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Halobaculum gomorrense gen. nov., sp. nov., a Novel Extremely Halophilic Archaeon from the Dead Sea

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A novel extremely halophilic archaeon was isolated from the Dead Sea. This isolate is rod shaped and, like *Halobacterium sodomense*, requires a relatively low level of sodium ions for growth and a very high level of magnesium; optimal growth occurs in the presence of 0.6 to 1.0 M Mg^{2+}. The new strain resembles members of the *Halobacterium saccharovorum-Halobacterium sodomense-Halobacterium trapanicum* group in many physiological properties. However, the polar lipid composition of this organism is characteristic of representatives of the genus *Haloferax*; a sulfated diglycosyl diether is present, and the glycerol diether analog of phosphatidylglycerosulfate is absent. The G+C content of the DNA is 70 mol%. We found that on the basis of 16S rRNA sequence data our new isolate occupies a position intermediate between the position of the *Halobacterium saccharovorum* group and the position of the genus *Haloferax* and is sufficiently different from the previously described members of the *Halobacteriaceae* to justify classification in a new species and a new genus. We propose the name *Halobaculum gomorrense* gen. nov., sp. nov. for this organism; the type strain is strain DSM 9297.

Halophilic archaea have been found in the Dead Sea since the first studies of the biology of the lake in the 1930s. At times these organisms are present in numbers high enough ($\geq 10^7$ cells per ml) to impart a reddish color to the water. Such a phenomenon occurred in 1963 and 1964 and again in 1980 (32).

The following four genera of halophilic nonalkaliphilic archaea have been described previously: *Halobacterium*, *Haloferax*, *Haloarcula*, and *Halococcus* (9, 40). In the past enrichment cultures in which Dead Sea water or sediment was used as the inoculum have yielded isolates of at least three novel halophilic archaea, *Halobacterium sodomense* (29), *Haloferax volcanii* (27), and *Haloarcula marismortui* (36).

A new bloom of halophilic archaea developed in the Dead Sea in the summer of 1992 (34). The results of polar lipid analyses suggested that the organism which dominated this community was related to the genus *Haloferax*. One major glycolipid was found in polar lipid extracts of the bacterial community collected from the Dead Sea during the bloom. This glycolipid coeluted with the major glycolipid of *Haloferax volcanii* and *Haloferax mediterranei* on both one-dimensional and two-dimensional thin-layer chromatograms (37). Moreover, phosphatidylglycerosulfate, a polar lipid present in all halophilic archaea except *Haloferax* species, was not detected in lipid extracts of the Dead Sea biomass.

Attempts to identify the numerically dominant archaeal species in the Dead Sea samples by isolating and characterizing the bacteria that developed on agar plates or in liquid media were not very successful. The numbers obtained were typically 2 or more orders of magnitude lower than the numbers observed microscopically. The highest estimates of the numbers of culturable bacteria were obtained by preparing dilution series in a liquid medium suitable for the growth of *Halobacterium sodomense*. The bacteria obtained in this way were motile rods whose morphology and polar lipid pattern were identical to the morphology and polar lipid pattern of *Halobacterium sodomense*. However, in one case, a sample collected on 28 July 1992 from a depth of 4 m, the organism that grew at the highest dilution (designated strain DS2807^T [T = type strain]) was a pleomorphic rod-shaped bacterium with a glycolipid composition similar to the glycolipid composition of extracts of biomass collected from the Dead Sea.

In this paper we describe the properties of isolate DS2807^T. We found that this strain occupies a distant position that is intermediate between the position of the group formed by *Halobacterium saccharovorum*, *Halobacterium sodomense*, and related species and the position of the genus *Haloferax* and that it is sufficiently different from the previously described members of the family *Halobacteriaceae* to justify classification in a new species and a new genus.

MATERIALS AND METHODS

Bacterial strains. Dead Sea strain DS2807^T was isolated from a sample collected on 28 July 1992 at a depth of 4 m at the deepest part of the Dead Sea, about 8 km east of Ein Gedi (37). This strain grew in the highest positive tube of a dilution series in the medium described below. *Halobacterium sodomense* ATCC 33755^T, *Halobacterium saccharovorum* ATCC 29252^T, *Haloferax volcanii* ATCC 29605^T, *Haloferax mediterranei* ATCC 33500^T, *Haloferax denitrificans* ATCC 35960^T, and *Haloarcula marismortui* ATCC 43049^T were used as reference strains in biochemical tests.

