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

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Abstract

A Gram-stain-negative, non-motile and aerobic bacterial strain, designated L1 8-17^T, was isolated from a marine alga, *Porphyridium marinum*, in South Korea. Cells of strain L1 8-17^T were found to be oxidase- and catalase-positive cocci without flagella. Growth of strain L1 8-17^T 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 $C_{18:1}\omega7c$/$C_{18:1}\omega6c$) and $C_{16:0}$ were detected as major cellular fatty acids. The major polar lipids of strain L1 8-17^T 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 8-17^T was most closely related to *Marimonas arenosa* CAU 1311^T, *Tropicibacter naphthalenivorans* C02^T and *Donghicola eburneus* SW-277^T with 96.68, 96.68 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 8-17^T represents a novel genus of the family *Rhodobacteraceae*, for which the name *Aquicoccus porphyridii* gen. nov., sp. nov. is proposed. The type strain of the type species is L1 8-17^T (KACC 18806^T=JCM 31543^T).

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 $C_{18:1}\omega7c$/$C_{18:1}\omega6c$ 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 8-17^T 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 $MgSO_4$, 4.53 g $MgCl_2{\cdot}6H_2O$, 0.64 g KCl and 1.75 g $CaCl_2{\cdot}2H_2O$ 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 *Hae*III and *Hha*I, 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

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Abbreviations: ASW, artificial sea water; MA, marine agar 2216; MB, marine broth; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; Q-10, ubiquinone-10.
The GenBank accession number for the 16S rRNA gene sequence of strain L1 8-17^T is MF113254.
One supplementary table and three supplementary figures are available with the online version of this article.

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 *Rhodobacteraceae*, designated strain L1 8-17^T, was selected for further phenotypic and phylogenetic analysis. Strain L1 8-17^T was routinely cultured aerobically on MA at 37 °C for 3 days. Strain L1 8-17^T was preserved at −80 °C in marine broth (MB; BD) containing 15 % (v/v) glycerol. *Marimonas arenosa* CAU 1311^T, *Tropicibacter naphthalenivorans* DSM 15961^T and *Donghicola eburneus* KCTC 12735^T 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 8-17^T 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^T 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 8-17^T and closely related type strains were aligned using the fast secondary-structure aware infernal aligner available in Ribosomal Database Project (RDP) [14]. Phylogenetic trees based on the neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) algorithms with bootstrap values (1000 replications) were reconstructed using MEGA6 software under the default options [15].

Pairwise sequence alignment based on the 16S rRNA gene sequences revealed that strain L1 8-17^T was most closely related to *M. arenosa* CAU 1311^T (96.68 %), *T. naphthalenivorans* C02^T (96.68 %) and *D. eburneus* SW-277^T (96.60 %). A phylogenetic analysis using the NJ algorithm revealed that strain L1 8-17^T 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 8-17^T formed a phylogenetic lineage distinct from the closely related members within the family *Rhodobacteraceae*. (Fig. S1, available in the online version of this article). The phylogenetic analyses suggest that strain L1 8-17^T may represent a novel genus of the family *Rhodobacteraceae*.

Growth of strain L1 8-17^T 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 8-17^T 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, Na_2HPO_4–NaH_2PO_4, Tris-HCl and Na_2CO_3/$NaHCO_3$ buffers, respectively [16]. After autoclaving (121 °C, 15 min), the pH values were adjusted again if necessary. Growth of strain L1 8-17^T 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 H_2O_2 [17]. Cell morphology was investigated using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL). Anaerobic growth of strain L1 8-17^T 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 % CO_2) 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 8-17^T 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.

Strain L1 8-17^T 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 8-17^T 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 8-17^T oxidized α-ketoglutaric acid, succinamic acid, L-alaninamide, uridine, L-histidine, L-leucine, α-cyclodextrin, formic 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, β-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-L-phosphate, but did not oxidize other carbon compounds in Biolog GN2 MicroPlate (Table S1). The phenotypic characteristics of strain L1 8-17^T 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 8-17^T from other closely related genera (Table 1).

