Marivita cryptomonadis gen. nov., sp. nov. and Marivita litorea sp. nov., of the family Rhodobacteraceae, isolated from marine habitats

Chung Y. Hwang,¹ Gi D. Bae,¹ Wonho Yih² and Byung C. Cho¹

¹School of Earth and Environmental Sciences and Research Institute of Oceanography, Seoul National University, San 56-1, Shillim-dong, Kwanak-gu, Seoul 151-742, Republic of Korea
²Department of Oceanography, Kunsan National University, San 68, Miryong-dong, Gunsan 573-701, Republic of Korea

Two strictly aerobic, Gram-negative, rod-shaped bacteria containing photosynthesis-related genes, designated strains CL-SK44T and CL-JM1T, were isolated from a culture of the marine phytoplankton Cryptomonas sp. and coastal seawater from Korea, respectively. Phylogenetic analysis of 16S rRNA gene sequences revealed that the two strains were related to members of the genera Thalassobius (95.3–96.7 % similarity), Pelagibacula (95.3–96.0 %) and Donghicola (95.6 %) in the family Rhodobacteraceae. However, the two novel strains did not form a robust clade with any species of the Roseobacter clade, forming a distinct clade. The major polar lipids of the strains were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, an unidentified aminolipid and an unidentified lipid, profiles that were distinguishable from those of the related genera examined. Although the level of 16S rRNA gene sequence similarity between strains CL-SK44T and CL-JM1T was very high (99.1 %), DNA–DNA relatedness between the strains was 13 %, suggesting that they represent genomically distinct species. In addition, the two strains could be differentiated based on the presence of a minor polar lipid, on the hydrolysis of gelatin and the utilization of carbon sources. Based on the data from the present study, strains CL-SK44T and CL-JM1T are considered to represent separate novel species of a new genus of the family Rhodobacteraceae, for which the names Marivita cryptomonadis gen. nov., sp. nov. (type species) and Marivita litorea sp. nov. are proposed. The type strains of Marivita cryptomonadis and Marivita litorea are CL-SK44T (=KCCM 90070T=JCM 15447T) and CL-JM1T (=KCCM 90071T=JCM 15448T), respectively.

The Roseobacter clade within the family Rhodobacteraceae is a major group in the class Alphaproteobacteria (Garrity et al., 2005). At the time of writing, the Roseobacter clade comprises more than 39 genera (see List of Prokaryotic Names with Standing in Nomenclature, http://www.bacterio.cict.fr). Members of the Roseobacter clade are physiologically diverse (Buchan et al., 2005); one of their distinct characteristics is aerobic anoxygenic phototrophy. Aerobic anoxygenic phototrophs were discovered by Shiba et al. (1979). The first described aerobic anoxygenic phototrophs of the Roseobacter clade were Roseobacter litoralis and Roseobacter denitrificans, both of which contain bacteriochlorophyll a (Shiba, 1991). Several members containing bacteriochlorophyll a or genes for the photosynthetic apparatus, namely the pufL and pufM genes, which code for proteins of the photosynthetic reaction centre, have since been discovered in members of the Roseobacter clade (e.g. Dinoroseobacter shibae, Roseovarius mucosus, Staleyaguttiformis and Thalassobacter stenotrophicus; Oz et al., 2005, Biebl et al., 2005a, b, Yi & Chun, 2006). In the present study, two bacteria containing the pufL and pufM genes were isolated from marine habitats and subjected to a polyphasic taxonomic analysis.

A clonal culture of the marine phytoplankton Cryptomonas sp. CR-MAL01 was established and maintained as described in Yih et al. (2004). An aliquot (100 µl) of the culture in the exponential phase was taken and spread on a marine agar 2216 (MA; Difco) plate, which was then incubated at 20 °C for 1 week. Bacterial strain CL-SK44T was
was isolated and subsequently purified on MA at 20 °C four times.

