

## *Pedobacter xinjiangensis* sp. nov., from the Desert, Xinjiang

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**A Gram-negative, rod-shaped, gliding, aerobic bacterium, designated 12157<sup>T</sup>, was isolated from the desert of Xinjiang, China and subjected to a polyphasic taxonomic study. The strain 12157<sup>T</sup> grew optimally at pH 7.0 and 30°C. MK-7 was the predominant respiratory menaquinone. The DNA G+C content was 42.0 mol%. Phylogenetic analysis based on the 16S rRNA gene sequences showed that the isolate was mostly related to members of the genus *Pedobacter*, with similarities ranging from 90.0% to 93.7%. Phylogenetic evidence and the results of phenotypic and genotypic analyses support the establishment of a novel species, *Pedobacter xinjiangensis* sp. nov., with strain 12157<sup>T</sup> (=CCTCC AB 208092<sup>T</sup>=NRRL B-51338<sup>T</sup>) as the type strain.**

**Keywords:** *Pedobacter xinjiangensis* sp. nov., polyphasic taxonomy, desert, 16S rRNA gene

The genus *Pedobacter* was proposed by Steyn *et al.* [22] to accommodate species characterized by Gram-negative rods that produce heparinase, are obligately aerobic, with or without gliding motility, and are negative for urease, lipase, gelatinase, arginine dihydrolase, indole production, and nitrate reduction. At the time of writing, the genus comprised 29 recognized species [1, 2, 9–14, 16, 17, 19, 22, 25, 26, 29–33], including the recently described species *Pedobacter composti* [13], *P. daechungensis* [1], *P. nyackensis*, *P. alluvionis*, and *P. borealis* [10]. In the course of our study on desert microbial diversity, a rod-shaped bacterial strain, designated as 12157<sup>T</sup>, was isolated by the standard dilution plating technique at 30°C on 0.1×tryptic soy (10-fold diluted TSB; BBL) agar plates [5] and was subjected to a polyphasic taxonomic research.

Cells of the strain 12157<sup>T</sup> grown on 0.1×TSB agar or R2A at 30°C for three days were used for the physiological

and biochemical tests. Cell morphology was examined by both phase-contrast microscopy (Olympus BX51) and transmission electron microscopy (HITACHI-8100). For transmission electron microscopy observation, cells were negatively stained with 1% (w/v) phosphotungstic acid after air drying. Gram reaction was determined by a nonstaining method using 3% KOH solution [4]. Gliding motility was tested by phase-contrast microscopy with 17-h incubated cells on microscopic slides coated with R2A agar (0.7% agar) according to the method described previously [3]. The pH range (pH 3–13, at intervals of 0.5), growth temperature (0–45°C), and NaCl tolerance [0%, 1%, 2%, 3%, 5%, 10% (w/v)] were investigated on 0.1×TSB agar for up to a week. Catalase activity was determined using 3% (v/v) hydrogen peroxide solution. Oxidation of *p*-aminodimethylaniline oxalate was used to determine oxidase activity. Hydrolysis experiments including casein, tyrosine, cellulose, chitin, and DNA were tested as the standard methods [20]. Other physiological and biochemical tests were performed by using API 20 E, API 20 NE, API ID 32 GN, and API 50 CH (bioMérieux). Enzyme activities were analyzed using an API ZYM kit (bioMérieux) according to the manufacturer's instructions. Strains *Pedobacter ginsengisoli* Gsoil 104<sup>T</sup> and *P. heparinus* DSM 2366<sup>T</sup> were obtained as the reference strains for biochemical features comparison under the same laboratory conditions.

