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NOTE

Pedobacter yonginense sp. nov., Isolated from a Mesotrophic Artificial Lake in Korea[§]

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A non-motile red-pigmented bacterium, designated strain HMD1002^T, was isolated from an artificial lake located on the campus of Hankuk University of Foreign Studies, South Korea. The major fatty acids were iso-$C_{15:0}$ (29.6%), Summed Feature 3 (comprising $C_{16:1}$ $\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 17.5%) and iso-$C_{17:0}$ 3-OH (12.5%). The major isoprenoid quinone was menaquinone-7 (MK-7). The DNA G+C content was 41.0 mol%. A phylogenetic tree based on 16S rRNA gene sequences showed that strain HMD1002^T formed a lineage in the genus *Pedobacer* and was closely related to *Pedobacer terrae* (96.3%) and *Pedobacer suwonensis* (95.8%) in sequence similarity. On the basis of the evidence presented in this study, strain HMD1002^T represents a novel species of the genus *Pedobacter*, for which the name *Pedobacter yonginense* sp.nov. is proposed. The type strain is HMD1002^T (=KCTC 22721^T =CECT 7544^T).

Keywords: *P. yonginense*, taxonomy, 16S rRNA gene sequence

The genus *Pedobacter* is a member of the family *Sphingobacteriaceae*. The genus *Pedobacter* was first described by Steyn *et al.* (1998) through the re-classification of 2 *Sphingobacterium* species and the description of 2 novel species. Recently the genus *Pedobacter* has consisted of 26 species (http://www.bacterio.cict.fr). Strains belonging to this genus have been found in terrestrial environments, such as eutrophic ponds (Baik *et al.*, 2007; An *et al.*, 2009), soil (Ten *et al.*, 2006; Yoon *et al.*, 2007a, 2007b; Roh *et al.*, 2008; Gordon *et al.*, 2009), and drinking water (Gallego *et al.*, 2006). The genus *Pedobacter* is characterized chemotaxonomically with MK-7 as the predominant menaquinone and $C_{16:1}$ $\omega7c$, iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, iso-$C_{15:0}$ 3-OH, $C_{16:0}$, $C_{16:1}$ $\omega5c$, $C_{16:1}$ $\omega7c$, $C_{16:0}$ 3-OH, iso-$C_{17:0}$ 3-OH, and iso-$C_{17:1}$ $\omega9c$ as the major fatty acids.

In the course of a study on the microbial diversity of a mesotrophic artificial lake located within the campus of Hankuk University of Foreign Studies, in Yongin, Gyeonggi, South Korea (37°20′18″N, 127°16′11″E), a novel bacterial strain was isolated on R2A agar (Difco, USA) as reddish-pigmented colonies using the standard dilution plating technique following incubation for 48 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.

Almost complete sequences of the 16S rRNA gene were obtained for strain HMD1002^T as described previously (Cho and Giovannoni, 2003). Identification of phylogenetic neighbors and calculation of pairwise 16S rRNA gene sequence similarity were performed using the EzTaxon server [http://147.47.212.35:8080/index.jsp or http://www.eztaxon.org; (Chun *et al.*, 2007)], closely related to members of the genus *Pedobacter*. To clarify the phylogenetic position of the strain, aligned nucleotide positions were used for phyloenetic analyses. The phylogenetic relationships between strain HMD1002^T and representative type strains of *Pedobacter* species were defined by MEGA4 (Tamura *et al.*, 2007). Phyloenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971), and neighbor-joining algorithms (Saitou and Nei, 1987). The robustness of the topologies for the maximum-likelihood and neighbor-joining trees was evaluated by means of bootstrap analysis (Felsenstein, 1985), based on 100 and 1,000 re-samplings of the sequences, respectively. Strain HMD1002^T formed a coherent clade with *Pedobacer terrae* (96.3%) and *Pedobacer suwonensis* (95.8%) within the phylogenetically well-resolved *Pedobacter* clade. This phylogenetic inference, together with the level of 16S rRNA gene sequence similarity (Wayne *et al.*, 1987) between strain HMD1002^T and the other *Pedobacer* species (<97%) suggest that the strain represents a novel species of the genus *Pedobacter*.

