Cyclobacterium caenipelagi sp. nov., isolated from a tidal flat sediment, and emended description of the genus Cyclobacterium

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A Gram-staining-negative, aerobic, non-flagellated, non-gliding and ring-like or horseshoe-shaped bacterial strain, designated HD-17T, was isolated from a tidal flat sediment in the Korean peninsula. Strain HD-17T grew optimally at pH 7.0–8.0, at 25 °C and in the presence of 2.0 % (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain HD-17T fell within the clade comprising species of the genus Cyclobacterium. Strain HD-17T exhibited 16S rRNA gene sequence similarity values of 93.8–98.8 % to the type strains of species of the genus Cyclobacterium. Strain HD-17T contained MK-7 as the predominant menaquinone and iso-C15 : 0, summed feature 3 (C16 : 1ω6c and/or C16 : 1ω7c), anteiso-C15 : 0 and iso-C15 : 1G as the major fatty acids. The major polar lipids of strain HD-17T were phosphatidylcholine, phosphatidylethanolamine and two unidentified lipids. The DNA G+C content of strain HD-17T was 43.8 mol% and its DNA–DNA relatedness values with Cyclobacterium amurskyense KCTC 12363T, Cyclobacterium qasimii KCTC 23011T and Cyclobacterium marinum KCTC 2917T were 10.4, 7.6 and 5.3 %, respectively. The phylogenetic and genetic distinctiveness and several differentiating phenotypic properties revealed that strain HD-17T was separate from other species of the genus Cyclobacterium. On the basis of the data presented, strain HD-17T represents a novel species of the genus Cyclobacterium, for which the name Cyclobacterium caenipelagi sp. nov. is proposed. The type strain is HD-17T (=KCTC 32178T = CCUG 63247T). An emended description of the genus Cyclobacterium is also provided.

The genus Cyclobacterium, a member of the family Cyclobacteriaceae, was first proposed by Raj & Maloy (1990) with the description of Cyclobacterium marinum as the sole recognized species. Subsequently, three further species of the genus Cyclobacterium with validly published names, Cyclobacterium amurskyense (Nedashkovskaya et al., 2005), Cyclobacterium lianum (Ying et al., 2006) and Cyclobacterium qasimii (Shivaji et al., 2012), have been described. In this study, a novel bacterial strain, designated HD-17T, which was isolated from a tidal flat sediment of the Yellow Sea, South Korea, is described. Comparative 16S rRNA gene sequence analysis indicated that strain HD-17T was phylogenetically most closely related to members of the genus Cyclobacterium. The aim of the present work was to determine the exact taxonomic position of strain HD-17T by using a polyphasic approach that included determination of the phenotypic properties and a detailed phylogenetic investigation based on 16S rRNA gene sequences and genetic analysis.

The standard dilution plating technique was used for isolation of bacterial strains from the tidal flat sediment sample. Strain HD-17T was isolated on marine agar 2216 (MA; BD) at 25 °C and cultivated routinely under the same conditions. Cyclobacterium amurskyense KCTC 12363T, C. lianum JCM 14011T, C. marinum KCTC 2917T and C. qasimii KCTC 23011T were used as reference strains for DNA–DNA hybridization, phenotypic characterization and the analyses of fatty acids and polar lipids. Cell morphology was examined by using light microscopy (BX51; Olympus) and transmission electron microscopy (JEM1010; JEOL). The presence of flagellum was determined by using...

A supplementary figure and a supplementary table are available with the online version of this paper.
transmission electron microscopy on cells from an exponentially growing MA culture. For this purpose, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. Gliding motility was investigated as described by Bowman (2000). The Gram reaction was investigated using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Growth under anaerobic conditions was determined after incubation for 10 days in an anaerobic jar (MGC) with AnaeroPack (MGC) on MA; the jar was kept overnight at 4 °C to make anoxic conditions before incubation at 25 °C. Growth at 4, 10, 20, 25, 30, 37 and 38 °C was measured on MA. Growth in the absence of NaCl and in the presence of 0.5, 1.0 and 2.0% (w/v) NaCl was investigated in trypticase soy broth prepared according to the formula of the BD medium except that NaCl was excluded and that 0.45% (w/v) MgCl₂.6H₂O was added. Growth in the presence of 2.0–11.0% (w/v) NaCl (in increments of 1.0%) as final concentration was investigated in marine broth 2216 (MB; BD). Catalase and oxidase activities were determined as described by Lánya (1987). Hydrolysis of casein, starch, hypoxanthine, Tween 20, 40, 60 and 80, L-tyrosine and xanthine was tested on MA using the substrate concentrations described by Barrow & Feltham (1993). Hydrolysis of gelatin and urea was investigated by using nutrient gelatin and urea agar base media (BD), respectively, with the modification that artificial seawater was used for the preparation of media. Hydrolysis of aesculin and nitrate reduction were investigated as described previously (Lánya 1987) with the modification that artificial seawater was used for preparation of media. The artificial seawater contained (l⁻¹ distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂, 6H₂O, 5.94 g MgSO₄.7H₂O and 1.3 g CaCl₂.2H₂O (Bruns et al., 2001). H₂S production was tested as described previously (Bruns et al., 2001). The pH range for growth, acid production from carbohydrates and utilization of various substrates for growth were determined as described by Park et al. (2010). Susceptibility to antibiotics was tested on MA plates using antibiotic discs (Advantec) containing the following (µg per disc unless stated otherwise): ampicillin (10), carbenicillin (100), cephalothin (30), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), novobiocin (5), oleandomycin (15), penicillin G (20 U), polymyxin B (100 U), streptomycin (50) and tetracycline (30). Enzyme activities were determined, after incubation for 8 h at 25 °C, by using the API ZYM system (bioMérieux).

