Hymenobacter yonginensis sp. nov., isolated from a mesotrophic artificial lake

Yochan Joung,¹ Sung-Heun Cho,² Haneul Kim,¹ Seung Bum Kim² and Kiseong Joh¹

Correspondence
Kiseong Joh
ksjoh@hufs.ac.kr

¹Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Wangsan, Yongin, Gyeonggi 449-791, Republic of Korea

²Department of Microbiology, School of Bioscience and Biotechnology, Chungnam National University, 220 Gung-dong, Yuseong, Daejeon 305-764, Republic of Korea

A non-motile, red-pigmented bacterium, designated strain HMD1010^T, was isolated from an artificial lake located within the campus of Hankuk University of Foreign Studies, Yongin, Korea. A phylogenetic tree based on 16S rRNA gene sequences showed that strain HMD1010^T formed a lineage within the genus *Hymenobacter* and was closely related to the type strains of *Hymenobacter rigui* (96.7% sequence similarity) and *H. gelipurpurascens* (95.6%). The major fatty acids were C_{16:1ω5c} (21.9%), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c}; 17.2%), iso-C_{15:0} (14.5%) and summed feature 4 (anteiso-C_{17:1} B and/or iso-C_{17:1} I; 11.9%). The DNA G+C content was 60.4 mol%. On the basis of the evidence presented in this study, strain HMD1010^T represents a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter yonginensis* sp. nov. was proposed. The type strain is HMD1010^T (=KCTC 22745^T =CECT 7546^T).

The genus *Hymenobacter* was described by Hirsch *et al.* (1998), and members of the genus have high DNA G+C contents (55–65 mol%) compared with other members of the family *Flexibacteraceae*. At the time of writing, the genus *Hymenobacter* included 14 species: *Hymenobacter roseosalivarius* (the type species; Hirsch *et al.*, 1998), *H. actinoscleris* (Collins *et al.*, 2000), *H. aerophilus* (Buczolits *et al.*, 2002), *H. norwichensis*, *H. ocellatus*, *H. gelipurpurascens* and *H. chitinivorans* (Buczolits *et al.*, 2006), *H. xinjiangensis* (Zhang *et al.*, 2007), *H. soli* (Kim *et al.*, 2008), *H. psychrotolerans* (Zhang *et al.*, 2008), *H. deserti* (Zhang *et al.*, 2009), *H. daecheongensis* (Xu *et al.*, 2009) and *H. psychrophilus* (Zhang *et al.*, 2011). Members of the genus have been isolated from various environments including deserts (in China and Antarctica) and vegetated soils, sandstone and wetlands.

In the course of a study on the microbial diversity of a mesotrophic freshwater lake (surface water, 17 °C) within the campus of Hankuk University of Foreign Studies, Yongin, Republic of Korea, a strain forming red-pigmented colonies was isolated using the standard dilution plating technique. Strain HMD1010^T was incubated on R2A agar (Difco) for 24 h at 30 °C. The isolate was routinely cultured on the same medium at 30 °C and the culture was suspended in aqueous glycerol (20%, w/v) for storage at –80 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HMD1010^T is GU808562.

An almost-complete sequence of the 16S rRNA gene (1392 bp) was obtained for strain HMD1010^T as described previously (Cho & Giovannoni, 2003). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were performed by using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic and molecular evolutionary analyses were conducted by using MEGA version 4 (Tamura *et al.*, 2007). Sequence comparisons showed that strain HMD1010^T was closely related to members of the genus *Hymenobacter*. Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) algorithms. The robustness of the topologies for the maximum-likelihood and neighbour-joining trees was evaluated by means of bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the sequences. The trees generated in this study (Fig. 1) indicated that strain HMD1010^T belonged to the genus *Hymenobacter*. Strain HMD1010^T formed a coherent clade with the type strains of *H. rigui* (96.2% similarity) and *H. gelipurpurascens* (95.6%), within the phylogenetically well-resolved *Hymenobacter* clade. This phylogenetic inference, coupled with 16S rRNA gene sequence similarities of <97% (Wayne *et al.*, 1987) between strain HMD1010^T and strains of other *Hymenobacter* species, suggested that the strain should be assigned to the genus *Hymenobacter* as a representative of a novel species.

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain HMD1010^T and strains of the genus *Hymenobacter*. Percentages at nodes are levels of bootstrap support (>50%) based on neighbour-joining analyses of 1000 resampled datasets. Filled circles indicate nodes recovered by all three treeing methods. The sequence of *Flavobacterium aquatile* ATCC 11947^T was used as the outgroup. Bar, 0.02 substitutions per nucleotide position.

