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Marinobacter shengliensis sp. nov., a moderately halophilic bacterium isolated from oil-contaminated saline soil

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Abstract Two moderately halophilic strains, designated SL013A34A2^T and SL013A24A, were isolated from oil-contaminated saline soil from Shengli Oilfield, eastern China. Cells were found to be Gram-staining negative, aerobic, rod-shaped with a single polar flagellum. The isolates were found to grow at 10–40 °C (optimum 35 °C), pH 6.0–9.0 (optimum pH 8.0), and NaCl concentrations of 0.5–18.0 % (w/v) (optimum 3.0–6.0 NaCl). The 16S rRNA gene sequence analysis indicated that the isolates belong to the genus *Marinobacter*. Strain SL013A34A2^T shares the highest 16S rRNA gene sequence similarities with strain SL013A24A (99.3 %), followed by *M. hydrocarbonoclasticus* CGMCC 1.7683^T (97.8 %), *M. vinifirmus* CGMCC 1.7265^T (97.8 %), and *M. excellens* KMM 3809^T (97.4 %), respectively, but low similarities (93.8–96.4 %) with type strains of the other numbers of genus *Marinobacter*. DNA–DNA relatedness values of strain SL013A34A2^T with strains SL013A24A, *M. hydrocarbonoclasticus* CGMCC 1.7683^T, *M. vinifirmus* CGMCC 1.7265^T and *M. excellens* KMM 3809^T were 88.7, 29.2, 33.4 and 29.4 %, respectively. The major fatty acids of strain SL013A34A2^T were identified as $C_{18:1}\omega9c$, $C_{16:0}$, $C_{12:0}$3-OH, $C_{12:0}$, $C_{16:1}\omega9c$ and 10-methyl $C_{18:0}$. The major respiratory quinone of strain SL013A34A2^T was found to be ubiquinone-9, and its predominant polar lipids were identified as diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and unidentified glycolipid. The genomic DNA G + C content was found to be 56.1 mol %. Based on the phenotypic, genetic and chemotaxonomic characteristics, these two isolates are representatives of a novel species of the genus *Marinobacter*, for which the name *Marinobacter*

Yi-Jing Luo and Bai-Sheng Xie have contributed equally to this work.

Sequence deposited The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SL013A34A2^T and SL013A24A are KF307780 and KF307779, respectively.

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shengliensis sp. nov. is proposed. The type strain is SL013A34A2^T(=LMG 27740^T = CGMCC 1.12758^T).

Keywords *Marinobacter shengliensis* sp. nov. · Oil-contaminated saline soil

Introduction

The genus *Marinobacter*, belonging to the family *Alteromonadaceae*, in *Proteobacteria*, was first proposed by Gauthier et al. (1992). The type species is *Marinobacter hydrocarbonoclasticus*, which is Gram-staining negative, aerobic, flagellated, halophilic, hydrocarbon-degrading and rod-shaped bacterium (Gauthier et al. 1992). At the time of writing, 34 species have been validly named (http://www.bacterio.net/marinobacter.html), which were isolated from diverse environments, including seawater (Gauthier et al. 1992; Gorshkova et al. 2003; Yoon et al. 2003; 2004; Romanenko et al. 2005; Shivaji et al. 2005; Antunes et al. 2007; Roh et al. 2008; Huo et al. 2008; Zhang et al. 2008; Xu et al. 2008; Zhuang et al. 2009; Kharroub et al. 2011; Qu et al. 2011; Lee et al. 2012), marine sediment(Gorshkova et al. 2003; Romanenko et al. 2005; Kim et al. 2006; Guo et al. 2007; Montes et al. 2008; Liu et al. 2012; Gao et al. 2013), saline lake (Aguilera et al. 2009; Bagheri et al. 2013), saltern (Yoon et al. 2007; Wang et al. 2009), saline soil (Martín et al. 2003; Gu et al. 2007), hot spring (Shieh et al. 2003), hydrothermal sediment(Handley et al. 2009), wastewater (Liebgott et al. 2006) and others organisms (Romanenko et al. 2005; Green et al. 2006; Kaeppel et al. 2012). The DNA G+C content of this genus ranges from 52.7 to 59.6 mol %.The predominant fatty acids are $C_{18:1}\omega 9c$, $C_{16:0}$,$C_{16:1}\omega 9c$ and $C_{12:0}$3-OH. The major respiratory quinone is Q-9.

