Aqüicoccus porphyridii gen. nov., sp. nov., isolated from a small marine red alga, *Porphyridium marinum*

Tingye Feng,1 Kyung Hyun Kim,1 Sang Eun Jeong,1 Wonyong Kim2 and Che Ok Jeon1,*

Abstract
A Gram-stain-negative, non-motile and aerobic bacterial strain, designated L1 8-17T, was isolated from a marine alga, *Porphyridium marinum*, in South Korea. Cells of strain L1 8-17T were found to be oxidase- and catalase-positive cocci without flagella. Growth of strain L1 8-17T was observed at 20–40 °C (optimum, 37 °C), pH 6.0–10.0 (optimum, pH 7.0–8.0) and in the presence of 0–7 % (w/v) NaCl (optimum, 2–3 %). The isoprenoid quinone detected was only ubiquinone-10. Summed feature 8 (comprising C18:1ω7c/C18:1ω6c) and C16:0 were detected as major cellular fatty acids. The major polar lipids of strain L1 8-17T consisted of phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid, an unidentified phospholipid and an unidentified lipid. The G+C content of the genomic DNA was 59.3 mol%. Strain L1 8-17T was most closely related to *Marinomaras arenosa* CAU 1311T, *Tropicibacter naphthalenivorans* C02T and *Donghicola eburneus* SW-277T with 96.68, 96.60 and 96.60 % 16S rRNA gene sequence similarities, respectively, but the strain formed a phylogenetic lineage clearly distinct from them within the family *Rhodobacteraceae*. On the basis of phenotypic, chemotaxonomic and molecular properties, strain L1 8-17T represents a novel genus of the family *Rhodobacteraceae*, for which the name *Aqüicoccus porphyridii* gen. nov., sp. nov. is proposed. The type strain of the type species is L1 8-17T (KACC 18806T=JCM 31543T).

The family *Rhodobacteraceae* as a member of class Alphaproteobacteria was first proposed by Garrity et al. [1, 2]. Numerous novel genera belonging to the family *Rhodobacteraceae* have been isolated from various marine environmental habitats including seawater, sea tidal flats, sea ice, marine invertebrates and marine algae and they have been considered as one of the key players responsible for carbon, sulphur and nitrogen cycling in ocean [3–5]. At the time of writing, the family *Rhodobacteraceae* includes more than 160 genera and 300 species with highly diverse physiological and phenotypic properties [3]. Members of the family *Rhodobacteraceae* are Gram-stain-negative coccus or rod-shaped bacteria with ubiquinone-10 (Q-10) and C18:1ω7c/C18:1ω6c as the major respiratory quinone and cellular fatty acids [3–6]. The G+C content of genomic DNA ranges widely from 50 to 70 mol%. It has been known that bacteria living in algal spheres (near algal cells) intimately interact with algae through various metabolic functions such as nitrogen fixation, vitamin synthesis and nutrient mineralization and they also produce various useful compounds such as food additives and pharmaceutical compounds [7, 8]. Therefore, algal-associated bacteria have gained great attention to understand bacteria–algae interactions as well as to produce useful compounds [9–11]. In this study, we isolated a bacterial strain, presumably a novel genus of the family *Rhodobacteraceae*, from a red alga. Here we describe its taxonomic characteristics using a polyphasic approach.

Strain L1 8-17T was isolated from *Porphyridium marinum*, a small marine red alga, isolated from the Yellow Sea of South Korea (36° 54′ 15.9" N 126° 11′ 52.8" E). Briefly, the culture of *P. marinum*, which was cultivated as described previously [11], was serially diluted in artificial seawater (ASW; 20 g NaCl, 2.9 g MgSO4, 4.53 g MgCl2·6H2O, 0.64 g KCl and 1.75 g CaCl2·2H2O per litre), spread on marine agar 2216 (MA; BD) and incubated at 25 °C for 3 days under aerobic conditions. The 16S rRNA genes of colonies grown on MA were PCR-amplified using the universal primers F1 (5′-AGA GTT TGA TCM TGG CTC AG-3′) and R13 (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) and double-digested with restriction enzymes HaeIII and Hhal, and then representative PCR amplicons showing distinct fragment patterns were partially sequenced using the primer F1, as described previously [12]. The resulting 16S rRNA gene sequences were compared with those of validated all type
strains using the Nucleotide Similarity Search program in EzTaxon-e server (https://www.ezbiocloud.net/identify) [13] and a putative novel strain belonging to the family Rhabdobacteraceae, designated strain L1 8-17T, was selected for further phenotypic and phylogenetic analysis. Strain L1 8-17T was routinely cultured aerobically on MA at 37 °C for 3 days. Strain L1 8-17T was preserved at ~80 °C in marine broth (MB; BD) containing 15 % (v/v) glycerol. Marimonas arenosa CAU 1311T, Tropicibacter naphthalenivorans DSM 15961T and Donghicola eburneus KCTC 12735T were used as reference strains for the comparisons of phenotypic properties and the analysis of cellular fatty acids and polar lipids.

