Pelagibius litoralis gen. nov., sp. nov., a marine bacterium in the family *Rhodospirillaceae* isolated from coastal seawater

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A Gram-negative, strictly aerobic, slightly curved rod-shaped bacterial strain, designated CL-UU02^T, was isolated from coastal seawater off the east coast of Korea. 16S rRNA gene sequence analysis revealed a clear affiliation of this novel strain with the family *Rhodospirillaceae*. Strain CL-UU02^T formed a robust cluster with the type strains of species of the genus *Rhodovibrio* at 16S rRNA gene sequence similarity levels of 89.9–90.4 %. Strain CL-UU02^T shared no more than 89 % 16S rRNA gene sequence similarity with the type strains of other species in the family *Rhodospirillaceae*. Strain CL-UU02^T was able to grow in the presence of 2–6 % sea salts, and grew optimally at 28–30 °C and pH 7–8. The DNA G+C content of strain CL-UU02^T was 66.3 mol%. On the basis of phylogenetic analyses and chemotaxonomic and physiological data, strain CL-UU02^T is considered to represent a novel species of a new genus in the family *Rhodospirillaceae*, for which the name *Pelagibius litoralis* gen. nov., sp. nov. is proposed. The type strain of *Pelagibius litoralis* is CL-UU02^T (=KCCM 42323^T=JCM 15426^T).

The order *Rhodospirillales* currently comprises two families, *Rhodospirillaceae* and *Acetobacteraceae*, in the class *Alphaproteobacteria* (Garrity *et al.*, 2005). At the time of writing, the family *Rhodospirillaceae* comprises 16 genera, namely *Azospirillum*, *Caenispirillum*, *Defluviicoccus*, *Inquilinus*, *Magnetospirillum*, *Phaeospirillum*, *Rhodocista*, *Rhodospira*, *Rhodospirillum*, *Rhodovibrio*, *Roseospira*, *Skermanella*, *Telmatospirillum*, *Thalassobaculum*, *Thalassospira* and *Tistrella* (see http://www.bacterio.cict.fr).

Among 40 recognized species in the family *Rhodospirillaceae*, only seven species affiliated with the genera *Rhodovibrio* (Mack *et al.*, 1993), *Rhodospira* (Pfennig *et al.*, 1997), *Thalassospira* (López-López *et al.*, 2002; Liu *et al.*, 2007; Kodama *et al.*, 2008) and *Thalassobaculum* (Zhang *et al.*, 2008) have been recovered from marine environments. Other species in the family *Rhodospirillaceae* have been isolated from various non-marine habitats, such as freshwater, activated sludge biomass, air, soil and roots of plants, and cystic fibrosis patients (Coenye *et al.*, 2002; Garrity *et al.*, 2005; Weon *et al.*, 2007; Yoon *et al.*, 2007). In the present study, a novel bacterial strain, designated CL-UU02^T, affiliated with the family *Rhodospirillaceae* was isolated from urea-enriched seawater and was subjected to a polyphasic taxonomic analysis.

In February 2005, coastal seawater taken from the east coast of Korea was brought back to the laboratory for analysis. One hundred microlitres of seawater was inoculated in autoclaved seawater (500 ml) supplemented with urea (final concentration of 100 mM) and incubated at 20 °C in the dark. After about 8 months, 100 µl of the sample was taken and spread on a marine agar 2216 (MA; Difco) plate, which was then incubated aerobically at 30 °C for 2 weeks. Strain CL-UU02^T was isolated and subsequently streaked onto fresh MA plates at 30 °C under aerobic conditions. The purification procedure was repeated four times. Strain CL-UU02^T was maintained both on MA at 30 °C and 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 based on 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 an AccuPrep PCR purification kit (Bioneer) and was cloned by using pGEM T-Easy vector (Promega). Sequencing of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CL-UU02^T is DQ401091.

