**Ponticoccus litoralis** gen. nov., sp. nov., a marine bacterium in the family *Rhodobacteraceae*

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A Gram-negative, coccoid to rod-shaped, strictly aerobic bacterium, strain CL-GR66\(^T\), was isolated from coastal seawater collected off Korea. The strain grew optimally in the presence of 3–5% sea salts, at a temperature of 30 °C and at pH 7. The polar lipid profile of strain CL-GR66\(^T\) comprised phosphatidycholine, phosphatidylglycerol, phosphatidylethanolamine, two unidentified aminolipids, an unidentified glycolipid and an unidentified lipid. The major fatty acids were \(C_{18:1}\)ω7c (71.4%), 11-methyl \(C_{18:1}\)ω7c (11.3%), \(C_{16:0}\) (6.4%), \(C_{18:0}\) (4.6%) and \(C_{12:1}\) 3-OH (3.0%). Ubiquinone 10 was the major quinone. The DNA G+C content was 67.9 mol%. 16S rRNA gene sequence analysis revealed that strain CL-GR66\(^T\) belonged to the *Roseobacter* clade within the family *Rhodobacteraceae*. Strain CL-GR66\(^T\) was related most closely to the type strain of *Marinovum algicola* (95.5% 16S rRNA gene sequence similarity), and sequence similarities between strain CL-GR66\(^T\) and other type species of the *Roseobacter* clade ranged from 91.8 to 95.4%. Phylogenetic analyses of 16S rRNA gene sequences showed that strain CL-GR66\(^T\) was not associated with any known genus in the family *Rhodobacteraceae*. The distinct phylogenetic position of strain CL-GR66\(^T\), its phylogenetic distance from genera of the family *Rhodobacteraceae*, together with phenotypic data, suggest that the organism represents a novel species of a new genus, for which the name *Ponticoccus litoralis* gen. nov., sp. nov. is proposed. The type strain of *Ponticoccus litoralis* is CL-GR66\(^T\) (=KCCM 90028\(^T\) =DSM 18986\(^T\)).

The *Roseobacter* clade, which was named after the genus *Roseobacter* by Giovannoni & Rappe (2000), is classified within the family *Rhodobacteraceae*, which in turn belongs to subgroup 3 of the *Alphaproteobacteria* (Woese et al., 1984). At the time of writing, this clade contains more than 35 genera (Buchan et al., 2005). Bacterial species affiliated with the *Roseobacter* clade have been isolated from various marine environments, such as seawater, sediment, marine algae, hypersaline microbial mats and marine biofilms (Buchan et al., 2005; Choi & Cho, 2006a; Dai et al., 2006). Their physiological properties are highly diverse, including aerobic anoxygenic photosynthesis, aerobic sulfite oxidation, organic sulfur compound degradation, lignin degradation, methylo tropathy and antibiotic production (Buchan et al., 2005; Martens et al., 2006). Here we describe a bacterial strain isolated from coastal seawater that was characterized based on a polyphasic approach (Vandamme et al., 1996). On the basis of its chemotaxonomic, physiological and phylogenetic characteristics, we propose that this bacterium represents a novel species of a new genus in the family *Rhodobacteraceae*.

A coastal water sample was incubated with sand sediment in a 150 mm-diameter glass Petri dish at room temperature for approximately 15 months. Without disturbing the sediment, 100 µl surficial seawater was taken and spread on a marine agar 2216 (MA; Difco) plate, which was then incubated at 30 °C for 1 week. Strain CL-GR66\(^T\) was isolated and subsequently purified four times on MA at 30 °C. The strain was maintained both on MA at 30 °C and in marine broth 2216 (MB; Difco) supplemented with 30% (v/v) glycerol at −80 °C.

