Sphingopyxis ginsengisoli sp. nov., isolated from soil of a ginseng field in South Korea

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A Gram-negative, aerobic, motile, rod-shaped bacterium (strain Gsoil 250^T) was isolated from soil of a ginseng field in Pocheon province (South Korea) and was characterized using a polyphasic approach in order to determine its taxonomic position. Comparative analysis of 16S rRNA gene sequences showed that strain Gsoil 250^T belonged to the family *Sphingomonadaceae* of the class *Alphaproteobacteria*, and was related to *Sphingopyxis macrogoltabida* (98.7%), *Sphingopyxis chilensis* (98.2%), *Sphingopyxis alaskensis* (97.9%), *Sphingopyxis taejonensis* (97.9%) and *Sphingopyxis witflariensis* (97.8%). The phylogenetic distance from any other species with validly published names within the genus *Sphingopyxis* was greater than 3.8%. The G + C content of the genomic DNA of strain Gsoil 250^T was 69.2 mol%. Strain Gsoil 250^T contained Q-10 as the predominant respiratory lipoquinone and C_{18:1}ω7c and summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 34.6%) as the major fatty acids. No 3-hydroxy fatty acids were detected. Major polar lipids consisted of sphingoglycolipid, phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol and two unknown glycolipids. Phenotypic and chemotaxonomic data supported the affiliation of strain Gsoil 250^T to the genus *Sphingopyxis*. The results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain Gsoil 250^T from the eight recognized *Sphingopyxis* species. Strain Gsoil 250^T therefore represents a novel species, for which the name *Sphingopyxis ginsengisoli* sp. nov. is proposed, with the type strain Gsoil 250^T (=KCTC 12582^T=LMG 23390^T).

A major cultivation area of ginseng plant (*Panax ginseng* C.A. Meyer) in South Korea includes Pocheon, Kanghwa, Geumsan, Chonkok, Kimpo, Yongin, Anseong and Suhsan. During the course of a study on the culturable aerobic bacterial community in soil from a ginseng field in Pocheon province, a large number of novel bacterial strains were isolated (Im *et al.*, 2005). One of these isolates (strain Gsoil 250^T) appeared to be a member of the genus

Sphingopyxis in the *Alphaproteobacteria* lineage and was subjected to a taxonomic investigation.

The genus *Sphingopyxis* was first described by Takeuchi *et al.* (2001) and at present the genus comprises eight species: *Sphingopyxis macrogoltabida* (Takeuchi *et al.*, 1993, 2001), *Sphingopyxis terrae* (Takeuchi *et al.*, 1993, 2001), *Sphingopyxis witflariensis* (Kämpfer *et al.*, 2002), *Sphingopyxis alaskensis* (Vancanneyt *et al.*, 2001; Godoy *et al.*, 2003), *Sphingopyxis chilensis* (Godoy *et al.*, 2003), *Sphingopyxis flavimaris* (Yoon & Oh, 2005), *Sphingopyxis baekryungensis* (Yoon *et al.*, 2005) and *Sphingopyxis taejonensis* (Pal *et al.*, 2006).

In the present study, we conducted a phylogenetic analysis based on 16S rRNA gene sequences, DNA–DNA hybridization and an analysis of the phenotypic, genotypic and chemotaxonomical characteristics to determine the precise taxonomic position of strain Gsoil 250^T. On the basis of the results obtained in this study, we propose that strain Gsoil

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 250^T is AB245343.

Figures showing a transmission electron micrograph of a negatively stained single cell of strain Gsoil 250^T and a two-dimensional, thin-layer chromatograph of the polar lipids and a table detailing the cellular fatty acid profiles of strain Gsoil 250^T and related species of the genus *Sphingopyxis* are available as supplementary material with the online version of this paper.

250^T should be placed in the genus *Sphingopyxis* as the type strain of novel species.

Strain Gsoil 250^T was isolated originally from a soil sample from a ginseng field in Pocheon province (South Korea) by using modified R2A medium, as described previously (Ten *et al.*, 2006). Single colonies on the plates were purified by transferring them onto fresh plates of modified R2A or half-strength modified R2A agar and incubating again. Purified colonies were tentatively identified from partial sequences of the 16S rRNA gene (Im *et al.*, 2005). Strain Gsoil 250^T was one of the isolates that appeared on the modified R2A agar plates under aerobic conditions. The strain was cultured routinely on R2A agar (Difco) 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 using a Nikon light microscope at ×1000, with cells grown for 3 days at 30 °C on R2A agar. Catalase activity was determined by bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. Anaerobic growth, nitrate reduction and assimilation of single carbon sources were determined at 30 °C as reported previously (Ten *et al.*, 2006). Some physiological characteristics were determined using API 20E galleries according to the instructions of the manufacturer (bioMérieux). Tests for degradation of DNA [in which DNase agar (Scharlau) plates were flooded with 1 M HCl], casein, chitin, starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 5 days. Growth at different temperatures (4, 15, 25, 30, 37 and 42 °C) and various pH values (pH 5.0–10.0, in increments of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A medium supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar (TSA; Difco) and MacConkey agar was also evaluated at 30 °C.

