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

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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 quinone and $C_{18:1}\omega7c$ (59.0 %), $C_{18:1}\omega7c$ 11-methyl (11.3 %) 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 micro-organisms 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′-AGAGTTTGATCMTGGCTCAG-3′) and R13 (5′-TACGGYTACCTTGTTACGACTT-3′). The amplicons were double-digested with *Hae*III and *Hha*I. 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

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 G4^T, 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 gallaeciensis* BS107^T and *Leisingera methylohalidivorans* DSM 14336^T (purchased from DSMZ, Germany), *Marinovum algicola* DSM 10251^T (purchased from KCTC, Korea) and *Leisingera nanhaiensis* NH52F^T (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 G4^T 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 G4^T was closely related to *Phaeobacter gallaeciensis* BS107^T, *Phaeobacter inhibens* T5^T, *Leisingera nanhaiensis* NH52F^T, *Phaeobacter arcticus* 20188^T and *Marinovum algicola* ATCC 51440^T 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 G4^T formed a phyletic lineage with *Leisingera nanhaiensis* NH52F^T with a relatively low bootstrap value (65 %) within the family *Rhodobacteraceae* (Fig. 1). However, strain G4^T formed a clear distinct phylogenetic lineage from other type strains of the genus *Leisingera* including the type species of the genus, *L. methylohalidivorans* NB2^T. 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 G4^T within the family *Rhodobacteraceae*.

Growth of strain G4^T 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

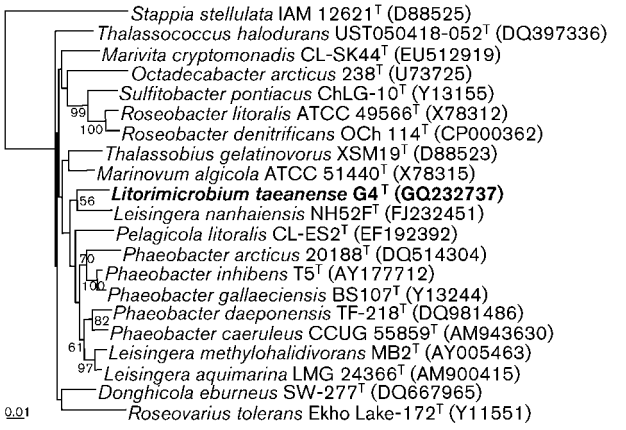

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strain G4^T and closely related taxa. Bootstrap values >50 %, based on 1000 replicates, are shown at branch points. *Stappia stellulata* IAM 12621^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

different pH values were prepared using appropriate biological buffers. Na_2HPO_4–NaH_2PO_4 buffer, Na_2CO_3–$NaHCO_3$ buffer and Na_2HPO_4–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 $MgSO_4.7H_2O$, 4.53 g $MgCl_2.6H_2O$, 0.64 g KCl and 1.3 g $CaCl_2$ 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 % CO_2) using a GasPak Plus system (BBL) at 30 °C for 20 days.

Strain G4^T 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 G4^T 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 G4^T from closely related members of the genera *Leisingera*, *Phaeobacter* and *Marinovum* (Table 1). Physiological and biochemical characteristics of strain G4^T are shown in Table 1 and in the species description.

The DNA G+C content of strain G4^T 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 extracted from cells of strain G4^T grown on MA plates at 30 °C for 3 days and analysed according to the instructions of the Sherlock Microbial Identification System (MIDI). For isoprenoid quinone and polar lipid analyses, cells of strain G4^T were cultivated in marine broth 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 G4^T was ubiquinone 10 (Q-10). The DNA G+C content of strain G4^T was 62.4 mol%. The major cellular fatty acids were $C_{18:1}\omega7c$ (59.0 %), $C_{18:1}\omega7c$ 11-methyl (11.3 %), $C_{12:1}$ 3-OH (9.8 %), $C_{16:0}$ 2-OH (7.3 %), $C_{16:0}$ (5.8 %), unknown 11.799 (5.4 %) and $C_{18:0}$ (1.3 %). Strain G4^T and closely related species contained $C_{18:1}\omega7c$ 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 $C_{12:1}$ 3-OH and the absence of $C_{10:0}$ 3-OH and $C_{12:0}$ 3-OH 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 G4^T contained PC, which distinguished the isolate from members of the genus *Leisingera* (Table 1). Based on physiological, biochemical and phylogenetic properties, strain G4^T represents a novel species of a novel genus within the

