Planktotalea arctica sp. nov., isolated from Arctic seawater
Kiwon Baek,1, 2 Ahyoung Choi,1, 3 Yung Mi Lee,4 Hong Kum Lee4 and Jang-Cheon Cho1,*

Abstract

A Gram-staining-negative, non-motile, non-pigmented, rod-shaped bacterium was isolated from an Arctic coastal seawater sample and was designated strain IMCC9565T. Analysis of the 16S rRNA gene sequence of strain IMCC9565T revealed that the closest phylogenetic neighbours of the strain were members of the genus Planktotalea. Furthermore, the strain formed a robust clade with Planktotalea frisia SH6-1T, with which it shared 97.9 % 16S rRNA gene sequence similarity. Determination of genomic relatedness based on average nucleotide identity and genome-to-genome distance showed that strain IMCC9565T was distantly related to P. frisia, meaning the Arctic strain represents a novel species. Optimum growth of strain IMCC9565T was observed at 20 °C, pH 7.0 and in the presence of 2 % (w/v) NaCl. The major respiratory isoprenoid quinone was ubiquinone-10 (Q-10) and the major polar lipids consisted of phosphatidylglycerol, phosphatidylcholine, one unidentified aminolipid and two unidentified lipids. The principal fatty acids were C18 : 1ω7c and/or C18 : 1ω6c, C18 : 1ω7c 11-methyl and C16 : 0, and the DNA G+C content was 57.1 mol%. Based on these data, Planktotalea arctica sp. nov. is proposed to accommodate the bacterial isolate and the type strain is IMCC9565T (=KACC 18009T=NBRC 110393T).

The genus Planktotalea [1] is a member of the family Rhodobacteraceae, one of the major phylogenetic assemblages found in the oceans [2]. At the time of writing, the genus includes only one species, Planktotalea frisia, which was isolated from a seawater sample collected from the North Sea during a phytoplankton bloom [1]. The genus Planktotalea is characterized by being Gram-staining-negative, non-pigmented, oxidase-positive, catalase-negative, aerobic bacteria with irregular rod-shaped cells. The DNA G+C content of P. frisia, the type species of the genus, is 53.8 mol%. P. frisia contains Q-10 as the major respiratory quinone, and phosphatidylcholine and phosphatidylglycerol as the major polar lipids. In the present study, strain IMCC9565T isolated from Arctic seawater is described as a representative of a novel species of the genus Planktotalea based on the taxonomic data obtained using polyphasic approaches.

Strain IMCC9565T was isolated as a single colony from a surface seawater sample collected off the coast of Kongsfjorden, Svalbard, in the Arctic (77° 00’ 07” N, 11° 18’ 33” E), by using a standard dilution plating method on marine agar 2216 (MA; BD Diagnostics) after 2 months of incubation at 8 °C. The optimum temperature for growth was determined and working cultures were thereafter maintained on MA or in marine broth 2216 (MB; BD Diagnostics) at 20 °C. Strain IMCC9565T was preserved in glycerol suspensions (20 % in MB, v/v) at −80 °C. The reference strain used in this study for phenotypic comparison, P. frisia DSM 23709T, was obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany) and maintained on MA at 20 °C.

For the 16S rRNA gene sequencing, genomic DNA of strain IMCC9565T was extracted using DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer’s instructions. The 16S rRNA gene was amplified and sequenced as described by Cho and Giovannoni [3]. The resulting almost complete 16S rRNA gene sequence was compared with those in the EzTaxon-e database [4]. Comparison of the 16S rRNA gene sequence showed that strain IMCC9565T was most closely related to Planktotalea frisia SH6-1T (with 97.9 % sequence similarity), followed by Litoreibacter meonggei MA1-1T (96.6 %) and Sulfitobacter marinus SW-265T (96.3 % sequence similarity). To find the phylogenetic position of strain IMCC9565T more clearly, 16S rRNA gene sequences aligned in the ARB database were transferred to MEGA 6.0 [5] where phylogenetic trees were generated based on the neighbor-joining method [6] using the algorithms

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Abbreviations: ANI, average nucleotide identity; GGDC, genome-to-genome distance calculation.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the draft whole genome sequence of strain IMCC9565T are KJ160495 and KUH00000000 respectively.