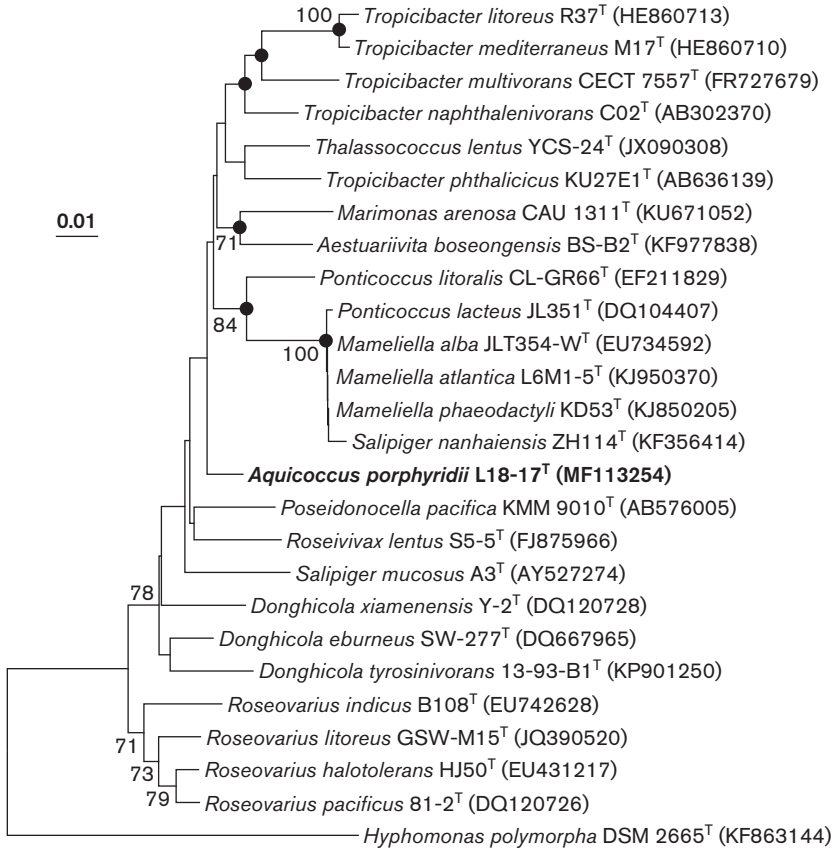

Fig. 1. A neighbour-joining tree showing the phylogenetic relationships between strain L1 8-17^T and closely related strains of the family *Rhodobacteraceae*, based on 16S rRNA gene sequences. The filled circles (●) indicate the corresponding nodes that were also recovered in the trees generated with the ML and MP algorithms. Bootstrap values are shown on nodes in percentages of 1000 replicates; when only values over 70 %. *Hyphomonas polymorpha* DSM 2665^T (KF863144) was used as an outgroup. The scale bar equals 0.01 changes per nucleotide position.

The isoprenoid quinones of strain L1 8-17^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 Í 4.6 mm, Kromasil; Akzo Nobel), as described by Komagata and Suzuki [20]. The DNA G+C content of strain L1 8-17^T was determined by a fluorometric method [21] using SYBR Green I and a real-time PCR thermocycler (Bio-Rad). Strain L1 8-17^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_{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^T, *T. naphthalenivorans* DSM 15961^T and *D. eburneus* KCTC 12735^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 Dittmer–Lester reagent (for phospholipids).

The only respiratory quinone detected from strain L1 8-17^T was Q-10, which was in line with all other members of the family *Rhodobacteraceae* [3–6]. The genomic DNA G+C content of strain L1 8-17^T was approximately 59.3 mol %, which was in the range of those of members of the family *Rhodobacteraceae* (Table 1) [3–6]. The major cellular fatty acids (>4.0 % of the total fatty acids) of strain L1 8-17^T were summed feature 8 (comprising $C_{18:1}\omega7c/C_{18:1}\omega6c$, 86.3 %) and $C_{16:0}$ (4.7 %), which were similar to those in the reference taxa of the family *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^T as the major polar lipids

Table 1. Comparison of phenotype characteristics of strain L1 8-17^T and closely related taxa of the family *Rhodobacteraceae*

Taxa: 1, strain L1 8-17^T (this study); 2, *Tropicibacter naphthalenivorans* DSM 15961^T [23]; 3, *Marimonas arenosa* CAU 1311^T [24]; 4, *Donghicola eburneus* KCTC 12735^T [25]. All strains are positive for the following characteristics: oxidase, activity* of esterase (C4), esterase lipase (C8), acid phosphatase, naphtol-AS-BI-phosphohydrolase and assimilation* of maltose. All strains are negative for the following characteristics: hydrolysis* of casein, Tween 80 and starch, activity* of crystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase and assimilation* of capric acid and trisodium citrate. +, Positive; –, negative.