Table 1. Phenotypic characteristics of strain G4^T and related species of the genera *Leisingera*, *Phaeobacter* and *Marinovum*

Strains: 1, G4^T (data from this study); 2, *Leisingera nanhaiensis* LMG 24841^T (Sun *et al.*, 2010); 3, *Leisingera methylohalidivorans* DSM 14336^T (Schaefer *et al.*, 2002; Martens *et al.*, 2006; Vandecandelaere *et al.*, 2008); 4, *Phaeobacter gallaeciensis* DSM 17395^T (Martens *et al.*, 2006; Vandecandelaere *et al.*, 2009); 5. *Marinovum algicola* DSM 10251^T (Lafay *et al.*, 1995; Ruiz-Ponte *et al.*, 1998). All strains were positive for the following characteristics: esterase (C4), leucine arylamidase, valine arylamidase and acid phosphatase activities (API ZYM) and growth in 1 % (w/v) NaCl. All strains were negative for Gram-reaction and nitrate reduction. +, Positive; −, negative; (+), weakly positive.

Characteristic	1	2	3	4	5
Morphology	Ovoid rods	Rods/ovoid rods	Rods/ovoid rods	Ovoid rods	Rods/ovoid rods
Colony colour	Creamy	Beige	Non-pigmented	Brown	Beige–pinkish-beige
Motility	−	+	+	+	+
Growth at/in:					
4 °C	−	+	(+)	−	−
37 °C	−	(+)	−	+	+
7 % (w/v) NaCl	−	−	+	+	+
Degradation of:*					
Aesculin	(+)	+	−	+	+
Gelatin	−	+	−	−	+
Enzyme activity*					
α-Chymotrypsin	−	+	(+)	(+)	+
α-Galactosidase	(+)	+	−	(+)	+
β-Galactosidase	(+)	(+)	−	(+)	+
Naphthol-AS-B1-phosphohydrolase	−	+	(+)	(+)	(+)
Polar lipids	PG, DPG, PC, AL, PL, L	PG, PE, PL, L, AL	PG, PE, PL, L, AL	PC, PG, PE, L, AL, PL	PC, PG, PE, L, AL, PL
DNA G+C content (mol%)	62.4	60.5	60.5	58.0	60.0

*Results from this study.

Table 2. Cellular fatty acid compositions (%) of strain G4^T and related members of the genera *Leisingera*, *Phaeobacter* and *Marinovum*

Strains: 1, G4^T; 2, *Leisingera nanhaiensis* LMG 24841^T; 3, *Leisingera methylohalidivorans* DSM 14336^T; 4, *Phaeobacter gallaeciensis* DSM 17395^T; 5, *Marinovum algicola* DSM 10251^T. All data from this study. Data are expressed as percentages of total fatty acids. Fatty acids <0.5 % not shown. ECL, Equivalent chain-length; −, not detected.