Morphological and physiological tests were performed as follows. Gram staining was performed according to Smibert & Krieg (1994). Cell motility was observed via the hanging-drop method (Suzuki et al., 2001). Cell morphology and the presence of flagella were observed by transmission electron microscopy (EX2; JEOL). Anaerobic growth was checked on MA and ZOF medium (Lemos et al., 1985) supplemented with additional agar.
(1.5 %) by using the GasPak anaerobic system (BBL) at 30 °C for 15 days. Poly-β-hydroxybutyrate (PHB) 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 with a spectrophotometer (Tulastec 2000; Pharmacia Biotech) for cells that had been grown in either the light or the dark for 7 days. The presence of photosynthetic reaction-centre genes pufL and pufM was determined by using PCR amplification with gene-specific primers (Allgaier et al., 2003) for strain CL-GR66T, with Porphyrobacter donghaensis SW-132T (= KCTC 12229T; Yoon et al., 2004) as a positive control.

The temperature range for growth was examined on the basis of colony formation on MA incubated at temperatures ranging from 5 to 40 °C, in increments of 5 °C. The pH range (3–10, in increments of 1 pH unit) for growth was determined by assessing changes in OD600 over the incubation period (up to 7 days) in MB at 30 °C. The final pH was adjusted by using 1 M NaOH and 1 M HCl. Tolerance of strain CL-GR66T to sea salts (Sigma) or NaCl was determined by assessing changes in OD600 in Zobell broth (5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate, 1 l distilled water) at sea salts or NaCl concentrations (0–10 % in increments of 1 %, and 15 %, w/v) at 30 °C.

Oxidase and catalase tests were performed according to the protocols described by Smibert & Krieg (1994). Gelatinase, amylase and nitrate reductase activities and degradation of Tween 80 were determined according to Hansen & Sørheim (1991). Other enzyme activities were assayed by using the API ZYM kit (bioMérieux) according to the manufacturer’s instructions, except that the cell suspension was prepared by using artificial seawater (24 g NaCl, 5.1 g MgCl2·6H2O, 4 g Na2SO4, 1.1 g CaCl2·2H2O, 0.7 g KCl, 0.2 g NaHCO3, 0.1 g KBr, 0.027 g H3BO3, 0.024 g SrCl2, 0.003 g NaF, 1 l distilled water; Lyman & Fleming, 1940). Carbon utilization was tested by using basal broth medium supplemented with yeast extract (23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl2·6H2O, 5.94 g MgSO4·7H2O, 1.3 g CaCl2·2H2O, 0.2 g NaNO3, 0.2 g NH4Cl, 0.05 g yeast extract, 1 l distilled water; Bruns et al., 2001) containing 0.4 % carbon source. Strain CL-GR66T was incubated for 4 weeks and carbon utilization was scored as negative when the growth rate was equal to or less than that in the negative control with no carbon source. Growth rate was measured by monitoring changes in OD600. Resistance to antibiotics was determined by the disc-diffusion plate method (Bauer et al., 1966).

Polar lipids were extracted by using the procedures described by Minnikin et al. (1984) and were identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Komagata & Suzuki, 1987). Fatty acid methyl esters in whole cells of strain CL-GR66T grown on MA at 30 °C for 4 days were analysed by GC according to the instructions of the Microbial Identification System (MIDI) at the Korean Culture Center of Microorganisms (KCCM), Seoul, Korea. The quinone system was determined according to Minnikin et al. (1984) and analysed by HPLC as described by Collins (1985). The DNA G+C content was analysed by HPLC (HP 100; Hewlett Packard) analysis of deoxyribonucleosides as described by Mesbah et al. (1989), after DNA extraction according to the method of Marmur (1961). Lambda DNA was used as a standard.

For 16S rRNA gene amplification by PCR, DNA was extracted from a single colony via a boiling method (Englen & Kelley, 2000). The crude extracts served as template DNA. Taq DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991) were used for PCR experiments. 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 (ABI3730XL) at Macrogen, Seoul, Korea. The almost-complete 16S rRNA gene sequence of strain CL-GR66T (1557 nt) was obtained and compared with available 16S rRNA gene sequences in GenBank by using BLASTN searches (Altschul et al., 1990). The sequence of strain CL-GR66T was manually aligned with those of species in the family Rhodobacteraceae, obtained from the GenBank and Ribosomal Database Project (Cole et al., 2003) databases, using known 16S rRNA secondary-structure information. Phylogenetic trees were obtained by using 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 tree topologies was assessed by bootstrap analyses based on 1000 replications for the neighbour-joining and maximum-parsimony methods. Alignment analysis was carried out by using the jhyphyid program (Jeon et al., 2005), and phylogenetic analyses were carried out by using MEGA 3 (Kumar et al., 2004).

