Mariniphaga sediminis sp. nov., isolated from coastal sediment

Feng-Qing Wang,1 Qi-Yao Shen,1 Guan-Jun Chen1,2 and Zong-Jun Du1,2

1College of Marine Science, Shandong University at Weihai, Weihai 264209, PR China
2State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, PR China

A Gram-stain-negative and facultatively anaerobic bacterium, SY21T, was isolated from marine sediments of the coastal area in Weihai, China (122°0’ 37”E 37°31’ 33” N). Cells of strain SY21T were 0.3–0.5 μm wide and 1.5–2.5 μm long, catalase- and oxidase-positive. Colonies on 2216E agar were transparent, beige- to pale-brown-pigmented, and approximately 0.5 mm in diameter. Growth occurred optimally at 33–37 °C, pH 7.0–7.5 and in the presence of 2–3 % (w/v) NaCl. Phylogenetic analysis of the 16S rRNA gene indicated that strain SY21T was a member of the genus Mariniphaga within the family Prolixibacteraceae. The closest described neighbour in terms of 16S rRNA gene sequences identity was Mariniphaga anaerophila Fu11-5T (94.7 %). The major respiratory quinone of strain SY21T was MK-7, and the dominant fatty acids were iso-C15 : 0, iso-C17 : 0 3-OH and anteiso-C15 : 0. The major polar lipids were phosphatidylethanolamine, aminolipid and an unidentified lipid, and the DNA G+C content was 37.9 mol%. The distinct phylogenetic position and phenotypic traits distinguished the novel isolate from M. anaerophila Fu11-5T. Phenotypic and genotypic analysis indicated that strain SY21T could be assigned to the genus Mariniphaga. The name Mariniphaga sediminis sp. nov. is proposed, with the type strain SY21T (=KCTC 42260T=MCCC 1H00107T).

As a large group, bacteria of the phylum Bacteroidetes, which can utilize dissolved organic matter in the marine ecosystem, especially by degrading various polymers, such as cellulose, chitin and pectin (Kirchman, 2002), play an important role in the ecosystem. In phytoplankton bloom, Bacteroidetes are dominant in absorbing algae-derived organic matter and can promote carbon, nitrogen and sulfur cycling (Giovannoni & Rappé, 2000). Owing to their significant role in ecological cycles, there has been growing interest in Bacteroidetes. At the time of writing, the order Bacteroidales (Krieg et al., 2011) comprised the families Bacteroidaceae, Marinilabiliaceae, Porphyromonadaceae, Prevotellaceae, Rikenellaceae, Porphyrales and Marinifilaceae. Recently, the genus Mariniphaga was classified in the family Porphyrales as a novel genus to accommodate Mariniphaga anaerophila Fu11-5T by Iino et al. (2014).

In our study, a coastal sediment sample was collected in the course of an investigation of Bacteroidetes in the coastal area of Weihai. We describe the isolation and identification of a novel species of the phylum Bacteroidetes, represented by SY21T, based on phenotypic and chemotaxonomic properties, and a detailed phylogenetic analysis of the 16S rRNA gene sequences.

The marine sediment samples were collected from marine sediments from the same site of the coastal area in Weihai, China (122°0’ 37”E 37°31’ 33” N), using a sterile plastic pipette at a depth of ≥5 cm. The samples were pooled as a single sample and stored on ice under transportation to the laboratory. The sample was then processed using the enrichment culture technique, as described previously (Du et al., 2014), and incubated at 28 °C in a 500 ml sealed glass bottle (completely filled with medium). After 2 months of incubation, 1 ml of liquid medium from the sealed glass bottle was diluted with 9 ml of sterilized saline solution and serially diluted up to 10-fold for inoculum preparation. The plates were incubated at 28 °C for 3–7 days after spreading the diluted sample (100 μl) on 2216E agar (Hopebio). A tiny, transparent, beige-pigmented colony was isolated and inoculated on 2216E agar to achieve purity. The strain, designated SY21T, was routinely incubated on 2216E agar at 33 °C and stored at −80 °C in 15 % (v/v) glycerol with 1 % (w/v) NaCl. M. anaerophila JCM 18693T, obtained from the Japan Collection of Microorganisms, was used as the reference strain for physiological tests and analysis of morphological, phylogenetic and chemotaxonomic properties.
characteristics (except polar lipid analysis). Unless otherwise stated, the samples of *M. anaerophila* JCM 18693\(^T\) and SY21\(^T\) were incubated at 33 °C on 2216E agar.

