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ORIGINAL PAPER

Salinivibrio sharmensis sp. nov., a novel haloalkaliphilic bacterium from a saline lake in Ras Mohammed Park (Egypt)

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Abstract A novel haloalkaliphilic, facultative anaerobic and Gram-negative *Salinivibrio*-like microorganism (designated strain BAG^T) was recovered from a saline lake in Ras Mohammed Park (Egypt). Cells were motile, curved rods, not spore-forming and occurred singly. Strain BAG^T grew optimally at 35°C (temperature growth range 25–40°C) with 10.0% (w/v) NaCl [NaCl growth range 6.0–16.0% (w/v)] and at pH 9.0 (pH growth range 6.0–10.0). Strain BAG^T had phosphatidylethanolamine (PEA) and phosphatidylglycerol (PG) as the main polar lipids, C16:0 (54.0%) and C16:1 (26.0%) as the predominant cellular fatty acids and Q-8 as the major respiratory quinone. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain BAG^T was a member of *Salinivibrio* genus, with the highest sequence similarities of 99.1, 98.4 and 98.1% to *Salinivibrio siamensis* JCM 14472^T, *Salinivibrio proteolyticus* DSM 19052^T and *Salinivibrio costicola* subsp. *alcaliphilus* DSM 16359^T, respectively. DNA–DNA hybridization values of strain BAG^T with members of *Salinivibrio* genus were lower than 55.0%. DNA G + C content was 51.0 mol%. On the basis of the polyphasic taxonomic results revealed in this study, strain BAG^T should be classified as a novel species of *Salinivibrio* genus, for which the name *Salinivibrio sharmensis* sp. nov. is proposed, with the type strain BAG^T (=ATCC BAA-1319^T = DSM 18182^T).

Keywords Phylogeny · *Salinivibrio* · Extremophilic · Haloalkaliphilic · Fatty acid methyl ester · Quinone

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16S rRNA gene sequence of *Salinivibrio sharmensis* is available in the GenBank/EMBL/DDBJ databases under the accession number AM279734.

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Introduction

Salinivibrio genus is included in the family *Vibrionaceae*, which belongs to the class *Gammaproteobacteria* of the phylum *Proteobacteria*. *Salinivibrio* genus was created for distinguishing *Salinivibrio costicola*, previously classified as *Vibrio costicola*, from other species of the *Vibrio* genus because of its physiological and molecular features (Mellado et al. 1996). Up to now, the *Salinivibrio* genus includes *S. costicola* subsp. *costicola* (Smith 1938; Mellado et al. 1996; Huang et al. 2000), *S. costicola* subsp. *vallismortis* (Huang et al. 2000), *S. costicola* subsp. *alcaliphilus* (Romano et al. 2005), *Salinivibrio proteolyticus* (Amoozegar et al. 2008) and *Salinivibrio siamensis* (Chamroensaksri et al. 2009).

Members of this genus are moderately halophilic bacteria distributed in salted meats, brines and several

hypersaline environments. Recent studies show clearly that halophilic species are good sources of biomolecules of great industrial interest, such as proteases used for laundry detergents (Rao et al. 1998). Sànchez-Porro et al. (2003, 2009) have reported the purification and biochemical characterization of a protease produced by the moderately halophilic microorganism *Pseudoalteromonas ruthenica* CP76. Lama et al. (2005) and Karbalaei-Heidari et al. (2008) have studied proteases from species belonging to *Salinivibrio* genus, such as *S. costicola* subsp. *alcaliphilus* and *S. proteolyticus*, respectively. Moreover, *S. costicola* has been used as model microorganism for studying osmoregulation and other physiological mechanisms in moderate halophiles (Oren 2002; Amoozegar et al. 2008).