Cells of strain CL-GR66T were Gram-negative, coccoid to rod-shaped and approximately 0.5–0.8 μm wide and 0.8–1.2 μm long (see Supplementary Fig. S1 in IJSEM Online). The cells were non-motile. After 3 days on MA at 30 °C, colonies were creamy and approximately 2 mm in diameter. Strain CL-GR66T was strictly aerobic (Table 1) and contained PHB granules. Bacteriochlorophyll a and the pufL and pufM genes were not detected. Other phenotypic characteristics of strain CL-GR66T are given in the genus and species descriptions and in Table 1.

The polar lipid profile of strain CL-GR66T comprised phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified glycolipid, two unidentified aminolipids and an unidentified lipid (Supplementary Fig. S2). The predominant fatty acid of strain CL-GR66T was C18:1ω7c (71.4 %), which is a feature of the vast majority of species within the Alphaproteobacteria (Martens et al., 2006), followed by 11-methyl C18:1ω7c (11.3 %), C16:0 (6.4 %), C18:0 (4.6 %) and C12:1 3-OH (3.0 %).
Table 1. Selected characteristics that differentiate strain CL-GR66\textsuperscript{T} from related genera in the family \textit{Rhodobacteraceae}

| Genera: 1, \textit{Ponticoccus} gen. nov. (strain CL-GR66\textsuperscript{T}); 2, \textit{Marinovum} (data from Lafay et al., 1995; Labrenz et al., 2000; Martens et al., 2006); 3, \textit{Pelagibaca} (Cho & Giovannoni, 2006); 4, \textit{Citreicella} (Sorokin et al., 2005); 5, \textit{Thalassofibius} (Arahal et al., 2005; Macián et al., 2005; Yi & Chun, 2006); 6, \textit{Sagittula} (Gonzalez et al., 1997); 7, \textit{Antarctobacter} (Labrenz et al., 1998, 2000); 8, \textit{Salipiger} (Martínez-Cañovas et al., 2004); 9, \textit{Thalassobius} (Arahal et al., 2005; Macián et al., 2005; Yi & Chun, 2006); 10, \textit{Sagittula} (Gonzalez et al., 1997); 11, \textit{Antarctobacter} (Labrenz et al., 1998, 2000); 12, \textit{Salipiger} (Martínez-Cañovas et al., 2004); 13, \textit{Citreicella} (Sorokin et al., 2005); 14, \textit{Thalassobius} (Arahal et al., 2005; Macián et al., 2005; Yi & Chun, 2006); 15, \textit{Sagittula} (Gonzalez et al., 1997); 16, \textit{Antarctobacter} (Labrenz et al., 1998, 2000); 17, \textit{Salipiger} (Martínez-Cañovas et al., 2004); 18, \textit{Roseovivax} (Suzuki et al., 1999); 19, \textit{Donghicola} (Yoon et al., 2007a); 20, \textit{Citreimonas} (Choi & Cho, 2006b); 21, \textit{Leisingera} (Schaefer et al., 2002); 22, \textit{Ruegeria} (Staley et al., 2005; Muramatsu et al., 2007); 23, \textit{Yangia} (Dai et al., 2006); 24, \textit{Shimia} (Choi & Cho, 2006a); 25, \textit{Phaeobacter} (Martens et al., 2006; Yoon et al., 2007b). +, Positive; w, weakly positive; −, negative; v, variable; ND, no data available; BChl, bacteriochlorophyll.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|
| Species (n)   | 111 | 1 | 31 | 11 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 2 | 4 | 4 | 1 | 1 | 3 |
| Cell morphology* | C–R | O–R | SR | O–SR | O–IR | R | R | R | R | C–R | OR | R–OR | R–OR | R | R–O | R | R | OR |
| Colony colour† | C | BG, ~P | C | WH | SP | C | BRY | C | P | 1 | BG | – | BG | BG | C | BG–PBG | R | FY | BG | Br, – |
| Anaerobic growth | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Growth at: 4°C | – | – | – | V | – | – | V | – | + | – | ND | – | – | – | – | – | – | – | V |
| 37°C | + | + | + | – | + | + | + | + | ND | + | + | – | + | + | + | – | – | – | V |
| Nitrate reduction | + | + | + | – | V | – | – | V | + | – | + | – | – | – | – | – | – | – | – |
| Hydrolysis of: | | | | | | | | | | | | | | | | | | | |
| Starch | + | W | ND | ND | – | – | V | – | – | – | – | + | – | – | – | – | – | – | + |
| Gelatin | + | + | + | – | V | – | – | + | – | ND | – | – | + | + | + | – | – | + | – |
| PHB | + | – | – | + | + | + | + | ND | ND | ND | – | – | ND | + | + | ND | – | – | ND |
| BChl a | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Succinate | – | – | + | + | ND | + | + | – | + | + | + | ND | + | + | + | ND | + | + | + |
| Acetate | + | + | + | + | ND | + | + | – | + | + | + | ND | + | + | + | ND | – | – | V |
| D-Glucose | + | + | + | + | ND | + | + | – | + | + | + | ND | + | + | + | ND | – | – | – |
| DNA G + C content (mol%) | 67.9 | 60–65 | 65.4 | 67.5–69.2 | 57–61 | 65.0 | 62–64 | 64.5 | 59.7–64.4 | 59.7 | 67.3 | 60.5 | 55–58.7 | 66.2–68 | 59–66 | 63.3 | 57.2 | 55.7–64.9 |

