Pedobacter daechungensis sp. nov., from freshwater lake sediment in South Korea

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A novel bacterial strain, designated Dae 13^T, was isolated from sediment from a freshwater lake in Daejeon, South Korea, and was characterized taxonomically by using a polyphasic approach. The isolate was Gram-negative, aerobic, non-motile, non-spore-forming and rod-shaped.

Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate belonged to the genus *Pedobacter* in the family *Sphingobacteriaceae* but was clearly separate from established species of this genus. The 16S rRNA gene sequence similarities between strain Dae 13^T and type strains of *Pedobacter* species with validly published names ranged from 91.6 to 97.5%. The G + C content of the genomic DNA was 33.8 mol%. Chemotaxonomic data, i.e. the presence of MK-7 as the major menaquinone and iso-C_{15:0}, C_{16:0} and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) as the major fatty acids, supported the affiliation of strain Dae 13^T to the genus *Pedobacter*. However, the results of physiological and biochemical tests allowed phenotypic differentiation of the isolate with respect to *Pedobacter* species with validly published names. Therefore, strain Dae 13^T represents a novel species within the genus *Pedobacter*, for which the name *Pedobacter daechungensis* sp. nov. is proposed. The type strain is Dae 13^T (=KCTC 12637^T=LMG 23489^T).

The genus *Pedobacter* was proposed by Steyn *et al.* (1998) to accommodate species characterized as obligately aerobic, heparinase-producing, Gram-negative rods with or without gliding motility, negative for urease, lipase, gelatinase, arginine dihydrolase, indole production and nitrate reduction and containing fatty acids iso-C_{15:0}, iso-C_{15:0} 2-OH, iso-C_{15:0} 3-OH, C_{16:0}, C_{16:1}ω5c, C_{16:1}ω7c, C_{16:0} 3-OH, iso-C_{17:0} 3-OH and iso-C_{17:1}ω9c. At the time of writing, the genus comprised 16 recognized species, including the recently described species *Pedobacter lentus* and *Pedobacter terricola* (Yoon *et al.*, 2007a). In this study, a *Pedobacter*-like strain, designated Dae 13^T, was isolated and characterized taxonomically.

Strain Dae 13^T was isolated from sediment from the Daechung freshwater lake near Daejeon in South Korea, using direct plating onto R2A agar (Difco). Single colonies on these plates were purified by transferring them onto

new plates and subjecting them to an additional incubation for 3 days at 30 °C. Purified colonies were tentatively identified using partial 16S rRNA gene sequences. Strain Dae 13^T was routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (20%, w/v) at –70 °C.

The Gram reaction was performed by using the non-staining method as described by Buck (1982). Cell morphology was observed at ×1000 magnification with a light microscope (Nikon), using cells grown on R2A agar for 3 days at 30 °C. Motility was tested by using the hanging drop technique. Catalase activity was determined by assessing bubble production in 3% (v/v) H₂O₂ and oxidase activity was determined using 1% (w/v) tetramethyl-*p*-phenylenediamine. Carbon-source utilization and enzyme activities were tested by using API 20E, API 20NE and API ZYM test kits (bioMérieux). Anaerobic growth was tested in serum bottles by adding sodium thioglycolate (1 g l^{–1}) to R2A broth (Difco) and substituting the upper air layer with nitrogen gas. Growth at

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Dae 13^T is AB267722.

different temperatures (4, 15, 20, 25, 30, 37 and 42 °C) and various pH values (pH 5.0–10.0, using increments of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested after 5 days incubation on R2A agar supplemented with 1–10% (w/v) NaCl. Growth on nutrient agar, trypticase soy agar (Difco) and MacConkey agar (Difco) was also evaluated, at 30 °C.

Cells of strain Dae 13^T were strictly aerobic, Gram-negative, non-motile, non-spore-forming and rod-shaped. Colonies grown on R2A agar plates for 4 days were sticky, smooth, circular, reddish orange in colour and 0.5–1 mm in diameter. The isolate grew well on nutrient agar and trypticase soy agar, but could not grow on MacConkey agar. Other physiological characteristics of strain Dae 13^T are summarized in the species description. Phenotypic characteristics that serve to differentiate the isolate from its closest phylogenetic relatives are listed in Table 1.

To measure the G + C content of the chromosomal DNA, genomic DNA was extracted from the novel strain and purified as described by Moore & Dowhan (1995) before being enzymically degraded into nucleosides and determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Isoprenoid quinones were extracted with

chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and re-extracted in *n*-hexane/water (1:1, v/v). The crude *n*-hexane-quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and subsequently analysed using HPLC as described by Hiraishi *et al.* (1996). Cellular fatty acid profiles were determined for strains grown on trypticase soy agar for 5 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed using a gas chromatograph (6890; Hewlett Packard) and identified with the Microbial Identification software package (Sasser, 1990).

