Sphingopyxis marina sp. nov. and Sphingopyxis litoris sp. nov., isolated from seawater

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Two yellow-pigmented, Gram-negative, aerobic bacterial strains, designated FR1087T and FR1093T, were isolated from surface seawater off Jeju Island, Republic of Korea. Both strains required sea salts for growth. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the two isolates belong to the genus Sphingopyxis, showing the highest level of sequence similarity with respect to Sphingopyxis flavimaris SW-151T (97.9 %). The two isolates shared 98.5 % sequence similarity. DNA–DNA hybridization between the isolates and the type strain of Sphingopyxis flavimaris clearly suggested that strains FR1087T and FR1093T represent two separate novel species in the genus Sphingopyxis. Several phenotypic characteristics served to differentiate these two isolates from recognized members of the genus Sphingopyxis. The data from the polyphasic study presented here indicated that strains FR1087T and FR1093T should be classified as representing novel species in the genus Sphingopyxis, for which the names Sphingopyxis marina sp. nov. and Sphingopyxis litoris sp. nov., respectively, are proposed. The type strain of Sphingopyxis marina sp. nov. is FR1087T (=KCTC 12763T=JCM 14161T) and the type strain of Sphingopyxis litoris sp. nov. is FR1093T (=KCTC 14133T=JCM 14182T).

Members of the genus Sphingopyxis are Gram-negative, non-fermentative, aerobic, non-spore-forming, yellow-pigmented or whitish-brown, motile and contain sphingoglycolipids. Takeuchi et al. (2001) proposed that Sphingomonas species should be divided into four different genera, namely Sphingobium, Novosphingobium, Sphingopyxis and Sphingomonas. The nomenclatural system of Takeuchi et al. (2001) has been generally accepted and used (Yabuuchi et al., 2002; Busse et al., 2003; Godoy et al., 2003; Yoon et al., 2005). In this study, we isolated two bacterial strains, designated FR1087T and FR1093T, from seawater and subjected them to a polyphasic analysis. The strains clearly differed from recognized Sphingopyxis species and from each other and therefore represent two novel species of the genus Sphingopyxis.

Strains FR1087T and FR1093T were isolated from a sample of coastal surface seawater collected off Jeju Island, Republic of Korea. The seawater sample was diluted with sterilized artificial seawater (Lyman & Fleming, 1940), spread onto a plate that contained marine agar 2216 (MA; Difco) and incubated at 30 °C. The isolates were routinely cultured on MA and maintained as a glycerol suspension (20 %, w/v) at −80 °C.

The 16S rRNA gene was amplified from a single colony by means of PCR with Taq polymerase (Takara). The PCR amplification and sequencing were performed as described previously (Chun & Goodfellow, 1995). The pairwise sequence similarity values were determined using the EzTaxon server (Chun et al., 2007). The regions available for all sequences (positions 47–1437; Escherichia coli numbering system) were used to construct the phylogenetic trees after the exclusion of ambiguous positions (positions 67–84). Phylogenetic analyses were performed by following the same procedure as that described previously by Yi et al. (2005), using jPHYDIT (Jeon et al., 2005; available at http://chunlab.snu.ac.kr/jphydit/) and PAUP 4.0 (Swofford, 1998). Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) methods. The resultant tree topologies were evaluated by means of bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Sequence comparisons with 16S rRNA gene sequences held in GenBank indicated that the two isolates were closely related to the genus Sphingopyxis. Strains FR1087T and FR1093T had a sequence similarity of 98.5 %. Sphingopyxis sequences of strains FR1087T and FR1093T are DQ781320 and DQ781321, respectively.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains FR1087T and FR1093T are DQ781320 and DQ781321, respectively.

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Table 1. Phenotypic characteristics that serve to differentiate strains FR1087<sup>T</sup> and FR1093<sup>T</sup> from recognized *Sphingopyxis* species

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td>Colony colour</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Orange</td>
<td>Whitis brown or yellow</td>
<td>Light or deep yellow</td>
<td>Yellow to beige</td>
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<td>Yellow</td>
<td>Pale yellow</td>
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<td>Motility</td>
<td>+</td>
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<td>v(+)</td>
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<td>ND</td>
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<td>ND</td>
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<td>C&lt;sub&gt;16&lt;/sub&gt;:0</td>
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*A*, Agmatine; *s*, spermidine.

