**Litorimicrobium taeanense** gen. nov., sp. nov., isolated from a sandy beach

Hyun Mi Jin, Hyo Jung Lee, Jeong Myeong Kim, Moon Su Park, Kangseok Lee and Che Ok Jeon

Department of Life Science and Research Center for Biomolecules and Biosystems, Chung-Ang University, Seoul 156-756, Republic of Korea

A Gram-reaction-negative, strictly aerobic, non-motile bacterium, designated strain G4\(T\), was isolated from a sandy beach of Taean in South Korea. Cells were ovoid rods and were catalase- and oxidase-positive. Growth of strain G4\(T\) was determined at 15–35 °C (optimum 25–30 °C) and pH 6–8 (optimum pH 6.5–7.5). Strain G4\(T\) contained Q-10 as the predominant isoprenoid quione and C\(_{18:1}\)ω7c (59.0%), C\(_{18:1}\)ω7c 11-methyl (11.9%) and C\(_{12:1}\) 3-OH (9.8%) as the major fatty acids. The major cellular polar lipids were identified as phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, an unidentified amino lipid, an unidentified phospholipid and an unidentified lipid. The DNA G+C content was 62.4 mol%. Phylogenetic and comparative analysis based on 16S rRNA gene sequences indicated that strain G4\(T\) fell within the family Rhodobacteraceae of Alphaproteobacteria and was most closely related to members of the genera Marinovum, Leisingera and Phaeobacter with 95.5–96.4% sequence similarities. On the basis of phenotypic, chemotaxonomic and molecular properties, strain G4\(T\) represents a novel species of a novel genus within the family Rhodobacteraceae, for which the name *Litorimicrobium taeanense* gen. nov., sp. nov. is proposed. The type strain is G4\(T\) (=KACC 13706\(T\) =DSM 22007\(T\)).

The so-called *Roseobacter* clade within the class Alphaproteobacteria described by Giovannoni & Rappé (2000) includes cultivated and uncultivated microorganisms found in marine environments all over the world (Buchan et al., 2005; Martens et al., 2006). Members of the *Roseobacter* clade have been isolated from various marine habitats such as a tidal flat, marine electroactive biofilms, the Arctic Ocean and the phycosphere of a dinoflagellate (Lafay et al., 1995; Yoon et al., 2007; Zhang et al., 2008; Vandecandelaere et al., 2008, 2009). Characterization of such isolates has demonstrated that they are physiologically and metabolically diverse with characteristics such as the ability to grow on methyl bromide (Schaefer et al., 2002), aerobic anoxygenic phototrophy (Shiba, 1991), aerobic sulfite oxidation (Moran et al., 2003) and aromatic monomers degradation (Buchan et al., 2000). Coastal seashores play very important roles in the restoration of ecosystems, micro-organisms and marine animals being valuable biological resources. Therefore, efforts have been made to isolate and characterize members of the bacterial community of the seashore of the Yellow Sea in South Korea (Kim et al., 2010). Here we describe the taxonomic characterization of a novel species of a novel genus belonging to the family Rhodobacteraceae, isolated from a sandy beach of the Yellow Sea in Korea.

Strain G4\(T\) was isolated from a sandy beach of Taean (36° 48′ 50″ N 126° 11′ 9″ E) in the Yellow Sea, South Korea, by using a previously described procedure (Kim et al., 2008) with some modifications. Briefly, a sand sample was dispersed in 0.9% (w/v) saline and serially diluted in 10-fold steps by adding 1 ml of the previous dilution to 9 ml saline. The diluted suspensions were spread on marine agar 2216 (MA; Difco) and incubated under aerobic conditions at 25 °C for 5 days. Colonies were selected at random and crude genomic DNA from respective colonies was prepared as described previously (Lu et al., 2006). PCR amplification of 16S rRNA genes was performed using universal primers, F1 (5′-AGAGTTTGTATCMTGGCTCAG-3′) and R13 (5′-TACTCGGYTACCTGTAGCACGAC-3′). The amplicons were double-digested with HaeII and HhaI. Restriction fragment length polymorphism (RFLP) patterns were analysed on 2.5% MetaPhore agarose (BioWhittaker, USA) gels and colonies were grouped according to their RFLP patterns. Representative PCR products containing distinct RFLP

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**Abbreviations:** AL, aminolipid; DPG, diphosphatidylglycerol; L, lipid; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PC, phosphatidylcholine; PG, phosphatidylglycerol; PL, phospholipid; RFLP, restriction fragment length polymorphism.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain G4\(T\) is GQ232737.

