Cyclobacterium amurskyense sp. nov., a novel marine bacterium isolated from sea water

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The taxonomic position of a novel marine, heterotrophic, aerobic, pigmented, non-motile bacterium isolated from sea water was determined. 16S rRNA gene sequence analysis revealed that strain KMM 6143T is a member of the genus Cyclobacterium. The results of DNA–DNA hybridization experiments supported by phenotypic and chemotaxonomic data showed that the isolate represents a novel species of the genus Cyclobacterium, for which the name Cyclobacterium amurskyense sp. nov. is proposed. The type strain is KMM 6143T (=KCTC 12363T =LMG 23026T).

The genus Cyclobacterium, comprising the single species Cyclobacterium marinum (Raj & Maloy, 1990), accommodates Gram-negative, strictly aerobic, heterotrophic, pink-pigmented and non-motile marine bacteria belonging to the phylum 'Bacteroidetes'. Strains of the genus can form ring-like cells. The genus Cyclobacterium forms a phylogenetic cluster with the recently described genera Belliella and Aquiflexum (Brettar et al., 2004a, b).

During June 2000 we isolated an unknown bacterium, designated KMM 6143T, from a sea-water sample collected in Amursky Bay, Gulf of Peter the Great. A polyphasic taxonomic study of the phenotypic, chemotaxonomic and genotypic characteristics and phylogenetic position of strain KMM 6143T, cultured on marine agar 2216 (Difco), indicated that the isolate represents a novel species of the genus Cyclobacterium.

Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene followed previously published procedures (Kim et al., 1998). To establish the precise taxonomic position of strain KMM 6143T, 1380 nucleotides of its 16S rRNA gene sequence was determined and 1365 bp of this sequence was used for comparative phylogenetic analysis. The sequence obtained was aligned with sequences of representative members of the family 'Flexibacteraceae' using PHYDIT version 3.2 (http://plaza.snu.ac.kr/~jchun/phydit/). 16S rRNA gene sequence analysis revealed that strain KMM 6143T was a member of the family ‘Flexibacteraceae’ and formed a distinct subline within the genus Cyclobacterium (Fig. 1).

The level of 16S rRNA gene sequence similarity between strains KMM 6143T and Cyclobacterium marinum LMG 13164T was 96.6 % (47 nucleotide differences). Phylogenetic trees were inferred using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the model of Jukes & Cantor (1969), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1993) algorithms. Bootstrap analysis was performed with 1000 resampled datasets, using SEQBOOT and CONSENSE programs of the PHYLIP package.
Genomic DNA was isolated by the method of Marmur (1961) and the G+C content of the DNA was determined by the thermal denaturation method (Marmur & Doty, 1962). The DNA G+C content of KMM 6143T was 41.3 mol%.

To detect whole-cell fatty acids, strain KMM 6143T and C. marinum LMG 13164T (= KCTC 2917T) were grown at 25 °C for 48 h on marine agar 2216 (Difco). The analysis of fatty acid methyl esters was carried out according to the standard protocol of the Microbial Identification System (Microbial ID Inc.). The predominant cellular fatty acids of KMM 6143T and C. marinum LMG 13164T were straight-chain unsaturated, branched-chain unsaturated and saturated, namely 15:0 iso (22.2 and 23.2 %, respectively), 15:0 anteiso (9.2 and 6.4 %), 15:1 iso (8.4 and 9.7 %), 17:1 iso ω9c (4.3 and 6.3 %), 17:0 iso 3-OH (10.7 and 12.7 %) and summed feature 3 (24.3 and 23.4 %), comprising 16:1o7c and/or 15:0 iso 2-OH.

Phenotypic features of the strain studied were tested as described previously (Nedashkovskaya et al., 2003, 2004). Physiological and biochemical properties of KMM 6143T and C. marinum LMG 13164T were also determined using API 20E, API 20NE, API ZYM and API 50 CH (bioMérieux) and the Biolog GN2 microplate system (Biolog Inc.) according to the manufacturers’ instructions. Gliding motility was determined as described by Bowman (2000). For determination of cell morphology, the samples were fixed in 2.5 % paraformaldehyde/glutaraldehyde mixture buffered with 0.1 M phosphate (pH 7.2) for 2 h and then fixed in 1 % osmium tetroxide in the same buffer for 1 h, dehydrated in graded ethanol and substituted with isoamyl acetate. Samples then underwent critical-point drying in CO2. Finally the samples were sputtered with gold in a sputter coater (SC502; Polaron) and observed using a scanning electron microscope (SEM 515; Philips). Cells of KMM 6143T were ring-like and horsehoe-shaped with an outer diameter ranging from 0.9 to 1.2 μm and a width of 0.3–0.4 μm (micrograph available as a supplementary figure in IJSEM Online).

The physiological, morphological and biochemical characteristics of the strains studied are given in the species description and Table 1. It should be noted that Raj & Maloy (1990) described C. marinum LMG 13164T as a halophilic bacterium; however, the results of our study indicated that this strain can grow without NaCl ions or sea water. Also, the G+C content of the DNA for the type strain of C. marinum was 41.9 mol% (thermal denaturation method), according to data obtained in this work, in contrast to 33.7 mol% reported by Raj & Maloy (1990) for this bacterium. Moreover, we found that C. marinum LMG 13164T demonstrated good growth at 42 °C. These results are different from those obtained by Raj & Maloy (1990). The similarities in the phenotypic characteristics, cellular fatty acid composition and G+C content of the DNA support the inclusion of strain KMM 6143T in the genus Cyclobacterium. However, strain KMM 6143T differed from C. marinum by an inability to grow at 42 °C, to hydrolyse Tween 40 and to utilize glucose 6-phosphate or mannitol. The presence of lipase (C14) and trypsin activities, acid production from L-arabinose, DL-xylene, starch and N-acetylgalcosamine, and the ability to utilize D-gluconate also distinguish strain KMM 6143T from C. marinum. Moreover, susceptibility to benzylpenicillin and kanamycin and resistance to streptomycin and tetracycline clearly separate strain KMM 6143T from C. marinum (Table 1).

