**Parapedobacter soli** sp. nov., isolated from soil of a ginseng field

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Strain DCY14ᵀ, a Gram-negative, non-spore-forming, rod-shaped, non-motile bacterium, was isolated from soil from a ginseng field in Korea and was characterized in order to determine its taxonomic position. 16S rRNA gene sequence analysis revealed that strain DCY14ᵀ belongs to the family Sphingobacteriaceae, the highest degree of sequence similarity being found with respect to Parapedobacter koreensis Jip14ᵀ (95.8%). Chemotaxonomic data revealed that strain DCY14ᵀ possesses MK-7 as the major menaquinone. The major fatty acids present were anteiso-C₁₃:₀, iso-C₁₅:₀, iso-C₁₇:₀ 3-OH and summed feature 4 (C₁₆:₁ω7c/iso-C₁₅:₀ 2-OH). The results of physiological and biochemical tests clearly demonstrated that strain DCY14ᵀ represents a distinct species. On the basis of these data, DCY14ᵀ represents a novel species of the genus Parapedobacter, for which the name Parapedobacter soli sp. nov. is proposed. The type strain is DCY14ᵀ (=KCTC 12984ᵀ =LMG 24069ᵀ).

The family Sphingobacteriaceae was proposed by Steyn et al. (1998). Typical features of the family Sphingobacteriaceae are the possession of sphingolipids, the presence of the MK-7 quinone system, a low G+C content (37–44 mol%) and the presence of the distinctive fatty acid iso-C₁₅:₀ 2-OH. At the time of writing, the family Sphingobacteriaceae consists of four genera, Sphingobacterium (Yabuuchi et al., 1983), Pedobacter (Steyn et al., 1998), Olivibacter (Ntougias et al., 2007) and Parapedobacter (Kim et al., 2007).

In a series of studies, we attempted to isolate microorganisms from soil samples in order to investigate the community structure, using a culture-dependent method. In this study, a strain was isolated from soil from a ginseng field in Daejeon in South Korea and was then characterized by means of a polyphasic approach designed to determine the precise taxonomic position of this organism, strain DCY14ᵀ. This approach included phylogenetic analyses based on 16S rRNA gene sequences, analysis of genomic relatedness and determination of the chemotaxonomic and phenotypic properties. The results obtained in this study indicated that DCY14ᵀ represents a novel member of the family Sphingobacteriaceae.

Strain DCY14ᵀ was isolated from soil from a ginseng field near Daejeon, 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 5 days at 30 °C. The purified colonies were tentatively identified by means of partial 16S rRNA gene sequences.

Cell morphology and motility were investigated with a Nikon light microscope (magnification, ×1000), using cells allowed to grow for 3 days at 30 °C on R2A agar. Gram reactions were determined using the non-staining method, as described by Buck (1982). Oxidase activity was evaluated from the oxidation of 1 % p-aminodimethylaniline oxalate. Catalase activity was determined from measurements of bubble production after the application of a 3 % (v/v) hydrogen peroxide solution. Growth at a variety of temperatures (4, 15, 25, 30, 37 and 42 °C) was assessed on R2A agar and growth at a variety of pH values was assessed in R2A broth. Growth at 30 °C on nutrient agar, trypticase soy agar and MacConkey agar was also evaluated. The API 20NE and API ID 32GN microtest systems were employed in these tests, according to the recommendations of the manufacturer (bioMérieux).

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified by TLC and subsequently analysed by HPLC, as described previously (Collins & Jones, 1981; Shin et al., 1996). For fatty acid methyl ester analysis, the strain was allowed to grow on trypticase soy agar for 48 h at 30 °C and then two loops of the well-grown cells were harvested. Fatty acid methyl esters were prepared, separated and identified with the Sherlock
Table 1. Phenotypic characteristics of strain DCY14T and related type strains

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<td>Growth at:</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>4 °C</td>
<td>-</td>
<td>-</td>
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<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>37 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+^*</td>
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<tr>
<td>42 °C</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>Enzyme activity</td>
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<td>+</td>
<td>ND</td>
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<td>+^*</td>
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<tr>
<td>Esterase (C8)</td>
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<td>+</td>
<td>ND</td>
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<tr>
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<td>+</td>
<td>-</td>
<td>V</td>
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<td>Glycogen</td>
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<td>+</td>
<td>ND</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>50.1</td>
<td>45.6</td>
<td>45.6</td>
<td>42.3–43.0</td>
<td>39.8</td>
</tr>
</tbody>
</table>

^*Data from Takeuchi & Yokota (1992).

Microbial Identification System (MIS; MIDI) (Sasser, 1990).