Many extremophilic bacteria have been isolated from saline and hypersaline environments in Egypt, as from the most famous Wadi el-Natrun (Hezayen et al. 2001, 2002; Asker and Ohta 2002; Krumbein et al. 2004). Friedman (1985) and Krumbein et al. (2004) performed studies on hypersaline microbial systems of sabkhas from the Red Sea, but the only report about the isolation and characterization of a new strain from a sabkha brine pool was by Oren et al. (1999), who classified it in the *Archaea* domain as *Haloarcula quadrata.* Up to now, to the best of our knowledge, there are no other studies, except for ours, about extremophilic microorganisms isolated from the lakes in Ras Mohammed Park in the south of the Sinai peninsula (Romano et al. 2007).

In this paper, a novel haloalkaliphilic, facultative anaerobic, *Salinivibrio*-like microorganism (designated strain BAG^T) isolated from a saline lake in Ras Mohammed Park (Egypt) is described. The phenotypic and genotypic characteristics of this strain, including 16S rRNA gene sequence analysis and DNA–DNA hybridization, have been determined. The data obtained strongly support the suggestion that strain BAG^T should be placed in the *Salinivibrio* genus as the type strain of a novel species, *Salinivibrio sharmensis* sp. nov.

Materials and methods

Sample site

The strain BAG^T was isolated from samples collected during spring 2005 in a small permanent saline lake in Ras Mohammed Park, located at Sharm el-Sheikh, Egypt (27°51′N 34°16′E). This lake, 200 m in diameter, was 1 km far from the sea, had a pH about 8.0 and total chlorides at a concentration of 70.0 g/L. The samples, a mixture of sediment and water, were collected at the edge of the lake and stored at 4°C until their use as inoculum for the enrichment media.

Reference strains

S. costicola subsp. *costicola* DSM 11403^T, *S. costicola* subsp. *vallismortis* DSM 8285^T and *S. proteolyticus* DSM 19052^T were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) and routinely cultured using media and conditions recommended by DSMZ. *S. costicola* subsp. *alcaliphilus* DSM 16359^T was grown according to Romano et al. (2005). *S. siamensis* JCM 14472^T was obtained from Japan Collection of Microorganisms (JCM, Saitama, Japan) and grown according to JCM instructions.

Enrichment, isolation and growth conditions

Several enrichment media (10 mL) were inoculated with sediment and water samples (1 mL) and incubated at different temperatures, ranging between 10 and 55°C, under aerobic condition in an orbital shaker. Visible growth was observed using medium 1 containing the following components (g/L): yeast extract 10.0, NaCl 100.0, Na_3-citrate 3.0, Na_2CO_3 3.0, KCl 2.0, $MgSO_4 \cdot 7\ H_2O$ 1.0, $MnCl_2 \cdot 4\ H_2O$ 0.00036, $FeSO_4$ 0.05. NaCl and Na_2CO_3 were separately sterilized; the resulting pH was 9.0. All media were autoclaved for 20 min at 121°C. Solid medium was prepared by adding agar 1.8% (w/v) to the medium 1 above described. For growth under anaerobic condition, aliquots (25 mL) of the following culture medium containing (g/L): glucose 5.0, yeast extract 3.0, NaCl 100.0, Na_3-citrate 3.0, Na_2CO_3 3.0, KCl 2.0, $MgSO_4 \cdot 7\ H_2O$ 1.0, $MnCl_2 \cdot 4\ H_2O$ 0.00036, $FeSO_4$ 0.05, cysteine 0.5 and resazurin 0.001 were dispensed into 120-mL serum bottles. The resulting pH was 9.0. Oxygen was removed by heating the batch-reactors until the solution was colourless and then flushing the medium under a stream of O_2-free N_2 gas. Then bottles were immediately capped with butyl rubber stoppers and sterilized by autoclaving for 5 min at 121°C.

Bacterial growth was directly monitored in a UV/Vis spectrophotometer DU 730 (Beckman Coulter) by utilizing the change in optical density at 540 nm. Cell morphology was examined by phase contrast microscopy (Nikon Eclipse E400), while colony morphology was analyzed on solid culture medium by a stereomicroscope (Leica Wild M8).