Cell size, morphology and motility of strain SY21\(^T\) were established by light microscopy (Ci-L; Nikon) after cultivation for 72 h on 2216E agar. The gliding motility of strain SY21\(^T\) cultured on 2216E agar was detected as described by Bowman (2000). Gram reaction, reduction of nitrate, formation of spores and hydrolysis of starch, agar, gelatin, Tween 80 and CM-cellulose were detected according to the methods of Smibert & Krieg (1994) and Dong & Cai (2001). The optimal growth temperature of strain SY21\(^T\) was determined after incubation on 2216E agar and shaking in 2216E liquid medium (Hopebio) at 4, 10, 15, 20, 25, 28, 30, 33, 37, 40 and 45 °C, respectively. Tolerance to NaCl was tested on 2216E agar and in 2216E liquid medium with 0–10 % (w/v) NaCl (seawater in 2216E agar and 2216E liquid medium was replaced with artificial seawater: 3.2 g MgSO\(_4\) \(\cdot\) 7H\(_2\)O l\(^{-1}\), 2.2 g MgCl\(_2\) \(\cdot\) 6H\(_2\)O l\(^{-1}\), 1.2 g CaCl\(_2\) \(\cdot\) 2H\(_2\)O l\(^{-1}\), 0.7 g KCl l\(^{-1}\), 0.2 g NaHCO\(_3\) l\(^{-1}\)). The novel isolate was shaken in 2216E liquid medium at pH 5.5 to 10.0. The pH was adjusted by adding MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) or CAPSO (pH 9.0, 9.5 and 10.0) at a concentration of 20 mM. Oxidase activity was tested using an oxidase reagent kit (bioMérieux) according to the manufacturer’s instructions. Catalase activity was determined by pouring 3 % \(\text{H}_2\text{O}_2\) solution on bacterial colonies and observing bubble production. Anaerobic growth of strain SY21\(^T\) was determined after incubation on 2216E agar at 33 °C for 2 weeks with or without 0.1 % (w/v) NaN\(_3\) in an anaerobic jar. To determine the utilization of cysteine as an alternative electron donor with oxygen, strain SY21\(^T\) and *M. anaerophila* JCM 18693\(^T\) were shaken, respectively, in test tubes (15 × 150 mm) using the method of Iino *et al.* (2014). Fermentative growth on sugars was tested using GXSm medium (Iino *et al.*, 2014) with addition of each sugar in place of D-glucose. *M. anaerophila* JCM 18693\(^T\) was used as a positive control. Bacterial growth was followed by determining the increase in turbidity at 660 nm using a spectrophotometer. Acid production was determined using the API 50CHB system (bioMérieux). Biolog GEN III MicroPlates were used to determine the utilization of organic substrates as sole carbon and energy sources. Various biochemical and additional enzyme activities were assayed using API 20E and API ZYM kits (bioMérieux). Tests using Biolog GEN III MicroPlates and API kits were conducted according to the manufacturers’ instructions, except that salinity was adjusted to 3 % (w/v) NaCl. Since strain SY21\(^T\) showed poor growth on Iso-Sensitest agar (Oxoid) and Mueller–Hinton agar (Hopebio), susceptibility to antibiotics was assessed on 2216E agar using the Kirby–Bauer disc diffusion method, according to Du *et al.* (2014), followed by the protocols of the Clinical and Laboratory Standards Institute (CLSI, 2012).

Genomic DNA obtained with a commercial gene extraction kit (MiniBEST Bacteria Genomic DNA Extraction kit version 3.0; TaKaRa) was used to determine the DNA G+C content via HPLC as described by Tamaoka & Komagata (1984) and Mesbah *et al.* (1989). \(\lambda\)DNA (Takara) was used as a standard. The 16S rRNA gene was amplified with two universal primers, 27f (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492r (5′-TACGGYTACCTTGTTACGAC-3′). The PCR products were ligated into the pGM-T vector (Tiangen) and cloned according to the method of Liu *et al.* (2014). Sequencing reactions were carried out using an ABI BigDye 3.1 sequencing kit (Applied BioSystems) and an automated DNA sequencer (model ABI3730; Applied BioSystems). The 16S rRNA gene sequence of strain SY21\(^T\) was compared with available sequences of species with validly published names from NCBI BLASTN. Multiple alignments with sequences of strain SY21\(^T\) and the most closely related taxa were carried out using CLUSTAL_X (version 1.81) (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed by the neighbour-joining method using MEGA (version 6.0) (Tamura *et al.*, 2013). The maximum-likelihood algorithm (Felsenstein, 1981) was included to estimate and verify the taxonomic positions of the novel isolate and reference strains on the tree.

