

## *Marinobacterium rhizophilum* sp. nov., isolated from the rhizosphere of the coastal tidal-flat plant *Suaeda japonica*

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A Gram-negative, strictly aerobic, marine bacterium, designated strain CL-YJ9<sup>T</sup>, was isolated from sediment closely associated with the roots of a plant (*Suaeda japonica*) inhabiting a coastal tidal flat. Cells of the novel strain were straight and rod-shaped and were motile by means of monopolar flagella. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CL-YJ9<sup>T</sup> belongs to the genus *Marinobacterium* and was most closely related to *Marinobacterium halophilum* mano11<sup>T</sup> (94.1 % sequence similarity) and to other members of the genus *Marinobacterium* (92.5–93.7 % sequence similarity). The strain grew with 1–5 % NaCl (optimum, 3 %) and at 5–30 °C (optimum, approx. 25 °C) and pH 6.0–9.0 (optimum, pH 7.0). The predominant cellular fatty acids were summed feature 3 (C<sub>16:1</sub>ω7c and/or iso-C<sub>15:0</sub> 2-OH, 40.3 %), C<sub>18:1</sub>ω7c (26.6 %), C<sub>16:0</sub> (16.6 %) and C<sub>10:0</sub> 3-OH (7.1 %). The major isoprenoid quinone was Q-8. The G + C content of the genomic DNA was 61 mol%. On the basis of the data from this polyphasic study, strain CL-YJ9<sup>T</sup> belongs to the genus *Marinobacterium* but is distinguishable from the recognized species. Strain CL-YJ9<sup>T</sup> therefore represents a novel species, for which the name *Marinobacterium rhizophilum* sp. nov. is proposed. The type strain is CL-YJ9<sup>T</sup> (=KCCM 42386<sup>T</sup> =DSM 18822<sup>T</sup>).

The genus *Marinobacterium* was first described with the single species *Marinobacterium georgiense*, which was isolated by González *et al.* (1997) from lignin-rich waste from a pulp mill. Later, *Pseudomonas stanieri* (Baumann *et al.*, 1983) and *Oceanospirillum jannaschii* (Bowditch *et al.*, 1984) were reclassified to the genus *Marinobacterium* as *Marinobacterium stanieri* and *Marinobacterium jannaschii* on the basis of phylogenetic analysis of 16S rRNA and *gyrB* genes (Satomi *et al.*, 2002). Recently, *Marinobacterium halophilum* was isolated from a tidal flat area of Korea (Chang *et al.*, 2007).

In this study, strain CL-YJ9<sup>T</sup> was isolated in November 2005 from sediment associated with the roots of a plant (*Suaeda japonica*) growing in a tidal flat of Youngjong Island, Korea; the sediment samples were diluted in sterile seawater and a 0.1 ml aliquot was spread onto marine agar 2216 (MA; Difco). The plate was incubated at 30 °C for 1 week. Strain CL-YJ9<sup>T</sup> was isolated from the plate and repeatedly purified four times on MA at 30 °C. The purified strain was maintained on MA at 4 °C and in

marine broth 2216 (Difco), supplemented with 30 % (v/v) glycerol, at –80 °C.

The 16S rRNA gene was amplified from a single colony by using a PCR with *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified using an AccuPrep PCR purification kit (Bioneer). Sequencing of 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-YJ9<sup>T</sup> (1371 nt) obtained was compared with 16S rRNA gene sequences available in GenBank, using BLASTN (Altschul *et al.*, 1990) searches. The sequence of strain CL-YJ9<sup>T</sup> was manually aligned with the sequences of the four recognized *Marinobacterium* species and of the type species of related genera obtained from GenBank and the Ribosomal Database Project (Cole *et al.*, 2003) databases, using known 16S rRNA secondary structure information. Phylogenetic trees were obtained using 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 using bootstrap analyses based on 1000 replications (for the neighbour-joining and maximum-parsimony approaches)

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CL-YJ9<sup>T</sup> is EF192391.

