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NOTE

Pedobacter soyangensis sp. nov., Isolated from Lake Soyang in Korea

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Strain HME6451^T was isolated from Lake Soyang in Korea. Phylogenetic tree based on 16S rRNA gene sequence showed that strain HME6451^T formed a lineage within the genus *Pedobacter*. The strain HME6451^T was closely related to *Pedobacter daechungensis* (95.4% sequence similarity), *Pedobacter lentus* (94.4%), and *Pedobacter glucosidilyticus* (93.8%). And strain HME6451^T was a Gram-staining-negative, short rod-shaped, strictly aerobic bacterium. The major fatty acids were iso-$C_{15:0}$ (41.2%), summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*; 23.1%), and iso-$C_{17:0}$-3OH (10.1%). The polar lipids of HME6451^T were consisted of one phosphatidylethanolamine, one unidentified aminolipid, one unidentified phospholipid and four unidentified polar lipids. The DNA G+C content was 36.0 mol%. On the basis of the evidence presented in this study, strain HME6451^T represent a novel species of the genus *Pedobacter*, for which the name *Pedobacter soyangensis* sp. nov., is proposed the type strain HME6451^T (=KCTC 23467^T =CECT 7865^T).

Keywords: *Pedobacter soyangensis* sp. nov., 16S rRNA gene

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 two *Sphingobacterium* species and the description of two novel species. The genus accommodates bacteria that are Gram-negative, obligately aerobic rods, non-motile and have a DNA G+C contents of 33.8–45 mol%. Strains in this genus have been found in terrestrial environments, such as fresh water (Baik *et al.*, 2007; An *et al.*, 2009), and soil environment (Ten *et al.*, 2006; Yoon *et al.*, 2007; Roh *et al.*, 2008; Gordon *et al.*, 2009).

During the course of a study on the microbial diversity of Lake Soyang, Republic of Korea (38° 02′24″ N, 128° 09′15″E), a novel bacterial strain of reddish-pigmented colonies was isolated on R2A agar (Difco, USA) and then following a 48 h incubation at 30°C. The isolate was routinely cultured on the same medium at 30°C and the culture was suspended in aqueous glycerol (20%, v/v) for storage at -80°C.

Genomic DNA was isolated by a genomic extract kit (Sogent, Korea) and the 16S rRNA gene sequence was analysed. Primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-AAGGAGGTGATCCAGCC-3′) were used for amplification of bacterial 16S rRNA genes. The PCR product was purified, and direct sequencing was performed by using sequencing primers 27F, 1492R, 518R, 785F (Solgent Inc., Korea). Phylogenetic neighbors were identified and pairwise 16S rRNA gene sequence similarity was calculated using the EzBioCloud server (Kim *et al.*, 2012). The phylogenetic relationships between strain HME6451^T and representative type strains of *Pedobacter* species were defined by MEGA5 (Tamura *et al.*, 2011). Phylogenetic 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 neighbor joining tree was evaluated by bootstrap analysis (Felsenstein, 1985), based on 1,000 re-samplings of the sequences. Strain HME6451^T formed a coherent clade with *Pedobacter daechungensis* (95.4% sequence similarity), *Pedobacter lentus* (94.4%), and *Pedobacter glucosidilyticus* (93.8%)-within the phylogenetically well-resolved *Pedobacter* clade (Fig. 1). This phylogenetic inference, together with the level of 16S rRNA gene sequence similarity (Wayne *et al.*, 1987) between strain HME6451^T and other *Pedobacter* species (<97%) suggests that the strain represents a novel species in the genus *Pedobacter*.

