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Sphingopyxis panaciterrae sp. nov., Isolated from Soil of Ginseng Field

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A Gram-negative, strictly aerobic, motile bacterial strain, designated Gsoil 124^T, was isolated from a soil sample taken from a ginseng field in Pocheon Province (South Korea). The isolate contained Q-10 as the predominant lipoquinone, plus $C_{18:1}$ ω7c and summed feature 4 ($C_{16:1}$ ω6c and/or iso-$C_{15:0}$ 2-OH) as the major fatty acids. The G+C content of the genomic DNA was 68.1 mol%, and the major polar lipids consisted of sphingoglycolipid, phosphatidylglycerol, phosphatidylcholine, and phosphatidylethanolamine. A comparative 16S rRNA gene sequence analysis showed that strain Gsoil 124^T was most closely related to *Sphingopyxis chilensis* (98.7%), *Sphingopyxis alaskensis* (98.2%), *Sphingopyxis witflariensis* (98.2%), *Sphingopyxis taejonensis* (98.0%), and *Sphingopyxis macrogoltabida* (97.6%). However, the DNA-DNA relatedness between strain Gsoil 124^T and its phylogenetically closest neighbors was less than 22%. Thus, on the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 124^T should be classified as representing a novel species in the genus *Sphingopyxis*, for which the name *Sphingopyxis panaciterrae* sp. nov. is proposed. The type strain is Gsoil 124^T (=KCTC 12580^T=LMG 24003^T).

Keywords: *Sphingopyxis panaciterrae* sp. nov, polyphasic taxonomy, ginseng-field soil

The genus *Sphingopyxis* was recently created by Takeuchi *et al.* [20] and presently comprises eight species: *Sphingopyxis macrogoltabida* [19, 20], *Sphingopyxis terrae* [19, 20], *Sphingopyxis witflariensis* [6], *Sphingopyxis alaskensis* [5, 23], *Sphingopyxis chilensis* [5], *Sphingopyxis flavimaris* [25], *Sphingopyxis baekryungensis* [26], and *Sphingopyxis taejonensis* [14]. While investigating the culturable aerobic bacterial community in soil from a ginseng field in Pocheon Province (South Korea), a large number of bacteria were isolated, with some being characterized and classified as novel species of the genera *Bacillus* [21], *Chitinophaga* [1], and *Cohnella* [27]. However, in this study, one of the isolates, strain Gsoil 124^T, is shown to be affiliated with the genus *Sphingopyxis* from *Alphaproteobacteria*-based phenotypic, chemotaxonomic, and phylogenetic analyses. Moreover, the data suggest that the isolate represents a novel species of the genus *Sphingopyxis*.

Strain Gsoil 124^T was isolated from a soil sample using a modified-R2A medium, as described previously [21]. The isolate was then routinely cultured on an R2A agar at 25–30°C and preserved in a glycerol solution (20%) at −70°C. The Gram reaction was performed using the nonstaining method, as described by Buck [2], and the cell morphology was observed under a light microscope (Model, Nikon) at ×1,000 using cells grown on an R2A agar for 3 days at 25°C. The assimilation of single carbon sources was determined at 25°C, as described previously [22]. Some physiological characteristics were determined with API 20E galleries according to the instructions of the manufacturer (bioMérieux). The catalase and oxidase activities, anaerobic growth, growth at different temperatures, degradation of natural biopolymers, and other characteristics were all tested as reported previously [21, 22]. The Gsoil 124^T cells were Gram-negative, strictly aerobic, heterotrophic, motile, and rod shaped (0.3–0.4 μm wide and 1.0–1.3 μm long). On an R2A agar, Gsoil 124^T was able to grow at 10–30°C, but not at 37°C. The strain did not require but tolerated up to 3% NaCl. Catalase and oxidase were produced; urease was negative. The isolate tested negative for the degradation of macromolecules such as casein, collagen, starch, chitin, CM-cellulose, and xylan. Acid was not produced from any of the tested substrates. The physiological characteristics of strain Gsoil 124^T are summarized in the species description below, and the selective characteristics that differentiate strain Gsoil 124^T from other members of the genus *Sphingopyxis* are listed in Table 1.