Three supplementary figures and one supplementary table are available with the online version of this paper.
patterns were sequenced. The resulting 16S rRNA gene sequences were analysed using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in GenBank. From the analysis, a novel strain belonging to the family *Rhodobacteraceae*, designated strain G4T, was selected for additional phenotypic and phylogenetic analysis. The strain was routinely grown aerobically on MA at 30 °C for 3 days, unless otherwise indicated. The strain was stored at −80 °C in marine broth (Difco) supplemented with 10 % (v/v) glycerol for preservation. *Phaeobacter galliciensis* BS107T and *Leisingera methylohalidivorans* DSM 14336T (purchased from DSMZ, Germany), *Marinovum algicola* DSM 10251T (purchased from KCTC, Korea) and *Leisingera nanhaiensis* NH52FT (purchased from BCCM/LMG, Belgium) were used as reference strains for phenotypic characterization and fatty acid analysis.

The 16S rRNA gene sequence (1386 nt) of strain G4T was checked manually for quality and for gaps. Sequence similarity values between the novel isolate and closely related bacteria were evaluated using the Nucleotide Similarity Search program in the EzTaxon server 2.1 (http://147.47.212.35:8080/; Chun et al., 2007) and aligned by using the CLUSTAL W program (Thompson et al., 1994). Phylogenetic trees were reconstructed using the neighbour-joining (NJ) and maximum-parsimony (MP) algorithms in PHYLIP version 3.6 software (Felsenstein, 2002). Tree topology was evaluated using bootstrap analysis based on 1000 resampled datasets. Maximum-likelihood (ML) analysis with bootstrap support was performed using RAxML-HPC in Abe (version 7.2.6) of the Cyberinfrastructure for Phylogenetic Research project (CIPRES, www.phylo.org) at the San Diego Supercomputer Center.

Comparative analysis of 16S rRNA gene sequences showed that strain G4T was closely related to *Phaeobacter galliciensis* BS107T, *Phaeobacter inhibens* T5T, *Leisingera nanhaiensis* NH52FT, *Phaeobacter arcticus* 20188T and *Marinovum algicola* ATCC 51440T with similarities of 96.6, 96.5, 96.1, 95.6 and 95.6 %, respectively. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain G4T formed a phyletic lineage with *Leisingera nanhaiensis* NH52FT with a relatively low bootstrap value (65 %) within the family *Rhodobacteraceae* (Fig. 1). However, strain G4T formed a clear distinct phylogenetic lineage from other type strains of the genus *Leisingera* including the type species of the genus, *L. methylohalidivorans* NB2T. The topology of phylogenetic trees reconstructed using the MP and ML algorithms also supported the distinct lineage from related taxa (Supplementary Fig. S1, available in IJSEM Online). In addition, analysis using the Ribosomal Database Project (RDP) classifier program (Wang et al., 2007) showed that there was no genus group with a clear phylogenetic relationship to strain G4T within the family *Rhodobacteraceae*.

Growth of strain G4T at 5–45 °C (5 °C intervals) and pH 5–10 (intervals of 0.5 pH units) was determined on MA agar and in marine broth, respectively. Media with different pH values were prepared using appropriate biological buffers. Na2HPO4–NaH2PO4 buffer, Na2CO3–NaHCO3 buffer and Na3HPO4–NaOH buffer were used for pH values of below 8, pH 8–10 and pH 11, respectively (Gomori, 1955). pH levels were checked again after sterilization. Gram staining was performed using a bioMérieux Gram Stain kit according to the manufacturer’s instructions. Cell morphology and the presence of flagella were determined using phase-contrast microscopy and transmission electron microscopy (JEM-1010, JEOL) with 2-day-old cells grown on MA as described previously (Jeon et al., 2004). Requirement and tolerance of NaCl were determined in nutrient broth (NB: 3.0 g beef extract and 5.0 g peptone per litre) supplemented with modified artificial seawater [ASW: 0–15 % (w/v) NaCl at 0.5 % intervals, 5.94 g MgSO4.7H2O, 4.53 g MgCl2.6H2O, 0.64 g KCl and 1.3 g CaCl2 per litre] (Kahng et al., 2009). Oxidase activity was tested by oxidation of 1 % (w/v) tetramethyl-p-phenylenediamine (Merck) and catalase activity was evaluated by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution (Smibert & Krieg, 1994). Antibiotic susceptibility tests were performed in duplicate using filter-paper discs (diameter, 8 mm) containing the following antibiotics (µg per disc unless stated otherwise): ampicillin (10), polymyxin B (100 IU), streptomycin (50), penicillin G (10 IU), gentamicin (30), chloramphenicol (100), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), carbenicillin (100) and novobiocin (50). Additional enzymic activities and biochemical features were determined using API ZYM and API 20 NE kits (bioMérieux) according to the manufacturer’s instructions except that the inocula were prepared by suspending cells in a 3 % (w/v) NaCl solution. Anaerobic growth was assessed by incubating on MA under...
anaerobic conditions (4–10 % CO₂) using a GasPak Plus system (BBL) at 30 °C for 20 days.

