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ORIGINAL PAPER

Halomonas sinaiensis sp. nov., a novel halophilic bacterium isolated from a salt lake inside Ras Muhammad Park, Egypt

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Abstract An alkalitolerant and halotolerant bacterium, designated strain Sharm was isolated from a salt lake inside Ras Muhammad. The morphological, physiological and genetic characteristics were compared with those of related species of the genus *Halomonas*. The isolate grew optimally at pH 7.0, 5–15% NaCl at 35°C. The cells were Gram-negative rods, facultative anaerobes. They accumulated glycine-betaine, as a major osmolyte, and ectoine and glutamate as minor components. The strain Sharm^T biosynthetised α-glucosidase. The polar lipids were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and a novel phosphoglycolipid as major components. Ubiquinone with nine repetitive unities (Q9) was the only quinone found and, *n*C16:0 and C19:0 with cyclopropane were the main cellular fatty acids, accounting for 87.3% of total fatty acids. The G + C content of the genomic DNA was 64.7 mol %. The 16S rRNA sequence analysis indicated that strain Sharm was a member of the genus *Halomonas*. The closest relatives of the strain Sharm were *Halomonas elongata* and *Halomonas eurihalina*. However, DNA–DNA hybridisation results clearly indicated that strain Sham was a distinct species of *Halomonas*. On the basis of the evidence, we propose to assign strain Sharm as a new species of the genus *Halomonas*, *H. sinaiensis* sp. nov, with strain Sharm^T as the type strain (DSM 18067^T; ATCC BAA-1308^T).

Keywords Egypt · Extremophiles · *Halomonas* · Halophiles · Lipids

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The EMBL accession number for the 16S rRNA sequence of *Halomonas sinaiensis* strain Sharm^T is AM238662.

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Introduction

Several saline and hypersaline environments in Egypt, other than the most famous Wadi Natrun, were studied and many halophiles were isolated from them (Asao et al. 2006; Asker and Ohta 2002; Grant 2004; Hezayen et al. 2001; Hezayen et al. 2002; Krumbein et al. 2004; Sorokin et al. 2002). On the best of our knowledge no studies have been conducted up now on microorganisms present in the lakes inside Ras Muhammad Park, the extreme promontory of Sinai peninsula.

The geology and sedimentology of costal desert and salt pan areas of the Red Sea were described by Friedman (1985) and Purser (1973, 1985), and a review of prior scientific exploitation was provided by Krumbein et al. (2004), which studied hypersaline microbial systems of sabkhas of the Red Sea. The only report of isolation and characterization of a new strain from the Gavish sabkha brine pool was by Oren et al. (1999), which classified it in the Archaea Domain as *Haloarcula quadrata.*

The present study is a part of large-scale investigation we are carrying out to analyse the presence and biodiversity of extremophiles, in particular that of halophilic and alkalihalophilic phenotypes in different continents.

Euryhaline halophiles are organisms which can grow in environments with a wide range of salinity up to 20–25% NaCl. Several studies on their molecular adaptation to conditions with different salt concentrations have increased interest in these bacteria (Oren 2002). Among euryhaline microrganisms, species of the genus *Halomonas* are well investigated and most of them produces biomolecules of biotechnological interest (Mellado and Ventosa 2003; Nieto and Vargas 2002; Oren 2002) or are of interest in decontamination of polluted saline habitats (Berendes et al. 1996; Garcia et al. 2004; Martínez-Cánovas et al. 2004; Mellado and Ventosa 2003). *Halomonas* members were isolated in every part of the world and from several sources (Garrity et al. 2005; Oren and Ventosa 2005 and references therein) forming a polyphyletic group possessing heterogeneous features (Arahal et al. 2002; Arahal and Ventosa 2005; Garrity et al. 2005; Vreeland 2005).

In this study, a new member of the genus *Halomonas* is proposed on the basis of polyphasic studies. The strain was isolated from samples collected from the saline lake inside Ras Muhammad Park (Egypt).

