Pedobacter glucosidilyticus sp. nov., isolated from dry riverbed soil

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Two Gram-staining-negative, rod-shaped, non-spore-forming bacterial strains, 1-2^T and 1-4 were isolated from dry riverbed soil collected from the Xietongmen area of Tibet, China. On the basis of 16S rRNA gene sequence similarity, the novel strains were shown to belong to the genus *Pedobacter*, sharing <95 % sequence similarity with all recognized species of the genus *Pedobacter*. The major respiratory quinone was MK-7 and the predominant cellular fatty acids were iso-C₁₅:0, iso-C₁₇:0 3-OH and summed feature 3 (comprising iso-C₁₆:1ω7c and/or C₁₆:1ω6c). The DNA G + C contents were 37.2–37.6 mol%. Chemotaxonomic data supported the affiliation of the two new isolates to the genus *Pedobacter* and the results of physiological and biochemical tests confirmed that the new strains differed significantly from the recognized species of the genus *Pedobacter*. Therefore, the new isolates represent a novel species within the genus *Pedobacter*, for which the name *Pedobacter glucosidilyticus* sp. nov. is proposed. The type strain is 1-2^T (=CCTCC AB 206110^T=KCTC 22438^T).

At the time the family *Sphingobacteriaceae* and the genus *Pedobacter* were proposed by Steyn *et al.* (1998), the genus comprised only four recognized species: *Pedobacter heparinus*, *Pedobacter piscium*, *Pedobacter africanus* and *Pedobacter saltans*. Many novel species have been recovered from various environments and, at the time of writing, the genus *Pedobacter* contained 26 recognized species (Steyn *et al.*, 1998; Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005; Gallego *et al.*, 2006; Hwang *et al.*, 2006; Ten *et al.*, 2006; Baik *et al.*, 2007; Kwon *et al.*, 2007; Muurholm *et al.*, 2007; Yoon *et al.*, 2006, 2007a, b, c, d; An *et al.*, 2009; Roh *et al.*, 2008; Lee *et al.*, 2009). The description of the genus *Pedobacter* has also been emended recently (Vanparys *et al.*, 2005; Gallego *et al.*, 2006; Hwang *et al.*, 2006).

In the course of a study of the cultivable microbial community of Tibet, China, a soil sample was collected from a dry riverbed in the Xietongmen area and used as a source for the isolation of bacterial strains. Over 50 colonies were isolated by means of the standard dilution plating technique at 28 °C on plates of 10-fold diluted tryptic soy agar (TSA, Difco). All strains were preserved as lyophilized cultures. Two cream to pink coloured, raised, translucent and slimy colonies developed on the plates inoculated with the terminal dilutions of the soil

suspension. They were picked up and purified and were designated strains 1-2^T and 1-4. The strains were routinely grown on 0.1 × TSA at 28 °C. Bacterial cells were observed by light and phase-contrast microscopy (BX51; Olympus) to ascertain their morphology. Gliding motility was assessed as described previously (Bowman, 2000). Cells were Gram-staining-negative, non-motile rods that occurred singly or in chains (see Supplementary Fig. S1 available at IJSEM online).

The genomic DNA of strains 1-2^T and 1-4 was isolated using a bacteria genomic kit (CASarray Co., Ltd). The fragments comprising the 16S rRNA gene were amplified by PCR using the primers described by Lane (1991). The PCR products were sequenced by Invitrogen Corporation and the 16S rRNA gene sequences (1386 bp) of the two strains were obtained. The identification of phylogenetic neighbours and the calculation of 16S rRNA gene sequence similarity were accomplished using the NCBI BLAST and the EzTaxon (<http://www.eztaxon.org/>; Chun *et al.*, 2007) servers.

The two strains shared 100 % 16S rRNA gene sequence similarity and showed the highest sequence similarity, 94.2 %, with *Pedobacter daechungensis* Dae 13^T, followed by *Pedobacter lentus* KCTC 12875^T (93.2 %). Sequence similarity with all other species of the genus *Pedobacter* was <93 %. Phylogenetic trees were constructed using the neighbour-joining and maximum-parsimony methods and the Kimura two-parameter model in MEGA version 3.1 (Kumar *et al.*, 2004). The topology of the phylogenetic tree

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 1-2^T is EU585748.

A light micrograph of cells of strain 1-2^T is available as supplementary material in the online version of this paper.

was evaluated by using the bootstrap resampling method with 1000 replicates. The new taxon formed a distinct branch within the genus *Pedobacter* in the neighbour-joining tree (Fig. 1). The maximum-parsimony tree showed essentially the same topology (data not shown).