Cell morphology was examined by light microscopy. Motility was investigated using motility test medium (Difco). Gram staining was determined using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Cellular pigments were extracted with acetone/methanol (1:1, v/v) and the absorption spectra was determined using a scanning UV/visible spectrophotometer (UV 6101A; Shimadzu, USA). The presence of flexirubin-type pigments was investigated

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using the bathochromatic shift test with a 20% (w/v) KOH solution (McCammon and Bowman, 2000; Bernardet *et al.*, 2002). Anaerobic growth was tested on R2A agar at 30°C by using a GasPak EZ Anaerobic Container System (BD) according to the manufacturer's instructions. Catalase and oxidase tests were performed according to standard methods (MacFaddin, 1980). The pH range for growth was determined in modified R2A broth (containg yeast extract 0.5 g, peptone 0.5 g, casamino acids 0.5 g, dextrose 0.5 g, starch 0.5 g, sodium pyruvate 0.3 g, dipotassium phosthate 0.3 g, magnesium sulfate 0.05 g per 1,000 ml DW) that was adjusted to various pH values (initial pH 5.0-10.0 at intervals of 1.0 pH units) at 30°C. Growth at various NaCl concentrations [0.5% (w/v) and 1.0-10.0% (w/v) at intervals of 1.0 % units] 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°C and 42°C). Hydrolysis of casein [3.0% skimmed milk (Difco) v/v], CM-cellulose [1.0% CM-cellulose (Sigma) w/v], and starch (1.0% w/v), were tested using modified R2A as the basal medium. MacConky agar (Difco) and DNase test agar (Difco) were used for growth and DNase assay, respectively. Basic biochemical tests, enzyme activity, and carbon-source-oxidation tests were performed using API 20NE and API ZYM strips (bioMérieux) and Biolog GN2 MicroPlates, according to manufacturers' instructions. Cell biomass for DNA extraction was obtained from R2A at 30°C after 48 h of incubation. The purification of chromosomal DNA was performed using a DNA Purification kit (Solgent, Korea) according to the manufacturer's instructions. The G+C content was determined using HPLC analysis of hydrolyzed DNA according to Tamaoka (1986) and Mesbah and Whitman (1989). Strain HMD1002^T was grown on R2A for 48 h at 30°C. The fatty acid methyl esters (FAMEs) were obtained from cells by saponification, methylation, and extraction. Analysis by gas chromatography was controlled by MIS software (Microbial ID), and peaks were automatically integrated and identified by the MICROBIAL DENTIFICATION software package (Sasser *et al.*, 1990). Polar lipids and isoprenoid quinones were isolated according to Minnikin *et al.* (1984) and analyzed by HPLC as described by Collins (1985). Polar lipids

Table 1. Phenotypic characteristics differentiating Strain HMD1002^T from related members of the genera *Pedobacter*
Strains: 1, HMD1002^T; 2, *Pedobacter suwonensis* KACC11317^T; 3, *Pedobacter roseus* KACC11594^T; 4, *Pedobacter sandarakinus* KACC11593^T; 5, *Pedobacter aquatilis* KACC 12695^T; 6, *Pedobacter agori* KACC13768^T. Data of 1 to 6 were obtained from in this study except the DNA G+C content and Quinone of the five reference strains. +, positive; -, negative; +^{w}, weak positive; NR, no reported.

Characteristic	1	2	3	4	5	6
Habit	Fresh water	hizosphere	hypertrophic pond	soil	drinking water	soil
G+C content (mol%)	41.0	44.2	41.3	39.7	38	41.4
Quinone	MK-7	NR	MK-7	MK-7	NR	NR
Growth at 37°C	+	-	-	+	-	-
Growth at pH 8	-	+	+	+	+	+
Growth with NaCl: 1.0(%)	-	+	+	-	+	+
Skim milk (3%)	-	+	-	-	-	+
API 20NE						
Nitrate reduction	-	+	-	-	-	-
Esculin hydrolysis	+	-	+	+	+	+
Gelatinase	-	-	+	-	-	+
API ZYM						
Esterase (C4)	+^{w}	+	+	+	+	+
Esterase lipase (C8)	+^{w}	+	+	+	+	+
Cystine arylamidase	-	-	+^{w}	+^{w}	+^{w}	+
α-Chymotrypsin	-	+	+	-	-	+
α-Galactosidase	-	+	+	-	-	+
β-Glucuronidase	-	+	+	-	-	+
β-Glucosidase	+	-	+	+	+	+
α-Mannosidase	-	-	+	+^{w}	+	+
Utilization of (GN2 Microplate)						
D-Arabitol	+	-	-	-	-	-
α-D-Lactose	+	-	+	-	-	+
L-Fucose	+	+	+	-	-	+
D-Psicose	+	-	+	-	+	+
Sucrose	+	-	+	+	+	+
α-Keto Valeric acid	+	-	-	-	-	-
Uridine	+	+	-	-	+	-
L-Proline	+	+	+	+	-	+
L-Serine	+	-	+	-	+	+