Extraction of the genomic DNA was achieved using a commercial genomic DNA extraction kit (Core Biosystem), and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). The full sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed by using the program CLUSTAL_X (Thompson *et al.*, 1997). Gaps were edited in the program BioEdit (Hall, 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in the program MEGA3 (Kumar *et al.*, 2004), with bootstrap values based on 1000 replications (Felsenstein, 1985).

To measure the G + C content of the chromosomal DNA, the genomic DNA of the strains was extracted and purified as described by Moore & Dowhan (1995) and enzymically degraded into nucleosides. The DNA G + C content was then 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 quinone in *n*-hexane was purified using Sep-Pak Vac cartridges (Waters) and was subsequently analysed by HPLC, as described previously (Hiraishi *et al.*, 1996). Cellular fatty acids were analysed in organisms grown on TSA for 2 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 then analysed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). Polar lipids were extracted using the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Komagata & Suzuki, 1987).

Cells of strain Gsoil 250^T were Gram-negative, aerobic, motile and rod-shaped (0.2–0.3 µm in diameter and 1.0–1.2 µm in length), with single polar flagella (see Supplementary Fig. S1, available in IJSEM online). The strain could not grow anaerobically supplemented with nitrate. Colonies grown on R2A agar plates for 4 days were smooth, circular, non-glossy, yellowish in colour and 1–3 mm in diameter. On R2A agar, strain Gsoil 250^T was able to grow at 4–42 °C, but not at 45 °C. Physiological characteristics of strain Gsoil 250^T are summarized in the species description and selected characteristics that differentiate it from related *Sphingopyxis* species are listed in Table 1.

The 16S rRNA gene sequence of strain Gsoil 250^T obtained was a continuous stretch of 1455 bp. Sequence similarity calculation after a neighbour-joining analysis indicated that the closest relatives of strain Gsoil 250^T were *S. macrogoltabida* NBRC 15033^T (98.7 %), *S. chilensis* LMG 20986^T (98.2 %), *S. alaskensis* LMG 18877^T (97.9 %), *S. taejonensis* KCTC 2884^T (97.9 %) and *S. witflariensis* DSM 14551^T (97.8 %). Lower sequence similarities (<96.2 %) were found with other established species of the genus *Sphingopyxis*. This relationship between strain Gsoil 250^T and other members of the genus *Sphingopyxis* was also evident in the phylogenetic tree (Fig. 1). Strain Gsoil 250^T and six closely related *Sphingopyxis* species (*S. macrogoltabida*, *S. chilensis*, *S. alaskensis*, *S. taejonensis*, *S. witflariensis* and *S. terrae*) formed a monophyletic clade with a high bootstrap value (97 %), which was supported by the two types of tree-making methods employed in this study.

DNA–DNA hybridization was performed between Gsoil 250^T and the type strains of recognized species of the genus *Sphingopyxis* according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes (Sigma)

Table 1. Differential phenotypic characteristics of strain Gsoil 250^T (*Sphingopyxis ginsengisoli* sp. nov.) and recognized *Sphingopyxis* species

Species: 1, Gsoil 250^T (data from this study); 2, *S. macrogoltabida* (*n*=6) (Takeuchi *et al.*, 1993, 1995; Kämpfer *et al.*, 1997; Lee *et al.*, 2001); 3, *S. alaskensis* (*n*=7) (Vancanneyt *et al.*, 2001); 4, *S. chilensis* (*n*=1) (Godoy *et al.*, 2003); 5, *S. taejonensis* (*n*=1) (Lee *et al.*, 2001); 6, *S. witflariensis* (*n*=1) (Kämpfer *et al.*, 2002); 7, *S. terrae* (*n*=6) (Takeuchi *et al.*, 1993, 1995; Kämpfer *et al.*, 1997; Lee *et al.*, 2001); 8, *S. baekryungensis* (*n*=1) (Yoon & Oh, 2005); 9, *S. flavimaris* (Yoon *et al.*, 2005) (*n*=1). All species are Gram-negative, rod-shaped, non-spore-forming, motile and catalase- and oxidase-positive. All species are negative for utilization of citrate. *n*, Number of strains; +, positive; −, negative; w, weakly positive; ND, not determined; v, variable reaction. Data in parentheses are for the type strain.