Cell morphology and gliding motility were examined by light microscopy on R2A at 30°C. Motility was investigated using motility test medium (Difco). Gram staining was conducted using the bioMérieux Gram Stain kit (France) according to the manufacturer's instructions. Cellular pigments were extracted with acetone-methanol (1:1, v/v), and absorption spectra were determined using a scanning UV/visible spectrophotometer (UV 6101A; Shimadzu, Japan). The presence of flexirubin-type pigments was investigated using the bathochromatic shift test with a 20% (w/v) KOH solution (Bernardet *et al.*, 2005). Anaerobic growth was tested on R2A agar at 30°C by using a GasPak EZ Anaerobic Container System (BD Science, USA) 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 (0.5 g yeast extract, 0.5 g peptone, 0.5 g casamino acids, 0.5 g dextrose, 0.5 g starch, 0.3 g sodium pyruvate, 0.3 g dipotas-

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sium phosphate, 0.05 g magnesium sulfate per 1,000 ml distilled water) adjusted with various pH buffers (initial pH 5.0–10.0 at intervals of 1.0 pH unit) at 30°C. Growth in 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 temperature 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, USA] w/v), and starch (Difco; 1.0% w/v), were tested using R2A as the basal medium. Growth was assessed and the DNase assay on marine agar 2216, trypticase soy, nutrient agar, blood agar. MacConky agar, and DNase test agar (all from Difco). Basic biochemical, enzyme activity, and carbon source utilization tests were performed using API 20NE, API 50CH, API ZYM (bioMérieux) and Biolog GN2 MicroPlates (USA), according to manufacturers' instructions. Cell biomasses for DNA extraction were obtained from R2A after 48 h incubation at 30°C. Chromosomal DNA was purified using a DNA purification kit (Solgent) according to the manufacturer's instructions. G+C content was determined by high performance liquid chromatography (HPLC) analysis of hydrolyzed DNA according to Tamaoka (1986) and Mesbah *et al.* (1989). Fatty acids were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, ver. 6.1) except that the incubation temperature was 30°C on R2A. Fatty acids were analyzed by gas chromatography (Hewlett Packard 7890; USA) and identified using the RTSBA6 database of the Microbial Identification System (Sasser, 1990). Polar lipids and isoprenoid quinones were extracted according to Minnikin *et al.* (1984) and isoprenoid quinones analyzed by HPLC as described by Collins (1985). Polar lipids were separated by two-dimensional thin-layer chromatography (TLC) (coated with silica gel, 10×10 cm; Merck, Germany). TLC plates were sprayed with various specific reagents to detect different polar lipids (Minnikin *et al.*, 1984).

The characteristics of strain HME6451^T compared with those of other related type strains of the genus *Pedobacter* are listed in Table 1 and in the species description. Strain HME6451^T exhibited a number of phenotypic similarities with respect to species of the genus *Pedobacter*, including that it was a Gram-negative, obligately aerobic rod that was non-motile and did not requirement of NaCl. Features of strain HME6451^T were typical *Pedobacter*. However, several characteristics of HME6451^T, such as its psychrotolerant nature and ability to produce indoles, clearly distinguished this strain from other strains of *Pedobacter* (Table 1). The DNA G+C content of strain HME6451^T was 36.0 mol%, which was within the range reported for the genus *Pedobacter*. The major fatty acids profile of strain HME6451^T included iso-$C_{15:0}$ (41.2%), summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*; 23.1%), and iso-$C_{17:0}$ 3-OH (10.1%). The complete fatty acid composition of strain HME6451^T is given in Table 2. The Major fatty acid profile of HME6451^T was similar to othat of ther *Pedobacer* species. The polar lipids of HME6451^T were consisted of one phosphatidylethanolamine, one unidentified aminolipid, one unidentified phospholipid and four unidentified polar lipids (Fig. 2). The major isoprenoid quinone in strain HME6451^T was major menaquinone-7 (MK-7), which was similar to that of other *Pedobacer* species (Hwang *et al.*, 2006). Therefore, strain

