Marinobacter mobilis sp. nov. and Marinobacter zhejiangensis sp. nov., halophilic bacteria isolated from the East China Sea

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Three Gram-negative, aerobic, motile, halophilic, rod-shaped strains (CN46T, CN71 and CN74T) were isolated from sediment of the East China Sea and subjected to a polyphasic taxonomic study. Strains CN46T and CN71 had identical 16S rRNA gene sequences and phenotypic characteristics. Strain CN46T was moderately halophilic. Growth of strain CN46T was observed between 0.5 and 10.0 % (w/v) NaCl (optimal growth at 3.0–5.0 %) and between pH 6.5 and 9.0. Strain CN74T grew over a wider range of pH (pH 6.0–9.5); the optimum NaCl concentration for growth was 1.0–3.0 %.

The major fatty acids of strain CN46T were C16 : 1ω9c, C16 : 0 and C12 : 0, whereas strain CN74T contained C16 : 0, C16 : 1ω9c, C18 : 1ω9c and C12 : 0. The DNA G+C contents of the three isolates were between 58.0 and 58.9 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strains CN46T, CN71 and CN74T grouped together within the cluster of Marinobacter species. 16S rRNA gene sequence similarities of the three strains with the type strains of Marinobacter species ranged from 94.0 to 97.1 %.

The DNA–DNA hybridization values of strain CN74T with strains CN46T and CN71 were 35.0 and 36.0 %, respectively. Levels of DNA–DNA relatedness between strains CN46T and CN74T and Marinobacter pelagius CGMCC 1.6775T, Marinobacter gudaonensis CGMCC 1.6294T and Marinobacter koreensis DSM 17924T were 15.3–45.2 %. The results of DNA–DNA hybridizations, fatty acid analysis, and physiological and biochemical tests allowed genotypic and phenotypic differentiation of the isolates from closely related species. Two novel species are proposed, named Marinobacter mobilis sp. nov. (type strain CN46T = CGMCC 1.7059T = JCM 15154T) and Marinobacter zhejiangensis sp. nov. (type strain CN74T = CGMCC 1.7061T = JCM 15156T).

The genus Marinobacter, which belongs to the family Alteromonadaceae, class Gammaproteobacteria, was first proposed by Gauthier et al. (1992) to accommodate aerobic, halophilic, rod-shaped bacteria that are capable of degrading a variety of hydrocarbons. The type species, Marinobacter hydrocarbonoclasticus, was isolated from seawater near a petroleum refinery. Over the past few years, a further 19 Marinobacter species have been described (Euzeby, 1997). Most of them have been isolated from saline environments, including seawater (Yoon et al., 2003, 2004; Shivaji et al., 2005), marine sediment (Gorschkova et al., 2003; Romanenko et al., 2005; Guo et al., 2007), saline soil (Martin et al., 2003; Gu et al., 2007), sea sand (Kim et al., 2006), a brine–seawater interface (Antunes et al., 2007), a coastal hot spring (Shieh et al., 2003) and a wastewater pond (Liebgott et al., 2006), although some have been isolated from animal tissue (Romanenko et al., 2005) and algae (Green et al., 2006). Three strains, CN46T, CN71 and CN74T, were isolated from sediment of the East China Sea. The aim of this study was to determine whether these isolates represent novel species within the genus Marinobacter by a polyphasic approach.

The sediment sample was collected by a multicorer from the East China Sea (27° 19’ 57” N 120° 34’ 29” E).
Approximately 100 mg sample was suspended in 3 ml sterile seawater and vortexed for 15 min. The dispersed sediment suspension was diluted and added to modified ZoBell medium (ZoBell, 1941). The modified ZoBell medium contained (per l distilled water): 19.45 g NaCl, 8.8 g MgCl₂, 3.24 g Na₂SO₄, 1.8 g CaCl₂, 0.55 g KCl, 0.16 g NaHCO₃, 0.1 g ferric citrate, 0.08 g KBr, 34 mg CsCl₂, 22 mg H₂BO₃, 4.0 mg Na₂SiO₃, 2.4 mg NaF, 1.6 mg NH₂NO₃, 8.0 mg Na₃PO₄, 0.5 g peptone (Difco) and 0.1 g yeast extract (Difco); pH 7.4. After 3 days of aerobic incubation at 25°C, three colonies, named CN46T, CN74 and CN74T, were picked. All strains were purified by repeated restreaking and maintained on halophilic medium (HM) (Ventosa et al., 1982) at 30°C. The HM medium contained (per 1 distilled water): 40.0 g NaCl, 2.0 g KCl, 1.0 g MgSO₄, 0.36 g CaCl₂·2H₂O, 0.23 g NaBr, 0.06 g NaHCO₃, trace FeCl₃, 10.0 g yeast extract (Difco), 5.0 g peptone (Difco), 1.0 g glucose; pH 7.2.

