Marinobacter oulmenensis sp. nov., a moderately halophilic bacterium isolated from brine of a salt concentrator

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A Gram-negative, aerobic, moderately halophilic bacterium, designated Set74^T, was isolated from brine of a salt concentrator at Ain Oulmene, Algeria. The strain grew optimally at 37–40 °C, at pH 6.5–7.0 and with 5–7.5 % (w/v) NaCl and used various organic compounds as sole carbon, nitrogen and energy sources. Ubiquinone 9 (Q-9) was the major lipoquinone. The main cellular fatty acids were C_{16:0}, C_{18:1}ω9c, summed feature 7 (ECL 18.846; C_{19:0} cyclo ω10c and/or C_{19:1}ω6c), C_{12:0} 3-OH, C_{16:1}ω9c, C_{18:0} and C_{12:0}. The major polar lipids were phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. The G + C content of the genomic DNA was 57.4 mol%. The 16S rRNA gene sequence analysis indicated that strain Set74^T was a member of the genus *Marinobacter*. The closest relatives of strain Set74^T were *Marinobacter santoriniensis* NKSG1^T (97.5 % 16S rRNA gene sequence similarity) and *Marinobacter koreensis* DD-M3^T (97.4 %). DNA–DNA relatedness between strain Set74^T and *M. santoriniensis* DSM 21262^T and *M. koreensis* DSM 17924^T was 45 and 37 %, respectively. On the basis of the phenotypic, chemotaxonomic and phylogenetic features, a novel species, *Marinobacter oulmenensis* sp. nov., is proposed. The type strain is Set74^T (=CECT 7499^T =DSM 22359^T).

The family *Alteromonadaceae* was created by Ivanova & Mikhailov (2001) to accommodate an assemblage of marine Gram-negative, chemoheterotrophic, straight or curved rods. Members of this group include the genera *Aestuariibacter*, *Agarivorans*, *Aliagarivorans*, *Alishewanella*, *Alteromonas*, *Bowmanella*, *Glaciecola*, *Haliea*, *Marinimicrobium*, *Marinobacter*, *Marinobacterium*, *Melitea*, *Microbulbifer*, *Saccharophagus* and *Salinimonas* (Baumann *et al.*, 1972; Bowman *et al.*, 1998; Ekborg *et al.*, 2005; Gauthier *et al.*, 1992; González *et al.*, 1997; Jean *et al.*, 2006, 2009; Jeon *et al.*, 2005; Kurahashi & Yokota, 2004; Lim *et al.*, 2006; Urios *et al.*, 2008a, b; Vogel *et al.*, 2000; Yi *et al.*, 2004). The genus *Marinobacter* was proposed by Gauthier *et al.* (1992) with the type species *Marinobacter hydrocarbonoclasticus*. Members of this genus can be found in different habitats, including marine environments, hypersaline lakes, saline

soils and hydrothermal sediments (Martín *et al.*, 2003; Yoon *et al.*, 2004; Handley *et al.*, 2009; Aguilera *et al.*, 2009).

A strain, designated Set74^T, was obtained from brine samples collected from the Ain Oulmene salt concentrator (35° 55' 23" N 5° 17' 36" E) in north-eastern Algeria. Isolation was performed in MH medium (containing per litre distilled water: 1 g glucose, 5 g proteose peptone, 5 g yeast extract) and supplemented with 7.5 % (w/v) total salts. The total salts stock solution was prepared at 30 % (w/v) as described by Subov (1931) (per litre distilled water: 234 g NaCl, 42 g MgCl₂ · 6H₂O, 60 g MgSO₄ · 7H₂O, 1 g CaCl₂ · 2H₂O, 6 g KCl, 0.2 g NaHCO₃, 0.7 g NaBr, 0.005 g FeCl₃). The medium was adjusted to pH 7.0 with NaOH and the medium was solidified with 2.0 % agar (Difco). Incubation was at 37 °C and a single colony was streaked at least three times on MH agar to obtain a pure culture. Unless otherwise stated, MH medium was routinely used for cultivation and phenotypic analysis.

Cellular morphology was examined by transmission electron microscopy as described previously (Kharroub

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Set74^T is FJ897726.