Materials and methods

Source of microorganism

During the summer 2005, samples of sand and water were collected on the edge of a small (about 200 m of diameter) salt lake inside Ras Muhammad Park in Egypt, about 1 km far from the sea. pH was around 8.0 and the total chlorides were about 70 g l^{-1}. *Halomonas elongata* (DSM 2581^T) *Halomonas eurihalina* (DSM 5270^T), *Halomonas halmophila* (DSM 5349^T), *Halomonas halophila* (DSM 4770^T), *Halomonas salina* (DSM 5928^T), were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany (DSMZ) (Arahal et al. 2002; Garrity et al. 2005; Mata et al. 2002; Quesada et al. 1990; Vreeland et al. 1980). *Halomonas koreensis* (KCTC 12127^T), was kindly provided by the authors (Lim et al. 2005), *Halomonas almeriensis* (CECT 7050^T, Martínez-Checa et al. 2005) was kindly provided by Prof. Quesada.

Isolation and culture conditions

Isolation of halophilic strains was carried out on a saline medium (medium 1) containing the following components per liter of deionized water:KCl, 2.0 g; $MgSO_4.7H_2O$, 20 g; NaCl, 150 g; sodium citrate, 3.0 g; yeast extract, 5.0 g; casamino acids, 5.0 g; $MnCl_2.4H_2O$, 0.36 mg; $FeSO_4$, 50 mg. Strains were isolated by the dilution-plating technique on solid medium 1**.** Growth on single carbon sources (medium 2) was tested on liquid media contained the following components per litre: (1) KCl, 2.0 g; $MgSO_4.7H_2O$, 1 g; $MgCl_2.6H_2O$, 16.4 g; $NaHCO_3$, 0.2 g ; NaCl, 150 g; $CaCl_2.2H_2O$, 2.3 g; (2) additionally NH_4Cl, 152 mg; K_2HPO_4, 33 mg; $FeCl_2.4H_2O$, 0.26 mg. The last three components, each prepared as a separate concentrated solution, were sterilised and added to solution (1) after cooling, to give the final concentration mentioned above, and (3) tested compounds, 10.0 g. Anaerobic growth was carried out both with and without nitrate as previously reported (Romano et al. 2005). Solid media for purification, physiological tests and maintenance of the strain was prepared by the addition of 1.8% agar to the medium 1. Routinely the strain was grown on liquid and/or solid medium 1. In liquid medium, growth was followed by measuring the absorbance at 540 nm. The strains from DSM were grown according to on line manual http://www.dsmz.de/microorganisms/html/bacteria.genus/halomonas.html, while *H. koreensis* (KCTC 12127 ^{T}) and *H. almeriensis* (CECT 7050^T) were grown according to Lim et al (2005) and Martínez-Checa et al. (2005), respectively.

Phenotypic characterization

Unless otherwise stated the metabolic and growth studies were carried out in the medium 1. The temperature range for growth was determined by incubating the isolates from 10 to 55°C (5 degrees intervals). For pH studies (pH range 5.0–11.0), the medium was adjusted with stock solutions of either KOH or HCl (1 M) to give the desired pH. All growth tests were done at the optimal growth temperatures (35°C) for 24 h. The microorganism was grown at different amounts of NaCl and KCl: 0, 2, 5, 10, 25% in minimal medium containing glucose.

Sensitivity of the strain to antibiotics was tested by using the solid medium 1 and sensi discs (6 mm, Oxoid) incubated for 48 h. The following antibiotics were used (μg): tetracycline (30, 50) chloramphenicol (10); erythromycin (5, 30); penicillin G (2; 10 iu); ampicillin (25); gentamicin (30); novobiocin (30); kanamycin (30); streptomycin (25); fusidic acid (10); neomycin (30); lincomycin (15); nystatin (100); vancomycin (30); bacitracin (10). The antibiotic tests were carried out according to Romano et al. (1996).

Casein, gelatine and starch hydrolysis, oxidase, tyrosinase, catalase, nitrate reduction, phenylalanine deamination were carried out as previously described (Gordon 1973; Mata et al. 2002; Romano et al. 2005). The Gram-staining was performed according to Dussault (1955). Hydrolysis of *N′*-benzoyl-arginine-*p*-nitroaniline (BAPNA) stereoisomers was tested according to Oren and Galinski (1994), the KOH test was performed according to Halebian et al. (1981). Hippurate hydrolysis, aminopetidase test and, *β*- and α-glucosidase were tested according to Romano et al. (1996, 2005).

Cellular morphology and spore formation were determined by phase contrast microscopy (Zeiss) and colony morphology was determined by Leica M8 stereomicroscope. For scanning electron microscopy (SEM), cells grown in liquid medium at the optimal growth conditions were treated as previously described (Romano et al. 1996).

