Myroides marinus sp. nov., a member of the family Flavobacteriaceae, isolated from seawater

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A Gram-negative, aerobic, non-motile, yellow-pigmented, rod-shaped bacterium (strain JS-08T) isolated from seawater was subjected to a polyphasic taxonomic study. 16S rRNA gene sequence analysis indicated that strain JS-08T belongs to the genus *Myroides*, a member of the phylum *Bacteroidetes*. Its closest phylogenetic relative was *Myroides odoratimimus* JCM 7460T, with which it shared 97.0 % 16S RNA gene sequence similarity. Strain JS-08T contained menaquinone-6 (MK-6) as the predominant menaquinone, and the dominant fatty acids were iso-C15 : 0, iso-C₁₇ : 0 3-OH and a summed feature consisting of iso-C₁₅ : 0 2-OH and/or C₁₆ : ₁₀7c. The DNA G+C content of strain JS-08T was 34.2 mol%. Based on phenotypic, genotypic and phylogenetic evidence, it is suggested that strain JS-08T represents a novel species of the genus *Myroides*, for which the name *Myroides marinus* sp. nov. is proposed. The type strain is JS-08T (=KCTC 23023T =JCM 16529T).

The genus *Myroides*, belonging to the family *Flavobacteriaceae* (Bernardet et al., 2002), was first described with the reclassification of *Flavobacterium odoratum* strains as *Myroides odoratus* and *Myroides odoratimimus* based on a polyphasic approach (Vancanneyt et al., 1996). At the time of writing, the genus *Myroides* comprised four recognized species, namely *M. odoratus*, *M. odoratimimus* (Vancanneyt et al., 1996), *M. pelagicus* (Yoon et al., 2006) and *M. profundii* (Zhang et al., 2008).

Strains of *Myroides* have been found in various terrestrial and aquatic environments. Most strains of *M. odoratus* and *M. odoratimimus* have been isolated from clinical specimens (Holmes et al., 1977), whereas those of the other species have been isolated from the marine environment (Yoon et al., 2006; Zhang et al., 2008), freshwater fish (González et al., 2000) and the insect gut (Spiteller et al., 2000).

A number of studies have focused on *Myroides* strains as causative agents in various infections (Bachmeyer et al., 2008; Douce et al., 2008; Green et al., 2001; Källman et al., 2006; Thomas et al., 2007; Yağcı et al., 2000). Attention has also been given to strains belonging to *Myroides* because novel types of metalloenzymes have been reported from various species of the genus (Chen et al., 2009; Mammeri et al., 2002) and biosurfactant compounds have been isolated from strains of *M. pelagicus* (Maneerat et al., 2008). These reports clearly indicate the wide distribution of members of the genus *Myroides* in nature and their potential as a source of industrially useful compounds, yet the diversity within the genus remains to be fully unravelled. In the present study, we report the description of a novel *Myroides* species isolated during a culture-dependent study of halophilic bacterial diversity associated with marine environments. The taxonomic status of the organism was determined by using a polyphasic approach.

Strain JS-08T was originally isolated by using the standard dilution plating technique on marine agar 2216 (MA; Difco) from seawater collected off Boryeong Province, Chungnam, Republic of Korea. Examination of the morphological and biochemical properties of strain JS-08T followed previously described procedures (Yoon et al., 2006; Zhang et al., 2008). The following phenotypic tests were performed: Gram stain, cell morphology, catalase and oxidase activities and hydrolysis of casein, cellulose, DNA, starch, Tweens 20, 40, 60 and 80 and urea. Growth at 4, 10, 20, 25, 30, 37 and 42 °C and at pH 4–12 (at intervals of 1.0 pH unit, adjusted by using 1 M HCl or NaOH) was examined in marine broth (Difco). Growth in the presence of 0, 3, 5, 10, 15 and 20 % NaCl was investigated by using a basal medium containing (per litre) 10 g peptone and 5 g yeast extract. Other biochemical and physiological properties were examined by using API 20E and API ZYM strips (bioMérieux) according to the manufacturer’s instructions. Utilization of 95 selected carbon sources was examined by using Biolog GN2 microplates according to the manufacturer’s instructions.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain JS-08T is GQ857652.
The isoprenoid quinone content was analysed via reversed-phase HPLC as described by Cho et al. (2008). Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Microbial Identification System (Microbial ID) (Kämpfer & Kroppenstedt, 1996). The DNA G+C content was determined by using the methods outlined by Cui et al. (2007).