Strain CL-JM1T was isolated from coastal seawater of the east coast of Korea; an aliquot (100 µl) of seawater was taken and spread on MA, which was then incubated at 30 °C for 1 week. Strain CL-JM1T was subsequently purified on MA at 30 °C four times. The two novel strains were maintained on MA at 30 °C as growth of strain CL-SK44T was faster at 30 than at 20 °C. The two strains were also maintained in marine broth 2216 (MB; Difco) supplemented with 30 % (v/v) glycerol at −80 °C.

For 16S rRNA gene amplification by PCR, DNA was extracted from a single colony by a boiling method (Englen & Kelley, 2000). The crude extracts served as the DNA template for PCRs, which included Taq DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified by using the AccuPrep PCR purification kit (Bioneer) and direct sequence determination of the purified 16S rRNA gene was performed with an Applied Biosystems automated sequencer (ABI 3730XL) at Macrogen, Seoul, Korea. The almost-complete 16S rRNA gene sequences of strains CL-SK44T (1376 nt) and CL-JM1T (1369 nt) were obtained and compared with available 16S rRNA gene sequences in the GenBank database by using BLASTN searches (Altschul et al., 1990). The sequences of strains CL-SK44T and CL-JM1T were aligned manually with those of species in the family Rhodobacteraceae, obtained from the GenBank and Ribosomal Database Project II (Cole et al., 2007) databases, by using known 16S rRNA gene secondary-structure information. Phylogenetic trees were obtained by use of the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of the tree topologies was assessed by bootstrap analyses based on 1000 replications for the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods. An evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of the tree topologies was assessed by bootstrap analyses based on 1000 replications for the neighbour-joining and maximum-parsimony methods. Alignment analysis was performed by using the jPHDIT program (Jeon et al., 2005) and phylogenetic analyses were carried out by using MEGA4 (Tamura et al., 2007). Levels of genomic DNA–DNA relatedness were determined by dot-blot hybridization (Kim et al., 2007b). Pre-hybridization, hybridization and detection were performed by using a DIG labelling and detection kit (Roche Molecular Biochemicals) according to the manufacturer’s instructions. The experiment was repeated on different days.

Morphological and physiological tests were performed as follows. Gram-staining was conducted as described by Smibert & Krieg (1994). Motility of the cells was observed via the hanging-drop method (Suzuki et al., 2001). Cell morphology and presence of flagellum were assessed by using transmission electron microscopy (EX2; JEOL). Anaerobic growth was checked on MA and ZOF medium (Lemos et al., 1985) supplemented with agar (1.5 %) by using the GasPak anaerobic system (BBL) at 30 °C for 15 days. Poly-β-hydroxybutyrate granules were observed by epifluorescence microscopy (BX60; Olympus) after Nile blue A staining (Ostle & Holt, 1982).

Bacteriochlorophyll a production was determined in 90 % acetone extracts by using an Ultraspec 2000 spectrophotometer (Pharmacia Biotech) for cells that had been grown in MB or a defined medium (Biebl et al., 2005b) either in the light (~10 µE m−2 s−1) or in the dark for 7 days at 30 °C. Attempts to induce bacteriochlorophyll a expression were further performed for cells that had been grown in a different defined medium (Yoon et al., 2004) either in the light (~40 µE m−2 s−1), in the dark or under a light (~40 µE m−2 s−1; 12 h)/dark (12 h) cycle for 14–21 days at 30 °C. The presence of photosynthetic reaction centre genes, pufL and pufM, was determined by using PCR amplification with specific primers (Allgaier et al., 2003) for strains CL-SK44T and CL-JM1T. Porphyrobacter donghaensis SW-132T (−KCTC 12229T; Yoon et al., 2004) served as a positive control. Furthermore, the presence of bch genes (i.e. bchl and bchX) coding for enzymes in the bacteriochlorophyll biosynthetic pathway was determined by using PCR amplification with specific primers (Oz et al., 2005) for the above strains. The PCR products for the pufL and pufM genes (~1500 nt) and the bchl and bchX genes (~350 nt) were cloned by using the pGEM Easy TA vector (Promega) and were sequenced to clarify identities.

