Marinobacter pelagius sp. nov., a moderately halophilic bacterium

Xue-Wei Xu,[1,2,3] Yue-Hong Wu,[3] Chun-Sheng Wang,[1,2] Jun-Yi Yang,[1,2] Aharon Oren[4] and Min Wu[3]

Correspondence
Min Wu
wumin@zju.edu.cn

[1]Laboratory of Marine Ecosystem and Biogeochemistry, State Oceanic Administration, Hangzhou 310012, People's Republic of China

[2]Second Institute of Oceanography, State Oceanic Administration, Hangzhou 310012, People's Republic of China

[3]College of Life Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China

[4]Institute of Life Sciences, and the Moshe Shilo Minerva Center for Marine Biogeochemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

A Gram-negative, aerobic, neutrophilic, moderately halophilic bacterial strain, HS225^T, was isolated from seawater samples around the Zhoushan Archipelago, Zhejiang Province, China. The isolate grew optimally in media with 5.0 % NaCl, at pH 7.0–8.0 and at 25–30 °C. The predominant fatty acids were $C_{16:0}\omega9c$, $C_{16:0}$, $C_{12:0}$ 3-OH and $C_{18:1}\omega9c$. The genomic DNA G+C content was 59.0 mol%. Based on 16S rRNA gene sequence analysis, the isolate was found to be affiliated to the genus *Marinobacter*. Strain HS225^T exhibited closest phylogenetic affinity to *Marinobacter koreensis* DD-M3^T (98.1 % 16S rRNA gene sequence similarity). DNA–DNA relatedness data and DNA G+C contents as well as physiological and biochemical test results allowed the genotypic and phenotypic differentiation of strain HS225^T from closely related species. Therefore, it is proposed that strain HS225^T represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter pelagius* sp. nov. is proposed. The type strain is HS225^T (=CGMCC 1.6775^T =JCM 14804^T)

The genus *Marinobacter* was first proposed by Gauthier *et al.* (1992) with the description of *Marinobacter hydrocarbonoclasticus*. This organism, isolated from Mediterranean seawater taken near a petroleum refinery, was aerobic, extremely halotolerant and hydrocarbon-degrading. A second species of the genus, *Marinobacter aquaeolei*, was proposed by Huu *et al.* (1999) to accommodate a moderately halophilic bacterium that was isolated from an oil-producing well at an offshore platform in southern Vietnam. *M. aquaeolei*, however, was later suggested to be a heterotypic synonym of *M. hydrocarbonoclasticus* (Márquez & Ventosa, 2005). At the time of writing, the genus *Marinobacter* comprises a further 14 recognized species, namely *Marinobacter litoralis* (Yoon *et al.*, 2003), *M. lutaoensis* (Shieh *et al.*, 2003), *M. lipolyticus* (Martín *et al.*, 2003), *M. excellens* (Gorshkova *et al.*, 2003), *M. flavimaris* and *M. daepoensis* (Yoon *et al.*, 2004), *M. bryozoorum* and *M. sediminum* (Romanenko *et al.*, 2005), *M. maritimus* (Shivaji *et al.*, 2005), *M. algicola* (Green *et al.*, 2006), *M. vinifirmus* (Liebgott *et al.*, 2006), *M. koreensis* (Kim *et al.*, 2006), *M. gudaonensis* (Gu *et al.*, 2007) and *M. salsuginis* (Antunes *et al.*, 2007). Here, we describe a moderately halophilic bacterium that represents a novel species of the genus *Marinobacter*.

Strain HS225^T was isolated from coastal seawater samples collected from the Zhoushan Archipelago, Zhejiang Province, China. The marine environment of the Zhoushan Archipelago was described previously (Xu *et al.*, 2007a). Halophilic medium (HM) used for isolation and maintenance of the strains was that described by Ventosa *et al.* (1982). The medium contained (per litre distilled water): 40.0 g NaCl, 2.0 g KCl, 1.0 g $MgSO_4$, 0.36 g $CaCl_2.2H_2O$, 0.23 g NaBr, 0.06 g $NaHCO_3$, trace $FeCl_3$, 10.0 g yeast extract (Difco), 5.0 g peptone (Difco) and 1.0 g glucose (pH 7.2). Water samples were filtered through 0.45- and 0.22-μm filters in sequence, and the 0.22-μm membranes were then suspended in HM broth. After vigorous shaking for 15 min, liquids were plated by using a tenfold dilution series method. Plates were incubated aerobically at 25 °C. After 3–7 days inoculation, representative colonies were picked and maintained at

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HS225^T is DQ458821.

