Marinicauda algicola sp. nov., isolated from a marine red alga Rhodosorus marinus

Sang Eun Jeong,1 Seung Heon Jeon,2 Byung Hee Chun,1 Dong-Woon Kim3 and Che Ok Jeon1,*

Abstract

An aerobic Gram-stain-negative prosthecate bacterium, designated RMAR8-3T, was isolated from a marine red alga Rhodosorus marinus in the Republic of Korea. Cells were dimorphic rods with a single polar prostheca (non-motile) or flagellum (motile) showing catalase- and oxidase-positive reactions. Growth of strain RMAR8-3T was observed at 15–45°C (optimum, 30°C), at pH 6.0–9.0 (optimum, pH 7.0) and in the presence of 0–10% (w/v) NaCl (optimum, 2%). Ubiquinone-10 was detected as the sole isoprenoid quinone and C18:0 summed feature 8 (comprising C18:1ω7c and/or C18:1ω6c), C17:0, C12:0 3-OH and C16:0 were identified as the major cellular fatty acids. The major polar lipids were sulfo-quinovosyldiacylglycerol, glucuronopranosyldiglyceride and monoglycosyldiglyceride. The G+C content of the genomic DNA was 66.3 mol%. Strain RMAR8-3T was most closely related to Marinicauda pacifica P-1 km-3T with a 97.6% 16S rRNA gene sequence similarity. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain RMAR8-3T formed a tight phylogenetic lineage with M. pacifica P-1 km-3T within the family Hyphomonoadaeae. On the basis of phenotypic, chemotaxonomic and molecular features, strain RMAR8-3T clearly represents a novel species of the genus Marinicauda, for which the name Marinicauda algicola sp. nov. is proposed. The type strain is RMAR8-3T (=KACC 18990T=JCM 31718T).

The genus Marinicauda, belonging to the family Hyphomonadaceae of the class Alphaproteobacteria, was first proposed by Zhang et al. [1]. At the time of writing, the genus Marinicauda comprises only one species, with the validly published name Marinicauda pacifica and which was isolated from deep seawater of the Pacific Ocean. Zhang et al. [1] described cells of the genus Marinicauda as being Gram-negative, aerobic, catalase- and oxidase-positive, dimorphic prosthecate rod bacteria with a single polar prostheca (non-motile) or flagellum (motile). They also reported that the genus Marinicauda contains ubiquinone-10 (Q-10) and sulfo-quinovosyldiacylglycerol (SQDG), glucuronopyranosyldiglyceride (GUDG) and monoglycosyldiglyceride (MGDG) as the major respiratory quinone and polar lipids, respectively. In this study, we isolated one more putative novel species strain belonging to the genus Marinicauda, designated strain RMAR8-3T, from a marine micro-red alga and characterized it further taxonomically using a polyphasic approach.

Strain RMAR8-3T was isolated from a marine red alga Rhodosorus marinus in the Republic of Korea. Briefly, R. marinus, which was isolated from the Yellow (West) Sea of the Republic of Korea (36° 54’ 15.9” N 126° 11’ 52.8” E), was cultivated in L1 medium [2] at 25°C for 4 weeks under light and dark conditions. Cells of R. marinus were homogenized using a homogenizer for 1 min and then serially diluted in artificial seawater (ASW; 20 g NaCl, 2.9 g MgSO4, 4.53 g MgCl2•6H2O, 0.64 g KCl, 1.75 g CaCl2•2H2O per litre). The aliquots of each serial dilution were spread on one-fifth-strength marine agar 2216 (MA; BD) supplemented with NaCl to a final concentration of 2% (w/v) and incubated aerobically at 25°C for 3 days. The 16S rRNA genes of colonies grown on the one-fifth-strength MA were PCR-amplified using the universal primers, F1 and R13, and double-digested with HaeIII and HhaI, as described previously [3], and the representative PCR amplicons showing distinct fragment patterns were partially sequenced using the universal primer 340F (5’-CCT ACG GGA GGC AGC AG-3’) and 907R (5’-CCG TCA TGT AGC CAC CAG-3’). The resulting 16S rRNA gene sequences were compared with those of all reported type strains using the Nucleotide Similarity Search program in the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/). From the analysis, a putative novel strain belonging to the genus Marinicauda, designated RMAR8-3T, was selected for further phenotypic and phylogenetic analyses. Strain RMAR8-3T was routinely cultured aerobically on MA at 40°C for 2 days, except where
indicated, and stored at −80 °C in marine broth (MB; BD) supplemented with 15 % (v/v) glycerol for a long-term preservation. *M. pacifica* KACC 16526T was purchased from a culture collection centre (Korean Agricultural Culture Collection, Republic of Korea) as a reference strain for the comparison of phenotypic properties and fatty acid compositions.