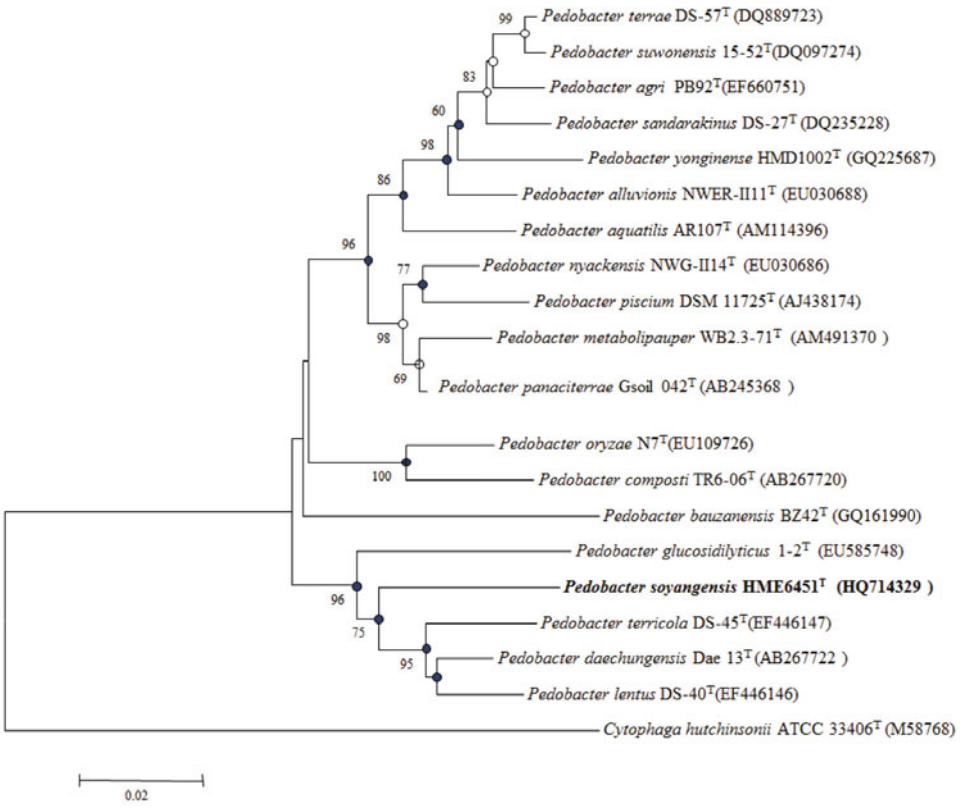

Fig. 1. Neighbor-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain HME6451^T and representative members of the genus *Pedobacter*. Percentages at nodes are levels of bootstrap support (>50%) based on neighbor-joining analyses of 1,000 re-sampled data sets. 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 substitutions per position.

Table 1. Differential characteristics differentiating strain HME6451^T from related members of the genus *Pedobacter*

Strains: 1, HME6451^T; 2, *Pedobacter lentus* KCTC 12875^T; 3, *Pedobacter daechungensis* KACC 14960^T; 4, *Pedobacter glucosidilyticus* KCTC 22438^T. Data of 1 to 4 were obtained from this study except the DNA G+C of the three reference strains. +, Positive; –, negative.

