Description of *Kordiimonas aquimaris* sp. nov., isolated from seawater, and emended descriptions of the genus *Kordiimonas* Kwon *et al.* 2005 emend. Xu *et al.* 2011 and of its existing species

Seong-Hyun Yang, 1 Mi-Ree Kim, 1 Hyun-Seok Seo, 1 Sung Hyuk Lee, 1, 2 Jung-Hyun Lee, 1, 2 Sang-Jin Kim, 1, 2 and Kae Kyoung Kwon 1, 2

1Marine Biotechnology Research Center, Korea Institute of Ocean Science & Technology, PO Box 29, Ansan 425-600, Republic of Korea
2University of Science and Technology, Daejeon 305-333, Republic of Korea

A Gram-negative, aerobic, rod-shaped (0.6–0.9×0.7–1.2 μm), motile marine bacterium, designated MEBiC06554 T, was isolated from seawater collected from the East Sea, Korea (also known as the Sea of Japan). 16S rRNA gene sequence analysis revealed that strain MEBiC06554 T was affiliated to the order *Kordiimonadales* and showed high similarity to *Kordiimonas gwangyangensis* GW14-5 T (95.4 %), but formed a distinct phyletic line. Growth was observed at 10.5–35.0 °C (optimum 20 °C), at pH 5.0–8.0 (optimum pH 7.0) and with 0–13 % (w/v) NaCl (optimum 3.0–3.5 %). The predominant cellular fatty acids were iso-C 15 : 0 (14.9 %), iso-C 17 : 0 3-OH (44.6 %), iso-C 17 : 0 9c (6.3 %) and summed feature 3 (comprising iso-C 15 : 0 2- OH and/or C 16 : 1 ω7c; 13.9 %). The DNA G+C content was 50.3 mol%. The major respiratory quinone was Q-10. Phosphatidylethanolamine, two unidentified glycolipids, six unidentified aminolipids, three unidentified lipids and one unidentified aminophospholipid were detected as major polar lipids. On the basis of the data from our polyphasic taxonomic study, strain MEBiC06554 T should be classified within a novel species of the genus *Kordiimonas*, as *Kordiimonas aquimaris* sp. nov. The type strain is MEBiC06554 T (=KCCM 42940 T =JCM 16665 T). Emended descriptions of the genus *Kordiimonas* and of its species *Kordiimonas gwangyangensis* and *Kordiimonas lacus* are also proposed.

At the time of writing, the genus *Kordiimonas* is the only member of the order *Kordiimonadales* in the class *Alphaproteobacteria*. The genus *Kordiimonas* Kwon *et al.* 2005 emend. Xu *et al.* 2011 comprises a group of Gram-negative, motile, rod-shaped, oxidase- and catalase-positive, non-fermentative, weakly halophilic, obligately aerobic marine bacteria. *Kordiimonas gwangyangensis*, the type species, isolated from sediments of Gwangyang Bay, Republic of Korea (Kwon *et al.*, 2005), and *Kordiimonas lacus*, isolated from a ballast water tank (Xu *et al.*, 2011), are the currently recognized members of the genus. Kwon *et al.* (2005) reported that the genomic DNA G+C content of *K. gwangyangensis* GW14-5 T was 39.3 mol% and the major respiratory quinone was MK-5. However, the major respiratory quinone was corrected to Q-10 by Xu *et al.* (2011) and was further confirmed in this study. However, the G+C content was not verified. In the present study, the taxonomic position of a *Kordiimonas*-like, rod-shaped bacterial strain, MEBiC06554 T (Fig. S1, available in IJSEM Online), was confirmed by a polyphasic taxonomic approach by comparison with previously reported members of the genus *Kordiimonas*; as a result, in addition to the description of a novel species of the genus *Kordiimonas*, a further emendation of the description of the genus *Kordiimonas*, in addition to the emendation by Xu *et al.* (2011), is given. Emended descriptions of the previously reported members of the genus are also given.

Strain MEBiC06554 T was isolated from seawater collected near Anmok Port (37° 46′ N 128° 56′ E) by the dilution-to-extinction cultivation method (Connon & Giovannoni, 2002). An aliquot (100 μl) of culture broth after 9 months at 15 °C was spread onto twofold-diluted marine agar 2216 solid medium (DMA; Difco) supplemented with seawater and incubated at 15 °C for 4 months. Individual colonies were isolated from DMA and a morphologically distinct strain, MEBiC06554 T, was selected and characterized further. After primary isolation, the appropriate medium
and optimum temperature of strain MEBiC06554T were confirmed as marine agar 2216 (MA; Difco) and 20 °C and the strain was thereafter cultivated under these conditions. For long-term storage, the strain was maintained at −80 °C in sterilized seawater supplemented with 20 % (v/v) glycerol. For phenotypic comparisons, K. gwangyangensis GW14-5T and K. lacus JCM 16261T were grown on MA at 25 °C.