The above-mentioned phenotypic features (Table 1) in association with molecular differences allow the differentiation of strain KMM 6143T from Cyclobacterium marinum. Thus, we propose that strain KMM 6143T should be placed in the genus Cyclobacterium, as the type strain of Cyclobacterium amurskyense sp. nov.

**Description of Cyclobacterium amurskyense sp. nov.**

*Cyclobacterium amurskyense* (a.mur.sky.en’se. N.L. neut. adj. amurskyense pertaining to Amursky Bay, in which the type strain was isolated).

Cells are Gram-negative, strictly aerobic with respiratory metabolism, chemo-organotrophic, non-motile, asporogenic, ring-like and horsehoe-shaped with an outer diameter...
of 0.9–1.2 μm and cell width of 0.3–0.4 μm. Oxidase-, catalase-, β-galactosidase- and alkaline phosphatase-positive. Colonies are circular, low-convex, shiny with entire edges, 1–3 mm in diameter on marine agar 2216. Produces non-diffusible pink pigments. Grows at 0–10 % NaCl. Flexirubin pigments are absent. Growth occurs at 4–40 °C. Degradates aesculin. Does not hydrolyse agar, casein, starch, cellulose (CM-cellulose and filter paper), chitin, urea, and Tweens 20 and 80, adonitol, D-arabitol, glycerol, myo-inositol, mannitol, malate, fumarate and citrate; utilization of glycerol, i-erythritol, adonitol, dulcitol, myo-inositol, D-sorbitol, glycogen, xylitol, D-arabitol, L-arabitol, gentiobiose, 2-ketogluconate, 5-ketogluconate, caprate, adipate, malate, citrate and phenylacetate; and susceptibility to gentamicin, neomycin and polymyxin B.

### Table 1. Phenotypic characteristics of *Cyclobacterium amurskyense* sp. nov. KMM 6143<sup>T</sup>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>C. amurskyense</em> KMM 6143&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>C. marinum</em> LMG 13164&lt;sup&gt;T&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipase (C14), trypsin activities</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Degradation of Tween 40</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 42 °C</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid from L-arabinose, DL-xylene, starch, N-acetyl-D-glucosamine</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose 6-phosphate, mannitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Gluconate</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Susceptibility to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzylpenicillin, kanamycin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Streptomycin, tetracycline</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content DNA (mol%)</td>
<td>41.3</td>
<td>41.9</td>
</tr>
</tbody>
</table>

Both strains were positive for following: oxidase, catalase, β-galactosidase, alkaline and acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, z- and β-galactosidase, z- and β-glucosidase, N-acetyl-β-glucosaminidase and z-mannosidase activities; growth at 0–10 % NaCl and at 4–40 °C; hydrolysis of aesculin; acid formation from D-cellobiose, L-fucose, D-galactose, D-lactose, L-raffinose, D-melibiose, L-rhamnose, D-tyrhalose, D-maltose and D-sucrose; susceptibility to ampicillin, carbenicillin, lincomycin and oleandomycin; and utilization of glucose, D-mannose, N-acetyl-D-glucosamine, D-fructose, methyl z-D-mannoside, methyl z-D-glucoside, myo-inositol, arbutin, salicin, inulin, melezitose, gentiobiose, D-turanose, lyxose, tagatose, D-fucose, L-fucose, ribose, sorbose, D-xylene and methyl β-D-xylosate. Both strains were negative for following: z-chymotrypsin, β-glucuronidase, z-fucosidase, arginine dihydrolase, lysozyme and ornithine decarboxylase activities; gliding motility; Na<sup>+</sup> requirement for growth; requirement for organic growth factors; nitrate reduction; flexirubin pigments; H<sub>2</sub>S, indole and acetoin production; degradation of agar, casein, gelatin, DNA, starch, cellulose (CM-cellulose, filter paper), chitin, urea, and Tweens 20 and 80; acid production from D-glucose, L-sorbose, adonitol, dulcitol, glycerol, myo-inositol, mannitol, malate, fumarate and citrate; utilization of glycerol, i-erythritol, adonitol, dulcitol, myo-inositol, D-sorbitol, glycogen, xylitol, D-arabitol, L-arabitol, gentiobiose, 2-ketogluconate, 5-ketogluconate, caprate, adipate, malate, citrate and phenylacetate; and susceptibility to gentamicin, neomycin and polymyxin B.
straight-chain unsaturated, branched-chain unsaturated and saturated fatty acids, namely 15:0 iso (22.2 %), 15:0 anteiso (9.2 %), 15:1 iso (8.4 %), 17:1 iso ν9c (4.3 %), 17:0 iso 3-OH (10.7 %) and summed feature 3 (24.3 %), comprising 16:1 ν7c and/or 15:0 iso 2-OH fatty acids. The G + C content of the DNA is 41.3 mol%.

The type strain is KMM 6143T ( = KCTC 12363T = LMG 23026T), isolated from sea water, collected in Amursky Bay, Gulf of Peter the Great, East Sea (also known as the Sea of Japan).

Acknowledgements

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References