Characteristic	1	2	3	4	5	6	7	8	9
Colony colour	Yellow	Whitish-brown or yellow	Yellow to beige	Yellow	Pale-yellow	Yellow	Light or deep-yellow	Orange	Yellow
Motility	+	+	+	+	+	+	v (+)	+	+
Reduction of nitrate to nitrite	−	−	−	−	−	−	−	+	−
Hydrolysis of:									
Aesculin	+	v (+)	+	+	−	−	v (−)	+	−
Gelatin	−	v (−)	−	−	−	−	−	−	−
Urea	−	−	v (+)	−	−	−	−	−	−
Utilization of:									
Acetate	+	(+)	−	−*	−*	−	(−)	+	−
L-Arabinose	−	+	−	−	−	−	−	+	−
Cellobiose	+	+	v (−)	+	+	−	+	+	+
Fructose	−	−	−	+	−*	−	−	−	−
D-Galactose	+	(−)	v (+)	+	−*	−	(−)	−	−
D-Glucose	+	(+)	+	+	+	+	(−)	+	+
Malate	+	−	+	+	+	−	+	w	−
Maltose	+	+	+	+	−	+	+	−	−
Mannose	−	−	−	+	−	−	−	+	−
Trehalose	−	+	+	+	−*	−	+	−	−
Xylose	−	−	−	w	−*	−	−	−	+
Nucleotide signature of 16S rRNA gene:									
134†	G	G	G	G	G	G	G	G	A
990:1215*	U:G	U:G	U:G	U:G	U:G	U:A	U:G	U:A	U:A
Major fatty acids	C _{18:1ω7c} , C _{16:1ω7c/iso} -C _{15:0} 2-OH, C _{16:0}	C _{18:1ω7c} , C _{16:1ω7c/iso} -C _{15:0} 2-OH, C _{17:1ω6c}	C _{17:1ω6c} , C _{18:1ω7c}	C _{18:1ω7c} , C _{17:1ω6c}	C _{18:1ω7c} /C _{18:1ω9t} , C _{18:1ω12t} , C _{16:0}	C _{17:1ω6c} , C _{16:1ω7c/iso} -C _{15:0} 2-OH	C _{17:1ω6c} , C _{18:1ω7c}	C _{18:1ω7c} , C _{17:1ω6c}	C _{18:1ω7c} , C _{16:1ω7c/iso} - C _{15:0} 2-OH, C _{17:1ω6c}
DNA G + C content (mol%)	69.2	63–65	65	66	63	ND	63–65	63	58

*Data from this study.

†*Escherichia coli* 16S rRNA numbering system was used.

Fig. 1. Phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationship of strain Gsoil 250^T (*Sphingopyxis ginsengisoli* sp. nov.) with other related species. Bootstrap values (expressed as a percentage of 1000 replications) $\geq 65\%$ are shown at branch points. Solid circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.01 substitutions per nucleotide position.

and microdilution wells (Greiner). The levels of DNA–DNA relatedness between strain Gsoil 250^T and recognized species of the genus *Sphingopyxis* were 9–29% (15% to *S. macrogoltabida* NBRC 15033^T, 29% to *S. chilensis* LMG 20986^T, 10% to *S. alaskensis* LMG 18877^T, 13% to *S. taejonensis* KCTC 2884^T and 12% to *S. witflariensis* DSM 14551^T, values that are low enough to assign strain Gsoil 250^T as representing a novel species of the genus *Sphingopyxis* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

The DNA G+C content of strain Gsoil 250^T was 69.2 mol%. The predominant respiratory lipoquinone type (Q-10) of strain Gsoil 250^T was the same as those of recognized species of the genus *Sphingopyxis* (Takeuchi *et al.*, 2001; Kämpfer *et al.*, 2002; Yoon *et al.*, 2005). The fatty acid profile of strain Gsoil 250^T was characterized by the occurrence of C_{18:1}ω7c (46.4%), summed feature 4 (C_{16:1}ω7c/iso-C_{15:0} 2-OH; 34.6%) and C_{16:0} (9.7%) as major fatty acids, and C_{14:0} 2-OH (5.1%) as a 2-hydroxy fatty acid and the absence of 3-hydroxy fatty acids. This cellular fatty acid profile was similar to that of the phylogenetically closest relative *S. macrogoltabida* NBRC 15033^T and those of some other members of the genus *Sphingopyxis* (see Supplementary Table S1, available in IJSEM online). The lack of 3-hydroxy fatty acids and the presence of 2-hydroxy myristic acid are characteristics that are also shared by all recognized species of the genus *Sphingopyxis* (Takeuchi *et al.*, 2001; Kämpfer *et al.*, 2002; Godoy *et al.*, 2003; Yoon & Oh, 2005; Yoon *et al.*, 2005). However, some qualitative and quantitative differences in

fatty acid content could be observed between strain Gsoil 250^T and its phylogenetically close relatives. In particular, strain Gsoil 250^T could be differentiated from some other *Sphingopyxis* species by a higher content of C_{18:1}ω7c, the absence of C_{18:0} and by a smaller amount of C_{17:1}ω6c. Significant quantitative variation of the latter fatty acid was reported previously by Kämpfer *et al.* (2002) and was also observed between different species of the genus *Sphingomonas* (Kämpfer *et al.*, 1997; Asker *et al.*, 2007).