* C, Cocci; O, ovoid; R, rods; IR, irregular rods; OR, ovoid rods; SR, short rods.
† BG, Beige; BR, brown; BRY, brownish yellow; C, cream; FY, faint yellowish; I, ivory; P, pink; ~P, pinkish; PBG, pinkish beige; R, red; SP, salmon pink; WH, white; −, non-pigmented.
‡ Data from Choi & Cho (2006b).
(Supplementary Table S1). The major isoprenoid quinone was ubiquinone 10. The DNA G+C content was 67.9 mol%.

Analysis of the 16S rRNA gene sequence of strain CL-GR66T revealed a clear affiliation with the Roseobacter clade in the family Rhodobacteraceae (Fig. 1). Strain CL-GR66T was related most closely to the type strains of Marinovum algicola (95.5% 16S rRNA gene sequence similarity), Pelagibaca bermudensis (95.4%), Citreicella thiooxidans (95.3%), Oceanicola batsensis (95.1%), Thalassobius mediterraneus (95.1%) and Sagittula stellata (95.0%); levels of 16S rRNA gene sequence similarity to the type strains of other type species of the Roseobacter clade were between 91.8 and 94.9%. In spite of a rather high level of sequence similarity (>95%) between strain CL-GR66T and the type strains of the above species, strain CL-GR66T did not form a robust clade with these or any other species in the Roseobacter lineage. In the phylogenetic tree including other genera in the family Rhodobacteraceae, strain CL-GR66T was weakly related to Antarctobacter and Sagittula (Fig. 1). Again, the cluster comprising the genera Antarctobacter and Sagittula plus strain CL-GR66T was not robustly supported in the phylogenetic tree in which most species in the family Rhodobacteraceae were included (Supplementary Fig. S3). Thus, strain CL-GR66T formed a distinct lineage in all 16S rRNA gene sequence-based phylogenetic trees (Fig. 1, Supplementary Fig. S3). Given mean sequence divergence values of >4.1% (sd 1.6%; data not shown) among established genera of the Roseobacter clade, the observed sequence divergence of >4.5% between strain CL-GR66T and genera of this clade suggests that the lineage represented by strain CL-GR66T is equivalent in rank to the genera of the Roseobacter clade.

