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# *Pelagicola litoralis* gen. nov., sp. nov., isolated from coastal water in Korea

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A Gram-negative, strictly aerobic, non-motile, club-shaped bacterial strain, designated CL-ES2$^T$, was isolated from coastal water from the east coast of Korea. Phylogenetic analysis of 16S rRNA gene sequences revealed that strain CL-ES2$^T$ was related to the genera *Phaeobacter* (95.0–96.6 % similarity to the type strains), *Leisingera* (96.1 %) and *Marinovum* (95.6 %) in the family *Rhodobacteraceae*. However, strain CL-ES2$^T$ did not form a robust clade with any species of the *Roseobacter* clade, instead forming a distinct subline. The optimum temperature and pH for growth were 25 °C and pH 7. Strain CL-ES2$^T$ was able to grow with sea salts at concentrations in the range 2–6 %, with optimum growth occurring at 3–4 %. The major fatty acid was $C_{18:1}\omega 7c$ (75.2 %). The polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and three unidentified lipids. The isoprenoid quinone was Q-10. The G+C content of the DNA was 47.0 mol%. On the basis of the data from the polyphasic analysis, strain CL-ES2$^T$ represents a novel genus and species, for which the name *Pelagicola litoralis* gen. nov., sp. nov. is proposed. The type strain of *Pelagicola litoralis* is CL-ES2$^T$ (=KCCM 42274$^T$ =DSM 18290$^T$).

The *Roseobacter* clade (family *Rhodobacteraceae*) is a major group in the class *Alphaproteobacteria* (Garrity *et al.*, 2005). Currently, the *Roseobacter* clade comprises at least 38 genera (List of Prokaryotic Names with Standing in Nomenclature; http://www.bacterio.cict.fr/). The members of the *Roseobacter* clade are physiologically diverse, including micro-organisms demonstrating aerobic anoxygenic phototrophy, aerobic sulfite oxidation, methylotrophy, organic sulfur compound degradation and antibiotic production (Buchan *et al.*, 2005; Martens *et al.*, 2006). In this study, a bacterium, designated strain CL-ES2$^T$, was isolated from coastal water from the east coast of Korea, subjected to a polyphasic analysis and identified as representing a novel genus and species within the family *Rhodobacteraceae*.

Strain CL-ES2$^T$ was isolated from coastal water from the east coast of Korea, using a standard dilution plating method on 100-fold-diluted R2A seawater agar medium (Lanoil *et al.*, 2000); the plate was incubated at 25 °C for 15 days. The strain was able to grow on marine agar 2216 (MA; Difco) and was subsequently purified four times on MA at 25 °C. The strain was maintained both on MA at 4 °C and in marine broth 2216 (Difco) supplemented with 30 % (v/v) glycerol at −80 °C. Strain CL-ES2$^T$ normally grows well on MA; however, an unexpected loss of viability occurred during our study. Notably, the viability seemed to be recoverable on MA supplemented with glucose (0.5 %, w/v), after which the strain could be subsequently cultivated on MA.

For PCR amplification of the 16S rRNA gene, DNA was extracted from a single colony by using a boiling method (Englen & Kelley, 2000). The crude extracts served as the DNA template for the PCRs, which involved *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified using the AccuPrep PCR Purification kit (Bioneer). Direct sequence determination of the 16S rRNA gene was performed with an Applied Biosystems automated sequencer (ABI3730XL) at Macrogen Corp. (Seoul, Republic of Korea). The almost-complete 16S rRNA gene sequence of the strain (1382 bp) was obtained and compared with 16S rRNA gene sequences available in the GenBank database by using BLASTN searches (Altschul *et al.*, 1990). The 16S rRNA gene sequence was manually aligned with those of members of the family *Rhodobacteraceae* using the jPHYDIT program (Jeon *et al.*, 2005). Phylogenetic trees were obtained by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. An evolutionary distance

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CL-ES2$^T$ is EF192392.

An extended neighbour-joining phylogenetic tree, a TLC of the polar lipids, micrographs of DAPI-stained cells and details of the whole-cell fatty acid composition of strain CL-ES2$^T$ and related strains are available as supplementary material with the online version of this paper.

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matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of tree topologies was assessed by means of bootstrap analyses based on 1000 replications (neighbour-joining and maximum-parsimony methods) or 100 replications (maximum-likelihood method). Phylogenetic analyses were carried out using MEGA3 (Kumar *et al.*, 2004) and PAUP 4.0 (Swofford, 1998). Likelihood parameters were estimated using the hierarchical ratio tests in MODELTEST 3.04 (Posada & Crandall, 1998). The DNA G+C content was determined using HPLC analysis (Tamaoka & Komagata, 1984) at the Korean Culture Center of Microorganisms (Seoul, Republic of Korea).