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During investigation into microbial communities in petroleum related environments by both culture-dependent and independent analyses, we found numerous novel bacterial lineages (Gu et al. 2007; Wang et al. 2007a; Wang et al. 2007b; Wu et al. 2009; Wang et al. 2010; Cai et al. 2011a; Cai et al. 2011b; Lv et al. 2014; Pan et al. 2014) and several isolates closely related to *Marinobacter* (Tang et al. 2012; Sun et al. 2014). In this study, we describe two novel *Marinobacter* strains isolated from an oil polluted saline soil in Shengli Oilfield in eastern China. The physiological, biochemical, and phylogenetic analyses revealed that the isolates represent a novel species of the genus *Marinobacter*.

Materials and methods

Isolation and cultivation

Strains SL013A34A2^T and SL013A24A were isolated from oil-contaminated saline soil in Gudao product (118°50′E; 37°53′N), Shengli Oilfield, China. The two strains were isolated by 10-fold dilution plating technique on the oil production water agar (OPWA, 15 g agar in 985 ml oil production mixture)plates, which were incubated at 30 °C for 7 days (Wang et al. 2007a). Single colonies were streaked on artificial seawater (ASW) agar plate containing (l^{-1}): 5 g peptone; 1 g yeast extract; 24 g NaCl; 4 g Na_2SO_4; 0.68 g KCl; 0.1 g KBr; 0.025 g H_3BO_3; 5.4 g $MgCl_2$·H_2O; 1.5 g $CaCl_2$·$2H_2O$; 0.024 g $SrCl_2$·$6H_2O$; 0.2 g $NaHCO_3$; 0.04 g Na_2HPO_4; 0.5 g NH_4Cl and 0.002 g NaF, 15 g agar; pH 8.0) (Eguchi et al. 1996) to obtain pure cultures. The reference strains *Marinobacter vinifirmus* CGMCC 1.7265^T and *Marinobacter hydrocarbonoclasticus* CGMCC 1.7683^T were obtained from China General Microbiological Culture Collection Center (CGMCC). *Marinobacter excellens* KMM 3809^T was kindly presented by Prof. E. P. Ivanova, Swinburne University of Technology.

Morphological, physiological, and chemotaxonomic tests

After growth on Luria–Bertani (LB) agar (l^{-1}: 10 g peptone; 5 g yeast extract; 10 g NaCl and 15 g agar; pH 8.0) for 2 days at 30 °C, cell morphology and

flagellum were examined by using transmission electron microscopy (TEM, JEM-1230; JEOL).The optimal temperature was assessed in LB (pH 8.0) broth by testing bacterial growth at 4,10,15,20,25,30,35, 40,45,50 and 55 °C while NaCl concentration was kept at 3 %. Requirement of NaCl for growth was determined using LB medium supplemented with different concentrations of NaCl (0, 0.5, 1.0, 2.0, 3.0, 4.0,6.0, 8.0, 10.0, 12.0, 14.0, 16.0,18.0 and 20.0 %, w/v),while pH value and temperature were kept at 8.0 and 35 °C, respectively. Requirement of pH for growth was tested with the pH ranged between 4.0 and 10.0 (at 1 pH unit interval) while NaCl concentration and temperature were kept at 3.0 % and 35 °C, respectively. For maintaining the pH of the medium, the sodium phosphate/citric acid buffer (10 mM, for pH 3-8) and glycine/NaOH buffer (10 mM, for pH 8.6–10) were used. All these growth tests were made in triplicates, and growth was determined with optical density (OD_{600}).