Several strains were selected by means of the dilution-plating technique; culture purity was checked by the uniformity of colony morphology and by the examination of single cells in phase contrast microscopy. In this paper the

taxonomic properties of one of these microorganisms, strain BAG^T, will be presented.

Morphological, physiological and biochemical tests

For the new isolate the optimum parameters for growth were determined after 24–48–72 h of incubation in culture medium 1 by varying the temperature from 10 to 55°C and sodium chloride concentration from 0 to 30.0% (w/v). The specific requirement for NaCl was examined by substituting KCl at the same concentrations tested for NaCl. The pH dependence for growth of strain BAG^T was tested in the pH range 4.0–11.0 using medium 1 without Na_2CO_3 and properly buffered.

Gram staining was performed according to Dussault (1955), while Gram reaction was performed assaying aminopeptidase activity by Bactident-Merck and by the KOH lysis method according to Halebian et al. (1981).

Motility study was performed using culture medium 1 solidified with agar 0.4% (w/v). For spore formation test, enrichment medium 1 plus $MnCl_2 \cdot H_2O$ 0.01 g/L was used.

The ability to utilize a variety of substrates was determined using a liquid medium containing (g/L): NaCl 100.0, K_2HPO_4 7.0, KH_2PO_4 2.0, Na_2CO_3 3.0, $(NH_4)_2SO_4$ 1.0, $MgSO_4 \cdot 7\ H_2O$ 0.1, thiamine hydrochloride 0.05 mg and the tested substrate (final concentration 10.0 g/L). Acid production from carbohydrates was assessed as recommended by Ventosa et al. (1982), but modifying NaCl concentration to 6.0% (w/v).

To test antibiotic sensitivity, Sensi-discs (6 mm, Oxoid) of erythromycin (5 μg), fusidic acid (10 μg), chloramphenicol (10 μg), lincomycin (15 μg), streptomycin (25 μg), ampicillin (25 μg), vancomycin (30 μg), novobiocin (30 μg), neomycin (30 μg), gentamicin (30 μg), tetracycline (30 μg), penicillin G (10 units) and bacitracin (10 units) were used on medium 1 plus 1.8% (w/v) agar (Romano et al. 1993).

Casein (5.0% w/v), gelatin (12.0% w/v) and hippurate (1.0% w/v) hydrolyses, catalase, oxidase and tyrosinase activities, nitrate reduction and indole test were evaluated in medium 1 (Romano et al. 2005). Starch hydrolysis was assayed using solid medium 1 plus starch 0.2% (w/v). Methyl-red and Voges-Proskauer tests were done using BactoMarine broth (Difco n.2216) modified with NaCl 60.0 g/L and glucose 5.0 g/L. The extraction and purification of intracellular solutes were obtained according to Motta et al. (2004).

If not otherwise indicated, all tests were performed in triplicate using medium 1 and incubating the samples at the optimal growth temperature (35°C) for the required time.

Chemotaxonomic studies

Lipid analysis and hydrolysis, identification of the fatty acid methyl esters, quinone purification and its analysis were obtained as reported by Romano et al. (2001, 2005). G + C DNA content was performed as previously published (Poli et al. 2009; Romano et al. 2010).

Phylogenetic analyses

Genomic DNA extraction, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Romano et al. 2007, 2010). Sequences of related taxa were obtained from GenBank/EMBL/DDBJ databases. Phylogenetic analysis was performed using the program PAUP* version 4.0b10 (Swofford 2002) after multiple alignment of the data via CLUSTAL_X (Thompson et al. 1997). Multiple sequence alignment was revised visually to identify positions with uncertain alignments, mainly at the ends of the sequences, to be corrected or omitted from further analysis. The values for pairwise 16S rRNA gene sequence similarity among the closest species were determined using the EzTaxon server (http://www.Eztaxon.org) (Chun et al. 2007). All positions containing gaps and missing data were eliminated from the dataset. Neighbour-Joining (NJ; with Kimura's two-parameter correction), Maximum-Parsimony (MP; heuristic search option) and Maximum-Likelihood (ML) analyses were done for 16S rRNA gene sequences. For ML, the optimal model of nucleotide substitution was estimated through the program jModelTest (Posada 2008) using the Akaike Information Criterion (AIC). Bootstrap analyses were performed using 1,000 replications for NJ and MP, and 500 replications for ML.