To obtain a longer sequencing information, the 16S rRNA gene of strain L1 8-17T was cloned into the pCR2.1 vector using a TOPO cloning kit (Invitrogen) according to the manufacturer’s instructions and sequenced using the M13 reverse and T7 primers from Macrogen’s TOPO cloning kit. The 16S rRNA gene sequence similarities between strain L1 8-17 and closely related type strains were calculated using the Nucleotide Similarity Search program in the EzTaxon-e server. The 16S rRNA gene sequences of strain L1 8-17T and closely related type strains were aligned using the fastMEGA6 software under the default options [15].

Pairwise sequence alignment based on the 16S rRNA gene sequences revealed that strain L1 8-17T was most closely related to M. arenosa CAU 1311T (96.68 %), T. naphthalenivorans CO2T (96.68 %) and D. eburneus SW-277T (96.60 %). A phylogenetic analysis using the NJ algorithm revealed that strain L1 8-17T formed a phylogenetic lineage distinct from members of the genera Marimonas, Tropicibacter and Donghicola (Fig. 1). Phylogenetic trees reconstructed by the ML and MP algorithms also supported that strain L1 8-17T formed a phylogenetic lineage distinct from the closely related members within the family Rhabdobacteraceae.

Growth of strain L1 8-17T was tested at 37 °C for 3 days on R2A (BD) agar, Luria–Bertani (LB) agar, nutrient agar (BD), tryptic soy agar (BD) and MA, which were adjusted with NaCl to have approximately 2 % (w/v) final NaCl concentrations. Growth of strain L1 8-17T at different temperatures (4, 10, 15, 20, 25, 30, 37, 40 and 45 °C) and pH values (4.0–11.0 at 1.0 pH unit intervals) was evaluated in MB for 3 days. MB broth media with below pH 5.0, pH 6.0–7.0, pH 8.0–9.0 and 10.0–11.0 were prepared using citrate, Na2HPO4–NaH2PO4, Tris-HCl and Na2CO3/NaHCO3 buffers, respectively [16]. After autoclaving (121 °C, 15 min), the pH values were adjusted again if necessary. Growth of strain L1 8-17T at different NaCl concentrations (0–10 % at 1 % intervals) was tested in MB prepared in the laboratory according to the BD formula. The following physiological and biochemical tests were conducted using cells grown on MA for 3 days at 37 °C. Gram staining was investigated using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Oxidase activity was evaluated by the oxidation of 1 % (w/v) tetramethyl-p-phenylenediamine (Merck), and catalase activity was tested by the production of oxygen bubbles in 3 % (v/v) aqueous H2O2 [17]. Cell morphology was investigated using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL). Anaerobic growth of strain L1 8-17T was assessed on MA and MA supplemented with sodium nitrate (10 mM), sodium nitrite (5 mM), dimethyl sulfoxide (10 mM) or disodium fumarate (10 mM) under the anaerobic (with 4–10 % CO2) condition using the GasPak Plus system (BBL) at 37 °C for 21 days. Bacteriochlorophylls were extracted from lyophilized cells by the addition of methanol for 60 min and the methanol extract was analysed using a UV–visible spectrophotometer (BioTek). The following properties of strain L1 8-17T and three reference strains were investigated under the same conditions in parallel. Hydrolysis of casein, starch, aesculin, tyrosine, Tween 20 and Tween 80 was tested on MA according to the methods described previously [17, 18]. Nitrate reduction was assessed in MB according to the method described previously [18]. Additional enzymatic activities, biochemical features and oxidation of carbon sources were evaluated using the API ZYM (bioMérieux), API 20NE (bioMérieux) and GN2 MicroPlate (Biolog) testing systems, respectively. The tests were performed according to the instructions of the manufacturers, except that cells resuspended in ASW were used as the inocula and the test strains were incubated at their optimal growth temperatures.