The isoprenoid quinones were extracted and purified according to the method reported previously [1] and

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Table 1. Comparison of phenotypic characteristics of *Sphingopyxis panaciterrae* sp. nov. and phylogenetically related *Sphingopyxis* species.

Characteristics	1	2	3	4	5	6	7	8	9
Hydrolysis of									
Aesculin	–	+	+	–	–	V (+)	V (–)	+	–
Gelatin	–	–	–	–	–	V (–)	–	–	–
Urea	–	–	V (+)	–	–	–	–	–	–
Utilization of									
Acetate	+	+	–	–	–	(+)	(–)	+	–
L-Arabinose	–	–	–	–	–	+	–	+	–
Cellobiose	+	ND	V (–)	–	ND	+	+	+	+
Fructose	–	+	–	–	ND	–	–	–	–
D-Galactose	–	ND	V (+)	–	ND	(–)	(–)	–	–
D-Glucose	+	+	+	+	+	(+)	(–)	+	+
Malate	+	+	+	–	+	–	+	w	–
Maltose	–	+	+	+	–	+	+	–	–
Mannose	+	+	–	–	–	–	–	+	–
Trehalose	–	ND	+	–	ND	+	+	–	–
Xylose	–	W	–	–	ND	–	–	–	+
DNA G+C content (mol%)	68.1	66	65	ND	63	63–65	63–65	63	58

Species: 1, *Sphingopyxis panaciterrae* sp. nov. (n=1) (data from this study); 2, *Sphingopyxis chilensis* (n=1) [5, 7]; 3, *Sphingopyxis alaskensis* (n=7) [23]; 4, *Sphingopyxis witflariensis* (n=1) [6]; 5, *Sphingopyxis taejonensis* (n=1) [7, 10]; 6, *Sphingopyxis macrogoltabida* (n=6) [6, 7, 10, 19]; 7, *Sphingopyxis terrae* (n=6) [6, 7, 10, 19]; 8, *Sphingopyxis baekryungensis* sp. nov. (n=1) [26]; 9, *Sphingopyxis flavimaris* sp. nov. (n=1) [25]. +, Positive; –, negative; w, weakly positive; ND, not determined; V, variable reaction; n, number of strains. Data in parentheses are for the type strain.

analyzed by HPLC, as described by Shin *et al.* [17], the cellular fatty acids extracted and analyzed according to the instructions of the Sherlock Microbial Identification System (MIDI) [16], and the polar lipids extracted as described by Minnikin *et al.* [12] and identified using two-dimensional TLC [8]. The predominant respiratory lipoquinone type (Q-10) of strain Gsoil 124^T was the same as that of the genus *Sphingopyxis* [6, 19, 20]. The major fatty acids were summed feature 4 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH), $C_{18:1}$ω7c, $C_{16:0}$, and $C_{14:0}$ 2-OH, plus the profile was similar to those previously reported for members of the genus [5, 6, 10, 19, 25, 26]. However, some differences in the proportions of the fatty acid content were found between strain Gsoil 124^T and the phylogenetically closest relatives, perhaps due to differences in the cultivation conditions. The major polar lipids detected in strain Gsoil 124^T were sphingoglycolipid, phosphatidylglycerol, phosphatidylcholine, and phosphatidylethanolamine with a minor amount of diphosphatidylglycerol, reflecting a similar polar lipid composition to that for *Sphingopyxis* species [6, 25, 26]. To measure the G+C content of the chromosomal DNA, the genomic DNA of strain Gsoil 124^T was extracted and purified as described by Moore [13], and then enzymatically degraded into nucleotides. The resulting G+C content, determined as described by Mesbah *et al.* [11], was 68.1 mol%, which was also within the range of G+C contents for *Sphingopyxis* species [5, 6, 14, 25, 26].