The 16S rRNA gene of strain JS-08\(^T\) was amplified by PCR from genomic DNA and sequenced as described previously (Cho et al., 2008). The resultant 16S rRNA gene sequence was aligned with corresponding sequences of the type strains of recognized Myroides species by using the PHYDIT program, version 3.1 (http://plaza.snu.ac.kr/~jchun/phydit/). Phylogenetic trees were inferred by using the Fitch–Margoliash, maximum-likelihood, maximum-parsimony and neighbour-joining methods following procedures described previously (Kim et al., 2003).

Strain JS-08\(^T\) formed yellow-to-orange, circular, convex colonies with entire margins on MA at 30 \(^\circ\)C within 48 h. Growth occurred at 10–37 \(^\circ\)C. Cells were aerobic, Gram-negative, non-spore-forming, non-motile rods. Other phenotypic characteristics are given in the species description and in Table 1. Strain JS-08\(^T\) could be readily distinguished phenotypically from recognized species of the genus Myroides. Notably, the novel strain was in general positive for more enzyme activities and had a wider carbon source utilization spectrum, implying that strain JS-08\(^T\) had a wider metabolic capability than the representative Myroides strains investigated.

Menaquinone-6 (MK-6) was the predominant quinone isoprenologue. Strain JS-08\(^T\) contained iso-C\(_{15}:0\) (24.2 % of the total fatty acid composition), iso-C\(_{17}:0\) 3-OH (18.1 %) and a summed feature consisting of iso-C\(_{15}:0\) 2-OH and/or C\(_{16}:1\)\(^\text{v}_{7}\)c (16.8 %) as the predominant fatty acids. Table 2 shows the detailed fatty acid profile of strain JS-08T in comparison with those of recognized Myroides species. The presence of C\(_{18}:1\)\(^\text{v}_{6}\)c (1.8 %) and C\(_{17}:0\) 3-OH (1.3 %) was characteristic of strain JS-08\(^T\). The DNA G+C content of strain JS-08\(^T\) was 34.2 mol%, within the range reported for the genus (33.8–36.7 mol%).

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain JS-08\(^T\) is a member of the family Flavobacteriaceae and forms a coherent cluster with species of the genus Myroides (Fig. 1). Strain JS-08\(^T\) was related

### Table 1. Differential phenotypic characteristics between strain JS-08\(^T\) and the type strains of recognized Myroides species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Seawater</td>
<td>Wound swab</td>
<td>Deep-sea sediment</td>
<td>Seawater</td>
<td>Faeces of patients</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Short rods</td>
<td>Rods</td>
<td>Spindle-shaped rods</td>
<td>Short rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Pigment</td>
<td>Yellow to orange</td>
<td>Pale yellow</td>
<td>Pale yellow, diffusible</td>
<td>Yellow to orange</td>
<td>Yellow</td>
</tr>
<tr>
<td>Growth temperature ((^\circ)C)</td>
<td>10–37</td>
<td>10–37</td>
<td>10–42</td>
<td>10–37</td>
<td>20–37</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tween 20</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tween 80</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Activity of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(\alpha)-Chymotrypsin</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of (Biolog):</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>L-Alanine</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(\alpha)-Hydroxybutyric acid</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(\gamma)-Hydroxybutyric acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(\alpha)-Ketoglutaric acid</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Pyruvic acid methyl ester</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Succinamic acid</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thymidine</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>34.2</td>
<td>33.8 (31.7)</td>
<td>33.9 (33)</td>
<td>35.8 (33.6)</td>
<td>36.7 (34.7)</td>
</tr>
</tbody>
</table>
Table 2. Fatty acid contents of strain JS-08T and the type strains of recognized Myroides species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
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<th>5</th>
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<tr>
<td>Straight-chain saturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>1.3</td>
<td>1.4</td>
<td>1.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Branched saturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C13:0</td>
<td>–</td>
<td>2.0</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C14:0</td>
<td>2.6</td>
<td>1.5</td>
<td>1.7</td>
<td>3.3</td>
<td>–</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>24.2</td>
<td>44.7</td>
<td>27.6</td>
<td>29.3</td>
<td>43.7</td>
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<tr>
<td>anteiso-C15:0</td>
<td>1.1</td>
<td>3.8</td>
<td>–</td>
<td>–</td>
<td>81.0</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>3.4</td>
<td>2.7</td>
<td>4.3</td>
<td>8.7</td>
<td>1.2</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>–</td>
<td>–</td>
<td>2.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>anteiso-C17:0</td>
<td>–</td>
<td>–</td>
<td>3.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C17:0 10:0c</td>
<td>–</td>
<td>–</td>
<td>2.3</td>
<td>–</td>
<td>–</td>
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<tr>
<td>C18:1 09:0c</td>
<td>1.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C17:10:09c</td>
<td>2.4</td>
<td>2.8</td>
<td>3.3</td>
<td>5.7</td>
<td>13.2</td>
</tr>
<tr>
<td>Hydroxy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15:0 2-OH</td>
<td>2.8</td>
<td>–</td>
<td>1.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C15:5 3-OH</td>
<td>2.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
</tr>
<tr>
<td>iso-C15:5 3-OH</td>
<td>4.7</td>
<td>3.6</td>
<td>4.0</td>
<td>5.3</td>
<td>4.4</td>
</tr>
<tr>
<td>C16:0 3-OH</td>
<td>6.9</td>
<td>6.5</td>
<td>5.2</td>
<td>4.5</td>
<td>3.0</td>
</tr>
<tr>
<td>iso-C16:0 3-OH</td>
<td>3.0</td>
<td>2.2</td>
<td>2.2</td>
<td>4.3</td>
<td>1.3</td>
</tr>
<tr>
<td>C17:0 2-OH</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.7</td>
</tr>
<tr>
<td>C17:0 3-OH</td>
<td>1.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C17:0 3-OH</td>
<td>18.1</td>
<td>16.9</td>
<td>14.5</td>
<td>16.5</td>
<td>18.8</td>
</tr>
<tr>
<td>Summed feature*</td>
<td>16.8</td>
<td>9.2</td>
<td>15.4</td>
<td>8.0</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*Comprising C16:10:07c and/or iso-C15:0 2-OH.