An extended neighbour-joining tree based on 16S rRNA gene sequences showing the position of strain CL-UU02^T among members of the family *Rhodospirillaceae* is available as supplementary material with the online version of this paper.

the 16S rRNA gene was performed with an Applied Biosystems automated sequencer (ABI3730XL) at Macrogen Corp. (Seoul, Korea). The almost-complete 16S rRNA gene sequence of strain CL-UU02^T (1422 nt) was obtained and compared with available 16S rRNA gene sequences in the GenBank database by using BLASTN searches (Altschul *et al.*, 1990). The sequence of strain CL-UU02^T was manually aligned with all available 16S rRNA gene sequences of recognized species in the family *Rhodospirillaceae*, obtained from GenBank and Ribosomal Database Project II (Cole *et al.*, 2007), by using known 16S rRNA secondary-structure information. Phylogenetic trees were constructed according to the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) 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 and 100 replications for the maximum-likelihood method. Alignment analysis was carried out by using the JPHYDIT program (Jeon *et al.*, 2005) and phylogenetic analyses were carried out by using MEGA 4 (Tamura *et al.*, 2007) and PAUP 4.0 (Swofford, 1998). Likelihood parameters were estimated by using the hierarchical ratio test in MODELTEST version 3.04 (Posada & Crandall, 1998).

Fatty acid methyl esters in whole cells of strain CL-UU02^T grown on MA at 30 °C for 13 days were analysed by GC according to the instructions of the Microbial Identification System (MIDI) at the Korean Culture Center of Micro-organisms (KCCM) in Seoul, Korea. Isoprenoid quinones were isolated according to the method of Minnikin *et al.* (1984) and were analysed by HPLC as described by Collins (1985) at KCCM. The DNA G+C content was determined by HPLC analysis (Tamaoka & Komagata, 1984) at KCCM.

Morphological and physiological characteristics were determined as follows. Gram-staining was performed as described by Smibert & Krieg (1994). Cell motility was determined based on the hanging drop method (Suzuki *et al.*, 2001). Cell morphology and the presence of flagellum was determined by using transmission electron microscopy (EX2; JEOL). Anaerobic growth was investigated on MA by using the GasPak anaerobic system (BBL). Poly-β-hydroxybutyrate granules were identified by using epifluorescence microscopy (BX60; Olympus) after Nile blue A staining (Ostle & Holt, 1982). Bacteriochlorophyll *a* production was determined in 90 % acetone extracts by using a spectrophotometer (Ultraspec 2000; Pharmacia Biotech) for cells that had been grown either in the light or in the dark for 7 days. 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-UU02^T and *Porphyrobacter donghaensis* SW-132^T (=KCTC 12229^T; Yoon *et al.*, 2004), the latter serving as a positive control strain.

The temperature range for growth was examined on the basis of colony formation on MA incubated at 5–45 °C at increments of 5 °C. The temperature range for optimal growth was further determined by assessing changes in OD_{600} with time in MB at 20, 23, 25, 28, 30 and 33 °C. Tolerance of strain CL-UU02^T to sea salts was determined by using synthetic ZoBell broth (per litre distilled water: 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate; Yi & Chun, 2004) with various concentrations of sea salts (Sigma) [0–10 % (w/v) at increments of 1 %, as well as 15, 20 and 25 %]. The pH range (pH 5–12, at increments of 1 pH unit) for growth was determined by assessing changes in OD_{600} with time in synthetic ZoBell broth. The final pH was adjusted by using 6 M NaOH and 6 M HCl solutions.

Oxidase and catalase tests were performed according to the protocols described by Smibert & Krieg (1994). Amylase, gelatinase and nitrate reductase activities and degradation of casein, hypoxanthine, Tween 80, L-tyrosine and xanthine were determined according to Hansen & Sørheim (1991). Other enzyme activities were assayed by using the 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 $MgCl_2.6H_2O$, 4 g Na_2SO_4, 1.5 g $CaCl_2.2H_2O$, 0.7 g KCl, 0.2 g $NaHCO_3$, 0.1 g KBr, 0.027 g H_3BO_3, 0.03 g $SrCl_2.6H_2O$, 0.003 g NaF; Lyman & Fleming, 1940). Carbon utilization was tested by using basal broth medium supplemented with yeast extract (per litre distilled water: 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, 0.05 g yeast extract; Bruns *et al.*, 2001) containing 0.4 % carbon source. Carbon utilization was scored as negative when growth was equal to or less than that in the negative control with no carbon source. Growth was measured by assessing changes in OD_{600} following incubation at 30 °C for 25 days.