Growth at 5, 15, 28, 30, 32, 37, 42 and 45 °C, at pH 5.5, 6, 7, 8, 9, 9.5, 10, 10.5 and 11 (adjusted using 0.1 M HCl and NaOH and sterilized through a 0.2 µm filter) and in the presence of 0, 0.5, 1, 2, 3 and 5 % NaCl (w/v) was assessed using 0.1 × tryptic soy broth (TSB, Difco) as the basal medium. Growth on MacConkey agar was also tested. Flexirubin pigments were detected by means of a colour shift after exposure to a 20 % (w/v) KOH solution (Reichenbach, 1992). Catalase and oxidase activities and hydrolysis of starch, casein, tyrosine and Tween 40 were assessed as described by Smibert & Krieg (1994) using 0.1 × TSA as the basal medium. Heparinase activity was detected as described previously (Zimmermann *et al.*, 1990). The utilization of various compounds as sole carbon sources was tested using Biolog GN2 MicroPlates (Biolog) in accordance with the manufacturer's instructions. The plates were incubated at 30 °C for 48 h and scored with a microplate reader using Microlog 4.2 computer software

for automated reading. *P. lentus* KCTC 12875^T, *Pedobacter terricola* KCTC 12876^T and *P. daechungensis* KCTC 12637^T were used as reference strains and studied in parallel with strains 1-2^T and 1-4 using API 20NE, API 20E, API 50CH and API ZYM strips (bioMérieux) according to the manufacturer's instructions.

Based on physiological and biochemical properties, strains 1-2^T and 1-4 showed characteristics consistent with the original and emended descriptions of the genus *Pedobacter* (Steyn *et al.*, 1998; Vanparys *et al.*, 2005; Gallego *et al.*, 2006; Hwang *et al.*, 2006). The few discrepancies that were noted between the two novel isolates, and the phenotypic characteristics that differentiated them from their closest phylogenetic neighbours are shown in Table 1.

For cellular fatty acid analysis, strain 1-2^T, *P. terricola* KCTC 12876^T and *P. daechungensis* KCTC 12637^T were grown on R2A agar (Difco) for 72 h at 30 °C. The cellular fatty acids were extracted, esterified and analysed by GC (6890N; Agilent) according to the instructions of the Sherlock Microbial Identification System (MIDI). The fatty acid pattern of strain 1-2^T was consistent with those of other species of the genus *Pedobacter*; the quantitative

Fig. 1. Neighbour-joining phylogenetic tree showing the relationship between strain 1-2^T, other species of the genus *Pedobacter* and representatives of related taxa. Numbers at branch nodes are bootstrap values based on 1000 resamplings; only values >50 % are given. *Flavobacterium lindanitolerans* IP-10^T was used as the outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Differential characteristics of strain 1-2^T, 1-4 and phylogenetically related species of the genus *Pedobacter*

Taxa: 1, strains 1-2^T and 1-4; 2, *Pedobacter lentus* KCTC 12875^T; 3, *P. terricola* KCTC 12876^T; 4, *P. daechungensis* KCTC 12637^T. All API data are from this study. All strains are positive for the following characteristics: hydrolysis of aesculin, activities of catalase, oxidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α -glucosidase (except *P. daechungensis*), valine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-biphosphohydrolase, acid production from maltose, D-mannose, L-arabinose, D-xylose, D-galactose (only *P. daechungensis* was negative for acid production from the above sugars) and D-glucose, and for susceptibility to chloramphenicol and tetracycline. All strains are negative for the following characteristics: production of H₂S and indole, nitrate reduction, activities of urease, arginine dihydrolase, β -glucuronidase, trypsin, α -chymotrypsin, α -galactosidase and α -mannosidase, acid production from erythritol, ribose, adonitol, inulin, raffinose, inositol, D-sorbitol, turanose, L-fucose, D-lyxose, D-fucose, L-arabitol, D-arabitol, 2-ketogluconate, L-xylose and mannitol. +, Positive; –, negative; w, weakly positive; v, variable (i.e. variable results between different strains of the same species or variable results observed in three independent experiments).

Characteristic	1	2	3	4
Colony colour	Cream to pink	Pale yellow to pale orange	Pale orange	Reddish orange
Maximum growth temperature (°C)	37	31	34	30
Enzyme activities (API ZYM)				
N-Acetyl- β glucosaminidase	+	+	+	–
α -Fucosidase	+	–	–	–
β -Galactosidase	w	+	+	–
β -Glucosidase	+	–	–	+
Lipase	w	–	–	w
Acid from (API 50CH)				
N-Acetylglucosamine	+	–	–	–
Amygdalin	+	–	+	–
Arbutin	+	–	–	–
Cellobiose	+	–	+	+
D-Fructose	+	–	–	v
Glycerol	w	–	+	v
D-Lactose	+	–	–	–
Maltose	+	v	w	–
Melibiose	w	–	–	–
Methyl α -D-glucopyranoside	v	v	w	–
Methyl α -D-mannopyranoside	v	–	–	–
Methyl α -D-xylopyranoside	–	–	+	–
L-Rhamnose	w	w	w	–
Salicin	+	–	–	–
L-Sorbose	–	+	+	–
DNA G + C content (mol%)	37.2–37.6	36.0	36.8	33.8

differences between its closest phylogenetic neighbours are shown in Table 2. The DNA G + C contents of the two isolates were determined by HPLC (UltiMate 3000, Dionex) (Mesbah *et al.*, 1989). The G + C contents of strains 1-2^T and 1-4 were 37.2 and 37.6 mol%, respectively. The respiratory quinones were extracted and identified by HPLC as described by Komagata & Suzuki (1987). Menaquinone-7 was detected as the predominant menaquinone.