Characteristic	1*	2	3	4
Morphology	Coccus	Rod	Rod	Rod/Coccus
Motility	–	+	–	–
Catalase activity	+	–	+	+
Nitrate reduction	–	+	–	+
Optimal temperature (°C)	37	37	30	37
Growth range of NaCl (%, w/v)	0–7.0	1.0–15.0	0–6.0	0.5–11.0
Hydrolysis* of:				
Tween 20	–	–	–	+
Tyrosine	+	–	–	–
Aesculin	–	+	–	+
Enzyme activity (API ZYM)* of:				
Alkaline phosphatase	+	+	–	–
Lipase (C14)	–	–	–	+
Leucine arylamidase	+	+	+	–
Valine arylamidase	+	–	–	+
β-Glucosidase	–	+	–	–
Assimilation (API 20NE)* of:				
L-Arabinose	+	–	–	+
D-Mannose	–	–	–	+
D-Mannitol	–	–	+	+
N-Acetyl-glucosamine	–	+	–	–
Potassium gluconate	–	+	+	+
Adipic acid	–	+	+	–
Malic acid	+	–	+	+
Phenylacetic acid	+	+	–	–
Major polar lipids†	PC, PG, PE, PL, AL, L	PC, PG, AL, L*	PC, PG, PE, AL, GL	PG, PE, PL, L*
DNA G+C content (mol%)	59.3	64.6	60.7	59.7

*Data from this study.

†PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanol-amine; AL, unidentified aminolipid; PL, unidentified phospholipid; L, unidentified lipid; GL, unidentified glycolipid.

(Fig. S3). The polar lipid profile of strain L1 8-17^T was clearly different from those of reference strains (Table 1). For example, PC, PG and PE were detected from strain L1 8-17^T as the major polar lipids, while PE and PC were not detected from *T. naphthalenivorans* DSM 15961^T and *D. eburneus* KCTC 12735^T, respectively. In conclusion, the physiological and chemotaxonomic features and the phylogenetic analysis clearly support that strain L1 8-17^T represents a novel genus of the family *Rhodobacteraceae*, for which the name *Aquicoccus porphyridii* gen. nov., sp. nov. is proposed.

DESCRIPTION OF *AQUICOCCUS* GEN. NOV.

Aquicoccus (A.qui.coc′cus. L. fem. n. *aqua* water; *coccus* from Gr. n. *kokkos*, a berry; N.L. masc. n. *Aquicoccus* a coccus-shaped bacterium from the water).

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 $C_{18:1}\omega7c/C_{18:1}\omega6c$) and $C_{16:0}$. The major polar lipids are PC, PG and PE. The type species is *Aquicoccus porphyridii*.

DESCRIPTION OF *AQUICOCCUS 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

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

Taxa: 1, strain L1 8-17^T; 2, *Tropicibacter naphthalenivorans* DSM 15961^T; 3, *Marimonas arenosa* CAU 1311^T; 4, *Donghicola eburneus* KCTC 12735^T. All data were obtained from this study. Data are expressed as percentages of the total fatty acids and fatty acids amounting to less than 0.2 % in all strains are not shown. Major components (>4.0 %) are highlighted in bold. TR, trace amount (<0.2 %); –, not detected.

Fatty acid	1	2	3	4
Saturated:				
$C_{9:0}$	–	0.3	–	0.7
$C_{10:0}$	0.4	0.6	0.5	0.7
$C_{16:0}$	**4.7**	2.9	3.4	**6.1**
$C_{17:0}$	0.4	0.9	1.4	0.5
$C_{18:0}$	1.5	**6.1**	1.7	1.4
Unsaturated:				
$C_{18:1}\omega 9c$	–	–	–	0.8
$C_{20:1}\omega 7c$	0.2	0.6	0.2	TR
11-methyl $C_{18:1}\omega 7c$	0.3	1.0	3.1	2.3
anteiso-$C_{17:1}\omega 9c$	0.8	–	–	–
cyclo-$C_{19:0}\omega 8c$	0.4	–	0.6	–
Hydroxy:				
$C_{8:0}$ 3-OH	TR	0.4	–	0.4
$C_{10:0}$ 3-OH	0.3	–	0.3	0.3
$C_{12:0}$ 3-OH	0.4	–	2.9	–
$C_{12:1}$ 3-OH	1.9	2.4	TR	**7.0**
Branched:				
iso-$C_{10:0}$	0.2	0.5	0.4	0.6
iso-$C_{18:0}$	0.5	–	–	–
Summed feature*:				
3	0.4	0.9	TR	0.9
8	**86.3**	**82.5**	**83.9**	**76.5**

*Summed features represent groups of two fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3, $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$; summed feature 8, $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$.

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, Tween 80, aesculin and starch. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are positive, but lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are negative. Assimilation of L-arabinose, maltose, malic acid and phenylacetic acid is positive, but assimilation of D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid and trisodium citrate is negative. The polar lipids consist of PC, PG, PE, an unidentified aminolipid, an unidentified phospholipid and an unidentified lipid.