Oxidase reagent (bioMérieux, Kovacs 1956) was used for testing oxidase activity, and catalase activity was determined via bubble production after addition of 3 % (v/v) hydrogen peroxide solution (Muurholm et al. 2007). H_2S production, and hydrolysis of starch, Tween 80 and gelatin were tested according to the methods described by Williams et al. (1983) and Lányí (1988). Tests of some enzyme activities and other physiological and biochemical properties were carried out by using the API ZYM and API-20NE systems according to manufacturer's instructions, respectively. The Biolog GEN III MicroPlate System (Biolog Inc., Hayward, CA)was used to perform the 94 phenotypic tests according to the manufacturer's instruction (http://www.biolog.com), including the assays for utilization of 71 carbon sources and sensitivities to 23 chemicals. Simultaneously, the two reference type strains were also tested, and acid production was determined as described elsewhere (Shivaji et al. 2005).Antibiotic resistance tests were performed on LB (pH 8.0) using strip with the antibiotics according to the method described by Andrews (2008).

For respiratory quinone analysis, strain SL013 A34A2^T were harvested from LB (pH 8.0) broth cultivated at 30 °C for 48 h. Cells were washed, lyophilized and extracted with chloroform/methanol (2:1, v/v). Respiratory quinones were then analyzed by a high performance liquid chromatography(HPLC) with a reversed-phase column as described by Komagata and Suzuki (1987).Total lipids were extracted by a chloroform/methanol system and analyzed by two-dimensional thin layer chromatography (TLC), as described previously (Kates 1986). The TLC plate (silica gel 60 F_{254}, Merck) dotted with sample was subjected to two-dimensional development, with the first solvent of chloroform/methanol/water (65:25:4, v/v) followed by second solvent of chloroform–methanol-acetic acid–water (85:12:15:4, v/v). For fatty acid analysis, the cells of SL013A34A2^T, SL013A24A, *M. hydrocarbonoclasticus* CGMCC 1.7683^T and *M. vinifirmus* CGMCC 1.7265^T, were firstly cultured on MA (2216E) medium (BD, U S A.; l^{-1} distilled water: Bacto peptone 5.00 g, Bacto yeast extract 1.00 g, Fe(III) citrate 0.10 g, NaCl 19.45 g, $MgCl_2$ (anhydrous) 5.90 g, Na_2SO_4 3.24 g, $CaCl_2$ 1.80 g, KCl 0.55 g, $NaHCO_3$ 0.16 g, KBr 0.08 g, $SrCl_2$ 34.00 mg, H_3BO_3 22.00 mg, Na_2SiO_3 4.00 mg, NaF 2.40 mg, NH_4NO_3 1.60 mg, Na_2HPO_4 8.00 mg, 18 g agar for solid medium, pH 8.0) (Power and Johnson 2009) at 30 °C for 2 days. Fatty acids were then extracted, methylated, detected by a gas chromatography (6890; Hewlett Packard). Peaks were automatically computed using the standard MIDI procedure (Microbial Identification, Sherlock version 6.0).

Phylogenetic analysis

Chromosomal DNA was extracted and purified according to standard methods (Marmur 1961). The 16S rRNA gene was PCR amplified with universal bacterial primers 8F (5′-AGA GTT TGA TCC TGG CTC AG) and 1492R (5′-GGT TAC CTT GTT ACG ACT T) (Embley 1991) and sequenced. Multiple sequence alignments, and phylogenetic analysis of 16S rRNA gene sequence from strains SL013A34A2^T and SL013A24A with 34 validly published species of the genus *Marinobacter*, were performed by MEGA version 5.0 (Tamura et al. 2011). Phylogenetic trees were reconstructed using neighbor-joining method. The neighbor-joining (NJ) algorithm used a matrix of pairwise distances estimated under the Tamura and Nei (1993) model for nucleotide sequences. Maximum-parsimony (Tamura et al. 2011) and maximum likelihood (Felsenstein 1981) algorithms were also used to evaluate the stability of the tree topology. The genome DNA G + C content was determined from