Media and growth conditions. The standard medium used for strain DS2807^T and *Halobacterium sodomense* contained (per liter) 125 g of NaCl, 160 g of $MgCl_2 \cdot 6H_2O$, 5.0 g of K_2SO_4, 0.1 g of $CaCl_2 \cdot 2H_2O$, 1.0 g of yeast extract (Difco), 1.0 g of Casamino Acids (Difco), and 2.0 g of soluble starch (BDH). The pH of the medium was adjusted to 7.0 with NaOH. This medium was modified with respect to salt concentrations and nutrient and inhibitor contents as described below. Other reference strains were grown in suitable media, as described previously (27, 36, 38, 40). In most experiments, cells were grown in a horizontal shaking water bath (100 strokes per min) at 35°C in 100-ml Erlenmeyer flasks containing 50 ml of medium. To prepare agar plates, the media were solidified with 20 g of agar per liter. The media were sterilized by autoclaving.

Miscellaneous diagnostic tests. Gram staining was performed by using acetic acid-fixed samples as described by Dussault (5). Tests for catalase and oxidase activities, starch hydrolysis, formation of indole from tryptophan, and nitrate

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TABLE 1. Organisms used for comparison in this study

Taxon	Strain[a]	Nucleotide sequence accession no.	Reference
Methanospirillum hungatei	DSM 864	M60880	45
Natronococcus occultus	NCIMB 2192	Z28378	24
Natronobacterium magadii	NCIMB 2190	X72495	22
Halobacterium salinarium (*Halobacterium halobium*)	DSM 671	M38280	23
Haloferax volcanii	ATCC 29605	K00421	10
Haloferax mediterranei	ATCC 33500	D11107	15
Haloferax gibbonsii	ATCC 33959		16
Haloferax denitrificans	ATCC 35960		16
Halococcus morrhuae	ATCC 17082	X00662	20
Haloarcula marismortui (*rrnA* gene)	Ginzburg strain 11C8	X61688	28
Haloarcula marismortui (*rrnB* gene)	Ginzburg strain 111110	X61689	28
"*Haloarcula sinaiiensis*" (major gene)	ATCC 33800		16
"*Haloarcula sinaiiensis*" (minor gene)	ATCC 33800		16
Halobacterium sodomense[b]	ATCC 33755	X82169	25
Halobacterium trapanicum[b]	NRC 34021	X82168	25
Halobacterium saccharovorum[b]	NCIMB 2081	X82167	25
Halobacterium lacusprofundi[b]	ACAM 34	X82170	25
Halobaculum gomorrense	DS2807^T (= DSM 9297^T)	L37444	

[a] ACAM, Australian Collection of Antarctic Microorganisms, Department of Agricultural Science, University of Tasmania, Hobart, Tasmania, Australia; ATCC, American Type Culture Collection, Rockville, Md.; DSM, DSM-Deutsche Sammlung von Microorganismen und Zellkulturen GmbH, Braunschweig, Germany; NCIMB, National Collection of Marine and Industrial Bacteria, Ltd., Aberdeen, Scotland; NRC, National Research Council, Ottawa, Canada.

[b] Transfer to the genus *Halorubrum* gen. nov. has been proposed by McGenity and Grant (25).

reduction were performed by using standard procedures (8, 29). Carotenoid pigments were extracted in methanol-acetone (1:1, vol/vol), and the absorption spectrum of the extract was determined with a Hewlett-Packard model 8452A diode array spectrophotometer. To test for induction of formation of bacteriorhodopsin, cultures were grown under oxygen-limited conditions in the light (29). The presence of poly-β-hydroxybutyrate was determined by extracting cells with chloroform, hydrolyzing the extracted material at 100°C with concentrated sulfuric acid, and assessing the formation of crotonic acid on the basis of its absorption maximum at 230 nm (21). The utilization of sugars and other compounds as carbon sources and acid production from these compounds were determined in standard medium modified as follows: starch was omitted, and the yeast extract and Casamino Acids concentrations were reduced to 0.25 g/liter each or yeast extract and Casamino Acids were omitted, as described below. In the latter case, the media were amended with 0.1 g of NH_4Cl per liter and 0.01 g of KH_2PO_4 per liter. Each potential carbon source was added to a final concentration of 5 g/liter from a concentrated sterile solution. Growth was monitored by determining the optical density of each culture at 600 nm, and the pH of each culture was compared with the pH of a control culture. A decrease in the pH to a value that was less than 6.0 was considered evidence of acid production. In a number of experiments, 20 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)]-NaOH buffer was added to the media. Starch hydrolysis was tested by flooding colonies grown on agar plates containing the standard growth medium with an iodine solution. For anaerobic growth determinations, cells were inoculated into medium that was or was not supplemented with 5 g $NaNO_3$ or L-arginine hydrochloride per liter in completely filled 20-ml glass tubes closed with butyl rubber stoppers. Susceptibility to antibiotics and other inhibitors was determined in liquid medium.