DNA–DNA hybridization, REP and RAPD-DNA fingerprints

DNA extraction and purification, DNA–DNA hybridization and DNA fingerprint using REP [$(GTG)_5$ primer, Versalovic et al. 1994] and RAPD (OPR2 primer, Ronimus et al. 1997)-PCRs were performed as previously published (Romano et al. 2007, 2010; Poli et al. 2009).

Results

Isolation

A haloalkaliphilic culture, designated strain BAG^T, was obtained by inoculating the medium 1 with sample recovered from a saline lake in Ras Mohammed Park (Egypt). Cell growth was observed after incubation at 35°C for 24 h and microscopic examination revealed bacterial populations composed of singly curved rods. Axenic culture of strain BAG^T was obtained by the dilution-plating technique; this microorganism was the only colony-forming

organism at the highest dilutions and was the predominant one in enrichment culture medium 1.

Morphological, physiological and biochemical tests

The main characteristics of strain BAG^T were summarized and compared with those of other type strains of *Salinivibrio* genus in Table 1. Although the novel strain showed various morphological, physiological and biochemical properties strictly related to *Salinivibrio* genus, it could be distinguished from its congeners.

Optimal temperature for growth was 35°C, while no growth was observed below 25°C or above 40°C; moreover, growth occurred at optimum pH 9.0 in the presence of NaCl concentration from 6.0 to 16.0% (w/v) with an optimum at 10.0% (w/v). Strain BAG^T had a specific requirement for NaCl, because K^+ did not support the growth.

Table 1 Salient characteristics of strain BAG^T and other type strains of *Salinivibrio* genus

Characteristic	1	2	3	4	5	6
Cell morphology	Curved rods	Curved rods	Curved rods	Curved rods	Curved rods	Curved rods
Colony pigmentation	Cream-coloured	Cream-coloured	Creamy white	Creamy pink	Cream-coloured	Creamy white
Growth conditions						
Temperature range (°C)	25–40	10–45[a]	10–45[a]	10–40[a]	10–45[a]	20–50[a]
Temperature optimum	35	37[a]	35[a]	30[a]	35[a]	37[a]
pH range	6.0–10.0	5.0–9.0[a]	5.0–9.5[a]	7.0–10.5[a]	5.0–10.0[a]	5.5–8.5[a]
pH optimum	9.0	8.0[a]	8.0[a]	9.0[a]	7.5[a]	7.5[a]
NaCl range (%, w/v)	6.0–16.0	1.0–22.0[a]	1.0–17.0[a]	2.0–25.0[a]	0.5–20.0[a]	0.0–12.0[a]
NaCl optimum	10.0	10.0[a]	5.0[a]	10.0[a]	10.0[a]	2.0[a]
Anaerobic growth	+	+	+	–	+	+
Substrates						
Glucose	+	–[a]	+[a]	+[a]	+[a]	+[a]
Fructose	+	–[a]	+[a]	+[a]	–[a]	+[a]
Ribose	+	–[a]	+[a]	–[a]	–[a]	–[a]
Mannose	–	+[a]	–[a]	+[a]	–[a]	+[a]
Xylose	–	+[a]	–[a]	–[a]	+[a]	+[a]
Sucrose	+	–[a]	–[a]	+[a]	+[a]	+[a]
Lactose	–	–[a]	–[a]	+[a]	–[a]	–[a]
Sodium acetate	–	+[a]	+[a]	+[a]	+[a]	–[a]
Sodium propionate	–	+[a]	+[a]	+[a]	+[a]	–[a]
Acid production from						
Glucose	+	+[a]	+[a]	+[a]	+[a]	+[a]
Fructose	–	+[a]	+[a]	–[a]	–[a]	+[a]
Xylose	–	+[a]	+[a]	–[a]	–[a]	–[a]
Galactose	+	–[a]	+[a]	–[a]	+[a]	+[a]
Methyl-red test	–	–[a]	–[a]	–[a]	–[a]	–[a]
Voges-Proskaeur test	+	+[a]	+[a]	+[a]	+[a]	+[a]
Tyrosinase activity	+	+[a]	–[a]	+[a]	–[a]	–[a]
Nitrate reduction	+	+[a]	–[a]	+[a]	–[a]	–[a]
Hydrolysis of						
Casein	+	+[a]	+[a]	+[a]	+[a]	+[a]
Gelatin	+	+[a]	+[a]	+[a]	+[a]	+[a]
Starch	–	+[a]	+[a]	–[a]	–[a]	+[a]

