Sphingopyxis panaciterrulae sp. nov., isolated from soil of a ginseng field

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A Gram-negative, rod-shaped, motile bacterium was isolated from the soil of a ginseng field in Daejeon, South Korea, and characterized in order to determine its taxonomic position. Phylogenetic analysis based on 16S rRNA gene sequence analysis revealed that strain DCY34^T belonged to the family *Sphingomonadaceae*, and the highest degree of sequence similarity was found with *Sphingopyxis wittlariensis* W-50^T (97.1%), *Sphingopyxis ginsengisoli* Gsoil 250^T (97.0%), *Sphingopyxis chilensis* S37^T (96.9%), *Sphingopyxis macrogoltabida* IFO 15033^T (96.8%), *Sphingopyxis alaskensis* RB2256^T (96.7%) and *Sphingopyxis taejonensis* JSS54^T (96.7%). Chemotaxonomic data revealed that strain DCY34^T possessed ubiquinone Q-10 as the predominant respiratory lipoquinone, which is common to members of the genus *Sphingopyxis*. The predominant fatty acids were C_{18:1}ω7c (27.5%), summed feature 4 (C_{16:1}ω7c and/or C_{15:0} iso 2-OH; 18.6%), C_{16:0} (15.6%) and summed feature 8 (C_{19:1}ω6c and/or unknown 18.864; 15.4%). The major polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, sphingoglycolipid and an unknown polar lipid. The results of physiological and biochemical tests clearly demonstrated that strain DCY34^T represented a separate species and supported its affiliation to the genus *Sphingopyxis*. Based on these data, the new isolate represents a novel species, for which the name *Sphingopyxis panaciterrulae* sp. nov. is proposed. The type strain is DCY34^T (=KCTC 22112^T=JCM 14844^T).

The genus *Sphingomonas* has been subdivided into three different genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* in addition to the genus *Sphingomonas sensu stricto* (Takeuchi *et al.*, 2001). Yabuuchi *et al.* (2002) suggested that there was no phenotypic and phylogenetic evidence to divide the genus *Sphingomonas* into the four separate genera, but the nomenclature of Takeuchi *et al.* (2001) is commonly used (Kämpfer *et al.*, 2002; Busse *et al.*, 2003; Fujii *et al.*, 2003; Godoy *et al.*, 2003; Ushiba *et al.*, 2003; Sohn *et al.*, 2004). At the time of writing, the genus *Sphingopyxis* comprised eleven species with validly published names.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCY34^T is EU075217.

Tables showing the cellular fatty acid profile of strain DCY34^T and the results of DNA–DNA hybridization experiments and an image showing the polar lipid profile of strain DCY34^T are available with the online version of this paper.

In a series of studies based on a culture-dependent method, we attempted to isolate micro-organisms from soil in order to investigate the structure of the community. Strain DCY34^T was one of the isolates that was dominant on one tenth-strength R2A agar (Difco) plates incubated in aerobic conditions. The strain was characterized by a polyphasic taxonomic approach, including 16S rRNA gene sequence analysis, genotypic relatedness and chemotaxonomic and phenotypic properties. The results obtained in this study indicated that DCY34^T represented a new member of the genus *Sphingopyxis*.

Cell morphology and motility were observed with a Nikon light microscope (×1000 magnification) using cell material taken from 3-day-old colonies grown on standard R2A agar (Difco) at 30 °C. Tests for the Gram reaction were performed 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 testing for bubble

production after the addition of 3% (v/v) hydrogen peroxide solution (Cappuccino & Sherman, 2002). Growth at different temperatures (4, 15, 18, 25, 30, 37 and 42 °C) and pH values (5.0–10.0 at intervals of 1 pH unit) was assessed with R2A broth. Anaerobic growth was tested using serum bottles by adding thioglycolate (1 g l⁻¹) to R2A broth and substituting the upper air layer with nitrogen gas. The API 20NE, API 50CH, API ZYM and API ID32 GN microtest systems were used according to the manufacturer's instructions (bioMérieux).