For a phylogenetic analysis of strain Gsoil 124^T, the genomic DNA was extracted using a commercial genomic DNA extraction kit (Solgent Co. Ltd., Korea). The PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* [7], and the full 16S rRNA gene sequences were compiled using SeqMan software (DNASTAR, Madison, WI, U.S.A.). The 16S rRNA gene sequences for related taxa were obtained from the GenBank database (accession numbers shown in Fig. 1). Phylogenetic trees (based on

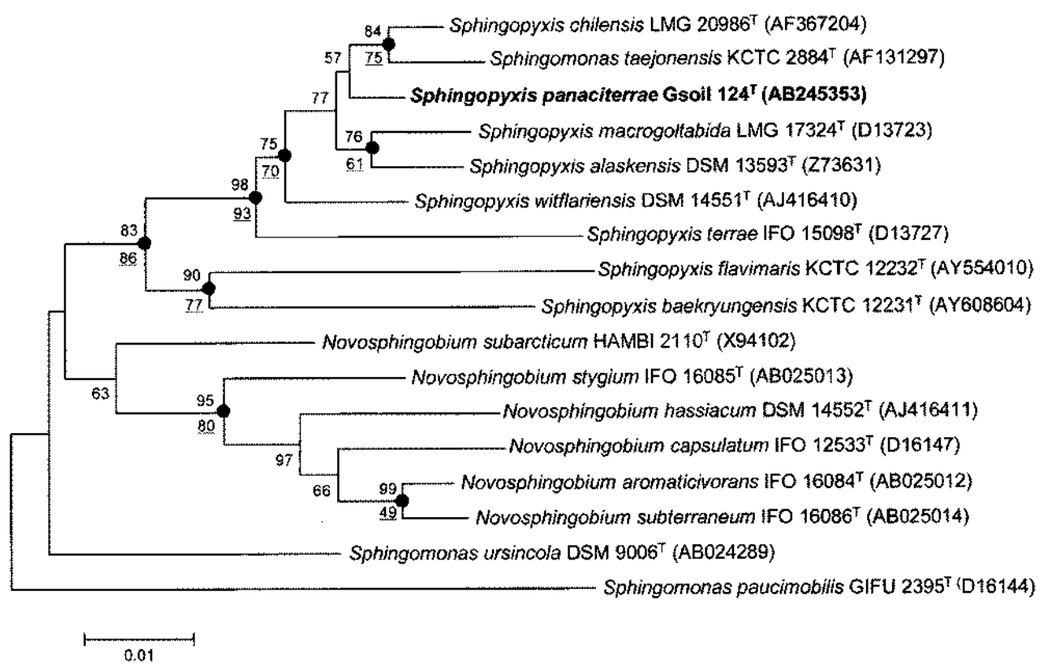

Fig. 1. Neighbor-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain Gsoil 124^T and some other related taxa.

The numbers at the branch nodes are bootstrap values (1,000 resamplings, only values over 50% are given). Dots indicate that the corresponding nodes were also recovered in a tree generated using the maximum-parsimony algorithm (underlined numbers are bootstrap values; 1,000 resamplings). Bar, 1% sequence divergence.

1,397 bp) were constructed based on the neighbor-joining [15] and maximum-parsimony [4] methods using the MEGA3 Program [9]. Other details of the phylogenetic analysis were as reported previously [1, 21, 22]. The 16S rRNA gene sequence for strain Gsoil 124^T determined in this study was a continuous stretch of 1,449 bp (base position 11-1,512 with respect to the *Escherichia coli* numbering system). A sequence similarity calculation after the neighbor-joining analysis indicated that the closest relatives to strain Gsoil 124^T were *Sphingopyxis chilensis* LMG 20986^T (98.7%), *Sphingopyxis alaskensis* DSM 13593^T (98.2%), *Sphingopyxis witflariensis* DSM 14551^T (98.2%), *Sphingopyxis taejonensis* KCTC 2884^T (98.0%), and *Sphingopyxis macrogoltabida* LMG 17324^T (97.6%). Lower sequence similarities (less than 95.4%) were also found with other validly described species in the genus *Sphingopyxis*. The relationship between strain Gsoil 124^T and other members of the genus *Sphingopyxis* was also evident in the phylogenetic tree (Fig. 1). Strain Gsoil 124^T and six closely related *Sphingopyxis* species formed a monophyletic clade with a high bootstrap value (98%), which was supported by both of the tree-making methods used in this study. The generally accepted criteria for delineating bacterial species are that strains with a 16S rRNA gene sequence dissimilarity above 3% or with a DNA-DNA relatedness value below 70% are considered as belonging to separate species [18, 24]. Thus, when taking this definition into account, the above-mentioned data indicate that strain Gsoil 124^T is clearly a separate species from other *Sphingopyxis* spp., with the exception of the 5 above-mentioned species of the genus. Therefore, to differentiate strain Gsoil 124^T from these 5 closely related species, DNA-DNA hybridization was performed fluorometrically using the method of Ezaki *et al*. [3]. The resulting DNA-DNA relatedness values for strain Gsoil 124^T with species in the genus *Sphingopyxis* ranged from 9% to 21% (21% with *S. chilensis* KCTC 12166^T, 17% with *S. alaskensis* KCTC 12916^T, 13% with *S. witflariensis* KCTC 12115^T, 9% with *S. macrogoltabida* KACC 10927^T, and 14% with *S. taejonensis* KCTC 2884^T), which were low enough [18, 24] to assign strain Gsoil 124^T as a novel species of the genus *Sphingopyxis*.