The G + C content of genomic DNA of strain Dae 13^T was 33.8 mol%. The predominant menaquinone was MK-7. The fatty acid profile of strain Dae 13^T comprised branched-chain fatty acids iso-C_{15:0} (22.4%), iso-C_{17:0} 3-OH (1.5%), iso-C_{17:1ω9c} (3.4%), iso-C_{15:0} 3-OH (2.1%) and iso-C_{17:0} (0.9%), unsaturated fatty acid C_{16:1ω5c} (0.6%), saturated fatty acids C_{16:0} (10.7%) and C_{15:0} (1.2%), hydroxyl fatty acid C_{16:0} 3-OH (2.6%) and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c}) (48.1%). This fatty acid profile, including the major fatty acids [iso-C_{15:0}, C_{16:0} and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c})], is

Table 1. Comparison of phenotypic characteristics of strain Dae 13^T and related species of the genus *Pedobacter*

Taxa: 1, strain Dae 13^T (*Pedobacter daechungensis* sp. nov.); 2, *P. lentus* DS-40^T; 3, *P. terricola* DS-45^T; 4, *Pedobacter heparinus*. Data for taxa 2–4 are from Yoon *et al.* (2007a). All strains were positive for catalase, oxidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, hydrolysis of aesculin and assimilation of L-arabinose, D-glucose, D-mannose, L-rhamnose, maltose and N-acetylglucosamine. All were negative for Gram stain, sporulation, urease, lipase (C14), trypsin, β-glucuronidase, α-fucosidase, production of H₂S and indole, nitrate reduction, growth on MacConkey medium and assimilation of D-arabinose, D-fucose, L-arabitol, adipate, caprate, phenylacetate, ribose, D-sorbitol, salicin, melibiose, citrate and malate.

Characteristic	1	2	3	4
Cell shape	Short rods	Pleomorphic	Pleomorphic	Short rods
Colony colour	Reddish orange	Pale yellow to pale orange	Pale yellow to pale orange	Translucent yellow
Maximum growth temperature (°C)	30	31	34	37
Motility	—	—	—	Gliding
Arginine dihydrolase	+	—	—	—
Assimilation of:				
Gluconate	+	—	—	—
Inositol	+	—	—	—
Mannitol	+	—	—	+
Sucrose	+	—	—	—
API ZYM tests				
Acid phosphatase	—	+	+	+
α-Chymotrypsin	—	+	+	+
Naphthol-AS-BI-phosphohydrolase	—	+	+	+
α-Galactosidase	—	—	—	+
β-Galactosidase	—	+	+	+
α-Glucosidase	—	+	+	+
β-Glucosidase	+	—	—	+
N-Acetyl-β-glucosaminidase	—	+	+	+
α-Mannosidase	—	—	—	+
DNA G + C content (mol%)	33.8	36.0	36.8	42.3–43.0

similar to those of *Pedobacter* species, although there are differences in the proportions of some fatty acids, perhaps because of differences in the extraction and cultivation conditions (Shivaji *et al.*, 2005; Vanparys *et al.*, 2005; Yoon *et al.*, 2006, 2007a, b; Hwang *et al.*, 2006; Ten *et al.*, 2006).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out as described by Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with the CLUSTAL_X program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed with the neighbour-joining method (Saitou & Nei, 1987), using the MEGA3 program (Kumar *et al.*, 2004) and bootstrap values based on 1000 replications (Felsenstein, 1985). DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed using five replications for each sample. The highest and lowest values obtained for each sample were excluded and the remaining three values were used to calculate similarity values. The DNA hybridization values quoted are the means of these three values.

An almost-complete 16S rRNA gene sequence (1467 bp) for Dae 13^T was determined and subjected to phylogenetic analysis, which indicated that the strain belongs to the genus *Pedobacter* (Fig. 1). Strain Dae 13^T showed the highest 16S rRNA gene sequence similarity with respect to *P. lentus* DS-40^T (97.5 %) and showed 33 % DNA–DNA hybridization; this was followed by *P. terricola* DS-45^T (95.9 %) and other species in the genus *Pedobacter* (<94 %). These values (<97 %) and the DNA–DNA hybridization values were sufficiently low to classify strain Dae 13^T as representing a novel species within the genus *Pedobacter* (according to Stackebrandt & Goebel, 1994).

