Thalassobaculum litoreum gen. nov., sp. nov., a member of the family *Rhodospirillaceae* isolated from coastal seawater

Gwang I. Zhang, Chung Y. Hwang and Byung C. Cho

Correspondence
Byung Cheol Cho
bccho@snu.ac.kr

School of Earth and Environmental Sciences and Research Institute of Oceanography, Seoul National University, San 56-1, Shillim-dong, Kwanak-gu, Seoul 151-742, Republic of Korea

A Gram-negative, facultatively anaerobic strain with slightly curved and straight rod-shaped cells, strain CL-GR58^T, was isolated from coastal seawater (near Gori, Korea). Analyses of the 16S rRNA gene sequence revealed that strain CL-GR58^T belonged to the family *Rhodospirillaceae* with *Azospirillum lipoferum* as its closest relative (gene sequence similarity of 90.9%).

Phylogenetic analyses of the 16S rRNA gene sequences showed that strain CL-GR58^T was not associated with any known genera in the family *Rhodospirillaceae*. The novel strain grew in the presence of 1–10% sea salts, optimally at 30–35 °C and pH 8. The major cellular fatty acids consisted of C_{18:1}ω7c (48.5%), C_{16:0} (14.8%), C_{17:0} (12.2%), C_{19:0} cyclo ω8c (6.3%) and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, 6.0%). Among the phylogenetically related genera, the fatty acid C_{17:0} was found only in strain CL-GR58^T. The DNA G + C content of the novel strain was 68.0 mol%. According to phylogenetic analyses of the 16S rRNA gene sequence, fatty acid content and the physiological data, strain CL-GR58^T represents a novel species in a new genus of the family *Rhodospirillaceae*, for which the name *Thalassobaculum litoreum* gen. nov., sp. nov. is proposed. The type strain of the type species is CL-GR58^T (=KCCM 42674^T=DSM 18839^T).

The family *Rhodospirillaceae*, belonging to subgroup 1 (Woese *et al.*, 1984) of the class *Alphaproteobacteria*, currently comprises 14 genera (<http://www.bacterio.cict.fr>). Members of the family *Rhodospirillaceae* exhibit a variety of modes of growth: the genera *Rhodovibrio* and *Phaeospirillum* and some species in three other genera (i.e. *Rhodocista centenaria*, *Rhodospirillum photometricum* and *Roseospira marina*) are capable of both photo- and chemoheterotrophic growth under anoxic conditions in the light and under microoxic to oxic conditions in the dark, respectively (Nissen & Dundas, 1984; Mack *et al.*, 1993; Garrity *et al.*, 2005). The other species in the genera *Rhodospirillum* and *Roseospira* (i.e. *Rhodospirillum rubrum*, *Roseospira mediosalina* and *Roseospira navarrensis*) are capable of photolithoautotrophic growth (with hydrogen or sulfide as an electron donor) as well as the above growth modes (Guyoneaud *et al.*, 2002; Garrity *et al.*, 2005). Other species in the genus *Rhodocista* (i.e. *Rhodocista pekingensis*) can grow photo- and chemolitho-heterotrophically (with hydrogen as an electron donor) under anoxic conditions in the light and under oxic conditions in the dark, respectively (Zhang *et al.*, 2003). The genus *Rhodospira* is capable of photolitho- (with sulfide as an electron donor) and chemoheterotrophic growth under anoxic conditions in the light

and under microoxic conditions in the dark, respectively (Pfennig *et al.*, 1997). The genus *Telmatospirillum* is capable of chemoheterotrophic growth under anoxic to microoxic conditions and both chemolithoheterotrophic and lithoautotrophic growth (with hydrogen as an electron donor), under microoxic conditions (Sizova *et al.*, 2007). Furthermore, chemoheterotrophic genera, *Azospirillum* (except *Azospirillum oryzae* which can grow chemolitho-heterotrophically utilizing hydrogen as an electron donor; Xie & Yokota, 2005), *Skermanella*, *Inquilinus*, *Tistrella*, *Thalassospira*, *Defluviococcus* and *Magnetospirillum* have been reported (Sly & Stackebrandt, 1999; Coenye *et al.*, 2002; López-López *et al.*, 2002; Shi *et al.*, 2002; Garrity *et al.*, 2005; Maszenan *et al.*, 2005).

