T contained 1457 bp. On the basis of 16S rRNA gene sequence similarity, the closest relative of strain HY02T was Adhaeribacter terreus DNG6T (98.08 %) [2]. Levels of sequence similarity with other Adhaeribacter species were less than 95.5 %. The phylogenetic position of the new isolate, determined by various tree-making algorithms, revealed that strain HY02T was a member of the genus Adhaeribacter, clustering with A. terreus DNG6T with high bootstrap values of 98–100 % by each of the methods mentioned above (Fig. 1). The generally accepted criteria for delineating bacterial species state that strains with DNA–DNA relatedness of less than 70 % (as measured by hybridization) or showing 16S rRNA gene sequence dissimilarity greater than 3 % are considered to belong to separate species [12, 13].

The Gram reaction with strain HY02T was examined by a staining method [14]. The morphology of strain HY02T cells, grown for 3 days at 25 °C on R2A agar, was observed under an Olympus light microscope (×1000 magnification) and a Hitachi HT7700 transmission electron microscope. Motility was investigated on 0.5 % (w/v) semisolid R2A agar [15] and gliding motility was assessed by the microscopic hanging drop technique [16, 17]. Catalase activity was determined by bubble production in 3 % (v/v) H2O2. Oxidase activity was determined using 1 % (w/v) tetramethyl-p-phenylenediamine [18]. Growth was assessed on R2A agar (Difco), Luria–Bertani agar (LB, Difco), nutrient agar (NA; Difco) and trypticase soy agar (TS; Difco). Hydrolysis of casein, starch, Tween 20 and Tween 80 were assayed as previously described by Tindall et al. [19]. Growth at different pH levels (pH 4–10, in 1 unit increments) and NaCl concentrations (0.5, 1, 1.5, 2, 3, 4, 5 and 10 %, w/v) was evaluated on R2A broth for 5 days. The pH of the medium was adjusted by using acetate buffer (pH 4–6), phosphate buffer (pH 7–8) and Tris buffer (pH 9–10). Enzyme activities, assimilation of carbon sources, acid production from substrates and other physiological characteristics of the isolate HY02T and A. terreus KACC 14257T as the reference strain were determined using API ZYM, API 20NE, API ID 32 GN and API 50 CH kits according to the manufacturer’s instructions (bioMérieux). Phenotypic characteristics of strain HY02T are given in Table 1 and in the species description.

The biochemical characteristics of strain HY02T were similar to those reported for members of the genus Adhaeribacter [1–3], i.e. it was positive for oxidase, acid phosphatase, alkaline phosphatase and leucine arylamidase activities, and negative for Gram reaction, nitrate reduction, indole production, α-fucosidase and β-glucuronidase. Phenotypic characteristics that differentiated strain HY02T from its closest neighbour in the genus Adhaeribacter are listed in Table 1. In particular, strain HY02T could be differentiated from A. terreus based on its ability to produce α-chymotrypsin, esterase (C4) and trypsin, for acid production from l-arabinose, d-arabitol and l-fucose, and its inability for growth at pH 9, urease production, l-arabinose utilization, and to produce acid from d-glucose and 5-ketogluconate.

Genomic DNA of strain HY02T and the most closely related strain, A. terreus KACC 14257T, were extracted according to the standard CTAB/NaCl protocol [20]. The genomic DNA G+C content of strain HY02T was determined by a reverse-phase high performance liquid chromatography (HPLC) analysis of individual nucleosides resulting from DNA hydrolysis and dephosphorylation using nuclease P1 and alkaline phosphatase [21]. Single-stranded DNA from salmon testes (D7656; Sigma; DNA G+C content, 41.2 mol%) was used as a standard. The genomic DNA G+C content of strain HY02T was 44.0 mol%, which lies within the range for recognized members (40.4–48.1 %) of the genus Adhaeribacter [1–3].

Strain HY02T was grown on R2A agar at 30 °C for 3 days and then approximately two full loops of cells were harvested for analysis of fatty acids. Fatty acid methyl esters (FAMEs) were prepared using a process of saponification, methylation and extraction described by Sasser [22]. The FAME mixtures were separated, analysed and identified by gas chromatography using the Sherlock Microbial Identification System (TSBA, Version 6.0; MIDI) with Microbial Identification software [22]. The major fatty acids of strain HY02T were C18:0 (24.5 %), iso-C15:0 (19.0 %), summed feature 4 (anteiso-C17:1, B iso-C17:1, i) (17.5 %) and C16:0 (13.5 %), representing 74.5 % of total fatty acids (Table 2). Iso-C15:0 and summed feature 4 are also the major fatty acids in the other four members of the genus Adhaeribacter, but they do not produce C18:0 [1–3]. In addition, certain qualitative and quantitative differences in fatty acid content were observed between strain HY02T and its closest relative, A. terreus DNG6T [2]. Specifically, strain HY02T could be differentiated by the presence of C16:0 N alcohol and by its higher C16:0 content.

