Pedobacter koreensis sp. nov., isolated from fresh water

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A motile, rod-shaped bacterium, designated strain WPCB189^T, was isolated from fresh water collected from the Woopo wetland (Republic of Korea). The cells were found to be Gram-negative, aerobic and catalase- and oxidase-positive. A phylogenetic analysis based on 16S rRNA gene sequences showed that this isolate forms a lineage within the genus *Pedobacter*, showing sequence similarities of 89.7–96.4 % with respect to recognized species of the genus, and represents a novel member of this genus. The major fatty acids were iso-C_{15:0} 2-OH and/or C_{16:1} ω7c (summed feature 3, 29.7 %), iso-C_{15:0} (26.4 %) and iso-C_{17:1} 3-OH (10.1 %). The DNA G + C content was 38.0 mol%. On the basis of data obtained from this polyphasic taxonomic study, strain WPCB189^T represents a novel species of the genus *Pedobacter*, for which the name *Pedobacter koreensis* sp. nov. is proposed. The type strain is WPCB189^T (=KCTC 12536^T=NBRC 101153^T).

The genus *Pedobacter* was first described by Steyn *et al.* (1998) and, at the time of writing, the genus comprises 12 species: *Pedobacter heparinus*, *Pedobacter piscium*, *Pedobacter africanus*, *Pedobacter saltans* (Steyn *et al.*, 1998), *Pedobacter cryoconitis* (Margesin *et al.*, 2003), *Pedobacter himalayensis* (Shivaji *et al.*, 2005), *Pedobacter caeni* (Vanparys *et al.*, 2005), *Pedobacter sandarakinus* (Yoon *et al.*, 2006), *Pedobacter roseus* (Hwang *et al.*, 2006), *Pedobacter aquatilis* (Gallego *et al.*, 2006), *Pedobacter ginsengisoli* (Ten *et al.*, 2006) and *Pedobacter panaciterrae* (Yoon *et al.*, 2007). In the course of our study on wetland microbial diversity, a rod-shaped bacterial strain, designated strain WPCB189^T, was isolated from a sample of fresh water and was the subject of a polyphasic taxonomic investigation. On the basis of the data obtained, strain WPCB189^T represents a novel species of the genus *Pedobacter*.

Strain WPCB189^T was isolated from a sample of fresh water collected from the Woopo wetland (35° 33' N 128°

25' E) in the Republic of Korea during July 2000, using the standard dilution plating technique. Isolation was achieved using PYGV (Staley, 1968) and R2A agar (Oxoid) at 25 °C for 7 days. The isolate was routinely cultured on R2A and maintained as a glycerol suspension (20 %, w/v) at –80 °C.

Bacterial DNA preparation and PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The resultant sequence from strain WPCB189^T was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were inferred from the regions available for all sequences (positions 47–1380; *Escherichia coli* numbering system) using the Fitch–Margoliash (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPHYDIT program (available at <http://plaza.snu.ac.kr/~jchun/phydit/index.php>) and PAUP 4.0 (Swofford, 1998) as described previously (Chun *et al.*, 2000).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WPCB189^T is DQ092871.

Preliminary sequence comparisons with 16S rRNA gene sequences held in GenBank indicated that the novel isolate was closely related to the genus *Pedobacter*. The newly determined sequence was then aligned manually against representatives of the genus *Pedobacter* on the basis of bacterial 16S rRNA secondary structures. Strain WPCB189^T showed the highest 16S rRNA gene sequence similarity with *P. sandarakinus* KCTC 12559^T (96.4%), followed by *P. caeni* LMG 22862^T (95.3%), *P. roseus* KCCM 42272^T (95.1%) and *P. piscium* DSM 11725^T (95.0%). These distant relationships between the novel isolate and other members of the genus *Pedobacter* were also supported in the phylogenetic trees. When analysed with respect to all species of the genus *Pedobacter* with validly published names, strain WPCB189^T was found to represent a distinct subline within the genus *Pedobacter* in all trees inferred in this study (Fig. 1). On the basis of the 16S rRNA gene similarity data and the data from the phylogenetic analysis, it is clear that the new isolate represents a novel genomic species within the genus *Pedobacter*.