For phenotypic and chemotaxonomic analyses, strain CL-ES2$^T$ was routinely cultivated on MA. Gram staining was performed as described by Smibert & Krieg (1994). The motility of the cells was determined using the hanging-drop method (Suzuki *et al.*, 2001). Cell morphology was examined using phase-contrast microscopy (BX50; Olympus), epifluorescence microscopy (BX50; Olympus) and transmission electron microscopy (EX2; JEOL) with cells in the exponential phase (i.e. at 5–7 days) and stationary phase (i.e. at approx. 1 month) at 25 °C on MA. Anaerobic growth was assessed on MA and ZOF medium (Lemos *et al.*, 1985) with additional agar content (1.5 %) by using the GasPak anaerobic system (BBL) at 25 °C for 21–40 days. Accumulation of poly-$\beta$-hydroxybutyrate granules was determined by using Nile blue A staining (Ostle & Holt, 1982). Catalase and oxidase activities were determined according to the protocols described by Smibert & Krieg (1994). Bacteriochlorophyll *a* production was determined spectrophotometrically in 90 % acetone extracts from cells cultured either in the dark or in the light. Hydrolysis of hypoxanthine, starch, Tween 80 and xanthine was determined according to the protocols described by Hansen & Sørheim (1991). In addition, arginine dihydrolase, aesculin hydrolysis, $\beta$-galactosidase, gelatinase, indole production, nitrate reduction and urease were tested using the API 20NE kit (bioMérieux) according to the manufacturer's instructions, except that the cell suspension was prepared using artificial seawater (24 g NaCl, 10.88 g $MgCl_2.6H_2O$, 4 g $Na_2SO_4$, 1.46 g $CaCl_2.2H_2O$, 0.7 g KCl, 0.2 g $NaHCO_3$, 0.1 g KBr, 0.027 g $H_3BO_3$, 0.04 g $SrCl_2.6H_2O$, 0.003 g NaF, 1 l distilled water; Lyman & Fleming, 1940) as the suspension medium. The temperature range for growth was determined on the basis of colony formation on MA incubated at 5–45 °C. The pH range (pH 4–12) for growth was determined from changes in the $OD_{600}$ over time in marine broth 2216. Tolerance of sea salts was determined by assessing changes in the $OD_{600}$ in synthetic ZoBell medium (5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate, 1 l distilled water; Yi & Chun, 2004) with different concentrations (0–10 % in increments of 1 % and 15 %, w/v) of sea salts (Sigma) at 25 °C. Carbon utilization was tested on basal agar medium supplemented with yeast extract (23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2.6H_2O$, 5.94 g $MgSO_4.7H_2O$, 1.3 g $CaCl_2.2H_2O$, 0.2 g $NaNO_3$, 0.2 g $NH_4Cl$, 15 g Bacto agar, 0.05 g yeast extract, 1 l distilled water; Choi & Cho, 2006) containing 0.2 % carbon source. Growth was scored as negative when growth was equal to, or less than, that in the negative control (lacking a carbon source) after 30 days incubation at 25 °C. All of the experiments were performed under aerobic conditions.

Isoprenoid quinones were isolated according to Minnikin *et al.* (1984) and analysed using HPLC as described by Collins (1985). The fatty acid methyl esters in whole cells grown on MA at 25 °C for 5 days were analysed with gas chromatography, according to the instructions of the Microbial Identification System (MIDI), at the Korean Culture Center of Microorganisms. Polar lipids were extracted using the procedures described by Minnikin *et al.* (1984) and were identified with two-dimensional TLC followed by spraying with appropriate detection reagents (Komagata & Suzuki, 1987).

On the basis of 16S rRNA gene sequence similarity, strain CL-ES2$^T$ was found to be most closely related to the genera *Phaeobacter* (95.0–96.6 % similarity to the type strains), *Leisingera* (96.1 %) and *Marinovum* (95.6 %). However, a phylogenetic analysis of the 16S rRNA gene sequence of CL-ES2$^T$ showed that the strain did not form a robust clade with those related genera, but instead formed a distinct subline in the *Roseobacter* clade (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online). The G+C content of the DNA was 47.0 mol%. The major isoprenoid quinone in strain CL-ES2$^T$ was Q-10. The major fatty acid was $C_{18:1}\omega 7c$ (75.2 %; Supplementary Table S1, available in IJSEM Online). The polar lipids found in strain CL-ES2$^T$ were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and three unidentified lipids (Supplementary Fig. S2). Other phenotypic and physiological characteristics of strain CL-ES2$^T$ are given in the genus and species descriptions; micrographs of cells of strain CL-ES2$^T$ following DAPI staining are shown in Supplementary Fig. S3.