For analysis of polar lipids and respiratory isoprenoid quinones, cells of strain HY02T were cultured, collected and freeze-dried. Polar lipids were extracted according to the method described by Minnikin et al. [23] and identified by two-dimensional thin-layer chromatography and spraying with appropriate detection substances [24]. Similar to A. terreus KACC 14257T [25], strain HY02T contained a large amount of phosphatidylethanolamine (PE) and moderate amounts of unknown lipids (L1 and L2). In addition, the polar lipid profile of the isolate included minor quantities of three unknown aminophospholipids (APL1–APL3), an
unknown glycolipid (GL₁), an unknown phospholipid (PL) and two unknown lipids (L₃ and L₄) (Fig. S2).

The respiratory isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and the solvent was evaporated in a vacuum rotary evaporator. Quinones were re-extracted in hexane and purified using Sep-Pak Vac silica cartridges (Waters) in hexane/diethyl ether (98:2, v/v), re-evaporated, dissolved in acetone and finally analysed by HPLC as described previously [26]. The predominant isoprenoid quinone in strain HY02ᵀ was menaquinone MK-7, which is also the major respiratory quinone found in other members of the genus Adhaeribacter [1–3].

DNA–DNA hybridization was performed fluorometrically by using the method of Ezaki et al. [27] with photobiotin-labelled DNA probes and microdilution wells. The highest and lowest values from five replications for each sample were excluded, and the means of the remaining three values were recorded as the DNA–DNA hybridization values. Standard deviations were also calculated based on these three values. DNA from strain HY02ᵀ showed relatively low DNA–DNA relatedness with A. terreus KACC 14257ᵀ, with a value of 16.3±3.5 %, which is significantly below the recommended cut-off threshold of 70 % for the identification of bacterial species [12]. This result indicated that the two strains differed from each other at the species level.

The phenotypic and phylogenetic characteristics of strain HY02ᵀ indicated that it was a member of the genus Adhaeribacter. However, on the basis of phylogenetic distances from established Adhaeribacter species, the low level of DNA–DNA relatedness with the most closely related Adhaeribacter strain and its specific combination of

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**Fig. 1.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between strain HY02ᵀ and related species. Bootstrap values of more than 70 % (percentages of 1000 replications) are shown at branching points. Flavobacterium aquatile ATCC11947ᵀ (M62797) was used as an outgroup. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood algorithm. Bar, 0.02 substitutions per nucleotide position.
phenotypic characteristics (Table 1), it is clear that strain HY02\textsuperscript{T} is not affiliated with any recognized species in the genus *Adhaeribacter*. These results support the conclusion that strain HY02\textsuperscript{T} represents a novel species of the genus *Adhaeribacter*, for which the name *Adhaeribacter terrae* sp. nov. is proposed.

### Table 1. Differential phenotypic characteristics of strain HY02\textsuperscript{T} and its closest phylogenetic relative in the genus *Adhaeribacter*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Red</td>
<td>Pink</td>
</tr>
<tr>
<td>Temperature range for growth (°C)</td>
<td>15–30 (25)</td>
<td>16–33 (28)*</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>7–8 (7)</td>
<td>6–9 (7.0–7.5)*</td>
</tr>
<tr>
<td>Assimilation of l-arabinose</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM, API 20 NE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>Trypsin</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>Urease</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from (API CH 50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-Arabinose</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>d-Arabinose</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>d-Arabitol</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>d-Adonitol</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>d-Fructose</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>d-Fucose</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>l-Fucose</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>Glycerol</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>d-Glucose</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>2-Ketogluconate</td>
<td>−</td>
<td>w</td>
</tr>
<tr>
<td>5-Ketogluconate</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>d-Lysozyme</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>d-Xylose</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>44.0</td>
<td>48.1*</td>
</tr>
</tbody>
</table>

*Data from Zhang et al. [2].