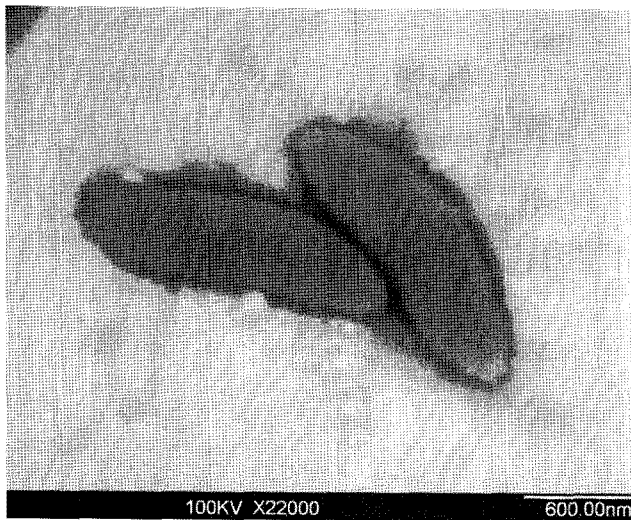
Strain 12157<sup>T</sup> was oxidase- and catalase-positive. Cells of the isolate were 0.4–0.6×1.2–2.0 μm, as shown in Fig. 1. Growth of the strain occurred at 4–37°C. The NaCl concentrations and pH range of the isolate for growth were 0–2% (w/v) and 6.0–8.0, respectively. Strain 12157<sup>T</sup> hydrolyzed DNA, but not starch, casein, cellulose, chitin, or tyrosine. The physiological characteristics of strain 12157<sup>T</sup> are summarized in the species description below, and the selective characteristics that differentiate strain 12157<sup>T</sup> from recognized members of the genus *Pedobacter* are listed in Table 1.

Biomass for molecular systematic and chemotaxonomic studies was obtained after incubation at 30°C for three

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**Fig. 1.** Transmission electron micrographs of negatively stained cells of strain 12157<sup>T</sup> (Bar, 600 nm).

days in shake flasks containing 0.1×TSB. Genomic DNA of the bacterial strain 12157<sup>T</sup> was prepared according to a modification of the procedure of Wilson [27]. The DNA G+C content was determined by HPLC [15]. Isoprenoid quinones were isolated from lyophilized cells by using the method of Collins *et al.* [7], and were analyzed by HPLC (UltiMate 3000; Dionex) as described by Xie and Yokota [28]. Polar lipids were extracted and analyzed by the methods of Tindall [24] by using two-dimensional TLC (Merck DC silica gel 60 F254 plates, layer thickness 0.2 mm; Art. 5554). To determine the whole cellular fatty acid methyl ester composition, strain 12157<sup>T</sup> was grown at 30°C for 48 h on R2A agar. Analysis of the fatty acid methyl esters was carried out by GC (Hewlett Packard 6890) according to the instructions of the Sherlock Microbial Identification System (MIDI). The DNA G+C content of strain 12157<sup>T</sup> was 42.0 mol%. Menaquinone 7 (MK-7) was the predominant isoprenoid quinone and sphingolipids were present. Both features were in agreement with members of the genus *Pedobacter*. The isolate displayed a fatty acid profile very similar to type strains of *Pedobacter* species [1, 2, 13, 17, 22, 30–33], having iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3-OH, C<sub>16:0</sub>, summed feature 3 (C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c) as the major fatty acids.

For a phylogenetic analysis of strain 12157<sup>T</sup>, its 16S rRNA gene was amplified with bacterial universal primers **27f** 5'-GAGTTTGATCCTGGCTCAG-3' and **1527r** 5'-AGAAAGGAGGTGATCCAGCC-3', which were also used for sequencing. The PCR product was sequenced by Invitrogen Biotechnology Co. Ltd. Preliminary phylogenetic analysis was done in the Eztaxon database [6]. Selected sequences were loaded into the software package MEGA version 4.0 [23], and phylogenetic trees were constructed using the neighbor-joining method [18], and the close-neighbor-

interchange (search level=2, random additions=100) was applied in the maximum-parsimony analysis. The topology of the neighbor-joining tree was evaluated by bootstrap analysis on the basis of 1,000 replications [8]. The almost-complete 16S rRNA gene sequence (1,437 nt) of strain 12157<sup>T</sup> was determined and compared with those of representatives of the phylum *Bacteroidetes*. Sequence-similarity calculations indicated that strain 12157<sup>T</sup> displayed the greatest degree of sequence similarities with members of the genus *Pedobacter*, ranging from 89.8% (with *Pedobacter saltans* DSM 12145<sup>T</sup>) to 93.6% (*Pedobacter ginsengisoli* Gsoil 104<sup>T</sup>). The neighbor-joining tree (Fig. 2) based on 16S rRNA gene sequences also showed that the isolate formed a distinct branch within the clade of the genus *Pedobacter*. DNA–DNA hybridization between strain 12157<sup>T</sup> and its nearest neighbors was not arranged, since strains differing by >3% at the 16S rRNA gene sequence level are unlikely to exhibit >70% relatedness at the whole genome level [21].

