Sphingopyxis baekryungensis sp. nov., an orange-pigmented bacterium isolated from sea water of the Yellow Sea in Korea

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A Gram-negative, motile, slightly halophilic bacterial strain, SW-150^T, was isolated from sea water of the Yellow Sea, Korea, and was characterized by a polyphasic taxonomic approach. Strain SW-150^T grew optimally at 25–30 °C and in the presence of 2% (w/v) NaCl. The isolate could be distinguished from other *Sphingopyxis* species in producing an orange pigment. It contained ubiquinone-10 as the predominant respiratory lipoquinone and C_{18:1}ω7c and C_{17:1}ω6c as the major fatty acids. No 3-hydroxy fatty acids were detected. Major polar lipids were sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The DNA G+C content was 63 mol%. Comparative 16S rRNA gene sequence analyses showed that strain SW-150^T was phylogenetically affiliated to the genus *Sphingopyxis* of the family *Sphingomonadaceae*. Similarity values between the 16S rRNA gene sequences of strain SW-150^T and the type strains of *Sphingopyxis* species ranged from 91.6 to 94.2%, making it possible to categorize strain SW-150^T as a species that is separate from previously described *Sphingopyxis* species. On the basis of phenotypic properties and phylogenetic distinctiveness, SW-150^T (=KCTC 12231^T=DSM 16222^T) should be classified as the type strain of a novel *Sphingopyxis* species, for which the name *Sphingopyxis baekryungensis* sp. nov. is proposed.

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 sensu stricto*. Although Yabuuchi *et al.* (2002) proposed that there is no phenotypic and phylogenetic evidence to divide the genus *Sphingomonas* into the four genera, the nomenclature of Takeuchi *et al.* (2001) is in common use (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 present the genus *Sphingopyxis* comprises six 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) and

Sphingopyxis flavimaris (Yoon & Oh, 2005). Recently, an orange-pigmented *Sphingopyxis*-like bacterium, SW-150^T, was isolated from sea water of the Yellow Sea, Korea. To date, all members allocated to the genus *Sphingopyxis* are yellow-pigmented (Takeuchi *et al.*, 1993; Kämpfer *et al.*, 2002; Godoy *et al.*, 2003; Yoon & Oh, 2005). Accordingly, the aim of the present work was to determine the exact taxonomic position of strain SW-150^T by using a polyphasic taxonomic characterization.

Sea water collected from Baekryung Island in the Yellow Sea in Korea was used as a source for isolation of bacterial strains. Strain SW-150^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 (TEM). The presence of flagella was investigated by TEM, using cells from exponentially growing cultures. The Gram-reaction was determined by using the bioMérieux Gram Stain kit, according to the manufacturer's instructions. Growth at various NaCl concentrations was investigated in marine broth 2216 (MB; Difco) or in trypticase soy broth (Difco).

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SW-150^T is AY608604.

The tree from which Fig. 1 was taken is available as supplementary material in IJSEM Online.

Growth in the absence of NaCl was investigated in trypticase soy broth without NaCl. Growth at various temperatures (4–40 °C) was measured on MA. The pH range for growth was determined in MB that was adjusted to various pH values (pH 4.5–9.5 at intervals of 0.5 pH units). Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MA and on MA supplemented with nitrate, both of which 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 investigated 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 (Lanyi, 1987), except that artificial sea water was used for preparation of media. The artificial sea water contained (per litre distilled water): 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). H₂S production was tested as described previously (Bruns *et al.*, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested according to the method of Baumann & Baumann (1981), supplemented with 2% (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1% (v/v) vitamin solution (Staley, 1968). For *in vivo* pigment-absorption spectrum

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

Species: 1, *Sphingopyxis baekryungensis* 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, *S. witflariensis* (*n*=1) (Kämpfer *et al.*, 2002); 7, *Sphingopyxis flavimaris* (*n*=1) (Yoon & Oh, 2005); 8, *Sphingomonas taejonensis* (*n*=1) (Lee *et al.*, 2001). 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
Colony colour	Orange	Whitish-brown or yellow	Light- or deep-yellow	Yellow to beige	Yellow	Yellow	Yellow	Pale-yellow
Motility	+	+	v (+)	+	+	+	+	+
Nitrate reduction to nitrite	+	–	–	–	–	–	–	–
Hydrolysis of:								
Aesculin	+	v (+)	v (–)	+	+	–	–	–
Gelatin	–	v (–)	–	–	–	–	–	–
Urea	–	–	–	v (+)	–	–	–	–
Utilization of:								
Acetate	+	(+)	(–)	–	ND	–	–	ND
L-Arabinose	+	+	–	–	–	–	–	–
Cellobiose	+	+	+	v (–)	ND	–	+	ND
Fructose	–	–	–	–	+	–	–	ND
D-Galactose	–	(–)	(–)	v (+)	ND	–	–	ND
D-Glucose	+	(+)	(–)	+	+	+	+	ND
Malate	w	–	+	+	+	–	–	+
Maltose	–	+	+	+	+	+	–	–
Mannose	+	–	–	–	+	–	–	–
Trehalose	–	+	+	+	ND	–	–	ND
Xylose	–	–	–	–	w	–	+	ND
Nucleotide signature of 16S rRNA gene:								
134*	G	G	G	G	G	G	A	G
990:1215*	U:A	U:G	U:G	U:G	U:G	U:A	U:A	U:G
Major fatty acids†	C _{18:1} ω7c, C _{17:1} ω6c	C _{18:1} ω7c, iso C _{15:0} 2-OH/ C _{16:1} ω7c	C _{17:1} ω6c, C _{18:1} ω7c	C _{17:1} ω6c, C _{18:1} ω7c	C _{18:1} ω7c, C _{17:1} ω6c	C _{17:1} ω6c, iso C _{15:0} 2-OH/ C _{16:1} ω7c	C _{18:1} ω7c, iso C _{15:0} 2-OH/ C _{16:1} ω7c, C _{17:1} ω6c	C _{18:1} ω7c/C _{18:1} ω9t/C _{18:1} ω12t, C _{16:0}
DNA G+C content (mol%)	63	63–65	63–65	65	66	ND	58	63