The dominant fatty acid of strain CL-UU02^T was $C_{18:1}\omega7c$ (48.5 % of the total), which is a feature of the vast majority of species within the *Alphaproteobacteria* (Labrenz *et al.*, 2000), followed by $C_{18:0}$ 3-OH (17.5 %), $C_{19:0}$ cyclo $\omega8c$ (10.3 %), 11-methyl $C_{18:1}\omega7c$ (7.0 %), 10-methyl $C_{19:0}$ (4.6 %), $C_{18:1}$ 2-OH (3.1 %), $C_{18:0}$ (3.0 %), $C_{18:1}\omega9c$ (1.6 %), $C_{16:0}$ (1.5 %) and an unknown fatty acid (ECL 14.959; 1.2 %). Trace amounts (<1 %) of $C_{17:0}$, $C_{16:0}$ 2-OH and $C_{17:0}$ 3-OH were found. The major isoprenoid quinone was ubiquinone 10 (Q-10). The genomic DNA G+C content was 66.3 mol%.

Cells of strain CL-UU02^T were Gram-negative, slightly curved or straight rods that were approximately 0.5–1.0 μm wide and 1.2–2.5 μm long. Cells were motile by means of a polar flagellum. Colonies were circular, convex and creamy on MA plates. Strain CL-UU02^T was strictly aerobic and contained poly-β-hydroxybutyrate granules. Bacteriochlorophyll *a* production and the *pufL* and *pufM* genes were not detected. Other phenotypic characteristics

Table 1. Selected differential characteristics between strain CL-UU02^T and other phylogenetically related species in the family *Rhodospirillaceae*

Taxa: 1, strain CL-UU02^T (*Pelagibius litoralis* gen. nov., sp. nov.); 2, *Rhodovibrio sodomensis* (data from Mack *et al.*, 1993; Imhoff *et al.*, 1998; Garrity *et al.*, 2005); 3, *Rhodovibrio salinarum* (Nissen & Dundas, 1984; Imhoff *et al.*, 1998; Garrity *et al.*, 2005); 4, *Tistrella mobilis* (Shi *et al.*, 2002); 5, *Defluviicoccus vanus* (Maszenan *et al.*, 2005); 6, *Thalassobaculum litoreum* (Zhang *et al.*, 2008); 7, *Inquilinus limosus* (Coenye *et al.*, 2002). +, Positive; W, weakly positive; −, negative; V, variable; NA, data not available. All taxa are Gram-negative and catalase-positive (data for catalase were not available for the genus *Rhodovibrio*).

Characteristic	1	2	3	4	5	6	7
Habitat	Coastal seawater	Seawater	Ponds of solar saltern	Wastewater	Sludge	Coastal seawater	Cystic fibrosis patients
Colony colour	Cream	Pink	Red	NA	Beige	Cream-yellow	Pink
Cell shape	Slightly curved rod	Vibrioid, spiral	Rod to spiral	Rod	Coccus	Slightly curved and straight rod	Rod
Cell size (width × length; μm)	0.5–1.0 × 1.2–2.5	0.6–0.7 × 1.6–2.5	0.8–0.9 × 1.0–3.5	0.7–1.0 (width)	1.5–4.5	0.3–0.5 × 1.3–1.5	NA
Flagella*	MP	MP	BP	MP	Absent	MP	NA
Temperature range (optimum) (°C)	15–33 (28–30)	25–47 (35–40)	20–45 (42)	20–40 (30)	20–30 (25–30)	10–35 (30–35)	25–42 (NA)
pH range (optimum)	6–11 (7–8)	NA (7)	NA (7.5–8)	5–9 (7.4)	5–8.5 (7.5–8)	7–9 (8)	NA (NA)
Salt tolerance (%)	2–6	6–20	3–24	<1	NA	1–10	<6
Bacteriochlorophyll *a*	−	+	+	−	NA	−	NA
Oxidase	+	NA	NA	+	−	+	V
Urease	−	NA	NA	−	W	NA	V
Gelatin hydrolysis	−	NA	NA	+	W	+	V
Carbon source utilization							
N-Acetylglucosamine	−	NA	NA	+	+	−	+
L-Arabinose	+	NA	NA	+	+	+	−
D-Glucose	+	NA	−	NA	+	−	−
Inositol	+	NA	NA	NA	NA	−	−
D-Mannitol	+	NA	−	+	NA	−	−
D-Ribose	−	NA	NA	NA	NA	+	NA
Sucrose	−	NA	−	NA	NA	+	−
Major quinone	Q-10	NA	Q-10, MK-10	Q-10	NA	Q-10	NA
DNA G+C content (mol%)	66.3	66.2–66.6	67.4–68.1	67.5	66	68.0	70.9

*BP, Bipolar; MP, monopolar.

of strain CL-UU02^T are given in the genus and species descriptions below and in Table 1.