The cellular fatty acid compositions of strain CL-YJ9<sup>T</sup> and type strains of *Marinobacterium* species are available as supplementary material with the online version of this paper.

or 100 replications (for the maximum-likelihood approach). Alignment analysis was carried out using the jPHYDIT program (Jeon *et al.*, 2005) and phylogenetic analysis was 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 from HPLC analysis (Tamaoka & Komagata, 1984) performed at the Korean Culture Center of Microorganisms, Seoul, Korea.

For phenotypic and chemotaxonomic analyses, strain CL-YJ9<sup>T</sup> was routinely cultivated on YTSS agar (4 g yeast extract, 2.5 g tryptone, 20 g sea salts, 18 g agar and 1 l distilled water; González *et al.*, 1997). Anaerobic growth was tested by using the GasPak anaerobic system in a GasPak anaerobic jar with an anaerobic indicator (BBL) and checking colony formation on MA at 30 °C for 1 month. Cellular morphology and motility were examined by using phase-contrast microscopy (BX50; Olympus) and transmission electron microscopy (EX2; JEOL) with cells from exponentially growing cultures. Gram-staining and catalase, oxidase and amylase activities were assayed as described by Smibert & Krieg (1994) and poly-β-hydroxybutyric acid accumulation was tested as described by Ostle & Holt (1982). Degradation of Tween 80 was tested as described by Hansen & Sørheim (1991). Other enzyme activities were also assayed using API 20NE and API ZYM (bioMérieux) kits according to the manufacturer's instructions, except that cell suspensions were prepared using a 3% (w/v) NaCl solution. Acid production from different carbohydrates was determined by employing the API 50 CH system (bioMérieux) according to the manufacturer's instructions. All suspension media were supplemented with 3% NaCl (final concentration). For the API 20NE, API ZYM and API 50 CH analyses, *M. georgiense* KW-40<sup>T</sup> was employed as a reference strain. The temperature range for growth was determined on the basis of colony formation on YTSS agar incubated at temperatures ranging from 5 to 40 °C (using increments of 5 °C). The tolerance of the isolate to NaCl was determined by using YTSS agar supplemented with various NaCl concentrations (0, 1, 3, 5, 7, 10, 13, 15, 20 and 25%, w/v). The pH range (5–11, using increments of 0.5 pH units) for growth was determined by monitoring changes in the OD<sub>600</sub> over time in YTSS broth (the same composition as YTSS agar but without agar). The final pH was adjusted using NaOH and HCl solutions. Carbon utilization was tested on basal agar medium [Tris/HCl, 50 mM, pH 7.5; NH<sub>4</sub>Cl, 19 mM; K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 0.33 mM; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.1 mM; 1.5% Bacto agar (Difco) in artificial seawater (NaCl, 400 mM; MgSO<sub>4</sub>·7H<sub>2</sub>O, 100 mM; KCl, 20 mM; CaCl<sub>2</sub>·2H<sub>2</sub>O, 20 mM)] containing 0.2% carbon source, as described by Baumann & Baumann (1981) but with the slight modification that artificial seawater was used instead of half-strength artificial seawater. Growth was scored as negative when it was equal to or less than that in the negative control (lacking a carbon source) after 7 days incubation at 30 °C. All

of the experiments were performed under aerobic conditions.

Isoprenoid quinones were extracted according to Minnikin *et al.* (1984) and analysed by HPLC as described by Collins (1985). For whole-cell fatty acid analyses, the strain was grown on MA at 25 °C for 5 days. Fatty acid extraction, conversion to methyl esters, gas chromatographic analysis and identification were performed at the Korean Culture Center of Microorganisms according to the protocols of the Microbial Identification System (Microbial ID).