The 16S rRNA gene amplicon of strain RMAR8-3T that was PCR-amplified by the F1 and R13 primers was further sequenced using the universal primers 518R (5′-ATT ACC GCG GCT GCT GG-3′) and 805F (5′-GAT TAG ATA CCC TGG TAG TC-3′) at Macrogen (Republic of Korea) to obtain an almost-complete 16S rRNA gene sequence (1392 nucleotides). The 16S rRNA gene sequences of strain RMAR8-3T and closely related type strains were aligned using the Infernal fast secondary-structure aware aligner from the Ribosomal Database Project [5]. Phylogenetic relationships between strain RMAR8-3T and closely related type strains were inferred using the DNADIST and DNAPARS programs based on the neighbour-joining (NJ) algorithm with the Kimura two-parameter model and the maximum-parsimony (MP) algorithm through a heuristic search, respectively, in PHYLIP software (version 3.69, [6]) and their tree topologies were evaluated through bootstrap analyses based on 1000 resamplings. Maximum-likelihood (ML) analysis with bootstrap values was conducted under default options using the MEGA6 software [7].

Comparative analysis based on the 16S rRNA gene sequences revealed that strain RMAR8-3T was most closely related to *M. pacifica* P-1 kim-3T with a 97.6 % sequence similarity. The 16S rRNA gene sequence similarities of strain RMAR8-3T with other validly reported type strains were less than 95.9 %. Phylogenetic analysis based on the ML algorithm showed that strain RMAR8-3T formed a tight phylogenetic lineage with the type strain of *M. pacifica* with a 100 % bootstrap value within the family *Hypphonomonadaceae* (Fig. 1). Phylogenetic trees reconstructed using the NJ (Fig. S1, available with the online Supplementary Material) and MP (not shown) algorithms also supported that strain RMAR8-3T formed a tight phylogenetic lineage with the type strain of *M. pacifica*. Less than 70 % genomic DNA relatedness between two strains is the gold standard for differentiating two strains in the species level and less than 97 % 16S rRNA gene sequence similarity has been widely used as an alternative threshold value for bacterial species delineation without laborious DNA–DNA hybridization (DDH) experiments [8, 9]. However, it was recently suggested that 98.65–98.7 % of 16S rRNA gene sequence similarity can be considered as a new alternative threshold value to avoid DDH experiments for the bacterial species delineation because the 98.65–98.7 % of 16S rRNA gene sequence similarity equates to 70 % genomic DNA relatedness between two strains [9–11]. The 16S rRNA gene sequence similarities between strain RMAR8-3T and closely related type strains were much lower than the new threshold value for the bacterial species delineation, which suggests that strain RMAR8-3T can represent a novel species of the genus *Marinicauda* without performing DDH experiments.