Chemotaxonomy

The analysis of respiratory quinones was done by EI/MS and H^1 NMR spectra according to Romano et al. (2001).

Lipid analyses were performed using freshly harvested cells (5 g) of Egyptian isolate, *H. elongata*, *H. eurihalina*, *H. halmophila*, *H. halophila*, *H. salina*, *H. koreensis*, *H. almeriensis* were grown under optimal conditions, lyophilised, extracted, purified and analysed according to Nicolaus et al. (2001) and to Romano et al. (2001). Mass spectra were recorded on a QTof-/micro/mass spectrometer (Waters) equipped with an ESI source in negative ion mode. Fatty acid methyl esters (FAME) were analysed according to Romano et al. (2001, 2005).

The extraction and identification of intracellular solutes from cells grown in complex media were carried out according to Romano et al. (2001). β-polyhydroxybutyrate (PHB) was extracted from cells grown in complex medium, according to Sykes (1971). Exopolysaccharide (EPS) analysis was carried out in medium 2 with sucrose according Poli et al. (2007).

Genetic analysis

The % G + C of DNA was determined by the HPLC method, and the value that is given, is the mean of three independent analyses of the same DNA sample (Mesbah and Whitman 1989; Romano et al. 1996, 2005). Genomic DNA extraction, PCR mediated amplification of the 16S rDNA and purification of the PCR products were carried out as described previously (Romano et al. 2005). Purified PCR products were sequenced using the CEQ^{TM}DTCS-Quick Start Kit (Beckmann Coulter) as directed in the manufacturer's protocol. Sequence reactions were electrophoresed using the CEQ^{TM} 8000 Genetic Analysis System. The multiple sequence alignment program Clustal W (Chenna et al. 2003) was used to align the 16S rRNA sequence of our isolates with the sequences of representative 16S rRNA gene of organisms belonging to the γ-*Proteobacteria*. Sequences of rRNA genes, for comparison, were obtained from the EMBL data base or RPD. Evolutionary distance matrices were calculated by using the algorithm of the Kimura two-parameter model (Kimura 1980) with the DNADIST program within the PHYLIP package, version 3.6 (Felsenstein 2004). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou and Nei 1987). The stability of relationships was assessed by a bootstrap analysis of 1000 datasets by using the programs SEQBOOT, DNADIST, NEIGHBOUR and CONSENSE of the PHYLIP package.

Quantitative DNA-DNA hybridisation and homology percentage calculation

DNA was extracted and purified from bacterial cell culture (about 250 mg of dry pellet for each strain) using the Genomic-DNA-Buffer Set and the Genomic-tip-100/G columns (Qiagen SpA, Milano, Italy), according to manufacturer's instructions with minor modifications. DNA was dissolved in TE buffer (10 mM Tris pH 8, 1 mm EDTA) and serial diluted to obtain a working solution (WS) of 50 µg/ml, as evaluated by UV-absorbance using a BioPhotometer® (Eppendorf, Germany). WS DNA concentration was confirmed by fluorimetric measurements using the Quant-iT DNA assay Kit (Invitrogen, Milano, Italy); DNA size was estimated by 0.8% DNA-grade agarose (Bio-Rad, Segrate-Milano, Italy) electrophoresis using λ DNA as molecular weight marker (DNAs size > 32 kDa). WS solutions were diluted to a final concentration of 1 ng/ml in 0.1 × SSC containing 2.5 ng/ml herring sperm DNA. DNA was denatured by 10 min at 100°C followed by quick immersion in water-ice bath. An amount of 50–80 ng/dot of DNA from each strain to screening, were blotted in quadruplicate on nylon membrane positively charged (Roche, Germany) by using a Dot-blot apparatus (Bio-Rad,) connected to a soft vacuum. Dots were washed twice by 0.1 × SSC. A standard curve 20 to 120 ng DNA/dot from the strain to the probe was included in the analysis to estimate the linearity response of assay. The DNA was cross-linked to nylon by 3 min UV exposure and by 1 h backing under-vacuum at 120°C. Membranes were frozen at –20°C until analysis.

