Robiginitomaculum antarcticum gen. nov., sp. nov., a member of the family Hyphomonadaceae, from Antarctic seawater

Kiyoungh Lee,1 Hong Kum Lee,2 Tae-Hwan Choi1 and Jang-Cheon Cho1

1Division of Biology and Ocean Sciences, Inha University, Incheon 402-751, Republic of Korea
2Polar BioCenter, Korea Polar Research Institute, KOPRI, Songdo Techno Park, Incheon 406-840, Republic of Korea

A seawater bacterium, designated IMCC3195T, was isolated from the Antarctic coast. Cells of the novel strain were Gram-negative, rusty-coloured, strictly aerobic, chemoheterotrophic, non-budding and non-motile rods or vibrioids that possessed a thin prostheca. Based on 16S rRNA gene sequence comparisons, the novel strain was most closely related to the genera Hyphomonas (89.4–90.9 %), Maricaulis (90.1–90.4 %), Hirschia (89.0 %) and Oceanicaulis (87.9 %) of the family Hyphomonadaceae. Phylogenetic analyses also showed the Antarctic isolate to be only distantly related to the genera of stalked bacteria of marine origin in the family Hyphomonadaceae. The DNA G+C content of the novel strain was 60.3 mol% and the predominant cellular fatty acids were C18:1ω7c (41.9 %), C17:1ω8c (21.4 %) and C17:0 (14.3 %). The major quinone was Q-10. Several phenotypic and chemotaxonomic characteristics, including optimum temperature and salinity range for growth, cell morphology, pigmentation and fatty acid content, differentiated the novel strain from other related genera in the family Hyphomonadaceae. From the taxonomic evidence collected in this study, it is suggested that strain IMCC3195T (=KCCM 42687T =NBRC 103098T) represents a new genus and novel species in the family Hyphomonadaceae, for which the name Robiginitomaculum antarcticum gen. nov., sp. nov. is proposed.

The family Hyphomonadaceae in the order Rhodobacterales was proposed by Lee et al. (2005) based on phylogenetic analyses of 16S rRNA gene sequences. The family encompasses several members of prostheta-bearing, motile, obligately aerobic, chemoheterotrophic bacteria that have been isolated from diverse marine environments, including surface seawater, brackish water, deep-sea, warm water hydrothermal vents and dinoflagellates (Abraham et al., 1999; Moore et al., 1984; Schlesner et al., 1990; Strömpel et al., 2003). The family is currently composed of four well-defined genera: Hyphomonas (Moore et al., 1984), Maricaulis (Abraham et al., 2002), Hirschia (Schlesner et al., 1990) and Oceanicaulis (Strömpel et al., 2003). The present study focuses on the taxonomy of strain IMCC3195T, isolated from Antarctic surface seawater. Data obtained from the polyphasic approach adopted in this study indicated that strain IMCC3195T represents a novel genus and species. Therefore, we propose the inclusion of this novel isolate in a fifth genus of the family Hyphomonadaceae.

Strain IMCC3195T was isolated from a seawater sample collected from the coast of King George Island, Weaver Peninsula, West Antarctica (62°14’S 58°47’E), using a standard dilution-plating method on an oligotrophic medium, R2A agar (Difco), diluted 1:10 in aged seawater (v/v, 1/10R2A). Strain IMCC3195T, initially grown on 1/10R2A, was further purified on marine agar 2216 (MA; Difco) after culture at 20 °C for 2 weeks. After the optimum growth temperature for the novel strain had been determined, cultures were maintained routinely on MA at 20 °C for characterization studies and preserved as a glycerol suspension (10 %, v/v) at −75 °C.