were separated by two-dimensional TLC (coated with silica gel, 10×10 cm; Merck) using chloroform/methanol/water (65:25:3.8) in the first direction, followed by chloroform/acetic acid/methanol/water (40:7.5:6:1.8) in the second direction. Plates were sprayed with various specific reagents for detection of different polar lipids (Minnikin *et al.*, 1984).

Morphological, cultural, physiological, and biochemical characteristics of strain HMD1002^T are listed in Table 1 and in the species description. Cells of strain HMD1002^T were Gram-negative, reddish-pink, aerobic, chemoheterotrophic, and non-motile. Strain HMD1002^T exhibited a number of phenotypic similarities with respect to species of the genus *Pedobacer*, including cell morphology, reddish-colored series pigments, a NaCl requirement for growth and obligately aerobic growth. Features of strain HMD1002^T were typical of members of the genus *Pedobacer*; however, several characteristics of HMD1002^T, such as its psychrotolerant nature, its ability to reduce nitrate, and its macromolecule-degradation profile, clearly differentiated this strain from the other strains of *Pedobacer* species (Table 1). The DNA G+C content of strain HMD1002^T was 41.0 mol% a value within the range reported for the genus *Pedobacer*. The fatty acid profile of strain HMD1002^T comprised iso-$C_{15:0}$ (29.6%), Summed Feature 3 (comprising $C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH; 17.5%) and iso-$C_{17:0}$ 3-OH (12.5%). The complete fatty acid of strain HMD1002^T composition is given in Table 2. This profile with iso-$C_{15:0}$, Summed Feature 3 and iso-$C_{17:0}$ 3-OH as major fatty acids was similar to other *Pedobacer* species. The polar lipid profiles of HMD1002^T and *Pedobacter aquatilis* KACC 12695^T consist of the predominant compound phosphatidy-lethanolamine, 3 unknown aminophosphlipid (AL1, AL2, and AL3) and unknown polar lipid (L1) (Supplementary data Fig. 1). So far, polar lipid analysis of HMD1002^T and *Pedobacter aquatilis* KACC 12695^T show similar polar lipid profiles. The major isoprenoid quinone in strain HMD1002^T was menaquinone-7 (MK-7), similar to those of other *Pedobacer* species (Hwang *et al*., 2006). Therefore, strain HMD1002^T should be classified in the genus *Pedobacer* as a member of a novel species, for which the name *Pedobacer yonginense* sp nov. is proposed.

Table 2. Cellular fatty acid compositions (%) of strain HMD1002^T and closely related strains

Strains: 1, HMD1002^T; 2, *Pedobacter suwonensis* KACC 11317^T; 3, *Pedobacter roseus* KACC11594^T; 4, *Pedobacter sandarakinus* KACC 11593^T; 5, *Pedobacter aquatilis* KACC 12695^T; 6, *Pedobacter agori* KACC 13768^T. Data of 1 to 6 were obtained from in this study. Data of 1 to 6 were obtained from in this study under the same experimental conditions. All the strains were grown under the same growth conditions (R2A agar, 20°C, 48 h of incubation). Only fatty acids that account for more than 1% for 1 of the strains are indicated. tr, trace.