Most species in the family *Rhodospirillaceae* have been isolated from various non-marine habitats such as freshwater, activated sludge biomass, soil and roots of plants and cystic fibrosis patients (Coenye *et al.*, 2002; Garrity *et al.*, 2005). Only limited numbers of members in the family *Rhodospirillaceae* have been discovered in marine environments (López-López *et al.*, 2002): only five species affiliated with the genera *Rhodovibrio* (*Rhodovibrio salinarum* and *Rhodovibrio sodomensis*; Nissen & Dundas, 1984; Mack *et al.*, 1993) and *Thalassospira* (*Thalassospira lucentensis*, *Thalassospira profundimaris* and *Thalassospira xiamenensis*, López-López *et al.*, 2002; Liu *et al.*, 2007) in

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CL-GR58^T is EF203900.

the family *Rhodospirillaceae* have been isolated from seawater samples.

Here, we describe a chemoheterotrophic bacterium, strain CL-GR58^T, isolated from coastal seawater in Gori, Korea. In August 2005, coastal seawater and sediment samples were brought back to the laboratory and incubated in a 150 mm diameter glass Petri dish for around 15 months at room temperature. Without disturbing the sediment, a 100 µl sample of seawater was removed from the surface and spread on a marine agar 2216 (MA; Difco) plate, which was then incubated at 30 °C for 1 week. Strain CL-GR58^T was isolated and subsequently purified on MA at 30 °C four times. The strain was maintained both on MA at 30 °C and in marine broth 2216 (MB; Difco) supplemented with 30 % (v/v) glycerol at –80 °C.

The 16S rRNA gene was amplified from a single colony by using PCR with *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified by using the AccuPrep PCR purification kit (Bioneer) and direct sequence determination of the purified 16S rRNA gene was performed with an Applied Biosystems automated sequencer (ABI3730XL) at Macrogen, Seoul, Korea. The almost-complete 16S rRNA gene sequence of strain CL-GR58^T (1336 nucleotides) was obtained and compared with available 16S rRNA gene sequences in GenBank using BLASTN searches (Altschul *et al.*, 1990). The sequence of strain CL-GR58^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 (Cole *et al.*, 2003) databases using known 16S rRNA secondary structure information. Phylogenetic trees were constructed by 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 the 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 using the jPHYDIT program (Jeon *et al.*, 2005) and phylogenetic analyses were carried out using MEGA 3 (Kumar *et al.*, 2004) and PAUP 4.0 (Swofford, 1998). Likelihood parameters were estimated by using the hierarchical ratio test in MODELTEST, version 3.04 (Posada & Crandall, 1998).

The fatty acid methyl esters in whole cells of strain CL-GR58^T grown on MA at 30 °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 (KCCM) in Seoul, Korea. The DNA G+C content was analysed by HPLC (HP 100; Hewlett Packard) analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989), after DNA extraction according to the method of Marmur (1961).

Lambda DNA was used as a standard. The quinone system was determined according to Minnikin *et al.* (1984) and analysed by HPLC as described by Collins (1985).

Morphological and physiological characteristics were determined. Gram-staining was performed as described by Smibert & Krieg (1994). Cell motility was observed by the hanging drop method (Suzuki *et al.*, 2001). Cell morphology and presence of flagella was observed using transmission electron microscopy (EX2; JEOL). Anaerobic growth was checked on MA using the GasPak anaerobic system (BBL). Poly-β-hydroxybutyrate granules were observed by epifluorescence microscopy (BX60; Olympus) after Nile blue A staining (Ostle & Holt, 1982). Bacteriochlorophyll *a* production was determined in 90 % acetone extracts 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 temperature range for growth was examined on the basis of colony formation on MA incubated at temperatures ranging from 5 to 40 °C, using increments of 5 °C. The pH range (pH 3–10, using increments of 1 pH unit) for growth was determined by assessing changes in OD₆₀₀ over the incubation period (up to 7 days) in MB. The final pH was adjusted using 1 M NaOH and 1 M HCl solutions. The tolerance of strain CL-GR58^T to sea salts was determined both on the basis of colony formation on synthetic Zobell agar (5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate, 15 g Bacto agar, 1 l distilled water) and assessing changes in OD₆₀₀ in Zobell broth with various concentrations (0–10 % increments of 1 % and 15 %, w/v) of sea salts (Sigma). Growth in a medium containing NaCl as the sole salt was determined both on the basis of colony formation on synthetic Zobell agar and by assessing changes in OD₆₀₀ in Zobell broth with different concentrations of NaCl (0–10 % increments of 1 % and 15 %, w/v). The ability to fix dinitrogen was tested, under anoxic conditions, on NFB medium (5.0 g malate, 0.5 g K₂HPO₄, 0.2 g MgSO₄·7H₂O, 0.1 g NaCl, 0.02 g CaCl₂·2H₂O, 2 ml 0.5 % bromothymol blue in 0.2 M KOH, 1 ml sterile filtered vitamin solution, 2 ml sterile filtered micronutrient solution, 4 ml 1.64 % FeEDTA solution, 4.5 g KOH, 1 l distilled water and 15 g l^{–1} Bacto agar, at pH 6.8; Eckert *et al.*, 2001), using *Azospirillum dobereineriae* KCTC 12904^T as the reference strain. Further, the presence of the *nifH* gene fragment was determined by using PCR amplification with specific primers (PolF/PolR; Poly *et al.*, 2001) for both strain CL-GR58^T and *A. dobereineriae* KCTC 12904^T. The oxidase and catalase tests were performed according to the protocols described by Smibert & Krieg (1994). Gelatinase, amylase and nitrate reductase activities and degradation of Tween 80 were determined according to Hansen & Sørheim (1991). In addition, other enzyme activities were assayed using the API ZYM kit (bioMérieux) according to the manufacturer's instructions, except that the cell suspension was prepared using artificial seawater (24 g NaCl, 5.1 g MgCl₂, 4 g Na₂SO₄, 1.1 g CaCl₂, 0.7 g KCl, 0.2 g NaHCO₃, 0.1 g