Growth on various standard bacteriological media such as nutrient agar (Difco), tryptone soy agar (TSA; Oxoid) and R2A agar was tested according to the manufacturers' instructions. Cells of strain WPCB189^T grown on TSA at 28 °C for 3–7 days were used for the physiological and biochemical tests. Motility was examined by observing the cells grown in wet mounts using a phase-contrast microscope (Axioskop 4; Zeiss). The pH range (pH 4–12) and temperature range (4–42 °C) for growth, the NaCl tolerance (0, 1, 2, 3, 5, 10%, w/v) and the ability to grow in an anaerobic chamber (CO₂/H₂/N₂, 10:10:80; Sheldon Manufacturing) were checked using cells grown on TSA for up to 1 week. Catalase and oxidase activities were

determined using 3% (v/v) hydrogen peroxide and Kovács reagent (Kovács, 1956), respectively. Other physiological and biochemical tests were performed using API 20E, API 20NE, and API 50 CH kits (bioMérieux). Enzymic activities were tested using an API ZYM kit (bioMérieux) according to the manufacturer's instructions. Antibiotic resistance was determined with the disc diffusion method. The results were interpreted according to the guidelines set forth by the National Committee for Clinical Laboratory Standards (NCCLS, 2003).

Detailed results from the physiological and biochemical analyses are given in Table 1 and in the species description. It is evident that there are several phenotypic characteristics that serve to distinguish strain WPCB189^T from phylogenetically related species.

The cellular fatty acids of strain WPCB189^T were analysed, as methyl esters, by using GLC according to the instructions of the Microbial Identification System (MIDI). The G+C content of the DNA was determined by using the thermal denaturation method of Marmur & Doty (1962). The cellular fatty acid profile of strain WPCB189^T is presented in Table 2. The DNA G+C ratio of strain WPCB189^T was 38.0 mol%.

The distinctive phyletic line formed by strain WPCB189^T within the genus *Pedobacter* indicates that this strain can be assigned novel species status in that genus. In addition, a number of physiological and chemotaxonomic characteristics clearly distinguished the novel isolate from other phylogenetically related species (Tables 1 and 2). Therefore strain WPCB189^T represents a novel species within the genus *Pedobacter*, for which the name *Pedobacter koreensis* sp. nov. is proposed.

Fig. 1. Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing the relationships between strain WPCB189^T, members of the genus *Pedobacter* and *Spingobacterium multivorum*. Bootstrap percentages, based on neighbour-joining analyses of 1000 resampled datasets, are shown at the nodes. The sequence of *Hymenobacter rigui* KCTC 12533^T (GenBank accession no. DQ089669) was used as an outgroup (not shown). Bar, 0.1 nucleotide substitution per position.

Table 1. Characteristics that differentiate strain WPCB189^T from other *Pedobacter* species

Strains: 1, strain WPCB189^T; 2, *P. heparinus* DSM 2366^T; 3, *P. africanus* DSM 12126^T; 4, *P. piscium* DSM 11725^T; 5, *P. saltans* DSM 12145^T; 6, *P. cryoconitis* DSM 14825^T; 7, *P. himalayensis* JCM 12171^T; 8, *P. caeni* LMG 22862^T; 9, *P. sandarakinus* KCTC 12559^T; 10, *P. roseus* KCCM 42272^T; 11, *P. aquatilis* JCM 13454^T; 12, *P. ginsengisoli* KCTC 12576^T; 13, *P. panaciterrae* KCTC 12594^T. Data are from Takeuchi & Yokota (1992), Steyn *et al.* (1998), Margesin *et al.* (2003), Shivaji *et al.* (2005), Vanparys *et al.* (2005), Yoon *et al.* (2006), Hwang *et al.* (2006), Gallego *et al.* (2006), Ten *et al.* (2006) and Yoon *et al.* (2007). All species are positive for catalase, oxidase and the utilization of D-mannose, D-glucose, D-lactose and maltose. All species are negative for Gram stain, sporulation, indole production, urease, reduction of nitrate to nitrite and the utilization of L-sorbose, dulcitol, citrate and malate. +, Positive; –, negative; w, weakly positive; NA, data not available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Maximum growth temperature (°C)	37	37	37	30	30	25	25	37	33	33	30	25	25
Growth on MacConkey medium	–	–	–	–	–	–	+	NA	–	+	–	–	–
Motility	+	+	–	–	+	+	–	–	–	–	–	–	–
Arginine dihydrolase	–	–	–	NA	–	–	+	–	–	–	–	–	–
Lysine decarboxylase	–	+	+	+	+	–	–	NA	–	–	–	–	–
Tryptophan deaminase	–	–	–	NA	–	–	+	NA	–	NA	NA	–	–
Gelatinase	–	–	NA	–	–	+	+	–	–	+	–	–	–
Enzyme activity (API ZYM)													
Lipase (C14)	–	–*	–	–	–	NA	NA	–	–	–	–	–	–
β-Galactosidase	+	+	+	+	+	NA	NA	+	+	+	+	–	+
Assimilation of:													
N-Acetyl-β-glucosamine	–	–	–	–	+	–	+	–	–	–	–	+	+
Adonitol	–	–	–†	–	NA	–	+	–	–	+	–	–	+
Amygdalin	–	+	–†	+	+	+	+	+	–	+	+	–	+
D-Arabinose	–	–	–†	–	–	–	+	–	–	–	–	–	–
L-Arabinose	–	+	–†	+	+	+	+	–	–	+	–	+	+
Cellobiose	–	–	–	–	–	–	+	+	–	+	–	+	+
Fructose	–	+	–	–	+	–	+	–	–	–	–	–	–
Galactose	+	+	+	NA	+	+	+	–	+	+	+	+	–
Glycerol	–	+	–†	+	NA	+	+	–	–	+	+	+	–
Glycogen	+	+	+	NA	+	–	+	–	–	+	+	–	–
Inositol	–	–	–	–	–	–	–	–	–	–	–	–	+
Inulin	–	+	–	–	–	–	+	–	–	–	–	–	–
Mannitol	–	+	–	–	–	–	+	–	–	–	–	–	–
Melibiose	+	+	+	+	+	NA	NA	–	+	+	+	+	+
Raffinose	–	+	+	+	+	+	NA	+	+	+	+	+	+
Rhamnose	–	+	–†	+	+	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	–	+	+	+	+	+	+	+	+
Salicin	–	+	+	+	NA	+	+	+	+	+	+	+	+
Sorbitol	–	+	+	+	NA	+	+	+	+	+	+	+	+
Starch	–	–	–	–	–	w	+	–	–	+	–	+	+
Trehalose	–	–	–	+	–	+	+	+	–	+	+	+	+
Xylitol	+	–	–†	+	–	+	+	+	+	+	+	–	–
D-Xylose	+	–	–	–	–	+	–	–	+	+	–	–	–
L-Xylose	–	–	–	–	–	–	–	+	–	–	–	–	–
DNA G + C content (mol%)	38.0	42.3	43.7	40.4	36.9	43.4	41.0	42.7	39.7	41.3	38.0	43.6	44.2