In addition, several phenotypic and chemotaxonomic characteristics can be used to differentiate strain CL-GR66T from related genera in the family Rhodobacteraceae. Strain CL-GR66T can be differentiated from members of the genus Antarctobacter based on the presence of 11-methyl C18:1ω7c (11.3%; Supplementary Table S1) and the presence of phosphatidylethanolamine and an additional aminolipid (Supplementary Fig. S2; Labrenz et al., 1998). These taxa are also distinguished by the ability to grow at 4°C, different optimal temperature for growth (i.e. 30 and 16–26°C for strain CL-GR66T and Antarctobacter...
heloithermus, respectively) and utilization of succinate and L-rhamnose (Table 1; Labrenz et al., 1998). Strain CL-GR66T is distinguishable from members of the genus Marinovum, including its closest phylogenetic relative, based on motility, the ability to reduce nitrate, PHB production and utilization of L-arabinose, myo-inositol, L-lysine, d-mannitol and D-ribose (Table 1; Labrenz et al., 1995). An additional aminolipid was uniquely detected in strain CL-GR66T, although phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified aminolipid are commonly found in members of the genus Marinovum (Supplementary Fig. S2; Martens et al., 2006). In addition, combinations of phenotypic and chemotaxonomic characteristics can be used to differentiate strain CL-GR66T from other related genera in the family Rhodobacteraceae (Table 1, Supplementary Table S1). The results of the present polyphasic study therefore indicate that strain CL-GR66T represents a novel species of a new genus, for which the name Ponticoccus litoralis gen. nov., sp. nov. is proposed.

**Description of Ponticoccus gen. nov.**

Ponticoccus (Pon.ti.co. cus. L. n. pontus the sea; N.L. masc. n. coccus berry; N.L. masc. n. Ponticoccus coccus from the sea).

Cells are Gram-negative, coccolid to rod-shaped. Non-motile. Growth is strictly aerobic. Oxidase- and catalase-positive. Bacteriochlorophyll a is not detected. Polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified glycolipid, two unidentified aminolipids and an unidentified lipid. Dominant cellular fatty acids are C₁₈:1ω7c, 11-methyl C₁₈:1ω7c, C₁₆:0, C₁₈:0 and C₁₂:1 3-OH. The isoprenoid quinone is Q-10. The DNA G+C content of the type strain of the type species is 67.9 mol%. Phylogenetically, the genus is a member of the family Rhodobacteraceae. The type species is Ponticoccus litoralis.

**Description of Ponticoccus litoralis sp. nov.**

Ponticoccus litoralis (li.to. ra’lis. L. masc. adj. litoralis of the shore).

Displays the following properties in addition to those given in the genus description. After 3 days on MA plates at 30 °C, colonies are creamy and approximately 2 mm in diameter. Cells are approximately 0.5–0.8 μm wide and 0.8–1.2 μm long. Grows at 10–37 °C (optimum at 30 °C) and pH 6–8 (optimum at pH 7). Growth occurs at sea salt concentrations of 1–15% (w/v) (optimum 3–5%), but no growth occurs in media containing NaCl as the only salt. Growth occurs in media containing NaCl as the only salt.

Weakly positive for valine arylamidase, but negative for lipase (C14), cystine arylamidase, trypsin, x-chymotrypsin, x-galactosidase, β-galactosidase, x-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, x-mannosidase, x-fucosidase and β-glucuronidase. Utilizes acetate, L-alanine, L-arabinose, L-arginine, D-glucose, myo-inositol, L-lysine, D-mannitol, peptone, L-proline, D-ribose and sucrose, but not ascorbate, DL-cysteine, dulcitol, erythritol, ethanol, glycogen, x-ketobutyric acid, lactose, L-rhamnose, oxalic acid, raffinose or succinate as sole carbon source. Cells are sensitive to (μg per disc) ampicillin (10), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), penicillin G (10), streptomycin (10), tetracycline (30) and vancomycin (30), but resistant to nalidixic acid (30) and polymyxin B (25). The type strain, CL-GR66T (=KCCM 90028T =DSM 18986T), was isolated from coastal seawater, Korea.

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