Consequently, while the phenotypic and phylogenetic characterizations indicated that strain Gsoil 124^T belonged to the genus *Sphingopyxis*, the phylogenetic distinctiveness, together with the DNA-DNA hybridization data, confirmed that strain Gsoil 124^T represented a species that is distinct

Table 2. Fatty acid composition of *Sphingopyxis panaciterrae* sp. nov. and phylogenetically related *Sphingopyxis* species[a].

Fatty acid	1	2	3	4	5	6	7	8[b]	9[b]
Straight-chain saturated									
$C_{14:0}$	–[c]	<1.0	<1.0	–	–	–	–	–	1.2
$C_{15:0}$	–	1.0	3.0	7.0	2.3	–	5.9	1.5	2.6
$C_{16:0}$	11.6	9.0	7.0	5.8	23.0	12.8	4.5	9.1	9.7
$C_{17:0}$	–	1.0	3.0	3.8	1.6	–	3.8	2.1	3.9
Hydroxy									
$C_{14:0}$ 2-OH	8.2	4.0	1.0	1.0	4.3	3.0	0.8	2.8	8.9
$C_{15:0}$ 2-OH	–	4.0	5.0	7.0	2.3	–	4.9	6.1	4.2
$C_{16:0}$ 2-OH	1.3	2.0	1.0	0.8	1.5	2.9	–	4.9	1.1
Mono-unsaturated									
$C_{15:1}\omega6c$	–	–	–	0.4	–	–	–	1.3	–
$C_{16:1}\omega5c$	1.8	1.0	1.0	1.2	1.6	2.9	2.1	–	–
$C_{17:1}\omega8c$	–	3.0	8.0	6.2	1.8	–	7.9	3.2	2.4
$C_{17:1}\omega6c$	–	18.0	33.0	43.0	11.8	4.5	48.0	17.8	18.8
$C_{18:1}\omega5c$	–	<1.0	<1.0	0.4	–	0.9	0.6	1.7	–
$C_{18:1}\omega7c$	35.3	34.0	27.0	9.0	–	–	–	24.0	33.8
$C_{18:1}\omega7c$ 11-methyl	–	2.0	1.0	2.8	–	–	–	4.5	5.6
Summed feature 4[d]	41.8	18.0	8.0	11.4	21.5	31.8	8.0	20.3	–
Summed feature 7[d]	–	–	–	–	28.3	41.2	13.0	–	–

Strains: 1, *Sphingopyxis panaciterrae* Gsoil 124^T (data from this study); 2, *Sphingopyxis chilensis* LMG 20986^T [5]; 3, *Sphingopyxis alaskensis* LMG 18877^T [5]; 4, *Sphingopyxis witflariensis* DSM 14551^T [6]; 5, *Sphingopyxis taejonensis* [10]; 6, *Sphingopyxis macrogoltabida* IFO 15003^T [6]; 7, *Sphingopyxis terrae* IFO15098^T [6]; 8, *Sphingopyxis flavimaris* KCTC 12232^T [25]; 9, *Sphingopyxis baekryungensis* KCTC 12231^T [26]. Strains 1-7 were grown on TSA for 24 h (strains 2, 3, and 6) or 48 h (1, 4, 5, and 7) at 28°C (1–4, 6, and 7) or 30°C (5); strains 8 and 9 were grown on marine agar for 7 days at 30°C.