*flavimaris* showed the highest level of 16S rRNA gene sequence similarity with respect to strains FR1087<sup>T</sup> and FR1093<sup>T</sup> (97.9 % for both). Out of five signature nucleotides defined for the genus *Sphingopyxis* (Takeuchi et al., 2001), three were found in the two isolates. They, along with *Sphingopyxis flavimaris*, lacked the remaining two signature nucleotides (Table 1).

The levels of sequence similarity between strain FR1087<sup>T</sup> and other *Sphingopyxis* species were in the range 92.8–93.7 % and the corresponding values for strain FR1093<sup>T</sup> were 91.8–93.1 %. The neighbour-joining tree showed that the two isolates formed a monophyletic clade with the type strain of *Sphingopyxis flavimaris* (Fig. 1), with 100 % bootstrap support. This relationship was confirmed by all other tree-infering methods used in this study.

The close taxonomic relationship, i.e. the high levels of 16S rRNA gene sequence similarity among strains FR1087<sup>T</sup> and FR1093<sup>T</sup> and *Sphingopyxis flavimaris* SW-151<sup>T</sup>, led us to employ DNA–DNA relatedness experiments to obtain further genomic comparisons (Stackebrandt & Goebel, 1994). Genomic relatedness was determined using a Cary 300 Bio model UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6 x 6 multicell changer and a temperature controller (Varian). The experimental details for the DNA–DNA hybridization studies were as described elsewhere (Yi & Chun, 2006). The level of genomic relatedness observed between *Sphingopyxis flavimaris* and strain FR1087<sup>T</sup> was 21.2 %, while that between strain FR1093<sup>T</sup> was 36.3 %. The two isolates shared a low DNA–DNA relatedness value (20.7 %). All of these values are below the threshold (70 %) for determining bacterial species (Wayne et al., 1987); the finding strongly suggested that the two isolates belonged to novel genomic species in the genus *Sphingopyxis*.

Growth under anaerobic conditions was checked in an anaerobic chamber on MA and on nitrate-supplemented MA, both of which had been prepared anaerobically under nitrogen gas. Growth at various concentrations of NaCl and sea salts was investigated using sea-salt-free ZoBell’s (1941) agar (ZoBell, 1941; 15 g Bacto agar (Difco), 5 g Bacto
Sphingopyxis flavimaris SW-151<T> (AY554010)

Sphingopyxis baekryungensis SW-150<T> (AY608604)

Strain FR1093<T> (DQ761321)

Strain FR1087<T> (DQ761320)

Sphingopyxis terrae NBRC 15098<T> (D13727)

Sphingopyxis wittlantiensis W-50<T> (AJ416410)

Sphingopyxis macrogotliba NA 15033<T> (D13723)

Sphingopyxis taejonensis JSS54<T> (AF131297)

Sphingopyxis alaskensis RB2256<T> (AY337601)

Sphingopyxis chilensis S37<T> (AF367204)

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peptone (Difco), 1 g yeast extract, 0.1 g ferric citrate in 1 l distilled water. The temperature range for growth was determined optically using a temperature gradient incubator (TVS 126MA; Advantec) with marine broth 2216 (Difco) and temperatures of 4–45 °C (specifically at 4.0, 10.0, 15.1, 18.3, 21.2, 23.7, 26.5, 29.0, 31.8, 34.4, 37.3, 40.7 and 45 °C). Biochemical tests were performed using API 20NE and API ZYM kits (bioMérieux): the strips were each inoculated with a heavy bacterial suspension in artificial seawater or AUX medium (bioMérieux) supplemented with 2 % (w/v) sea salts. Hydrolysis of casein, starch, Tween 80, hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations described by Cowan & steel (1965). The two isolates showed differences in terms of the hydrolysis of gelatin and the utilization of glucose. Both isolates utilized xanthine and areopha, but not other phyllogenetic neighbours. The results of the biochemical and physiological tests are given in the species description and are shown in Table 1.