Growth of strain RMAR8-3T was assessed at 40 °C for 2 days on MA, R2A agar (BD), Luria–Bertani (LB; MP Biomedicals) agar, nutrient agar (NA; BD), laboratory prepared TYS (0.5 % tryptone and 0.1 % yeast extract in artificial seawater) agar and tryptic soy agar (TSA; BD), which were supplemented with NaCl to final concentrations of approximately 2 %. Growth of strain RMAR8-3T was tested on MA at different temperatures (5–50 °C at 5 °C intervals) and in MB at different pH values (5.0–10.0 at 0.5 pH unit intervals) for 2 days. MBs with pH values below 8.0 and pH 8.0–10.0 were prepared using Na2HPO4-NaH2PO4 and Tris-HCl buffers, respectively, and the pH values were adjusted again if necessary after sterilization (121 °C for 15 min). Growth at different NaCl concentrations (0–15 % at 1 % intervals) was assessed in MB, which was prepared in the laboratory according to the BD formula, for 2 days. Gram-staining was tested using the Gram-stain kit (bioMérieux) according to the manufacturer’s instructions. Anaerobic growths were assessed on MA and MA supplemented with potassium nitrate (0.2 %, w/v), potassium nitrite (0.2 %, w/v) or sodium fumarate (0.08 %, w/v) after 20 days of incubation at 40 °C under the anaerobic condition (with 4–10 % CO2) using the GasPak Plus system (BBL). Cell morphology and motility of strain RMAR8-3T were investigated using transmission electron microscopy (JEM-1010, JEOL) and phase-contrast microscopy with cells grown in MB at 40 °C for 2 days. Catalase and oxidase activities of strain RMAR8-3T were tested by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution and the oxidation of 1 % (w/v) tetramethyl-p-phenylenediamine (Merck), respectively [12]. The following properties of strain RMAR8-3T and the type strain of *M. pacifica* (KACC 16526T) were investigated in parallel under the same conditions at their optimum temperatures. Hydrolysis of Tween 20, Tween 80, casein, starch, tyrosine and aesculin was checked on MA, according to methods described previously [12, 13]. Additional enzymatic activities, biochemical features and carbon compound oxidations of strain RMAR8-3T were tested using the API ZYM and API 20NE kits (bioMérieux) and the GN2 MicroPlate system (Biolog), respectively, according to the instructions of the manufacturers, except that resuspended cells in artificial seawater were used as cell inocula.

Strain RMAR8-3T grew well on MA, but did not grow on R2A agar, LB agar, NA, TYS agar containing 2 % NaCl. Cells were Gram-stain-negative rods of approximately 0.5–0.6 µm in width and 1.2–3.6 µm in length and typical *Caulobacte ria*-type dimorphic prosthecate under the transmission electron microscope (Fig. S2). Cells possessed a polar prostheca and holdfast without motility or were motile non-prosthe cate by means of a polar flagellum. Strain RMAR8-3T grew in the range of 15–45 °C, with an optimum temperature of 40 °C, which was clearly higher than that of *M. pacifica* [1] (Table 1). Anaerobic growth was not observed on MA as well as MA with potassium nitrate, potassium nitrite or
sodium fumarate after 20 days of incubation at 40 °C. Phenotypic characteristics of strain RMAR8-3 T are presented in the species description and compared with those of the closely related type strain of *M. pacifica* in Tables 1 and S1. Some of the characteristics of strain RMAR8-3 T, such as Gram-stain-negative, dimorphic growth, oxidase and catalase activities and nitrate reduction, were in agreement with those considered to be characteristic of the genus *Marinicauda*, whereas others, such as growth ranges of NaCl and temperature and hydrolysis of Tween 20, aesculin and sodium fumarate after 20 days of incubation at 40 °C. Phenotypic characteristics of strain RMAR8-3 T are presented in the species description and compared with those of the closely related type strain of *M. pacifica* in Tables 1 and S1. Some of the characteristics of strain RMAR8-3 T, such as Gram-stain-negative, dimorphic growth, oxidase and catalase activities and nitrate reduction, were in agreement with those considered to be characteristic of the genus *Marinicauda*, whereas others, such as growth ranges of NaCl and temperature and hydrolysis of Tween 20, aesculin and casein, allowed the differentiation of strain RMAR8-3 T from the type strain of *M. pacifica*.