Analysis of the 16S rRNA gene sequence of strain CL-UU02^T revealed a clear affiliation with the family *Rhodospirillaceae* (Fig. 1). Strain CL-UU02^T was related most closely to the type strains of *Rhodovibrio sodomensis* (90.4 % 16S rRNA gene sequence similarity), *Rhodovibrio salinarum* (89.9 %), *Thalassobaculum litoreum* (88.7 %) and *Tistrella mobilis* (88.3 %); it showed levels of 16S rRNA gene sequence similarity of 85.6–88.2 % to the type strains of other type species of genera in the family *Rhodospirillaceae*. In the neighbour-joining, maximum-parsimony and maximum-likelihood phylogenetic trees, strain CL-UU02^T clearly formed a basal branch of the sister clade containing *Rhodovibrio* species, supported by high bootstrap values (97, 91 and 70 %, respectively). In phylogenetic trees containing environmental clones and uncharacterized isolates (see Supplementary Fig. S1, available in IJSEM Online), strain CL-UU02^T formed a distinct group that was clearly separated from that containing recognized *Rhodovibrio* species. The low levels of 16S rRNA gene sequence similarity with other bacteria (i.e. <91 %) and distinct phylogenetic position indicated that strain CL-UU02^T should be assigned to a novel species of a new genus in the family *Rhodospirillaceae*.

Furthermore, strain CL-UU02^T could be differentiated from members of the genus *Rhodovibrio* based on chemotaxonomic and phenotypic characteristics. The sole major respiratory quinone (Q-10) of strain CL-UU02^T differentiated this strain from *Rhodovibrio salinarum* (Q-10 and MK-10; Table 1). Strain CL-UU02^T was clearly distinguishable from the genus *Rhodovibrio* based on growth temperature range, salt tolerance range and the absence of bacteriochlorophyll *a* (Table 1). Furthermore,

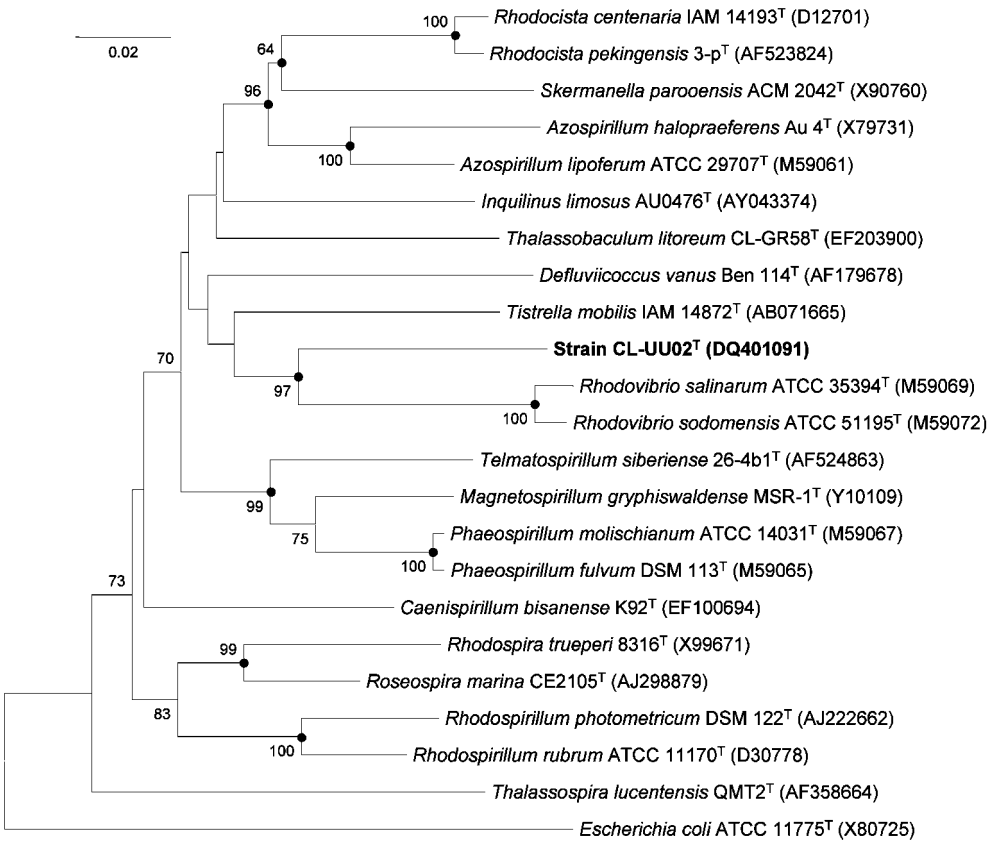

Fig. 1. Neighbour-joining tree derived from 16S rRNA gene sequences showing the position of strain CL-UU02^T among members of the family *Rhodospirillaceae*. *Escherichia coli* ATCC 11775^T was used as an outgroup. Numbers at nodes are bootstrap percentages (based on 1000 resamplings); only values >60 % are shown. Solid circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 0.02 nucleotide substitutions per site. An extended version of this tree is available as Supplementary Fig. S1 in IJSEM Online.