A scanning electron micrograph of cells of strain HS225^T, phylogenetic trees based on 16S rRNA gene sequences according to the maximum-parsimony and maximum-likelihood methods and a table detailing the fatty acid compositions of *Marinobacter* strains are available as supplementary material with the online version of this paper.

30 °C. Strains were purified by repeated restreaking; purity was confirmed based on uniformity of colony morphology. Cell morphology and motility were examined by optical microscopy (Olympus BX40) and scanning electron microscopy (Cambridge S260) (see Supplementary Fig. S1 available in IJSEM Online).

The optimal conditions for growth were determined in HM with different NaCl concentrations (0, 0.5, 1, 3, 5, 7.5, 10, 15, 20, 25 and 30 %, 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) and CHES (pH 9.0–10.0) to HM at a concentration of 25 mM. The temperature range for growth was determined by incubating over the range 4–48 °C.

Single carbon source assimilation tests were performed by using a minimal medium (Shivaji *et al.*, 2005). This medium contained (per litre distilled water): 40.0 g NaCl, 6.15 g $MgCl_2$, 0.75 g KCl, 1.45 g $CaCl_2$, 1.0 g NH_4Cl, 0.075 g K_2HPO_4, 0.028 g $FeSO_4$ and 25 mM PIPES/NaOH (pH 7.2). The corresponding filter-sterilized sugar (0.2 %), alcohol (0.2 %), organic acid (0.1 %) or amino acid (0.1 %) was added to liquid medium. Acid production was investigated by using modified MOF medium supplemented with 1 % sugars or alcohols (Leifson, 1963). The medium contained (per litre distilled water): 40.0 g NaCl, 2.5 g $MgCl_2 . 2H_2O$, 1.0 g $MgSO_4 . 7H_2O$, 0.5 g KCl, 0.25 g $CaCl_2$, trace $FeSO_4$, 0.5 g $(NH_4)_2SO_4$, 1.0 g casitone (Difco), 0.1 g yeast extract (Difco), 0.5 g Tris and 0.01 g phenol red (pH 7.5). The substrates used for single carbon source assimilation and acid production tests are listed in the species description below.

Phenotypic characteristics, including oxidase and catalase reactions, H_2S production, hydrolysis of aesculin, gelatin, casein, DNA, starch, Tweens 20 and 80, tyrosine and urea, indole production, phenylalanine deamination and antimicrobial susceptibility, were tested in HM according to the methods given in Mata *et al.* (2002) as described by Xu *et al.* (2007b). Sensitivity to antimicrobial agents was determined in HM containing each antimicrobial agent at 50 mg l^{-1} for at least 3 days. Antimicrobial agents used were ampicillin, bacitracin, carbenicillin, cefotaxime, chloramphenicol, erythromycin, kanamycin, nalidixic acid, neomycin, nitrofurantoin, novobiocin, nystatin, penicillin, polymyxin B, rifampicin, streptomycin and tetracycline. *M. koreensis* DSM 17924^T and *M. gudaonensis* CGMCC 1.6294^T were used as controls in these tests. Detailed results are given in the species description.

The 16S rRNA gene of strain HS225^T was amplified as described by Xu *et al.* (2007b). The sequence was compared with closely related sequences of reference organisms from the FASTA network service. Sequence data were aligned with CLUSTAL W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed by using the neighbour-joining and maximum-parsimony methods with the MEGA 3 program package (Kumar *et al.*, 2004) and the maximum-likelihood method with the PHYLIP 3.6 program. Fatty acid methyl esters were obtained from cells grown in HM for 36 h at 30 °C and analysed by using GC/MS (Kuykendall *et al.*, 1988). The DNA G+C content was determined by thermal denaturation (T_m) (Marmur & Doty, 1962) with *Escherichia coli* K-12 DNA as a calibration standard. DNA–DNA hybridizations were performed by the thermal denaturation and renaturation method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983), by using a Beckman DU 800 spectrophotometer.

