Sphingopyxis soli sp. nov., isolated from landfill soil

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A Gram-negative, aerobic, rod-shaped, motile, oxidase-positive, catalase-negative bacterium, designated strain BL03^T, was isolated from landfill soil in Pohang, Republic of Korea. Colonies on Luria–Bertani agar plates were yellow. The strain grew in the presence of 0–3 % (w/v) NaCl, at 15–42 °C and at pH 5.0–9.5. The predominant ubiquinone was Q-10, and the major cellular fatty acids were $C_{17:1}\omega6c$, $C_{15:0}$ 2-OH and $C_{18:1}\omega7c$. Polar lipids detected were phosphatidylmonomethylethanolamine, diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, sphingoglycolipid and an unknown glycolipid. Spermidine was identified as the major polyamine component. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BL03^T belongs to the genus *Sphingopyxis* with high sequence similarity to *Sphingopyxis taejonensis* JSS54^T (97.8 %), *Sphingopyxis alaskensis* RB2256^T (97.4 %) and *Sphingopyxis chilensis* S37^T (96.9 %). Levels of DNA–DNA relatedness between strain BL03^T and the above three type strains were only 10.3–40.3 %. The DNA G+C content of strain BL03^T was 65.9 mol%. Based on the data presented, strain BL03^T is considered to represent a novel species of the genus *Sphingopyxis*, for which the name *Sphingopyxis soli* sp. nov. is proposed. The type strain is BL03^T (=KCTC 22405^T =JCM 15910^T).

The genus *Sphingopyxis* was proposed by Takeuchi *et al.* (2001) and classified in the family *Sphingomonadaceae*. At the time of writing, the genus comprises 11 recognized species: *Sphingopyxis alaskensis* (Vancanneyt *et al.*, 2001), *S. baekryungensis* (Yoon *et al.*, 2005), *S. chilensis* (Godoy *et al.*, 2003), *S. flavimaris* (Yoon & Oh, 2005), *S. ginsengisoli* (Lee *et al.*, 2008), *S. litoris* (Kim *et al.*, 2008), *S. macrogoltabida* (Takeuchi *et al.*, 2001), *S. marina* (Kim *et al.*, 2008), *S. taejonensis* (Pal *et al.*, 2006), *S. terrae* (Takeuchi *et al.*, 2001) and *S. witflariensis* (Kämpfer *et al.*, 2002). Members of the genus *Sphingopyxis* are yellow- or whitish-brown-pigmented, Gram-negative, aerobic, non-spore-forming, motile or non-motile and with DNA G+C contents of 58–69.2 mol% (Lee *et al.*, 2008). They are chemotaxonomically characterized by the presence of 2-OH fatty acids but the absence of 3-OH fatty acids, and the presence of ubiquinone Q-10 and spermidine. Sphingoglycolipids have been detected in all strains of the genera *Sphingomonas sensu strictu*, *Sphingobium*, *Novosphingobium* and *Sphingosinicella* (Pal *et al.*, 2006; Takeuchi *et al.*, 2001). In this paper, we describe the morphological, biochemical and phylogenetic characteristics of a *Sphingopyxis*-like strain, BL03^T, employing *Sphingopyxis taejonensis* JSS54^T, *Sphingopyxis alaskensis* RB2256^T and *Sphingopyxis chilensis* S37^T as reference strains in parallel tests.

Strain BL03^T was isolated from landfill soil in Pohang, Korea, by plating the sample on Luria–Bertani broth (LB; BBL) agar plates at 30 °C for 3 days. On LB agar plates, strain BL03^T formed colonies that were yellow, circular, convex and entire. For phylogenetic analysis, genomic DNA was extracted as described by Sambrook *et al.* (1989). The 16S rRNA gene of strain BL03^T was amplified by using the colony PCR method with two universal bacterial primers (Baker *et al.*, 2003). 16S rRNA gene sequence analysis was carried out by using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) and an automated DNA analyser system (PRISM 3730XL DNA analyser; Applied Biosystems) according to the manufacturer's instructions. The 16S rRNA gene sequence of strain BL03^T was compared with known sequences in the GenBank database of the NCBI, and was aligned by using the multiple sequence alignment program CLUSTAL X (1.83) (Thompson *et al.*, 1997). The phylogenetic relationships between strain BL03^T and representative species of the genus *Sphingopyxis* were determined by using the program MEGA 4 (Tamura *et al.*, 2007). A phylogenetic consensus tree was reconstructed by means of bootstrap analysis based on the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Felsenstein, 1981) and maximum-

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BL03^T is FJ599671.