A supplementary figure is available with the online version of this paper.

et al., 2006). Gram staining was performed using a Gram stain kit (Sigma). Growth was tested with 0, 0.5, 1, 3, 5, 7.5, 10, 15 and 20 % (w/v) NaCl, at pH 5.0–10.0 and at 10, 15, 20, 25, 30, 37, 40, 45 and 50 °C. Tests for catalase, oxidase and hydrolysis of aesculin, DNA, gelatin, starch and Tween 80 were conducted according to the methods of Smibert & Krieg (1981). Growth on MacConkey agar supplemented with 7.5 % (w/v) NaCl was assessed. Tyrosine hydrolysis was revealed by clear zones on medium containing 5 g L-tyrosine l⁻¹. Pigment production was also determined. Simmons' citrate test and β -galactosidase, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase activities were tested according to the procedure of Larpent & Larpent-Gourgaud (1985) with medium supplemented with 7.5 % (w/v) NaCl. Urea hydrolysis and indole production were assessed as described by Ventosa *et al.* (1982). Respiration of nitrate and nitrite, oxidation and fermentation of glucose and acid production from and utilization of organic compounds as sole sources of carbon or nitrogen and energy were determined as described by Mata *et al.* (2002). Sensitivity to antibiotics was determined using standard disc assays. Analysis of isoprenoid quinones, fatty acid methyl ester profiles and polar lipids was carried out by the DSMZ Identification Service (Braunschweig, Germany).

Genomic DNA was extracted according to Marmur (1961). The 16S rRNA gene of strain Set74^T was amplified by PCR using primers as described by Saiki *et al.* (1988). Identification of phylogenetic neighbours and calculation of pairwise sequence similarities were carried out using a BLAST search of sequences in GenBank (Altschul *et al.*, 1997). Multiple sequence alignments were performed using CLUSTAL W version 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in the MEGA4 package (Tamura *et al.*, 2007). The resultant tree topologies were evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Genomic DNA G+C content was determined from the mid-point value (T_m) of the thermal denaturation profile (Marmur & Doty, 1962). DNA–DNA hybridization studies were performed using the non-radioactive method described by Ziemke *et al.* (1998). DNA was double-labelled using DIG-11-dUTP and biotin-16-dUTP (Boehringer Mannheim). The labelling reaction was carried out using the Boehringer Mannheim nick-translation kit (Roche Applied Science).

Cells of strain Set74^T were Gram-staining-negative motile rods. Colonies on MH medium were cream, small, circular, smooth and 2–3 mm in diameter after incubation for 3 days at 37 °C. Strain Set74^T grew in media containing 1–15 % (w/v) NaCl (optimum 5–7.5 % NaCl), at pH 5.5–9.0 (optimum pH 6.5–7.0) and at 30–45 °C (optimum 37–40 °C). The detailed phenotypic features of strain Set74^T are presented in the species description and Table 1.

Table 1. Differential characteristics of strain Set74^T and type strains of closely related species of the genus *Marinobacter*

Strains: 1, *Marinobacter oulmenensis* sp. nov. Set74^T; 2, *M. santoriniensis* DSM 21262^T; 3, *M. koreensis* DSM 17924^T. Data were obtained in this study unless indicated. All strains have rod-shaped cells and are positive for motility, catalase, oxidase and utilization of acetate. All strains are negative for indole production, arginine dihydrolase, glucose fermentation, hydrolysis of aesculin and gelatin and assimilation of glucose and arabinose.

Characteristic	1	2	3
NaCl concentration for growth (% w/v)			
Range	1–15	0.5–16	0.5–20
Optimum	5–7.5	5–10	3–8
pH for growth			
Range	5.5–9	5.5–9	5–9
Optimum	6.5–7	7–8	6–8
Temperature for growth (°C)			
Range	30–45	25–45	10–45
Optimum	37–40	35	28
Reduction of nitrate to nitrite	–	+	+
Hydrolysis of Tween 80	–	–	+
Enzyme activities			
β -Galactosidase	+	–	–
Urease	–	+	–
Utilization of:			
D-Mannitol	+	–	–
Citrate	+	–	–
Phenylacetic acid	–	+	+
Lactate	–	+	+
Succinate	+	+	–
DNA G+C content (mol%)	57.4	58.1 ^{a*}	54.1 ^b

*Data taken from: a, Handley *et al.* (2009); b, Kim *et al.* (2006).

The predominant quinone of strain Set74^T was ubiquinone Q-9; a small amount (4 %) of Q-8 was also present. The fatty acid profile of strain Set74^T contained saturated and unsaturated straight-chain fatty acids. The major fatty acids were C_{16:0} (29 % of total fatty acids), C_{18:1 ω 9c} (19.6 %), C_{12:0} 3-OH (9.4 %), C_{16:1 ω 9c} (8.6 %), C_{18:0} (7.2 %) and C_{12:0} (6.2 %). These fatty acids are also predominant components of other members of the genus *Marinobacter* (Martín *et al.*, 2003; Yoon *et al.*, 2004; Romanenko *et al.*, 2005; Antunes *et al.*, 2007). Additionally, strain Set74^T contained summed feature 7 (ECL 18.846, C_{19:0} cyclo ω 10c and/or C_{19:1 ω 6c}; 12.6 %), which was also reported for the type strains of *Marinobacter lutaoensis*, *Marinobacter bryozoorum* and *Marinobacter segnicrescens* (Shieh *et al.*, 2003; Romanenko *et al.*, 2005; Guo *et al.*, 2007). The polar lipids of strain Set74^T were phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine, which are reported to predominate in *M. segnicrescens*, *Marinobacter excellens* and *Marinobacter szutsaonensis* (Gorshkova *et al.*, 2003; Guo *et al.*, 2007; Wang *et al.*,

2009). The isolate was also shown to possess two unknown phospholipids and two unknown phosphoglycolipids.