Characteristic	1	2	3	4
Growth :				
at 30°C	+	-	+	+
at 10°C	+	-	-	-
at pH 9	+	-	-	+
at 1.5% NaCl conc.	+	+	-	-
API 20NE:				
Indole production	-	-	+	-
Enzyme activity: (API ZYM)				
α-Chymotrypsin	-	+	-	-
β-Galactosidase	-	+	-	-
β-Glucosidase	-	-	-	+
N-Acetyl-β-glucosaminidase	+	+	-	+
β-Fucosidase	-	-	-	+
Utilzation of: (BIOLOG GN2)				
Dextrin	+	-	-	+
N-Acetyl-D-glucosamine	+	-	-	+
D-Fructose	+	-	-	+
D-Galactose	+	+	-	-
α-D-glucose	+	+	-	+
Lactulose	+	-	-	+
D-Mannose	+	-	-	-
D-Melibiose	+	-	-	-
Turanose	+	-	-	-
D-Glucuronic acid	+^{w}	+	-	-
α-keto Butyric acid	+^{w}	-	+	+
L-Glutamic acid	+	+	+	-
Acid production of: (API 50CH)				
D-Arabinose	+	-	-	+
L-Arabinose	+	-	-	+
D-Xylose	+	-	-	+
D-Galactose	+	-	-	+
D-Glucose	+	-	-	+
Methyl-α D-mannopyranoside	+	-	-	-
Methyl-α D-glucopyranoside	+	-	-	-
N-Acetylglucosamine	+	-	+	+
D-Cellobiose	+	-	+	+
D-Maltose	+	-	+	-
D-Melibiose	+	-	-	-
Xylitol	+	-	-	-
D-Turanose	+	-	-	-
DNA G+C content (mol%)	36.0	36.0	33.8	37.2

HME6451^T should be classified as novel species in *Pedobacer*, for which the name *Pedobacer soyangensis* sp. nov. is proposed.

Description of *Pedobacer soyangensis* sp. nov.

(*so yang en sis*. N.L. fem. adj. *soyangensis* of *soyang*, Korea, from where the type strain was isolated)

Cells are Gram-negative, non-motile, non-gliding aerobic rods. Colonies are convex, circular, and smooth, with entire margins, reddish-pink in color, and approximately 5 mm in diameter after a 48 h incubation at 30°C on R2A agar. Good growth occurs on TSA, R2A agar, nutrient agar, and blood agar. No growth occurs on marine agar, or MacConkey agar. Growth occurs in the presence of 0–1.5% (w/v) NaCl (optimum, 1.0%), at pH 7 (optimum, pH 7) and 10–30°C (optimum, 30°C). Oxidase and catalase activities are present, but arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, β-galactosidase, and L-phenylalanine deaminase activities are absent. DNA, CM-cellulose, dextran, casein, and starch are not hydrolyzed. Flexirubin-type pigments are not produced. Esculin is hydrolyzed (API 20NE). Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl-β-glucosaminidase, and α-glucosidase activities are present in the API ZYM gallery, but lipase (C14), trypsin, α-chymotrypsin, β-glucosidase, α-galactosidase, β-glucuronidase, β-galactosidase, α-mannosidase, and α-fucosidase activities are absent. Acid is produced frrm an API 50CH strip after a 3 day of incubation, D-arabinose, L-arabinose, D-xylose, D-galactose, D-glucose, methyl-α D-mannopyranoside, methyl-α D-glucopyranoside, *N*-acetyl-glucosamine, esculin ferric citrate, D-cellobiose, D-maltose, D-melibiose, xylitol, and D-turanose are substrate assimilated. The following compounds are utilized as sole carbon sources on GN2 MicroPlates: dextrin, *N*-acetyl-D-glucosamine, D-cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, lactulose, D-mannose, sucrose, turanose, L-glutamic acid, D-melibiose, D-glucuronic acid, and α-keto butyric acid. The following carbon sources are not utilized: α-cyclodextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, *i*-erythritol, L-fucose, m-inositol, α-D-lactose, maltose, D-man-