One supplementary table and two supplementary figures are available with the online Supplementary Material.
of Jukes-Cantor distance [7], maximum-parsimony [8] and maximum-likelihood [9]. The robustness of the neighbour-joining, maximum-likelihood and maximum-parsimony trees were confirmed by bootstrap analyses based on 1000 random resamplings. In all phylogenetic trees, strain IMCC9565<sup>T</sup> and <i>P. frisia</i> formed a robust clade with high bootstrap values (≥99%), indicating that this strain represented a member of the genus <i>Planktotalea</i> (Fig. 1).

Since the 16S rRNA gene sequence similarity between strains IMCC9565<sup>T</sup> and <i>P. frisia</i> SH6-1<sup>T</sup> exceeded 97%, genomic relatedness between the two strains was determined on the basis of the genome sequence information. For the comparison of genome relatedness, a draft genome sequence of strain IMCC9565<sup>T</sup> was obtained using the Illumina MiSeq sequencing platform. The detail of the draft genome is summarized in Table S1 (available in the online Supplementary Material).
level of pairwise genome-based similarity was estimated based on both the average nucleotide identity (ANI) value following the BLAST-based ANI calculation method described by Goris et al. [10] and the genome-to-genome distance calculation (GGDC) method described by Auch et al. [11]. The ANI value calculated between strains IMCC9565\(^T\) and \(P. frisia\) SH6-1\(^T\) was 79.6 %, which was below the proposed cut-off ANI values of 95–96 % for demarcating bacterial species [10, 12]. Consistently, digital DNA–DNA hybridization values estimated by GGDC ranged from 20.4–21.6 % between the two strains, indicating that strain IMCC9565\(^T\) represents a novel species of the genus \(Planktotaëa\) [13]. The presence of the photosynthetic reaction centre genes, \(puf\) and \(pufM\), were determined by PCR amplification according to Beja et al. [14] and confirmed by searching the genes from the draft genome sequence of strain IMCC9565\(^T\). In contrast to \(P. frisia\) SH6-1\(^T\), no \(puf\)LM genes were detected either from PCR or in the genome sequence of strain IMCC9565\(^T\). The genomic DNA G+C content was calculated directly from the genome sequence and was determined to be 57.1 mol % for IMCC9565\(^T\).

Phenotypic characteristics were determined for strain IMCC9565\(^T\) and the reference strain, \(P. frisia\) SH6-1\(^T\), using bacterial cultures grown under the same culture conditions. Cellular morphology and cell size were examined by phase-contrast microscopy (Nikon 80i) and transmission electron microscopy (TEM) (CM200; Philips) by staining the bacterial cells with 2.0 % uranyl acetate on a carbon-coated copper grid. The cellular motility was observed in wet mounts, using the hanging drop method. Growth under anaerobic conditions was examined for 2 weeks on MA culture plates, using the MGC anaerobic system (Mitsubishi Gas Chemical). The temperature range and optimum for growth were determined in MB at temperatures ranging from 4 to 42 °C (4, 10, 15, 20, 25, 30, 37 and 42 °C). To determine the pH range and optimum, the strains were maintained in artificial seawater medium (ASW), as described by Choo et al. [15], supplemented with 0.5 % peptone and 0.1 % yeast extract at different pH ranging from 5.0 to 10.0 (at intervals of 0.5 pH units). The buffers MES (pH 5.0–6.0), MOPS (pH 6.5–7.0), HEPES (pH 7.5–8.0), Tris (pH 8.5–9.0) and CHES (pH 9.5–10.0) were used at a final concentration of 0.05 M to maintain the pH. The requirement for and tolerance of NaCl were determined by culturing the strains in NaCl-free artificial seawater (ASW) containing 0.5 % peptone and 0.1 % yeast extract, with different concentrations of NaCl (0–5 % of NaCl at intervals of 0.5 %; 5.0–15.0 % of NaCl at intervals of 2.5 %). The increase in turbidity of each culture was monitored using a spectrophotometer (Optizen 2120UV; Mechasis Co.) every day for 10 days. Gram staining was performed using a Gram-staining kit (BioMérieux). Production of H\(_2\)S was investigated using triple sugar iron agar (BD Difco) supplemented with 2.0 % NaCl. Hydrolysis of Tweens 20, 40, 60 and 80 (each 1.0 %, v/v) was tested on MA supplemented with each component according to the method described in Smibert and Krieg [16]. Hydrolysis of casein (10 % skimmed milk, w/v) and starch (1 %, w/v) was determined based on the formation of clear zones around colonies after applying the suitable staining solutions [17]. Degradation of DNA was evaluated using DNase test agar (BD Difco). Decomposition of hypoxanthine and xanthine (each 1 %, w/v) was tested on MA supplemented with each component based on the instructions by Gordon et al. [18]. Other biochemical tests and carbon source utilization tests were performed using API 20NE, API ZYM (BioMérieux) and GN2 microplates (Biolog) according to the manufacturers’ manuals. Production of bacteriochlorophyll \(a\) (BChl\(a\)) and carotenoids was determined by spectrophotometric analysis as described by Martens et al. [19].

