Pedobacter roseus sp. nov., isolated from a hypertrophic pond, and emended description of the genus *Pedobacter*

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A Gram-negative, pink-coloured, rod-shaped, non-flagellated bacterium, designated CL-GP80^T, was isolated from a hypertrophic pond located within the campus of Seoul National University, Korea. Analysis of its 16S rRNA gene sequence revealed that strain CL-GP80^T belongs to the family *Sphingobacteriaceae* and is closely related to *Pedobacter heparinus* ATCC 13125^T (95.8% sequence similarity) and to other members of the genus *Pedobacter* (90.8–95.3% similarity). Temperature and pH ranges for growth were 5–33 °C and pH 6–8, respectively. The DNA G + C content was 41.3 mol%. The major fatty acids were iso-C_{15:0} (37.0%), iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (24.5%), and iso-C_{17:0} 3-OH (11.3%). Phenotypic, chemotaxonomic and phylogenetic analyses indicated that strain CL-GP80^T could be assigned to the genus *Pedobacter*, but distinguished from recognized species of the genus. Strain CL-GP80^T (=KCCM 42272^T = JCM 13399^T) is therefore proposed as the type strain of a novel species, for which the name *Pedobacter roseus* sp. nov. is proposed.

The original description of the genus *Pedobacter* included the description of three bacterial species isolated from soil (*Pedobacter heparinus*, *Pedobacter africanus* and *Pedobacter saltans*) and one isolated from fish (*Pedobacter piscium*) (Steyn *et al.*, 1998). Thereafter, novel species affiliated to this genus have been isolated from an alpine glacier cryoconite (vertical, cylindrical melt hole) in the Tyrolean Alps (*Pedobacter cryoconitis*; Margesin *et al.*, 2003), a glacier in the Himalayas (*Pedobacter himalayensis*; Shivaji *et al.*, 2005) and a nitrifying inoculum (*Pedobacter caeni*; Vanparys *et al.*, 2005). In this study, we isolated a pink-coloured bacterium from a hypertrophic pond. 16S rRNA gene sequence analysis suggested that this strain, designated CL-GP80^T, was related to species of the genus *Pedobacter*. We additionally performed phenotypic, chemotaxonomic and phylogenetic analyses. The data obtained suggest that the strain represents a novel species of the genus *Pedobacter*.

A freshwater sample from a hypertrophic pond, Gongdae pond, located within the campus of Seoul National University, was spread on a plate containing R2A agar (Difco), which was then incubated at 20 °C for 1 week. Strain CL-GP80^T, colonies of which were pink in colour, was isolated on the plate and subsequently purified four times on

R2A agar at 20 °C. The strain was stored at –80 °C in R2A broth (Difco) supplemented with 30% (v/v) glycerol until further analysis.

The temperature range for growth was determined on the basis of colony formation on R2A plates that were incubated at 5–45 °C at intervals of 5 °C. The pH range (from pH 5 to 11 at intervals of 1 pH unit) for growth was determined by changes in OD₆₀₀ with incubation (up to 7 days) in R2A broth. The final pH was adjusted using NaOH and HCl solutions. To test for salt tolerance, R2A agar (Difco) and nutrient agar (Difco) containing different concentrations of NaCl [0, 0.5, 1, 2, 3, 5 and 7% (w/v)] were used. Growth of the isolate occurred between 5 and 33 °C with optimal growth at about 25–30 °C (Table 1). The isolate grew on R2A agar and nutrient agar supplemented with 0–2% (w/v) NaCl, with optimal growth observed with no NaCl. The isolate grew at pH values from 6 to 8; optimal growth occurred at pH 7.

Morphological and physiological tests were also performed. Gram-staining was performed as described by Smibert & Krieg (1994). Cellular morphology and motility of the isolate were observed by using phase-contrast microscopy. The presence of flagella was observed via transmission electron microscopy (Jeol EX2). Anaerobic growth was tested on R2A agar using the GasPak anaerobic system (BBL). The pigment absorption spectrum between 200 and 800 nm was analysed by using an Ultraspec 2000 spectrophotometer (Pharmacia Biotech) following ethanol

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CL-GP80^T is DQ112353.

Details of the cellular fatty acid compositions of strain CL-GP80^T and other *Pedobacter* species are available as supplementary material in IJSEM Online.

