The genus *Aestuariibacter* was described by Yi et al. (2004) and comprises two species, *Aestuariibacter halophilus* JC2043\(^T\) and *Aestuariibacter salexigens* JC2042\(^T\), which were isolated from a getbol sediment sample collected from Ganghwa Island, Korea. Here we report the phenotypic and phylogenetic characterization of a Gram-negative, aerobic, flagellated bacterium (designated strain KMM 3894\(^T\)), isolated from a sandy sediment sample collected offshore of the Sea of Japan, were investigated. Comparative 16S rRNA gene sequence analysis revealed that strain KMM 3894\(^T\) belonged to the genus *Aestuariibacter* and was most closely related to *Aestuariibacter halophilus* JC2043\(^T\) (95.5 % sequence similarity). Fatty acid analysis showed C\(_{16:1}\) \(\omega_{9c}\), C\(_{18:1}\) \(\omega_{7c}\), and C\(_{18:0}\) as the dominant components. Strain KMM 3894\(^T\) could be differentiated from recognized species of the genus *Aestuariibacter* by its ability to grow at 4 °C and at 30 °C, the optimum temperature for growth, and its inability to utilize most carbohydrates. On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain KMM 3894\(^T\) is considered to represent a novel species of the genus *Aestuariibacter*, for which the name *Aestuariibacter litoralis* sp. nov. is proposed. The type strain is KMM 3894\(^T\) (=NRIC 0754\(^T\)=JCM 15896\(^T\)).

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Abbreviations: ASW, artificial seawater base; MP, maximum-parsimony; NJ, neighbour-joining; SWM, seawater medium.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMM 3894\(^T\) is AB473549.

A maximum-parsimony phylogenetic tree based on 16S rRNA gene sequences showing the position of strain KMM 3894\(^T\) and related species is available as supplementary material with the online version of this paper.

The sodium ion requirement and tolerance of various NaCl concentrations (0–20 %) were examined on SWM prepared with an artificial seawater base (ASW) (Lyman & Fleming, 1940), supplemented with an appropriate amount of NaCl. The SWM medium contained: 5.0 g peptone, 2.5 g yeast extract, 1.0 g glucose, 0.2 g K\(_2\)HPO\(_4\), 0.05 g MgSO\(_4\), and 15.0 g agar, 750 ml natural seawater/250 ml distilled water. The sodium ion requirement was tested using various concentrations (0–20 %) were examined on SWM prepared with an artificial seawater base (ASW) (Lyman & Fleming, 1940), supplemented with an appropriate amount of NaCl. The SWM medium contained: 5.0 g peptone, 2.5 g yeast extract, 1.0 g glucose, 0.2 g K\(_2\)HPO\(_4\), 0.05 g MgSO\(_4\), and 15.0 g agar, 750 ml natural seawater/250 ml distilled water. The sodium ion requirement was tested using various concentrations (0–20 %) were examined on SWM prepared with an artificial seawater base (ASW) (Lyman & Fleming, 1940), supplemented with an appropriate amount of NaCl. The SWM medium contained: 5.0 g peptone, 2.5 g yeast extract, 1.0 g glucose, 0.2 g K\(_2\)HPO\(_4\), 0.05 g MgSO\(_4\), and 15.0 g agar, 750 ml natural seawater/250 ml distilled water. The sodium ion requirement was tested using various concentrations (0–20 %) were examined on SWM prepared with an artificial seawater base (ASW) (Lyman & Fleming, 1940), supplemented with an appropriate amount of NaCl. The SWM medium contained: 5.0 g peptone, 2.5 g yeast extract, 1.0 g glucose, 0.2 g K\(_2\)HPO\(_4\), 0.05 g MgSO\(_4\), and 15.0 g agar, 750 ml natural seawater/250 ml distilled water.

The oxidation/fermentation medium of Leifson (1963) for marine bacteria was used to test acid production from carbohydrates, with 1 % (w/v) of each compound. Growth at various temperatures and pH values and in the presence of various NaCl concentrations and antibiotic resistance were studied as described previously (Romanenko et al., 2003, 2004). In addition, biochemical tests were carried out using API 20E, API 20E and API 50 CH test kits (bioMérieux), according to the