The optimal conditions for growth were determined in HM with different salt concentrations (0, 0.5, 1, 3, 5, 7.5, 10, 15 and 20%, w/v). The pH range for growth was determined by adding MES (pH 5.0–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5), CAPSO (pH 9.0–9.5) or CAPS (pH 10.0–10.5) to HM at a concentration of 40 mM. The temperature range for growth was determined by incubating cultures at 4–48°C. Cell morphology and motility were examined by optical microscopy (Olympus BX40) and transmission electron microscopy (JEM-1230). Strains CN46T and CN74T were motile by a polar flagellum (see Supplementary Fig. S1 available in IJSEM Online).

Physiological and biochemical characteristics were determined using previously described methods (Xu et al., 2008; Mata et al., 2002). Susceptibility to antibiotics was detected on HM plates by using antibiotic discs containing the following: amoxicillin (10 μg), ampicillin (10 μg), bacitracin (0.04 IU), carbenicillin (100 μg), cefoxitin (30 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), nitrofurantoin (300 μg), novobiocin (30 μg), nystatin (100 μg), penicillin (10 μg), polymyxin B (300 IU), streptomycin (10 μg), tobramycin (10 μg) and tetracycline (30 μg). Detailed results are given in the species descriptions.

Fatty acid methyl esters, prepared from lipids that had been extracted from cells grown in HM plates for 48 h at 30°C, were analysed by using GC-MS (Kuykendall et al., 1983), using a Beckman DU 800 spectrophotometer. The tests were carried out in triplicate. The DNA–DNA hybridization values of strain CN74T with CN46T and CN71 were 35.0 and 36.0%, respectively. DNA–DNA hybridization values between strain CN46T and the type strains of 

Marinobacter pelagius, 

Marinobacter gudaonensis, 

and 36.0 %, respectively. DNA–DNA hybridization values of strain CN74T to 

Marinobacter pelagius 

Marinobacter koreensis 

d NA contents were determined by thermal denaturation and renaturation method of De Ley et al. (1970) as modified by HuB et al. (1983), using a Beckman DU 800 spectrophotometer. The tests were carried out in triplicate. The DNA–DNA hybridization values of strain CN74T with CN46T and CN71 were 35.0 and 36.0%, respectively. DNA–DNA hybridization values between strain CN46T and 

Marinobacter pelagius CGMCC 1.6775T, 

M. gudaonensis CGMCC 1.6294T and 

M. koreensis DSM 17924T were 32.6, 45.2 and 37.0%, respectively. The DNA–DNA hybridization values of strain CN74T to 

Marinobacter pelagius CGMCC 1.6775T, 

M. gudaonensis CGMCC 1.6294T and 

M. koreensis DSM 17924T were 15.3, 32.5 and 31.0%,
Table 1. Differential phenotypic characteristics of strains CN46^T and CN74^T and the type strains of related Marinobacter species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<tbody>
<tr>
<td>Colony pigmentation</td>
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<td>Cream</td>
<td>Cream</td>
<td>Cream</td>
<td>Off-white</td>
<td>White</td>
<td>White</td>
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<td>Range for growth</td>
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<td>0.5–10.0</td>
<td>0.5–15.0</td>
<td>0.5–20.0</td>
<td>0–15</td>
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<td>6.0–9.5</td>
<td>6–9</td>
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<td>Nitrate reduction</td>
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<tr>
<td>Lecithinase</td>
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<td>+</td>
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<td>Utilization of:</td>
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<td>Cellobiose</td>
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<td>–</td>
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<td>+</td>
<td>ND</td>
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<td>Succinate</td>
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<td>+</td>
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<td>–</td>
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<td>ND</td>
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<tr>
<td>Sucrose</td>
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<td>–</td>
<td>+</td>
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<tr>
<td>DNA G + C content (mol%)</td>
<td>58.0–58.9</td>
<td>58.4</td>
<td>59.0</td>
<td>54.1</td>
<td>57.9</td>
<td>63.5</td>
<td>59.6</td>
<td>52.7</td>
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</table>

*All values were determined using the T_m method except that of M. koreensis DSM 17924^T, which was determined by HPLC.

respectively. All the values are sufficiently low to classify strains CN46^T and CN74^T as representatives of two genotypically distinct species within the genus Marinobacter.