melting point (T_m) curves (Mandel and Marmur 1968) obtained by using a Lambda35 UV/Vis spectrophotometer (Perkin Elmer) equipped with a temperature program controller (PTP-1 Peltier System). *Escherichia coli* strain K-12 DNA was used as a control (Marmur and Doty 1962).Homoduplex and heteroduplex DNA–DNA hybridizations were performed in triplicate as described by De Ley et al. (1970) and Huß et al. (1983). The optimum renaturation temperatures (T_{or}) of the heteroduplex genomic DNA solution were performed at 74.0 °C.

Results and Discussion

The two isolates exhibited similar phenotypic features. Cells were found to be Gram-staining negative, rod-shaped, motile with a single polar flagellum and multiplied by binary fission (Fig. 1). The cell size was found to be 1.5–2.0 μm in length and 0.5 to 0.8 μm in width. Colonies of both of the isolates are smooth, circular, convex and creamy on LB agar (pH 8.0; 3.0 % NaCl). Growth of strain SL013A34A2^T occurs at 0.5–18.0 % (w/v) NaCl (optimum 3.0–6.0 % NaCl), pH 6.0–9.0 (optimum pH 8.0) and 10–45 °C (optimum 35 °C),while strain SL013A24A grows at 0.5–16.0 % (w/v) NaCl (optimum 1.0–3.0 % NaCl), pH 6.0–9.0 (optimum pH 8.0) and 15–40 °C (optimum 30 °C). The detailed phenotypic and physiological differences are presented in Table 1, which clearly differentiate the two isolates from the close phylogenetic neighbours. Both strains were found to be resistant to vancomycin, fusidic acid, rifamycin SV and aztreonam. Sensitive to amikacin, amoxicillin, ampicillin, carbenicillin, cefalexin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, lincomycin, minocycline, nalidixic acid, norfloxacin, ofloxacin, roxithromycin, penicillin G, polymyxin B, streptomycin, sulfamethoxazole, tetracycline and tobramycin.

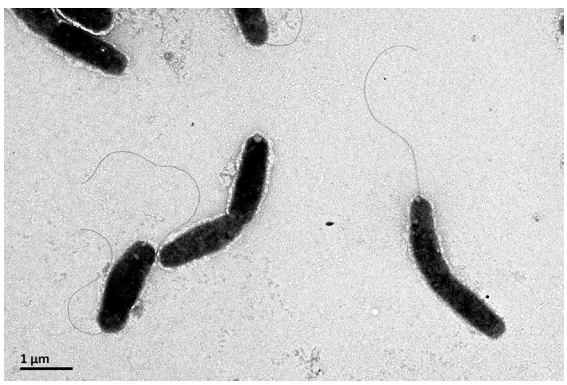

Fig. 1 Transmission electron micrograph of strain SL013A34A2^T grown on LB agar containing 3 % NaCl at 30 °C, pH 8.0 for 2 days. Bar, 1 μm

The 16S rRNA gene sequences (1,463 bp) of strains SL013A34A2^T and SL013A24A show 99.3 % similarity to each other and strain SL013A34A2^T was found to have the highest 16S rRNA gene sequence similarities to *M. hydrocarbonoclasticus* CGMCC 1.7683^T (97.8 %), *M. vinifirmus* CGMCC 1.7265^T (97.8 %) and *M. excellens* KMM 3809^T (97.4 %), while those to the type strains of the other recognized species of the genus *Marinobacter* are between 93.8 and 96.4 %. Phylogenetic analysis showed that the two strains form a separate cluster within a subgroup containing *M. vinifirmus* CGMCC 1.7265^T, *M. excellens* KMM 3809^T, *M. litoralis* SW-45^T, *M. daepoensis* SW-156^T and *M. hydrocarbonoclasticus* CGMCC 1.7683^T (Fig. 2 and Supplementary Fig. S1 & S2).The bootstrap resampling analysis showed that the association was relatively stable.