Cellular fatty acids from the test strains were analysed by GLC according to the instructions of the Microbial Identification System (MIDI). For analysis of the cellular fatty acid methyl esters, cells were grown on MA at 30 °C for 6 days. The two strains differed substantially in terms of their major fatty acids: details are given in the species description and are shown in Table 1. Polar lipids were extracted using the procedures described by Minnikin et al. (1984) and were identified by using two-dimensional TLC followed by spraying with the appropriate detection reagents (Komagata & Suzuki, 1987). Sphingoglycolipid was found as a major polar lipid in both isolates. Polyamines were extracted and analysed according to the methods of Busse et al. (1989) and Flores & Galston (1982). Strain FR1087T contained spermidine [8.6 μmol (g dry wt)<sup>−1</sup>] and agmatine [4 μmol (g dry wt)<sup>−1</sup>] as major components, whereas strain FR1093T contained only spermidine [17.78 μmol (g dry wt)<sup>−1</sup>] as the major component.

On the basis of the data from the polyphasic study presented here, it is evident that strains FR1087T and FR1093T represent separate novel species in the genus Sphingopyxis, for which the names Sphingopyxis marina sp. nov. and Sphingopyxis litoris sp. nov., respectively, are proposed.

**Description of Sphingopyxis marina sp. nov.**

Sphingopyxis marina (ma.ri’na. L. fem. adj. marina of the sea, marine).

Gram-negative and aerobic. Oxidase-positive. Colonies are circular, convex and yellow in colour and 0.7–1.0 mm in diameter after cultivation for 6 days at 30 °C on MA. Spores are not formed. Does not grow without sea salts. Grows at 3–7 % (w/v) sea salts (optimum, 3–5 %) and 15–34.4 °C (optimum, 23.7–31.8 °C). Does not grow under anaerobic conditions on MA or MA supplemented with nitrate. Tyrosine is hydrolysed, but Tween 80, starch, hypoxanthine and casein are not. Positive reactions are obtained for arginine dihydrolase, urease, protease and the assimilation of glucose, arabinose, adiopate, maltate and citrate. Weakly positive for β-glucosidase (with API 20NE). With API ZYM, positive reactions are obtained for alkaline phosphatase, leucine arylamidase and trypsin, weakly positive reactions are obtained for esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative reactions are obtained for z-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Sphingoglycolipid is present as the major polar lipid. Major fatty acids are C<sub>17:0</sub> (20.5 %), C<sub>17:1ω6c</sub> (15.38 %), C<sub>17:1ω6c</sub> (14.04 %) and C<sub>18:1ω7c</sub> (13.6 %). Spermidine and agmatine are present as major polyamine compounds, whereas cadaverine, putrescine and spermine are detected only in small amounts.

The type strain, FR1087T (=IMSNU 14132T=KCTC 12763T=JCM 14161T), was isolated from surface seawater collected off Jeju Island, Republic of Korea.

**Description of Sphingopyxis litoris sp. nov.**

Sphingopyxis litoris (li.to’ris. L. gen. n. litoris of the seashore, of the coast).
Gram-negative and aerobic. Oxidase- and catalase-positive. Colonies are circular, convex and yellow in colour and 0.7–1.0 mm in diameter after 6 days cultivation at 30 °C on MA. Spores are not formed. Does not grow without sea salts. Grows at 2–7% (w/v) sea salts (optimum, 3.5–5%) and 15–34.4 °C (optimum, 23.7–31.8 °C). Does not grow under anaerobic conditions on MA or MA supplemented with nitrate. Tyrosine and starch are hydrolysed, but Tween 80, xanthine, hypoxanthine and casein are not. Positive reactions are obtained for arginine dihydrolase, urease, β-galactosidase and assimilation of malate and citrate. Weakly positive for β-glucosidase (with API 20NE). With API ZYM, positive reactions are obtained for alkaline phosphatase, leucine arylamidase, esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase, β-galactosidase and β-galactosidase and negative reactions are obtained for β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, x-maninosidase and x-fucosidase. Sphingoglycolipid is present as the major polar lipid. Major cellular fatty acids are C_{18:1}ω7c (47.82%) and iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (14.1%); the two fatty acids cannot be separated by GLC with the MIDI system). Spermidine is present as the major polyamine component, whereas agmatine, cadaverine, putrescine and spermine are detected only in small amounts.

The type strain, FR1093^T (=KCTC 12764^T=JCM 14162^T), was isolated from surface seawater collected off Jeju Island, Republic of Korea.

Acknowledgements

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References