Growth of strain L1 8-17T grew well on MA, and grew slowly on R2A agar and NA containing 2 % NaCl, but did not grow on tryptic soy agar and LB agar containing 2 % NaCl. Cells of strain L1 8-17T were Gram-stain-negative and non-motile cocci without flagella (0.6–0.8 µm in diameter) (Fig. S2). Anaerobic growth was not observed under all tested electron acceptor conditions. In the Biolog GN2 MicroPlate, strain L1 8-17T oxidized α-ketoglutaric acid, succinamic acid, L-alaninamide, uridine, L-histidine, L-leucine, α-cyclohexanecarboxylic acid, L-ornithine, thymidine, α-ketovaleric acid, L-phenylalanine, phenylethylamine, L-glutamic acid, L-threonine, D,L-carnitine, L-pyroglutamic acid, γ-aminobutyric acid, L-asparagine, L-alanine, 2-aminoethanol, L-hydroxybutyric acid, glycyl-L-aspartic acid, succinic acid, D,L-lactic acid, L-serine, glycyl-L-glutamic acid, α-hydroxybutyric acid, L-aspartic acid, succinic acid, monomethyl ester and α-D-glucose-1-phosphate, but did not oxidize other carbon compounds in Biolog GN2 MicroPlate (Table S1). The phenotypic characteristics of strain L1 8-17T are presented in Table 1 and in the genus description. Many properties such as morphology, motility, catalase activity, nitrate reduction and tyrosine hydrolysis allowed the differentiation of strain L1 8-17T from other closely related genera (Table 1).
The isoprenoid quinones of strain L1 8-17\textsuperscript{T} were extracted according to the method of Minnikin et al. [19] and analysed using a model LC-20A high-performance liquid chromatography system (Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250 \( \times \) 4.6 mm, Kromasil; Akzo Nobel), as described by Komagata and Suzuki [20]. The DNA G+C content of strain L1 8-17\textsuperscript{T} was determined by a fluorometric method [21] using SYBR Green I and a real-time PCR thermocycler (Bio-Rad). Strain L1 8-17\textsuperscript{T} and three reference strains were cultivated in MB at their respective optimal temperatures and their microbial cells were harvested at the same growth stage (exponential phase, OD\textsubscript{600}=0.6–0.8) for the cellular fatty acid analysis. The cellular fatty acids of the microbial cells were saponified, methylated and extracted using the standard protocol of MIDI. Fatty acid methyl esters were prepared and separated according to the standard protocol described in the Microbial Identification System (Microbial ID), and identified by MIDI version 6.0 and the RTSBA6 database. The polar lipids of strain L1 8-17\textsuperscript{T}, \textit{T. naphthalenivorans} DSM 15961\textsuperscript{T} and \textit{D. eburneus} KCTC 12735\textsuperscript{T} were analysed by thin-layer chromatography using cells harvested during the exponential growth phase, according to the procedure described by Minnikin et al. [22]. The following spraying reagents were used to detect different polar lipids: 10\% ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids) and Lester reagent (for phospholipids).

The only respiratory quinone detected from strain L1 8-17\textsuperscript{T} was Q-10, which was in line with all other members of the family \textit{Rhodobacteraceae} [3–6]. The genomic DNA G+C content of strain L1 8-17\textsuperscript{T} was approximately 59.3 mol \%, which was in the range of those of members of the family \textit{Rhodobacteraceae} (Table 1) [3–6]. The major cellular fatty acids (>4.0\% of the total fatty acids) of strain L1 8-17\textsuperscript{T} were summed feature 8 (comprising C\textsubscript{18:1}\textit{w}7c/C\textsubscript{18:1}\textit{w}6c, 86.3\%) and C\textsubscript{16:0} (4.7\%), which were similar to those in the reference taxa of the family \textit{Rhodobacteraceae} (Table 2). Phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), an unidentified aminolipid, an unidentified phospholipid and an unidentified lipid were detected from strain L1 8-17\textsuperscript{T} as the major polar lipids.
The polar lipid profile of strain L1 8-17T was clearly different from those of reference strains (Table 1). For example, PC, PG and PE were detected from strain L1 8-17T as the major polar lipids, while PE and PC were not detected from *T. naphthalenivorans* DSM 15961T and *D. eburneus* KCTC 12735T, respectively. In conclusion, the physiological and chemotaxonomic features and the phylogenetic analysis clearly support that strain L1 8-17T represents a novel genus of the family *Rhodobacteraceae*, for which the name *Aquicoccus porphyridii* gen. nov., sp. nov. is proposed.