The temperature range for growth was examined on the basis of colony formation on MA incubated at temperatures ranging from 5 to 45 °C, by using increments of 5 °C, and at 33 °C. The pH range (pH 5–11, increments of 1 pH unit) for growth was determined by assessing changes in OD600 over the incubation period (up to 9 days) in MB at 30 °C. The final pH was adjusted by using 1 M NaOH and 1 M HCl solutions. The tolerances of strains CL-SK44T and CL-JM1T to sea salts (Sigma) or NaCl were determined by assessing changes in OD600 in synthetic ZoBell broth (per litre distilled water: 2 g Bacto peptone, 1 g yeast extract and 0.1 g ferric citrate; Yi & Chun, 2004) with concentrations of 0–10 % (increments of 1 %) and 15 % (w/v) sea salts or NaCl at 30 °C.

Oxidase and catalase tests were performed according to the protocols described by Smibert & Krieg (1994). Amylase and nitrate reductase activities and degradation of Tween 40 and 80 were determined according to Hansen & Sørheim (1991). Other enzyme activities were assayed by using API ZYM and API 20NE kits (bioMérieux) according to the manufacturer’s instructions, except that the cell suspension was prepared by using artificial seawater (per litre distilled water: 24 g NaCl, 10.9 g MgCl2·6H2O, 4 g Na2SO4·10H2O, 1.5 g CaCl2·2H2O, 0.7 g KCl, 0.2 g NaHCO3, 0.1 g KBr, 0.027 g H3BO3, 0.03 g SrCl2·6H2O and 0.003 g NaF; Lyman & Fleming, 1940). Carbon utilization was tested by using the basal broth medium supplemented with yeast extract (per litre distilled water: 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl2·6H2O, 5.94 g MgSO4·7H2O, 1.3 g
The level of 16S rRNA gene sequence similarity between DNA was used as a standard. Described by Mesbah (1989), after DNA extraction based on 16S rRNA gene sequences showed that strains CL-SK44T and CL-JM1T belonged to the Rhodobacteraceae (Fig. 1). In spite of a high level of sequence similarity between the novel strains and members of their phylogenetically closest genus Thalassobius, the novel strains did not form a robust clade with the genus Thalassobius or with any other recognized species in the family Rhodobacteraceae, but instead formed a distinct clade in the neighbour-joining and maximum-parsimony phylogenetic trees (Fig. 1 and Supplementary Fig. S1 in IJSEM Online). The level of DNA–DNA relatedness between strains CL-SK44T and CL-JM1T was on average 13 %, which was much lower than the level accepted as delineating separate species (Vandamme et al., 1996). Based on these results, the novel strains are considered to represent two separate species in a new genus within the family Rhodobacteraceae.

Cells of strains CL-SK44T and CL-JM1T were Gram-negative, motile rods approximately 0.4–1.2 × 1.9–3.5 μm and 0.3–0.9 × 1.0–3.5 μm in size, respectively. The two strains were strictly aerobic and contained poly-β-hydroxybutyrate granules (Table 1). The pufL and pufM genes and the bchL and bchX genes were detected in both strains CL-SK44T and CL-JM1T (data not shown). However, bacteriochlorophyll a was not detected in either strain. It is not uncommon that strains containing the pufL and pufM genes do not produce bacteriochlorophyll a (Allgäuer et al., 2003; Kim et al., 2007a), probably as a result of inappropriate culture conditions employed (Biebl et al., 2006). Other phenotypic characteristics of the two novel strains are given in the genus and species descriptions below and in Table 1. Most biochemical characteristics tested were similar between strains CL-SK44T and CL-JM1T except gelatin hydrolysis (Table 1). However, carbon source utilization patterns were significantly (six of 19 tested sources) different between the two novel strains.

The polar lipid profile of strain CL-SK44T comprised phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid, diphosphatidylglycerol and an unidentified lipid (see Supplementary Fig. S2 in IJSEM Online). A similar polar lipid profile was detected for strain CL-JM1T except for the presence of an additional unidentified lipid (Supplementary Fig. S2). The fatty acid profiles were generally similar between the two strains (Supplementary Table S1); the predominant fatty acids were C18:1ω7c (73.5–76.1 % of the total) and 11-methyl C18:1ω7c (9.0–11.4 %). The two strains had ubiquinone 10 as a major isoprenoid quinone. The DNA G+C contents of strains CL-SK44T and CL-JM1T were 58.6 and 61.0 mol%, respectively (Table 1).