A measure of 1 µg of DNA from the strain to probe, sheared by ultrasonic treatment (Branson mod B-12, Genéve, Switzerlandat), was digoxigenin-dUTP labelled over-night in a 20 µl reaction mixture using the hexanucleotide random priming procedure (Dig DNA Labeling kit, Roche) according to manufacturer's instructions. Membranes were pre-hybridized for 3 h at 41°C in DIG Easy-Hyb solution (Roche) and hybridized over-night at 41°C, using a roller-tube hybridization incubator (GFL, Germany), in DIG Easy-Hyb solution containing 20 pg/ml of Dig-labelled probe, heat-denatured as above described or by 10 min at 68°C in DIG Easy-Hyb solution. Stringency washes were: twice for 5 min at room temperature in 2 × SSC solution containing 0.1 × SDS, twice for 15 min at 68°C in 0.1 × SSC solution containing 0.1 × SDS. Immune-detection was performed using the anti-Digoxigenin-AP antibody (anti-digoxigenin FAB fragment conjugated to alkaline-phosfatase) the

CDP-Star chemiluminescent substrate and the DIG Wash and Block buffer set Kit, all reagents and relative instructions were from Roche. Chemiluminescence was quantified in condition of time-exposure linearity by using a Versa-DOC 4000 apparatus (Bio-Rad) equipped by the Quantity-one software version 4.6. The DNA–DNA homology percentage was calculated according to Jahnke (1994) by putting as 100% the media of the chemilumiscence values (adjusted volume intensity × mm^2) from the homologous DNA dots, taking in account the linear response of the DNA standard curve. The media standard deviation of replicate samples did not exceeded 5%. Cross-experiments (probe A vs. B, probe B vs. A) showed variation coefficients in the homology values within 10%.

Results

Enrichment and isolation

Strain Sharm was isolated from samples of water with sand taken from saline lake inside Ras Muhammad Park in the promontory of Sinai Peninsula in Egypt. An axenic culture of the halophilic strain was obtained by repeated transfer of single colonies through agar dilution plates and the pure culture obtained given the designation strain Sharm.

Phenotypic features

The new isolate was a motile Gram-negative, not sporulating rod with a length of 1.8–2.2 μm and with a diameter of 0.7–1.0 μm (Fig. 1). Colonies on enrichment medium 1 were white, circular convex with entire margin, bright.

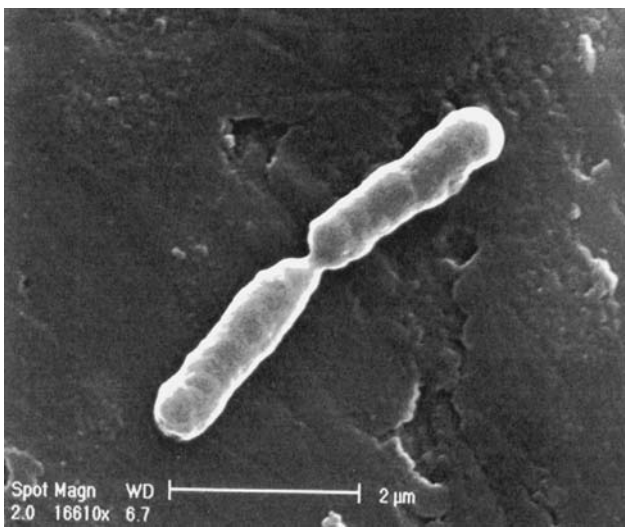

Fig. 1 Electronmicrographs of *Halomonas sinaiensis* (Sharm) under SEM. (*Bar*, 2.0 μm)

The strain Sharm grew aerobically in enrichment medium 1 containing 0 to 30% of NaCl with an optimum at 5.0–15% NaCl. The strain was a facultative anaerobe when it was grown in the presence of NO_3. The temperature growth range was 25–50°C with an optimum growth at 35°C. The pH growth range was from 6.0 to 9.0 with an optimum at pH 7.0. At the optimal growth conditions Sharm had a doubling time of 50 min. The Sharm strain had no a specific requirement for NaCl. In fact, KCl, at the same concentration, supported the growth and the range tolerance for Mg^{+2} was 0–1.0 M. The isolate grew in the presence of ampicillin (25), erythromycin (30), tetracycline (50), kanamycin (30), streptomycin (25), but it was sensitive to chloramphenicol (10), penicillin G (10), nystatin (100) and fusidic acid. The microorganism gave negative tests for oxidase, starch, gelatin, casein, indole production and phenylalanine deamination. The isolate was catalase positive and was able to hydrolyse hippurate, to decompose tyrosine, to reduce nitrate. The isolate Sharm possessed α-glucosidase activity, but was unable to hydrolyse L- and D-BAPA. The isolates grew well in minimal media containing trehalose, lactose, cellobiose, maltose, fructose, sucrose, citrate and lysine and produced acid from glucose, galactose and mannose.