DNA–DNA hybridization experiments were performed between strains 1-2^T and 1-4 using the fluorometric method of Ezaki *et al.* (1989); the two strains shared a DNA–DNA relatedness of 94 %. All the evidence gathered in this study indicates that strains 1-2^T and 1-4 belong to the same species and that they can be differentiated from other species of the genus *Pedobacter* by a number of phenotypic characteristics. The low 16S rRNA gene sequence similarity of the novel strains with recognized species of the genus *Pedobacter*

confirmed that strains 1-2^T and 1-4 represent a novel species, for which the name *Pedobacter glucosidilyticus* sp. nov. is proposed with strain 1-2^T (=CCTCC AB 206110^T=KCTC 22438^T) as the type strain.

Description of *Pedobacter glucosidilyticus* sp. nov.

Pedobacter glucosidilyticus (glu.co.si.di.ly'ti.cus. N.L. n. *glucosidum* glucoside; Gr. adj. *lutikos* able to loosen, able to dissolve; N.L. adj. *lyticus* -a -um dissolving; N.L. masc. adj. *glucosidilyticus* glucoside-dissolving).

Cells stain Gram-negative and are non-spore-forming, non-flagellated and non-motile rods of 0.6–0.7 μ m diameter and length 1.2–5.0 μ m. Colonies on 0.1 $\times$ TSA (Difco) plates are circular, convex, smooth, cream to pink coloured and 1.0–2.0 mm in diameter after incubation for 3 days at 28 °C. Growth occurs from 15 to 37 °C (optimum,

Table 2. Cellular fatty acid contents (%) of strain 1-2^T, *P. terricola* KCTC 12876^T and *P. daecheungensis* KCTC 12637^T

Taxa: 1, 1-2^T; 2, *Pedobacter terricola* KCTC 12876^T; 3, *P. daecheungensis* KCTC 12637^T. All strains were grown on R2A agar for 72 h at 30 °C. All data are from this study. —, Not detected; tr: trace (<0.5 %). Summed feature 3 comprised iso-C_{16:1}ω7c and/or C_{16:1}ω6c. Fatty acids amounting to <0.5 % of the total fatty acids in all strains tested are not listed.

Fatty acid	1	2	3
Branched chain			
iso-C _{15:0} 3-OH	2.2	2.8	1.2
iso-C _{15:0}	31.5	30.7	20.4
anteiso-C _{15:0}	4.9	4.2	—
iso-C _{16:0}	0.9	1.8	2.2
iso-C _{17:0} 3-OH	13.1	12.3	7.3
iso-C _{17:1} ω9c	3.5	2.0	1.9
anteiso-C _{17:0}	tr	2.7	2.0
Unsaturated			
C _{15:1} ω6c	3.3	2.3	—
C _{16:1} ω5c	1.3	tr	1.6
Saturated			
C _{16:0}	5.4	7.6	9.6
C _{15:0}	—	—	1.3
Hydroxyl			
C _{16:0} 3-OH	1.9	—	4.1
Summed feature 3	12.5	11.5	13.4

30 °C), but not at 42 °C. Growth occurs from pH 5.5 to 9 (optimum, 7.5). Growth occurs in the presence of 0–3 % NaCl (w/v) (optimum, 0.5 %), but not in the presence of 5 % NaCl. No growth occurs on MacConkey agar. Oxidase, catalase, β-glucosidase and α-fucosidase activities are present, but DNase, heparinase, amylase and urease activities are absent. Negative for nitrate reduction, production of H₂S and indole and for hydrolysis of tyrosine, casein and Tween 40. Flexirubin-type pigments are absent. Dextrin, N-acetyl-D-glucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannose, sucrose, trehalose, turanose, pyruvic acid methyl ester, D-glucuronic acid and α-D-glucose 1-phosphate are utilized in the Biolog GN2 microplate. Acid is produced from N-acetylglucosamine, arbutin, D-fructose, D-lactose and salicin. The predominant menaquinone is MK-7. The major fatty acids (>10 %) are iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (comprising iso-C_{16:1}ω7c and/or C_{16:1}ω6c). The DNA G+C content is 37.2–37.6 mol% (HPLC).

The type strain, 1-2^T (=CCTCC AB 206110^T=KCTC 22438^T), was isolated from soil of the Tietongmen area in Tibet, China. A reference strain, 1-4 (=CCTCC AB 206111), was isolated from the same source.

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