Temperatibacter marinus gen. nov., sp. nov., a mesophilic bacterium isolated from surface seawater and description of Temperatibacteraceae fam. nov. in the class Alphaproteobacteria

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A Gram-stain-negative, motile, mesophilic, aerobic, rod-shaped bacterium, strain 5-11¹, was isolated from surface seawater at Muroto city, Kochi prefecture, Japan. The strain exhibited a narrow growth temperature range of 20–30 °C. Phylogenetic analyses based on 16S rRNA gene sequences showed that the strain fell within the order Kordiimonadales in the class Alphaproteobacteria and was related most closely to the genus Kordiimonas (up to 91.2 % similarity to the type strains of species of the genus) but branched deeply from species of Kordiimonas. The major fatty acids were iso-C₁₇:0 3-OH, iso-C₁₅:0 2-OH, and C₁₆:1 3-OPC and/or iso-C₁₅:0 2-OH. Ubiquinone-10 (Q-10) was detected as the sole isoprenoid quinone. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and one unidentified aminolipid. Although strains of Kordiimonas have been shown to contain unidentified glycolipids, they were not detected from strain 5-11¹. The DNA G+C content of strain 5-11¹ was 44.3 mol%, a value that was lower than those of strains of Kordiimonas (50–58 mol%) and was relatively low for the members of the class Alphaproteobacteria. On the basis of phenotypic, genotypic and chemotaxonomic data, it is proposed that strain 5-11¹ represents a novel species of a new genus, Temperatibacter marinus gen. nov., sp. nov., within a new family Temperatibacteraceae fam. nov. The type strain of Temperatibacter marinus is 5-11¹ (=NBRC 110045T =LMG 28278T).

Strain 5-11¹ was obtained from seawater by direct plating on DSW5 medium, which comprised (per litre) 1 litre deep seawater (from the coastal area of Muroto city; 33° 18′ N 134° 14′ E, water depth of 320 m), 15 g agar, 64 mg urea, 17 mg K₂HPO₄, 18 mg FeCl₂·4H₂O, 0.1 g yeast extract (Bacto yeast extract; BD), 100 mg sodium acetate and 100 mg sodium pyruvate.

Strain 5-11¹ was cultivated at 28 °C for 48 h on a dMB1 plate, which contained (per litre) 7.48 g marine broth 2216 (MB; BD), 15 g agar, 0.2 litres distilled water and 0.8 litres artificial seawater (ASW; Marine Art SF-1; Tomita Pharmaceutical). Phase-contrast microscopy (BX50F4; Olympus) showed that the strain was motile. Gram-staining tests (Favor G kit; Nissui) showed that the strain was Gram-stain-negative. The strain was non-sporulating, and formed smooth, convex, opaque-yellow circular colonies with a wavy edge that had a butyrous consistency on the dMB1 plate. The strain was oxidase- and catalase-positive (Barrow & Feltham, 1993), and negative for acid production from D-glucose on modified Hugh–Leifson’s O-F medium (Leifson, 1963). The API 20 NE kit (bioMérieux) was used for assessing biochemical characteristics according to the manufacturer’s instructions, with the exception that the

Muroto city, Kochi prefecture, Japan, is one of the rare areas where deep seawater is upwelling. Deep seawater is relatively rich in nitrogen and phosphorus (DeLong et al., 2006; Hansman et al., 2009), which are the two main growth-limiting factors in marine environments. Therefore, surface seawater around Muroto may be more nutrient-rich than surface seawater in other areas, suggesting that rich microbial resources might be found in the Muroto surface seawater. The present study describes a novel bacterium, designated strain 5-11¹, isolated from the Muroto surface seawater (33° 18′ N 134° 11′ E, water depth of 0.5 m) in summer 2011. The growth temperature of strain 5-11¹ was very limited (20–30 °C), and this strain could thus inhabit a limited area around Japan. This feature, the narrow temperature spectrum for growth, could be of practical value, i.e. make the strain a useful host for processes, including bioaugmentation, that can be controlled by temperature (Teramoto et al., 2010).
cells were suspended in ASW. In addition, API ZYM tests (bioMérieux) were conducted to examine enzyme activities as described in the manufacturer’s manual, with the exceptions that the inoculum was prepared using ASW and the incubation time was extended to 24 h at 28 °C. The strain was negative for reduction of nitrate to nitrite, indole production from tryptophan, fermentation of glucose, arginine dihydrolase, urease, acetaldehyde, gelatinase and β-galactosidase. Other detailed phenotypic features of strain 5-11T are given in the species description.