Strain G4T grew at 15–35 °C (optimum 25-30 °C) but not at 10 or 37 °C. The novel strain also grew at pH 6–8 (optimum pH 6.5–7.5) on MA at 30 °C and in NB supplemented with modified artificial seawater containing 1–6 % (w/v) NaCl (optimum 2–4 % NaCl). Cells of strain G4T were Gram-reaction-negative, non-motile, ovoid rods, 0.8–1.2 × 1.2–1.8 μm in size (Supplementary Fig. S2). The motility of these cells clearly differentiated strain G4T from closely related members of the genera Leisingera, Phaeobacter and Marinovum (Table 1). Physiological and biochemical characteristics of strain G4T are shown in Table 1 and in the species description.

The DNA G+C content of strain G4T was determined using reversed-phase HPLC (GROM-SIL 100 ODS-2FE, GROM) according to the method of Tamaoka & Komagata (1984). Fatty acid methyl esters were analysed according to the instructions of the Sherlock Microbial Identification System (MIDI). For isoprenoid quinone and polar lipid analyses, cells of strain G4T grown on MA plates at 30 °C for 3 days and then harvested by centrifugation. Isoprenoid quinones were analysed using a HPLC (model LC-20A, Shimadzu) equipped with a diode array detector (SPD-M20A, Shimadzu) and a reversed-phase column (250 × 4.6 mm, AkzoNobel–Kromasil) as described previously (Komagata & Suzuki, 1987). Polar lipids were determined by TLC as described by Minnikin et al. (1977). The major respiratory lipoquinone of strain G4T was ubiquinone 10 (Q-10). The DNA G+C content of strain G4T was 62.4 mol%. The major cellular fatty acids were C18:1ω7c (59.0 %), C18:1ω7c 11-methyl (11.3 %), C12:1 3-OOH (9.8 %), C16:0 2-OOH (7.3 %), C16:0 (5.8 %), unknown 11.799 (5.4 %) and C18:0 (1.3 %). Strain G4T and closely related species contained C18:1ω7c as the predominant fatty acid, which is a feature of the majority of species within the class Alphaproteobacteria (Martens et al., 2006), but the presence of C12:1 3-OOH and the absence of C10:0 3-OOH and C12:0 3-OOH clearly differentiated the novel isolate from closely related members of the genera Leisingera, Phaeobacter and Marinovum (Table 2). The major cellular polar lipids were identified as phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylcholine (PC), an unidentified amino lipid (AL), an unidentified phospholipid (PL) and unidentified lipid (L) (Supplementary Fig. S3). Strain G4T contained PC, which distinguished the isolate from members of the genus Leisingera (Table 1). Based on physiological, biochemical and phylogenetic properties, strain G4T represents a novel species of a novel genus within the

### Table 1. Phenotypic characteristics of strain G4T and related species of the genera Leisingera, Phaeobacter and Marinovum

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>2</th>
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<th>5</th>
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<tr>
<td><strong>Morphology</strong></td>
<td></td>
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<tr>
<td>Colony colour</td>
<td>Ovoid rods Creamy</td>
<td>Rods/ovoid rods Beige</td>
<td>Rods/ovoid rods Non-pigmented</td>
<td>Ovoid rods Brown</td>
<td>Rods/ovoid rods Beige–pinkish-beige</td>
</tr>
<tr>
<td>Motility</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Growth at/in:</td>
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<tr>
<td>4 °C</td>
<td>−</td>
<td>+</td>
<td>(+)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>37 °C</td>
<td>−</td>
<td>(+)</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7 % (w/v) NaCl</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Degradation of:*</td>
<td></td>
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<tr>
<td>Aesculin</td>
<td>(+)</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Gelatin</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td><strong>Enzyme activity</strong></td>
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<tr>
<td>α-Chymotrypsin</td>
<td>−</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>(+)</td>
<td>+</td>
<td>−</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>(+)</td>
<td>(+)</td>
<td>−</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Naphthol-AS-B1-phosphohydrolase</td>
<td>−</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>62.4</td>
<td>60.5</td>
<td>60.5</td>
<td>58.0</td>
<td>60.0</td>
</tr>
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</table>