phenotypic characteristics could also be used to differentiate strain CL-UU02^T from other related genera in the family *Rhodospirillaceae*. Temperature range for growth distinguished strain CL-UU02^T (15–33 °C) from the genera *Tistrella* (20–40 °C) and *Inquilinus* (25–42 °C; Table 1). In addition, the salt tolerance range distinguished strain CL-UU02^T (2–6 %) from the genus *Tistrella* (<1 %; Table 1). Finally, cell morphology and the presence of oxidase differentiated strain CL-UU02^T from the genus *Defluviicoccus* (Table 1).

In conclusion, evidence from the present polyphasic study indicated that strain CL-UU02^T represents a novel species of a new genus, for which the name *Pelagibius litoralis* gen. nov., sp. nov. is proposed.

Description of *Pelagibius* gen. nov.

Pelagibius (Pe.la.gi.bi′us. L. n. *pelagus* the sea; N.L. masc. n. *bius* from Gr. n. *bios* life; N.L. masc. n. *Pelagibius* sea life).

Cells are Gram-negative, strictly aerobic, non-fermentative heterotrophs. Salt is required for growth. Oxidase- and catalase-positive. The dominant fatty acids are $C_{18:1}\omega7c$, $C_{18:0}$ 3-OH and $C_{19:0}$ cyclo $\omega8c$. The major isoprenoid quinone is ubiquinone 10 (Q-10). Phylogenetically, the genus is a member of the family *Rhodospirillaceae*. The type species is *Pelagibius litoralis*.

Description of *Pelagibius litoralis* sp. nov.

Pelagibius 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. Cells are slightly curved or straight rods, approximately 0.5–1.0 μm in width and 1.2–2.5 μm in length. Cells are motile by means of a polar flagellum. Colonies are circular, convex and creamy on MA plates. After 5 days on MA at 30 °C, colonies are approximately 0.3 mm in diameter. Grows at 15–33 °C (optimum 28–30 °C) and pH 6–11 (optimum pH 7–8). Growth occurs at sea salt concentrations of 2–6 % (w/v) (optimum 3–4 %). Cells contain poly-β-hydroxybutyrate granules. Bacteriochlorophyll *a* is not present. Amylase and

gelatinase are not produced. L-Tyrosine is hydrolysed, but casein, hypoxanthine, Tween 80 and xanthine are not. Nitrate is reduced to nitrite. According to the API ZYM system, positive for acid phosphatase, alkaline phosphatase, esterase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and β-galactosidase, but negative for *N*-acetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, esterase lipase (C8), α-fucosidase, α-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase, trypsin and valine arylamidase. According to the API 20NE system, positive for aesculin hydrolysis, β-galactosidase and nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase and urease. Utilizes L-arabinose, D-galactose, D-glucose, inositol, inulin, D-mannitol, D-mannose, pyruvic acid, succinate, tartrate and D-xylose, but not acetate, *N*-acetylglucosamine, L-arginine, L-ascorbate, L-asparagine, DL-aspartate, benzoate, cellobiose, citrate, DL-cysteine, D-fructose, L-glutamate, glycerol, glycine, glycogen, α-ketobutyric acid, lactose, L-leucine, L-lysine, L-ornithine, L-proline, raffinose, L-rhamnose, D-ribose, D-salicin, D-sorbitol, sucrose or trehalose as sole carbon source. The DNA G+C content of the type strain is 66.3 mol%.

The type strain, CL-UU02^T (=KCCM 42323^T=JCM 15426^T), was isolated from seawater off the east coast of Korea.

Acknowledgements

We acknowledge the expert technical support of Ms In-Sung Lee (electron microscopy) of the National Center for Inter-university Research Facilities at Seoul National University. This work was supported in part by the BK21 project of the Korean Government, the Ministry of Maritime Affairs and Fisheries (the Korea EAST-1 program) and the research program (PE98312, PM53901) of KORDI.

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