KBr, 0.027 g H₃BO₃, 0.024 g SrCl₂, 0.003 g NaF, 1 l distilled water; Lyman & Fleming, 1940).

Carbon utilization was tested using the basal broth medium supplemented with yeast extract (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, 1 l distilled water; Bruns *et al.*, 2001) containing 0.4 % carbon source. Strain CL-GR58^T was incubated for 4 weeks and carbon utilization was scored as negative when the growth rate was equal to, or less than, that in the negative control with no carbon source. Growth rate was measured by monitoring changes in OD₆₀₀. Resistance to antibiotics was determined by the disc diffusion plate method (Bauer *et al.*, 1966).

The 16S rRNA gene sequence of strain CL-GR58^T showed 90.9 % similarity to the type strains of *Azospirillum lipoferum*, 89.8 % to *Azospirillum oryzae*, 89.7 % to *Azospirillum canadense*, 89.5 % to *A. doebereineriae* and 79.3–89.5 % to the other type species of the family

Rhodospirillaceae. Although strain CL-GR58^T was closely related to species of the genus *Azospirillum* on the basis of 16S rRNA gene sequence similarity, the novel strain was not included in the clade of the genera *Azospirillum*, *Rhodocista* or *Skermanella* (Fig. 1). Further, the lineage of strain CL-GR58^T was not associated with any other genera of the family *Rhodospirillaceae*. Therefore, phylogenetically, strain CL-GR58^T should be recognized as representing a distinct genus in the family *Rhodospirillaceae*.

The fatty acid profile of strain CL-GR58^T was composed of C_{18:1}ω7c (48.5 %), C_{16:0} (14.8 %), C_{17:0} (12.2 %), C_{19:0} cyclo ω8c (6.3 %), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, 6.0 %), 11-methyl C_{18:1}ω7c (3.0 %), C_{18:0} (2.0 %), C_{15:0} (1.5 %) and an unknown fatty acid (ECL 18.814; 1.4 %) (Table 1). Fatty acid C_{18:1}ω7c was commonly found as a major component in strain CL-GR58^T and related genera (Table 1); a feature shared by members of the class *Alphaproteobacteria* (Labrenz *et al.*, 2000). However, a significant amount of C_{17:0} (12.2 %)

Fig. 1. Neighbour-joining tree derived from 16S rRNA gene sequences for strain CL-GR58^T and members of the family *Rhodospirillaceae* with *Escherichia coli* as an outgroup. Only bootstrap values above 60 % are shown (1000 resamplings) at the branching points. Solid circles indicate that the corresponding nodes were also recovered in maximum-likelihood and maximum-parsimony trees. Bar, 0.02 nucleotide substitutions per site.

Table 1. Cellular fatty acid content of strain CL-GR58^T and other phylogenetically related genera of the family *Rhodospirillaceae*

Taxa: 1, strain CL-GR58^T; 2, *Azospirillum* (Schenk & Werner, 1988; López-López *et al.*, 2002; Xie & Yokota, 2005; Peng *et al.*, 2006; Mehnaz *et al.*, 2007); 3, *Rhodovibrio* (Imhoff *et al.*, 1998); 4, *Tistrella* (Shi *et al.*, 2002); 5, *Inquilinus* (Coenye *et al.*, 2002). ND, Not detected; tr, trace (<1.0 %); ECL, equivalent chain-length; SF2, summed feature 2, C_{12:0} aldehyde, C_{16:1} iso I and/or C_{14:0} 3-OH; SF3, summed feature 3, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Data were not available for the genera *Rhodocista*, *Defluviicoccus* or *Skermanella*.