Chemotaxonomy

The isolate possessed three phospholipids, 1,2 diacylglycero-3-phosphorylethanolamine (PEA 13%), 1,2 diacylglycero-3-phosphoryl-glycerol (PG 51.4%) and 1,2 diacylglycero-3-phosphoryl-glycero-phosphoryl-1,2 diacylglycerol (DPG 0.7%) and a new phosphoglycolipid (33.0%). This last compound was present together with PEA, PG and DPG in all *Halomonas* species here studied, although in different proportions depending upon the species analysed.

*n*C16:0 and C19:0 with cyclopropane (54.7% of total fatty acids) are the main cellular fatty acids, accounting for 87.3% of total fatty acids, minor components are C17:0, family of C18:1, C18:0, and C20:0. Acyl C16:0 and C18:1 were, generally the most abundant fatty acids in *H. elongata*, in *H. eurihalina*, *H. salina* in *H. halophila*, in *H. halmophila;* in *H. koreensis* were present in a quite similar amount also C17:0, C19:cp, while in *H. almeriensis* the major is C18:1, C16:0 was *ca* a half of C18:1. The LC-MS analysis of quinone gave a molecular peak corresponding to UQ-9 also confirmed by ^{1}H-NMR spectrum.

The Sharm strain produced PHB under standard growth conditions expressed as mg per g of cells wet weight (0.65) and EPS when they were grown in the presence of sucrose (see "Materials and Methods"). *H. elongata*, *H. eurihalina, H. halophila* and *H. halmophila* under growth conditions described in "Materials and Methods", produced a trace

amount of PHB, less than 0.1. In contrast, *H. koreensis*, *H. salina* produced 9.63 and 1.8, respectively.

The new isolate accumulated as osmoprotectants, identified by NMR studies (Motta et al. 2004), glycine betaine, ectoine and hydroxyectoine, while in *H. elongata* and *H. eurihalina* were present glycine betaine in a large amount and ectoine as minor component; in other species studied was observed only glycine betaine.

Sharm strain had a G + C DNA content of 64.7 mol%. The 16S rRNA gene sequence (EMBL database accession number AM238662) was determined and compared with all sequences currently available for members of the Genus *Halomonas* and related taxa. The results were presented as a phylogenetic dendrogram (Fig. 2) showing that Sharm strain was member of the genus *Halomonas*. The species *H. elongata* and *H. eurihalina* were the most related (16S rRNA gene similarities >97.5% in both cases). DNA–DNA reassociation values found between strain Sharm and the close strains *H. elongata* and *H. eurihalina* were 45 and 38%, respectively. *H. halmophila*, *H. halophila*, *H. almeriensis*, *H salina*, and *H. koreensis*, that followed in the same group (Arahal et al. 2002), had a DNA–DNA reassociation values with Sharm strain of 30.0, 15.0, 14.0, 11.0, 8.6%, respectively. Homology values lower that 25–30% were often observed by DNA–DNA hybridization on membrane filters (Jahnke 1994) or microplate (Ezaki et al. 1989; Goris et al. 1998) unlike the spectroscopic DNA–DNA renaturation rate method of De Ley et al. (1970), that presents low resolution power (estimated background 25–30%) at low homology values (Huss et al. 1983; Jahnke 1994). The method used in this study is a chemiluminescent-quantitative approach (see "Materials and Methods") that utilizes the Digoxigenin-dUTP labelling and detection procedures developed by the Roche (the method can be used to single copy gene detection on human genomic DNA Southern blots). The *H. halmophila*, *H. halophila*, *H. almeriensis*, *H salina*, and *H. koreensis*, phylogeny based on 16S rRNA sequences was compatible to DNA–DNA hybridization data. Because, some researchers have suggested that the genus *Halomonas* may be dissected into several genera, probably most species require reclassification in future (Lim et al. 2005).