*Data from Takeuchi & Yokota (1992).

†Data from Yoon *et al.* (2006).

Description of *Pedobacter koreensis* sp. nov.

Pedobacter koreensis (ko.re.en'sis. N.L. masc. adj. *koreensis* pertaining to Korea, from where the organism was isolated).

Cells are rod-shaped, Gram-negative, motile, aerobic bacteria. Best growth is observed on media such as TSA,

plate count agar and R2A, but grows only weakly on nutrient agar. Does not grow on MacConkey agar. Colonies on TSA agar are translucent, low-convex, circular, smooth, wet, slimy and approximately 3.0 mm in diameter after 5 days incubation at 25 °C (pH 7). Growth occurs in 0–2 % (w/v) NaCl (optimum, 0 %). Growth occurs at pH 6–11 (optimum, pH 7) and at 4–37 °C (optimum,

Table 2. Fatty acid content (%) of strain WPCP189^T and type strains of *Pedobacter* species

Strains: 1, strain WPCB189^T; 2, *P. heparinus* DSM 2366^T; 3, *P. africanus* DSM 12126^T; 4, *P. piscium* DSM 11725^T; 5, *P. saltans* DSM 12145^T; 6, *P. cryoconitis* DSM 14825^T; 7, *P. himalayensis* JCM 12171^T; 8, *P. caeni* LMG 22862^T; 9, *P. sandarakinus* KCTC 12559^T; 10, *P. roseus* KCCM 42272^T; 11, *P. aquatilis* JCM 13454^T; 12, *P. ginsengisoli* KCTC 12576^T; 13, *P. panaciterrae* KCTC 12594^T. Data are from Steyn *et al.* (1998), Margesin *et al.* (2003), Shivaji *et al.* (2005), Vanparys *et al.* (2005), Yoon *et al.* (2006), Hwang *et al.* (2006), Gallego *et al.* (2006), Ten *et al.* (2006) and Yoon *et al.* (2007). tr, Trace amount (<1 %); –, not detected; NA, not applicable (fatty acids are components of unresolved summed feature 3); ECL, equivalent chain-length.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
Straight-chain saturated													
C _{14:0}	2.0	1.1	1.5	1.3	tr	1.0	1.0	2.5	–	–*	1.0	–	–
C _{15:0}	tr	1.1	tr	–	1.4	–	–	–	4.2	–*	1.6	–	tr
C _{16:0}	3.8	3.0	3.8	3.3	4.0	9.1	7.3	3.0	–	–*	1.9	15.5	8.4
C _{14:0} 2-OH	tr	–	–	–	–	–	–	–	–	–*	–	–	tr
C _{15:0} 2-OH	tr	–	–	–	–	–	–	–	1.2	–*	–	1.0	tr
C _{16:0} 3-OH	3.0	1.5	3.1	4.5	tr	–	–	2.8	–	–*	3.5	2.1	2.5
C _{17:0} 2-OH	3.2	–	tr	–	–	–	–	1.3	–	–*	tr	2.9	2.3
Branched saturated													
iso-C _{15:0}	26.4	28.2	26.6	26.2	31.4	15.0	33.0	21.3	35.8	37.0	28.9	21.5	24.3
iso-C _{17:0}	tr	–	–	–	–	–	–	–	1.8	–*	–	–	–
iso-C _{15:0} 2-OH	NA	10.4	10.4	10.7	8.9	2.0	2.0	NA	NA	NA	NA	10.9	NA
iso-C _{15:0} 3-OH	2.1	2.5	2.1	2.5	2.8	1.9	1.5	3.3	3.0	2.7	2.7	3.6	2.2
iso-C _{16:0} 3-OH	1.8	–	–	–	–	–	–	–	–	–*	–	1.1	–
iso-C _{17:0} 3-OH	10.1	15.2	14.7	9.2	12.7	4.2	6.0	12.1	17.7	11.3	13.6	13.1	11.6
anteiso-C _{15:0}	1.2	tr	tr	2.6	2.9	–	–	tr	–	1.9	tr	1.2	tr
Monounsaturated													
C _{15:1} ω6c	tr	–	tr	–	1.4	–	–	–	1.6	1.6	2.1	–	–