The isoprenoid quinone of strain RMAR8-3 T was analysed by using a high-performance liquid chromatography (model LC-20A, Shimadzu) system equipped with a reversed-phase column (250 mm 4.6 mm, Kromasil, Akzo Nobel) and a diode array detector (SPD-M20A, Shimadzu) using methanol-isopropanol (2 : 1, v/v) as an eluent (1 ml min

![Fig. 1. A maximum-likelihood tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain RMAR8-3 T and closely related taxa.](image-url)

Filled circles (●) indicate that the corresponding nodes were also recovered in the trees reconstructed by the NJ and MP algorithms. Brevundimonas aveniformis EMB102 T (DQ372984) was used as an outgroup. Scale bar, 0.02 changes per nucleotide position.
acid profile of strain RMAR8-3T was similar to that of
M. pacifica, there were some differences in the respective
compositions of some fatty acid components, as shown in
Table 2. The DNA G+C content of strain RMAR8-3T was
66.3 mol%, which was similar to that of M. pacifica
(66.0 mol%) [1]. In conclusion, the phenotypic and chemo-
taxonomic features of strain RMAR8-3T and the phylo-
genetic inference support its assignment to a novel species of
the genus Marinicauda, for which the name Marinicauda
algicola sp. nov. is proposed.

**DESCRIPTION OF MARINICAUDA ALGICOLA
SP. NOV**

*Marinicauda algicola* (al.gi’co.la. L. fem. n. alga a seaweed;
L. suffix. -cola, (from L. masc. or fem. n. incola) a dweller;
N.L. fem. n. algicola an alga dweller).

Cells are Gram-stain-negative, strictly aerobic, dimorphic
prosthecatc rods; they possess a polar prostheca without
motility or are motile non-prosthecte by means of a polar
flagellum. Colonies on MA are white, round, creamy and
convex with smooth surfaces. Growth occurs at 15–45°C
(optimum, 40°C) and pH 6.0–9.0 (optimum, pH 7.0) and in

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Red algae</td>
<td>Deep seawater</td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (optimum, °C)</td>
<td>15–45 (40)</td>
<td>6–40 (30)</td>
</tr>
<tr>
<td>pH (optimum)</td>
<td>6.0–9.0 (7.0)</td>
<td>6.0–9.5 (7.0)</td>
</tr>
<tr>
<td>NaCl (optimum, %)</td>
<td>0–10 (2)</td>
<td>0.5–12 (2)</td>
</tr>
<tr>
<td>Hydrolysis of*:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein, aesculin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tween 20</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM) of*:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Glucosidase, arginine</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>dihydrolyase, urease</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Valine arylamidase, trypsin,</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-chymotrypsin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>66.3</td>
<td>66.0</td>
</tr>
</tbody>
</table>

*These analyses were conducted under the same conditions in this
study.

**Table 1. Phenotypic comparisons of strain RMAR8-3T and the
type strain of Marinicauda pacifica**

Strains: 1, strain RMAR8-3T; 2, *M. pacifica* KACC 16526T [1]. All strains
were positive for the following characteristics: dimorphic growth, fla-
gella motility, nitrate reduction*, activity* of oxidase, catalase, alkaline
phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine
arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-
BI-phosphohydrolase and assimilation* of maltose, potassium gluco-
nate, capric acid. All strains were negative for the following character-
istics: Gram-reaction, activity* of α-galactosidase, α-mannosidase,
N-acetyl-β-glucosaminidase, β-galactosidase, β-glucuronidase and β-
glucosidase, indole production*, hydrolysis* of starch, tyrosine, gelatin
and Tween 80 and assimilation* of D-glucose, L-arabinose, d-mannose,
d-mannitol, N-acetyl-glucosamine, adipic acid, malic acid, trisodium
citrate and phenylacetic acid. +, Positive; –, negative.