Fatty acid	1	2	3	4	5
Saturated					
$C_{12:0}$	−	1.0	1.9	1.4	−
$C_{16:0}$	5.8	3.6	3.4	8.6	3.4
$C_{18:0}$	1.3	0.9	−	1.8	2.3
Hydroxy					
$C_{10:0}$ 3-OH	−	9.4	6.5	7.8	1.6
$C_{12:0}$ 3-OH	−	4.5	6.4	4.7	1.6
$C_{12:1}$ 3-OH	9.8	−	−	−	0.9
$C_{16:0}$ 2-OH	7.3	8.0	10.3	5.6	−
Unsaturated					
$C_{17:1}\omega 8c$	−	−	−	−	1.1
$C_{18:1}\omega 7c$	59.0	59.5	58.1	56.4	77.5
$C_{18:1}\omega 7c$ 11-methyl	11.3	3.6	3.5	6.0	8.6
$C_{19:0}$ 10-methyl	−	−	−	−	0.5
Branched					
anteiso-$C_{17:0}$	−	−	−	−	0.7
iso-$C_{17:1}\omega 9c$	−	−	−	−	0.5
Unknown ECL 11.799	5.4	7.8	9.9	7.9	−
Summed feature 4*	−	1.7	−	−	−

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 comprised iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

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

Description of *Litorimicrobium* gen. nov.

Litorimicrobium (Li.to.ri.mi.cro′bi.um. L. n. *litus -oris* sand beach; N.L. neut. n. *microbium* 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 $C_{18:1}\omega 7c$. 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 $C_{18:1}\omega 7c$, $C_{18:1}\omega 7c$ 11-methyl, $C_{12:1}$ 3-OH, $C_{16:0}$ 2-OH, $C_{16:0}$ 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).

Description of *Litorimicrobium taeanense* sp. nov.

Litorimicrobium taeanense (ta.e.a.nen′se. 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 phenylacetic acid (API 20 NE). Positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and acid phosphatase activities but negative for α-chymotrypsin, α-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannose and α-fucosidase activities. Weakly positive for lipase (C14), trypsin, alkaline phosphatase, cystine arylamidase, α-galactosidase, β-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, G4^T (=KACC 13703^T =DSM 22007^T), 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).

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References

Buchan, A., Collier, L. S., Neidle, E. L. & Moran, M. A. (2000). Key aromatic-ring-cleaving enzyme, protocatechuate 3,4-dioxygenase, in the ecologically important marine *Roseobacter* lineage. *Appl Environ Microbiol* **66**, 4662–4672.

Buchan, A., González, J. M. & Moran, M. A. (2005). Overview of the marine *Roseobacter* lineage. *Appl Environ Microbiol* **71**, 5665–5677.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

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

Giovannoni, S. J. & Rappé, M. (2000). Evolution, diversity, and molecular ecology of marine prokaryotes. In *Microbial Ecology of the Oceans*, pp. 47–84. Edited by D. L. Kirchman. New York: Wiley.

Gomori, G. (1955). Preparation of buffers for use in enzyme studies. *Methods Enzymol* **1**, 138–146.

Jeon, C. O., Park, W., Ghiorse, W. C. & Madsen, E. L. (2004). *Polaromonas naphthalenivorans* sp. nov., a naphthalene-degrading bacterium from naphthalene-contaminated sediment. *Int J Syst Evol Microbiol* **54**, 93–97.

Kahng, H.-Y., Chung, B. S., Lee, D.-H., Jung, J.-S., Park, J. H. & Jeon, C. O. (2009). *Cellulophaga tyrosinoxydans* sp. nov., a tyrosinase-producing bacterium isolated from seawater. *Int J Syst Evol Microbiol* **59**, 654–657.

Kim, J. M., Le, N. T., Chung, B. S., Park, J. H., Bae, J.-W., Madsen, E. L. & Jeon, C. O. (2008). 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* **74**, 7313–7320.

Kim, J. M., Lee, S. H., Jung, J. Y. & Jeon, C. O. (2010). *Marinobacterium lutimaris* sp. nov., isolated from a tidal flat. *Int J Syst Evol Microbiol* **60**, 1828–1831.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lafay, B., Ruimy, R., de Traubenberg, C. R., Breittmayer, V., Gauthier, M. J. & Christen, R. (1995). *Roseobacter algicola* sp. nov., a new marine bacterium isolated from the phycosphere of the toxin-producing dinoflagellate *Prorocentrum lima*. *Int J Syst Bacteriol* **45**, 290–296.