The phenotypic characteristics of strain IMCC9565\(^T\) are presented in the species description and in Table 1. A transmission electron micrograph of cells showing a rod-shaped morphology is also presented in Fig. S1. Analyses of differential characteristics (Table 1) showed that strain IMCC9565\(^T\) and \(P. frisia\) DSM 23709\(^T\) differed from each other in multiple characteristics including cell shape, NaCl tolerance, presence of \(puf\)LM genes and hydrolysis of macromolecules.

Fatty acid methyl esters (FAME) of strain IMCC9565\(^T\) and \(P. frisia\) DSM 23709\(^T\) were extracted from cultures grown to the late exponential phase (5 days) on MA at 20 °C. Analysis was performed according to the method described by the Sherlock Microbial Identification System version 6.1 (MIDI) using the TSBA6 database. The most abundant

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tr>
<td>Cell shape</td>
<td>Rod</td>
<td>Irregular</td>
</tr>
<tr>
<td>NaCl requirement (optimum) (% w/v)</td>
<td>1.0–5.0</td>
<td>1.0–8.0</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>Slight*</td>
</tr>
<tr>
<td>Enzyme activities (API ZYME)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Valine arylamidase, cystine arylamidase, naphthol-AS-Bl-phosphohydrolase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of carbon sources (Biolog GN2): α-Hydroxybutyric acid, α-alaminamide, α-alanyl-glycine, α-glutamic acid, glycy1-3-aspartic acid, glycy1-3-glutamic acid, α-pyroglutamic acid, inosine, uridine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80, α-fructose, α-galactose, maltose, α-mannitol, α-mannose, sucrose, succinic acid mono-methyl-ester, acetic acid, citric acid, β-hydroxybutyric acid, α-aspartic acid</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of: hypoxanthine</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tween 60, Tween 80</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Presence of (puf)LM</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (%)</td>
<td>57.1</td>
<td>53.8*</td>
</tr>
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</table>

*Data were taken from Hahnke et al. [1].
cellular fatty acid of strain IMCC9565T was summed feature 8 (C18:1ω7c and/or C18:1ω6c, Table 2) that was identical to that of P. frisia DMS 23709T and the two strains were very similar in the composition of fatty acids except for the presence or absence of C10:0 3-OH and summed feature 3 (C16:1ω7c and/or C16:1ω6c).

The respiratory isoprenoid quinones were purified by TLC according to the protocol in Minnikin et al. [20] and analysed using HPLC [21]. The respiratory quinone detected in IMCC9565T was Q-10, the major quinone generally observed in members of the family Rhodobacteraceae. Polar lipids of strain IMCC9565T were extracted from lyophilized bacterial cells and examined using two dimensional TLC, followed by detection with the reagents molybdoephosphoric acid, ninhydrin, molybdenum blue, α-naphthol, Dragendorff’s solution and Schiff’s solution [20, 22]. The polar lipids of strain IMCC9565T were determined to be phosphatidylglycerol (PG), phosphatidylcholine (PC), one unidentified aminolipid (AL) and two unidentified lipids (L), which were very similar to those of P. frisia SH6-1T [1] (Fig. S2).