Cells of strain SY21\(^T\) were harvested at the late-exponential growth phase in 2216E liquid medium at 33 °C for characterization of isoprenoid quinones, cellular fatty acids and polar lipids. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v) from lyophilized cells (300 mg) and purified using HPLC (Minnikin *et al.*, 1984). Analysis of fatty acid composition was carried out using the microbial identification system (MIDI) according to the method of Sasser (1990). Polar lipids were extracted from 100 mg freeze-dried cell material using a chloroform/methanol/0.3 % (w/v) aqueous NaCl mixture (1 : 2 : 0.8, by vol.), modified after Bligh & Dyer (1959), recovered into the chloroform phase by adjusting the chloroform/methanol/0.3 % (w/v) aqueous NaCl mixture to a ratio of 1 : 1 : 0.9 (by vol.) and separated by two-dimensional silica gel TLC (no. 818 135; Macherey-Nagel). The first direction was developed in chloroform/methanol/water (65 : 25 : 4, by vol.), and the second in chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.). Total lipid material was detected using molybdato.phosphoric acid and specific functional groups were detected using spray reagents specific for defined functional groups (Tindall *et al.*, 2007). Polar lipid analysis was performed by the Identification Service of the DSMZ, Braunschweig, Germany.

Colonies on 2216E agar were transparent, beige- to pale-brown-pigmented and around 0.5 mm in diameter after 72 h of incubation at 33 °C. Cells of strain SY21\(^T\) were approximately 0.3–0.5 \(\mu\)m wide and 1.5–2.5 \(\mu\)m long, Gram-stain-negative, short rods, non-gliding and non-motile. No spores were evident. Growth was observed at 15–40 °C and in the presence of 1–7 % (w/v) NaCl. The pH range for cell growth was pH 6.0–9.5. Optimal growth was detected at 33–37 °C, pH 7.0–7.5 and 2–3 % (w/v) NaCl. Starch, agar, gelatin, Tween 80 and CM-cellulose were not hydrolysed. Strain SY21\(^T\) was facultatively anaerobic, negative
for reduction of nitrate, and positive for catalase and oxidase. Further data on the morphological, physiological and biochemical characteristics of strain SY21<sup>T</sup> are presented in the species description and Table 1.

### Table 1. Differential characteristics of strain SY21<sup>T</sup> and *Mariniphaga anaerophila* JCM 18693<sup>T</sup>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Beige- to pale-brown</td>
<td>Beige</td>
</tr>
<tr>
<td>Cell size (μm)</td>
<td>0.3–0.5 × 1.5–2.5</td>
<td>0.5–0.6 × 1.9–6.9</td>
</tr>
<tr>
<td>Growth range:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>15–40</td>
<td>20–42</td>
</tr>
<tr>
<td>NaCl (%, w/v)</td>
<td>1–7</td>
<td>0–9</td>
</tr>
<tr>
<td>pH</td>
<td>6.0–9.5</td>
<td>6.0–8.5</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of starch</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>utilization of D-glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzymic activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Fucosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetylglucosaminidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>37.9</td>
<td>41.7*</td>
</tr>
</tbody>
</table>

*Data taken from Iino *et al.* (2014).

The almost full-length 16S rRNA gene sequence (1443 bp) of strain SY21<sup>T</sup> was obtained. The 16S rRNA gene sequence similarity was 94.7% to *M. anaerophila* Fu11-5<sup>T</sup>, which is lower than the borderline value used for definition of bacterial species (i.e. 97%) as proposed by Stackebrandt & Goebel (1994). As shown in Fig. 1, strain SY21<sup>T</sup> clustered with *M. anaerophila* Fu11-5<sup>T</sup> and formed a branch separate from other type strains. A phylogenetic tree reconstructed with the maximum-likelihood method (data not shown) had similar topological structure.

The major respiratory quinone of strain SY21<sup>T</sup> was MK-7, compatible with *M. anaerophila* Fu11-5<sup>T</sup> and most other members of the family *Prolixibacteraceae*. The following compositions of cellular fatty acids (<5 %) were obtained: iso-C<sub>15 : 0</sub> (20.6%), iso-C<sub>17 : 0</sub> 3-OH (13.5%), anteiso-C<sub>15 : 0</sub> (11.3%), iso-C<sub>16 : 0</sub> (9.2%), C<sub>15 : 0</sub> (6.5%) and iso-C<sub>16 : 0</sub> 3-OH (5.9%). The major fatty acids were consistent with those of *M. anaerophila* Fu11-5<sup>T</sup>, except for some differences in the proportions and types of fatty acids. The DNA G+C content of strain SY21<sup>T</sup> was 37.9 mol%, which could be distinguished from that of *M. anaerophila* Fu11-5<sup>T</sup> (41.7 mol%) (Iino *et al.*, 2014). The major polar lipids of strain SY21<sup>T</sup> were phosphatidylethanolamine, aminolipid and an unidentified lipid. Minor amounts of other aminolipids, unknown polar lipids, phospholipid and glycolipid were additionally found (Fig. S1, available in the online Supplementary Material). Both of the strains, SY21<sup>T</sup> and Fu11-5<sup>T</sup>, contained phosphatidylethanolamine as the major polar lipid. However, strain SY21<sup>T</sup> contained several kinds of lipid that were not present in strain Fu11-5<sup>T</sup>. The polar lipid pattern of *M. anaerophila* Fu11-5<sup>T</sup> comprised only phosphatidylethanolamine. As a result, there were differences between strain SY21<sup>T</sup> and Fu11-5<sup>T</sup> in major polar lipid components.