Fatty acid	1	2	3	4	5
C _{12:0}	ND	ND–5.8	ND	4.1	ND
C _{14:0}	ND	ND–2.3	1.0	ND	ND
C _{15:0}	1.5	ND–1.5	ND	ND	ND
C _{16:0}	14.8	ND–12.3	7.4	2.6	tr
C _{17:0}	12.2	tr	ND	ND	tr
C _{18:0}	2.0	ND–3.6	23.0	1.4	tr
C _{16:0} 2-OH	ND	ND	ND	ND	5.1
C _{16:0} 3-OH	ND	ND–4.8	ND	tr	3.7
C _{17:0} 2-OH	ND	ND	ND	ND	5.0
C _{17:0} 3-OH	tr	tr	ND	ND	4.3
C _{18:0} 3-OH	tr	tr	ND	1.9	9.0
C _{18:1} 2-OH	ND	ND–5.5	ND	16.1	15.5
C _{17:1} ω6c	tr	ND–7.1	ND	ND	ND
C _{17:1} ω8c	tr	ND–3.4	ND	tr	ND
C _{18:1} ω7c	48.5	15.3–75.1	35.2*	43.4	35.6
C _{19:0} cyclo ω8c	6.3	ND–3.8	ND	5.0	7.9
C _{20:1} ω9c	tr	ND	ND	ND	ND
11-methyl C _{18:1} ω7c	3.0	ND	ND	8.5	ND
ECL 18.814	1.4	ND	ND	ND	ND
SF2	tr	ND–6.7	ND	8.6	ND
SF3	6.0	ND–31.4†	tr‡	1.2	ND

*C_{18:1}.†C_{16:1}ω7c and/or C_{16:1}ω6c.‡C_{16:1}.

was only found in strain CL-GR58^T and was not found at significant levels in the related genera *Azospirillum*, *Rhodovibrio*, *Tistrella* or *Inquilinus* (Table 1). Furthermore, the absence of fatty acids C_{16:0} 2-OH, C_{16:0} 3-OH, C_{17:0} 2-OH and C_{18:1} 2-OH clearly differentiated strain CL-GR58^T from the genera *Azospirillum*, *Tistrella* and *Inquilinus* (Table 1). A significant concentration of C_{18:1} 2-OH (15.5–16.1 %) observed in the genera *Tistrella* and *Inquilinus* was not detected in strain CL-GR58^T or in the genus *Rhodovibrio*. Absence of fatty acid C_{19:0} cyclo ω8c differentiated the genus *Rhodovibrio* from other related genera. Therefore, the fatty acid pattern of strain CL-GR58^T differs distinctly from those of related genera in the family *Rhodospirillaceae*. The major quinone in strain CL-GR58^T was ubiquinone 10 (Q-10), which is also found in the genera *Azospirillum*, *Tistrella* and *Skermanella*; however

in the genera *Rhodocista* and *Rhodovibrio*, Q-9, and Q-10 and MK-10 were found, respectively (Table 2). The G+C content of the DNA was 68.0 mol% (Table 2).

Cells of strain CL-GR58^T were Gram-negative, slightly curved and straight rod-shaped and approximately 0.3–0.5 µm wide and 1.3–1.5 µm long. Cells were motile by means of a polar flagellum. Colonies were circular, convex and cream–yellow on MA plates. After 10 days on MA at 30 °C, colonies were approximately 1 mm in diameter. Strain CL-GR58^T was heterotrophic and facultatively anaerobic. Cells contained poly-β-hydroxybutyrate granules. Bacteriochlorophyll *a* was not detected. The optimum growth temperature was 30–35 °C; the optimum pH was 8. Growth occurred at sea salts concentrations of 1–10 % (w/v; optimum 2–4 %), but no growth occurred in media containing only NaCl as a salt. Strain CL-GR58^T was unable to fix dinitrogen and did not possess a *nifH* gene, whereas *A. doebereineriae* was able to grow on the NFB medium by fixing nitrogen and had a *nifH* gene (data not shown). Oxidase, catalase, gelatinase, nitrate reductase and amylase activities were positive. Tween 80 was not hydrolysed. According to API ZYM tests, alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-glucosidases and *N*-acetyl-β-glucosaminidase activities were positive and were weakly positive for esterase lipase (C8), lipase (C14), α- and β-galactosidases, α-mannosidase and α-fucosidase. No activities were found for cystine arylamidase, trypsin, α-chymotrypsin and β-glucuronidase. Growth occurred on D-ribose, sucrose, L-arabinose and yeast extract. No growth occurred on acetate, α-ketobutyric acid, citrate, fructose, galactose, glucose, mannitol, raffinose, salicin, trehalose, ethanol, inositol, inulin, lactose, L-lysine, L-rhamnose, *N*-acetylglucosamine, oxalic acid, pyruvic acid, succinate or L-proline. Strain CL-GR58^T was susceptible to streptomycin, gentamicin, vancomycin, kanamycin, penicillin, erythromycin, tetracycline, chloramphenicol, ciprofloxacin and ampicillin, but resistant to mitomycin C, nalidixic acid and polymyxin B.