Table 1. Selected phenotypic characteristics of strain CL-GP80^T and other *Pedobacter* species

Strains/species: 1, *P. roseus* CL-GP80^T (data from this study); 2, *P. heparinus* (data from Steyn *et al.*, 1998 unless indicated); 3, *P. caeni* LMG 22862^T (Vanparys *et al.*, 2005); 4, *P. africanus* (Steyn *et al.*, 1998); 5, *P. himalayensis* HHS 22^T (Shivaji *et al.*, 2005); 6, *P. cryoconitis* A37^T (Margesin *et al.*, 2003); 7, *P. piscium* (Steyn *et al.*, 1998); 8, *P. saltans* (Steyn *et al.*, 1998). *P. roseus* is distinguishable from presently described species in the genus *Pedobacter* by its colony colour. All strains are negative for the following: reduction of nitrate to nitrite, indole production, urease activity and utilization of inositol, L-malate and citric acid. All strains form round colonies and are positive for the following: aerobic growth, catalase and oxidase activities, aesculin hydrolysis, acid and alkaline phosphatases and utilization of D-glucose, lactose and D-mannose. +, Positive; −, negative; NA, no data available; V, variable.

Characteristic	1	2	3	4	5	6	7	8
Habitat	Hypertrophic freshwater	Soil	Nitrifying inoculum	Soil, sludge	Glacier water	Glacier cryoconite	Fish	Soil
Colony diameter (mm)	1–4.5	1–4	0.5–8	2–4	1.6–2	1.6–2	2–4	2–4
Colony shape	Convex, smooth, entire	Convex or slightly umbonate, entire	Convex, smooth, slimy	Convex, smooth, entire scalloped margins	Convex, mucoid, entire	Convex, mucoid, entire	Convex, opaque, smooth, entire	Convex, entire
Colony colour	Pink	Translucent yellow	Creamish white or yellow	Translucent yellow	Pale white	Creamish white	Yellow or creamish white	Light yellow
Cell shape	Short rods	Short rods	Long rods	Rods	Long rods	Long rods	Long rods	Rods
Motility	Non-motile	Gliding	Non-motile	Non-motile	Non-motile	Gliding	Non-motile	Gliding
Temperature range (°C)	5–33	5–30*	NA	NA	4–25	1–25	5–30	NA
Maximum temperature (°C)	33	37†	37	37	25	25	25–30	25–30
pH range	6–8	NA	NA	NA	6–10	5–8	NA	NA
DNA G + C content (mol%)	41.3	42–43	42.7	43–45	41.0	43.0	40–43	36–38
Biochemical tests								
Arginine dihydrolase	−	−	−	−	+	−	−	−
Gelatinase	+‡	−	−	V	+	+	−	−
β-Glucosidase	−	V	−	V	+	+	+	+
β-Galactosidase	V§	+	+	+	+	+	+	+
Utilization of carbon sources								
L-Rhamnose	+	+	−	+	+	−	V	+
D-Cellobiose	+	+	+	+	+	+	+	−
D-Fructose	+	+	−	V	+	+	+	V
D-Galactose	+	+	−	V	+	+	V	+
Glycerol	+	−	−	−	+	−	−	+
Glycogen	+	−	−	−	−	+	−	−
Inulin	+	−	−	−	+	+	−	−
D-Raffinose	+	−	+	−	+	+	+	V
L-Xylose	+	−	+	−	+	−	−	−
L-Arabinose	+	+	−	V	+	V	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8
Trehalose	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
L-Arginine	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Gluconate	+	+	+	+	+	+	+	+

*Range given for [*Flavobacterium*] *heparinum* by Reichenbach (1989).

†Reported as positive and negative by Takeuchi & Yokota (1992) and Steyn *et al.* (1998), respectively.

‡This test was also performed by using the API 20NE kit, and gave a congruent result.

§Positive and negative responses given in API 20NE and API ZYM, respectively.

extraction (Gosink *et al.*, 1998). The sample was further alkalized with 0.1 vols 0.1 M NaOH, and the absorption spectrum was obtained again to check for the presence of flexirubin pigments (Gosink *et al.*, 1998). Cells were Gram-negative, obligately aerobic, short and non-motile rods with no flagellum. Other morphological characteristics are given in Table 1. Pigment extracts of strain CL-GP80^T showed an absorption maximum at 481–482 nm and a shoulder at 499–500 nm; this profile differs from that for *Pedobacter heparinus* KCTC 12437^T (=LMG 10339^T), which has two absorption peaks at 452 and 477 nm (determined in this study; data not shown). Flexirubin pigments were not detected for either of these strains.