In addition to the phylogenetic separation of strain CL-ES2$^T$ from the related genera (i.e. *Phaeobacter*, *Leisingera* and *Marinovum*) in the *Roseobacter* clade (Fig. 1), some phenotypic and chemotaxonomic characteristics can serve to differentiate the strain from these genera; there was a considerable difference between the DNA G+C content of strain CL-ES2$^T$ (47.0 mol%) and those of the related genera (55.7–64.9 mol%; Table 1). The absence of an unidentified phospholipid, the absence of motility and the presence of Tween 80 hydrolysis in strain CL-ES2$^T$ clearly distinguished the strain from the related genera *Phaeobacter*, *Leisingera* and *Marinovum* (Table 1). Strain CL-ES2$^T$ can be differentiated from the genus *Phaeobacter* using other phenotypic traits, including amylase activity and the utilization of certain carbon sources (glycerol, leucine and succinate; Table 1). Strain CL-ES2$^T$ can be distinguished from the genus *Leisingera* by the presence of

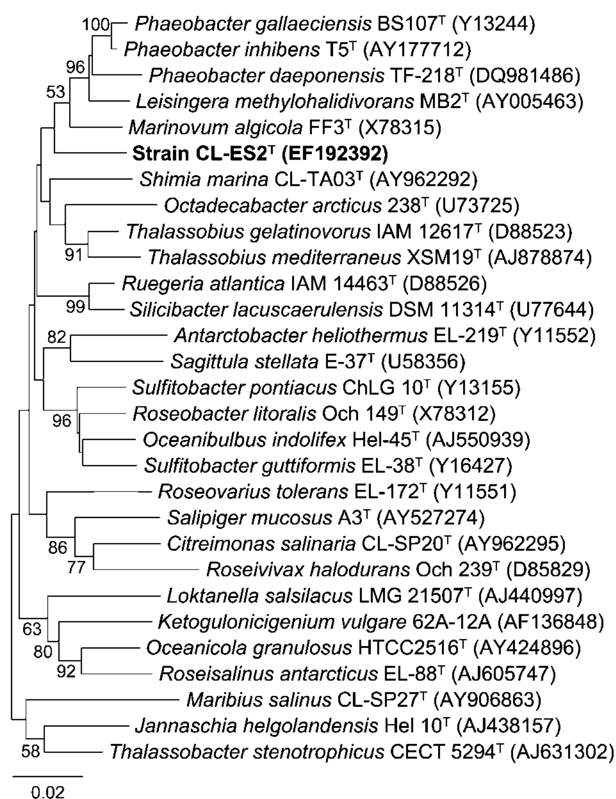


**Fig. 1.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain CL-ES2$^T$ with respect to related taxa. Bootstrap percentages (based on 1000 resamplings) >50 % are shown at branching points. *Alteromonas marina* SW-47$^T$ (GenBank accession no. AF529060) was used as an outgroup (not shown). Bar, 0.02 nucleotide substitutions per site. An extended version of this tree is available as Supplementary Fig. S1.

phosphatidylcholine and the utilization of certain carbon sources (acetate, dimethyl sulfate, glucose and serine; Table1). Furthermore, strain CL-ES2$^T$ can be differentiated from the genus *Marinovum* on the basis of gelatinase activity and the utilization of certain carbon sources (citrate, serine, sucrose and trehalose; Table 1). In conclusion, phylogenetic analyses based on 16S rRNA gene sequences, DNA G+C contents, phenotypic features and the components of polar lipids suggest that strain CL-ES2$^T$ represents a novel genus and species, for which the name *Pelagicola litoralis* gen. nov., sp. nov. is proposed.

**Table 1.** Differential phenotypic characteristics of strain CL-ES2$^T$ and members of the genera *Phaeobacter*, *Leisingera* and *Marinovum*