For transmission electron microscopy (H7600; Hitachi), strain 5-11T was grown at 20–28 °C for 7 days on a dMB plate (composition same as for the dMBI plate except that Muroto surface seawater was used instead of ASW), and negatively stained with 2 % uranyl acetate. Cells of strain 5-11T were curved rods (0.5–0.8 × 1.5–3.4 μm) with a polar flagellum (Fig. S1, available in the online Supplementary Material).

Anaerobic growth was tested on the dMB plate at 20 °C in a GasPak anaerobic jar (BD) for 2 months. Strain 5-11T did not grow under anaerobic conditions. Growth temperature was tested on dMB plates at 4, 10, 15, 20, 22, 25, 28, 30, 35, and 40 °C for 2 months. Strain 5-11T grew at 20–30 °C (optimally at 22–28 °C). NaCl requirement of strain 5-11T was tested at 28 °C for 1 week on modified MB medium (pH 7.6) supplemented with 0–7 % (w/v) NaCl (at intervals of 0.5 %). The modified MB medium contained (per litre distilled water) 5 g peptone (bacteriologically neutralized; Oxoid), 1 g yeast extract (Oxoid), 0.1 g ferric citrate, 1.8 g CaCl2, 2H2O and 5.0 g MgCl2.6H2O. Strain 5-11T grew with 1.0–4.5 % (w/v) NaCl (optimally with 3.0–3.5 %). The pH range for growth (tested at pH 2–11, intervals of 1 pH unit) was determined at 20 °C for 4 weeks with shaking in filter-sterilized dMB medium, which contained (per litre) 7.48 g MB, 0.2 litres distilled water and 0.8 litres Muroto surface seawater. Strain 5-11T grew at pH 6–11 (optimally at pH 7–9).

The almost full-length 16S rRNA gene sequence of strain 5-11T (1362 bp) was obtained as described previously (Teramoto et al., 2009) and aligned with related sequences of members of the class Alphaproteobacteria available in public databases using CLUSTAL X (ver. 2.1) (Larkin et al., 2007). The alignments were manually modified where necessary, and trimming of gaps was performed. Phylogenetic trees were inferred from the aligned sequences of 1230 bp using the neighbour-joining algorithm (Saitou & Nei, 1987) by CLUSTAL X with default parameters (including Kimura’s correction) and maximum-likelihood algorithm (Felsenstein, 1981) by MEGA 6.06 (Tamura et al., 2013) and analysed using bootstrapping (Felsenstein, 1985) based on 1000 resamplings. The neighbour-joining tree is shown in Fig. 1. Strain 5-11T and two closely related clones (GenBank accession numbers AF030779 and EU268103) formed a coherent and distinct cluster that was supported by a high bootstrap value (100 %) within the order Kordiimonadales (Kwon et al., 2005). Strain 5-11T, together with the two closely related clones, branched deeply from a cluster of the type strains of species of the most closely related genus Kordiimonas within the order Kordiimonadales and from the type strain of the single species of the second closely related genus Eilatimonas, which was supported by high bootstrap values of 92 and 100 %, respectively. Furthermore, 16S rRNA gene sequence similarities between strain 5-11T and the type strains of species of the genus Kordiimonas were very low (89.9–91.2 %). These results indicate that strain 5-11T could be a representative of a novel family of the order Kordiimonadales.

The following nucleotides or regions in the 16S rRNA gene sequence conserved for strains of Kordiimonas could be signatures for a family containing the strains of Kordiimonas (‘Kordiimonadaceae’ in Fig. 1): 166–167 (G–T), 461 (A), 743–747 (A–G), 859 (A), 1010–1011 (C–C), 1018–1020 (G–A), 1117–1120 (G–T), 1131–1134 (T–no nucleotide), 1138–1140 (A–T), 1151–1153 (A–A), 1310–1311 (T–T), 1326 (A), 1434–1442 (C–G) and 1464 (G). These nucleotides or regions differentiated strains of Kordiimonas from strain 5-11T and Eilatimonas milleporae MD21.

