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

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Strain DCY14^T, 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 DCY14^T belongs to the family *Sphingobacteriaceae*, the highest degree of sequence similarity being found with respect to *Parapedobacter koreensis* Jip14^T (95.8%). Chemotaxonomic data revealed that strain DCY14^T possesses MK-7 as the major menaquinone. The major fatty acids present were anteiso-C_{13:0}, iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 4 (C_{16:1}ω7c/iso-C_{15:0} 2-OH). The results of physiological and biochemical tests clearly demonstrated that strain DCY14^T represents a distinct species. On the basis of these data, DCY14^T represents a novel species of the genus *Parapedobacter*, for which the name *Parapedobacter soli* sp. nov. is proposed. The type strain is DCY14^T (=KCTC 12984^T =LMG 24069^T).

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-C_{15:0} 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 micro-organisms 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 DCY14^T. 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 DCY14^T represents a novel member of the family *Sphingobacteriaceae*.

Strain DCY14^T 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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCY14^T is EF151805.

Table 1. Phenotypic characteristics of strain DCY14^T and related type strains

Strains: 1, DCY14^T (data from this study); 2, *Parapedobacter koreensis* Jip14^T (data from this study); 3, *Olivibacter sitiensis* AW-6^T (Ntougias *et al.*, 2007); 4, *Pedobacter heparinus* LMG 10339^T (Steyn *et al.*, 1998); 5, *Sphingobacterium spiritivorum* LMG 8347^T (Steyn *et al.*, 1998). All of the strains are positive for the presence of catalase and oxidase and for the assimilation of D-glucose and sucrose. All of the strains are negative for the Gram-stain reaction and for the assimilation of acetate, citrate and L-proline. The following tests were positive for all strains (no data available for *O. sitiensis* AW-6^T): N-acetyl- β -glucosaminidase, acid phosphatase, alkaline phosphatase, α -glucosidase, β -galactosidase (PNPG), leucine arylamidase, D-mannose, mannitol, salicin and growth on nutrient agar. All of the strains were negative for indole production, protease (gelatin hydrolysis) and L- α -alanine. +, Positive; –, negative; v, variable; ND, no data available.

Characteristic	1	2	3	4	5
Motility	–	–	–	Gliding	–
Growth at:					
4 °C	–	–	+	ND	–
37 °C	+	+	+	–	+
42 °C	–	+	+	–	–
Enzyme activity					
Chymotrypsin	–	+	ND	–	+
Cystine arylamidase	–	+	ND	–	+
Esterase (C8)	+	–	ND	+	+
α -Fucosidase	–	–	ND	–	+
α -Galactosidase	+	+	ND	–	+
β -Glucosidase	+	+	ND	–	+
β -Glucuronidase	–	–	ND	–	+
Lipase (C14)	–	–	ND	–	+
α -Mannosidase	+	–	ND	–	+
Trypsin	–	+	ND	–	+
Urease	–	–	ND	–	+
Valine arylamidase	–	+	ND	–	+
Assimilation of:					
L-Arabinose	+	–	ND	+	–
L-Fucose	–	+	ND	+	–
Maltose	+	+	–	+	+
Melibiose	+	–	ND	+	+
Rhamnose	+	–	ND	+	+
Ribose	+	+	ND	–	–
Inositol	–	+	–	–	–
Sorbitol	+	+	+	+	–
L-Histidine	–	–	+	–	–
L-Serine	–	+	–	v	–
Glycogen	+	–	ND	–	–
DNA G + C content (mol%)	50.1	45.6	45.6	42.3–43.0	39.8

*Data from Takeuchi & Yokota (1992).

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

For the determination of G + C content, genomic DNA of strain DCY14^T was extracted and purified with Qiagen

Table 2. Comparison of the cellular fatty acid profiles of strain DCY14^T and related type strains

Strains: 1, DCY14^T (data from this study); 2, *Parapedobacter koreensis* Jip14^T (data from this study); 3, *O. sitiensis* AW-6^T (Ntougias *et al.*, 2007); 4, *Pedobacter heparinus* LMG 10339^T (Steyn *et al.*, 1998); 5, *S. spiritivorum* LMG 8347^T (Steyn *et al.*, 1998). Values are percentages of total fatty acids; components making up more than 10 % of the total fatty acids are highlighted in bold. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* isomers are indicated by the suffix *c*. tr, Trace amount; ECL, equivalent chain length; –, not detected/not reported.