In addition to the phylogenetic divergence of strains CL-SK44T and CL-JM1T from members of related genera in the family Rhodobacteraceae, several phenotypic and chemotaxonomic characteristics could be used to differentiate these novel strains from other related genera. Strains CL-SK44T and CL-JM1T could be differentiated from members of their phylogenetically closest genus Thalassobius by an inability to grow at 37 °C and utilization of certain carbon sources (i.e. acetate, l-arginine, citrate and succinate; Table 1). Several phenotypic characteristics (e.g. the presence of motility, inability to grow at 37 °C, inability to reduce nitrate and utilization of certain carbon sources) could be used to differentiate strains CL-SK44T and CL-JM1T from members of the genera Donghicola, Oceanicola and Pelagibaca (Table 1). The presence of an unidentified lipid and the absence of an unidentified phospholipid distinguished strains CL-SK44T and CL-JM1T from members of the genus Sulfitobacter (Table 1). Several phenotypic characteristics (e.g. the presence of motility, inability to grow at 37 °C and utilization of certain carbon sources) and chemotaxonomic characteristics (e.g. polar lipids or DNA G+C contents) differentiated strains CL-SK44T and CL-JM1T from members of the genera Marinovum, Oceanubulbus and Pseudoruegeria (Table 1). Based on data collected using a polyphasic approach, strains CL-SK44T and CL-JM1T are considered to represent separate novel species of a new genus, for which the names Marivita cryptomonadis gen. nov., sp. nov. and Marivita litorea sp. nov. are proposed, respectively.
Description of Marivita gen. nov.

Marivita (Ma.ri.vi’ta. L. neut. n. mare the sea; L. fem. n. vita life; N.L. fem. n. Marivita sea life).

Cells are Gram-negative rods. Motile by means of a polar flagellum. Growth is strictly aerobic. Oxidase- and catalase-positive. Photosynthesis-related genes are present. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, an unidentified aminolipid and an unidentified lipid. The predominant cellular fatty acids are C₁₈:₁ω₇c and 11-methyl C₁₈:₁ω₇c. The isoprenoid quinone is ubiquinone 10. The G+C content of the DNA is 58.6–61.0 mol%. Phylogenetically, the genus is a member of the family Rhodobacteraceae. The type species is Marivita cryptomonadis.

Description of Marivita cryptomonadis sp. nov.

Marivita cryptomonadis (cryp.to.mo.na’dis. N.L. gen. n. cryptomonadis of the generic name of the Cryptomonas sp. from which the type strain was isolated).

Displays the following properties in addition to those given in the genus description. After 5 days on MA plates at 30 °C, colonies are creamy and approximately 1 mm in diameter. Cells are approximately 0.4–1.2 μm wide and 1.9–3.5 μm long. Grows at 15–35 °C (optimum, 30 °C) and pH 6–10 (optimum, pH 7–9). Growth occurs at sea salt concentrations of 2–10 % (w/v) (optimum, 3–5 %), but no growth occurs in media containing NaCl as the only salt. Cells contain poly-beta-hydroxybutyrate granules. Amylase is not produced. Tweens 40 and 80 are hydrolysed. Nitrate is not reduced to nitrite. Positive for the

Fig. 1. Neighbour-joining tree derived from 16S rRNA gene sequences for strains CL-SK44ᵀ, CL-JM1ᵀ and related members in the family Rhodobacteraceae; Alteromonas marina SW-47ᵀ served as the outgroup. Bootstrap values (based on 1000 resamplings) are shown at branch points (only values >60 % are shown). Solid circles indicate that the corresponding nodes were also recovered in the maximum-parsimony tree. Bar, 0.02 nt substitutions per site.
Table 1. Selected differential characteristics between strains CL-SK44<sup>T</sup> and CL-JM1<sup>T</sup> and related taxa in the family Rhodobacteraceae