The 1415 bp sequence of the 16S rRNA gene for strain IMCC3195T was obtained as described previously (Cho & Giovannoni, 2003). Phylogenetic analyses, including multiple alignment of 16S rRNA gene sequences, determination of sequence similarity and generation of phylogenetic trees, were performed with the ARB (Ludwig et al., 2004) and PAUP software packages (Swofford, 2002) as described by Cho & Giovannoni (2006). Reference sequences of more than 1300 bp were included in the phylogenetic analysis and 1171 unambiguously aligned nucleotide positions were used. Sequence comparisons in the ARB database and

Abbreviation: PHA, poly-β-hydroxyalkanoate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC3195T is EF495229.
BLASTN search results showed that strain IMCC3195T was only distantly related to members of the family Hyphomonadaceae. The novel strain showed the highest sequence similarity with coastal alphaproteobacterium 26III/A02/215 (93.9 %, GenBank accession no. AY576758, Agouge et al., 2005). The most closely related members of the family Hyphomonadaceae with validly published names were the genera Hyphomonas (89.4–90.9 %), Maricau-lis (90.1–90.4 %), Hirschia (89.0 %) and Oceanicaulis (87.9 %). No other recognized species showed greater than 91 % 16S rRNA gene sequence similarity with strain IMCC3195T. To further reveal the phylogenetic position of the strain, phylogenetic trees were generated by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. The robustness of the neighbour-joining and maximum-parsimony trees was evaluated by bootstrap analyses based on 1000 resamplings. In all the phylogenetic trees generated in this study, strain IMCC3195T did not form any robust phylogenetic clade with other genera of the order Rhodobacterales, but formed an independent monophyletic clade within the family Hyphomonadaceae (Fig. 1). The low gene sequence similarity and distinct phylogenetic relationship between the novel strain and other genera of the family Hyphomonadaceae revealed that the novel strain could not be assigned to any of the recognized genera. Consequently, strain IMCC3195T was considered to represent a new genus in the family Hyphomonadaceae.

Phenotypic and physiological characterization was carried out according to a previous study (Choo et al., 2007) and standard methods (Smibert & Krieg, 1994) using MA as the basal medium at 20 °C, unless otherwise specified. The morphology and sizes of cells and colonies were examined from cultures grown aerobically on MA for 5 days. The presence of poly-β-hydroxyalkanoate (PHA) granules was checked by epifluorescence microscopy after staining the cells with Nile blue A (Ostle & Holt, 1982). For detecting bacteriochlorophyll a and carotenoids, pigments of strain IMCC3195T were extracted with acetone/methanol (1:1, v/v) and the absorption spectra were determined using a scanning UV/visible spectrophotometer (Optizen 2120UV; Mechasis). Additionally the genetic potential for anoxygenic phototrophy was determined by PCR amplification of the photosynthetic reaction centre genes (pufLM) using the pufLf and pufMr primer set (Béja et al., 2002). Growth temperature range and optimum temperature were tested at 3–42 °C. The pH range and optimum for growth were examined on MA adjusted to pH values of 4.0–12.0 with 0.1 M HCl and 0.1 M NaOH. The NaCl concentration range and optimum level for growth were determined in NaCl-free artificial seawater medium (ASW; Choo et al., 2007), supplemented with 5.0 g peptone, 1.0 g yeast extract and various concentrations of NaCl (0–15 %, w/v). The temperature, pH and NaCl concentrations ranges for growth were monitored for 2 weeks on MA or MA adjusted to various pH and NaCl concentrations. Biochemical and carbon source utilization tests were carried out with API 20NE and API ZYM strips (bioMérieux) and in GN2 microplates (Biolog), according to the manufacturer’s instructions, and inoculated with bacterial suspensions in ASW. Ten different antimicrobial agents (listed in the species description) were tested by the.

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**Fig. 1.** Neighbour-joining 16S rRNA gene sequence phylogenetic tree showing the relationships between strain IMCC3195T and representatives of the families Hyphomonadaceae and Rhodobacteraceae. Bootstrap proportions over 50 % from both neighbour-joining (above nodes) and maximum-parsimony (below nodes) are shown. Closed circles indicate the nodes that were recovered reproducibly by all treeing methods. Open circles represent nodes that were recovered by two treeing methods. Bar, 0.01 substitutions per nucleotide position.
diffusion plate method (Jorgensen et al., 1999). The DNA G + C content was analysed by using HPLC (Mesbah et al., 1989). Cellular fatty acid methyl esters were prepared from cultures grown on MA 20 °C for 5 days and analysed according to the MIDI Microbial Identification System by the Korean Culture Center of Micro-organisms (KCCM). Respiratory quinones were analysed by the KCCM using a reverse-phase HPLC.