Lanes: (1) strain BAG^T (this study), (2) *S. siamensis* (Chamroensaksri et al. 2009), (3) *S. proteolyticus* (Amoozegar et al. 2008), (4) *S. costicola* subsp. *alcaliphilus* (Romano et al. 2005), (5) *S. costicola* subsp. *costicola* (Mellado et al. 1996; Huang et al. 2000), (6) *S. costicola* subsp. *vallismortis* (Huang et al. 2000)

+ positive, – negative

[a] Data from our laboratory

After 24 h of incubation at 35°C on solid medium 1, the colony area appeared round, creamy, bright and had a diameter of 2.0–2.3 mm. In liquid medium 1, in the exponential growth phase, the strain BAG^T formed singly motile curved rod-shaped cells, 3.0–3.5 μm in length and 0.4–0.6 μm in width. Strain BAG^T was a facultative anaerobic Gram-negative bacterium.

In the culture medium plus Mn^{2+}, no spores were observed by light microscopy examination after 24, 48 and 72 h of incubation.

Glucose, fructose, ribose, sucrose, maltose, trehalose, glycerol and Tween 40 were able to sustain the growth of strain BAG^T; no growth occurred when xylose, galactose, mannose, cellobiose, lactose, raffinose, sodium acetate, sodium citrate, sodium propionate and ethanol (0.1% v/v) were used.

Acid production was observed using glucose, galactose, mannose, sucrose, maltose and trehalose, but not with xylose, fructose, ribose, cellobiose, lactose and raffinose.

Strain BAG^T was sensitive to erythromycin, fusidic acid, chloramphenicol, lincomycin, streptomycin, ampicillin, novobiocin, gentamicin, penicillin G and resistant to vancomycin, neomycin, tetracycline and bacitracin.

Strain BAG^T was able to produce catalase, oxidase and tyrosinase; casein, gelatin and Voges-Proskaeur tests were positive; nitrate reduction was observed. On the contrary, hippurate, starch, indole and methyl-red tests were negative. Strain BAG^T showed the capacity to accumulate glycine betaine as osmoprotectant.

Chemotaxonomic studies

Strain BAG^T contained complex lipids based on fatty acids. The total lipid content ranged between 12.0 and 15.0% of the total dry weight of cells grown under the optimal conditions in medium 1. In these conditions, phosphatidylethanolamine (PEA) and phosphatidylglycerol (PG) were the main lipids, while diphosphatidylglycerol (DPG) was completely absent. Glycolipids were not visualized and this could probably be due to their absence or presence in very low amount (Supplementary Table 1).

The fatty acid composition determined on strain BAG^T cells grown for 24 h at 35°C in medium 1 was characterized by the abundance of C16:0 and C16:1 (54.0 and 26.0%, respectively), while C12:0, C14:0, C18:0 and C18:1 were present in lower amounts (Supplementary Table 1).

The LC/MS as well as EI/MS analyses of the quinone content of strain BAG^T showed the presence of Q-8 as the major respiratory lipoquinone (Supplementary Table 1).