Cell biomass for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown at 25 °C in MB. Chromosomal DNA was extracted and purified according to the method described by Yoon et al. (1996), with the exception that RNase T₁ was used in combination with RNase A to minimize the contamination of RNA. The 16S rRNA gene was amplified by PCR as described previously (Yoon et al., 1998) using two universal primers and the PCR products were purified by using a QIAquick PCR purification kit (Qiagen).

Table 1. Differential phenotypic characteristics of strain HD-17T and the type strains of four species of the genus Cyclobacterium

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Optimum temperature for growth (°C)*</td>
<td>25</td>
<td>25</td>
<td>18</td>
<td>20–25</td>
<td>33</td>
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<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>l-Arabinose</td>
<td>+</td>
<td>w</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Susceptibility to (µg per disc):</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Carbenicillin (100)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Novobiocin (5)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Enzyme activity (API ZYM)</td>
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<tr>
<td>α-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Butyrate oxidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>43.8</td>
<td>41.3</td>
<td>40.5</td>
<td>41.9</td>
<td>45.2</td>
</tr>
</tbody>
</table>

*Data for the reference strains from Raj & Maloy (1990), Nedashkovskaya et al. (2005), Ying et al. (2006) and Shivaji et al. (2012).
Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain HD-17\textsuperscript{T}, the type strains of species of the genus *Cyclobacterium* and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >70% are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms (data not shown). Strain HD-17\textsuperscript{T} exhibited 16S rRNA gene sequence similarity values of 98.8, 98.7, 97.9 and 93.8% to *C. amurskyense* KMM 6143\textsuperscript{T}, *C. qasimii* KMM 6143\textsuperscript{T}, *C. caenipelagi* KMM 6143\textsuperscript{T} (JX515600), and *C. amurskyense* KMM 6143\textsuperscript{T} (AY960985).
M12-11BT, C. marinum DSM 745T and C. lianum HY9T, respectively and of less than 91.6% to those of other species used in phylogenetic analysis.

The predominant isoprenoid quinone detected in strain HD-17T was menaquinone-7 (MK-7) which is identical to that of C. marinum and C. qasimii (Raj & Maloy, 1990; Shivaji et al., 2012). The major fatty acids (>10% of the total fatty acids) found in strain HD-17T were iso-C$_{15:0}$ summed feature 3 (C$_{16:1}$ω6c and/or C$_{16:1}$ω7c), anteiso-C$_{15:0}$ and iso-C$_{15:1}$ G (Table S1). The fatty acid profile of strain HD-17T was similar to those of the type strains of the four species of the genus Cyclobacterium also analysed in this study, but there were differences in the proportions of some fatty acids (Table S1). The major polar lipids detected in strain HD-17T were phosphatidylcholine, phosphatidylycerethanolamine and two unidentified lipids (Fig. 2). The polar lipid profile of strain HD-17T was compared with those of C. amurskyense KCTC 12363T, C. lianum JCM 14011T, C. marinum KCTC 2917T and C. qasimii KCTC 23011T also analysed in this study (Fig. 2). The polar lipid profile of strain HD-17T was highly similar to the four reference strains in that phosphatidylcholine, phosphatidylycerethanolamine and one unidentified lipid (L1) were the major polar lipids. The DNA G+C content of strain HD-17T was 43.8 mol%, a value in the range reported for species of the genus Cyclobacterium (Ying et al., 2006; Shivaji et al., 2012).

