Sphingopyxis flavimaris sp. nov., isolated from sea water of the Yellow Sea in Korea

Jung-Hoon Yoon and Tae-Kwang Oh

Correspondence

Jung-Hoon Yoon
jhyoon@kribb.re.kr

Korea Research Institute of Bioscience and Biotechnology (KRIBB), PO Box 115, Yusong, Taejeon, Korea

A Gram-negative, motile, yellow-pigmented, slightly halophilic bacterial strain, SW-151^T, was isolated from sea water of the Yellow Sea in Korea, and subjected to a polyphasic taxonomic study. The isolate grew optimally at 30 °C and in the presence of 2–3 % NaCl. Strain SW-151^T was characterized chemotaxonomically as having Q-10 as the predominant respiratory lipoquinone and C_{18:1}ω7c, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH and C_{17:1}ω6c as the major fatty acids. Sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine were the major polar lipids. The DNA G+C content was 58 mol%. 16S rRNA gene sequence analysis showed that strain SW-151^T joins the evolutionary radiation enclosed by the genus *Sphingopyxis*. Similarities between the 16S rRNA gene sequences of strain SW-151^T and the type strains of *Sphingopyxis* species ranged from 92.3 to 94.3 %, which is low enough to categorize strain SW-151^T as a species distinct from previously described *Sphingopyxis* species. On the basis of phenotypic properties and phylogenetic distinctiveness, strain SW-151^T (=KCTC 12232^T=DSM 16223^T) should be classified as a novel *Sphingopyxis* species, for which the name *Sphingopyxis flavimaris* sp. nov. is proposed.

The genus *Sphingomonas* was proposed by Yabuuchi *et al.* (1990), with five species and two genomospecies. Subsequently, a large number of species have been included in the genus (e.g. Takeuchi *et al.*, 1993, 1995; Lee *et al.*, 2001; Busse *et al.*, 2003). Phylogenetic analyses based on 16S rRNA gene sequences have shown that the genus *Sphingomonas* and three related genera belong to the α -subclass of the *Proteobacteria* (Anzai *et al.*, 2000; Takeuchi *et al.*, 2001; Yabuuchi *et al.*, 2002). Takeuchi *et al.* (2001) proposed that *Sphingomonas* species should be assigned to three different genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, in addition to the genus *Sphingomonas*. However, Yabuuchi *et al.* (2002) showed that there is no phenotypic or phylogenetic evidence for dividing the genus *Sphingomonas* into the four genera and hence the genus *Sphingomonas* should remain undivided. These proposals have made the taxonomy of the genus *Sphingomonas* confused, but at present the nomenclature of Takeuchi *et al.* (2001) is generally 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). In this study, we report on the detailed taxonomic characterization of a slightly halophilic, yellow-pigmented, *Sphingopyxis*-like bacterial strain, SW-151^T, which was isolated from the water of the Yellow Sea in Korea.

Strain SW-151^T was isolated by the usual dilution-plating technique on marine agar 2216 (MA; Difco) at 30 °C. Cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy. For the latter, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and, after air-drying, the grids were examined by using a model CM-20 transmission electron microscope (Philips). The flagellation type was examined by transmission electron microscopy using cells from exponentially growing cultures. Growth at various NaCl concentrations was investigated in marine broth 2216 (Difco) or in trypticase soy broth (Difco). Growth in the absence of NaCl was investigated in trypticase soy broth without NaCl. Growth at various temperatures (4–45 °C) was measured on MA. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MA and on MA supplemented with nitrate that had been prepared anaerobically using nitrogen. Catalase and oxidase activities and hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA with the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were studied as described previously (Lányi, 1987) but with the modification that artificial sea water was used for preparation of media. The artificial sea water contained the following (l⁻¹ distilled water): 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g

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CaCl₂·2H₂O (Levring, 1946). H₂S production was tested as described previously (Bruns *et al.*, 2001). Acid production from carbohydrates was evaluated as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested according to the method of Baumann & Baumann (1981) using supplementation with 2% (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1% (v/v) vitamin solution (Staley, 1968).