	1	2	3	4	5	6
unknown 13.565	1.9	2.6	3.2	4.3	tr	2.7
iso $C_{15:0}$	29.6	37.6	38.2	33.7	34.9	40.0
anteiso $C_{15:0}$	2.4	3.0	3.2	2.9	1.2	1.3
$C_{15:1}$ *ω6c*	8.3	1.3	tr	4.0	1.8	1.1
$C_{15:0}$	5.3	0.9	tr	4.1	1.0	tr
iso $C_{16:1}$ H	1.0	tr	tr	tr	tr	tr
Summed Feature 3[a]	17.5	24.4	25.5	18.8	28.0	20.8
$C_{16:1}$ *ω5c*	tr	1.4	1.0	tr	1.8	1.0
$C_{16:0}$	1.8	1.6	1.5	1.1	2.7	2.6
$C_{15:0}$ iso 3OH	2.6	2.6	2.9	2.0	3.0	3.1
$C_{15:0}$ 2OH	1.1	tr	tr	tr	tr	tr
Summed Feature 9[b]	6.9	7.2	6.8	7.0	6.1	5.1
Summed Feature 4[c]	tr	1.4	tr	tr	1.0	1.0
$C_{15:0}$ 3OH	1.9	tr	tr	tr	tr	tr
iso $C_{17:0}$	tr	tr	tr	1.0	tr	tr
$C_{17:1}$ *ω8c*	1.5	tr	tr	1.1	tr	tr
$C_{17:1}$ *ω6c*	1.0	tr	tr	tr	tr	tr
iso $C_{16:0}$ 3OH	1.8	tr	tr	1.5	tr	tr
$C_{16:0}$ 3OH	tr	tr	tr	tr	1.1	tr
$C_{18:0}$	1.4	tr	tr	tr	2.2	tr
iso $C_{17:0}$ 3OH	12.5	10.0	11.0	13.9	11.2	12.5

[a] Summed Feature 3: $C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH
[b] Summed Feature 9: iso- $C_{17:1}$ *ω9c* and/or 10-methyl $C_{16:0}$
[c] Summed Feature 4: anteiso $C_{17:1}$ B and/or iso $C_{17:1}$ I

Description of *Pedobacer yonginense* sp. nov.

(*yong in en*. N.L. fem. adj. *yonginense* of *yongin*, Korea, from where the type strain was isolated)

Cells are Gram-negative, non-motile, aerobic rods 0.7-0.8 μm in diameter and 1.5-2.0 μm in length. Colonies on R2A agar are convex, circular, and smooth, with entire margins, red in color and approximately 5 mm in diameter after 48 h at 30°C. Good growth occurs on TSA, R2A agar, marine agar 2216, nutrient agar, and blood agar. No growth occurs on cetrimide agar or MacConkey agar. Growth occurs in the presence of 0-0.5% (w/v) NaCl (optimum 0.5%), at pH 7 (optimum pH 7) and 4-37°C (optimum 30°C). Oxidase, catalase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities are present, but β-galactosidase and L-phenylalanine deaminase activities are absent. DNA, detrain and cellulose are hydrolysed in growth. Casein and starch are not hydrolysed. Flexirubin-type pigments are not produced. Esculin and gelatin are hydrolysed (API 20NE). In the API ZYM gallery, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase activities are present, but lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, and α-mannosidase activities are absent. The following compounds are utilized as sole carbon sources in GN2 MicroPlates: α-cyclodextrin, dextrin, glycogen, D-arabitol, D-cellobiose, L-fucose, D-galactose, gentiobiose, α-D-Glucose, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, β-methyl-D-glucoside, D-psicose, D-raffinose, sucrose, pyruvic acid methyl ester, acetic acid, α-keto valeric acid, succinic acid, D-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-proline, L-serine, L-threonine, γ-amino butyric acid, uridine, 2-aminoethanol, α-D-glucose-1-phosphate, and D-glucose-6-phosphate. The following carbon sources are not utilized: tween 40, tween 80, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, i-erythritol, D-fructose, m-inositol, D-mannitol, L-rhamnose, D-sorbitol, D-trehalose, turanose, xylitol, succinic acid mono-methyl-ester, Cis-aconitic acid, citric acid, formic acid, D-