The morphological, physiological and biochemical characteristics of strain IMCC3195T are listed in the genus and species descriptions and in Table 1. In summary, cells of strain IMCC3195T were Gram-negative, rusty-coloured, obligately aerobic, chemoheterotrophic, non-motile, non-budding, prostheca-possessing and were rod or vibrioid-shaped (Fig. 2). The novel strain did not produce bacteriochlorophyll a or PHA granules. The absence of anoxygenic phototrophy was also supported by the PCR results indicating that pufLM genes were not amplified. The DNA G + C content of strain IMCC3195T was 60.3 mol%.

The only respiratory quinone detected was Q-10. The major fatty acids found in strain IMCC3195T, C18:1ω7c (41.9%), C17:1ω9c (21.4%), C17:0 (14.3%) and C17:1ω6c (7.7%), showed moderate differences when compared with the other genera in the family Hyphomonadaceae (Table 1). Several phenotypic characteristics, including cell morphology, pigmentation, optimum growth temperature and salinity range for growth, clearly differentiated the strain from other related genera in the family Hyphomonadaceae (Table 1).

As shown by the low 16S rRNA gene sequence similarity (<91%) with other genera, the distinct phylogenetic relationship (Fig. 1) and several differential phenotypic characteristics, strain IMCC3195T could not be assigned to any of the known genera in the family Hyphomonadaceae. These data, obtained by a polyphasic approach, demonstrate conclusively that the novel strain belongs to a new genus and species in the family Hyphomonadaceae of the order Rhodobacterales, for which the name Robiginitomaculum gen. nov., sp. nov. is proposed.

### Table 1. Differential characteristics of strain IMCC3195T and related genera of the family Hyphomonadaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td>Cell morphology:</td>
<td></td>
<td></td>
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<tr>
<td>Shape</td>
<td>Rod or vibrioid</td>
<td>Ovoid or rod</td>
<td>Ovoid or rod</td>
<td>Rod or vibrioid</td>
<td>Rod or vibrioid</td>
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<tr>
<td>Budding</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Flagella</td>
<td>–</td>
<td>+ *</td>
<td>+</td>
<td>+ #</td>
<td>+</td>
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<tr>
<td>Prostheca</td>
<td>+ d</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Pigmentation</td>
<td>Rusty orange</td>
<td>–, Grey, reddish brown</td>
<td>Yellow</td>
<td>–</td>
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<td>20–45</td>
<td>22–28</td>
<td>30–40§</td>
<td>30</td>
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<td>Growth at:</td>
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<tr>
<td>6.0 % NaCl</td>
<td>–</td>
<td>v</td>
<td></td>
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<td>ND</td>
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<tr>
<td>0.5 % NaCl</td>
<td>+</td>
<td>v ¥</td>
<td>ND</td>
<td>+ #</td>
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<td>v</td>
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<td>–</td>
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<td>Hydrolysis of gelatin</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
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<td>Major fatty acid composition (%)</td>
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<tr>
<td>C17:0</td>
<td>14</td>
<td>0–23</td>
<td>1</td>
<td>7–22</td>
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<tr>
<td>C17:1</td>
<td>29</td>
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<td>1</td>
<td>10–39</td>
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<tr>
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<td>4</td>
<td>–</td>
<td>2</td>
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<td>Major quinone</td>
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<td>Q-10/Q-11</td>
<td>Q-10</td>
<td>Q-10</td>
<td>ND</td>
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<td>DNA G + C content (mol%)</td>
<td>60.3</td>
<td>57–64</td>
<td>45.6</td>
<td>62.5–65.2</td>
<td>61.8</td>
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</table>

*Data for Hyphomonas adhaerens, Hyphomonas johnsonii and Hyphomonas rosenbergii.
†Data for M. maris. The other species are not determined.
‡Some cells have a prostheca with thin-tapered end.
§Data for M. parvimensis and M. salignorans.
||All species are positive except Hyphomonas polymorpha.
¥All species are negative except Hyphomonas polymorpha.
#Data for all species except M. maris.
Rhodobacterales. The type species of the genus is Robiginitomaculum antarcticum.