[a]Values are shown as a percentage of the total fatty acid content for each strain.

[b]Fatty acids that account for less than 1.0% of the total fatty acid content are not shown.

[c]Not detected.

[d]Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contained $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH. Summed feature 9 contained one or more of the following fatty acids: $C_{19:1}$ $\omega6c$, $C_{19:1}$ cyclo$\omega10c$ and/or unknown ECL 18.846.

from recognized *Sphingopyxis* species. Moreover, some phenotypic differences were noted between strain Gsoil 124^T and phylogenetically related *Sphingopyxis* species (Table 1). Accordingly, on the basis of the data presented, strain Gsoil 124^T should be classified within the genus *Sphingopyxis* as representing a novel species, for which the name *Sphingopyxis panaciterrae* sp. nov. is proposed.

Description of *Sphingopyxis panaciterrae* sp. nov.

Sphingopyxis panaciterrae (pa.na.ci.ter'rae. N.L. n. Panax-acis, scientific name of ginseng; L. n. terra, soil; N.L. gen. n. *panaciterrae*, of soil from a ginseng field). Colonies grown on R2A agar (Difco) for 2 days are smooth, circular, non-glossy, yellowish, and convex. Cells grow on TSA agar, but not on skim milk, chitin agar, starch agar, or MacConkey agar. The optimum temperature for growth is 25°C. The pH growth range is between pH 5.5 and 8.5, with an optimum between pH 6.5 and 7. Growth does not occur under anaerobic conditions on R2A agar. The isolate cannot reduce nitrate and nitrite. D-Mannose, D-lyxose, D-cellobiose, D-galactose, D-glucose, L-rhamnose, D-ribose, salicin, acetate, propionate, 3-hydroxybutylate, valerate, malate, suberate, fumarate, caprate, succinate, L-alanine, L-glutamate, L-glutamine, L-isoleucine, L-leucine, L-tryptophane, L-arginine, and L-phenylalanine are all utilized as sole carbon and energy sources, whereas D-maltose, D-fructose, D-fucose, L-xylose, L-sorbose, D-arabinose, L-arabinose, D-xylose, D-lactose, D-melibiose, D-sucrose, D-trehalose, D-raffinose, D-adonitol, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, glycerol, methanol, ethanol, *N*-acetyl-glucosamine, glycogen, inulin, dextran, pyruvate, formate, adipate, malate, phenylacetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, citrate, malonate, glutarate, tartrate, lactate, itaconate, oxalate, amygdalin, gluconate, L-asparagine, L-aspartate, L-histidine, L-proline, L-threonine, L-cysteine, glycine, L-lysine, L-methionine, L-serine, L-tyrosine, and L-valine are not utilized. In API 20E tests, indole and hydrogen sulfide production, gelatin hydrolysis, and the activity of β-galactosidase, ornithine decarboxylase, lysine decarboxylase, arginine dihydrolase, and urease are all negative. Acid is not produced from D-glucose, L-arabinose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, or amygdalin. The predominant respiratory ubiquinone is Q-10. The cellular fatty acids are summed feature 4 ($C_{16:1}\omega6c$ and/or iso-$C_{15:0}$ 2-OH) (41.8%), $C_{18:1}\omega7c$ (35.3), $C_{16:0}$ (11.6%), $C_{14:0}$ 2-OH (8.2%), $C_{16:1}$ ω5c (1.8%), and $C_{16:0}$ 2-OH (1.3). The G+C content of the genomic DNA is 68.1 mol%.

The type strain Gsoil 124^T (= KCTC 12580^T=LMG 24003^T) was isolated from soil taken from a ginseng field in Pocheon Province (South Korea). The 16S rRNA gene sequence for strain Gsoil 124^T has been deposited in the NCBI GenBank under the accession number AB245353.

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