Cell morphology was examined by light microscopy. Gram staining was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Anaerobic growth was tested on R2A agar at 30 °C by using the GasPak EZ anaerobic container system (BD) according to the manufacturer's instructions. Cellular pigments were extracted with acetone/methanol (1:1, v/v) and their absorption spectra were determined using a scanning UV/visible spectrophotometer (UV 6101A; Shimadzu). The presence of flexirubin-type pigments was investigated by using the bathochromic shift test with NaOH solution (Bernardet *et al.*, 2005). Catalase and oxidase tests were performed according to standard methods (MacFaddin, 1980). The pH range for growth was determined in R2A broth at 20 °C. The medium was adjusted to pH 5.0–10.0 (at intervals of 1.0 pH unit) using 0.1 mM sodium acetate buffer (pH 5.0–7.0), 0.1 mM phosphate buffer (pH 7.0–8.0) and 0.1 mM sodium carbonate buffer (pH 9.0–10.0). Growth at various NaCl concentrations [0.5% (w/v) and 1.0–10.0% (w/v) at intervals of 1.0%] was investigated in modified R2A broth. The temperature range and optimum for growth were measured in R2A broth at 4–42 °C (at 4 °C, 10–30 °C at 5 °C intervals, 37 and 42 °C). Hydrolysis of casein [3.0%, v/v, skimmed milk (Difco)], CM-cellulose [1.0%, w/v, CM-cellulose (Sigma)] and starch (1.0%, w/v) was tested using R2A agar as the basal medium. Growth was assessed on marine agar 2216 (MA), cetrimide agar, trypticase soy agar (TSA) and MacConkey agar (all from Difco). DNase test agar (Difco) was used to assess production of DNase. Basic biochemical tests, enzyme activity tests and carbon source oxidation tests were performed using API 20E and API ZYM strips (bioMérieux) and Biolog GN3 MicroPlates according to the manufacturers' instructions. The DNA G+C content was determined by using HPLC (Mesbah & Whitman, 1989). For fatty acid methyl ester analysis, biomass of strain HMD1010^T and

the reference strains *H. aerophilus* KACC 12071^T, *H. gelipurpurascens* KACC 12069^T, *H. rigui* KACC 11614^T and *H. psychrotolerans* KACC 13767^T was harvested from cells grown on R2A agar at 30 °C for 48 h. Fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System.

Morphological, cultural, physiological and biochemical characteristics of strain HMD1010^T are listed in Table 1 and in the species description. Cells of strain HMD1010^T were Gram-staining-negative, red-pigmented, strictly aerobic and non-motile. Strain HMD1010^T exhibited a number of phenotypic similarities to species of the genus *Hymenobacter* (Hirsch *et al.*, 1998; Buczolits *et al.*, 2006). However, test results for growth in 2% NaCl concentration, growth at pH 8 and utilization of D-mannitol clearly differentiated strain HMD1010^T from related *Hymenobacter* species (Table 1).

The fatty acid profile of strain HMD1010^T included the major components C_{16:1}ω5c (21.9%), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; 17.2%), iso-C_{15:0} (14.5%) and summed feature 4 (anteiso-C_{17:1} B and/or iso-C_{17:1} I; 11.9%) (Table 2). This fatty acid profile was similar to those of other *Hymenobacter* species, although there were differences in the proportions of some fatty acids, including summed feature 4 and summed feature 2 (iso-C_{16:1} I and/or C_{14:0} 3-OH) (Table 2). The DNA G+C content of strain HMD1010^T was 60.4 mol%, a value within the range reported for members of the genus *Hymenobacter* (54–65 mol%). Chemotaxonomic data confirm the phylogenetic affiliation of strain HMD1010^T as a member of the genus *Hymenobacter*. Therefore, strain HMD1010^T should be classified in the genus *Hymenobacter* as a member of a novel species, for which the name *Hymenobacter yonginensis* sp. nov. is proposed.

Table 1. Differential characteristics of strain HMD1010^T and closely related type strains of genus *Hymenobacter*

Strains: 1, HMD1010^T; 2, *H. aerophilus* KACC 12071^T; 3, *H. gelipurpurascens* KACC 12069^T; 4, *H. rigui* KACC 11614^T; 5, *H. psychrotolerans* KACC 13767^T. Data were generated in this study except for the DNA G+C contents of the four reference strains (data from Buczolits *et al.*, 2002, 2006; Zhang *et al.*, 2008; Baik *et al.*, 2006). All strains were negative for utilization of L-histidine (Biolog GN3).

Characteristic	1	2	3	4	5
Growth at pH 8	+	–	–	+	+
Growth in 2% (w/v) NaCl	+	–	–	–	–
Gelatinase (API 20E)	+	–	+	+	+
Utilization of (Biolog GN3):					
Dextrin	+	+	+	+	–
Maltose	–	+	+	+	+
Trehalose	–	–	+	+	–
Cellobiose	–	–	+	+	–
N-Acetylneuraminic acid	+	–	–	–	–
D-Fructose	–	+	+	+	+
D-Galactose	–	+	+	+	–
D-Fucose	+	+	–	+	–
D-Sorbitol	–	–	–	+	+
D-Mannitol	+	–	–	–	–
D-Arabitol	+	–	+	+	–
L-Alanine	+	–	+	+	+
L-Arginine	–	–	–	+	–
L-Aspartic acid	–	+	+	+	+
L-Glutamic acid	–	+	+	+	+
L-Pyroglutamic acid	–	+	–	–	+
L-Serine	+	+	–	–	+
D-Gluconic acid	+	+	–	+	+
D-Glucuronic acid	–	+	+	–	–
DNA G+C content (mol%)	60.4	60	63.1	57–58	65

Description of *Hymenobacter yonginensis* sp. nov.