Two-dimensional TLC analysis of polar lipids extracted from strain Gsoil 250^T (see Supplementary Fig. S2, available in IJSEM Online) showed that strain Gsoil 250^T contained a sphingoglycolipid, clearly suggesting that the bacterium belongs to subgroup 4 of the *Alphaproteobacteria*. The overall polar lipid pattern of strain Gsoil 250^T corresponded to those reported for sphingomonads (Busse *et al.*, 1999), in particular, for the genus *Sphingopyxis* (Kämpfer *et al.*, 2002; Yoon & Oh, 2005; Yoon *et al.*, 2005), as it was composed of phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol and sphingoglycolipid. Small amounts of phosphatidylmonomethylethanolamine, which has been detected in the majority of members of the genus *Sphingopyxis*, were also present in strain Gsoil 250^T. However, Gsoil 250^T has several significant differences in polar lipid composition from that of its closest relative, *S. macrogoltabida* NBRC 15033^T (Busse *et al.*, 1999). In contrast to *S. macrogoltabida*, strain Gsoil 250^T produced large amounts of two unknown glycolipids, GL2 and GL3, minor amounts of aminolipid AL and unknown lipids L1

and L2. The unique combination of six unknown polar lipids as well as the proportion of common polar lipids was very useful for differentiation of strain Gsoil 250^T from other species of the genus *Sphingopyxis* (Kämpfer *et al.*, 2002; Yoon & Oh, 2005; Yoon *et al.*, 2005).

All of the characteristics determined for strain Gsoil 250^T were in accordance with those of members of the genus *Sphingopyxis*. However, on the basis of phylogenetic distances from established *Sphingopyxis* species, low levels of DNA–DNA relatedness, and the combination of unique phenotypic characteristics (Table 1), it is clear that strain Gsoil 250^T is not affiliated with any recognized species of the genus *Sphingopyxis*. Therefore, based on the data presented, strain Gsoil 250^T should be placed in the genus *Sphingopyxis* as representing a novel species, for which the name *Sphingopyxis ginsengisoli* sp. nov. is proposed.

Description of *Sphingopyxis ginsengisoli* sp. nov.

Sphingopyxis ginsengisoli (gin.sen.gi.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil of a ginseng field, the source of the organism).

Cells are Gram-negative, aerobic, positive for catalase and oxidase, and rod-shaped (0.2–0.3 µm in diameter and 1.0–1.2 µm in length after 4 days culture on R2A agar). Cells are motile by means of single polar flagella. Colonies grown on R2A agar plates for 4 days are smooth, circular, non-glossy, yellowish in colour and 1–3 mm in diameter. Growth occurs at 4–42 °C and pH 5.0–8.5. Growth occurs in the absence of NaCl and in the presence of 5.0 % (w/v) NaCl. Does not grow anaerobically in R2A broth medium supplemented with nitrate. Xylan, chitin, starch, cellulose and DNA are not degraded. Growth occurs on TSA and nutrient agar, but not on MacConkey agar. Substrate utilization, enzyme production and other physiological characteristics are given in Table 1. In addition, the following substrates are utilized for growth: D-lyxose, L-xylose, D-fucose, L-rhamnose, propionate, 3-hydroxybutyrate, valerate, lactate, adipate, suberate, fumarate, succinate, amygdalin, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-glutamine, L-histidine, L-isoleucine, L-leucine, L-proline and L-tryptophan. The following substrates are not utilized for growth: L-sorbose, D-arabinose, D-ribose, N-acetylglucosamine, pyruvate, formate, caprate, maleic acid, phenylacetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, salicin, citrate, malonate, glutarate, tartrate, itaconate, oxalate, D-lactose, melibiose, sucrose, raffinose, gluconate, D-adonitol, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, glycerol, methanol, ethanol, glycogen, inulin, dextran, L-arginine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tyrosine and L-valine. H₂S and indole are not produced. Arginine dihydrolase and tryptophan deaminase are produced, but not lysine decarboxylase, ornithine decarboxylase or urease. Predominant respiratory lipoquinone is Q-10. Major fatty acids are C_{18:1ω7c} (46.4 %), C_{16:1ω7c}/iso-C_{15:0} 2-OH (34.6 %); 2-hydroxy fatty acid is also present.

Major polar lipids are sphingoglycolipid, phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol and two unknown glycolipids. The DNA G + C content of the type strain is 69.2 mol%.

The type strain, Gsoil 250^T (=KCTC 12582^T=LMG 23390^T), was isolated from soil of a ginseng field in Pocheon province, South Korea.

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