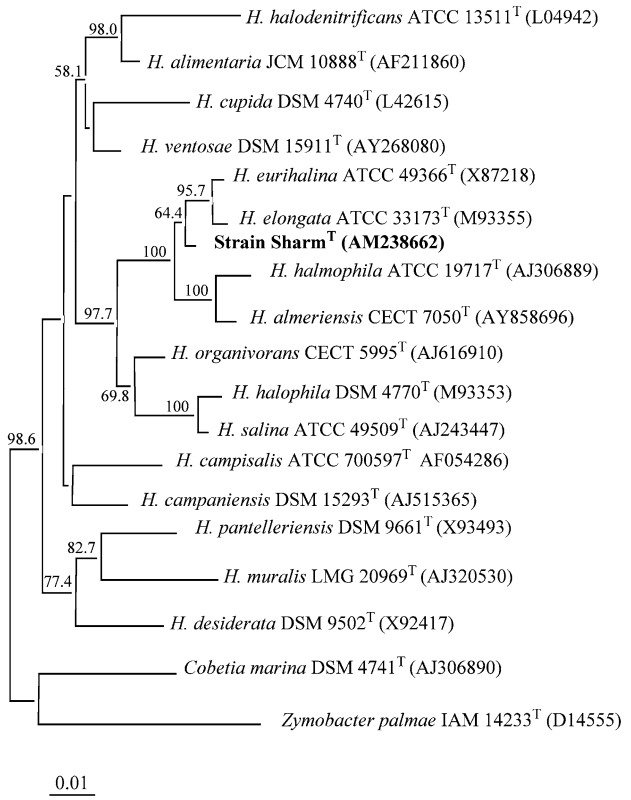

Fig. 2 Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain Sharm^T and other related taxa. Numbers at branching nodes are bootstrap values (percentages of 1000 replications); only values greater than 50% are indicated. *Bar*, 0.01 substitutions per nucleotide position

The genetic analyses let us to assign the isolate Sharm as a new species, for which *H. sinaiensis* was proposed.

The phenotypic differences (Table 1) among Sharm and the related species further support that *Halomonas* Sharm is a novel species, according to the taxonomy of Halomonadaceae (Arahal et al. 2002b; Arahal and Ventosa 2005; Garrity et al. 2005; Oren and Ventosa 2005; Vreeland 2005).

Discussion

The new isolate as well as the most related strains *H. elongata* (DSM 2581^T) (Vreeland et al. 1980), *H. eurihalina* (DSM 5270^T) (Quesada et al. 1990) and *H. almeriensis* (CECT 7050 ^{T}) (Martínez-Checa 2005), were non-motile rods, Gram-negative by staining and bactident test, but all these species showed a Gram-positive reaction with KOH test. In Table 1 are reported the differential characteristics among Sharm isolate and the related species *H. elongata, H. eurihalina*, *H. almeriensis, H. halmophila, H. koreensis, H. salina, H. halophila*. The strains were all alkalitolerant and most of them are moderate halophilic requiring NaCl for the growth. As reported in Table 1, many morphological and physiological characteristics differentiated the species, such as colony colour, acid production in the presence of sugars and some enzymatic tests. All strains produced PHB under their optimal growth conditions, but Sharm strain produced also EPS, that was not found in *H. elongata*, *H. salina* and *H. halmophila*. Sharm isolate and *H. elongata* were facultatively anaerobic when they were grown in the presence of nitrate.

Table 1 Differential characteristics among isolate Sharm and related species of genus *Halomonas*