Catalase and oxidase activities were determined according to the protocols described by Smibert & Krieg (1994), and gelatinase, amylase and nitrate reductase activities and the Voges–Proskauer test were examined as described by Hansen & Sørheim (1991). Heparinase activity was detected by the method of Zimmermann *et al.* (1990). Utilization of organic compounds as sole carbon sources was tested on the basis of colony formation on minimal medium [1.05 % (w/v) K₂HPO₄, 0.45 % (w/v) KH₂PO₄, 0.1 % (w/v) (NH₄)₂SO₄, 1.5 % (w/v) agar; Shivaji *et al.* (2005)] containing 0.1 % carbon compound. Growth was scored as negative when growth was equal to or less than that in a negative control without any carbon source, after 1 month incubation at 25 °C. Additional biochemical tests were performed using the API 20NE, API ZYM and API 50CH kits (bioMérieux) according to the manufacturer's instructions. Susceptibility to antibiotics was examined by the disc-diffusion plate method (Bauer *et al.*, 1966). Strain CL-GP80^T showed positive responses in tests for catalase, oxidase, gelatinase and amylase, but negative responses in heparinase and Voges–Proskauer tests. Other biochemical and physiological characteristics are given in Table 1 and in the species description below.

Isoprenoid quinones were isolated according to Minnikin *et al.* (1984) and analysed by HPLC as described by Collins (1985). The major isoprenoid quinone in strain CL-GP80^T was menaquinone-7 (MK-7). The DNA G + C content of strain CL-GP80^T, determined by HPLC analysis (Mesbah *et al.*, 1989), was 41.3 mol%. This is within the range of 36–45 mol% for recognized species of the genus *Pedobacter* (Table 1). For sphingolipid analysis, strain CL-GP80^T and *P. heparinus* KCTC 12437^T were cultured in trypticase soy broth at 25 °C with shaking at 120 r.p.m. for 2 days. Cells were harvested by centrifugation and lyophilized. Extraction and chromatography of sphingolipids were performed according to Kato *et al.* (1995). The presence of sphingolipids was confirmed by two-dimensional TLC. Strain CL-GP80^T and *P. heparinus* contained sphingolipids, which is a distinct feature of members of the genus *Pedobacter* (Steyn *et al.*, 1998). Fatty acid methyl esters in whole cells grown on trypticase soy agar (TSA) at 25 °C for 2 days were analysed by GC according to the instructions of the Microbial Identification System (MIDI) at MicroID (Daejeon, Korea).

The fatty acid profile of strain CL-GP80^T was dominated by iso-C_{15:0} (37.0%), iso-C_{15:0} 2-OH and/or C_{16:1 ω 7c} (24.5%), and iso-C_{17:0} 3-OH (11.3%) (see Supplementary Table S1 in IJSEM Online), which are known to be the main cellular fatty acids in recognized members of the genus *Pedobacter* (Steyn *et al.*, 1998).

To establish phylogenetic relationships within the genus *Pedobacter*, the 16S rRNA gene sequence of strain CL-GP80^T was determined: the gene was PCR-amplified from a single colony using *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified using the AccuPrep PCR purification kit (Bioneer), and direct sequence determination of the purified 16S rRNA gene was performed with an Applied Biosystems automatic sequencer (ABI3730XL) at Macrogen Corp. (Korea). The almost-complete 16S rRNA gene sequence of strain CL-GP80^T (1381 bp) was obtained. This was compared with 16S rRNA gene sequences available in GenBank by using a BLASTN search (Altschul *et al.*, 1990). The sequence of strain CL-GP80^T was manually aligned with sequences from seven recognized species of the genus *Pedobacter* and the type species of other genera belonging to the family *Sphingobacteriaceae* obtained from the GenBank and Ribosomal Database Project (Cole *et al.*, 2003) databases using known 16S rRNA gene secondary structure information. Phylogenetic trees were constructed according to the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of tree topologies was assessed by bootstrap analyses based on 1000 replications for the neighbour-joining and maximum-parsimony methods and 100 replications for the maximum-likelihood method. Alignment analysis was carried out using the jPHYDIT program (Jeon *et al.*, 2005), and phylogenetic analyses were carried out by using MEGA 3 (Kumar *et al.*, 2004) and PAUP* 4.0 (Swofford, 1998). Likelihood parameters were estimated by the hierarchical ratio tests in MODELTEST version 3.04 (Posada & Crandall, 1998). Strain CL-GP80^T was phylogenetically related most closely to *P. heparinus* ATCC 13125^T (95.8% 16S rRNA gene sequence similarity) and to other members of the genus *Pedobacter* (90.8–95.3% similarity; Fig. 1). Phylogenetic analyses based on the 16S rRNA gene sequence revealed that strain CL-GP80^T formed a robust cluster with species of the genus *Pedobacter* (Fig. 1). The low sequence similarity values (90.8–95.8%) and its placement in the phylogenetic trees indicated that strain CL-GP80^T does not belong to any recognized species, but rather represents a novel species of the genus *Pedobacter* (Stackebrandt & Goebel, 1994; Keswani & Whitman, 2001).