Description of Robiginitomaculum antarcticum sp. nov.

Robiginitomaculum antarcticum (ant.arc’ti.cum. L. neut. adj. antarcticum of the Antarctic environment, from where the organism was isolated).

In addition to the characteristics reported for the genus, cells are 1.3–4.5 μm long and 0.4–1.0 μm wide, with a tapered end. Colonies on MA are circular, smooth, convex, viscid, rusty-coloured and 0.3–1.0 mm in diameter. Growth occurs at 3–25 °C, optimally at 20 °C, but not above 30 °C. Growth occurs at pH 5–10 and 0.5–5.0 % NaCl, optimally at pH 7 and at 2.0–2.5 % NaCl. Oxidase-negative and catalase-positive. In API 20NE strips, positive for nitrate reduction, aesculin hydrolysis and β-galactosidase activity (substrate, p-nitrophenyl-β-D-galactopyranoside). Negative for urea hydrolysis, indole production, acid production from glucose, gelatin liquefaction and arginine dihydrolase. In the API ZYM system, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase and cystine arylamidase activities are present. Negative for esterase (C4), acid phosphatase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase, lipase (C14), trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase (substrate, 2-naphthyl-β-D-galactopyranoside), β-glucuronidase, α-galactosidase, α-glucosidase and α-fucosidase activities. In tests with GN2 microplates (Biolog), oxidizes the following carbon substrates: TWEEN 40 and TWEEN 80, D-galactose, myo-inositol, trehalose, turanose, succinic acid monomethyl ester, D-gluconic acid, D-gluconic acid, α-ketoglutaric acid, quinic acid, succinic acid, L-alanine, L-alanyl glycine, L-asparginase, L-glutamic acid, glycol L-aspartic acid, glycyld L-glutamic acid, L-ornithine, L-proline, L-proteoglutaric acid, D-serine, L-serine, glycerol and DL-α-glycerol phosphate. Does not utilize the following carbon substrates: x-cyclodextrin, dextrin, glycerogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, D-arabinose, D-arabitol, D-cellobiose, i-erythritol, D-fructose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, methyl β-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, xylitol, pyruvic acid methyl ester, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, γ-oxohydroxybutyric acid, p-hydroxy phenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, D-saccharic acid, sebacic acid, succinic acid, bromo-succinic acid, glucuronamide, L-alaninamide, D-alanine, L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-threonine, DL-carntnine, γ-amino-butyric acid, urocanic acid, inosine, uridine, thymidine, phenyl-ethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, α-
d-glucose 1-phosphate and d-glucose 6-phosphate. Susceptible to chloramphenicol (25 μg), erythromycin (15 μg), rifampicin (50 μg) and tetracycline (30 μg), but resistant to ampicillin (10 μg), gentamicin (10 μg), kanamycin (30 μg), penicillin G (10 μg), streptomycin (10 μg) and vancomycin (30 μg). The cellular fatty acids comprise C<sub>18:1ω7c</sub> (41.9 %), C<sub>12:0</sub> (21.4 %), C<sub>17:0</sub> (14.3 %), C<sub>17:1ω6c</sub> (7.7 %), C<sub>18:0</sub> (3.5 %), C<sub>16:1ω7c</sub> and/or iC<sub>15:0</sub> 2-OH (3.0 %) and C<sub>18:1ω9c</sub> (2.8 %). Traces (<1 %) of the following fatty acids are also present: C<sub>16:0</sub>, C<sub>10:0 3-OH</sub>, C<sub>11:0 3-OH</sub>, C<sub>15:1ω8c</sub>, C<sub>16:1ω9c</sub>, C<sub>15:0 3-OH</sub> and C<sub>15:1ω9c</sub>. The type strain, IMCC3195<sup>T</sup> (=KCCM 42687<sup>T</sup>=NBRC 103098<sup>T</sup>), was isolated from surface seawater of Maxwell Bay, King George Island, West Antarctica.

Acknowledgements

We are grateful to Dr Soon-Gyu Hong and Dr Il-Chan Kim for providing Antarctic seawater samples. This research was supported by the research grant (grant no. PE07050) from the Korea Polar Research Institute (KOPRI).

References