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified via TLC and analysed by HPLC, as described previously (Collins & Jones, 1981; Shin *et al.*, 1996). In order to analyse the methyl esters of the cellular fatty acids, the strain was grown on trypticase soy agar (TSA, Difco) for 48 h at 30 °C and two loopfuls of cell material was harvested. Fatty acid methyl esters were prepared, separated and identified according to the protocol of the Sherlock Microbial Identification System (MIS, MIDI, Inc.) (Sasser, 1990). Total lipids were extracted three times from 50 mg dried cells with 5 ml chloroform/methanol (2:1, v/v), dried and redissolved in methanol. A portion of the extracted lipids was subjected to alkaline hydrolysis with 1 M KOH in methanol (2:1, v/v) for 2 h at 40 °C. The lipids were analysed according to Yabuuchi *et al.* (1990) by TLC with a solvent system composed of chloroform, methanol and water (70:30:5, v/v/v). To determine the polar lipid profile, total cellular polar lipids were extracted and examined by two-dimensional TLC (Minnikin *et al.*, 1977). For the detection of glycolipid spots, orcinol-sulfuric acid reagent (0.2% orcinol in 2 M sulfuric acid) was sprayed and charred at 120 °C until the maximum purple colour developed.

For the determination of the DNA G + C content, genomic DNA was extracted and purified with the Qiagen Genomic-tip system 100/G (Qiagen) and enzymically degraded into nucleosides. The nucleosides were analysed using HPLC as described previously (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). DNA–DNA hybridization experiments were performed fluorometrically, according to the method developed by Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and micro-dilution wells. Hybridization was performed at 49.8 °C in the presence of 50% (v/v) formamide with the ionic strength of 0.3 M NaCl. The hybridizations were carried out reciprocally with five replications per sample. The highest and lowest values obtained for each sample were excluded; the remaining three values were utilized for the calculation of DNA–DNA hybridization values. The DNA relatedness values quoted are expressed as the means of these three values.

The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer set, fD1 and rP1 (Weisburg *et al.*, 1991) and the purified PCR product was sequenced by Genotec (Kim *et al.*, 2005). The full sequence of the 16S rRNA gene was compiled with SeqMan software and 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). The phylogenetic tree was reconstructed via the neighbour-joining method (Saitou & Nei, 1987) with the MEGA3 program (Kumar *et al.*, 2004). Bootstrap analysis with 1000 replicates was also conducted in order to obtain confidence levels for the branches (Felsenstein, 1985). The closest relatives of strain DCY34^T, the type strains of all of the members of the genus *Sphingopyxis* and closely related type species of the order *Sphingomonadales* were included in the phylogenetic tree.

When cultured on R2A agar (Difco) at 30 °C, strain DCY34^T yielded pale yellow, circular colonies. The cells were found to be Gram-negative, motile and rod-shaped. When grown on R2A agar and LB agar at 30 °C for 3 days, cells were 0.2–0.5 µm in diameter and 0.3–0.9 µm in length. Strain DCY34^T was able to grow at 25–37 °C, but not at 4 °C or 42 °C. The optimum growth temperature range was 25 to 30 °C. The results of the physiological tests for strain DCY34^T are summarized in the species description. The characteristics of strain DCY34^T are compared with those of related type strains of species of the genus *Sphingopyxis* in Table 1.

The cellular fatty acid contents of strain DCY34^T included C_{18:1ω7c} (27.5%), summed feature 4 (C_{16:1ω7c} and/or C_{15:0} iso 2-OH; 18.6%), C_{16:0} (15.6%), summed feature 8 (C_{19:1ω6c} and/or unknown 18.864; 15.4%) and C_{17:1ω6c} (7.3%). The cellular fatty acid profiles of strain DCY34^T and those of closely related type strains are shown in Supplementary Table S1 (available with IJSEM Online). The fatty acid profile of the strain DCY34^T was very similar to those of other type strains, but contained different amounts of fatty acids. The presence of C_{18:1ω7c}, C_{16:0} and summed feature 4 as the major fatty acids is a characteristic feature of the genus *Sphingopyxis* (Takeuchi *et al.*, 2001). The characteristic difference between strain DCY34^T and the other type strains of the genus *Sphingopyxis* was the absence of the minor fatty acid C_{16:0} 2-OH, which is generally found in members of this genus (see Supplementary Table S1). Strain DCY34^T contained ubiquinone Q-10 as the predominant respiratory lipoquinone. Alkaline stable sphingoglycolipid was identified in strain DCY34^T which clearly suggested that the bacterium belonged to the α-4 subclass of the *Proteobacteria*. The overall polar lipid pattern of strain DCY34^T corresponded to those reported previously for sphingomonads (Busse *et al.*, 1999) and was composed of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, sphingoglycolipid and an unknown polar lipid (see Supplementary Fig. S1 in IJSEM Online).

An almost-complete sequence (1432 bp) of the 16S rRNA gene of strain DCY34^T was used for phylogenetic analysis.