Based on the polyphasic data presented, strain CL-GP80^T is proposed as the type strain of a novel species in the genus *Pedobacter*, for which the name *Pedobacter roseus* sp. nov. is proposed.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between strain CL-GP80^T and members of the genus *Pedobacter* and other related genera belonging to the *Sphingobacteriaceae*. Only bootstrap values (expressed as percentage of 1000 replications) greater than 70% are shown at nodes. Solid circles indicate that the corresponding nodes are also recovered in maximum-likelihood and maximum-parsimony trees. The sequence of *Flectobacillus major* ATCC 29496^T (M62787) was used as outgroup (not shown). Bar, 0.02 nucleotide substitutions per site.

Description of *Pedobacter roseus* sp. nov.

Pedobacter roseus (ro.se'us. L. masc. adj. *roseus* rose-coloured, pink).

Gram-negative, obligately aerobic, short rods, approximately 0.5–0.7 µm wide and 0.8–1.1 µm long. Cells are non-motile with no flagellum. Colonies on R2A agar after 2 days are pink and round (1–4.5 mm in diameter), convex and smooth and with entire margins. Growth occurs between 5 and 33 °C and pH 6 and 8; optimal temperature and pH for growth are 25–30 °C and pH 7, respectively. Grows well without NaCl, and growth occurs in the presence of up to 2% (w/v) NaCl. Grows on R2A medium, nutrient medium, TSA medium and MacConkey's medium. Positive for catalase, oxidase, gelatinase and amylase activities; negative for heparinase and Voges–Proskauer tests. Positive for aesculin hydrolysis, gelatinase, acid and alkaline phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, naphthol-phosphohydrolase, α-galactosidase, α-glucosidase and *N*-acetyl-β-glucosaminidase; negative for indole production, glucose fermentation, arginine dihydrolase, urease, lipase (C14), cystine arylamidase, β-glucuronidase, α-fucosidase and α-mannosidase. Acid is produced from DL-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-glucopyranoside, amygdalin, aesculin, D-maltose, D-lactose, D-melibiose, sucrose, inulin, D-raffinose, starch, gentiobiose and D-turanose, but not from glycerol, erythritol, D-ribose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, *N*-acetylglucosamine, arbutin, salicin, D-cellobiose, D-trehalose, D-melezitose, glycogen, xylitol, D-lyxose, D-tagatose, DL-fucose, DL-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. Utilizes L-rhamnose, D-cellobiose,

D-fructose, D-galactose, glycogen, inulin, D-raffinose, L-xylose, L-glutamic acid, pyruvate, sodium succinate, L-arabinose, trehalose, D-glucose, D-lactose, salicin, sucrose, D-mannose, N-acetylglucosamine and maltose as sole carbon sources, but not L-arginine, ribose, sorbitol, mannitol, gluconate, L-aspartic acid, L-proline, inositol, L-lysine, caprate, adipate, L-malate, citric acid or phenylacetate. Cells are sensitive to (μg per disc) chloramphenicol (30), ciprofloxacin (5) and tetracycline (30), but resistant to penicillin (10), ampicillin (10), gentamicin (10), kanamycin (30) and vancomycin (30). The DNA G+C content is 41.3 mol%. The major isoprenoid is MK-7 and sphingolipids are present. Fatty acids comprising >1 % of the total are iso-C_{15:0} (37.0 %), iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c (24.5 %), iso-C_{17:0} 3-OH (11.3 %), iso-C_{17:1} ω 9c (7.6 %), iso-C_{15:0} 3-OH (2.7 %), anteiso-C_{15:0} (1.9 %), C_{15:1} ω 6c (1.6 %), unknown ECL (equivalent chain length) 13:565 (1.6 %), unknown ECL 16:582 (1.5 %), C_{16:1} ω 5c (1.3 %) and iso-C_{17:1} I and/or anteiso-C_{17:1} B (1.1 %).

The type strain, CL-GP80^T (=KCCM 42272^T=JCM 13399^T), was isolated from a hypertrophic pond in Seoul, Korea.

Emended description of the genus *Pedobacter*

In addition to the properties given in descriptions of the genus *Pedobacter* in earlier studies (Steyn *et al.*, 1998; Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005), the colony colour of strains belonging to the genus is whitish, yellowish or pinkish on TSA and/or on nutrient agar.

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