Taxa: 1, strain CL-ES2$^T$; 2, *Phaeobacter inhibens* (data from Martens *et al.*, 2006); 3, *Phaeobacter gallaeciensis* (data from Ruiz-Ponte *et al.*, 1998 unless indicated); 4, *Phaeobacter daeponensis* (Yoon *et al.*, 2007); 5, *Leisingera methylohalidivorans* (Schaefer *et al.*, 2002); 6, *Marinovum algicola* (Lafay *et al.*, 1995). All of these organisms are positive for catalase and oxidase and contain phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and unidentified lipids. All organisms are negative for Gram staining and bacteriochlorophyll *a*. +, Positive; −, negative; W, weakly positive; V, variable; ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Motility | − | + | + | + | + | + |
| pH optimum | 7.0 | 7.5 | 7.0 | 7.0–8.0 | 7.7 | 7.5 |
| **Growth at:** | | | | | | |
| 4 °C | − | + | − | + | +[a*] | − |
| 37 °C | − | − | + | + | −[a] | + |
| Nitrate reduction | − | − | − | + | − | − |
| **Hydrolysis of:** | | | | | | |
| Gelatin | − | − | − | − | −[a] | + |
| Starch | + | − | − | − | + | + |
| Tween 80 | + | − | − | − | −[a] | − |
| **Utilization of:** | | | | | | |
| Acetate | + | + | V | + | − | V |
| Arabinose | − | − | − | − | −[a] | −/+[b] |
| Betaine | + | + | − | ND | + | ND |
| Cellobiose | − | + | + | − | −[a] | −/+[b] |
| Citrate | − | + | − | + | − | + |
| Dimethyl sulfate | − | − | −[a] | ND | + | ND |
| Glucose | W | + | + | + | − | + |
| Glycerol | − | + | + | + | − | − |
| Leucine | − | + | + | + | −[a] | −[b] |
| Rhamnose | − | − | − | ND | −[a] | −/+[b] |
| Serine | + | + | − | + | − | −[b] |
| Succinate | − | + | + | + | −[a] | − |
| Sucrose | − | + | + | − | −[c] | +[d] |
| Trehalose | − | + | + | − | −[a] | + |
| Tween 80 | + | − | −[a] | ND | −[a] | −[b] |
| **Polar lipids** | | | | | | |
| Phosphatidylcholine | + | + | +[a] | + | −[a] | +[a] |
| Unidentified phospholipid | − | + | +[a] | + | +[a] | +[a] |
| DNA G+C content (mol%) | 47.0 | 55.7 | 57.6–58.0 | 64.9 | 60.5 | 60.0[d] |

*Data taken from the following studies: *a*, Martens *et al.* (2006); *b*, Labrenz *et al.* (1999); *c*, Yoon *et al.* (2007); *d*, Ruiz-Ponte *et al.* (1998).

## Description of *Pelagicola* gen. nov.

*Pelagicola* [Pe.la.gi.co′la. L. n. *pelagus* the sea; L. suff. -*cola* (from L. n. *incola*) inhabitant; N.L. masc. n. *Pelagicola* inhabitant of the sea].

Cells are Gram-negative, non-motile, strictly aerobic, club-shaped rods. Not able to accumulate poly-$\beta$-hydroxybutyrate granules. Catalase- and oxidase-positive. Cells do not contain bacteriochlorophyll *a*. The predominant isoprenoid quinone is Q-10. The predominant fatty acid is $C_{18:1}\omega7c$. The DNA G+C content of the type strain of the type species is 47.0 mol%. The cellular polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and three unidentified lipids. The type species is *Pelagicola litoralis*.

### Description of *Pelagicola litoralis* sp. nov.

*Pelagicola litoralis* (li.to.ra'lis. L. masc. adj. *litoralis* of the shore).

Exhibits the following properties in addition to those given in the genus description. After 10 days on MA medium at 25 °C, colonies are circular, convex, creamy and approximately 0.7 mm in diameter. Cells appear singly, in pairs or in groups of three and are approximately 0.5–1.4 μm wide and 1.1–7.0 μm long during the exponential growth phase. In old cultures (approx. 1 month), cells are cocci (1.0–1.4 μm in diameter) or short rods (0.5–0.9 μm wide and 1.2–1.8 μm long). The optimum sea salts concentration for growth is 3–4 % (w/v), with a range of 2–6 % (at pH 7). Growth is observed at 20–30 °C (optimum, 25 °C) and at pH 6–8 (optimum, pH 7). Positive for hydrolysis of starch and Tween 80. Negative for hydrolysis of hypoxanthine and xanthine. With the API 20NE system, hydrolysis of aesculin and $\beta$-galactosidase (PNPG) activity occur, but arginine dihydrolase, hydrolysis of gelatin, indole production, nitrate reductase and urease are absent. Acetate, betaine, glucose, L-lysine, peptone, L-proline, serine and Tween 80 are utilized as sole carbon sources, but *N*-acetyl-D-glucosamine, arabinose, ascorbate, cellobiose, citrate, DL-cysteine, dimethyl sulfate, glycerol, glycogen, leucine, pyruvic acid, rhamnose, succinate, sucrose and trehalose are not utilized.

The type strain, CL-ES2$^T$ (=KCCM 42274$^T$ =DSM 18290$^T$), was isolated from coastal water from the east coast of Korea.

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