Strain CL-GR58^T could be differentiated phenotypically from the genus *Azospirillum* by its inability to perform nitrogen fixation and from the genera *Rhodocista* and *Rhodovibrio* by the absence of bacteriochlorophyll *a* (Table 2). Strain CL-GR58^T could be differentiated from members of the genus *Defluviicoccus* by some phenotypic features (presence of flagella, positive activity for oxidase) and from the genus *Skermanella* by the positive activity for gelatinase. The growth temperature range distinguished strain CL-GR58^T (10–35 °C) from members of the genus *Inquilinus* (25–42 °C). In addition, the salt tolerance range distinguished strain CL-GR58^T (1–10 %) from the genera *Azospirillum* (<5 %), *Tistrella* (<1 %), *Skermanella* (<5 %) and *Inquilinus* (<6 %) (Table 2).

In conclusion, phylogenetic analyses based on 16S rRNA gene sequences, chemotaxonomic data (fatty acid profiles,

Table 2. Selected characteristics that differentiate strain CL-GR58^T from other phylogenetically related genera in the family Rhodospirillaceae

Taxa: 1, strain CL-GR58^T; 2, *Azospirillum* (Tarrand *et al.*, 1978; Reinhold *et al.*, 1987; Khammas *et al.*, 1989; Sly & Stackebrandt, 1999; Eckert *et al.*, 2001; Xie & Yokota, 2005; Peng *et al.*, 2006; Mehnaz *et al.*, 2007); 3, *Rhodocista* (Favinger *et al.*, 1989; Kawasaki *et al.*, 1992; Imhoff *et al.*, 1998; Zhang *et al.*, 2003); 4, *Rhodovibrio* (Nissen & Dundas, 1984; Mack *et al.*, 1993; Imhoff *et al.*, 1998; Garrity *et al.*, 2005); 5, *Deftuviococcus* (Maszenan *et al.*, 2005); 6, *Tistrella* (Shi *et al.*, 2002); 7, *Skermanella* (Sly & Stackebrandt, 1999); 8, *Inquilinus* (Coenye *et al.*, 2002). +, Positive; w, weakly positive; –, negative; v, variable; MP, monopolar; BP, bipolar; NA, not available. All strains are Gram-negative and catalase-positive (data for catalase were not available for the genera *Rhodocista* and *Rhodovibrio*).

Characteristic	1	2	3	4	5	6	7	8
Habitat	Coastal seawater	Soil, root, fresh water	Fresh water, wastewater	Seawater, ponds of solar salt	Sludge	Wastewater	Lake water	Cystic fibrosis patients
Colony colour	Cream–yellow	Pink, white	Red, pink	Pink, red	Beige	NA	Apricot-coloured	Pink
Cell size (width × length; µm)	0.3–0.5 × 1.3–1.5	0.6–0.9 × 2–30	1–2 × 3.0	0.6–0.9 × 1.0–3.5	1.5–4.5	0.7–1.0 (width)	1–1.5 × 2–3	NA
Cell shape	Slightly curved and straight rod	Plump, vibrioid, straight rod	Vibrioid, spiral	Vibrioid, spiral	Coccus	Rod	Rod	Rod
Flagella	MP	V	MP	MP, BP	Absent	MP	MP	NA
Temperature range (°C)	10–35	4–41	25–47	20–47	20–30	20–40	10–37	25–42
Temperature optimum (°C)	30–35	20–41	31–45	35–42	25–30	30	28	NA
pH range	7–9	5–8.5	5.7–8	7–8	5–8.5	5–9	NA	NA
pH optimum	8	5–7.2	6.8–7	7–8	7.5–8	7.4	NA	NA
Salt tolerance (% w/v)	1–10	<5	NA	3–24	NA	<1	<5	<6
Poly-β-hydroxybutyrate	+	v	+	+	+	+	+	NA
Bacteriochlorophyll <i>a</i>	–	–	+	+	NA	–	–	NA
Oxidase	+	+	NA	NA	–	+	+	v
Gelatinase	+	v	NA	NA	w	+	–	v
Major quinone	Q-10	Q-10	Q-9	Q-10, MK-10	NA	Q-10	Q-10	NA
DNA G + C content (mol%)	68.0	64–71	68.3–69.9	66.2–68.1	66	67.5	67.2	70.9
Utilization of carbon sources:								
<i>N</i> -Acetylglucosamine	–	v	NA	NA	+	+	–	+
L-Arabinose	+	v	NA	NA	+	+	+	–
D-Glucose	–	v	–	–	+	NA	+	–
Citrate	–	v	–	–	NA	NA	+	–
Inositol	–	v	NA	NA	NA	NA	–	–
D-Mannitol	–	v	–	–	NA	+	+	–
L-Rhamnose	–	v	NA	NA	NA	NA	+	–
D-Ribose	+	v	NA	NA	NA	NA	+	NA
Sucrose	+	v	–	–	NA	NA	NA	–

quinones) and phenotypic traits indicated that strain CL-GR58^T should be classified as a novel genus and species, for which the name *Thalassobaculum litoreum* gen. nov., sp. nov. is proposed.