Table 1. Differential phenotypic characteristics between strain DCY34^T and closely related species of the genus *Sphingopyxis*

Strain: 1, DCY34^T (data from this study); 2, *S. alaskensis* KCTC 12916^T; 3, *S. chilensis* KACC 12321^T; 4, *S. ginsengisoli* KCTC 12582^T (Lee *et al.*, 2008); 5, *S. macrogoltabida* KACC 10927^T; 6, *S. taejonensis* KCTC 2884^T; 7, *S. witflariensis* KCTC 12115^T. All strains are motile and catalase and oxidase-positive. All strains are Gram-negative, cannot produce indole and are negative for the assimilation of caprate and mannitol. Data were obtained during this study under identical growth conditions, except where indicated otherwise. +, Positive; –, negative; V, variable; w, weakly positive; B, beige; BY, bright yellow; Y, yellow; PY, pale yellow; WB, whitish brown; WG, weak growth; NA, not available.

Characteristic	1	2	3	4	5	6	7
Morphology	Rod	Short rod	Rod	Rod	Rod	Rod	Rod
Colony colour	PY	Y–B	Y	Y	WB–Y	PY	BY
Growth at (R2A):							
4 °C	–	+	WG	+	–	WG	–
42 °C	+	+	WG	+	+	+	–
pH 11	+	–	–	–	+	–	–
Enzyme activity (API ZYM):							
N-Acetyl-β-glucosaminidase	+	+	–	–	+	–	–
Acid phosphatase	+	+	+	w	–	+	+
Alkaline phosphatase	+	w	+	w	w	+	+
α-Chymotrypsin	–	w	–	–	+	–	–
Cystine arylamidase	+	–	+	w	–	w	–
α-Galactosidase	–	+	w	w	w	w	w
α-Glucosidase (starch hydrolysis)	+	w	+	+	–	+	+
β-Galactosidase (PNPG)	–	+	–	–	+	–	+
β-Glucosidase (aesculin hydrolysis)	+	+	+	+	+	w	–
Leucine arylamidase	+	w	+	+	+	+	w
Lipase (C14)	–	+	w	–	w	–	–
α-Mannosidase	–	–	+	+	–	+	–
Naphthol-AS-BI-phosphohydrolase	+	–	+	+	+	+	+
Trypsin	+	–	+	w	–	w	+
Urease	–	+	–	–	–	–	–
Valine arylamidase	+	+	+	w	+	w	w
Fermentation of sugars (API 50CH):							
N-Acetylglucosamine	+	–	+	w	–	–	–
Aesculin	+	+	+	w	–	–	–
Mannitol	–	–	–	–	–	w	–
D-Mannose	–	–	+	w	–	–	–
Assimilation tests (API ID32 GN):							
Acetate	–	–	–	–	+	–	–
N-Acetyl-D-glucosamine	+	–	–	+	–	w	–
Adipate	–	+	+	w	–	+	–
L-Histidine	–	+	w	+	w	–	+
3-Hydroxybenzoate	–	+	w	w	+	+	+
4-Hydroxybenzoate	–	+	–	–	w	–	–
DL-3-Hydroxybutyrate	–	+	w	+	–	–	w
Itaconate	+	–	+	–	+	+	w
DL-Lactate	–	–	w	+	+	–	–
L-Malate	+	+	+	w	–	+	–
Malonate	–	w	–	–	w	–	+
D-Mannose	–	–	+	w	–	–	–
L-Proline	+	w	+	w	–	–	+
Propionate	–	+	–	–	+	+	–
L-Rhamnose	+	–	+	w	w	w	–
D-Ribose	w	–	–	w	–	–	–
Salicin	–	–	+	+	–	+	–
L-Serine	–	w	+	+	–	+	–
Suberate	–	w	w	w	w	w	+
Sucrose	+	–	w	–	+	+	–
n-Valerate	–	+	–	–	w	–	–

Table 1. cont.

Characteristic	1	2	3	4	5	6	7
Quinone*	Q-10	Q-10 ^a	Q-10 ^b	Q-10 ^a	Q-10 ^d	Q-10 ^e	Q-10, 8 ^f
DNA G + C content*	62.3	65.0 ^c	66.0 ^c	69.2 ^c	63–65 ^c	63.0 ^c	NA

*Data from other studies as indicated: a, Vancanneyt *et al.* (2001); b, Godoy *et al.* (2003); c, Lee *et al.* (2008); d, Takeuchi *et al.* (2001); e, Pal *et al.* (2006); f, Kämpfer *et al.* (2002).