Taxa: 1, strain CL-SK44<sup>T</sup>; 2, strain CL-JM1<sup>T</sup>; 3, *Thalassobius gelatinovorans* (data from Rüger & Höfte, 1992; Arahal et al., 2005); 4, *Thalassobius aestuarii* (Yi & Chun, 2006); 5, *Thalassobius mediterraneus* (Arahal et al., 2005); 6, *Donghicola eburneus* (Yoon et al., 2007a); 7, *Oceanicola* (Cho & Giovannoni, 2004; Gu et al., 2007; Lin et al., 2007); 8, *Sulfitobacter* (Pukall et al., 1999; Labrenz et al., 2000; Ivanova et al., 2004; Park et al., 2007; Yoon et al., 2007b); 9, *Pelagibaca bermudensis* (Cho & Giovannoni, 2006); 10, *Marinovum algicola* (Lafay et al., 1995; Martens et al., 2006); 11, *Oceanibulbus indolifex* (Wagner-Döbler et al., 2004); 12, *Pseudoruegeria aquimaris* (Yoon et al., 2007c). +, Positive; −, negative; V, variable; W, weakly positive; NA, data not available.

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<td>Irregular rod-shaped</td>
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<td>61.0</td>
<td>59</td>
<td>61</td>
<td>57</td>
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<td>65.4</td>
<td>60</td>
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</table>

*Growth occurred at 35 °C but not at 40 °C (Yi & Chun, 2006).
†AL, an unidentified aminolipid; DPG, diphosphatidylglycerol; GL, an unidentified glycolipid; L, an unidentified lipid PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, an unidentified phospholipid.
following enzyme activities as tested with the API ZYM system: alkaline phosphatase, acid phosphatase and leucine arylamidase. Weakly positive for esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase and valine arylamidase, but negative for N-acetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase and trypsin. Positive for the following enzyme activities as tested with the API 20NE system: aesculin hydrolysis, β-galactosidase (PNPG) and gelatinase. Negative for indole production, nitrate reductase and urease. Utilizes L-arabinose, D-cellobiose, D-galactose, D-glucose, lactose, trehalose and xylose, but not acetate, L-arginine, citrate, D-fructose, glycerol, myo-inositol, D-mannitol, D-mannose, L-ornithine, L-phenylalanine, L-salicin, sorbitol, succinate or sucrose as sole carbon source.

The type strain, CL-SK44T (=KCCM 90070T=JCM 15447T), was isolated from a culture of the marine phytoplankton *Cryptomonas* sp.

**Description of Marivita litorea sp. nov.**


Displays the following properties in addition to those given in the genus description. After 5 days on MA plates at 30 °C, colonies are creamy and approximately 1 mm in diameter. Cells are approximately 0.3–0.9 μm wide and 1.0–3.5 μm long. Grows at 15–33 °C (optimum, 30 °C) and pH 6–10 (optimum, pH 7–8). Growth occurs at sea salt concentrations of 1–10 % (w/v) (optimum, 3–5 %), but no growth occurs in media containing NaCl as the only salt. Cells contain poly-β-hydroxybutyrate granules. Amylase is not produced. Tweens 40 and 80 are hydrolysed. Nitrate is not reduced to nitrite. Positive for the following enzyme activities as tested with the API ZYM system: esterase lipase (C8) and leucine arylamidase. Weakly positive for alkaline phosphatase, acid phosphatase, esterase (C4), naphthol-AS-BI-phosphohydrolase and valine arylamidase, but negative for N-acetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase and trypsin. Positive for the following enzyme activities as tested with the API 20NE system: aesculin hydrolysis and β-galactosidase (PNPG). Negative for gelatinase, indole production, nitrate reductase and urease. Acetate, L-arabinose, L-arginine, cellobiose, citrate, D-fructose, D-galactose, D-glucose, glycerol, myo-inositol, lactose, D-mannitol, D-mannose, L-ornithine, L-phenylalanine, L-salicin, sorbitol, succinate, sucrose, trehalose and xylose are not utilized as sole carbon source. In addition to the polar lipids listed in the genus description, a minor amount of an additional unidentified lipid is present.

The type strain, CL-JM1T (=KCCM 90071T=JCM 15446T), was isolated from coastal seawater of Korea.

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**References**


