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-150T, was isolated from sea water of the Yellow Sea, Korea, and was characterized by a polyphasic taxonomic approach. Strain SW-150T 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 C18:1ω7c and C17:1ω6c 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-150T was phylogenetically affiliated to the genus Sphingopyxis of the family Sphingomonadaceae. Similarity values between the 16S rRNA gene sequences of strain SW-150T and the type strains of Sphingopyxis species ranged from 91.6 to 94.2 %, making it possible to categorize strain SW-150T as a species that is separate from previously described Sphingopyxis species. On the basis of phenotypic properties and phylogenetic distinctiveness, SW-150T (= KCTC 12231T = DSM 16222T) should be classified as the type strain of a novel Sphingopyxis species, for which the name Sphingopyxis baekryungensis sp. nov. is proposed.

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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 phyllogenetic 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-150T, 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-150T 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-150T 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-150T 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, 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 (Brüns et al., 2001). H₂S production was tested as described previously (Brüns 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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Orange</td>
<td>Whitish-brown or yellow</td>
<td>Light- or deep-yellow</td>
<td>Yellow to beige</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Pale-yellow</td>
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<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>V (+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>Nitrate reduction to nitrite</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Aesculin</td>
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<td>V (+)</td>
<td>V (−)</td>
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<tr>
<td>Gelatin</td>
<td>−</td>
<td>V (−)</td>
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<td>+</td>
<td>V (−)</td>
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<td>ND</td>
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<td>D-Galactose</td>
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<td>+</td>
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<td>+</td>
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<td>−</td>
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<td>−</td>
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<td>990 : 1215*</td>
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<td>Major fatty acids†</td>
<td>C₁₈₋₇₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₆₋₁₀₇₀</td>
<td>C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₆₋₁₀₇₀</td>
<td>C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₆₋₁₀₇₀</td>
<td>C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₆₋₁₀₇₀</td>
<td>C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₆₋₁₀₇₀</td>
<td>C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₆₋₁₀₇₀</td>
<td>C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₆₋₁₀₇₀</td>
<td>C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₈₋₇₀, C₁₈₋₇₀, C₁₅₋₀, 2-OH/ C₁₆₋₁₀₇₀</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>63</td>
<td>63–65</td>
<td>63–65</td>
<td>65</td>
<td>66</td>
<td>58</td>
<td>63</td>
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</table>

*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.
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-150T 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-150T 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-150T 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-150T 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-150T were unsaturated fatty acids C_{18:1ω7c} (33.8%), C_{17:1ω6c} (18.8%), 10-methyl-C_{18:1ω7c} (5.6%) and C_{17:1ω8c} (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; Vancanneyt et al., 2001; Godoy et al., 2003; Yoon & Oh, 2005) (Table 1). 3-Hydroxy fatty acids were not detected in strain SW-150T, which was also found
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; Vancannet et al., 2001; Busse et al., 2003; Godoy et al., 2003). The major polar lipids detected in strain SW-150T were siringoglycolipid, 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-150T 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 rDNA gene sequences (Takeuchi et al., 1993; Kämpfer et al., 2002; Vancannet et al., 2001; Godoy et al., 2003).

There are not enough phenotypic, particularly chemotaxonomic, properties to distinguish strain SW-150T from the genus Sphingopyxis (Takeuchi et al., 1993; Lee et al., 2001; Vancannet et al., 2001; Godoy et al., 2003). The orange pigmentation of strain SW-150T is not observed in other Sphingopyxis species (Stackebrandt & Goebel, 1994). Accordingly, it seems reasonable to assign strain SW-150T within the genus Sphingopyxis. The phylogenetic distinctiveness shown in this study is low enough to exclude the possibility of assigning strain SW-150T to a Sphingopyxis species with a validly published name, despite the lack of DNA-DNA hybridization experiments between strain SW-150T and other Sphingopyxis species (Stackebrandt & Goebel, 1994). There are differences between strain SW-150T 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-150T 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 x 1.5–2.5 μm. Gram-negative. Non-spor-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 extract shows 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-xylene. The predominant respiratory lipoquinone is Q-10. The major fatty acids are C18:1ω7c (33.8%) and C17:1ω8c (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-150T (=KCTC 12231T =DSM 16222T), was isolated from sea water at Baekryung Island in the Yellow Sea in Korea.

Acknowledgements

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References