C _{16:1} ω5c	4.7	1.4	2.1	3.5	tr	–	–	2.5	–	1.3	3.2	–	3.0
C _{16:1} ω7c	NA	20.2	23.7	31.4	19.6	27.0	20.0	NA	NA	NA	NA	23.3	NA
C _{17:1} ω8c	–	–	–	–	–	–	–	–	1.1	–*	–	–	–
iso-C _{17:1} ω9c	2.7	6.3	4.4	1.6	6.6	8.6	4.0	2.2	3.8	7.6	2.6	2.4	3.7
anteiso-C _{17:1} ω9c	tr	–	–	1.2	–	–	–	tr	–	–*	–	–	tr
C _{18:1} ω5c	tr	–	–	–	–	–	–	–	–	–*	–	–	1.2
Summed feature 3†	29.7	–	–	–	–	–	–	39.9	21.7	24.5	30.7	–	35.7
Unknown													
ECL 13.566	tr	5.0	2.6	tr	1.1	–	–	–	–	1.6	–	–	–
ECL 13.961	–	–	–	–	–	–	–	–	–	–*	–	1.4	–
ECL 16.580	1.0	1.0	1.0	tr	1.2	–	–	–	–	1.5	–	–	–

*Fatty acids comprising < 1 % of total volume or not detected.

†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system; summed feature 3 contained iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

25 °C). Oxidase- and catalase-positive. Does not produce H₂S or acetoin. Produces *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, aesculin hydrolase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase. Does not produce α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-mannosidase, ornithine decarboxylase or trypsin. Utilizes the following substrates as sole carbon and energy sources: arabinose, β-gentiobiose and maltose. Does not utilize the following substrates: D-arabitol, L-arabitol, arbutin, erythritol, aesculin, D-fucose, L-fucose, mannitol, melezitose, methyl α-D-mannoside, methyl α-D-glucoside, methyl β-D-xyloside, D-turanose, D-tagatose, adipate, caprate, 2-ketogluconate,

5-ketogluconate and phenylacetate. Sensitive to the following antibiotics (μg per disc, unless otherwise indicated): cefotaxime (30), chloramphenicol (30), ciprofloxacin (5), gentamicin (10), imipenem (10), isepamicin (30), lincomycin (2), novobiocin (30), piperacillin (100), rifampicin (5), tetracycline (30), teicoplanin (30) and vancomycin (30). Resistant to the following antibiotics (μg per disc, unless otherwise indicated): amikacin (30), ampicillin (10), aztreonam (30), bacitracin (10 U), cefepime (30), cefmetazole (30), ceftazidime (30), moxalactam (30), oxacillin (1), penicillin (10 U), streptomycin (100) and tobramycin (10). Other physiological and biochemical characteristics are given in Table 1. Major fatty acids are iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (29.7 %), iso-C_{15:0} (26.4 %) and iso-C_{17:1}

3-OH (10.1 %); the complete fatty acid content is given in Table 2. The DNA G + C content is 38.0 mol%.

The type strain, WPCB189^T (=KCTC 12536^T=NBRC 101153^T), was isolated from fresh water from the Woopo wetland, Republic of Korea.

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