most closely to M. odoratinimines and M. profundii, but formed an independent phylogenetic line. Levels of 16S rRNA gene sequence similarity between strain JS-08T and the type strains of M. odoratinimus and M. profundii were 97.0% (40 differences out of 1349 bp) and 96.8% (43 differences out of 1349 bp), respectively, values that can be considered low enough for distinction at the species level.

Thus, the phylogenetic and phenotypic evidence presented indicates clearly that strain JS-08T represents a novel species of the genus Myroides, for which the name Myroides marinus sp. nov. is proposed.

Description of Myroides marinus sp. nov.

Myroides marinus (ma.ri’nu.s. L. masc. adj. marinus of the sea, marine).

Cells are Gram-negative, aerobic, non-spore-forming, non-motile, short rods (0.6–1.0 μm long and 0.2–0.3 μm wide). Colonies are yellowish orange, circular and convex with entire margins on MA. The optimal temperature for growth is 30 °C. Growth occurs at 10–37 °C but not at 4 or 42 °C. The pH range for growth is pH 5–9, and optimal growth occurs at pH 6–7. Growth occurs in the presence of 0–5% (w/v) NaCl within 7 days. The predominant menaquinone is MK-6. The major fatty acids are iso-C15:0, iso-C17:0 3-OH and a summed feature comprising iso-C15:0 2-OH and/or C16:1ω7c. Catalase- and oxidase-positive. Voges-Proskauer test is positive. Citrate is utilized. Indole is produced weakly. Nitrate and nitrite are not reduced, and hydrogen sulphide is not produced. Hydrolyses DNA and Tweens 40 and 60, but not casein, cellulose, starch or Tweens 20 or 80. Acid is not produced from amygdalin, arabinose, glucose, inositol, mannitol, melibiose, rhamnose, sorbitol or sucrose. As determined from API ZYM and API 20E strips, positive for alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, gelatinase and urease activities, but negative for lipase (C14), α-galactosidase, β-glucuronidase, β-galactosidase, β-glucosidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase. In Biolog GN2 microplates, the following compounds exhibit positive reactions: Tweens 40 and 80, succinic acid monomethyl ester, acetic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketobutyric acid, α-ketovaleric acid, DL-lactic acid, propionic acid, succinic acid, bromosuccinic acid, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycylic L-aspartic acid, glycylic L-glutamic acid, L-leucine, L-proline, L-serine, L-threonine, inosine, uridine and...
2,3-butanediol. The DNA G+C content of the type strain is 34.2 mol%.

The type strain, JS-08T (=KCTC 23023T =JCM 16529T), was isolated from seawater collected off Boryeong Province, Chungnam, Republic of Korea.

Acknowledgements

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References