Strain DCY34^T belonged to the class *Alphaproteobacteria*, the order *Sphingomonadales* and the family *Sphingomonadaceae*. The highest degree of sequence similarity was found with *Sphingopyxis witflariensis* W-50^T (97.1 %), *Sphingopyxis ginsengisoli* Gsoil 250^T (97.0 %), *Sphingopyxis chilensis* S37^T (96.9 %), *Sphingopyxis macrogoltabida* IFO 15033^T (96.8 %), *Sphingopyxis alaskensis* RB2256^T (96.7 %) and *Sphingopyxis taejonensis* JSS54^T (96.7 %). As shown in the phylogenetic tree (Fig. 1), strain DCY34^T clearly belonged to the genus *Sphingopyxis* lineage, as evidenced by the high bootstrap value. Based on 16S rRNA gene sequencing, the phylogenetic position of strain DCY34^T among members of the family *Sphingomonadaceae* was unique and distinct. The G + C content of the genomic DNA of strain DCY34^T was 62.3 mol% and was similar to that found for other species of the genus *Sphingopyxis* (58–66 mol%). DNA–DNA hybridization studies were performed to differentiate strain DCY34^T from the closely related species of the genus *Sphingopyxis*. Strain DCY34^T exhibited relatively low levels of DNA–DNA relatedness (see Supplementary Table S2) with respect to *S. alaskensis* KCTC 12916^T (19%), *S. chilensis* KACC 12321^T

(4 %), *S. ginsengisoli* KCTC 12582^T (39 %), *S. macrogoltabida* KACC 10927^T (1 %), *S. taejonensis* KCTC 2884^T (6 %) and *S. witflariensis* KCTC 12115^T (48 %), thus indicating that it constituted a separate species (Wayne *et al.*, 1987).

The results of the phenotypic and phylogenetic characterizations indicated that strain DCY34^T belonged to the genus *Sphingopyxis*. The phylogenetic distinctiveness, together with DNA–DNA hybridization data, confirmed that the new strain represented a novel species that was distinct from recognized members of the genus *Sphingopyxis*. Therefore, on the basis of the data presented, strain DCY34^T should be classified as representing a novel species of the genus *Sphingopyxis*, for which the name *Sphingopyxis panaciterrulae* sp. nov. is proposed.

Description of *Sphingopyxis panaciterrulae* sp. nov.

Sphingopyxis panaciterrulae (pa.na.ci.ter.ru'la.e. N.L. n. *Panax* -*acis* scientific name of ginseng; L. fem. n. *terrula* a field; N.L. gen. n. *panaciterrulae* of a ginseng field).

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain DCY34^T and related species (all species of the genus *Sphingopyxis* and other related genera). The neighbour-joining method was used. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at the branch points. Filled circles indicate generic branches that were also recovered by using maximum-parsimony algorithms. Bar, 0.02 substitutions per nucleotide position.

Gram-negative, motile rod when grown on R2A agar (Difco) at 30 °C for 3 days. Colonies are circular and pale yellow in colour. Optimal growth temperature is 25–30 °C and the optimum pH is 7.0–8.0. Both oxidase and catalase are positive. Acid is produced from D-glucose. Indole is not produced and nitrate is not reduced. Does not grow anaerobically in R2A broth medium supplemented with nitrate. Assimilates *N*-acetylglucosamine, D-glucose, itaconate, malate, maltose, L-rhamnose and sucrose. The following carbon sources are not assimilated: adipate, L-alanine, L-arabinose, caprate, citrate, L-fucose, gluconate, glycogen, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyric acid, inositol, lactate, D-mannitol, mannose, melibiose, phenylacetate, potassium 5-ketoglucuronate, propionate, potassium 2-ketoglucuronate, L-proline, D-ribose, salicin, L-serine, sodium acetate, sodium malonate, D-sorbitol, suberate and valerate. Produces acid phosphatase, *N*-acetyl- β -glucosaminidase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), α -glucosidase, β -glucosidase (aesculin hydrolysis), naphthol-AS-BI-phosphohydrolase, leucine arylamidase, trypsin and valine arylamidase, but does not produce arginine dihydrolase, α -chymotrypsin, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, lipase (C14), α -mannosidase, protease (gelatin hydrolysis) or urease. Ubiquinone Q-10 is the predominant respiratory lipoquinone. The cellular fatty acids include C_{18:1} ω 7c, summed feature 4 (C_{16:1} ω 7c and/or C_{15:0} iso 2-OH), C_{16:0}, summed feature 8 (C_{19:1} ω 6c and/or unknown 18.864) and C_{17:1} ω 6c (7.3 %). The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, sphingoglycolipid and an unknown polar lipid.

The type strain, DCY34^T (=KCTC 22112^T=JCM 14844^T), was isolated from soil of a ginseng field in Daejeon, South Korea. The DNA G+C content of the type strain is 62.3 mol% as determined by HPLC.

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