DNA–DNA relatedness values of strain SL013A34A2^T with strains SL013A24A, *M. hydrocarbonoclasticus* CGMCC 1.7683^T, *M. vinifirmus* CGMCC 1.7265^T and *M. excellens* KMM 3809^T were 88.7 ± 1.3, 29.2 ± 2.9, 33.4 ± 0.3 and 29.4 ± 3.0 %, respectively. These values are below the DNA–DNA hybridization threshold value of 70 % which is used for separate species delineation by Wayne et al. (1987). Strains SL013A34A2^T and SL013A24A showed 88.7 ± 1.3 % DNA–DNA relatedness with each other, which further confirmed that they belong to the same species.

The genomic DNA G + C contents of the strains SL013A34A2^T and SL013A24A were found to be 56.1 and 55.7 mol % (Table 1), respectively, compared to 53.8–59.9 mol % for the closest type species.

The major cellular fatty acids of the two isolates were identified as $C_{18:1}\omega 9c$ (37.6–37.7 %), $C_{16:0}$ (27.3–28.9 %), $C_{12:0}$3-OH(7.5–8.2 %), $C_{12:0}$(5.6–6.0 %) and $C_{16:1}\omega 9c$ (4.3–5.4 %), which are in line with those of strains *M. hydrocarbonoclasticus* CGMCC 1.7683^T and *M. vinifirmus* CGMCC 1.7265^T. The 10-methyl $C_{18:0}$ (5.0–5.3 %) fatty acid was found to be present only in the two isolates, while $C_{17:1}\omega 8c$ fatty acid, which is found in other reference strains, was found to be absent in the two isolates

Table 1 Differential characteristics of strains SL013A34A2^T and SL013A24A, and their closely related strains in genus *Marinobacter*

Characteristics	1	2	3	4	5	6	7
Cell size (μm)	1.5–2.0 × 0.5–0.8	1.5–2.0 × 0.5–0.8	2.0–3.0 × 0.3–0.6[a]	1.0–2.0 × 0.5–0.7[b]	1.0–8.0 × 0.6–1.4	1.5–3.0 × 0.6–0.8	1.5–3.0 × 0.5–0.8
Salinity range (%, w/v)	0.5–18.0	0.5–16.0	0.5–18.0	0.5–18.0	1.0–15.0	ND	0.5–18.0
Optimal salinity (%, w/v)	3.0–6.0	1.0–3.0	3.0–6.0	3.0–6.0	ND	2.0–6.0	2.0–7.0
Growth temperature (°C)	10–45	15–40	15–45	15–40	10–41	ND	4–46
Optimal temperature (°C)	35	30	35	25	20–25	30–37	30–37
Nitrate reduction	−	−	+	+	+	−	−
Hydrolysis of							
Tween 80	+	+	+	−	+	+	+
Tween 40	+	+	+	−		+	
Starch	+	+	−	−	+	−	−
Glycerol	+	+	−	−	ND	ND	ND
Pectin	w	−	−	+	ND	ND	ND
Utilization[c] of							
D-Maltose	+	+	−	−	+	ND	ND
D-Lactose	−(+[d])	−	w	+	w	ND	ND
D-Mannose	−	w	−	−	−	+	ND
D-Fructose	−	−	w	w	+	−	−
D-Mannitol	−	−	−	−		−	−
L-Glutamate	+	+	+	−		−	+
Succinate	−	−	+	+	−	+	+
L-Proline	+	+	w	−	+	ND	ND
Aspartate	w	w	−	−	w	−	ND
D-Cellobiose	−	−	−	−	−	+	−
Sucrose	−	−	−	−	−	+	−
Glycerol	+	+	−	−	−	−	−
L-Alanine	+	w	−	−	−	−	−
API ZYM							
Alkaline phosphatase	+	+	−	−	+	+	+
Acid phosphatase	+	+	−	−	−	+	+
α-glucosidase	+	+	−	−	−	−	−