Chemotaxonomic examination, together with phylogenetic analysis, effectively revealed that strain SY21<sup>T</sup> is affiliated with the genus *Mariniphaga*. Despite common features,
strain SY21<sup>T</sup> displayed substantial differences from <i>M. anaerophila</i> Fu11-<sup>5</sup><sup>T</sup>. For example, strain SY21<sup>T</sup> could not grow without NaCl and was positive for catalase and oxidase, unlike <i>M. anaerophila</i> Fu11-<sup>5</sup><sup>T</sup>. Further differences were observed in terms of enzyme activities (Table 1). Strain SY21<sup>T</sup> could also be distinguished based on antibiotic sensitivity. Moreover, strain SY21<sup>T</sup> did not utilize D-glucose to produce acid. In view of the collective phenotypic, physiological and chemotaxonomic characteristics and phylogenetic position, strain SY21<sup>T</sup> appears to represent a novel species of the genus <i>Mariniphaga</i>, for which the name <i>Mariniphaga sediminis</i> sp. nov. is proposed.

**Description of <i>Mariniphaga sediminis</i> sp. nov**

<i>Mariniphaga sediminis</i> (se.di.mi’nis. L. n. sedimen -inis sediment; L. gen. n. sediminis of sediment).

Cells are 0.3–0.5 μm in width and 1.5–2.5 μm in length. Colonies on 2216E agar are transparent, beige- to pale-brown-pigmented, and approximately 0.5 mm in diameter after 72 h of incubation at 33 °C. Growth occurs at 15–40 °C (optimum 33–37 °C), pH 6.0–9.5 (optimum pH 7.0–7.5) and in the presence of 1–7 % (w/v) NaCl (optimum 2.0–3.0 %). Cells of strain SY21<sup>T</sup> are Gram-stain-negative, catalase- and oxidase-positive, facultatively anaerobic and non-gliding. Reduction of nitrate, motility and spore formation are negative. L-Cysteine is used as an alternative electron donor in oxidative fermentation and growth. Nitrate is reduced in the presence of L-cysteine. Acid is not produced from mannose, glucosamine, n-acetylmuramide. L-rhamnose, methyl a-D-pyran mannitol glycoside, methyl 3-D-pyran mannoside, methyl a-D-pyran glycoside, a-D-glucoside or a-D-glucosidase or a-D-glucosidase, and 5-ketotassoglucaronamide. Acid is not produced from utilization of d-glucose. With Biolog GEN III MicroPlates, the following substrates are utilized as sole carbon and energy sources: raffinose, Tween 40, d-salicin, N-acetyl-D-glucosamine, a-D-glucoside, methyl beta-D-glucoside, glycerol, L-aspartic acid, L-glutamic acid, D-glucuronic acid, D-fructose, D-mannose, L-malic acid and acetic acid. Enzymic activities detected with API ZYM include alkaline phosphatase, acid phosphatase, leucine arylamidase, a-chymotrypsin, naphthol-AS-BI-phosphohydrolase, a-galactosidase, N-acetylglucosaminidase and beta-fucosidase, but not esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, beta-galactosidase, beta-glucuronidase, a-glucosidase, beta-galactosidase or a-glucosidase. Cells of the type strain are susceptible to acetylsyriramycin, erythromycin, lincomycin, chloramphenicol, vancomycin, penicillin, sulphamethoxazole, latamoxef, cefotaxime sodium and ceftriaxone, and resistant to tetacycline, streptomycin, tobramycin, gentamicin, nalidixic acid, neomycin and kanamycin. The major menaquinone is MK-7. The major fatty acids are iso-C<sub>15</sub>:0, iso-C<sub>17</sub>:0 3-OH and anteiso-C<sub>15</sub>:0<sup>9</sup>, and the major polar lipids are phosphatidylethanolamine, aminolipid and an unidentified lipid.

The type strain, SY21<sup>T</sup>(=KCTC 42260<sup>T</sup>=MCCC 1H00107<sup>T</sup>), was isolated from a sediment sample collected from the coastal area of Weihai, China. The Genomic DNA G + C content of the type strain is 37.9 mol%.

**Acknowledgements**

This work was supported by the National Natural Science Foundation of China (31370057, 31290231), the National Science and Technology Major Project of China (2013ZX10004217), and the China Ocean Mineral Resources R&D Association Special Foundation (DY125-15-T-05).

**References**