In conclusion, strain IMCC9565T formed a well-supported phylogenetic clade with P. frisia SH6-1T (Fig. 1), and based on their similar chemotaxonomic characteristics, strain IMCC9565T could be assigned to the genus Planktotalea. The low genomic relatedness between strain IMCC9565T and P. frisia DMS 23709T and several differential phenotypic and genotypic characteristics (Table 1) suggest that strain IMCC9565T represents a novel species of the genus Planktotalea. Therefore, the name Planktotalea arctica sp. nov. is proposed for the type strain IMCC9565T.

**DESCRIPTION OF PLANKTOTALEA ARCTICA SP. NOV.**

Planktotalea arctica (arc’ti.ca. L. fem. adj. arctica of the Arctic, the environment from where the type strain was isolated).

**Table 2. Cellular fatty acid composition (%) of strain IMCC9565T and P. frisia DSM23709T**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>C16:0</td>
<td>5.7</td>
<td>2.0</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.2</td>
<td>3.6</td>
</tr>
<tr>
<td>C10:0 3-OH</td>
<td>–</td>
<td>1.4</td>
</tr>
<tr>
<td>C12:1 3-OH</td>
<td>4.3</td>
<td>3.7</td>
</tr>
<tr>
<td>C18:1ω7c 11-methyl</td>
<td>6.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Unknown 11.799</td>
<td>3.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Summed features*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (C18:1ω7c and/or C18:1ω6c)</td>
<td>1.4</td>
<td>–</td>
</tr>
<tr>
<td>8 (C18:1ω7c and/or C18:1ω6c)</td>
<td>76.3</td>
<td>78.7</td>
</tr>
</tbody>
</table>

*Summed features represent groups of two fatty acids that cannot be separated by the MIDI system.

Gram-staining-negative, oxidase positive and catalase negative, non-pigmented, non-motile, non-gliding and aerobic. Cells are rod-shaped (0.8–1.0×2.2–2.8μm). Colonies are circular, convex, shiny with entire margins and approximately 1–2 mm in diameter on MA plates after 5 days of incubation at 20°C. Growth occurs at 4–30°C (optimum 20°C), pH 6.0–9.0 (optimum 7.0) and in the presence of 1.0–5.0 % NaCl (optimum 2.0 %, tested in NaCl-free artificial seawater medium supplemented with 0.5 % peptone and 0.1 % yeast extract). Tween 20, Tween 40 and hypoxanthine are hydrolysed, but starch, DNA, casein, colloidal chitin, Tween 60, Tween 80 and xanthine are not hydrolysed. H2S is not produced. Positive for aesculin hydrolysis and PNPG (β-galactosidase), but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatin liquefaction (In API 20NE). With API ZYM, positive for alkaline phosphatase, esterase (C4), lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for esterase lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Positive for formic acid, α-hydroxybutyric acid, D,L-lactic acid, succinic acid, succinamic acid, L-alaninamide, L-alanine, L-alanylglutamic acid, L-glutamic acid, glycol-L-aspartic acid, glycol-L-glutamic acid, L-proline, L-prolylglutamic acid, L-serine, inosine and uridine in Biolog GN2 carbon source oxidation test. The isoprenoid quinone detected is Q-10. The major polar lipids constitute phosphatidylglycerol, phosphatidylcholine, one unidentified aminolipid and two unidentified lipids. The major cellular fatty acids are C18:1ω7c and/or C18:1ω6c, C18:1ω7c 11-methyl and C16:0.

The type strain is IMCC9565T (=KACC 18009T =NBRC 110393T), isolated from a coastal seawater sample of the Arctic. The genomic DNA G+C content of the type strain is 57.1 mol%.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