Table 1 continued

Characteristics	1	2	3	4	5	6	7
Major polar lipids	DPG, PC, PE, PG, GL	DPG, PC, PE, PG, GL	DPG, PC, PE, PG, GL, AL	DPG, PC, PE, PG, GL	PE, PG, DPG	ND	ND
DNA G + C content (mol %)	56.1	55.7	53.8	58.9	56.0	57.0	55.0

All strains are positive for catalase, oxidase, esterase (C4), esterase lipase (C8), leucine arylamidase, phosphohydrolase and *N*-acetyl-*β*-glucosaminidase activities. All strains are negative for activities of H_2S production, urease, *β*-galactosidase, and glucose fermentation, hydrolysis of gelatin, arginine dihydrolase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, *α*-mannosidase and *α*-fucosidase. All strains could not utilize D-glucose, L-serineas the sole carbon source. All data are from the present study and tested under the identical conditions except for the data of cell size. Utilization of carbohydrates was not tested for *M. excellens* KMM3809^T, *M. daepoensis* SW-156^T or *M. litoralis* SW-45^T

Taxa *1 M. shengliensis* SL013A34A2^T, *2 M. shengliensis* SL013A24A, *3 M. hydrocarbonoclasticus* CGMCC 1.7683^T, *4 M. vinifirmus* CGMCC 1.7265^T, *5 M. excellens* KMM3809^T, *6 M. daepoensis* SW-156^T (Yoon et al. 2004), *7 M. litoralis* SW-45^T (Yoon et al. 2003), + positive, − negative, *w* weakly positive, *ND* not detected

[a] Data from Gauthier et al. (1992)

[b] Data from Liebgott et al. (2006)

[c] According to the Biolog GEN III MicroPlate System

[d] According to the method described by Shivaji et al. (2005)

(Table 2). The predominant respiratory quinone of strains SL013A34A2^T and SL013A24A were determined to be Q-9, which is typical for *Marinobacter* species except for *M. lutaoensis*, which contains Q-8 (Shieh et al. 2003).The polar lipid profiles of strains SL013A34A2^T and SL013A24A was found to consist of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and unidentified glycolipid(GL); strain SL013A34A2^T also has phosphatidylcholine (PC), while strain SL013A24A does not. Strains SL013A34A2^T and SL013A24A are different from *M. hydrocarbonoclasticus* CGMCC 1.7683^T with regatd to an unidentified aminolipid, which was absent from the two isolates. The polar lipid profiles for both new isolates was found to be similar to that of *M. vinifirmus* CGMCC 1.7265^T (Table 1 and Supplementary Fig. S3).

The phylogenetically coherent clustering and chemotaxonomic characteristics revealed that the two isolates are members of genus *Marinobacter*. Based on the low DNA–DNA relatedness to the members of closely-related taxa, their unique branching position in phylogenetic analyses, and differences in physiological characteristics including the NaCl and temperature ranges for growth, ability of nitrate reduction, hydrolysis of certain substrates, the carbon utilization pattern and the enzyme activities, strains SL013A34A2^T and SL013A24A represent a novel species within the genus *Marinobacter*, family *Alteromonadaceae*, for which the name *Marinobacter shengliensis* sp. nov. is proposed.

Description of *Marinobacter shengliensis* sp. nov

Marinobacter shengliensis (sheng.li.en'sis. N.L. masc. adj. *shengliensis* pertaining to Shengli oilfield, China, from where the type strain was firstly isolated.)