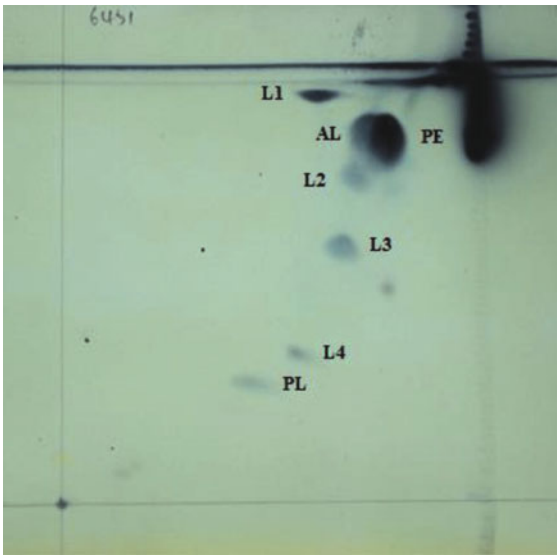

Fig. 2. The total polar lipids of strain HME6451^T. PE, Phosphatidylethanolamine; AL, unidentified aminolipid; PL, unidentified phospholipids; L1–4, unidentified polar lipids.

Table 2. Cellular fatty acid compositions (%) of strain HME6451^T and closely related strains

Strains: 1, HME6451^T; 2, *Pedobacter lentus* KCTC 12875^T; 3, *Pedobacter daechungensis* KACC 14960^T; 4, *Pedobacter glucosidilyticus* KCTC 22438^T. Data of 1 to 4 were obtained from this study. All the strains were grown under the same growth conditions (R2A agar, 25°C, 48 h of incubation). Values are percentages of total fatty acids, and only fatty acids representing more than 1% for at least one of the strains are shown. nd, not detected; tr, trace amounts (<1%).

Fatty acids	1	2	3	4
iso-$C_{11:0}$	nd	tr	1.4	1.7
iso-$C_{14:0}$	nd	2.3	nd	nd
$C_{14:0}$	tr	tr	1.1	tr
iso-$C_{15:0}$	41.2	27.8	15.8	37.3
anteiso-$C_{15:0}$	4.7	3.4	6.3	5.4
$C_{15:1}$ *ω6c*	2.3	1.4	2.3	3.7
iso-$C_{16:1}$ H	nd	tr	1.9	tr
iso-$C_{16:0}$	tr	1.1	3.9	1.1
$C_{16:1}$ *ω5c*	6.0	3.5	3.4	2.7
$C_{16:0}$	1.5	3.3	6.9	3.4
iso-$C_{15:0}$ 3OH	2.4	3.3	1.8	2.0
$C_{15:0}$ 2OH	tr	1.1	1.9	tr
$C_{17:1}$ *ω8c*	tr	tr	2.9	1.1
iso-$C_{16:0}$ 3OH	tr	1.2	4.4	tr
$C_{16:0}$ 3OH	1.3	tr	tr	tr
iso-$C_{17:0}$ 3OH	10.1	14.4	10.4	10.7
$C_{17:0}$ 2OH	tr	tr	2.0	tr
Summed feature*				
3	23.1	26.5	27.6	17.9
9	1.3	3.0	4.4	5.0

* Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 3 comprised $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c* and summed feature9 comprised iso-$C_{17:1}$ *ω9c* and/or 10-methyl $C_{16:0}$.

nitol, β-methyl-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, D-trehalose, xylitol, pyruvic acid methyl ester, succinic acid mono-methyl-ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxy phenlyacetic acid, itaconic acid, α-keto glutaric acid, α-keto valeric acid, D,L-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, D,L-carnitine, γ-amino butyric acid, urocanic acid, inosine, uridine, thymidine, phenylethyl-amine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol,D,L,α-glycerol phosphate, α-D-glucose-1-phosphate and D-glucose-6-phosphate. The major quinone is MK-7. The major fatty acids are iso-$C_{15:0}$, summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and iso-$C_{17:0}$ 3-OH. The complete fatty acid content is given in Table 2. The polar lipids of HME6451^T consist of one phosphatidylethanolamine, one unidentified aminolipid, one unidentified phospholipid and four unidentified polar lipids. The DNA G+C content is 36.0 mol%.

The type strain, HME6451^T (=KCTC 23467^T =CECT 7865^T), was isolated from Lake *Soyang*, Republic of Korea (38° 02′24″ N, 128° 09′15″E).

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