Fatty acid	1	2	3	4	5
anteiso-C _{13:0}	11.4	–	–	–	–
C _{14:0}	–	1.1	0.5	1.1	1.0
C _{15:0}	–	–	–	1.1	–
C _{15:0} 3-OH	–	1.7	–	–	–
anteiso-C _{15:0}	0.6	–	–	tr	tr
iso-C _{15:0}	29.3	33.6	29.3	28.2	30.1
iso-C _{15:0} 3-OH	3.7	2.3	1.9	2.5	2.2
iso-C _{15:1} F	–	–	0.3	–	–
C _{15:1} ω 6c	–	1.1	–	–	–
C _{16:0}	2.7	6.0	5.5	3.0	3.5
C _{16:0} 10-methyl	–	7.1	–	–	–
C _{16:0} 2-OH	–	1.1	0.9	–	–
C _{16:0} 3-OH	2.3	1.9	0.7	1.5	2.7
anteiso-C _{16:0}	–	0.5	–	–	–
C _{16:1} 2-OH	–	–	0.6	–	–
C _{16:1} ω 5c	–	–	1.3	1.4	tr
anteiso-C _{17:0}	3.1	–	–	–	–
iso-C _{17:0}	–	–	0.4	–	–
iso-C _{17:0} 3-OH	16.3	14.4	11.2	15.2	12.5
iso-C _{17:1} ω 9c	7.1	–	2.0	6.3	1.7
C _{18:0}	1.2	–	–	–	–
Summed features*					
iso-C _{15:0} 2-OH/C _{16:1} ω 7c	19.4	27.8	43.2	30.6	42.6
iso-C _{17:1} I/anteiso-C _{17:1} B	0.8	–	0.5	–	–
C _{19:1} ω 11c/unknown ECL 18.756	1.2	–	–	–	–
ECL 13.566	–	0.8	0.9	5.0	tr
ECL 16.580	1.1	–	–	1.0	tr
ECL 16.582	–	0.7	1.1	–	–

*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 DCY14^T 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 DCY14^T 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 DCY14^T grew at 15–37 °C, but did not grow at 4 or 42 °C. Physiological features of strain DCY14^T 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 DCY14^T and related taxa are shown in Table 2. The major cellular fatty acids in strain DCY14^T included the following: anteiso-C_{13:0} (11.4 %), iso-C_{15:0} (29.3 %), iso-C_{17:1ω9c} (7.1 %), iso-C_{17:0} 3-OH (16.3 %) and summed feature 4 (C_{16:1ω7c}/iso-C_{15:0} 2-OH, 19.4 %). Minor amounts of saturated fatty acids were present: 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 %).

Strain DCY14^T 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 DCY14^T was a continuous stretch of 1468 nt. The 16S rRNA gene sequences of related taxa were obtained from GenBank. Strain DCY14^T was shown to belong to the class *Sphingobacteria* (order *Sphingobacteriales*, family *Sphingobacteriaceae*). The highest level of sequence similarity was found with respect to *Parapedobacter koreensis* Jip14^T (95.8 % similarity). The phylogenetic tree (Fig. 1) shows clearly that strain DCY14^T 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 DCY14^T among members of the family *Sphingobacteriaceae* is unique and distinct.

The G + C content of the genomic DNA of strain DCY14^T 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 DCY14^T 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'li. 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 DCY14^T and related species. Bar, 0.01 substitutions per nucleotide position.

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_{13:0} (11.4%), iso-C_{15:0} (29.3%), iso-C_{17:1 ω 9c} (7.1%), iso-C_{17:0} 3-OH (16.3%) and summed feature 4 (C_{16:1 ω 7c}/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.

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