On the basis of the data described above, strain Dae 13^T should be assigned to the genus *Pedobacter* as the type strain of a novel species, for which the name *Pedobacter daechungensis* sp. nov. is proposed.

Description of *Pedobacter daechungensis* sp. nov.

Pedobacter daechungensis (dae.chun.gen'sis. N.L. masc. adj. *daechungensis* pertaining to Daechung lake).

Cells are Gram-negative, strictly aerobic, non-motile and 0.3–0.4 µm wide and 0.8–1.3 µm long after 4 days culture on R2A agar. Colonies grown on R2A agar for 4 days are sticky, smooth, circular, convex and reddish orange in colour. Good growth occurs at 30 °C and at pH 7.0.

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain Dae 13^T and related species. The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with a Kimura two-parameter distance matrix (Kimura, 1983) and pairwise deletion. Bootstrap values (expressed as percentages of 1000 replications) greater than 60 % are shown at branch points. Bar, 0.02 substitutions per nucleotide position.

Growth is observed at 15–30 °C, at pH 5.0–8.0 and with 0–1 % (w/v) NaCl. Nitrate is not reduced. Anaerobic growth does not occur. The substrates utilized, the enzymes produced and other physiological characteristics are indicated in Table 1. In addition, citrate, L-lactate, mannitol and L-serine are utilized as sole carbon sources. The following are not utilized as sole carbon sources: acetate, L-alanine, glycogen, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, itaconate, 2-ketogluconate, malonate, propionate, L-proline, suberate and valerate. The predominant menaquinone is MK-7. The major fatty acids are iso-C_{15:0}, C_{16:0} and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c). The G+C content of the genomic DNA of the type strain is 33.8 mol%. Other phenotypic characteristics are given in Table 1.

The type strain, Dae 13^T (=KCTC 12637^T=LMG 23489^T), was isolated from sediment collected from a freshwater lake in Daejeon, South Korea.

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References

- Buck, J. D. (1982). Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hiraishi, A., Ueda, Y., Ishihara, J. & Mori, T. (1996). Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* **42**, 457–469.
- Hwang, C. Y., Choi, D. H. & Cho, B. C. (2006). *Pedobacter roseus* sp. nov., isolated from a hypertrophic pond, and emended description of the genus *Pedobacter*. *Int J Syst Evol Microbiol* **56**, 1831–1836.
- Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005). *Sphingopyxis granuli* sp. nov., a β-glucosidase producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Moore, D. D. & Dowhan, D. (1995). Preparation and analysis of DNA. In *Current Protocols in Molecular Biology*, pp. 2–11. Edited by F. W. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shivaji, S., Chaturvedi, P., Reddy, G. S. N. & Suresh, K. (2005). *Pedobacter himalayensis* sp. nov., from the Hamta glacier located in the Himalayan mountain ranges of India. *Int J Syst Evol Microbiol* **55**, 1083–1088.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Steyn, P. L., Segers, P., Vancanneyt, M., Sandra, P., Kersters, K. & Joubert, J. J. (1998). Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae* fam. nov. *Int J Syst Bacteriol* **48**, 165–177.
- Ten, L. N., Im, W.-T., Kim, M.-K., Kang, M.-S. & Lee, S.-T. (2004). Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* **56**, 375–382.
- Ten, L. N., Liu, Q.-M., Im, W.-T., Lee, M., Yang, D.-C. & Lee, S.-T. (2006). *Pedobacter ginsengisoli* sp. nov., a DNase-producing bacterium isolated from soil of a ginseng field in South Korea. *Int J Syst Evol Microbiol* **56**, 2565–2570.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Vanparys, B., Heylen, K., Lebbe, L. & De Vos, P. (2005). *Pedobacter caeni* sp. nov., a novel species isolated from a nitrifying inoculum. *Int J Syst Evol Microbiol* **55**, 1315–1318.
- Yoon, J.-H., Lee, M.-H., Kang, S.-J., Park, S.-Y. & Oh, T.-K. (2006). *Pedobacter sandarakinus* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 1273–1277.
- Yoon, J.-H., Kang, S.-J., Park, S. & Oh, T.-K. (2007a). *Pedobacter lentus* sp. nov. and *Pedobacter terricola* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **57**, 2089–2095.
- Yoon, M.-H., Ten, L. N., Im, W.-T. & Lee, S.-T. (2007b). *Pedobacter panaciterrae* sp. nov., isolated from soil in South Korea. *Int J Syst Evol Microbiol* **57**, 381–386.