16S rRNA gene sequence analysis indicated that strain HS225^T clustered within the genus *Marinobacter* (Fig. 1; see also Supplementary Figs S2 and S3). Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that strain HS225^T formed a coherent cluster with *M. koreensis* DD-M3^T with high bootstrap resampling values (98 % by the neighbour-joining method and 94 % by the maximum-parsimony method). Strain HS225^T exhibited highest 16S rRNA gene sequence similarity to the type strains of *M. koreensis* and *M. gudaonensis* (98.1 and 97.7 %, respectively). 16S rRNA gene sequence similarity between strain HS225^T and the type strains of other recognized *Marinobacter* species, however, was below 97.0 %. Several phenotypic properties, such as growth and biochemical characteristics, were similar between strain HS225^T and recognized *Marinobacter* species, but many differences, especially hydrolysis of certain substrates, allowed them to be distinguished from each other (Table 1).

The major fatty acids of strain HS225^T were $C_{16:0}\omega 9c$ and $C_{16:0}$. These fatty acids were also predominant components of *M. gudaonensis* CGMCC 1.6294^T and *M.*

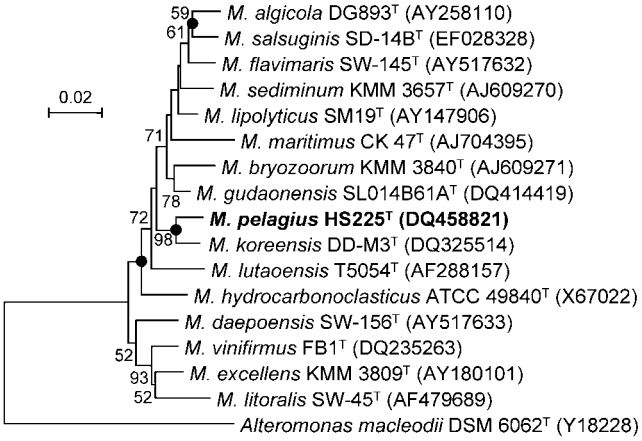

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of strain HS225^T and members of the genus *Marinobacter*. Bootstrap values are percentages based on 1000 replicates; only values >50 % are shown. Dots indicate branches of the tree that were also formed with the maximum-parsimony and maximum-likelihood methods (see Supplementary Figs S2 and S3). Bar, 0.02 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics between strain HS225^T and related *Marinobacter* species

Taxa: 1, strain HS225^T; 2, *M. koreensis* DSM 17924^T; 3, *M. gudaonensis* CGMCC 1.6294^T (data in columns 1–3 from this study unless indicated); 4, *M. algicola*; 5, *M. bryozoorum*; 6, *M. hydrocarbonoclasticus*; 7, *M. lipolyticus*; 8, *M. maritimus*; 9, *M. sediminum*. Data for reference species were derived from Gauthier *et al.* (1992), Martín *et al.* (2003), Romanenko *et al.* (2005), Shivaji *et al.* (2005), Green *et al.* (2006), Kim *et al.* (2006) and Gu *et al.* (2007). +, Positive; −, negative; ND, no data available. DNA G+C contents were determined by the T_m method unless indicated.

Characteristic	1	2	3	4	5	6	7	8	9
NaCl growth range (%)	0.5–15	0.5–20	0–15	1–12	1–18	0.5–20	1–15	1–13	0.5–18
Growth temperature range (°C)	4–48	10–45	10–45	5–40	7–42	10–45	10–41	4–37	4–42
Nitrate reduction	+	+	+	−	+	+	−	−	−
Urease	−	−	−	+	−	−	−	+	−
Amylase	−	−	+	+	−	−	−	−	−
Hydrolysis of Tween 80	+	+	+	+	−	ND	+	+	+
Utilization of:									
L-Arabinose	−	−	−	−	+	−	−	ND	+
Cellobiose	−	−	−	−	+	−	−	−	+
D-Fructose	−	−	+	+	−	−	+	+	ND
Glucose	−	−	+	+	−	−	+	ND	+
Maltose	−	−	+	+	+	ND	+	−	+
Mannitol	−	−	−	+	+	−	+	+	+
Sucrose	−	−	+	−	+	−	−	−	+
Glycerol	−	−	+	+	+	−	−	−	+
Acetate	+	+	+	+	−	+	−	ND	−
L-Alanine	+	−	+	+	−	−	−	+	−
Citrate	−	−	+	+	−	+	−	+	−
Gluconate	−	−	−	+	+	−	+	−	+
Glutamate	+	+	+	+	−	+	−	−	−
Succinate	+	−	+	+	−	+	−	−	ND
DNA G+C content (mol%)	59.0	54.1 (HPLC)[a*]	57.9[b]	55	59.6	52.7	57.0	58.0	56.5

