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

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A novel bacterial strain, designated Dae 13T, 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 13T 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-C15:0, C16:0 and summed feature 3 (iso-C15:0 2-OH and/or C16:1ω7c) as the major fatty acids, supported the affiliation of strain Dae 13T 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 13T represents a novel species within the genus Pedobacter, for which the name Pedobacter daechungensis sp. nov. is proposed. The type strain is Dae 13T (=KCTC 12637T=LMG 23489T).

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-C15:0, iso-C15:0 2-OH, iso-C15:0 3-OH, C16:0 C16:1ω9c, C16:1ω7c, C16:0 3-OH, iso-C17:0 3-OH and iso-C17:0ω9c. 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 13T, was isolated and characterized taxonomically. Strain Dae 13T 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 13T 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...
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 13T 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 13T 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 13T was 33.8 mol%. The predominant menaquinone was MK-7. The fatty acid profile of strain Dae 13T comprised branched-chain fatty acids iso-C15:0 (22.4 %), iso-C17:0 3-OH (1.5 %), iso-C17:1ω9c (3.4 %), iso-C15:0 3-OH (2.1 %) and iso-C17:0 (0.9 %), unsaturated fatty acid C16:1ω5c (0.6 %), saturated fatty acids C16:0 (10.7 %) and C15:0 (1.2 %), hydroxyl fatty acid C16:0 3-OH (2.6 %) and summed feature 3 (iso-C15:0 2-ΟΗ and/or C16:1ω7c) (48.1 %). This fatty acid profile, including the major fatty acids [iso-C15:0, C16:0 and summed feature 3 (iso-C15:0 2-ΟΗ and/or C16:1ω7c)], is

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Cell shape</td>
<td>Short rods</td>
<td>Pleomorphic</td>
<td>Pleomorphic</td>
<td>Short rods</td>
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<tr>
<td>Colony colour</td>
<td>Reddish orange</td>
<td>Pale yellow to pale orange</td>
<td>Pale yellow to pale orange</td>
<td>Translucent yellow</td>
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<td>Maximum growth temperature (°C)</td>
<td>30</td>
<td>31</td>
<td>34</td>
<td>37</td>
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<tr>
<td>Motility</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Gliding</td>
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<td>Arginine dihydrolase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
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<tr>
<td>Gluconate</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>Inositol</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<td>Sucrose</td>
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<td>–</td>
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<td>+</td>
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<td>α-Glucosidase</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>β-Glucosidase</td>
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<td>–</td>
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<tr>
<td>N-Acetyl-β-glucosaminidase</td>
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<tr>
<td>α-Mannosidase</td>
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<td>–</td>
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<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>33.8</td>
<td>36.0</td>
<td>36.8</td>
<td>42.3–43.0</td>
</tr>
</tbody>
</table>

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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_W 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 13T was determined and subjected to phylogenetic analysis, which indicated that the strain belongs to the genus *Pedobacter* (Fig. 1). Strain Dae 13T showed the highest 16S rRNA gene sequence similarity with respect to *P. lentus* DS-40T (97.5 %) and showed 33 % DNA–DNA hybridization; this was followed by *P. terricola* DS-45T (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 13T as representing a novel species within the genus *Pedobacter* (according to Stackebrandt & Goebel, 1994).

On the basis of the data described above, strain Dae 13T 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 13T and related species. The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with a Kimura two-parameter distance matrix (Kumura, 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.](http://ijs.sgmjournals.org)
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-C₁₅:₀, C₁₆:₀ and summed feature 3 (iso-C₁₅:₀ 2-OH and/or C₁₆:₁ω7c). 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, Daee 13² (=KCTC 12637²=LMG 23489²T), was isolated from sediment collected from a freshwater lake in Daejeon, South Korea.

Acknowledgements

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References