Comparative 16S rRNA gene sequence analyses showed that strain 12157<sup>T</sup> is most closely related to the genus *Pedobacter* of the family *Sphingobacteriaceae* (Fig. 2). Chemotaxonomic properties support the monothetic phylogenetic classification. The isolate also exhibited sufficient phenotypic differentiation (especially in the detection of enzyme activities using API ZYM) and phylogenetic and genetic distinctiveness. All taken together, strain 12157<sup>T</sup> should represent a novel species of the genus *Pedobacter*, for which the name *Pedobacter xinjiangensis* is proposed and the type strain is 12157<sup>T</sup> (=CCTCC AB 208092<sup>T</sup>=NRRL B-51338<sup>T</sup>).

#### Description of *Pedobacter xinjiangensis* sp. nov.

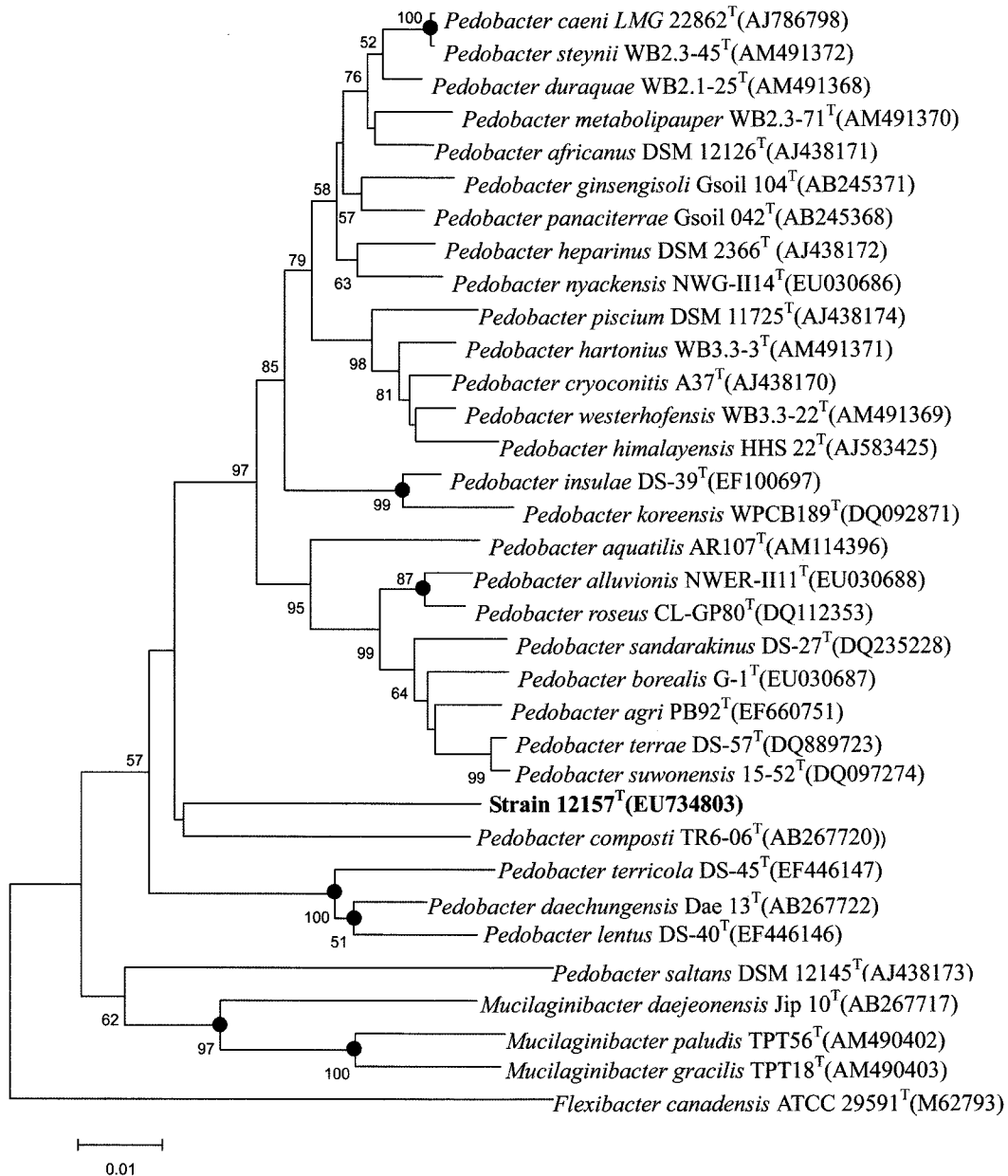
*Pedobacter xinjiangensis* (xin.jiang.en'sis. M. L. neut. adj. xinjiangensis pertaining to Xinjiang, an autonomous region of north-west China)