### Table 2. Cellular fatty acid profiles of strain HY02\textsuperscript{T} and closely related *Adhaeribacter* species

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>1.7</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>19.0</td>
<td>40.6</td>
</tr>
<tr>
<td>iso-C15:0-3-OH</td>
<td>1.4</td>
<td>2.3</td>
</tr>
<tr>
<td>C16:0</td>
<td>13.5</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>C18:1ω9c</td>
<td>1.6</td>
<td>−</td>
</tr>
<tr>
<td>C17:0</td>
<td>1.1</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>iso-C17:0-3-OH</td>
<td>2.6</td>
<td>4.4</td>
</tr>
<tr>
<td>C18:0</td>
<td>24.5</td>
<td>−</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C15:1 G</td>
<td>3.4</td>
<td>6.6</td>
</tr>
<tr>
<td>C16:1ω7c</td>
<td>1.2</td>
<td>4.8</td>
</tr>
<tr>
<td>C17:1ω6c</td>
<td>2.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>1.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Summed feature 4*</td>
<td>17.5</td>
<td>26.5</td>
</tr>
<tr>
<td>Summed feature 5*</td>
<td>1.2</td>
<td>TR</td>
</tr>
</tbody>
</table>

*As indicated by Montero-Calasanz et al. [28], summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLs (equivalent chain-lengths) as well as those where the ECLs are not reported separately. Summed feature 3 was listed as C11:0 3-OH and/or iso-C15:0 2-OH; summed feature 4 was listed as anteiso-C17:1 B and/or iso-C17:1 I; and summed feature 5 was listed as anteiso-C18:0 and/or C18:2ω6c.

### DESCRIPTION OF ADHAERIBACTER TERRAE SP. NOV.

*Adhaeribacter terrae* (ter’rae. L. gen. n. terrae of the soil).

The cells are Gram-stain-negative, short, rod-shaped and 0.7–0.9 µm in width and 1.5–2.3 µm in length (Fig. S1, available in the online Supplementary Material). Colonies are red in colour after growth on R2A agar at 25°C for 3 days. The cells grow between 15°C and 30°C (optimum 25°C) and at pH 7–8 (optimum pH 7). Cells tolerate up to 1% NaCl in R2A broth medium. Growth occurs on R2A agar, but not on NA, TSA or LB agar. Cells are oxidase positive and catalase negative. Casein and gelatin are hydrolysed, but starch, Tween 20 and Tween 80 are not. Based on API ZYM and API 20NE tests, cells were positive for acid phosphatase, alkaline phosphatase, cystine arylamidase, leucine arylamidase, protease (gelatin hydrolysis) and valine arylamidase. They were weakly positive for α-chymotrypsin, esterase (C4), esterase (C8), naphthol-AS-BI-phosphohydrolase, trypsin and aesculin hydrolysis; and they were

...
negative for N-acetyl-β-glucosaminidase, arginine dihy- 
lase, α-fucosidase, α-galactosidase, β-galactosidase, α-glu- 
cosidase, β-glucosidase, β-glucuronidase, lipase (C14), α- 
mannosidase and urease. Based on API ID 32 GN and API 
20NE tests, acetate, N-acetyl-D-glucosamine, adipate, L- 
alanine, L-arabinose, caprate, citrate, L-fucose, gluconate, D- 
glucose, glycogen, L-histidine, 3-hydroxybenzoate, 4- 
hydroxybenzoate, DL-3-hydroxybutyrate, m-inositol, ita- 
cone, 2-ketogluconate, 5-ketogluconate, DL-lactate, L-malate, 
malonate, maltose, D-mannitol, D-mannose, melibiose, phe- 
nyl acetate, L-proline, propionate, L-rhamnose, D-ribose, sal-
icin, L-serine, D-sorbitol, suberate, sucrose and n-valerate 
are not utilized by these cells. Based on the API 50 CH test, 
acid is produced from L-arabinose, D-arabitol and L-fucose, 
and is weakly produced from D-adonitol, aesculin, D-arabi-
nose, D-fructose, D-fucose, D-galactose, glycerol, inositol, D- 
lyxose, D-mannitol, D-ribose and D-xylene. Acid is not 
produced from N-acetyl-glucosamine, amygdalin, arbutin, 
L-arabitol, cellulbiose, dulcitol, erythritol, gentiobiose, glu-
conate, glycogen, D-glucose, inulin, 2-ketogluconate, 5- 
ketogluconate, lactose, maltose, D-mannose, melezitose, 
melibiose, methyl-α-D-glucopyranoside, methyl-α-D-mann-
opyranoside, methyl-β-D-xylpyranoside, raffinose, L- 
rhamnose, salicin, D-sorbitol, starch, sucrose, D- 
tagatose, trehalose, turanose, xylitol or L-xylene. The major 
cellular fatty acids are C18:0, iso-C15:0, summed feature 4 
(anteiso-C17:1 B/iso-C17:1 I) and C16:0. The predominant 
isoprenoid quinone is MK-7. Phosphatidylethanolamine is 
the major polar lipid. The DNA G+C content is 44.0 mol%. 
The type strain, HY02T (=KCTC 52512T=JCM 31652T), was 
isolated from a soil sample collected at Namyangju-si, 
Gyeonggi-do (N 37° 36′ 25.89″ E127° 10′ 07. 28′′), Republic 
of Korea.

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Korea.

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

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