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-UU02T, 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-UU02T 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-UU02T shared no more than 89 % 16S rRNA gene sequence similarity with the type strains of other species in the family Rhodospirillaceae. Strain CL-UU02T 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-UU02T was 66.3 mol%. On the basis of phylogenetic analyses and chemotaxonomic and physiological data, strain CL-UU02T 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-UU02T (=KCCM 42323T=JCM 15426T).

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, Defluvicoccus, 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 (Pfenning 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-UU02T, 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-UU02T was isolated and subsequently streaked onto fresh MA plates at 30 °C under aerobic conditions. The purification procedure was repeated four times. Strain CL-UU02T 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-UU02T is DQ401091.
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-UU02T (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-UU02T 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 jHYDIDT 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-UU02T 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-UU02T and Porphyrobacter donghaensis SW-132T (=KCTC 12229T; 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 OD600 with time in MB at 20, 23, 25, 28, 30 and 33 °C. Tolerance of strain CL-UU02T 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 % (v/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 OD600 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 MgCl2.6H2O, 4 g Na2SO4, 1.5 g CaCl2.2H2O, 0.7 g KCl, 0.2 g NaHCO3, 0.1 g KBr, 0.027 g H3BO3, 0.03 g SrCl2.6H2O, 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 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; 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 OD600 following incubation at 30 °C for 25 days.

The dominant fatty acid of strain CL-UU02T was C18:1ω7c (48.5 % of the total), which is a feature of the vast majority of species within the Alphaproteobacteria (Labrenz et al., 2000), followed by C18:0 3-OH (17.5 %), C19:0 cyclo ω8c (10.3 %), 11-methyl C18:1ω7c (7.0 %), 10-methyl C19:0 (4.6 %), C18:1 2-OH (3.1 %), C18:0 (3.0 %), C18:1ω9c (1.6 %), C16:0 (1.5 %) and an unknown fatty acid (ECL 14.959; 1.2 %). Trace amounts (<1 %) of C17:0, C16:0 2-OH and C17:0 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-UU02T 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-UU02T was strictly aerobic and contained poly-β-hydroxybutyrate granules. Bacteriochlorophyll a production and the pufL and pufM genes were not detected. Other phenotypic characteristics...
of strain CL-UU02<sup>T</sup> are given in the genus and species descriptions below and in Table 1.

Analysis of the 16S rRNA gene sequence of strain CL-UU02<sup>T</sup> revealed a clear affiliation with the family Rhodospirillaceae (Fig. 1). Strain CL-UU02<sup>T</sup> 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 Rhodovibrio. Furthermore, strain CL-UU02<sup>T</sup> 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<sup>T</sup> differentiated this strain from Rhodovibrio salinarum (Q-10 and MK-10; Table 1). Strain CL-UU02<sup>T</sup> was clearly distinguishable from the genus Rhodovibrio based on growth temperature range, salt tolerance range and the absence of bacteriochlorophyll a (Table 1). Furthermore,
phenotypic characteristics could also be used to differentiate strain CL-UU02T from other related genera in the family Rhodospirillaceae. Temperature range for growth distinguished strain CL-UU02T (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-UU02T (2–6%) from the genus Tistrella (<1%; Table 1). Finally, cell morphology and the presence of oxidase differentiated strain CL-UU02T from the genus Defluviococcus (Table 1).

In conclusion, evidence from the present polyphasic study indicated that strain CL-UU02T represents a novel species of a new genus, for which the name Pelagibius litoralis gen. nov., sp. nov. is proposed.

Description of Pelagibius litoralis 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 C₁₈₁ω7c, C₁₈₀3-OH and C₁₉₀cycoω8c. 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-glucuronidase, lipase (C14), α-mannosidase, trypsin and valine arylamidase. According to the API 20NE system, positive for ascorulhydrolysis, 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-xylolse, 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-ribose, D-ribose, D-salicin, sucrose or trehalose as sole carbon source. The DNA G+C content of the type strain is 66.3 mol%.

The type strain, CL-UU02T (=KCCM 4323T=JCM 15426T), was isolated from seawater off the east coast of Korea.

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