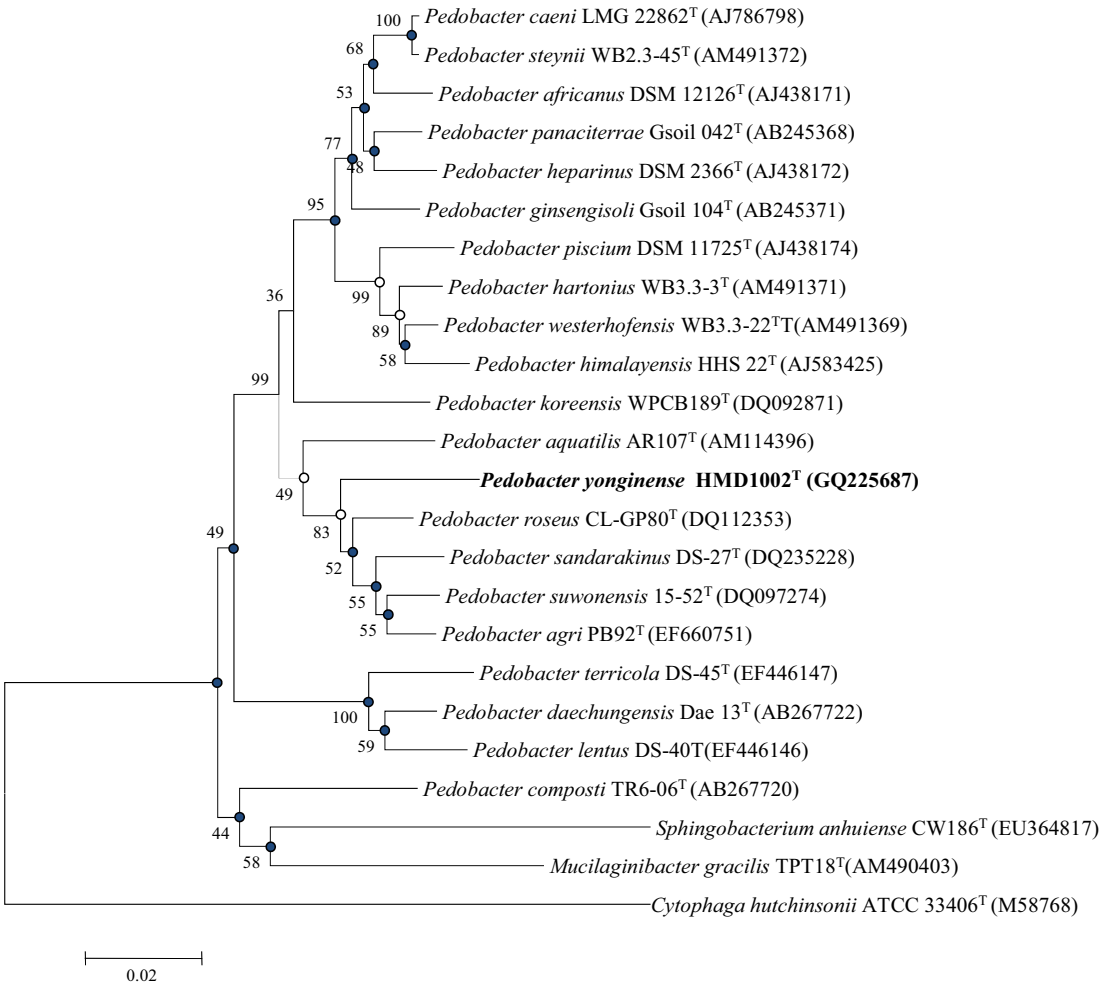

Fig. 1. Neighbor-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain HMD1002^T and representative members of the genus *Pedobacter*. Percentages at nodes are levels of bootstrap support (>50%) based on neighbor-joining analyses of 1,000 re-sampled datasets. Filled and open circles indicate nodes recovered by all three treeing methods (using MP, ML and NJ) or by two treeing methods (using ML and NJ or MP and NJ), respectively. *Cytophaga hutchinsonii* ATCC 33406^T (M58768) was used as an outgroup. Bar=0.02 nucleotide substitution per position.

galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, ρ-hydroxy Phenlyacetic acid, itaconic acid, α-keto butyric acid, α-keto glutaric acid, D,L-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, L-alanine, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, D,L-carnitine, urocanic acid, inosine, thymidine, phenylethyl-amine, putrescine, 2,3-butanediol, glycerol and D,L-glycerol phosphate. The major fatty acids are iso-$C_{15:0}$, Summed Feature 3 (comprising $C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2-OH) and iso-$C_{17:0}$ 3-OH. The complete fatty acid content is given in Table 2. The DNA G+C content is 41.0 mol%.

The type strain, HMD1002^T (=KCTC 22721^T =CECT 7544^T), was isolated from a mesotrophic artificial lake in Wangsan, Yongin, Gyeonggi, Republic of Korea (37°20′18″N, 127° 16′11″E).

The GenBank accession number for the 16S rRNA gene sequences of strain HMD1002^T is GQ225687.

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