The G + C content of BAG^T-DNA was 51.0 mol%, a value comparable to G + C content described in literature for its closest phylogenetic relatives (Table 2).

Table 2 Values of 16S rRNA gene sequence similarity, DNA–DNA hybridization and G + C content for strain BAG^T and its closest phylogenetic relatives

Species	16S rDNA (%)	DNA–DNA (%)	G + C (mol%)
Strain BAG^T	100	100	51.0
S. siamensis	99.1	55.0	49.0
S. proteolyticus	98.4	39.0	49.5
S. costicola subsp. *alcaliphilus*	98.1	26.0	49.3
S. costicola subsp. *costicola*	98.0	34.0	49.9
S. costicola subsp. *vallismortis*	97.9	24.0	50.0

Phylogenetic analyses

A nearly full-length 16S rRNA gene sequence (1,377 nt) of strain BAG^T was determined. As shown in the Neighbour-Joining phylogenetic tree (Fig. 1), strain BAG^T was affiliated to the *Salinivibrio* genus and formed a distinct lineage with respect to closely related species. This tree topology is supported by high bootstrap values and by its recurrence in Maximum-Parsimony and Maximum-Likelihood trees (Supplementary Figs. 1 and 2). According to pairwise sequence comparisons, strain BAG^T showed 99.1% gene sequence similarity to *S. siamensis* JCM 14472^T, 98.4% to *S. proteolyticus* DSM 19052^T, 98.1% to *S. costicola* subsp. *alcaliphilus* DSM 16359^T, 98.0% to *S. costicola* subsp. *costicola* ATCC 35508^T and 97.9% to *S. costicola* subsp. *vallismortis* DSM 8285^T (Table 2). These high interspecific distances are in agreement with other ones found within the *Salinivibrio* genus (Amoozegar et al. 2008; Chamroensaksri et al. 2009).

DNA–DNA hybridization, REP and RAPD-DNA fingerprints

The results of DNA–DNA hybridization experiments between strain BAG^T and other validated members of *Salinivibrio* genus showed reassociation values of 55.0% with *S. siamensis* and less than 39.0% with other strains (Table 2).

REP and RAPD-PCR fingerprints using the $(GTG)_5$ and OPR2 primers were able to discriminate within *Salinivibrio* species and produced relevant, distinct DNA-bands of high molecular weight. Fingerprint profiles of strain BAG^T were clearly different from those produced by its closest relatives (Supplementary Fig. 3).

Discussion

Several saline environments located in Sinai peninsula were yet unexplored from the point of view of the presence