A two-dimensional thin-layer chromatogram of polar lipids of strain BL03^T is available with the online version of this paper.

likelihood (Kluge & Farris, 1969) methods. The numbers of bootstrap replicates were set to 1000, 1000 and 300, respectively. Phylogenetic analysis indicated that strain BL03^T should be classified in the genus *Sphingopyxis* and was closely related to *Sphingopyxis taejonensis* JSS54^T (Fig. 1). Strain BL03^T showed highest levels of 16S rRNA gene sequence similarity to *Sphingopyxis taejonensis* JSS54^T (97.8 %), *Sphingopyxis alaskensis* RB2256^T (97.4 %) and *Sphingopyxis chilensis* S37^T (96.9 %).

Genomic DNA was extracted from strain BL03^T, the three reference strains, and *Escherichia coli* K-12, as a calibration reference, according to Sambrook *et al.* (1989). The DNA G+C content was determined by the fluorometric method (Gonzalez & Saiz-Jimenez, 2002) by using SYBR Green I and a real-time PCR thermocycler (Bio-Rad). The DNA G+C content of strain BL03^T was 65.9 mol%, within the range reported for members of the genus *Sphingopyxis*. DNA–DNA hybridizations were performed by using the method of Ezaki *et al.* (1989) as amended by Hirayama *et al.* (1996) with dry-adsorption immobilization. The results showed that strain BL03^T had low levels of DNA–DNA relatedness to *Sphingopyxis taejonensis* JSS54^T (40.3 %), *Sphingopyxis alaskensis* RB2256^T (34.7 %) and *Sphingopyxis chilensis* S37^T (10.3 %).

The cell morphology of strain BL03^T was determined by light microscopy (ECLIPSE 80*i*; Nikon). The Gram reaction was performed according to the non-staining method described by Buck (1982). The presence of flagella was determined by using the flagella staining method (Heimbrook *et al.*, 1989). To determine the physiologically optimal conditions for growth, strain BL03^T was incubated on LB agar for 2 days at various temperatures (4, 10, 15, 20, 25, 30, 37 and 42 °C), NaCl concentrations (0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 %, w/v) and pH (5.0–10.0 at increments of 0.5 pH units). Biochemical tests for enzyme activities and the utilization of substrates as sole carbon source were carried out by using API 20NE and API ZYM kits (bioMérieux) and Biolog GN2 microplate assays, according to the manufacturers' protocols. Hydrolysis of DNA and casein was determined by using DNase test agar (BBL) and skimmed milk (BBL), respectively, as described by Atlas (1993). Hydrolysis of cellulose was tested according to the method of Gerhardt *et al.* (1994). Catalase and oxidase activities were tested in 3 % (v/v) hydrogen peroxide solution (Hanker & Rabin, 1975) and 1 % (w/v) *p*-tetramethyl phenylenediamine (bioMérieux), respectively.

Cells of strain BL03^T were Gram-negative, non-flagellated rods. The strain grew at 15–42 °C, at pH 5.0–9.5 and in the presence of 0–3 % (w/v) NaCl. Optimal growth occurred at 30 °C, at pH 6.0 and in the presence of 0 % NaCl. Strain BL03^T was catalase-negative and oxidase-positive. DNA was hydrolysed but casein and cellulose were not. The biochemical characteristics of strain BL03^T are given in the species description below and in Table 1.

For fatty acid analysis, cells of strain BL03^T and the three *Sphingopyxis* reference strains were harvested from LB agar plates after incubation at 30 °C for 2 days. Cellular fatty acids were extracted according to the protocol of the Microbial Identification System (MIDI). The cellular fatty acid composition was analysed quantitatively by GC (Hewlett Packard 6890) and by using the MIDI System (Sasser, 1990). The predominant cellular fatty acids of strain BL03^T were $C_{17:1}\omega6c$ (44.2 % of the total), $C_{15:0}$ 2-OH (14.7 %) and $C_{18:1}\omega7c$ (10.6 %). The cellular fatty acid profile of strain BL03^T was similar to those of *Sphingopyxis*