**Table 2. Cellular fatty acid compositions (%) of strain RMAR8-3T and
the type strain of Marinicauda pacifica**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Saturated fatty acid:</td>
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</tr>
<tr>
<td>C6:0</td>
<td>0.7</td>
<td>–</td>
</tr>
<tr>
<td>C9:0</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>C12:0</td>
<td>1.6</td>
<td>0.9</td>
</tr>
<tr>
<td>C14:0</td>
<td>1.4</td>
<td>–</td>
</tr>
<tr>
<td>C16:0</td>
<td>5.9</td>
<td>2.0</td>
</tr>
<tr>
<td>C17:0</td>
<td>14.9</td>
<td>5.5</td>
</tr>
<tr>
<td>C18:1</td>
<td>23.0</td>
<td>27.6</td>
</tr>
<tr>
<td>C19:0</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Unsaturated fatty acid:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12:0,ω6c</td>
<td>4.9</td>
<td>0.6</td>
</tr>
<tr>
<td>C12:0,ω8c</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>11-methyl C18,ω7c</td>
<td>3.2</td>
<td>11.2</td>
</tr>
<tr>
<td>C19:0,ω8c</td>
<td>1.6</td>
<td>14.7</td>
</tr>
<tr>
<td>Hydroxyl fatty acid:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C11:0,3-OH</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>C12:0,3-OH</td>
<td>10.5</td>
<td>6.9</td>
</tr>
<tr>
<td>C12:1,3-OH</td>
<td>TR</td>
<td>1.3</td>
</tr>
<tr>
<td>Summed features*:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>2.4</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>1.7</td>
<td>1.0</td>
</tr>
<tr>
<td>8</td>
<td>22.1</td>
<td>20.9</td>
</tr>
</tbody>
</table>

*Summed features represent groups of two or three fatty acids that
cannot be separated by gas-liquid chromatography with the MIDI
system. Summed feature 1, C13:0 3-OH and/or C15:1 iso H; summed
feature 3, C16:1,ω6c and/or C16:1,ω7c; summed feature 4, C17:1 iso I
and/or C17:1 anteiso B; summed feature 8, C18:1,ω7c and/or C18:1,ω6c.

Taxa: 1, strain RMAR8-3T; 2, *M. pacifica* KACC 16526T. All data were
from this study and data are expressed as percentages of the total
fatty acids. Major fatty acid components (>5.0 %) are highlighted in
bold. –, Not detected; tr, trace amount (<0.5 %).
l-alanyl glycine, l-asparagine, l-histidine, hydroxyl-l-proline, D-serine, urocanic acid, inosine, uridine, thymidine, D, L-α-glycerol phosphate, glucose-6-phosphate, dextrin, i-erythritol, D-fructose, l-fucose, D-mannitol, D-mannose, methyl β-D-glucoside, D-psicose, α-D-glucose, D-sorbitol, sucrose, D-galacturonic acid, glucuronamide, L-α alaninamide, L-glutamic acid, L-proline, L-serine, putrescine, glycerol and glucose-1-phosphate, but does not oxidize α-cyclodextrin, L-arabinose, D-arabitol, gentiobiose, melibiose, raffinose, L-rhamnose, acetic acid, formic acid, D-glactonic acid lactone, α-hydroxy butyric acid, β-hydroxy butyric acid, γ-hydroxy butyric acid, succinic acid, L-aspartic acid, glycy1-L-aspartic acid, glycy1-L-glutamic acid, L-ornithine, L-threonine, γ-amino butyric acid, glycogen, Tween 40, cellobiose, D-galactose, lactose, lactulose, turanose, D-glucosaminic acid, p-hydroxy phenylacetic acid, itaconic acid, α-keto butyric acid, α-keto glutaric acid, α-keto valeric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, succinamic acid, L-leucine, L-phenylalanine, L-pyrogulactamic acid, D,L-carnitine, phenethylamine, 2-aminoethanol and 2,3-butanediol, and does not oxidize other carbon compounds in Biolog GN2 MicroPlate. The type strain is RMAR8-3 (=KACC 18990^T=ICM 31718^T), isolated from a marine red alga Rhodosorus marinus in South Korea.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


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