For the determination of G+C content, genomic DNA of strain DCY14T was extracted and purified with Qiagen for the assimilation of D-glucose and sucrose. All of the strains are positive for the presence of catalase and oxidase and for the assimilation of acetate, citrate and l-proline. The following tests were positive for all strains: 1, DCY14T (data from this study); 2, Parapedobacter koreensis Jip14T (data from this study); 3, O. sitiensis AW-6^T (Ntougias et al., 2007); 4, Pedobacter hepatus LMG 10339^T (Steyn et al., 1998); 5, Sphingobacterium spiritivorum LMG 8347^T (Steyn et al., 1998). All of the strains are positive for indole production, protease (gelatin hydrolysis) and L-xylanine. +, Positive; −, negative; V, variable; ND, No data available.

Table 2. Comparison of the cellular fatty acid profiles of strain DCY14T and related type strains

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<th>4</th>
<th>5</th>
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<tr>
<td>anteiso-C15:0</td>
<td>11.4</td>
<td>-</td>
<td>-</td>
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<tr>
<td>C14:0</td>
<td>1.1</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>C15:0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
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</tr>
<tr>
<td>C15:0 3-OH</td>
<td>-</td>
<td>1.7</td>
<td>-</td>
<td>-</td>
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<tr>
<td>anteiso-C15:0</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
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<td>tr</td>
</tr>
<tr>
<td>iso-C15:0</td>
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<td>33.6</td>
<td>29.3</td>
<td>28.2</td>
<td>30.1</td>
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<tr>
<td>iso-C15:0 3-OH</td>
<td>3.7</td>
<td>2.3</td>
<td>1.9</td>
<td>2.5</td>
<td>2.2</td>
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<tr>
<td>iso-C15:1 F</td>
<td>-</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
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<tr>
<td>C15:106c</td>
<td>-</td>
<td>1.1</td>
<td>-</td>
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<tr>
<td>C16:0</td>
<td>2.7</td>
<td>6.0</td>
<td>5.5</td>
<td>3.0</td>
<td>3.5</td>
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<tr>
<td>C16:0 10-methyl</td>
<td>7.1</td>
<td>-</td>
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<tr>
<td>C16:0 2-OH</td>
<td>-</td>
<td>1.1</td>
<td>0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C16:0 3-OH</td>
<td>2.3</td>
<td>1.9</td>
<td>0.7</td>
<td>1.5</td>
<td>2.7</td>
</tr>
<tr>
<td>anteiso-C16:0</td>
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<tr>
<td>C16:1 2-OH</td>
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<td>C16:105c</td>
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<td>1.4</td>
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<td>0.4</td>
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<tr>
<td>iso-C17:0 3-OH</td>
<td>16.3</td>
<td>14.4</td>
<td>11.2</td>
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<tr>
<td>iso-C17:109c</td>
<td>7.1</td>
<td>-</td>
<td>2.0</td>
<td>6.3</td>
<td>1.7</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Summed features*</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>iso-C15:0 2-OH/iso-C16:107c</td>
<td>19.4</td>
<td>27.8</td>
<td>43.2</td>
<td>30.6</td>
<td>42.6</td>
</tr>
<tr>
<td>iso-C17:1 l/anteiso-C17:1 B</td>
<td>0.8</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C19:111e/unknown ECL</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>18.756</td>
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<tr>
<td>ECL 13.566</td>
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<td>0.8</td>
<td>0.9</td>
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<tr>
<td>ECL 16.580</td>
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</tr>
<tr>
<td>ECL 16.582</td>
<td>-</td>
<td>0.7</td>
<td>1.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Summed features are fatty acids that could not be separated by GLC with the Microbial Identification System (MIDI).

Genomic-tip system 100/G and was then enzymically degraded into nucleosides, as described previously (Tamaoka & Komagata, 1984; Mesbah et al., 1989).

For 16S rRNA gene sequencing, genomic DNA was extracted and purified with a genomic DNA isolation kit (Core Bio System). The 16S rRNA gene was amplified from the chromosomal DNA of strain DCY14T using the universal bacterial primer set 9F and 1512R (Weisburg et al., 1991) and the purified PCR products were sequenced by Genotec (Daejeon, Korea) (Kim et al., 2005). The full
sequence of the 16S rRNA gene was compiled with SeqMan software, and the 16S rRNA gene sequences of the test strain were edited using the BioEdit program (Hall, 1999). 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), evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983) and the phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) in the MEGA2 program (Kumar et al., 2001). A bootstrap analysis (based on 1000 replicates) was conducted to obtain confidence levels for the branches of the phylogenetic tree (Felsenstein, 1985).