Cell biomass of strain SW-151^T for respiratory lipoquinone analysis and for DNA extraction was obtained from cultivation in marine broth at 30 °C. Respiratory lipoquinones were analysed as described previously (Komagata & Suzuki, 1987), using reversed-phase HPLC. Chromosomal DNA was isolated and purified according to a method described previously (Yoon *et al.*, 1996), with the exception that RNase T1 was used together with RNase A. For fatty acid methyl ester analysis, a loop of cell mass was harvested from agar plates after cultivation for 7 days at 30 °C on MA. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (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). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984), with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The 16S rRNA gene sequence was amplified by the PCR, using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003).

Morphological, cultural, physiological and biochemical properties of strain SW-151^T are shown in Table 1 or are given in the species description. The predominant respiratory lipoquinone detected in strain SW-151^T was Q-10, at a peak area ratio of approximately 92%. Strain SW-151^T had a cellular fatty acid profile that contained large amounts of unsaturated, straight-chain and hydroxy fatty acids; the components were C_{18:1}ω7c (24.0%), C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (20.3%), C_{17:1}ω6c (17.8%), C_{16:0} (9.1%), C_{15:0} 2-OH (6.1%), C_{16:0} 2-OH (4.9%), 11-methyl C_{18:1}ω7c (4.5%), C_{17:1}ω8c (3.2%), C_{14:0} 2-OH (2.8%), C_{17:0} (2.1%), C_{18:1}ω5c (1.7%), C_{15:0} (1.5%) and C_{15:1}ω6c (1.3%). The lack of 3-hydroxy fatty acids is a

Table 1. Differential phenotypic characteristics of *Sphingopyxis* species and *Sphingomonas taejonensis*

Species: 1, *Sphingopyxis flavimaris* sp. nov. (n=1); 2, *Sphingopyxis macrogoltabida* (n=6) (data from Takeuchi *et al.*, 1993, 1995; Kämpfer *et al.*, 1997; Lee *et al.*, 2001); 3, *Sphingopyxis terrae* (n=6) (Takeuchi *et al.*, 1993, 1995; Kämpfer *et al.*, 1997; Lee *et al.*, 2001); 4, *Sphingopyxis alaskensis* (n=7) (Vancanneyt *et al.*, 2001); 5, *Sphingopyxis chilensis* (n=1) (Godoy *et al.*, 2003); 6, *Sphingopyxis witflariensis* (n=1) (Kämpfer *et al.*, 2002); 7, *Sphingomonas taejonensis* (n=1) (Lee *et al.*, 2001). All species are rod-shaped, non-spore-forming, Gram-negative, positive for catalase and oxidase and negative for nitrate reduction and utilization of citrate. +, 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
Colony colour	Yellow	White or yellow	Light- or deep yellow	Yellow to beige	Yellow	Yellow	Pale yellow
Motility	+	+	v (+)	+	+	+	+
Hydrolysis of:							
Aesculin	–	v (+)	v (–)	+	+	–	–
Gelatin	–	v (–)	–	–	–	–	–
Urea	–	–	–	v (+)	–	–	–
Utilization of:							
L-Arabinose	–	+	–	–	–	–	–
Cellobiose	+	+	+	v (–)	ND	–	ND
Fructose	–	–	–	–	+	–	ND
Malate	–	–	+	+	+	–	+
Maltose	–	+	+	+	+	+	–
Mannose	–	–	–	–	+	–	–
Sucrose	–	–	–	–	ND	–	–
Trehalose	–	+	+	+	ND	–	ND
Xylose	+	–	–	–	W	–	ND
Nucleotide signatures of 16S rRNA:*							
134	A	G	G	G	G	G	G
990:1215	U:A	U:G	U:G	U:G	U:G	U:A	U:G
DNA G+C content (mol%)	58	63–65	63–65	65	66	ND	63

**E. coli* 16S rRNA numbering system was used.

characteristic also shared by *Sphingopyxis* and *Sphingomonas* species (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 1993, 1995; Lee *et al.*, 2001; Busse *et al.*, 2003). The major polar lipids found in strain SW-151^T were sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Phosphatidylmonomethylethanolamine, phosphatidylmethylethanolamine, an unidentified polar lipid and unidentified phospholipids were also present. The DNA G+C content of strain SW-151^T was 58 mol%. The 16S rRNA gene sequence of strain SW-151^T determined in this study comprised 1444 nucleotides, representing approximately 96% of the *Escherichia coli* 16S rRNA sequence. 16S rRNA gene sequence similarity searches with a public database (GenBank) revealed that strain SW-151^T is phylogenetically related to the genus *Sphingopyxis*. In the neighbour-joining tree based on 16S rRNA gene sequences, strain SW-151^T joined the cluster comprising *Sphingopyxis* species at a bootstrap resampling value of 66.9% (Fig. 1). Strain SW-151^T exhibited 16S rRNA gene sequence similarity levels of 92.3–94.3% to all *Sphingopyxis* species with validly published names. Sequence similarities to other species included in the phylogenetic analysis were in the range 85.5–93.6% (Fig. 1).