The 16S rRNA gene sequence of strain Set74^T (1464 nt) was found to be related to those of members of the *Gammaproteobacteria* and the strain shared a close phylogenetic relationship with members of the genus *Marinobacter* (Fig. 1), exhibiting highest sequence similarity with *Marinobacter santoriniensis* NKSG1^T (97.5 % 16S rRNA gene sequence similarity) and *M. koreensis* DD-M3^T (97.4 %). A phylogenetic tree based on maximum-parsimony analysis of 16S rRNA gene sequences is shown in Supplementary Fig. S1 (available in IJSEM Online).

The DNA G+C content of strain Set74^T was 57.4 mol% (T_m). DNA–DNA relatedness between strain Set74^T and its closest phylogenetic neighbours, *M. santoriniensis* DSM 21262^T and *Marinobacter koreensis* DSM 17924^T, was 45 and 37 %, respectively.

The phenotypic, chemotaxonomic and genotypic data presented above support the assignment of strain Set74^T to a novel species, for which the name *Marinobacter oulmenensis* sp. nov. is proposed.

Description of *Marinobacter oulmenensis* sp. nov.

Marinobacter oulmenensis (oul.me.nen'sis. N.L. masc. adj. *oulmenensis* belonging to Ain Oulmene salt concentrator, where the species was first isolated).

Cells are Gram-negative, non-spore-forming, motile rods, 0.3–0.5 µm wide and 1.6–3.0 µm long. Colonies are cream, small, circular and smooth and 2–3 mm in diameter after growth on MH medium containing 7.5 % (w/v) NaCl for 3 days at 37 °C. Moderately halophilic and capable of growth with 1–15 % (w/v) NaCl (optimum 5–7.5 %, w/v, NaCl). Grows at 30–45 °C (optimum 37–40 °C) and at

pH 5.5–9.0 (optimum pH 6.5–7.0). Catalase and oxidase are produced. Aesculin, DNA, gelatin, starch and Tween 80 are not hydrolysed. Indole is not produced. Grows on MacConkey agar and hydrolyses tyrosine with production of pigment. Positive for β-galactosidase, but negative for urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and glucose fermentation. Positive for Simmons' citrate test, but does not reduce nitrate or nitrite and negative for respiration of nitrate and nitrite. The following substrates are utilized for growth: D-mannitol, dulcitol, glycerol, acetate, malate, malonate, succinate, oxalate, formate, fumarate, D-glucuronic acid and arginine. Does not utilize D-glucose, D-fructose, D-galactose, cellobiose, L-arabinose, D-mannose, raffinose, sucrose, D-xylose, adonitol, glycerol, benzoate, phenylacetic acid, DL-lactate, L-cysteine, L-lysine, L-methionine, L-alanine, L-tyrosine, L-tryptophan, L-glycine, L-asparagine or L-glutamate. Acid is produced from D-mannitol and dulcitol, but not from D-glucose, D-fructose, D-galactose, cellobiose, L-arabinose, raffinose, D-mannose, sucrose, D-xylose, glycerol or adonitol. The type strain is sensitive to (µg per disc unless otherwise stated) chloramphenicol (30), kanamycin (30), penicillin G (10 U), gentamicin (10) and erythromycin (15), but resistant to ampicillin (10), clindamycin (2 U) and novobiocin (30). The predominant isoprenoid quinone is Q-9; a small amount of Q-8 is present. The major fatty acids are C_{16:0}, C_{18:1}ω9c, summed feature 7 (C_{19:0} cyclo ω10c and/or C_{19:1}ω6c), C_{12:0} 3-OH, C_{16:1}ω9c, C_{18:0} and C_{12:0}. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and unknown phospholipids and phosphoglycolipids. The DNA G+C content of the type strain is 57.4 mol% (T_m).

The type strain is Set74^T (=CECT 7499^T =DSM 22359^T), which was isolated from brine of a salt concentrator at Ain Oulmene, Algeria.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship of strain Set74^T with other members of the genus *Marinobacter*. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes. *Oceanospirillum linum* ATCC 11336^T was used as an outgroup. Bar, 1 substitution per 100 nucleotide positions.

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