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

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A micro-organism, DCY12^T, comprising Gram-negative, non-motile, pale-yellow rods was isolated from soil from a ginseng field in South Korea and was investigated to determine its taxonomic status. It grew optimally at 30 °C and at pH 7.0, the G + C content of its DNA was 40.5 mol%, the major components of the fatty acid profile were C_{16:0} and C_{18:1} and the major ubiquinone was Q-8. A phylogenetic analysis based on the 16S rRNA gene sequence revealed that the novel isolate was most closely related to *Hydrocarboniphaga effusa* AP103^T (89.2 %), *Nevskia ramosa* Soe1 (88.8 %) and *Pseudomonas aeruginosa* ATCC 10145^T (83.2 %). The phenotypic, physiological, metabolic and phylogenetic properties of DCY12^T suggest that it represents a novel genus (class *Gammaproteobacteria*) and species, for which the name *Solimonas soli* gen. nov., sp. nov. is proposed. The type strain of *Solimonas soli* is DCY12^T (=KCTC 12834^T =LMG 24014^T).

In a series of studies, we attempted to isolate micro-organisms from soil in order to investigate the community structure by using a culture-dependent method. In the course of this study, strain DCY12^T was isolated from soil from a ginseng field near Daejeon in South Korea and was characterized by using a polyphasic approach. The polyphasic approach, which included a phylogenetic analysis based on 16S rRNA gene sequences, genomic relatedness studies and the determination of various chemotaxonomic and phenotypic properties, was designed to elucidate the precise taxonomic position of this strain. The results obtained in this study indicated that DCY12^T represents a novel species of a novel genus within the *Gammaproteobacteria*.

Strain DCY12^T was isolated from surface soil from an agricultural field in which ginseng was growing. A soil sample (1 g) was immersed in 50 ml saline solution, vortexed and serially diluted and then an aliquot (100 µl) was inoculated onto 10-fold-diluted R2A agar (Difco). Single colonies on these agar plates were purified by

transferring them onto new plates and subjecting them to additional incubation for 5 days at 30 °C. The purified colonies were tentatively identified using a partial sequence of the 16S rRNA gene.

Cell morphology and motility were observed with a Nikon light microscope (× 1000 magnification), using cells grown on R2A agar at 30 °C for 5 days. Gram reactions were determined according to the non-staining method, as described by Buck (1982). Oxidase activity was evaluated via the oxidation of 1 % *p*-aminodimethylaniline oxalate. Catalase activity was determined by measuring bubble production after the application of a 3 % (v/v) hydrogen peroxide solution. Growth at various temperatures (4, 15, 25, 30, 37 and 42 °C) was assessed on R2A agar, while growth at different pHs was assessed in R2A broth. Growth on nutrient agar, Luria–Bertani (LB) agar and trypticase soy agar (TSA) was also evaluated at 30 °C. The API 20NE, API ID 32GN and API ZYM microtest systems were employed in these tests, according to the recommendations of the manufacturer (bioMérieux). Because strain DCY12^T was unable to assimilate any of the single growth substrates in the API 20NE and API ID 32GN tests, LB broth was added to the AUX medium (0.1 %, v/v) and then the microbial broth was inoculated; with LB broth as a growth factor, strain DCY12^T was then able to assimilate some substrates in these microtest systems.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCY12^T is EF067861.

The cellular fatty acid profile of strain DCY12^T is available as supplementary material with the online version of this paper.

Isoprenoid quinones were extracted using chloroform/methanol (2:1, v/v), purified via TLC and subsequently analysed by HPLC, as described previously (Collins & Jones, 1981; Shin *et al.*, 1996). In order to perform a fatty acid methyl ester analysis, the strain was allowed to grow on TSA for 48 h at 30 °C and then two loops of the well-grown cells were harvested. The fatty acid methyl esters were prepared, separated and identified with the Sherlock Microbial Identification System (MIS; MIDI) (Sasser, 1990).

To determine the G+C content, genomic DNA was extracted and purified with the Genomic-tip 100/G system (Qiagen) and was then enzymically degraded into nucleosides. The nucleosides were analysed using HPLC, as described previously (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989).