Cells are Gram-staining negative, aerobic, moderately halophilic, motile and rod-shaped (1.5–2.0 × 0.5–0.8 μm) with a single polar flagellum. Colonies grown on LB (pH 8.0) agar for 2 days are creamy, circular and convex with smooth surface (1.5–2.0 mm in diameter). Growth occurs at 0.5–18.0 % (w/v) NaCl, pH 6.0–9.0 and 10–45 °C with the optimum grow that pH 8.0, 35 °C and 3.0–6.0 %(w/v) NaCl. Activities of catalase, oxidase, and hydrolysis of Tween 80 and starch are positive, whereas activities of H_2S production, urease, *β*-galactosidase, glucose fermentation, nitrate reduction, indole production, arginine dihydrolase, and hydrolysis of gelatin are

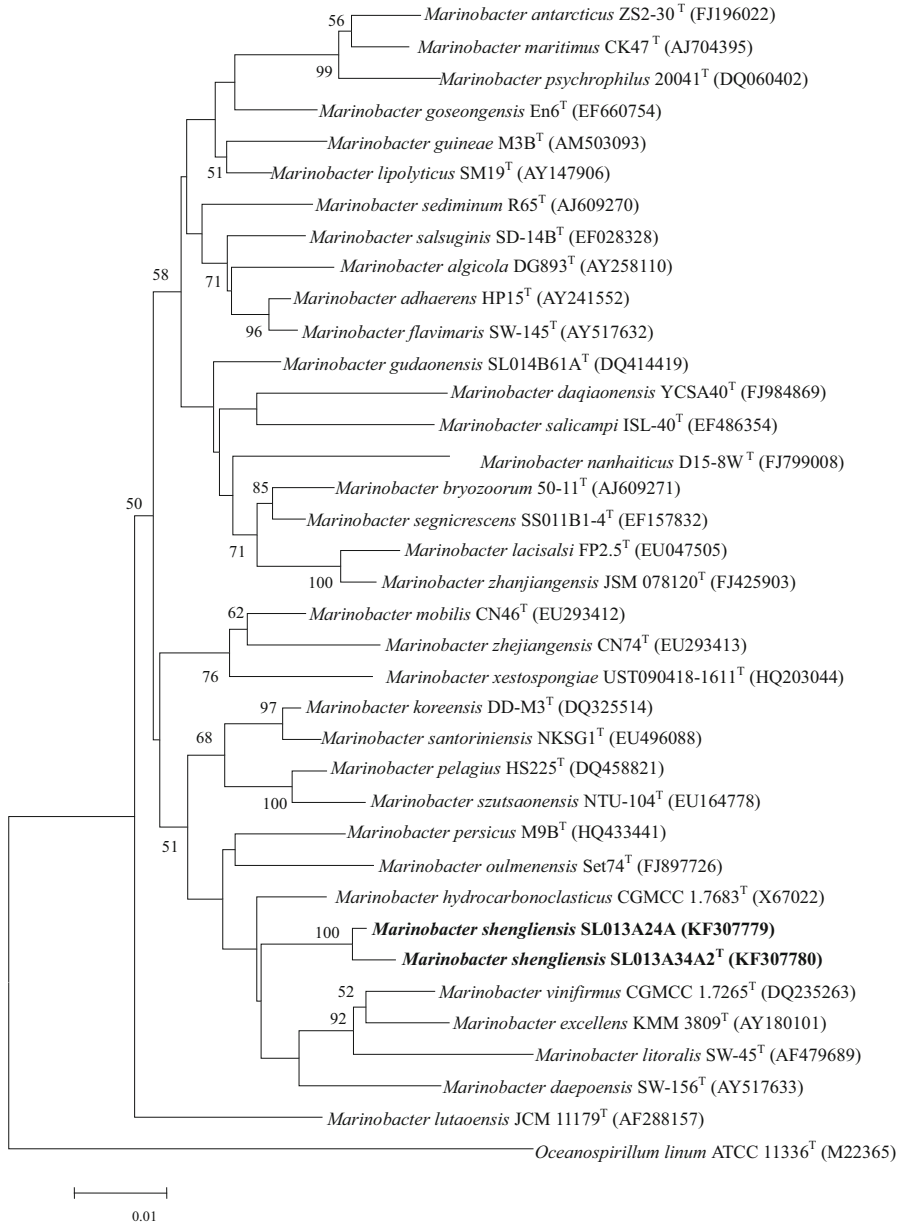

Fig. 2 Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences, showing the positions of strains SL013A34A2^T and SL013A24A, and all the type strains of genus *Marinobacter*. *Oceanospirillum linum* ATCC 11336^T was used as an out group. Bootstrap values >50 % are shown at nodes. *Bar*, 0.01 substitutions per nucleotide position

negative. Acid is not produced from fructose, xylose, mannitol, lactose, maltose, sucrose or mannose. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, phosphohydrolase, α-glucosidase and *N*-acetyl-β-glucosamindase activities. Negative for urease, arginine dihydrolase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase activities. Can utilise Tween 40, dextrin, glycerol, pectin, D-maltose, D, L-fucose, D-fructose-6-PO_4, L-alanine, L-glutamic acid, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid,

Table 2 Fatty acid compositions of strains SL013A34A2^T and SL013A24A, and their closest related type strains of genus *Marinobacter*

Fatty	1	2	3	4
Saturated				
$C_{10:0}$	–	–	1.3	–
$C_{12:0}$	5.6	6.0	4.8	6.5
$C_{12:0}$3-OH	7.5	8.2	9.8	10.9
$C_{14:0}$	1.0	1.2	2.7	1.4