The cellular fatty acid composition of strain 5-11T was analysed by using cells (from the third quadrant of the quadrant streaked plate) grown at 30 °C for 3 days on marine agar 2216 (BD) when growth had reached its maximum. Cellular fatty acid methyl esters were prepared and analysed by GC (7890A GC system; Agilent Technologies) according to the instructions given for the Microbial Identification System version 6.0 and were compared using the database TSBA40 (MIDI). The major cellular fatty acids of strain 5-11T were iso-C17:0 3-OH (28.0 % of the total), iso-C15:0 3-OH (15.8 %), and C16:0 3-OH and/or iso-C15:0 2-OH (13.7 %). Detailed results are given in Table 1 and compared with the results for the closest taxonomic relatives. The fatty acid composition of strain 5-11T seemed similar to those of species of the genus Kordiimonas but different from that of Eilatimonas milleporae MD21: iso-C15:0 3-OH (4.1 % versus not detected) and iso-C15:0 2-OH (15.8 % versus 2.6 %).

Isoprenoid quinones were extracted from cells of strain 5-11T grown in MB with shaking at 28 °C for 24 h as described previously (Nishijima et al., 1997) and were analysed by HPLC (Waters 600; Nihon Waters). Ubiquinone-10 (Q-10) was detected from strain 5-11T as the sole isoprenoid quinone. Q-10 is often the dominant isoprenoid quinone for members of the class Alphaproteobacteria.

For polar lipid analysis, cells of strain 5-11T were grown in MB with shaking at 28 °C for 2 days. Polar lipids were extracted, separated and revealed as described previously (Teramoto et al., 2011) except that the total lipid content was revealed by spraying with 10 % (w/v) molybdophosphoric acid in 2-propanol and incubating at 180 °C for 15 min. The polar lipid profile is shown in Fig. S2. The major polar lipids were phosphatidylyethanolamine (PE), phosphatidylglycerol (PG) and one unidentified aminolipid; two unidentified lipids, one unidentified phospholipid and lysophosphatidylyethanolamine (PE where one fatty acid chain is missing from the glycerol backbone) were found as
minor components. Unidentified glycolipid(s) have been found for the type strains of all species of the genera *Kordiimonas* and *Eilatimonas*, while they were not detected from strain 5-11\(^T\) (Table 2).

To determine the G+C content, genomic DNA was extracted and purified from cells of strain 5-11\(^T\) grown in MB with shaking at 28 °C for 2 days based on the protocol of Marmur (1961). DNA was first separated from protein by shaking with phenol as described by Saito & Miura (1963) before treatment with chloroform and isoamyl alcohol. The genomic DNA was digested to nucleotides with nuclease P1 using a DNA-GC kit (Seikagaku Kogyo) according to the procedures described by Katayama-Fujimura *et al.* (1984). The G+C content of the DNA was determined by HPLC (LC-10; Shimadzu) with an RP Aqueous column (4.6 × 250 mm; Nomura Chemical) and a UV–visual spectrophotometric detector (SPD-10AV; Shimadzu) at 270 nm. The DNA G+C content of strain 5-11\(^T\) was 44.3 mol%. This value was lower than those for the genera *Kordiimonas* (50–58 mol%; Yang *et al.*, 2013) and *Eilatimonas* (60.3 mol%; Paramasivam *et al.*, 2013) and was relatively low for members of the class Alphaproteobacteria (Garrity *et al.*, 2005).

Strain 5-11\(^T\) showed low 16S rRNA gene sequence similarity to the type strains of the phylogenetically closest genus *Kordiimonas* (89.9–91.2 %). Also, strain 5-11\(^T\) differed from the type strains of species of *Kordiimonas* based on DNA G+C content and major polar lipids (Table 2). In addition, strain 5-11\(^T\) differed from the type strain of the single species of the second closest genus *Eilatimonas* based on DNA G+C content, major polar lipids (Table 2) and fatty acid compositions (Table 1). On the basis of genotypic (Fig. 1) and chemotaxonomic (Tables 1 and 2) data as well...
as phenotypic characteristics (Table 2), we propose that strain 5-11T represents a novel species of a new genus, for which the name Temperatibacter marinus gen. nov., sp. nov. is proposed.