Description of *Thalassobaculum* gen. nov.

Thalassobaculum (Tha.las'so.ba.cu.lum. Gr. n. *Thalassa* the sea; L. neut. n. *baculum* stick; N.L. neut. n. *Thalassobaculum* rod-shaped bacterium from the sea).

Cells are Gram-negative, slightly curved and straight rod-shaped and are motile by means of a polar flagellum. Growth is heterotrophic and facultatively anaerobic. Oxidase- and

catalase-positive. Bacteriochlorophyll *a* is not detected. Unable to fix dinitrogen under anoxic conditions. Dominant cellular fatty acids are C_{18:1ω7c}, C_{16:0}, C_{17:0}, C_{19:0} cyclo ω8c and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH). The isoprenoid quinone is Q-10. The G + C content of the DNA is 68.0 mol%. Phylogenetically, the genus is a member of the family Rhodospirillaceae. The type species is *Thalassobaculum litoreum*.

Description of *Thalassobaculum litoreum* sp. nov.

Thalassobaculum litoreum (li.to.re'um. L. neut. adj. *litor-eum* of the shore).

Displays the following properties in addition to those given in the genus description. Colonies are circular, convex and cream–yellow on marine agar plates. After 10 days on MA at 30 °C, colonies are approximately 1 mm in diameter. Cells are approximately 0.3–0.5 µm wide and 1.3–1.5 µm long. Grows at between 10 and 35 °C (optimum of 30–35 °C) and pH 7–9 (optimum of pH 8). Growth occurs at sea salts concentrations of 1–10 % (w/v) (optimum 2–4 %), but no growth occurs in media containing only NaCl as a salt. Cells contain poly-β-hydroxybutyrate granules. Gelatinase and amylase are produced. Tween 80 is not hydrolysed. Nitrate is reduced to nitrite. Positive for the following enzyme activities as tested with the API ZYM system: alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-glucosidases and N-acetyl-β-glucosaminidase; weakly positive for esterase lipase (C8), lipase (C14), α- and β-galactosidases, α-mannosidase and α-fucosidase. No activities for cystine arylamidase, trypsin, α-chymotrypsin and β-glucuronidase. D-ribose, sucrose, L-arabinose and yeast extract are utilized as carbon sources. Negative for utilization of the following: acetate, α-ketobutyric acid, citrate, fructose, galactose, glucose, mannitol, raffinose, salicin, trehalose, ethanol, inositol, inulin, lactose, L-lysine, L-rhamnose, N-acetylglucosamine, oxalic acid, pyruvic acid, succinate and L-proline. Cells are sensitive to (µg per disc): streptomycin (10), gentamicin (10), vancomycin (30), kanamycin (30), penicillin (10), erythromycin (15), tetracycline (30), chloramphenicol (30), ciprofloxacin (5) and ampicillin (10).

The type strain, CL-GR58^T (=KCCM 42674^T=DSM 18839^T), was isolated from coastal seawater, Korea.

Acknowledgements

We would like to thank Ms Yeon-A Jin for helping with strain isolation. We also 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 and the Ministry of Maritime Affairs and Fisheries (the Korea EAST-1 program).