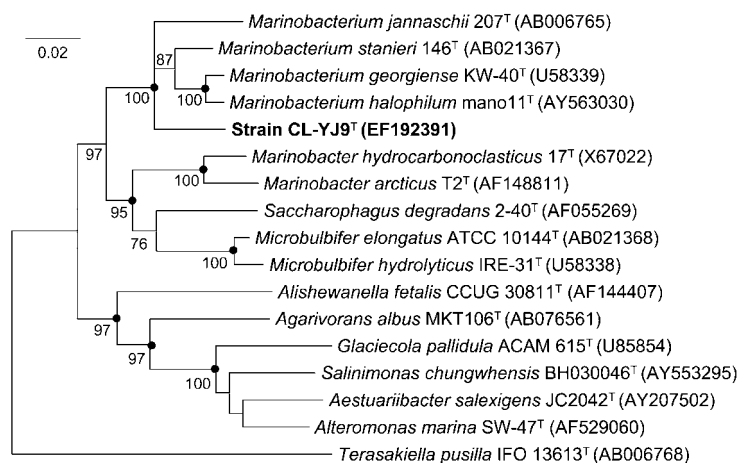
Cells of strain CL-YJ9<sup>T</sup> were found to be Gram-negative, rod-shaped, strictly aerobic, approximately 0.3–0.4 μm wide and 0.6–0.8 μm long and motile by means of monopolar flagella. The strain grew with 1–5% NaCl (optimum, 3%) and at 5–30 °C (optimum, approx. 25 °C) and pH 6.0–9.0 (optimum, pH 7.0). The results of the other biochemical and physiological tests are given in Table 1 and in the species description. The major isoprenoid quinone in strain CL-YJ9<sup>T</sup> was Q-8. The major fatty acids were summed feature 3 (C<sub>16:1</sub>ω7c and/or iso-C<sub>15:0</sub> 2-OH, 40.3%), C<sub>18:1</sub>ω7c (26.6%), C<sub>16:0</sub> (16.6%) and C<sub>10:0</sub> 3-OH (7.1%) (see Supplementary Table S1, available in IJSEM Online). Strain CL-YJ9<sup>T</sup> was most closely related to *M. halophilum* mano11<sup>T</sup> (94.1% sequence similarity) followed by other members of the genus *Marinobacterium* (92.5–93.7% sequence similarity).

**Table 1.** Differential characteristics of strain CL-YJ9<sup>T</sup> and type strains of *Marinobacterium* species

Strains: 1, CL-YJ9<sup>T</sup> (this study); 2, *M. halophilum* mano11<sup>T</sup> (Chang *et al.*, 2007); 3, *M. georgiense* KW-40<sup>T</sup> (González *et al.*, 1997); 4, *M. stanieri* 146<sup>T</sup> (Baumann *et al.*, 1983); 5, *M. jannaschii* 207<sup>T</sup> (Bowditch *et al.*, 1984). All strains are Gram-negative, rod-shaped, strictly aerobic, motile, catalase-positive, gelatinase-negative, lipase-negative (data from this study for lipase activity for *M. georgiense* KW-40<sup>T</sup>) and unable to utilize lactose. +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5
Growth at 35 °C	–	+	+	+	–
Poly-β-hydroxybutyrate accumulation	+	ND	–*	+	+
Nitrate reduction	–	–	–	–*	+
Hydrolysis of:					
Starch	+	+	–	–	–
Tween 80	+	+	+	ND	ND
Utilization of:					
Citrate	–	+	+	+	+
Glycine	+	–	–	–	+
N-Acetyl-D-glucosamine	+	–	–	–	–
Glucose	+	+	+	–	–
L-Arginine	+	–	–	–	+
Mannose	+	+	+	–	–
DNA G+C content (mol%)	61	ND	55	55–57	56–57

\*Positive according to Satomi *et al.* (2002).



**Fig. 1.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain CL-YJ9<sup>T</sup>, members of the genus *Marinobacterium* and other related genera. Bootstrap percentages >60% (based on 1000 resamplings) are shown at branching points. Solid circles indicate that the corresponding nodes are also recovered in the maximum-likelihood and maximum-parsimony trees. *Terasakiella pusilla* IFO 13613<sup>T</sup> was used as an outgroup. Bar, 0.02 nucleotide substitutions per site.