The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer set 9F and 1512R (Weisburg *et al.*, 1991) and the purified PCR product was sequenced by Genotec (Daejeon, Korea) (Kim *et al.*, 2005). The complete sequence of the 16S rRNA gene was compiled with SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank and edited using the BioEdit program (Hall, 1999). 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 a 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 also conducted in order to obtain confidence levels for the branches (Felsenstein, 1985). The closest relatives of strain DCY12^T and all of the type species representing all of the orders in the *Betaproteobacteria* and *Gammaproteobacteria* were included in the phylogenetic tree.

Strain DCY12^T was cultured on R2A agar at 30 °C, yielding pale-yellow, circular colonies. The strain was found to be an aerobic, Gram-negative, non-motile bacterium consisting of short rod-shaped cells. Strain DCY12^T was also shown to be able to grow at 25–37 °C, but it did not grow at 4 or 42 °C. Physiological data for strain DCY12^T are summarized in the species description; Table 1 lists the differential characteristics with respect to related type strains.

The major cellular fatty acids in strain DCY12^T included C_{12:0} (6.13%), C_{14:0} (7.12%), C_{16:0} (21.24%), C_{12:0} 2-OH (3.84%), C_{16:1}ω5c (4.99%), C_{18:1} (25.90%), C_{19:0} cyclo ω8c (8.59%), summed feature 3 (C_{16:1} iso I/C_{14:0} 3-OH; 13.25%) and summed feature 4 (C_{15:0} iso 2-OH/C_{16:1}ω7c; 3.65%). Minor amounts of saturated fatty acids C_{15:0} (1.36%), C_{17:0} (0.85%), C_{18:0} (0.62%), C_{16:0} 2-OH (1.31%), C_{16:0} 3-OH (0.92%) and summed feature 2 (C_{15:1} iso H/I/C_{13:0} 3-OH; 0.25%) were also present.

The strain contained a ubiquinone with eight isoprene units (Q-8) as the predominant isoprenoid quinone. Q-8 is

commonly found in species belonging to the *Gammaproteobacteria*.

The 16S rRNA gene sequence of DCY12^T was found to be a continuous stretch of 1491 nt, and the strain was found to belong to the class *Gammaproteobacteria*. The highest levels of sequence similarities for DCY12^T occurred with *Hydrocarboniphaga effusa* AP103^T (89.2%), *Nevskia ramosa* Soe1 (88.8%) and *Pseudomonas aeruginosa* ATCC 10145^T (83.2%). In the phylogenetic tree (Fig. 1), strain DCY12^T clearly belonged to the lineage containing the genera *Hydrocarboniphaga* and *Nevskia*, as evidenced by the high level of bootstrap support. On the basis of the 16S rRNA gene sequencing results, the phylogenetic position of

Table 1. Phenotypic characteristics of strain DCY12^T and related taxa

Data for *N. ramosa* Soe1 and *H. effusa* AP103^T are from Stürmeyer *et al.* (1998) and Palleroni *et al.* (2004), respectively. None of the strains assimilated L-alanine. Strains DCY12^T and AP103^T grew on nutrient agar, LB agar and TSA, but did not grow at 42 °C and did not assimilate 4-hydroxybenzoate, 2-ketogluconate, malonate, maltose or melibiose. Strains DCY12^T and Soe1 did not assimilate citrate. +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	DCY12 ^T	<i>N. ramosa</i> Soe1	<i>H. effusa</i> AP103 ^T
Motility	–	ND	+
Cell length (µm)	0.3–0.5	1.5–2.3	1.5–2
Cell width (µm)	0.2–0.4	0.7–1.1	0.75–0.85
Catalase activity	+	w	ND
Optimum growth temperature (°C)	30	ND	28
Growth at 37 °C	+	ND	–
Lipase activity	–	ND	+
Assimilation of:			
DL-3-Hydroxybutyrate	+	ND	–
Acetate	+	+	–
Adipate	+	ND	–
Caprate	–	ND	w
Gluconate	+	ND	–
DL-Lactate	–	+	+
Malate	–	–	+
Propionate	w	ND	+
D-Glucose	+	+	–
Mannose	–	ND	w
Ribose	–	+	ND
L-Rhamnose	–	ND	+
Sucrose	–	+	w
Mannitol	–	ND	+
L-Histidine	+	ND	w
L-Proline	w	ND	–
L-Serine	w	ND	–
DNA G+C content (mol%)	40.53	67.8	60

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain DCY12^T and related taxa. Bar, 0.01 substitutions per nucleotide position.