Phylogenetic analysis indicated that strain 5-11T, together with its closely related clones, branched deeply from a cluster of strains of the genus Kordiimonas within the order Kordiimonadales (Fig. 1). Also, strain 5-11T showed low 16S rRNA gene sequence similarity to strains of the genus Kordiimonas. Therefore, strain 5-11T is also proposed to belong to a novel family, Temperatibacteraceae fam. nov., of the order Kordiimonadales.

Description of Temperatibacter gen. nov.

Temperatibacter (Tem.per.at.i.bac.ter. L. adj. temperatus temperate, referring to the moderate growth temperature range of the type species; N.L. masc. n. bacter rod; N.L. masc. n. Temperatibacter the temperate rod).

According to 16S rRNA gene sequence analysis, belongs to the Alphaproteobacteria. Cells are Gram-stain-negative, motile, aerobic, curved rods. Predominant cellular fatty acids are iso-C17:1ω9c, iso-C15:0, and C16:1ω7c and/or iso-C15:0 2-OH. Q-10 is detected as the sole isoprenoid quinone. Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and one unidentified aminolipid. The DNA G+C content of a known strain of the type species is 44.3 mol%. The type species is Temperatibacter marinus.

Description of Temperatibacter marinus sp. nov.

Temperatibacter marinus (ma.ri’nus. L. masc. adj. marinus of the sea, marine).

Displays the following properties in addition to those given in the genus description. Cells are 0.5–0.8×1.5–3.4 μm and have a polar flagellum. Growth occurs at 20–30 °C (optimally at 22–28 °C), at pH 6–11 (optimally at pH 7–9) and with 1.0–4.5% (w/v) NaCl (optimally with 3.0–3.5%). Catalase- and oxidase-positive. In API 20 NE tests, negative for reduction of nitrate to nitrite, indole production from tryptophan, fermentation of glucose, arginine dihydrolase, urease, aesculin hydrolase, gelatinase, β-galactosidase, and assimilation of glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, maltose, potassium gluconate, n-caprate, adipate, DL-malate, sodium citrate and phenylacetate. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C9), leucine arylamidase, valine arylamidase, trypsin and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), cystine arylamidase, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

The type strain is 5-11T (=NBRC 110045T=LMG 28278T), isolated from surface seawater at Muroto city, Kochi prefecture, Japan. The DNA G+C content of the type strain is 44.3 mol%.

Description of Temperatibacteraceae fam. nov.

Temperatibacteraceae (Tem.per.at.i.bac.ter.a.ce.ae. N.L. masc. n. Temperatibacter the type genus of the family; suff. -aceae ending to denote a family; N.L. masc. pl. n. Temperatibacteraceae the family of the genus Temperatibacter).

The description is the same as that for the genus Temperatibacter. The family contains the type genus Temperatibacter.
Table 2. Selected differential characteristics between strain 5-11\textsuperscript{T} and its phylogenetically closest relatives in the order Kordiimonadales

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<tr>
<td>Growth temperature range (°C)</td>
<td>20–30</td>
<td>17–44*</td>
<td>10–43</td>
<td>15–40</td>
<td>10.5–35.0</td>
<td>20–37</td>
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<tr>
<td>Optimum temperature (°C)</td>
<td>22–28</td>
<td>37–40*</td>
<td>30–37</td>
<td>30</td>
<td>20</td>
<td>30</td>
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<td>Assimilation of:</td>
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<tr>
<td>Glucose</td>
<td>–</td>
<td>+*</td>
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<tr>
<td>Maltose</td>
<td>–</td>
<td>+*</td>
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<tr>
<td>(\alpha)-Chymotrypsin</td>
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<td>+†</td>
<td></td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Acid phosphatase</td>
<td>–</td>
<td>+†</td>
<td></td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>44.3</td>
<td>55.6–58.0\§</td>
<td>54.9</td>
<td>53.3</td>
<td>50.3</td>
<td>60.3</td>
</tr>
<tr>
<td>Major polar lipids</td>
<td>PE, PG, AL</td>
<td>PG, GL† (PE, GL, AL, APL)†</td>
<td>PG, GL</td>
<td>L, PE, AL</td>
<td>PE, GL, AL, APL</td>
<td>PG, PE, PL, GL, L, AL§</td>
</tr>
</tbody>
</table>

*From Kwon et al. (2005).
†From Xu et al. (2011).
§All polar lipids detected.

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References