Cells are Gram-negative, gliding and aerobic rods. Colonies on 0.1×TSB agar are circular, smooth, and pink-pigmented. Growth occurs at pH 6–8 (optimum pH 7), at 4–37°C (optimum 30°C), and in the presence of up to 2% NaCl concentration. Besides features listed in Table 1, additional physiological information of the species is as follows. Positive for hydrolysis of DNA and Voges–Proskauer tests. Negative for ornithine decarboxylase, and hydrolysis of casein, cellulose, chitin, starch, or gelatin. Acid is produced from arbutin, D-arabinose, D-fructose, D-galactose, D-ribose, methyl-α-D-glucopyranoside, methyl-β-D-xylopyranoside, and xylitol; but not from amygdalin, dulcitol, D-adonitol, D-arabitol, D-cellobiose, D-fucose, D-glucose, D-lactose, D-lyxose, D-maltose, D-mannitol, D-mannose, D-melezitose, D-melibiose, D-sorbitol, D-raffinose, D-tagatose, D-trehalose, D-turanose, D-xylose, erythritol, esculin, gentiobiose, glycerol, glycogen, inositol, inulin, L-arabinose, L-arabitol, L-fucose, L-rhamnose, L-sorbose, L-

**Table 1.** Comparison of phenotypic characteristics of strain 12157<sup>T</sup> and related species of the genus *Pedobacter*.

Characteristic	1	2	3	4	5	6	7
Cell shape	Rods	Rods	Rods	Short rods	Pleomorphic	Short rods	Short rods
Colony Color	Pink	White	White	Reddish orange	Pale yellow to pale orange	Pale orange	Translucent yellow
Maximum growth temperature (°C)	37	30	30	30	31	37	37
Motility	Gliding	-	-	-	-	-	Gliding
Arginine dihydrolase	-	+	-	-	-	-	-
Hydrolysis of aesculin	+	-	-	+	+	+	+
Enzyme activities (API ZYM)							
Acid phosphatase	+	-	+	+	+	+	+
Esterase lipase (C8)	w	+	+	+	+	+	+
Esterase (C4)	w	+	-	+	+	+	+
Valine arylamidase	w	w	+	+	+	+	+
Cystine arylamidase	-	+	-	+	+	+	+
α-Chymotrypsin	-	-	-	-	+	-	+
Naphthol-AS-BI-phosphohydrolase	+	+	+	-	+	-	+
α-Galactosidase	-	+	+	-	-	-	+
β-Galactosidase	+	-	+	-	+	+	+
α-Glucosidase	+	+	+	-	+	w	+
β-Glucosidase	-	-	-	+	-	+	+
N-Acetyl-β-glucosaminidase	+	+	+	-	+	+	+
α-Mannosidase	-	-	-	-	-	-	+
α-Fucosidase	-	-	w	-	-	-	-
Assimilation of:							
L-Arabinose	+	-	+	+	+	+	+
L-Rhamnose	+	-	-	+	+	+	+
Inositol	-	-	+	+	-	-	-
Salicin	-	+	+	-	-	-	+
Major fatty acids (>6%)	iso-C <sub>15:0</sub> ; iso-C <sub>17:0</sub> 3-OH; C <sub>16:0</sub> ; C <sub>16:1</sub> ω7c and/or C <sub>16:1</sub> ω6c	*C <sub>16:1</sub> ω7c; iso-C <sub>15:0</sub> ; C <sub>16:0</sub> ; iso-C <sub>17:0</sub> 3-OH; iso-C <sub>15:0</sub> 2-OH	C <sub>16:1</sub> ω7c and/or iso-C <sub>15:0</sub> 2-OH; iso-C <sub>15:0</sub> ; iso-C <sub>17:0</sub> 3-OH; C <sub>16:0</sub>	C <sub>16:1</sub> ω7c and/or iso-C <sub>15:0</sub> 2-OH; iso-C <sub>15:0</sub> ; C <sub>16:0</sub>	iso-C <sub>15:0</sub> ; iso-C <sub>17:0</sub> 3-OH; C <sub>16:1</sub> ω7c and/or iso-C <sub>15:0</sub> 2-OH	iso-C <sub>15:0</sub> ; iso-C <sub>17:0</sub> 3-OH; C <sub>16:1</sub> ω7c; anteI C <sub>15:0</sub> ; C <sub>15:0</sub>	*iso-C <sub>15:0</sub> ; iso-C <sub>17:0</sub> 3-OH; C <sub>16:1</sub> ω7c; iso-C <sub>17:1</sub> ω9c
DNA G+C content (mol %)	42.0	*43.6	44.2	33.8	36.0	42	*42.3%–43%