Strain DCY14T was cultured on R2A agar at 30 °C, yielding yellow, circular colonies. The strain was found to comprise aerobic, Gram-negative, non-motile, rod-shaped (0.8–1.0 × 0.2–0.4 µm) bacterial cells. Strain DCY14T grew at 15–37 °C, but did not grow at 4 or 42 °C. Physiological features of strain DCY14T are summarized in the species description, and a comparison of selective characteristics with those of related type strains is shown in Table 1.

The cellular fatty acid profiles of strain DCY14T and related taxa are shown in Table 2. The major cellular fatty acids in strain DCY14T included the following: anteiso-C13:0 (11.4 %), iso-C15:0 (29.3 %), iso-C17:1ω9c (7.1 %), iso-C17:0 3-0H (16.3 %) and summed feature 4 (C16:1ω7c(iso-C15:0 2-0H, 19.4 %)). Minor amounts of saturated fatty acids were present: anteiso-C15:0 (0.6 %), C16:0 (2.7 %), anteiso-C17:0 (3.1 %), C18:0 (1.2 %), C15:0 3-0H (3.7 %) and C16:0 3-0H (2.3 %).

Strain DCY14T contained menaquinones with seven and 13 isoprene units (MK-7 and MK-13), with MK-7 as the predominant quinone. MK-7 is commonly found amongst species of the family Sphingobacteriaceae (Steyn et al., 1998).

The 16S rRNA gene sequence determined for strain DCY14T was a continuous stretch of 1468 nt. The 16S rRNA gene sequences of related taxa were obtained from GenBank. Strain DCY14T was shown to belong to the class Sphingobacteria (order Sphingobacterales, family Sphingobacteriaceae). The highest level of sequence similarity was found with respect to Parapedobacter koreensis Jip14T (95.8 % similarity). The phylogenetic tree (Fig. 1) shows clearly that strain DCY14T belongs to the lineage of the family Sphingobacteriaceae, as evidenced by the high level of bootstrap support. On the basis of the 16S rRNA gene sequencing data, the phylogenetic position of strain DCY14T among members of the family Sphingobacteriaceae is unique and distinct.

The G+C content of the genomic DNA of strain DCY14T was found to be 50.1 mol%, this being higher than the contents for known species within the family Sphingobacteriaceae.

On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain DCY14T represents a novel species of the genus Pedobacter (family Sphingobacteriaceae), for which the name Pedobacter soli sp. nov. is proposed.

Description of Parapedobacter soli sp. nov.

Parapedobacter soli (so’lī. L. neut. gen. n. soli of soil, the source of the type strain).

Cells grown on R2A agar at 30 °C for 5 days are Gram-negative, aerobic, non-motile rods. Colonies grown on R2A agar for 5 days are circular and yellow. Optimal growth temperature on trypticase soy agar is 30 °C. Oxidase- and catalase-positive. Produces N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, esterase (C4), esterase (C8), α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase, α-mannosidase and naphthol-AS-BI-phosphohydrolase but does not

![Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain DCY14T and related species. Bar, 0.01 substitutions per nucleotide position.](http://ijs.sgmjournals.org)
produce arginine dihydrolase, α-chymotrypsin, cystine arylamidase, α-fucosidase, β-glucuronidase, lipase (C14), protease (gelatin hydrolysis), trypsin, urease or valine arylamidase. Assimilates L-arabinose, D-glucose, maltose, D-mannose, melibiose, L-rhamnose, D-ribose, sucrose, D-mannitol, D-sorbitol, N-acetyl-D-glucosamine, salicin and glycogen. Does not assimilate 2-ketogluconate, 3-hydroxybenzoate, 3-hydroxybutyrate, 4-hydroxybenzoate, 5-ketogluconate, acetate, adipate, caprate, citrate, gluconate, itaconate, DL-lactate, L-malate, malonate, phenylacetate, propionate, suberate, n-valerate, L-fucose, myo-inositol, L-alanine, L-histidine, L-proline or L-serine. DNA G+C content is 50.1 mol% (as determined by HPLC). The predominant quinone is MK-7. The major cellular fatty acids include anteiso-C_{15:0} (11.4 %), iso-C_{15:0} (29.3 %), iso-C_{17:0} anteiso (7.1 %), iso-C_{17:0} 3-OH (16.3 %) and summed feature 4 (C_{16:1}ω7c/iso-C_{15:0} 2-OH, 19.4 %). Minor saturated fatty acids anteiso-C_{15:0} (0.6 %), C_{16:0} (2.7 %), anteiso-C_{17:0} (3.1 %), C_{18:0} (1.2 %), C_{15:0} 3-OH (3.7 %) and C_{16:0} 3-OH (2.3 %) are present. Nitrate is not reduced to either nitrite or nitrogen gas.

The type strain, DCY14^{T} (=KCTC 12984^{T} =LMG 24069^{T}), was isolated from soil from a ginseng field near Daejeon, South Korea.

Acknowledgements
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References