Lu, S., Park, M., Ro, H.-S., Lee, D. S., Park, W. & Jeon, C. O. (2006). Analysis of microbial communities using culture-dependent and culture-independent approaches in an anaerobic/aerobic SBR reactor. *J Microbiol* **44**, 155–161.

Martens, T., Heidorn, T., Pukall, R., Simon, M., Tindall, B. J. & Brinkhoff, T. (2006). Reclassification of *Roseobacter gallaeciensis* Ruiz-Ponte *et al.* 1998 as *Phaeobacter gallaeciensis* gen. nov., comb. nov., description of *Phaeobacter inhibens* sp. nov., reclassification of *Ruegeria algicola* (Lafay *et al.* 1995) Uchino *et al.* 1999 as *Marinovum algicola* gen. nov., comb. nov., and emended descriptions of the genera *Roseobacter*, *Ruegeria* and *Leisingera*. *Int J Syst Evol Microbiol* **56**, 1293–1304.

Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.

Moran, M. A., Gonzalez, J. M. & Kiene, R. P. (2003). Linking a bacterial taxon to sulfur cycling in the sea: studies of the marine *Roseobacter* group. *Geomicrobiol J* **20**, 375–388.

Ruiz-Ponte, C., Cilia, V., Lambert, C. & Nicolas, J. L. (1998). *Roseobacter gallaeciensis* sp. nov., a new marine bacterium isolated from rearings and collectors of the scallop *Pecten maximus*. *Int J Syst Bacteriol* **48**, 537–542.

Schaefer, J. K., Goodwin, K. D., McDonald, I. R., Murrell, J. C. & Oremland, R. S. (2002). *Leisingera methylohalidivorans* gen. nov., sp. nov., a marine methylotroph that grows on methyl bromide. *Int J Syst Evol Microbiol* **52**, 851–859.

Shiba, T. (1991). *Roseobacter litoralis* gen. nov., sp. nov., and *Roseobacter denitrificans* sp. nov., aerobic pink-pigmented bacteria which contain bacteriochlorophyll a. *Syst Appl Microbiol* **14**, 140–145.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt. Washington, DC: American Society for Microbiology.

Sun, F., Wang, B., Liu, X., Lai, Q., Du, Y., Li, G., Luo, J. & Shao, Z. (2010). *Leisingera nanhaiensis* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* **60**, 275–280.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Vandecandelaere, I., Segaert, E., Mollica, A., Faimali, M. & Vandamme, P. (2008). *Leisingera aquimarina* sp. nov., isolated from a marine electroactive biofilm, and emended descriptions of *Leisingera methylohalidivorans* Schaefer *et al.* 2002, *Phaeobacter daeponensis* Yoon *et al.* 2007 and *Phaeobacter inhibens* Martens *et al.* 2006. *Int J Syst Evol Microbiol* **58**, 2788–2793.

Vandecandelaere, I., Segaert, E., Mollica, A., Faimali, M. & Vandamme, P. (2009). *Phaeobacter caeruleus* sp. nov., a blue-coloured, colony-forming bacterium isolated from a marine electroactive biofilm. *Int J Syst Evol Microbiol* **59**, 1209–1214.

Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73**, 5261–5267.

Yoon, J. H., Kang, S. J., Lee, S. Y. & Oh, T. K. (2007). *Phaeobacter daeponensis* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **57**, 856–861.

Zhang, D. C., Li, H. R., Xin, Y. H., Liu, H. C., Chi, Z. M., Zhou, P. J. & Yu, Y. (2008). *Phaeobacter arcticus* sp. nov., a psychrophilic bacterium isolated from the Arctic. *Int J Syst Evol Microbiol* **58**, 1384–1387.