Characteristics	Sharm	*H. elongata*	*H. eurihalina*	*H. almeriensis*	*H. halmophila*	*H. koreensis*	*H. salina*	*H. halophila*
Cell moprphology	Rod	Long rod	Short rod	Short rod	Rod	Short rod	Short rod	Rod
Colony colour	White bright	Cream-beige	Cream-coloured, opaque		Cream-white	Cream	Cream-yellow	Cream
NaCl range (%w/v)	0–30	0–25	3.5–25	5–25	3–25	1–20	2–20	2–30
NaCl optimum (%w/v)	5–15	11.0	2.0	7.5	7.5	1–12	5.0	7.5
pH range	6.0–9.0	5.0–9.0	5.0–10.0	6.0–10.0	5.0–9.0	5.5–10.0	5.0–10.0	5.0–10.0
Temperature range(°C)	25–50	15–45	4–45	15–37	15–45	10–47	4–45	10–45
Nitrate reduction	+	+	+	–	–	+	+	+
Respiration on nitrate	+	+	–	–	–	–	–	–
Oxydase	–	–	–	–	+	+	+	+
Hydrolysis of								
Gelatin	–	+	+	–	–	–	–	–
Urea	–	+	+	–	–	+	+	+
Tyrosine	+	–	+	–	–	+	+	–
EPS production	+	–	+	+	–	n.d	+	–
Acid from								
D-Glucose	+	+	–	–	+	+	–	+
L-Arabinose	–	+	–	–	+	–	–	+
D-Galatose	+	–	–	–	+	–	–	+
Lactose	–	+	–	–	+	–	–	–
Maltose	–	+	–	–	+	n.d	–	+
Mannose	+	–	–	–	+	n.d	–	+
Sucrose	–	+	–	–	+	–	–	–
Trehalose	–	+	–	–	+	–	–	+
Osmoprotectant[a]	GB,E,OH-E	GB, E	GB	GB	GB	GB	GB	GB
G + C content of DNA (mol%)	64.7	60.5	59.1–65.7	63.5	63.0	70.0	60.4–64.7	66.7

Data were from the present studies and for comparison from Arahal et al. (2002), Mata et al. (2002), Garrity et al. (2005), Lim et al. (2005), Martínez-Checa et al. (2005) and Vreeland (2005)

n.d. not determined

[a] GB: glycine betaine; E, ectoine; OH-E, hydroxyectoine

Together with PEA, PG and DPG, a new undescribed phosphoglycolipid at R_f lower than PG was detected. Interestingly, this new lipid seems to be present in all members of *Halomonas* rRNA group 1, according to the classification by Arahal et al. (2002a), confirming a relationship between these species. The glycine betaine, as often found in cells grew in presence of yeast extract, was present in a large amount in all species studied while in Sharm isolate were present also ectoine and hydroxyectoine, the latter not usually found in *Halomonas* genus.

Description of *Halomonas sinaiensis* sp. nov

Halomonas sinaiensis (si.nai.en'sis, N.L. fem. adj. sinaiensis belonging to Sinai peninsula where the microorganism was isolated). Alkalitolerant and halotolerant non-sporulating rod, Gram-negative, facultative anaerobe in the presence of NO_3.

Colonies on medium 1 are white, circular with entire margin, convex, bright. It is mesophilic exhibiting optimum growth temperature of 35°C, and is able to grow between 25 and 50°C. It presents an optimum pH at 7.0, a pH range of 6.0–9.0 and it tolerates up to 30% NaCl, and requires 5.0–15.0% of NaCl for optimal growth. The isolate grows well in minimal media containing trehalose, lactose, cellobiose, maltose, fructose, sucrose, citrate, lysine, and produces acid from glucose, galactose and mannose. Tyrosine and hippurate are hydrolysed and nitrate is reduced. Catalase is present. The new isolate gives negative tests for oxidase, casein, gelatine, starch hydrolysis, phenylalanine deamination and nitrite reduction. The strain is able to synthetize α-glucosidase, EPS and

PHB. UQ9 is the major ubiquinone and the predominant polar lipids are phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PEA) and a novel phosphoglycolipid.

C16:0 and C19:0 with cyclopropane are the main cellular fatty acids, minor components are C17:0, family of C18:1, C18:0, and C20:0. Strain Sharm^T accumulates glycine-betaine as major component, ectoine and hydroxyectoine are minor osmoprotectants. The mol% G + C content of DNA is 64.7 mol%, and this species is phylogenetically related to *H. elongata* and *H. eurihalina* on the basis of 16S rRNA gene sequence. The following antibiotics do not inhibit the growth of the strain Sharm^T:ampicillin (25), erytromycin (30), tetracycline (50), kanamycin (30), streptomycin (25), but it is sensitive to chloramphenicol (10), penicillin G (10), nystatin (100), and fusidic acid (10). *H. sinaiensis* was isolated from water with mat of the saline lake inside Ras Muhammad Park, Egypt.

Type strain Sharm^T was deposited in Deutsche Sammlung von Mikroorganismen Collection, Braunschweig, Germany (DSM^T 18067) and in American Type Culture Collection, University Boulevard, Manassas, USA (ATCC^T BAA-1308). The EMBL accession number for the 16S rRNA gene sequence of Sharm^T is AM238662.

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