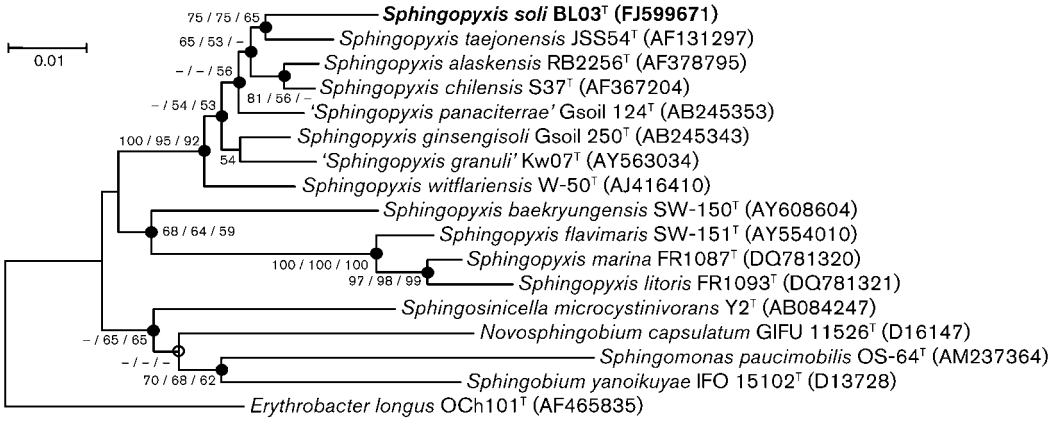

Fig. 1. Phylogenetic consensus tree based on 16S rRNA gene sequences showing the relationship between strain BL03^T and the type strains of closely related sphingomonads. The tree was reconstructed by using the neighbour-joining, maximum-parsimony and maximum-likelihood methods; numbers at nodes represent bootstrap values (based on 1000, 1000 and 300 resamplings, respectively). Filled circles and empty circles indicate generic branches that were found by using the neighbour-joining, maximum-parsimony and maximum-likelihood algorithms, and by using the neighbour-joining and maximum-likelihood algorithms, respectively. GenBank accession numbers are given in parentheses. *Pseudomonas antarctica* KJPB54^T was used as the outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

Table 1. Physiological and biochemical characteristics of strain BL03^T and the type strains of related species of the genus *Sphingopyxis*

Strains: 1, BL03^T; 2, *Sphingopyxis taejonensis* JSS54^T; 3, *S. alaskensis* RB2256^T; 4, *S. chilensis* S37^T. Data are from the present study. All strains were positive for hydrolysis of casein, D-glucose utilization, and alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities. All strains were negative for hydrolysis of gelatin and casein, utilization of arabinose, mannitol, acetylglucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid, reduction of nitrates to nitrites, indole production, glucose fermentation, and catalase, arginine hydrolase, galactosidase, lipase (C14), α-galactosidase, β-galactosidase, β-glucosidase, α-mannosidase and α-fucosidase activities. −, Negative; +, positive; W, weakly positive.

Characteristic	1	2	3	4
Hydrolysis of:				
Urea	+	−	−	−
Aesculin	+	−	−	+
DNA	+	+	+	−
Cellulose	−	+	−	−
Utilization of:				
Mannose	−	−	−	+
Maltose	+	−	+	+
Malic acid	−	+	+	+
Activity of:				
Valine arylamidase	+	+	−	W
Cystine arylamidase	−	W	W	−
Trypsin	+	W	W	−
α-Chymotrypsin	−	+	−	−
β-Glucuronidase	−	−	+	−
α-Glucosidase	+	−	+	W
N-acetyl-β-glucosaminidase	−	−	−	+

Table 2. Polyamine and fatty acid components of strain BL03^T and related reference strains

Strains: 1, BL03^T; 2, *Sphingopyxis taejonensis* JSS54^T; 3, *S. alaskensis* RB2256^T; 4, *S. chilensis* S37^T. Data are from the present study. t, <0.1 %; Tr, trace (<1.0 %); ND, not detected; fatty acids that comprised <1.0 % of the total in all strains are omitted.

Cellular component	1	2	3	4
Polyamines [μmol (g dry weight)$^{-1}$]				
1,4-Diaminopropane	t	t	t	t
Putrescine	0.6	0.4	0.5	0.7
Spermidine	18.2	13.9	9.2	13.3
Spermine	0.4	0.3	0.1	0.2
Fatty acids (%)				
$C_{15:0}$	3.9	1.5	4.4	6.8
$C_{16:0}$	4.7	12.8	3.0	3.1
$C_{17:0}$	4.5	Tr	5.4	3.0
$C_{14:0}$ 2-OH	1.3	7.4	1.3	2.0
$C_{15:0}$ 2-OH	14.7	3.6	22.7	25.2
$C_{16:0}$ 2-OH	1.1	3.5	1.3	Tr
$C_{16:1}\omega5c$	1.2	1.3	Tr	Tr
$C_{17:1}\omega8c$	6.9	1.0	8.3	6.8
$C_{17:1}\omega6c$	44.2	6.3	27.4	29.0
$C_{18:1}\omega7c$	10.6	30.3	14.9	10.5
$C_{18:1}\omega5c$	Tr	1.6	Tr	1.7
11-Methyl $C_{18:1}\omega7c$	2.0	9.1	6.4	4.2
Summed feature 3*	2.9	19.0	1.6	4.0
Summed feature 7*	ND	ND	2.1	Tr

*Summed feature 3 comprises $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; summed feature 7 comprises $C_{19:1}$ cyclo $\omega10c$ and/or $C_{19:1}\omega6c$.

alaskensis RB2256^T and *Sphingopyxis chilensis* S37^T. The detailed fatty acid profile of strain BL03^T is given in Table 2.