DCY12^T among members of the *Gammaproteobacteria* was unique and distinct.

In previous publications on the genera *Nevskia* and *Hydrocarboniphaga*, they were described as members of the *Gammaproteobacteria*, but no conclusive evidence was presented for this (Stürmeyer *et al.*, 1998; Palleroni *et al.*, 2004). In our study, 16S rRNA gene sequences of the type species representing all of the orders in the *Betaproteobacteria* and *Gammaproteobacteria* were collected from GenBank and a phylogenetic tree was constructed. In Fig. 1, the *Nevskia*–*Solimonas*–*Hydrocarboniphaga* cluster is grouped with the *Gammaproteobacteria*, although its position is not supported by high levels of bootstrap support.

The G + C content of the genomic DNA of strain DCY12^T was 40.5 mol%, which is much lower than those of related taxa (*N. ramosa*, 67.8–69.0 mol%; *H. effusa*, 60–61 mol%).

On the basis of the phenotypic, chemotaxonomic and phylogenetic data, we conclude that strain DCY12^T represents a novel species and genus, within the *Gammaproteobacteria*, for which the name *Solimonas soli* gen. nov., sp. nov. is proposed.

Description of *Solimonas* gen. nov.

Solimonas (So'li.mo'nas. L. n. *solum* soil; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Solimonas* a monad in soil).

Gram-negative, non-motile rods. Oxidase-negative and catalase-positive. The major ubiquinone is Q-8. The major cellular fatty acids are C_{16:0}, C_{18:1} and summed feature 3 (C_{16:1} iso I/C_{14:0} 3-OH). The DNA G + C content of the

type strain of the type species is 40.5 mol%. The type, and only, species is *Solimonas soli*.

Description of *Solimonas soli* sp. nov.

Solimonas soli (so'li. L. neut. gen. n. *soli* of soil, the source of the type strain).

General characteristics are given above under the genus description. Cells grown on R2A agar at 30 °C for 5 days are 0.3–0.5 × 0.2–0.4 µm, non-motile rods. Colonies grown on R2A agar plates for 5 days are pale yellow. Grows optimally at 30 °C and at pH 7.0; growth occurs at 25–37 °C and pH 7.0–9.0 but not at temperatures above 42 °C or below 4 °C and not above pH 11 or below pH 5. Negative for indole production and acid production from glucose. Acid phosphatase, alkaline phosphatase, esterase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and urease are produced, but *N*-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase, α-mannosidase, protease, trypsin and valine arylamidase are not produced. In the presence of LB broth as a growth factor, 3-hydroxybutyrate, acetate, adipate, gluconate, *n*-valerate, *D*-glucose and *L*-histidine are assimilated. Growth on propionate, *L*-arabinose, *L*-fucose, *D*-sorbitol, *L*-proline and *L*-serine, with R2A as a growth factor, is slow and very poor. No substrates are assimilated in the presence of yeast extract as a growth factor or in the absence of a growth factor. The following are not assimilated: 2-ketogluconate, 3-hydroxybenzoate, 4-hydroxybenzoate, 5-ketogluconate, caprate, citrate, itaconate, lactate, *L*-malate,

malonate, phenylacetate, suberate, maltose, D-mannose, D-melibiose, L-rhamnose, D-ribose, sucrose, *myo*-inositol, D-mannitol, L-alanine, *N*-acetyl-D-glucosamine, salicin and glycogen. Major cellular fatty acids include C_{16:0} (21.24%), summed feature 3 (C_{16:1} iso I/C_{14:0} 3-OH; 13.25%) and C_{18:1} (25.90%). Nitrate is not reduced to nitrite or nitrogen gas.

The type strain, DCY12^T (=KCTC 12834^T =LMG 24014^T), was isolated from soil from a ginseng field in South Korea.

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