Genomic DNA was obtained by using a MoBio DNA extraction kit (gene All). After PCR amplification, the 16S rRNA gene sequence was determined by using an ABI 3100 automatic DNA sequencer (ABI) according to the manufacturer’s instructions and a 1455 bp sequence was obtained. Phylogenetic analysis based on the 16S rRNA gene sequence was conducted according to the procedure described by Kwon et al. (2005). A partial 16S rRNA gene sequence (1349 nt) of strain MEBiC06554T was compared with those of representative members of the class Alphaproteobacteria. The neighbour-joining tree (Saitou & Nei, 1987) revealed that strain MEBiC06554T formed a coherent clade with the type strains of K. gwangyangensis and K. lacus (16S rRNA gene sequence similarity to both strains was 96.1 %) within the order Kordiimonadales (Fig. 1). This relationship was supported by a bootstrap value of 86 % and was also recovered in trees obtained with the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms. Strain MEBiC06554T showed less than 90 % 16S rRNA gene sequence similarity to other members of the class Alphaproteobacteria with validly published names. This relationship was also confirmed in a neighbour-joining tree reconstructed from the available sequences affiliated to the genus Kordimonas in GenBank (Fig. S2).

Unless otherwise stated, physiological and morphological characterization was conducted according to the methods of Kwon et al. (2005) and Yang et al. (2006). Growth was tested in marine broth (MB) in a temperature gradient incubator (TVS126MA; Advantec) for up to 3 days at 12 different temperatures from 5 to 50 °C. Tolerance of NaCl

![Fig. 1. Rooted neighbour-joining tree based on nearly complete 16S rRNA gene sequences (1329 or 1349 unambiguously aligned base pairs, depending on the v5 region) showing relationships between strain MEBiC06554T and members of the order Kordiimonadales in the class Alphaproteobacteria. Closed circles indicate nodes that were also found in both maximum-likelihood and maximum-parsimony trees with >70 % bootstrap support; open circles indicate nodes that were also found in both the maximum-likelihood and maximum-parsimony trees with >50 % bootstrap support. Bootstrap values based on 1000 replicates are shown. Bar, 0.01 substitutions per nucleotide position.](http://ijs.sgmjournals.org)
was tested in MB prepared with distilled water and supplemented with NaCl (Sigma) from 0 to 20 % (w/v). The tolerance of pH was determined from pH 4 to 10 in MB with the pH adjusted using biological buffers MES (pH 4–6), HEPES (pH 6–8) or AMPSO (pH 8–10) (each at 10 mM). The bacterial suspension used to inoculate API 20E, API 20NE and API ZYM test strips (bioMérieux) and the Microlog GN2 system (Biolog) was prepared in a 2 % sea salt (Sigma) solution. To detect anaerobic growth, cells were inoculated into MB in a serum vial and the vial was purged with deoxygenated nitrogen gas, capped with an aluminium seal and cultivated at 20 °C for 3 days. The physiological, biochemical and morphological characteristics of strain MEBiC0654T are given in the species description and in Table 1.

The profile of cellular fatty acids in cells grown on MA at 20 °C for 3 days was determined commercially by using the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990) with Sherlock version 4.02 according to the manufacturer’s instructions. Dominant fatty acids were iso-C15:0 (14.9 %), iso-C17:1ω9c (44.6 %), iso-C17:0