Hymenobacter yonginensis (yon.gi.nen'sis. N.L. masc. adj. *yonginensis* of or belonging to Yongin, Korea, from where the type strain was isolated).

Cells are Gram-staining-negative, non-motile, aerobic rods. Colonies on R2A are convex, circular and smooth with entire margins, reddish pink and approximately 5 mm in diameter after 2 days at 30 °C. Good growth occurs on R2A, blood agar and TSA. No growth occurs on MA, DNA test agar or MacConkey agar. Growth occurs in the presence of 0–3.0% (w/v) NaCl (optimum, 0.5%), at pH 7–8 (optimum, pH 7) and at 10–30 °C (optimum, 25 °C). Oxidase and catalase activities are present. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase activities are absent. Casein and starch are hydrolysed. Citrate utilization, H₂S production, indole production and acetylmethylcarbinol production are not observed. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine

Table 2. Cellular fatty acid compositions of strain HMD1010^T and closely related type strains

Strains: 1, HMD1010^T; 2, *H. aerophilus* KACC 12071^T; 3, *H. gelipurpurascens* KACC 12069^T; 4, *H. rigui* KACC 11614^T; 5, *H. psychrotolerans* KACC 13767^T. Data were generated in this study; strains were grown on R2A at 25 °C for 2 days. Values are percentages of total fatty acids; only fatty acids amounting to at least 1.0% of the total in at least one strain are shown. tr, Trace (<1%); ND, not detected.

Fatty acid	1	2	3	4	5
iso-C _{14:0}	ND	tr	tr	1.6	tr
iso-C _{15:0}	14.5	9.9	12.3	26.3	20.5
anteiso-C _{15:0}	3.0	13.9	7.6	8.5	8.1
iso-C _{16:1} H	ND	1.6	3.5	ND	2.0
iso-C _{16:0}	1.3	tr	2.7	1.1	1.5
C _{16:1} ω5c	21.9	11.3	24.6	16.6	16.6
C _{16:0}	2.4	1.7	3.3	2.1	2.9
iso-C _{15:0} 3-OH	5.3	2.0	3.6	5.2	2.9
anteiso-C _{17:1} ω9c	tr	1.1	tr	tr	tr
iso-C _{17:0}	1.6	4.5	1.7	2.0	0.5
anteiso-C _{17:0}	tr	1.8	0.6	0.7	0.5
C _{16:0} 3-OH	1.5	tr	1.5	1.0	1.3
iso-C _{17:0} 3-OH	4.3	4.4	3.7	4.9	2.1
Summed feature 1*	2.2	tr	tr	2.7	1.4
Summed feature 2*	3.2	tr	tr	2.3	tr
Summed feature 3*	17.7	29.1	22.2	17.2	21.1
Summed feature 4*	11.9	12.2	5.2	tr	11.6

*Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 1 comprised C_{13:0} 3-OH and/or iso-C_{15:1} H, summed feature 2 comprised iso-C_{16:1} I and/or C_{14:0} 3-OH, summed feature 3 comprised iso-C_{15:0} 2-OH and/or C_{16:1}ω7c and summed feature 4 comprised anteiso-C_{17:1} B and/or iso-C_{17:1} I.

arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and N-acetyl-β-glucosaminidase activities are present, but lipase (C14), α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase activities are absent. The following Biolog GN3 microplate compounds are utilized: dextrin, sucrose, lactose, melibiose, N-acetyl-β-D-mannosamine, N-acetylneuraminic acid, D-mannose, D- and L-fucose, L-rhamnose, D- and L-serine, D-mannitol, D-arabitol, D-glucose 6-phosphate, D-fructose 6-phosphate, D-aspartic acid, gelatin, glycyl L-proline, L-alanine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, glucuronamide, methyl pyruvate, L-lactic acid, α-ketoglutaric acid, L-malic acid, bromo-succinic acid, Tween 40, aminobutyric acid, β-hydroxy-DL-butyric acid, propionic acid, acetic acid and formic acid. The following carbon sources are not utilized: maltose, trehalose, cellobiose, gentiobiose, turanose, stachyose, raffinose, methyl β-D-glucoside, D-salicin, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, α-D-glucose, D-fructose, D-galactose, 3-methyl glucose, inosine, D-sorbitol, myo-inositol, glycerol,

L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, D-glucuronic acid, mucic acid, quinic acid, D-saccharic acid, *p*-hydroxyphenylacetic acid, D-lactic acid methyl ester, citric acid, D-malic acid, α -hydroxybutyric acid, α -ketobutyric acid and acetoacetic acid. The major fatty acids are C_{16:1} ω 5c, summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c), iso-C_{15:0} and summed feature 4 (anteiso-C_{17:1} B and/or iso-C_{17:1} I). The complete fatty acid profile of the type strain is given in Table 2. The DNA G + C content of the type strain is 60.4 mol%.

The type strain, HMD1010^T (=KCTC 22745^T =CECT 7546^T), was isolated from a mesotrophic freshwater pond in Yongin, Republic of Korea.

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