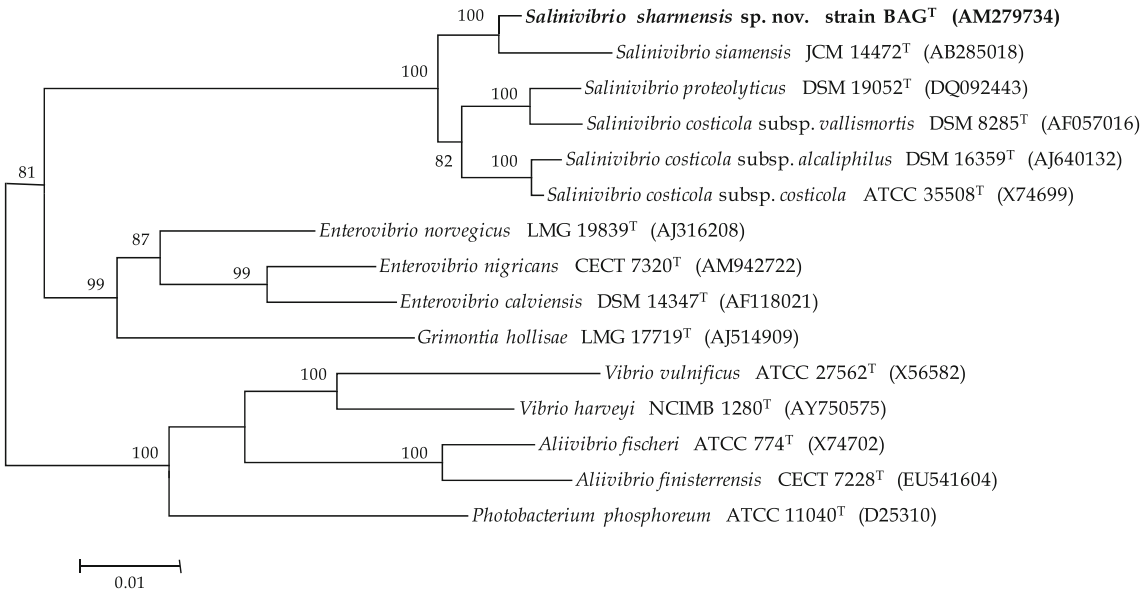

Fig. 1 Neighbour-Joining tree showing the phylogenetic position of strain BAG^T and some related *Vibrionaceae* species based on partial 16S rRNA gene sequences. Only bootstrap values above 75% are shown (1,000 resamplings) at branching points. Sequence accession numbers are given in *parentheses*. *Bar* 0.01 expected nucleotide substitution per site

and diversity of microbial life. This report is about the characterization of strain BAG^T, isolated from a saline lake located in one of these areas. 16S rRNA gene sequence analysis revealed that the closest relatives of this bacterium were the validated microorganisms classified in the *Salinivibrio* genus. It has been suggested that if the level of 16S rDNA similarity is greater than 97.0%, other additional phenotypic and/or genotypic characteristics should be used for taxonomic purposes (Stackebrandt and Goebel 1994). According to Wayne et al. (1987), less than 70.0% DNA–DNA relatedness is considered to be the threshold value for the delineation of genospecies. In our case low values for DNA–DNA hybridization experiments, not unusual, were obtained, as it was observed for other halophilic genera (Lim et al. 2004). On the basis of the DNA–DNA hybridization values strain BAG^T can be considered a new species of the *Salinivibrio* genus. Also, G + C DNA content (51.0 mol%) was in the range of values for members of this genus (Table 2).

Strain BAG^T exhibited differences with respect to previously described *Salinivibrio* genus members utilized for the comparative evaluations. As reported in Table 1, strain BAG^T was a facultative anaerobic bacterium and it was not able to grow below 25°C, unlike the other *Salinivibrio* members, which were usually able to grow at lower temperatures. The optimum pH for strain BAG^T growth was the highest one as compared to its closest relatives. Moreover, at least NaCl 6.0% (w/v) was necessary for obtaining strain BAG^T growth with respect to other *Salinivibrio* members, which were able to grow also at lower NaCl concentration. On the contrary, pH range and both optimum temperature and NaCl concentration requirements were similar between strain BAG^T and its congeners.

A biochemical difference was determined in the spectrum of substrates used for growth: strain BAG^T was able to grow only in the presence of glucose, fructose, ribose, sucrose, maltose, trehalose, glycerol and Tween 40 (Table 1).

All the members of *Salinivibrio* genus were positive for catalase, oxidase and Voges-Proskaeur tests and were able to hydrolyse casein and gelatin. On the contrary, strain BAG^T and all its congeners were negative for methyl-red and indole tests. Only strain BAG^T, *S. siamensis* and *S. costicola* subsp. *alcaliphilus* were able to reduce nitrate and to hydrolyse tyrosine. Moreover, strain BAG^T was not able to utilize starch, such as *S. costicola* subsp. *alcaliphilus* and *S. costicola* subsp. *costicola*, and unlike *S. siamensis, S. proteolyticus* and *S. costicola* subsp. *vallismortis*.