### Table 1. Differential phenotypic characteristics of strain MEBiC0654T and the type strains of Kordiimonas species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell width (µm)</td>
<td>0.6–0.9</td>
<td>0.25</td>
<td>0.5–0.8</td>
</tr>
<tr>
<td>Cell length (µm)</td>
<td>0.7–1.2</td>
<td>1.3–1.4</td>
<td>3–6</td>
</tr>
<tr>
<td>Colour of colonies on MA</td>
<td>Beige</td>
<td>Creamy white</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Colony diameter (mm)</td>
<td>0.5–1</td>
<td>2–3</td>
<td>1–2</td>
</tr>
<tr>
<td>Growth temperature (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>10.5–35</td>
<td>17–44</td>
<td>10–43</td>
</tr>
<tr>
<td>Optimum</td>
<td>20</td>
<td>37–41</td>
<td>30–37</td>
</tr>
<tr>
<td>Growth pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>5.0–8.0</td>
<td>6–8.5*</td>
<td>6–9*</td>
</tr>
<tr>
<td>Optimum</td>
<td>7</td>
<td>7*</td>
<td>7*</td>
</tr>
<tr>
<td>NaCl concentration for growth (% w/v)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0–13</td>
<td>0.5–4</td>
<td>0.5–10</td>
</tr>
<tr>
<td>Optimum</td>
<td>3–3.5</td>
<td>2</td>
<td>2–3</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>w</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetoin production</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
<td>+*</td>
</tr>
<tr>
<td>Hydrolysis of gelatin</td>
<td>–</td>
<td>–</td>
<td>+*</td>
</tr>
<tr>
<td>Enzyme activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>–</td>
<td>+*</td>
<td>+*</td>
</tr>
<tr>
<td>α-Glucosidase, N-acetyl-β-glucosaminidase</td>
<td>–</td>
<td>+*</td>
<td>–*</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>w</td>
<td>–*</td>
<td>+*</td>
</tr>
<tr>
<td>N-Acetyl-D-glucosamine, l-arabinose, glycyll-glutamic acid, l-phenylalanine, succrose</td>
<td>+</td>
<td>+*</td>
<td>–*</td>
</tr>
<tr>
<td>Erythritol, D-fructose, D-galactose, lactose, lactulose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, turanose, monomethyl succinate, cis-aconitic acid, citric acid, D-galacturonic acid, D-gluconic acid, α-ketoglutaric acid, propionic acid, quinic acid, succinic acid, succinamic acid, l-asparagine, glycyll-1-aspartic acid, l-ornithine, γ-aminobutyric acid, glucose 1-phosphate, glucose 6-phosphate</td>
<td>+</td>
<td>–*</td>
<td>–*</td>
</tr>
<tr>
<td>myo-Inositol, α-ketoveric acid, inosine, uridine, thymidine</td>
<td>–</td>
<td>–*</td>
<td>+*</td>
</tr>
<tr>
<td>α-Cyclodextrin, β-hydroxybutyric acid, l-serine</td>
<td>–</td>
<td>+*</td>
<td>+*</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>50.3</td>
<td>58.0*</td>
<td>54.9</td>
</tr>
</tbody>
</table>

*Data from this study.
Table 2. Major fatty acids of strain MEBiC06554T and the type strains of Kordiimonas species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15:0</td>
<td>2.2</td>
<td>1.6</td>
<td>2.8</td>
</tr>
<tr>
<td>C16:0</td>
<td>1.6</td>
<td>2.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Saturated branched-chain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>14.9</td>
<td>18.1</td>
<td>22.2</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>6.3</td>
<td>11.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Unsaturated branched-chain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C15:1</td>
<td>1.2</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>iso-C17:1ω9c</td>
<td>44.6</td>
<td>43.1*</td>
<td>34.5</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C17:0ω8c</td>
<td>4.5</td>
<td>3.6</td>
<td>2.4</td>
</tr>
<tr>
<td>C17:0ω6c</td>
<td>2.2</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>C18:0ω7c</td>
<td>1.9</td>
<td>3.0</td>
<td>3.9</td>
</tr>
<tr>
<td>Hydroxy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C17:0 3-OH</td>
<td>tr</td>
<td>tr</td>
<td>1.0</td>
</tr>
<tr>
<td>Summed features‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed feature 3</td>
<td>13.9</td>
<td>10.5</td>
<td>14.6</td>
</tr>
<tr>
<td>Summed feature 4</td>
<td>tr</td>
<td>1.2</td>
<td>tr</td>
</tr>
</tbody>
</table>

*Identified as iso-C17:1 by Kwon et al. (2005).
†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained iso-C15:0 2-OH and/or C16:1ω7c; Summed feature 4 contained iso-C17:1 I and/or anteiso-C17:1 B.

(6.3%) and summed feature 3 (comprising iso-C15:0 2-OH and/or C16:1ω7c; 13.9%) (Tables 2 and S1). Polar lipids were extracted using a chloroform/methanol system and separated by two-dimensional TLC using silica gel 60 F254 aluminium-backed thin-layer plates (Merck) (Minnikin et al., 1984; Kämpfer et al., 2003). The solvent systems chloroform/methanol/water (65:24:4, by vol.) in the first dimension and chloroform/glacial acetic acid/methanol/water (40:7.5:6:1, by vol.) in the second dimension were used. Separated components were visualized by treating the plates with 10% (w/v) molybdophosphoric acid followed by heating at 150 °C for 10 min. Glycolipids were detected by spraying the plate with 0.5% (w/v) α-naphthol in methanol/water (1:1) and then with sulfuric acid/ethanol (1:1) followed by heating at 100 °C for 5 min; phospholipids were detected with Zinnadze reagent. *Ochrobactrum gallinifaciens* Iso196T was used as a reference. Phosphatidylethanolamine, an unidentified glycolipid and an unidentified aminophospholipid were detected as major polar lipids (Fig. S3); several unidentified aminolipids, lipids and aminophospholipids were also detected (Fig. S3). The major respiratory quinone was determined to be Q-10 by HPLC analysis according to the method described by Collins (1985). The DNA G+C content was 50.3 mol%, as determined by HPLC using a Symmetry reversed-phase C18 column (Stackebrandt & Liesack, 1993).