Strain HD-17T exhibited mean DNA–DNA relatedness values of 10.4, 7.6 and 5.3% to C. amurskyense KCTC 12363T, C. qasimii KCTC 23011T and C. marinum KCTC 2917T, respectively. Strain HD-17T was distinguishable from the type strains of the four species of the genus Cyclobacterium by differences in several phenotypic characteristics as shown in Table 1. The phylogenetic and

![Fig. 2. Thin layer chromatograms of the total polar lipids of strain HD-17T (a), C. amurskyense KCTC 12363T (b), C. qasimii KCTC 23011T (c), C. marinum KCTC 2917T (d) and C. lianum JCM 14011T (e). Spots were revealed by spraying the plates with 10% ethanolic molybdophosphoric acid. PC, Phosphatidylcholine; PE, phosphatidylycerethanolamine; L1–L3, unidentified lipids.](http://ijs.sgmjournals.org)
genetic distinctiveness and differential phenotypic properties of strain HD-17<sup>T</sup> were sufficient to support that the novel strain was separate from recognized species of the genus Cyclobacterium (Wayne et al., 1987; Stackebrandt & Goebel, 1994). On the basis of these data, strain HD-17<sup>T</sup> represents a novel species of the genus Cyclobacterium, for which the name Cyclobacterium caenipelagi sp. nov. is proposed. The description of the genus Cyclobacterium is also emended.

**Emended description of the genus Cyclobacterium Ray and Maloy 1990 emend. Ying et al. 2006**

The description is as given by Ray & Maloy (1990) and Ying et al. (2006) with the following amendment. The major polar lipids are phosphatidylcholine, phosphatidylethanolamine and one unidentified lipid. The type species is *Cyclobacterium marinus*.

**Description of Cyclobacterium caenipelagi sp. nov.**

*Cyclobacterium caenipelagi* (ca.e.ni.pe’la.gi. L. n. caenum -i mud; L. n. pelagus -i the sea; N.L. gen. n. caenipelagi of mud of the sea, from which the type strain was isolated).

Cells are Gram-staining-negative, non-flagellated, non-gliding and ring-like or horseshoe-shaped, 0.2–0.5 μm in diameter and 0.8–1.0 μm in length. Colonies on MA are irregular, convex, smooth, glistening, yellowish-pink in colour and 0.5–1.5 mm after incubation for 5 days at 25 °C. The optimal growth temperature is 25 °C; growth occurs at 4 and 37 °C, but not at 38 °C. The optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.0, but not at pH 4.5. Growth occurs in the presence of 1.0–10.0 % (w/v) NaCl with an optimum of approximately 2.0 % (w/v) NaCl. Mg<sup>2+</sup> ions are required for growth. Anaerobic growth does not occur on MA. Catalase- and oxidase-positive. Nitrate reduction is negative. Aesculin and Tween 20 are hydrolysed, but casein, gelatin, hypoxanthine, starch, L-tyrosine, Tween 40, 60 and 80, urea and xanthine are not. L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, sucrose, trehalose, D-xylene and salicin are utilized, but acetate, benzoate, citrate, formate, L-malate, pyruvate, succinate and L-glutamate are not. Acid is produced from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, melezitose, melibiose, raffinose, D-rhamnose, D-ribose, sucrose, trehalose, D-xylene and myo-inositol, but not from D-mannitol or D-sorbitol. Susceptible to (μg per disc unless otherwise stated) carbencillin (100), cephalothin (30), chloramphenicol (100), lincomycin (15), oleandomycin (15) and penicillin G (20 μ), but not to ampicillin (10), gentamicin (30), kanamycin (30), neomycin (30), novobiocin (5), polymyxin B (100 μ), streptomycin (50) or tetracycline (30). In the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase activities are present and β-galactosidase activity is weakly present, but lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase and α-fucosidase activities are absent. The predominant menaquinone is MK-7. The major fatty acids (>10 % of total fatty acids) are iso-C<sub>15:0</sub>, summed feature 3 (C<sub>16:1</sub>ω6c and/or C<sub>16:1</sub>ω7c), anteiso-C<sub>15:0</sub> and iso-C<sub>15:1</sub> G. The major polar lipids are phosphatidylcholine, phosphatidylethanolamine and two unidentified lipids.

The type strain, HD-17<sup>T</sup> (=KCTC 32178<sup>T</sup>=CCUG 63247<sup>T</sup>), was isolated from a tidal flat sediment of Hwang-do at the Yellow Sea, South Korea. The DNA G+C content of the type strain is 43.8 mol% (determined by HPLC).

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**References**