The result of the phylogenetic analysis based on 16S rRNA gene sequences suggests two possibilities for the classification of strain SW-151^T: membership of the genus

Sphingopyxis or membership of a novel genus. The predominant respiratory lipoquinone type of strain SW-151^T is the same as that of the genus *Sphingopyxis* (Takeuchi *et al.*, 1993; Kämpfer *et al.*, 2002). The cellular fatty acid profile of strain SW-151^T is similar to those of *Sphingopyxis* species, particularly *Sphingopyxis chilensis*, although there are differences in the compositions of some fatty acids, which may be caused by different cultivation conditions (Takeuchi *et al.*, 1993; Lee *et al.*, 2001; Vancanneyt *et al.*, 2001; Godoy *et al.*, 2003). There are not enough phenotypic properties to distinguish strain SW-151^T from *Sphingopyxis* species (Takeuchi *et al.*, 1993; Lee *et al.*, 2001; Vancanneyt *et al.*, 2001; Godoy *et al.*, 2003). It seems reasonable to assign strain SW-151^T to the genus *Sphingopyxis*. There is a widely recognized criterion for species definition in current bacteriology that suggests that strains with more than 3% difference in 16S rRNA gene sequences be considered as representing different species (Stackebrandt & Goebel, 1994). Despite the lack of DNA–DNA hybridization experiments, the phylogenetic distinctiveness shown in this study is sufficient to separate strain SW-151^T from other *Sphingopyxis* species with validly published names (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain SW-151^T should be placed in the genus *Sphingopyxis* as a member of a novel species, for which the name *Sphingopyxis flavimaris* sp. nov. is proposed.

Description of *Sphingopyxis flavimaris* sp. nov.

Sphingopyxis flavimaris (fla.vi.ma'ris. L. adj. *flavus* yellow; L. gen. n. *maris* of the sea; N.L. gen. n. *flavimaris* of the Yellow Sea).

Cells are rods, 0.7–0.9 × 1.5–2.5 µm. Gram-negative. Non-spore-forming. Motile by means of a single polar flagellum. Colonies are circular, convex, yellow in colour and 0.8–1.0 mm in diameter after 7 days cultivation at 30 °C on MA. Growth occurs at 4 °C but not at 37 °C. Optimal pH for growth is 7.0–8.0; growth occurs at pH 5.0 but not at pH 4.5. Optimal growth occurs in the presence of 2–3% (w/v) NaCl. Growth occurs in the presence of 6% (w/v) NaCl but not without NaCl or in the presence of >8% (w/v) NaCl. Growth does not occur under anaerobic conditions on MA or MA supplemented with nitrate. Tweens 20, 40, 60 and 80 and tyrosine are hydrolysed. Casein, hypoxanthine, starch and xanthine are not hydrolysed. H₂S is not produced. D-Glucose and pyruvate are utilized as sole carbon and energy sources. D-Galactose, lactose, acetate, succinate, benzoate, formate and L-glutamate are not utilized. Acid is not produced from the following substrates: adonitol, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, D-melezitose, melibiose, *myo*-inositol, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, sucrose, D-trehalose and D-xylose. The predominant respiratory lipoquinone is Q-10. The major fatty acids are C_{18:1}ω7c, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH and C_{17:1}ω6c. The major polar lipids are sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine;

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequence data, showing the phylogenetic position of strain SW-151^T and representatives of some related taxa. Bootstrap values (1000 replications) are shown as percentages at each node only if they are 50% or greater. Scale bar, 0.01 substitutions per nucleotide position. *Rhodospirillum rubrum* ATCC 11170^T was used as the outgroup.

minor components are phosphatidylmonomethylethanolamine, phosphatidylmethylethanolamine, an unidentified polar lipid and unidentified phospholipids. The DNA G + C content is 58 mol% (determined by HPLC).

The type strain, SW-151^T (=KCTC 12232^T=DSM 16223^T), was isolated from sea water at Baekryung Island in the Yellow Sea in Korea.

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