*Results from this study.
**Description of Litorimicrobium taeanense gen. nov., sp. nov.**

*Litorimicrobium taeanense* (ta.e.a.nen’s. N.L. neut. adj. *taeanense* of or belonging to Taean, from where the organism was isolated).

In addition to the characters described for the genus, the species is characterized by the following properties. Cells are strictly aerobic and 0.8–1.2 × 1.2–1.8 μm. Colonies on MA are creamy white, convex and round with entire margins. Growth occurs at 15–35 °C (optimum 25–30 °C) and pH 6–8 (optimum pH 6.5–7.5). Aesculin and urea are hydrolysed. Gelatin is not hydrolysed. Negative for assimilation of D-glucose, L-arabinose, D-mannose, maltose, D-mannitol, malic acid, potassium gluconate, N-acetylglucosamine, capric acid, adipic acid, trisodium citrate and phyllocadecic acid (API 20 NE). Positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and acid phosphatase activities but negative for x-chymotrypsin, x-glucosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, x-mannose and x-fucosidase activities. Weakly positive for lipase (C14), trypsin, alkaline phosphatase, cystine arylamidase, x-galactosidase, x-galactosidase, β-glucosidase and naphthol-AS-B1-phosphohydrolase activities (API ZYM). The major isoprenoid quinone is Q-10. Resistant to polymyxin B, gentamicin, kanamycin, novobiocin, oleandomycin, lincomycin, ampicillin, tetracycline and carbenicillin but sensitive to streptomycin, penicillin G and chloramphenicol.

The type strain, G4T (=KACC 13703T =DSM 22007T), was isolated from a sandy beach of the coast of Taean, South Korea. The DNA G+C content of the type strain is 62.4 mol% (HPLC).

**Acknowledgements**

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**References**


Felsenstein, J. (2002). *PHYLP* (phylogeny inference package), version 3.6a. Distributed by the author, Department of Genome Sciences, University of Washington, Seattle, USA.

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**Table 2. Cellular fatty acid compositions (%) of strain G4T and related members of the genera *Leisingera*, *Phaeobacter* and *Marinovum***

<table>
<thead>
<tr>
<th>Strains</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C12:0</td>
<td>C16:0</td>
<td>C18:0</td>
<td>Hydroyx</td>
<td>Unsaturated</td>
</tr>
<tr>
<td>G4T</td>
<td>—</td>
<td>—</td>
<td>0.9</td>
<td>—</td>
<td>1.3</td>
</tr>
<tr>
<td>KACC 13703T</td>
<td>0.9</td>
<td>1.3</td>
<td>2.3</td>
<td>1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Family Rhodobacteraceae of the class Alphaproteobacteria, for which the name Litorimicrobium taeanense gen. nov., sp. nov. is proposed.

### Description of Litorimicrobium taeanense gen. nov.

*Litorimicrobium* (Li.to.ri.mi.cro’bi.um. L. *n. litus- oris* sand beach; N.L. neut. *n. micro*bium microbe; N.L. neut. *n. Litorimicrobium* microbe living in a sand beach).

Cells are Gram-reaction-negative, non-motile, ovoid rods. Oxidase- and catalase-positive. Nitrate is reduced to nitrite. The isoprenoid quinone detected is Q-10. The predominant fatty acid is C18:1ω7c. The major cellular polar lipids are PG, DPG, PC, an unidentified AL, an unidentified PL and an unidentified L. The major cellular fatty acids are C18:1ω7c, C18:1ω7c 11-methyl, C12:1ω6 3-OH, C16:0 2-OH, C16:0 and unknown ECL 11.799. Phylogenetically, the genus belongs to the family Rhodobacteraceae of the class Alphaproteobacteria. The type species is Litorimicrobium taeanense. The DNA G+C content of the type strain of the type species is 62.4 mol% (HPLC).


