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

Chung Yeon Hwang, Dong Han Choi and Byung Cheol Cho

A Gram-negative, pink-coloured, rod-shaped, non-flagellated bacterium, designated CL-GP80T, 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-GP80T belongs to the family *Sphingobacteriaceae* and is closely related to *Pedobacter heparinus* ATCC 13125T (95.8% 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-C15:0 (37.0%), iso-C17:0 2-OH and/or C16:0 3-OH (24.5%), and iso-C17:0 3-OH (11.3%). Phenotypic, chemotaxonomic and phylogenetic analyses indicated that strain CL-GP80T could be assigned to the genus *Pedobacter*, but distinguished from recognized species of the genus. Strain CL-GP80T (=KCCM 42272T = JCM 13399T) 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-GP80T, 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-GP80T, 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, with optimal growth observed with no NaCl. The DNA G+C content was 41.3 mol%. The major fatty acids were iso-C15:0 (37.0%), iso-C17:0 2-OH and/or C16:0 3-OH (24.5%), and iso-C17:0 3-OH (11.3%). Phenotypic, chemotaxonomic and phylogenetic analyses indicated that strain CL-GP80T could be assigned to the genus *Pedobacter*, but distinguished from recognized species of the genus. Strain CL-GP80T (=KCCM 42272T = JCM 13399T) is therefore proposed as the type strain of a novel species, for which the name *Pedobacter roseus* sp. nov. is proposed.

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Table 1. Selected phenotypic characteristics of strain CL-GP80T and other *Pedobacter* species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tbody>
<tr>
<td>Habitat</td>
<td>Hypertrophic freshwater</td>
<td>Soil</td>
<td>Nitrifying inoculum</td>
<td>Soil, sludge</td>
<td>Glacier water</td>
<td>Glacier cryoconite</td>
<td>Fish</td>
<td>Soil</td>
</tr>
<tr>
<td>Colony diameter (mm)</td>
<td>1–4•5</td>
<td>1–4</td>
<td>0–5–8</td>
<td>2–4</td>
<td>1–6–2</td>
<td>1–6–2</td>
<td>2–4</td>
<td>2–4</td>
</tr>
<tr>
<td>Colony shape</td>
<td>Convex, smooth, entire</td>
<td>Convex, smooth, slimy</td>
<td>Convex, smooth, entire, scalloped margins</td>
<td>Convex, smooth, entire</td>
<td>Convex, mucoid, entire</td>
<td>Convex, mucoid, entire</td>
<td>Convex, opaque, smooth, entire</td>
<td>Convex, entire</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Pink</td>
<td>Creamish white or yellow</td>
<td>Translucent yellow</td>
<td>Translucent yellow</td>
<td>Pale white</td>
<td>Creamish white</td>
<td>Yellow or creamish white</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Short rods</td>
<td>Short rods</td>
<td>Long rods</td>
<td>Rods</td>
<td>Long rods</td>
<td>Long rods</td>
<td>Long rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Motility</td>
<td>Non-motile</td>
<td>Gliding</td>
<td>Non-motile</td>
<td>Non-motile</td>
<td>Non-motile</td>
<td>Non-motile</td>
<td>Gliding</td>
<td>Gliding</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>5–33</td>
<td>5–30•*</td>
<td>NA</td>
<td>NA</td>
<td>4–25</td>
<td>1–25</td>
<td>5–30</td>
<td>NA</td>
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<tr>
<td>Maximum temperature (°C)</td>
<td>33</td>
<td>37†</td>
<td>37</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25–30</td>
<td>25–30</td>
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<tr>
<td>pH range</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>6–10</td>
<td>4–25</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>41•3</td>
<td>42–43</td>
<td>42•7</td>
<td>43–45</td>
<td>41•0</td>
<td>43•0</td>
<td>40–43</td>
<td>36–38</td>
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<tr>
<td>Biochemical tests</td>
<td>Arginine dihydrolase</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td></td>
<td>Gelatinase</td>
<td>+‡</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>β-Glucosidase</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>V§</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Utilization of carbon sources</td>
<td>1-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>V</td>
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<td></td>
<td>D-Cellobiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>D-Fructose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>D-Galactose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td></td>
<td>Glycerol</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td></td>
<td>Glycogen</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td></td>
<td>Inulin</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>D-Raffinose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>L-Xylose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>L-Arabinose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>V</td>
<td>+</td>
</tr>
</tbody>
</table>

*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.
The sample was further alkalinized 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-GP80T showed an absorption maximum at 481–482 nm and a shoulder at 499–500 nm; this profile differs from that for Pedobacter heparinus KCTC 12437T (= LMG 10339T), 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-GP80T 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-GP80T was menaquinone-7 (MK-7). The DNA G+C content of strain CL-GP80T, 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-GP80T and P. heparinus KCTC 12437T 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-GP80T 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-GP80T was dominated by iso-C\textsubscript{15:0} (37.0\%), iso-C\textsubscript{17:0} 3-OH and/or C\textsubscript{16:1} \textit{9c} (24.5\%), and iso-C\textsubscript{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\textsuperscript{T} was determined: the gene was PCR-amplified from a single colony using 

\textit{Tag} DNA polymerase (Bioneer) and primers 27\textit{F} and 1492\textit{R} (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 gene was performed with an Applied Biosystems automatic 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 gene was performed with an Applied Biosystems automatic.

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D-fructose, D-galactose, glycerogen, inulin, D-raffinose, L-xylene, 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, glucurate, 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 sphenolipids are present. Fatty acids comprising > 1% of the total are iso-C\textsubscript{15}:0 (37.0%), iso-C\textsubscript{15}:0 2-OH and/or C\textsubscript{16}:1\textsubscript{o7c} (24.5%), iso-C\textsubscript{17}:0 3-OH (11.3%), iso-C\textsubscript{17}:1\textsubscript{o9c} (7.6%), iso-C\textsubscript{15}:0 3-OH (2.7%), anteiso-C\textsubscript{15}:0 (1.9%), C\textsubscript{15}:1\textsubscript{o6c} (1.6%), unknown ECL (equivalent chain length) 13\textsubscript{565} (1.6%), unknown ECL 16:582 (1.5%), C\textsubscript{16}:1\textsubscript{o5c} (1.3%) and iso-C\textsubscript{17}:1 I and/or anteiso-C\textsubscript{17}:1 B (1.1%).

The type strain, CL-GP80\textsuperscript{T} (=KCCM 42272\textsuperscript{T} = JCM 13399\textsuperscript{T}), was isolated from a hypotrophic 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; Shiwaïi *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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**References**


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