*Data from: *a*, Kim *et al.* (2006); *b*, Gu *et al.* (2007).

koreensis DSM 17924^T (see Supplementary Table S1). However, $C_{17:0}$ was detected in strain HS225^T but not in *M. koreensis* DSM 17924^T. The DNA G+C content of strain HS225^T (59.0 mol%, by T_m) was notably higher than that of *M. koreensis* DD-M3^T (54.1 mol%, by HPLC) (Kim *et al.*, 2006). DNA–DNA hybridization experiments were carried out at 76 °C. Levels of DNA–DNA relatedness between strain HS225^T and *M. koreensis* DSM 17924^T and *M. gudaonensis* CGMCC 1.6294^T were 46 and 44 %, respectively. Therefore, based on 16S rRNA gene sequence analysis, as well as DNA–DNA hybridization data and differential phenotypic properties, we suggest that strain HS225^T represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter pelagius* sp. nov. is proposed.

Description of *Marinobacter pelagius* sp. nov.

Marinobacter pelagius (pe.la′gi.us. L. masc. adj. *pelagius* of the sea, marine).

Cells are Gram-negative and motile. Young cultures show rod-like cells (2.0–4.0 × 0.4–0.8 μm), occurring singly or in pairs. Colonies are 1–2 mm in diameter, smooth, circular, elevated and cream coloured after 48 h at 30 °C. Moderately halophilic. No growth occurs in the absence of salt. Growth occurs at NaCl concentrations of 0.5–15 % (w/v), with optimum growth at 5.0 % NaCl. The pH and temperature ranges for growth are pH 6.0–9.0 and 4–48 °C (optimum growth at pH 7.0–8.0 and 25–30 °C). Oxidase- and catalase-positive. Nitrate is reduced. Tweens 20 and 80 are hydrolysed. Aesculin, casein, DNA, gelatin, starch, tyrosine and urea are not hydrolysed. Tests for indole production and phenylalanine deamination are negative. H_2S is produced from thiosulfate. Chemo-organotrophic. The following substrates are utilized for growth: acetate, L-alanine, fumarate, L-glutamate, L-isoleucine, lactate, malate, propionate, pyruvate, succinate and L-valine. The following compounds are not utilized as sole carbon sources: L-arabinose, L-arginine, L-aspartate, cellobiose, citrate, L-cysteine, ethanol, formate, D-fructose, D-galactose, gluconate, glucose, glycerol, glycine, L-histidine, *myo*-inositol, lactose, malonate, maltose, mannitol, D-mannose, L-methionine, L-ornithine, raffinose, ribose, L-serine, L-sorbitol, sorbose, starch, sucrose and xylose. Acid is not produced from the following carbohydrates: L-arabinose, D-fructose, D-galactose, glucose, *myo*-inositol, lactose, maltose, mannitol, D-mannose, L-sorbitol, sorbose, sucrose or trehalose. Susceptible to ampicillin, carbenicillin,

cefotaxime, chloramphenicol, erythromycin, nalidixic acid, nitrofurantoin, penicillin, polymyxin B, novobiocin and tetracycline, but not to bacitracin, kanamycin, neomycin, nystatin, rifampicin or streptomycin. Principal fatty acids are $C_{16:0}\omega9c$ (23.5 %), $C_{16:0}$ (19.5 %), $C_{12:0}$ 3-OH (8.3 %), $C_{18:1}\omega9c$ (7.4 %) and $C_{14:0}$ (4.7 %). The DNA G+C content of the type strain is 59.0 mol% (T_m).

The type strain, HS225^T (=CGMCC 1.6775^T =JCM 14804^T), was isolated from seawater samples of the Zhoushan Archipelago, Zhejiang Province, China.

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