The results of phylogenetic analysis based on 16S rRNA gene sequences suggest that strain MEBiC06554T is affiliated to the genus Kordiimonas (Fig. 1) and should be assigned to a novel species.

Differential characteristics between strain MEBiC06554T and *K. gwangyangensis* GW14-5T and *K. lacus* S3-22T are given in Table 1. The properties of strain MEBiC06554T are consistent with the general features of the genus *Kordiimonas*; cells are oxidase- and catalase-positive, non-fermentative, halophilic, aerobic, Gram-negative, motile rods. In addition, members of the genus *Kordiimonas* share the ability to hydrolyse Tweens 40 and 80 and gelatin but cannot produce indole, lipase, galactosidases or fucosidase (Table 1). However, strain MEBiC06554T exhibits distinguishing properties, such as its optimal growth temperature and salinity, the absence of naphthol-AS-BI-phosphohydrolase activity and the range of carbon sources used compared with other members of the genus (Table 1). The optimal growth temperature of strain MEBiC06554T was lower than those of other members of the genus *Kordiimonas* (Table 1), and this seemed to be reflected in the composition of fatty acids; the proportion of iso-C17:1ω9c in strain MEBiC06554T was much higher than that found in other members of the genus (Table 2). On the basis of the evidence from this polyphasic taxonomic study, strain MEBiC06554T should be assigned to a novel species in the genus *Kordiimonas*, for which we propose the name *Kordiimonas aquimaris* sp. nov. Emended descriptions of the genus *Kordiimonas* and its existing species are also given.

**Emended description of the genus Kordiimonas**

Kwon et al. 2005 emend. Xu et al. 2011

The description is as given by Kwon et al. (2005) and emended by Xu et al. (2011) with the following further amendments. The major polar lipids are phosphatidylethanolamine and unidentified glycolipids, aminophospholipids, aminolipids and lipids (Fig. S2). The DNA G+C content is 50–58 mol%.

**Emended description of Kordiimonas gwangyangensis**

Kwon et al. 2005

In addition to the description given by Kwon et al. (2005), the DNA G+C content should be emended to 55.6–58.0 mol%. Other characteristics are described in Tables 1 and 2.

**Emended description of Kordiimonas lacus**

Xu et al. 2011

In addition to the description by Xu et al. (2011), it utilizes glycojen, α-cyclodextrin, methylpyruvate, alaninamide, l-leucine, l-proline, l-αlanyl glycine, β-hydroxybutyric
acid, α-ketovaleric acid, inosine, uridine and thymidine. Can reduce nitrate to nitrogen gas and produce acetoin.

**Description of Kordiimonas aquimaris sp. nov.**


Cells are Gram-negative, rod-shaped (0.6–0.9 × 0.7–1.2 μm) and motile and occur singly or in chains. Colonies are circular, convex, opaque and butyrous with entire edges, 0.5–1 mm in diameter on MA after cultivation for 2–3 days. Growth does not occur under anaerobic conditions on MA. Growth is observed at 10.5–35 °C (optimum 20 °C), pH 5.0–8.0 (optimum pH 7.0) and in the presence of 0–13% (w/v) NaCl (optimum 3.0–3.5%). Produces oxidase and catalase. When assayed with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin and acid phosphatase are present; activities of lipase (C14), naphthol-AS-BI-phosphohydrolase, phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are not detected. Degrades Tweens 40 and 80, N-acetyl-D-glucosamine, L-arabinose, cellobiose, erythritol, D-fructose, D-galactose, gentiobiose, α-D-glucose, lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, sucrose, trehalose, turanose, methylpyruvate, monomethyl succinate, cis-aconitic acid, citric acid, D-galacturonic acid, D-gluconic acid, α-ketoglutaric acid, propionic acid, quinic acid, succinic acid, succinic acid, alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, glycoll L-aspartic acid, glycoll 1-glutamatic acid, L-leucine, L-ornithine, L-phenylalanine, L-proline, γ-aminobutyric acid, glucose 1-phosphate and glucose 6-phosphate on Microlog GN2 plates. Dextrin, glycogen, D-psicose and raffinose are also utilized but the maximum likelihood approach. *J Mol Evol* 17, 368–376.


Acknowledgements

This work was supported by the Marine and Extreme Genome Research Center Program of the Ministry of Land, Transport and Maritime Affairs, Korea.

References