Taxa: 1. Strain 12157<sup>T</sup> (present study); 2. *Pedobacter ginsengisoli* Gsoil 104<sup>T</sup> (present study); 3. *P. panaciterrae* Gsoil 042<sup>T</sup> [29, 31]; 4. *P. daechungensis* Dae 13<sup>T</sup> [1]; 5. *P. lentus* DS-40<sup>T</sup> [29]; 6. *P. composti* TR 6-06<sup>T</sup> [13]; 7. *P. heparinus* DSM 2366<sup>T</sup> (present study). All strains were positive for oxidase, catalase, alkaline phosphatase, leucine arylamidase, and assimilation of N-acetyl-L-glucosamine, D-mannose, and D-glucose. All were negative for Gram stain, sporulation, production of H<sub>2</sub>S and indole, urease, lipase (C14), trypsin, β-glucuronidase, and nitrate reduction. Symbols: +, positive; w, weakly positive; -, negative. \*Data from [21, 23].



**Fig. 2.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing distant relationships between strain 12157<sup>T</sup> and some other related taxa.

Bootstrap values (1,000 replications) are shown as percentages at branch points only if they are 50% or greater. *Flexibacter canadensis* ATCC 29591<sup>T</sup> was used as the outgroup. Filled circles at each node indicated nodes recovered reproducibly by using maximum-parsimony algorithms. Bar, 0.01 substitutions per nucleotide position.

xylose, methyl- $\alpha$ -D-mannopyranoside, *N*-acetylglucosamine, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, salicin, starch, or sucrose. Unable to utilize capric acid, D-maltose, D-mannose, D-melibiose, D-mannitol, D-ribose, D-sorbitol, glycogen, 3-hydroxybenzoic acid, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, itaconic acid, lactic acid, L-alanine, L-fucose, L-histidine, L-proline, L-serine, potassium 2-ketogluconate, potassium 5-ketogluconate, propionic acid, sodium acetate, sodium malonate, suberic acid, sucrose, trisodium citrate, or valeric acid as sole

carbon sources. The predominant menaquinone is MK-7 and sphingolipids are present. The whole cellular fatty acids profile contains (>1%) iso-C<sub>15:0</sub> (24.87%), iso-C<sub>17:0</sub> 3-OH (15.14%), C<sub>16:0</sub> (7.15%), Sum In Feature 3 (C<sub>16:1</sub>  $\omega$ 7c and/or C<sub>16:1</sub>  $\omega$ 6c) (6.86%), Sum In Feature 9 (17:1 iso- $\omega$ 9c and/or C<sub>16:0</sub> 10-methyl) (5.71%), iso-G C<sub>15:1</sub> (5.47%), C<sub>18:0</sub> (4.74%), iso-C<sub>15:0</sub> 3-OH (4.13%), Sum In Feature 8 (C<sub>18:1</sub>  $\omega$ 7c and/or C<sub>18:1</sub>  $\omega$ 6c) (3.48%), C<sub>18:1</sub>  $\omega$ 9c (3.40%), iso-C<sub>16:1</sub>  $\omega$ 5c (8.31%), iso-C<sub>15:1</sub> (4.65%), C<sub>16:0</sub> (4.17%), C<sub>18:0</sub> (3.97%), Sum In Feature 4 (C<sub>17:1</sub> iso-I and/or anteiso-B) (2.83%),

Sum In Feature 1 (C<sub>13:0</sub> 3-OH and/or C<sub>15:1</sub> i H) (1.85%), iso-C<sub>15:1</sub> ω6c (1.38%), iso-C<sub>17:0</sub> (1.37%), and Sum In Feature 5 (C<sub>18:2</sub> ω6, 9c and/or C<sub>18:0</sub> ante) (1.30%) acids. DNA G+C content is 42.0 mol% for the type strain.

The type strain is 12157<sup>T</sup> (=CCTCC AB 208092<sup>T</sup>=NRRL B-51338<sup>T</sup>), isolated from the desert of Xinjiang, China.

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