$C_{16:0}$	27.3	28.9	25.6	26.0
$C_{17:0}$	–	–	2.1	–
$C_{18:0}$	2.6	2.3	2.7	2.5
10-methyl $C_{18:0}$	5.0	5.3	ND	ND
Unsaturated				
$C_{16:1}\omega 9c$	5.4	4.3	6.3	8.9
$C_{17:1}\omega 8c$	ND	ND	2.4	–
$C_{18:1}\omega 9c$	37.7	37.6	35.8	33.2
Summed features[a]				
Summed feature 3	1.2	–	3.8	6.4
Summed feature 8	–	–	1.1	ND
Summed feature 9	2.3	2.3	1.3	1.7

The four strains were cultured and investigated under same conditions with the same protocols. All data are from the present study

Taxa *1 M. shengliensis* SL013A34A2^T, *2 M. shengliensis* SL013A24A, *3 M. hydrocarbonoclasticus* CGMCC 1.7683^T, *4 M. vinifirmus* CGMCC 1.7265^T, – content less than 1 %, *ND* not detected

[a] Summed features represent groups of two or more fatty acids that could not be separated in the MIDI system. Summed feature 3 contained $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$. Summed feature 8 contained $C_{18:1}\omega 6c$ and/or $C_{18:1}\omega 7c$. Summed feature 9 contained iso-$C_{17:1}\omega 9c$ and/or 10-methyl $C_{16:0}$

glucuronamide, D-lactic acid methyl ester, L-lactic acid, D, L-malic acid, bromo-succinic acid, α-hydroxy-butryric acid, β-hydroxy-D, L-butyric acid, α-keto-butryric acid, acetoacetic acid, propionic acid and acetic acid as the sole carbon sources.

Q-9 is the predominant respiratory quinone. The major cellular fatty acids are $C_{18:1}\omega 9c$, $C_{16:0}$, $C_{12:0}$3-OH, $C_{12:0}$, $C_{16:1}\omega 9c$ and 10-methyl $C_{18:0}$. The major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and unidentified glycolipid. The DNA G + C content of the type strain is 56.1 mol %.

The type strain, SL013A34A2^T (=LMG 27740^T = CGMCC 1.12758^T), was isolated from oil-contaminated saline soil in Shengli oilfiled, China.

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