The phenotypic and phylogenetic characteristics of an aerobic, Gram-negative, motile, non-pigmented *Alteromonas*-like bacterium (designated strain KMM 3894\(^T\)), isolated from a sandy sediment sample collected offshore of the Sea of Japan, were investigated. Comparative 16S rRNA gene sequence analysis revealed that strain KMM 3894\(^T\) belonged to the genus *Aestuariibacter* and was most closely related to *Aestuariibacter halophilus* JC2043\(^T\) (95.5 % sequence similarity). Fatty acid analysis showed C\(_{16:1}\) \(\omega_{9c}\), C\(_{18:1}\) \(\omega_{7c}\), and C\(_{18:0}\) as the dominant components. Strain KMM 3894\(^T\) could be differentiated from recognized species of the genus *Aestuariibacter* by its ability to grow at 4 °C and at 30 °C, the optimum temperature for growth, and its inability to utilize most carbohydrates. On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain KMM 3894\(^T\) is considered to represent a novel species of the genus *Aestuariibacter*, for which the name *Aestuariibacter litoralis* sp. nov. is proposed. The type strain is KMM 3894\(^T\) (=NRIC 0754\(^T\)=JCM 15896\(^T\)).
manufacturer’s instructions, except that the culture was suspended in ASW. For comparative fatty acid analysis, strain KMM 3894<sup>T</sup> was cultivated on MA at 28 °C for 3 days, and lipids were extracted using the chloroform–methanol extraction method of Bligh & Dyer (1959). Fatty acid methyl esters (FAMEs) were obtained by using alkaline methanolysis (15 % NaOH/methanol). The resultant FAMEs were extracted with hexane and analysed using a GLC-MS Hewlett Packard model 6890 gas chromatograph equipped with a HP 5 MS 5 % phenyl methyl silicone capillary column (30 m × 250 μm × 0.25 μm) and connected to a Hewlett Packard model 5973 mass spectrometer. A 16S rRNA gene sequence of 1504 nucleotides was determined for strain KMM 3894<sup>T</sup>, as described by Shida et al. (1997). The sequence obtained was compared with 16S rRNA gene sequences retrieved from the GenBank/EMBL/DDBJ databases by using the FASTA program (Pearson & Lipman, 1988). Phylogenetic analysis of 16S rRNA gene sequences was performed using the software package MEGA 4 (Tamura et al., 2007) after multiple alignment of data by using CLUSTAL_X (version 1.83; Thompson et al., 1997). Phylogenetic trees were constructed by using the neighbour-joining (NJ) and maximum-parsimony (MP) methods and the distances were calculated according to the Kimura two-parameter model. The robustness of the phylogenetic trees was estimated by using a bootstrap analysis of 1000 replicates. Phylogenetic trees constructed using the NJ and MP methods based on the 16S rRNA gene sequence both gave similar results, as shown, respectively, in Fig. 1 and Supplementary Fig. S1 (available in IJSEM Online). FASTA searches displayed that strain KMM 3894<sup>T</sup> belonged to the genus Aestuariibacter; Aestuariibacter halophilus JC2043<sup>T</sup> and Aestuariibacter salixigens JC2042<sup>T</sup> were most closely related to the novel isolate, with 95.5 and 94.9 % sequence similarities, respectively. Strain KMM 3894<sup>T</sup> shared lower sequence similarities with other species, including Alteromonas genovensis LMG 24078<sup>T</sup> (94.4 %), Alteromonas hispanica F-32<sup>T</sup> (94.4 %), Alteromonas stellipolaris LMG 21861<sup>T</sup> (93.9 %) and Glaciecola chathamensis S18K6<sup>T</sup> (93.8 %). The 16S rRNA gene sequence similarities obtained for strain KMM 3894<sup>T</sup> were significantly lower than the threshold similarity value of 97 % proposed by Stackebrandt & Goebel (1994) and re-evaluated as 98.7 % by Stackebrandt & Ebers (2006), indicating that strain KMM 3894<sup>T</sup> could be assigned to the genus Aestuariibacter as an individual species. Strain KMM 3894<sup>T</sup> contained C<sub>16:1ω7c</sub>, C<sub>16:0</sub> and C<sub>18:1ω7c</sub> as the dominant fatty acids (Table 1), which is in accordance with data reported previously for Aestuariibacter species (Yi et al., 2004). The detailed fatty acid composition of strain KMM 3894<sup>T</sup> is given in Table 1. The differential phenotypic features of strain KMM 3894<sup>T</sup> and related species of the genus Aestuariibacter are listed in Table 2 and in the species.
Table 1. Cellular fatty acid compositions (%) of strain KMM 3894T and related Aestuariibacter species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10:0 3-OH</td>
<td>–</td>
<td>0.6</td>
<td>4.6</td>
</tr>
<tr>
<td>C12:0</td>
<td>2.3</td>
<td>1.8</td>
<td>3.0</td>
</tr>
<tr>
<td>C11:0 3-OH</td>
<td>–</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>C12:1 3-OH</td>
<td>1.3</td>
<td>2.2</td>
<td>1.1</td>
</tr>
<tr>
<td>C12:0 3-OH</td>
<td>1.6</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>C14:0</td>
<td>3.8</td>
<td>3.9</td>
<td>3.1</td>
</tr>
<tr>
<td>C15:0 iso-10:8c</td>
<td>–</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>C15:1</td>
<td>1.2</td>
<td>3.8</td>
<td>2.4</td>
</tr>
<tr>
<td>C16:0 10:7c alcohol</td>
<td>–</td>
<td>2.1</td>
<td>0.7</td>
</tr>
<tr>
<td>C12:0 16:0 3-OH</td>
<td>–</td>
<td>2.0</td>
<td>1.6</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>–</td>
<td>1.1</td>
<td>0.1</td>
</tr>
<tr>
<td>C16:10:8c</td>
<td>2.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C16:10:7c iso-C15:0</td>
<td>2-0</td>
<td>27.2</td>
<td>32.5</td>
</tr>
<tr>
<td>C16:10:7c</td>
<td>29.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C16:0</td>
<td>17.7</td>
<td>15.8</td>
<td>23.9</td>
</tr>
<tr>
<td>iso-C17:0 10:5c</td>
<td>–</td>
<td>–</td>
<td>1.6</td>
</tr>
<tr>
<td>C17:0 10:8c</td>
<td>–</td>
<td>6.8</td>
<td>3.1</td>
</tr>
<tr>
<td>C17:0 10:6c</td>
<td>3.4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C17:0</td>
<td>2.7</td>
<td>4.5</td>
<td>2.8</td>
</tr>
<tr>
<td>C18:10:7c</td>
<td>20.8</td>
<td>14.0</td>
<td>11.1</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.1</td>
<td>2.9</td>
<td>0.9</td>
</tr>
<tr>
<td>C19:10:6c/C19:0 10:10c cyclo/C19:0 10:6c</td>
<td>–</td>
<td>1.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Strains: 1, KMM 3894T (Aestuariibacter litoralis sp. nov.); 2, Aestuariibacter salexigens JC2042T (data from Yi et al., 2004); 3, Aestuariibacter halophilus JC2043T (Yi et al., 2004). −, Not detected.