For analysis of polyamines, cells were cultivated in nutrient agar medium at 30 °C. Polyamines were extracted according to Busse & Auling (1988) and were analysed by HPLC as described by Stolz *et al.* (2007). Strain BL03^T exhibited a polyamine pattern with spermidine as the predominant compound, and 1,3-diaminopropane, putrescine and spermine as minor components [<1.0 μmol (g dry weight)$^{-1}$]. The polyamine patterns of *Sphingopyxis taejonensis* JSS54^T, *Sphingopyxis chilensis* S37^T and *Sphingopyxis alaskensis* RB2256^T, which were examined concurrently, were similar (Table 2). These results are in good agreement with those reported by Takeuchi *et al.* (2001) and Busse *et al.* (1999) and support the classification of strain BL03^T within the genus *Sphingopyxis*.

Polar lipids were extracted according to Xin *et al.* (2000), and were identified by two-dimensional TLC, as described by Tindall (1990). Major polar lipids of strain BL03^T were diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine, sphingoglycolipid, an unknown phospholipid and an unknown glycolipid (Fig. 2 and Supplementary Fig. S1). The polar lipid profile of strain BL03^T corresponded to those reported for sphingomonads (Busse *et al.*, 1999), and in particular for the genus *Sphingopyxis* (Kämpfer *et al.*, 2002; Yoon *et al.*, 2005; Yoon & Oh, 2005).

On the basis of its phenotypic, genetic and phylogenetic characteristics, strain BL03^T is considered to represent a novel species of the genus *Sphingopyxis*, for which we propose the name *Sphingopyxis soli* sp. nov.

Description of *Sphingopyxis soli* sp. nov.

Sphingopyxis soli (so′li. L. gen. n. *soli* of soil, the source of the type strain).

Colonies are circular, convex, entire, yellow and 2.0–5.0 mm in diameter after cultivation for 5 days at 30 °C. Cells are Gram-negative, aerobic and oxidase-positive. Grows at 15–42 °C (optimum, 30 °C), in the presence of 0–3 % (w/v) NaCl (optimum, 0 %) and at pH 5–9.5 (optimum, pH 6.0). DNA is hydrolysed, but casein and cellulose are not. Positive reactions are obtained for aesculin,

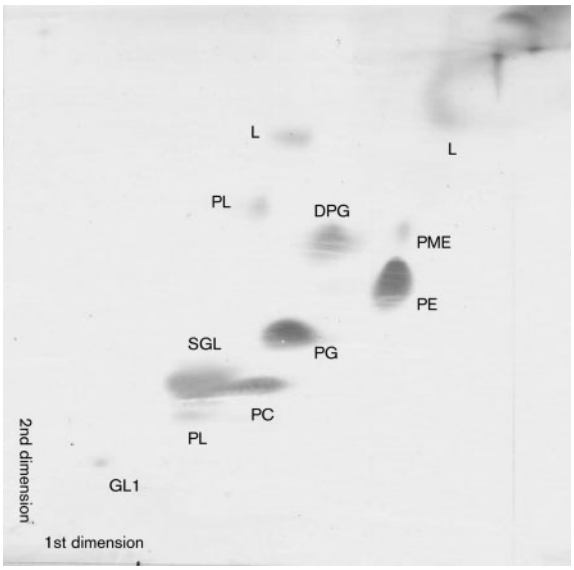

Fig. 2. Two-dimensional thin-layer chromatogram of polar lipids of strain BL03^T. PC, phosphatidylcholine; PME, phosphatidylmonomethylethanolamine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PL, unknown phospholipid; PE, phosphatidylethanolamine; SGL, sphingoglycolipid; GL1, unknown glycolipid; L, unknown lipids.

glucose and maltose (API 20NE). Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase, but negative for lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM). Utilizes dextrin, *N*-acetyl-D-glucosamine, adonitol, pyruvic acid methyl ester, β-hydroxybutyric acid, itaconic acid, DL-lactic acid, propionic acid, quinic acid, succinic acid, L-alanine, L-alanyl glycine, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, L-leucine, L-ornithine, L-pyroglutamic acid, L-serine, γ-aminobutyric acid and D-glucose 6-phosphate (Biolog GN2). Other organic substrates are not utilized. Major fatty acids are $C_{17:1}\omega6c$, $C_{15:0}$ 2-OH and $C_{18:1}\omega7c$. The DNA G+C content of the type strain is 65.9 mol%. Major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylmonomethylethanolamine and sphingoglycolipid. Spermidine is the major polyamine component.

The type strain, BL03^T (=KCTC 22405^T =JCM 15910^T), was isolated from soil taken from a landfill in Pohang, Republic of Korea.

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