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

†Data from Lee *et al.* (2001), Vancanneyt *et al.* (2001), Godoy *et al.* (2003), Yoon & Oh (2005) and this study.

analysis, the strain was cultivated aerobically in the dark at 30 °C in MB. The cultures were washed twice by centrifugation using MOPS buffer (MOPS/NaOH, 0.01 M; KCl, 0.1 M; MgCl₂, 0.001 M; pH 7.5), and were disrupted by sonication with a Branson Sonifier 450. After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined on a Beckman Coulter DU800 spectrophotometer. Other physiological and biochemical tests were performed with the API 20E system (bioMérieux). The morphological, cultural, physiological and biochemical properties of strain SW-150^T are shown in Table 1, or are given in the species description (see below). The sonicated cell extracts showed absorption maximum at 463–464 nm, and methanol-soluble pigment was characterized by absorption maximum at 462 nm.

The 16S rRNA gene was amplified by 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). The almost complete 16S rRNA gene sequence of strain SW-150^T determined in this study comprised 1446 nucleotides, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. Comparative 16S rRNA gene sequence analyses showed that strain SW-150^T is phylogenetically most closely related to *Sphingopyxis* species of the family *Sphingomonadaceae*, particularly forming a cluster with *S. flavimaris* with a relatively high bootstrap resampling value of 85.8 % (Fig. 1). The sequence contained four of five signature nucleotides defined for the genus *Sphingopyxis*, as described by Takeuchi *et al.* (2001) (Table 1). Similarity values between the 16S rRNA gene sequences of strain SW-150^T and the type strains of *Sphingopyxis* species ranged from 91.6 % (with *S. terrae*) to 94.2 % (with *S. macrogoltabida* and *S. chilensis*). Sequence similarities to other members used in the phylogenetic analysis were lower than 93.6 % (Fig. 1).

Cell biomass for respiratory lipoquinone and polar lipid analyses and for DNA extraction was obtained from cultivation in MB at 30 °C. Respiratory lipoquinones were analysed as described previously (Komagata & Suzuki, 1987), by using reversed-phase HPLC. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that ribonuclease T1 was applied in combination with ribonuclease A. For fatty acid methyl ester (FAME) analysis, a loop of cell mass was harvested from MA plates after cultivation for 7 days at 30 °C. The FAMES were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and were 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 the DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. Strain SW-150^T contained ubiquinone-10 (Q-10) as the predominant respiratory lipoquinone, at a peak area ratio of approximately 91 %. The major components (>1 %) of the fatty acids detected in strain SW-150^T were unsaturated fatty acids C_{18:1}ω7c (33.8 %), C_{17:1}ω6c (18.8 %), 10-methyl-C_{18:1}ω7c (5.6 %) and C_{17:1}ω8c (2.4 %), straight-chain fatty acids C_{16:0} (9.7 %), C_{17:0} (3.9 %), C_{15:0} (2.6 %) and C_{14:0} (1.2 %), and hydroxy fatty acids C_{14:0} 2-OH (8.9 %), C_{15:0} 2-OH (4.2 %) and C_{16:0} 2-OH (1.1 %). This cellular fatty acid profile was similar to those of some *Sphingopyxis* species, particularly *S. chilensis*, although there are differences in the compositions of some fatty acids, which may be caused by different cultivation conditions (Takeuchi *et al.*, 1993; Vancanney *et al.*, 2001; Godoy *et al.*, 2003; Yoon & Oh, 2005) (Table 1). 3-Hydroxy fatty acids were not detected in strain SW-150^T, which was also found

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence data showing the phylogenetic positions of strain SW-150^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. Bar, 0.01 substitutions per nucleotide position. *Rhodospirillum rubrum* ATCC 11170^T was used as an outgroup.