Lipid analyses. Cells that had been collected by centrifugation were suspended in 1 ml of 4 M NaCl and extracted with 3.75 ml of methanol-chloroform (2:1, vol/vol) for 4 h. The extracts were collected by centrifugation, and the pellets were reextracted with 4.75 ml of methanol-chloroform-water (2:1:0.8). Then 2.5 ml of chloroform and 2.5 ml of water were added, to the combined supernatants, and the chloroform phase was collected by centrifugation and dried in a stream of nitrogen. The lipids were separated by thin-layer chromatography by single development on silica gel plates (20 by 20 cm; Sigma) in a chloroform-methanol-acetic acid-water (85:22.5:10:4, by volume) solvent system. In addition, two-dimensional chromatography was performed by using chloroform-methanol-acetic acid-water (80:12:15:4) in the first dimension and chloroform-methanol-water (65:25:4) in the second dimension. Glycolipid spots were detected by spraying the plates with 0.5% α-naphthol in 50% methanol and then with 5% H_2SO_4 in ethanol and heating them at 150°C. Phospholipids were visualized with a ammonium molybdate-sulfuric acid spray (17).

DNA base composition. The G+C content of the DNA was determined by workers at the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). The DNA was isolated and purified by chromatography on hydroxyapatite. The G+C content was determined by using high-performance liquid chromatography (HPLC) as described by Mesbah et al. (26).

PCR amplification of the 16S rRNA gene coding sequence and sequencing. Nucleic acids were isolated by digesting cells with proteinase K-sodium dodecyl sulfate, extracting the preparations with phenol, and precipitating the nucleic acids with ethanol as previously described (39). The 16S rRNA gene was amplified by PCR by using primers 4F-Archaea (5′-TCCGGTTGATCCTGCCGG-3′; corresponding to *Escherichia coli* positions 4 to 21) and 1542R-Archaea/Bacteria (3′-ACCTAGTGGAGGAAA-5′; corresponding to *E. coli* positions 1528 to 1542). Each of the 30 PCR cycles started with 1 min of denaturation at 95°C, which was followed by 2 min of annealing at 40°C and 3 min of elongation at 71°C. Each reaction mixture (total volume, 50 μl) contained 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 200 μM dGTP, 200 μM dATP, 200 μM dTTP, 200 μM dCTP, 0.5 to 1.0 U of *Taq* DNA polymerase (SuperTaq; HT Biotechnology, Ltd.), and each primer at a concentration of 0.5 μM. The PCR products were analyzed by electrophoresis on horizontal 1% agarose gels in TAE buffer (38) by using a defined double-stranded 1.5-kb 16S rRNA PCR copy derived from *Desulfobulbus* sp. as the size marker. Each product examined was purified by cutting a small well in the agarose gel in front of the selected band. Electrophoresis was then continued until the PCR product migrated into the buffer-filled well, from which it was collected with a pipette. After precipitation with 0.1 volume of 5 M NaCl and 2.5 volumes of ethanol for 1 h at −80°C, the product was collected by centrifugation, dissolved in 50 μl of water, and sequenced.