Phylogenetic analysis of the 16S rRNA gene sequence revealed that strain CL-YJ9<sup>T</sup> formed a robust cluster with the members of the genus *Marinobacterium* (Fig. 1): thus, it is clear that our isolate belongs to this genus. However, the low levels of sequence similarity (<94.1%) between strain CL-YJ9<sup>T</sup> and the recognized species of the genus *Marinobacterium* indicated that strain CL-YJ9<sup>T</sup> represents a novel species of the genus (Stackebrandt & Goebel, 1994; Rosselló-Mora & Amann, 2001). There were differences in DNA G + C content between strain CL-YJ9<sup>T</sup> (61 mol%) and existing species of the genus *Marinobacterium* (55–57 mol%; no data are available for *M. halophilum*) (Table 1). Furthermore, strain CL-YJ9<sup>T</sup> can be differentiated from species of the genus *Marinobacterium* from the utilization patterns for some carbon sources (citrate and *N*-acetyl-D-glucosamine; Table 1). In conclusion, on the basis of the data from this polyphasic study, strain CL-YJ9<sup>T</sup> represents a novel species of the genus *Marinobacterium*, for which the name *Marinobacterium rhizophilum* sp. nov. is proposed.

### Description of *Marinobacterium rhizophilum* sp. nov.

*Marinobacterium rhizophilum* (rhi.zo.phi'lum. Gr. n. *rhiza* root; Gr. adj. *philos* loving; N.L. neut. adj. *rhizophilum* root-loving).

Cells are Gram-negative, strictly aerobic, straight rods, approximately 0.3–0.4 µm wide and 0.6–0.8 µm long, and motile by means of monopolar flagella. Colonies on MA are circular, smooth, entire, convex and creamy white. After 3 days on MA at 30 °C, colonies are approximately 1.0–3.0 mm in diameter. Growth occurs at temperatures in the range 5–30 °C (optimally at approx. 25 °C) and at pH 6.0–9.0 (optimum, pH 7.0). Growth occurs at NaCl concentrations of 1–5% (optimally at 3%, w/v). Catalase, oxidase, amylase and Tween 80 hydrolysis are present. In the API 20NE system, nitrate reductase, gelatinase, β-galactosidase (PNPG), urease, aesculin hydrolysis and indole production are absent. In the API ZYM system, alkaline and acid phosphatases, esterase (C4), leucine

arylamidase, naphthol phosphohydrolase and α-glucosidase activities are present, whereas esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidases, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. In the API 50CH system, acids are produced from L-arabinose, D-adonitol, D-galactose, D-glucose, D-fructose, inositol, D-mannitol, D-sorbitol, maltose, sucrose, raffinose, turanose, D-fucose, L-fucose, D- and L-arabitol and potassium 5-ketogluconate, are produced weakly from glycerol, methyl β-D-xylopyranoside, L-rhamnose, methyl α-D-glucopyranoside, trehalose, inulin, melezitose and xylitol and are not produced from erythritol, D-arabinose, D-ribose, D- or L-xylose, D-mannose, L-sorbose, dulcitol, methyl α-D-mannopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, D-lactose, melibiose, starch, glycogen, gentiobiose, D-lyxose, D-tagatose, potassium gluconate or potassium 2-ketogluconate. Utilizes L-arginine, L-asparagine, betaine, D-galactose, L- and D-leucine, glucose, glutamic acid, glycine, mannose, *N*-acetyl-D-glucosamine, L-arabinose, D-fructose, glycerol, mannitol, trehalose and sucrose as sole carbon sources, but does not utilize citrate, dimethyl sulfide, lactose, L-cysteine, maleic acid, *p*-hydroxyphenylacetate, succinate or urea. Major isoprenoid quinone is Q-8. Fatty acids comprising >1% of the total are summed feature 3 (C<sub>16:1</sub>ω7c and/or iso-C<sub>15:0</sub> 2-OH, 40.3%), C<sub>18:1</sub>ω7c (26.6%), C<sub>16:0</sub> (16.6%), C<sub>10:0</sub> 3-OH (7.1%), C<sub>12:0</sub> (4.0%) and C<sub>10:0</sub> (3.9%). The DNA G + C content of the type strain is 61 mol%.

The type strain, CL-YJ9<sup>T</sup> (=KCCM 42386<sup>T</sup> =DSM 18822<sup>T</sup>), was isolated from the rhizosphere of a coastal plant (*Suaeda japonica*) inhabiting a tidal flat of Youngjong Island, Korea.

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