Aestuariibacter litoralis (li.to.ri’lis. L. masc. adj. litoralis of the seashore).

Aerobic, Gram-negative, oxidase- and catalase-positive, motile, rod-shaped bacterium (approx. 2 μm in length). Colonies are non-pigmented, opaque, whitish and shiny, with wrinkled surface and regular edges of 2–3 mm in diameter on MA. Growth occurs at 4–40 °C, with optimum growth at 30 °C. Sodium ions are essential for growth. Growth occurs in medium containing 0.5–6 % NaCl without other sea salts added. No growth is observed on media containing 0.5–12 % (w/v) artificial sea salts, but is observed on media containing 20–100 % (w/v) artificial sea salts. pH range for growth is 5.5–10.5, with optimum growth at pH 6.5–8.0. Positive for Tween 80, starch, aesculin and DNA hydrolysis. Negative for casein and chitin hydrolysis, and for H2S production. Gelatin is slowly hydrolysed over 4–5 days routinely and in API 20NE, and 2 days in API 20E tests. Does not produce melanin-like pigments or clearance zone on medium containing L-lysine. Acid is not produced from D-glucose, D-mannitol, sucrose, D-lactose, maltose, D-galactose, D-mannose, cellobiose, D-xylene, D-sorbitol, L-arabinose or L-rhamnose. In addition, according to API 20E tests, positive for aesculin hydrolysis and nitrate reduction, and negative for indole production, glucose acidification, arginine dihydrolase, urease production and the β-nitrophenyl α-D-glucopyranoside test, and assimilation of D-glucose, D-mannitol, maltose, L-arabinose, D-mannose, N-acetylglucosamine, D-glucurate, caprate, adipate, L-malate, citrate and phenylacetate. In API 20E tests, positive for trisodium citrate utilization and negative for ONPG, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H2S production, urease, tryptophan deaminase, indole production, acetoin production, and utilization of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin and D-arabinose. In API 50 CH tests, weakly positive for utilization of potassium 5-ketogluro- nate and negative for utilization of glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylene, L-xylose, D-adonitol, methyl β-D-xylpyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-
mannopyranoside, methyl α-D-glucopyranoside, N-acetyl-
glucosamine, amygdalin, arbutin, aesculin, salicin, cello-
biose, maltose, D-lactose, melibiose, sucrose, trehalose,
inulin, melezitose, raffinose, starch, glycygen, xyitol, 
gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, 
L-fucose, L-arabitol, D-arabitol, potassium 2-ketogluconate 
and gluconate. Predominant fatty acids are C18 : 1

\[ \text{and C}_{16:0} \] (detailed fatty acid composition of the type 
strain is given in Table 2). Susceptible to the following 
antibiotics (content per disc): ampicillin (10 μg), vanco-
mycin (30 μg), gentamicin (10 μg), kanamycin (30 μg), 
carbenicillin (100 μg), chloramphenicol (30 μg), nalidixic 
acid (30 μg), oleandomycin (15 μg), ofloxacin (5 μg), 
polymyxin B (300 U), rifampicin (5 μg), streptomycin 
(30 μg), erythromycin (15 μg), tetracycline (30 μg), cepha-
zolin (30 μg) and cephalaxin (30 μg); and resistant to 
benzylenicillin (10 U), lincomycin (15 μg), neomycin 
(30 μg) and oxacillin (10 μg).

The type strain, KMM 3894T (=NRIC 0754T =JCM 
15896T), was isolated from a marine sandy sample, 
collected offshore of the Sea of Japan, Russia at a depth 
of 3 m.

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Presidium of RAS ‘Molecular and Cell Biology’.

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strategies for multiple sequence alignment aided by quality analysis 

nov., sp. nov. and Aestuariibacter halophilus sp. nov., isolated from 
tidal flat sediment, and emended description of Alteromonas 