When analysed using their optimal growth conditions, the total lipid content of strain BAG^T accounted for about 12.0–15.0% the total dry weight of cells, in accordance with *S. costicola* subsp. *alcaliphilus*, *S. costicola* subsp. *costicola* and *S. costicola* subsp. *vallismortis*. The validated members of *Salinivibrio* genus had phosphatidylethanolamine (PEA), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) as the main lipids. In particular, in *S. siamensis*, *S. proteolyticus*, *S. costicola* subsp. *costicola* and *S. costicola* subsp. *vallismortis* DPG was present in a lower amount with respect to PEA and PG, while in strain BAG^T it was completely absent.

In all the tested strains the fatty acid composition was determined on cells grown under standard conditions and

the predominant fatty acids were long saturated and monounsaturated acyl chains. In each strain, the sum of C16:0, C16:1 and C18:1 were more than 50.0% of the total cellular fatty acid content and C18:0, C19:1, C20:0, C20:1 were present in trace amounts. In particular, in strain BAG^T the most abundant was C16:0 (54.0%) and C12:0, C14:0, C18:0 were less than 10.0% of the total cellular fatty acids.

The presence of Q-8 as the major respiratory lipoquinone was showed by LC/MS as well as EI/MS analyses in all the tested strains (Supplementary Table 1).

On the basis of the phenotypic and genotypic differences between the new isolate and previously described species and subspecies within *Salinivibrio* genus, we propose to classify strain BAG^T as the type strain of a novel species of this genus with the name *Salinivibrio sharmensis* sp. nov.

Description of *Salinivibrio sharmensis* sp. nov

Salinivibrio sharmensis (sha.rm.en'sis N.L. masc. adj. sharmensis, referring to Sharm el-Sheikh, where the strain was isolated)

Cells are Gram-negative, non-spore-forming, motile singly curved rods, 0.4–0.6 × 3.0–3.5 μm. Form circular, with entire edges, bright, creamy-colonies (diameter 2.0–2.3 mm). Facultative anaerobic. Growth at 25–40°C (optimally at 35°C), at pH values of 6.0–10.0 (optimally at pH 9.0) and in 6.0–16.0% (w/v) NaCl (optimally in 10.0% NaCl). No growth occurred in the absence of sodium chloride in the culture medium and KCl cannot substitute NaCl. Utilizes glucose, fructose, ribose, sucrose, maltose, trehalose, glycerol and Tween 40, but does not utilize xylose, galactose, mannose, cellobiose, lactose, raffinose, sodium acetate, sodium citrate, sodium propionate and ethanol (0.1% v/v). Acid is produced from glucose, galactose, mannose, sucrose, maltose and trehalose, but not from xylose, fructose, ribose, cellobiose, lactose and raffinose.

The type strain growth is susceptible to erythromycin (5 μg), fusidic acid (10 μg), chloramphenicol (10 μg), lincomycin (15 μg), streptomycin (25 μg), ampicillin (25 μg), novobiocin (30 μg), gentamicin (30 μg) and penicillin G (10 units), but resistant to vancomycin (30 μg), neomycin (30 μg), tetracycline (30 μg) and bacitracin (10 units).

Positive in tests for catalase, oxidase, tyrosinase, hydrolysis of casein and gelatin, for nitrate reduction and for the Voges-Proskauer test. Negative results in tests for the hydrolysis of hippurate and starch, for indole production and for methyl-red test.

Glycine betaine is accumulated as osmoprotectant. Phosphatidylethanolamine (PEA) and phosphatidylglycerol (PG) are the main polar lipids. C16:0 and C16:1 are the most abundant cellular fatty acids. Q-8 is the predominant respiratory lipoquinone.

The type strain, BAG^T (=ATCC BAA-1319^T = DSMZ 18182^T), was isolated from sediment–water mixed samples collected in a saline lake in Ras Mohammed Park (Egypt). On the basis of 16S rRNA gene sequence analysis and DNA–DNA reassociation strain BAG^T is phylogenetically related to *Salinivibrio* species. The G + C DNA content is 51.0 mol%.

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