for other *Sphingopyxis* species and all species that were previously assigned to the genus *Sphingomonas* (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 1993, 1995; Lee *et al.*, 2001; Vancanneyt *et al.*, 2001; Busse *et al.*, 2003; Godoy *et al.*, 2003). The major polar lipids detected in strain SW-150^T were sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine; minor amounts of phosphatidylmonomethylethanolamine, an unidentified glycolipid and unidentified phospholipids were also present. This polar lipid composition is similar to those of four *Sphingopyxis* species, the data of which have been reported by Kämpfer *et al.* (1997, 2002) and Yoon & Oh (2005); phosphatidylcholine, which was detected in three *Sphingopyxis* species with the exception of *S. flavimaris*, was not present (Kämpfer *et al.*, 1997, 2002; Yoon & Oh, 2005). The DNA G + C content of strain SW-150^T was 63 mol%. The results obtained from chemotaxonomic analyses, showing the properties that are characteristic of the genus *Sphingopyxis*, were in agreement with the result of phylogenetic classification based on 16S rRNA gene sequences (Takeuchi *et al.*, 1993; Kämpfer *et al.*, 2002; Vancanneyt *et al.*, 2001; Godoy *et al.*, 2003).

There are not enough phenotypic, particularly chemotaxonomic, properties to distinguish strain SW-150^T from the genus *Sphingopyxis* (Takeuchi *et al.*, 1993; Lee *et al.*, 2001; Vancanneyt *et al.*, 2001; Godoy *et al.*, 2003). The orange pigmentation of strain SW-150^T is not observed in *Sphingopyxis* species. However, three orange-pigmented species, *Sphingomonas aurantiaca*, *Sphingomonas aerolata* and *Sphingomonas faeni*, were recently assigned to the genus *Sphingomonas*, which had previously comprised only yellow-pigmented species (Busse *et al.*, 2003). Accordingly, it seems reasonable to assign strain SW-150^T within the genus *Sphingopyxis*. The phylogenetic distinctiveness shown in this study is low enough to exclude the possibility of assigning strain SW-150^T to a *Sphingopyxis* species with a validly published name, despite the lack of DNA–DNA hybridization experiments between strain SW-150^T and other *Sphingopyxis* species (Stackebrandt & Goebel, 1994). There are differences between strain SW-150^T and the other six *Sphingopyxis* species in some phenotypic properties, including pigmentation, nitrate reduction and utilization of some substrates (Table 1). In particular, it is noteworthy that nitrate reduction has not been shown in other recognized *Sphingopyxis* species (Table 1). Therefore, on the basis of the data presented, strain SW-150^T should be placed in the genus *Sphingopyxis* in a novel species, for which the name *Sphingopyxis baekryungensis* sp. nov. is proposed.

Description of *Sphingopyxis baekryungensis* sp. nov.

Sphingopyxis baekryungensis (baek.ryung.en'sis. N.L. fem. adj. *baekryungensis* of Baekryung Island, an island of the Yellow Sea in Korea where the type strain was isolated).

Cells are ovoid, 0.7–0.9 µm × 1.5–2.5 µm. Gram-negative.

Non-spore-forming. Motile by means of a single polar flagellum. Colonies are circular, convex, glistening, orange in colour and 0.8–1.0 mm in diameter after 7 days cultivation at 30 °C on MA. The sonicated cell extracts show absorption maximum at 463–464 nm. Methanol-soluble pigment shows absorption maximum at 462 nm. Optimal growth temperature is 25–30 °C; growth occurs at 4 °C, but not at 37 °C. Optimal pH for growth is 7.0–8.0; growth occurs at pH 4.5, but not at pH 4.0. Optimal growth occurs in the presence of 2 % (w/v) NaCl; growth occurs in the presence of 10 % (w/v) NaCl, but not without NaCl and in the presence of > 11 % (w/v) NaCl. Growth does not occur under anaerobic conditions on MA and on MA supplemented with nitrate. Tweens 20, 40, 60 and 80 are hydrolysed. Casein, starch, hypoxanthine, tyrosine and xanthine are not hydrolysed. H₂S and indole are not produced. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. Pyruvate is utilized. Lactose, sucrose, succinate, benzoate, formate and L-glutamate are not utilized. Acid is produced from L-arabinose, D-galactose and D-glucose, and weakly produced from D-cellobiose and sucrose. No acid is formed from the following substrates: adonitol, D-fructose, lactose, maltose, D-mannitol, D-mannose, D-melezitose, melibiose, *myo*-inositol, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, D-trehalose or D-xylose. The predominant respiratory lipoquinone is Q-10. The major fatty acids are C_{18:1ω7c} (33.8 %) and C_{17:1ω6c} (18.8 %); 2-hydroxy fatty acids are detected. Major polar lipids are sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The DNA G + C content is 63 mol% (determined by HPLC). Other phenotypic properties are given in Table 1.

The type strain, SW-150^T (= KCTC 12231^T = DSM 16222^T), was isolated from sea water at Baekryung Island in the Yellow Sea in Korea.

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