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

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

References

1. Garrity GM, Bell JA, Lilburn T. Order III. *Rhodobacterales* ord. nov. In: Brenner DJ, Krieg NR, Staley JT and Garrity GM (editors). *Bergey's Manual of Systematic Bacteriology*, 2nd ed, vol. 2, part C. New York: Springer; 2005. pp. 161.
2. Garrity GM, Bell JA, Lilburn T. List of new names and new combinations previously effectively, but not validly, published. *Int J Syst Evol Microbiol* 2006;56:1–6.
3. Pujalte MJ, Lucena T, Ruvira MA, Arahal DR, Macian M *et al.* The family *Rhodobacteraceae*. In: *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria*; 2014. pp. 439–512.
4. Buchan A, González JM, Moran MA. Overview of the marine *Roseobacter* lineage. *Appl Environ Microbiol* 2005;71:5665–5677.
5. Simon M, Scheuner C, Meier-Kolthoff JP, Brinkhoff T, Wagner-Döbler I *et al.* Phylogenomics of *Rhodobacteraceae* reveals evolutionary adaptation to marine and non-marine habitats. *ISME J* 2017;11:1483–1499.
6. Yang LQ, Tang L, Liu L, Salam N, Li WJ *et al.* *Rubricella aquisinus* gen. nov., sp. nov., a novel member of the family *Rhodobacteraceae*. *Antonie van Leeuwenhoek* 2017;110:331–337.
7. Kazamia E, Czesnick H, Nguyen TT, Croft MT, Sherwood E *et al.* Mutualistic interactions between vitamin B_{12}-dependent algae and heterotrophic bacteria exhibit regulation. *Environ Microbiol* 2012; 14:1466–1476.
8. Kouzuma A, Watanabe K. Exploring the potential of algae/bacteria interactions. *Curr Opin Biotechnol* 2015;33:125–129.
9. Jeong SE, Jeon SH, Chun BH, Kim DW, Jeon CO. *Marinicauda algicola* sp. nov., isolated from a marine red alga *Rhodosorus marinus*. *Int J Syst Evol Microbiol* 2017;67:3423–3427.
10. Jeong SE, Kim KH, Baek K, Jeon CO. *Parasphingopyxis algicola* sp. nov., isolated from a marine red alga *Asparagopsis taxiformis* and emended description of the genus *Parasphingopyxis* Uchida *et al.* 2012. *Int J Syst Evol Microbiol* 2017;67:3877–3881.
11. Hyeon JW, Jeong SE, Baek K, Jeon CO. *Roseitalea porphyridii* gen. nov., sp. nov., isolated from a red alga, and reclassification of *Hoeflea suaedae* Chung *et al.* 2013 as *Pseudohoeflea suaedae* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2017;67:362–368.
12. Kim JM, Le NT, Chung BS, Park JH, Bae JW *et al.* Influence of soil components on the biodegradation of benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes by the newly isolated *bacterium Pseudoxanthomonas spadix* BD-a59. *Appl Environ Microbiol* 2008; 74:7313–7320.
13. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017; 67:1613–1617.
14. Nawrocki EP, Eddy SR. Query-dependent banding (QDB) for faster RNA similarity searches. *PLoS Comput Biol* 2007;3:e56.

15. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
16. **Gomori G.** Preparation of buffers for use in enzyme studies. *Methods Enzymol* 1955;1:138–146.
17. **Smibert RM, Krieg NR.** Phenotypic characterization. In: Gerhardt P (editor). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994. pp. 607–654.
18. **Lányi B.** Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 1987;19:1–67.
19. **Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al.*** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
20. **Komagata K, Suzuki K.** Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–208.
21. **Gonzalez JM, Saiz-Jimenez C.** A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* 2002;4:770–773.
22. **Minnikin DE, Patel PV, Alshamaony L, Goodfellow M.** Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* 1977;27:104–117.
23. **Harwati TU, Kasai Y, Kodama Y, Susilaningsih D, Watanabe K.** *Tropicibacter naphthalenivorans* gen. nov., sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from Semarang Port in Indonesia. *Int J Syst Evol Microbiol* 2009;59:392–396.
24. **Thongphrom C, Kim JH, Yoon JH, Bora N, Kim W.** *Marimonas arenosa* gen. nov., sp. nov., isolated from sea sand. *Int J Syst Evol Microbiol* 2017;67:121–126.
25. **Yoon JH, Kang SJ, Oh TK.** *Donghicola eburneus* gen. nov., sp. nov., isolated from seawater of the East Sea in Korea. *Int J Syst Evol Microbiol* 2007;57:73–76.