References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* **45**, 493–496.
- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* **51**, 1997–2006.
- Coenye, T., Goris, J., Spilker, T., Vandamme, P. & LiPuma, J. J. (2002). Characterization of unusual bacteria isolated from respiratory secretions of cystic fibrosis patients and description of *Inquilinus limosus* gen. nov., sp. nov. *J Clin Microbiol* **40**, 2062–2069.
- Cole, J. R., Chai, B., Marsh, T. L., Farris, R. J., Wang, Q., Kulam, S. A., Chandra, S., McGarrell, D. M., Schmidt, T. M. & other authors (2003). The ribosomal database project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**, 442–443.
- Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.
- Eckert, B., Weber, O. B., Kirchhof, G., Halbritter, A., Stoffels, M. & Hartmann, A. (2001). *Azospirillum doebereineriae* sp. nov., a nitrogen-fixing bacterium associated with the C4-grass *Miscanthus*. *Int J Syst Evol Microbiol* **51**, 17–26.
- Favinger, J., Stadtwald, R. & Howard, G. (1989). *Rhodospirillum centenum*, sp. nov., a thermotolerant cyst-forming anoxygenic photosynthetic bacterium. *Antonie van Leeuwenhoek* **55**, 291–296.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Garrity, G. M., Bell, J. A. & Lilburn, T. (2005). Family I. *Rhodospirillaceae* Pfennig and Trüper 1971, 17^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, *The Proteobacteria*, part C, *The Alpha-, Beta-, Delta-, and Epsilonproteobacteria*, pp. 1–40. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Guyoneaud, R., Mouné, S., Eatock, C., Bothorel, V., Hirschler-Réa, A., Willison, J., Duran, R., Liesack, W., Herbert, R. & other authors (2002). Characterization of three spiral-shaped purple nonsulfur bacteria isolated from coastal lagoon sediments, saline sulfur springs, and microbial mats: emended description of the genus *Roseospira* and description of *Roseospira marina* sp. nov., *Roseospira navarrensis* sp. nov., and *Roseospira thiosulfatophila* sp. nov. *Arch Microbiol* **178**, 315–324.
- Hansen, G. H. & Sørheim, R. (1991). Improved method for phenotypical characterization of marine bacteria. *J Microbiol Methods* **13**, 231–241.
- Imhoff, J. F., Petri, R. & Suling, J. (1998). Reclassification of species of the spiral-shaped phototrophic purple non-sulfur bacteria of the α-Proteobacteria: description of the new genera *Phaeospirillum* gen. nov., *Rhodovibrio* gen. nov., *Rhodothalassium* gen. nov. and *Roseospira* gen. nov. as well as transfer of *Rhodospirillum fulvum* to *Phaeospirillum fulvum* comb. nov., of *Rhodospirillum molischianum* to *Phaeospirillum molischianum* comb. nov., of *Rhodospirillum salinarum* to *Rhodovibrio salinarum* comb. nov., of *Rhodospirillum sodomense* to *Rhodovibrio sodomensis* comb. nov., of *Rhodospirillum salexigens* to *Rhodothalassium salexigens* comb. nov. and of *Rhodospirillum mediosalinum* to *Roseospira mediosalina* comb. nov. *Int J Syst Bacteriol* **48**, 793–798.
- Jeon, Y.-S., Chung, H., Park, S., Hur, I., Lee, J.-H. & Chun, J. (2005). jPHYDIT: a JAVA-based integrated environment for molecular phylogeny of ribosomal RNA sequences. *Bioinformatics* **21**, 3171–3173.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kawasaki, H., Hoshino, Y., Kuraiki, Y. & Yamasato, K. (1992). *Rhodocista centenaria* gen. nov., sp. nov., a cyst-forming anoxygenic photosynthetic bacterium and its phylogenetic position in the Proteobacteria alpha group. *J Gen Appl Microbiol* **38**, 541–551.
- Khammas, K. M., Ageron, E., Grimont, P. A. D. & Kaiser, P. (1989). *Azospirillum irakense* sp. nov., a nitrogen-fixing bacterium associated with rice roots and rhizosphere soil. *Res Microbiol* **140**, 679–693.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