Based on differential phenotypic properties, as well as 16S rRNA gene sequence analysis and DNA–DNA hybridization data, it is concluded that strains CN46^T and CN74^T represent two novel species within the genus Marinobacter.

Description of Marinobacter mobilis sp. nov.

Marinobacter mobilis (mo’bi.lis. L. masc. adj. mobilis motile).

Cells are Gram-negative and motile by a polar flagellum. Young cultures show rod-like cells (1.5–3.0 \times 0.5–0.8 \mu m). Colonies on HM agar are 1–2 mm in diameter, circular, smooth, elevated and transparent after 48 h at 30 °C. Moderately halophilic. No growth occurs in the absence of salt. Growth occurs at NaCl concentrations of 0.5–10.0% (w/v), with optimum growth at 3.0–5.0%.

Grows at pH 6.5–9.0 and 15–42 °C (optimum growth at pH 7.0–7.5 and 30–35 °C). Oxidase- and catalase-positive. Nitrate is reduced. Tweens 20 and 80 are hydrolysed. Aesculin, casein, DNA, gelatin, starch and tyrosine are not hydrolysed. Lecithinase-positive. Negative for gluconate oxidation, indole production, o-nitrophenyl-β-D-galactopyranosidase and urease. H$_2$S is produced from thiosulfate.

The following substrates are utilized for growth: acetate, glutamate, L-isoleucine, lactate, malate, propionate, pyruvate, succinate and valine. The following compounds are not utilized as sole carbon sources: L-alanine, L-arabinose, L-arginine, cellobiose, citrate, L-cysteine, ethanol, formate, D-fructose, fumarate, D-galactose, gluconate, glucose, glycerol, glycine, L-histidine, myo-inositol, lactose, lysine, malonate, maltose, mannitol, D-mannose, L-methionine, raffinose, rhamnose, ribose, L-serine, sorbitol, L-sorbose, sucrose, trehalose, tyrosine and D-xylose. Acid is not produced from L-arabinose, D-fructose, D-galactose, glucose, myo-inositol, lactose, malate, mannitol, D-mannose, rhamnose, sorbitol, L-sorbose, sucrose, trehalose or xylose.

Susceptible to amoxicillin, ampicillin, carbenicillin, cefoxitin, ceftiraxone, chloramphenicol, erythromycin, nitrofurantoin, novobiocin, penicillin, polymyxin B, tobramycin.
and tetracycline, but not to bacitracin, nystatin or streptomycin. Major fatty acids are C\text{16:1\omega9c}, C\text{16:0} and C\text{12:0}. The DNA G+C content is 58.0–58.9 mol\% (Tm).

The type strain is CN46\textsuperscript{T} (=CGMCC 1.7059\textsuperscript{T} =JCM 15154\textsuperscript{T}), isolated from a marine sediment sample, Zhejiang, China. Strain CN71, a reference strain, was isolated from the same source.

**Description of Marinobacter zhejiangensis sp. nov.**

*Marinobacter zhejiangensis* (zhe.ji.ang.en’si.s. N.L. masc. adj. *zhejiangensis* pertaining to Zhejiang province in China, where the type strain was isolated).

Cells are Gram-negative and motile. Young cultures show rod-like cells (1.0–2.5 × 0.4–0.8 μm), occurring singly or in pairs. Colonies on HM agar are 2–3 mm in diameter, circular and slightly irregular, elevated, semitransparent and cream-coloured after 48 h at 30°C. No growth occurs in the absence of salt. Growth occurs at NaCl concentrations of 0.5–10.0% (w/v), with optimum growth at 1.0–3.0%. Grows at pH 6.0–9.5 and 15–42°C (optimum growth at pH 7.0–7.5 and 30–35°C). Oxidase- and catalase-positive. Nitrate is reduced. Tweens 20 and 80 are hydrolysed. Aesculin, casein, sucrose. Susceptible to amoxicillin, ampicillin, carbenicillin, cefotaxime, ceftriaxone, chloramphenicol, erythromycin, nitrofurantoin, novobiocin, penicillin, polymyxin B, tobramycin and tetracycline, but not to bacitracin, nystatin or streptomycin. Major fatty acids are C\text{16:1\omega9c}, C\text{16:0} and C\text{12:0}. The DNA G+C content of the type strain is 58.4 mol\% (Tm).

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**References**