**DESCRIPTION OF AQUICOCUS GEN. NOV.**


Cells are Gram-stain-negative, strictly aerobic and non-motile cocci without flagella. Oxidase and catalase activities are positive. Nitrate is not reduced to nitrite. The predominant quinone is Q-10. The major cellular fatty acids are summed feature 8 (comprising C18:1ω7c/C18:1ω6c) and C16:0. The major polar lipids are PC, PG and PE. The type species is *Aquicoccus porphyridii*.

**DESCRIPTION OF AQUICOCUS PORPHYRIDII SP. NOV.**

*Aquicoccus porphyridii* (por.phy.ri’di.i. N.L. gen. n. *porphyridii* of *Porphyridium*, referring to the isolation of the type strain from the alga, *Porphyridium marinum*).

In addition to the characteristics given in the genus description above, this species has the following properties. Growth
occurs at 20–40 °C (optimum, 37 °C), at pH 6.0–10.0 (optimum, pH 7.0–8.0) and in the presence of 0–7.0 % (w/v) NaCl (optimum, 2–3 %). Does not produce bacteriochlorophylls. Hydrolyses tyrosine, but not casein, Tween 20, 80, aesculin and starch. Alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine

table 2. Cellular fatty acid compositions (%) of strain L1 8-17T and closely related taxa of the family Rhodobacteraceae

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6:0</td>
<td>–</td>
<td>0.3</td>
<td>–</td>
<td>0.7</td>
</tr>
<tr>
<td>C10:0</td>
<td>0.4</td>
<td>0.6</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>C16:0</td>
<td>4.7</td>
<td>2.9</td>
<td>3.4</td>
<td>6.1</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.4</td>
<td>0.9</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.5</td>
<td>6.1</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Unsaturated:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15:0ω9c</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.8</td>
</tr>
<tr>
<td>C20:1ω7c</td>
<td>0.2</td>
<td>0.6</td>
<td>0.2</td>
<td>TR</td>
</tr>
<tr>
<td>11-methyl C18:1ω7c</td>
<td>0.3</td>
<td>1.0</td>
<td>3.1</td>
<td>2.3</td>
</tr>
<tr>
<td>anteoiso-C17:1ω9c</td>
<td>0.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>cyclo-C19:ω8c</td>
<td>0.4</td>
<td>–</td>
<td>–</td>
<td>0.6</td>
</tr>
<tr>
<td>Hydroxy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C9:0 3-OH</td>
<td>TR</td>
<td>0.4</td>
<td>–</td>
<td>0.4</td>
</tr>
<tr>
<td>C10:0 3-OH</td>
<td>0.3</td>
<td>–</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>C12:0 3-OH</td>
<td>0.4</td>
<td>–</td>
<td>2.9</td>
<td>–</td>
</tr>
<tr>
<td>C12:1 3-OH</td>
<td>1.9</td>
<td>2.4</td>
<td>TR</td>
<td>7.0</td>
</tr>
<tr>
<td>Branched:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C11:0</td>
<td>0.2</td>
<td>0.5</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>iso-C18:0</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Summed feature*:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>0.9</td>
<td>TR</td>
<td>0.9</td>
</tr>
<tr>
<td>8</td>
<td>86.5</td>
<td>82.5</td>
<td>83.9</td>
<td>76.5</td>
</tr>
</tbody>
</table>

*Summed features represent groups of two fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3, C15:1ω7c and/or C16:1ω6c; summed feature 8, C18:1ω7c and/or C18:1ω6c.

The type strain is L1 8-17T (KACC 18806T=JCM 31543T), isolated from a red alga Porphyridium marinus in South Korea. The DNA G+C content is 59.3 mol%.

Funding information
This work was supported by the Program for Collection of Domestic Biological Resources from the National Institute of Biological Resources (NIBR No. 2017-02-001) of Ministry of Environment (MOE) and the National Research Foundation (2017M3C1B5019250) of Ministry of Science and ICT, Republic of Korea.

Acknowledgements
The authors would like to thank to nomenclature reviewers for the support regarding the nomenclature of the micro-organism.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.