- Labrenz, M., Tindall, B. J., Lawson, P. A., Collins, M. D., Schumann, P. & Hirsch, P. (2000). *Staleyia guttiformis* gen. nov., sp. nov. and *Sulfitobacter brevis* sp. nov., α -3-Proteobacteria from hypersaline, heliothermal and meromictic antarctic Ekho Lake. *Int J Syst Evol Microbiol* **50**, 303–313.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Liu, C., Wu, Y., Li, L., Ma, Y. & Shao, Z. (2007). *Thalassospira xiamenensis* sp. nov. and *Thalassospira profundimaris* sp. nov. *Int J Syst Evol Microbiol* **57**, 316–320.
- López-López, A., Pujalte, M. J., Benlloch, S., Mata-Roig, M., Rosselló-Mora, R., Garay, E. & Rodríguez-Valera, F. (2002). *Thalassospira lucentensis* gen. nov., sp. nov., a new marine member of the α -Proteobacteria. *Int J Syst Evol Microbiol* **52**, 1277–1283.
- Lyman, J. & Fleming, R. H. (1940). Composition of sea water. *J Mar Res* **3**, 134–146.
- Mack, E. E., Mandelco, L., Woese, C. R. & Madigan, M. T. (1993). *Rhodospirillum sodomense*, sp. nov., a Dead Sea *Rhodospirillum* species. *Arch Microbiol* **160**, 363–371.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Maszenan, A. M., Seviour, R. J., Patel, B. K. C., Janssen, P. H. & Wanner, J. (2005). *Defluviococcus vanus* gen. nov., sp. nov., a novel Gram-negative coccus/coccobacillus in the 'Alphaproteobacteria' from activated sludge. *Int J Syst Evol Microbiol* **55**, 2105–2111.
- Mehnaz, S., Weselowski, B. & Lazarovits, G. (2007). *Azospirillum canadense* sp. nov., a nitrogen-fixing bacterium isolated from corn rhizosphere. *Int J Syst Evol Microbiol* **57**, 620–624.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, K. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Nissen, H. & Dundas, I. D. (1984). *Rhodospirillum salinarum* sp. nov., a halophilic photosynthetic bacterium isolated from a Portuguese saltern. *Arch Microbiol* **138**, 251–256.
- Ostle, A. G. & Holt, J. G. (1982). Nile blue A as fluorescent stain for poly- β -hydroxybutyrate. *Appl Environ Microbiol* **44**, 238–241.
- Peng, G., Wang, H., Zhang, G., Hou, W., Liu, Y., Wang, E. T. & Tan, Z. (2006). *Azospirillum melinis* sp. nov., a group of diazotrophs isolated from tropical molasses grass. *Int J Syst Evol Microbiol* **56**, 1263–1271.
- Pfennig, N., Lünsdorf, H., Süling, J. & Imhoff, J. F. (1997). *Rhodospira trueperi* gen. nov., a new phototrophic Proteobacterium of the alpha group. *Arch Microbiol* **168**, 39–45.
- Poly, F., Monrozier, L. J. & Bally, R. (2001). Improvement in the RFLP procedure for studying the diversity of *nifH* genes in the communities of nitrogen fixers in soil. *Res Microbiol* **152**, 95–103.
- Posada, D. & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.
- Reinhold, B., Hurek, T., Fendrik, I., Pot, B., Gillis, M., Kersters, K., Thielemans, S. & De Ley, J. (1987). *Azospirillum halopraeferens* sp. nov., a nitrogen-fixing organism associated with roots of Kallar Grass (*Leptochloa fusca* (L.) Kunth). *Int J Syst Bacteriol* **37**, 43–51.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schenk, S. U. & Werner, D. (1988). Fatty acid analysis of four *Azospirillum* species reveals three groups. *Arch Microbiol* **149**, 580–582.
- Shi, B. H., Arunpairojana, V., Palakawong, S. & Yokota, A. (2002). *Tistrella mobilis* gen. nov., sp. nov., a novel polyhydroxyalkanoate-producing bacterium belonging to α -Proteobacteria. *J Gen Appl Microbiol* **48**, 335–343.
- Sizova, M. V., Panikov, N. S., Spiridonova, E. M., Slobodova, N. V. & Tourova, T. P. (2007). Novel facultative anaerobic acidotolerant *Telmatospirillum siberiense* gen. nov., sp. nov., isolated from mesotrophic fen. *Syst Appl Microbiol* **30**, 213–220.
- Sly, L. I. & Stackebrandt, E. (1999). Description of *Skermanella parooensis* gen. nov., sp. nov. to accommodate *Conglomeromonas largomobilis* subsp. *parooensis* following the transfer of *Conglomeromonas largomobilis* subsp. *largomobilis* to the genus *Azospirillum*. *Int J Syst Bacteriol* **49**, 541–544.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Suzuki, M., Nakagawa, Y., Harayama, S. & Yamamoto, S. (2001). Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. *Int J Syst Evol Microbiol* **51**, 1639–1652.
- Swofford, D. L. (1998). PAUP—phylogenetic analysis using parsimony, version 4. Sunderland, MA: Sinauer Associates.
- Tarrand, J. J., Krieg, N. R. & Döbereiner, J. (1978). A taxonomic study of the *Spirillum lipoferum* group, with description of a new genus, *Azospirillum* gen. nov., and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov., and *Azospirillum brasiliense* sp. nov. *Can J Microbiol* **24**, 967–980.
- Woese, C. R., Stackebrandt, E., Weisburg, W. G., Paster, B. J., Madigan, M. T., Fowler, V. J., Hahn, C. M., Blanz, P., Gupta, R. & other authors (1984). The phylogeny of purple bacteria: the alpha subdivision. *Syst Appl Microbiol* **5**, 315–326.
- Xie, C.-H. & Yokota, A. (2005). *Azospirillum oryzae* sp. nov., a nitrogen-fixing bacterium isolated from the roots of the rice plant *Oryza sativa*. *Int J Syst Evol Microbiol* **55**, 1435–1438.
- Zhang, D., Yang, H., Zhang, W., Huang, Z. & Liu, S.-J. (2003). *Rhodocista pekingensis* sp. nov., a cyst-forming phototrophic bacterium from a municipal wastewater treatment plant. *Int J Syst Evol Microbiol* **53**, 1111–1114.