The sequencing primers used were complementary to highly conserved regions of the 16S rRNA sequence (see Fig. 4). The procedure which we used was based on a previously described protocol (1), modified as follows: each reaction mixture contained 2 μl of 5× buffer (200 mM Tris-HCl [pH 7.5], 100 mM $MgCl_2$, 250 mM NaCl), 1 μl of a 5% Nonidet P-40 (Sigma) aqueous solution, 2 pmol of primer, and 10 to 100 ng of purified PCR product in a final volume of 10 μl. The PCR product was denatured by heating the preparation at 95°C for two 5-min periods with a centrifugation step in between. The sequencing reaction was started by adding 1 μl of 0.1 M dithiothreitol, 2 μl of a deoxynucleoside triphosphate solution (200 nM dGTP, 200 nM dATP, 200 nM dTTP), 0.5 μl of [α-^{33}P]dCTP (10 μCi/μl; 3,000 Ci/mmol), and 1 U of Sequenase 2.0 (United States Biochemical Corp.). After centrifugation to collect and mix the samples, the samples were incubated for 5 min at 37°C. The mixtures were then divided into four 3.5-μl portions and added to dideoxynucleotide termination solutions (2.5 μl) containing 80 μM dGTP, 80 μM dATP, 80 μM dTTP, 80 μM dCTP, and 8 μM dideoxynucleotide. After 5 min of incubation at 37°C, each reaction was terminated by adding 4 μl of a solution containing 96% formamide and 20 mM EDTA, and sequencing gels were electrophoresed by using standard procedures. The sequence obtained was compared with previously described 16S rRNA sequences of halophilic archaea (19); the strains used are listed in Table 1. The sequences were aligned by using CLUSTAL V (12). A phylogenetic tree was constructed from the molecular sequence data by using programs in version 3.4 of PHYLIP, the phylogenetic inference package of Felsenstein (6). Using the program DNADIST, we prepared a matrix of evolutionary distances from the sequence alignment data with the Jukes-Cantor model (14), which assumes that independent changes occur at all sites with equal probability. A phylogenetic tree was constructed from the distance matrix by using the program FITCH, which

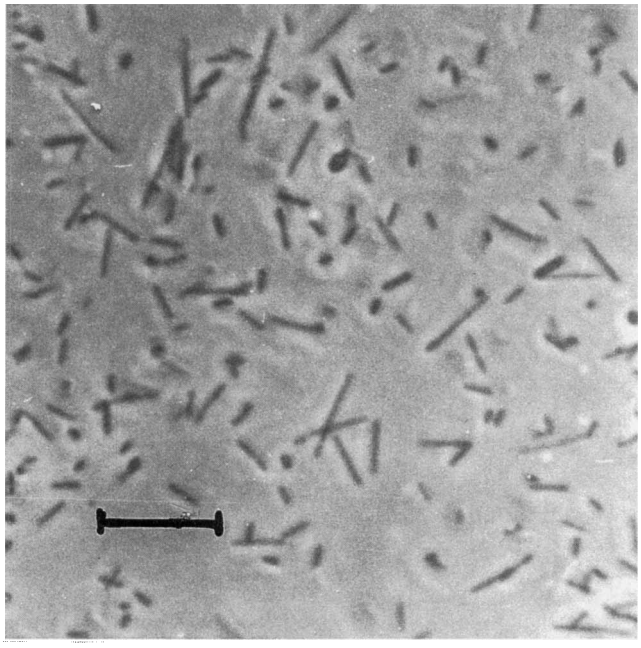

FIG. 1. Phase-contrast micrograph of strain DS2807^T cells grown in standard liquid medium. Bar = 10 μm.

uses the least-squares algorithm of Fitch and Margoliash (7); this tree was drawn by using DRAWTREE.

Nucleotide sequence accession numbers. The GenBank accession numbers for the 16S rRNA sequences of the organisms used in this study are shown in Table 1.

RESULTS

Cultural characteristics. When strain DS2807^T was grown in the standard growth medium described above, the cells were rod shaped (5 to 10 by 0.5 to 1 μm) and lacked gas vacuoles (Fig. 1). The cells stained gram negative. Motile cells were occasionally observed. The cultures were orange-red. This organism formed small, round, translucent colonies on agar plates. Starch was not required, but was highly stimulatory. No growth was observed on other media that have been described as suitable for the growth of *Halobacterium*, *Haloferax*, and *Haloarcula* species. These media contain much higher concentrations of yeast extract (5 to 10 g/liter), which inhibited strain DS2807^T. Increasing the yeast extract concentration and/or the Casamino Acids concentration in the standard medium to more than 2.5 g/liter resulted in poor growth.

Strain DS2807^T required high salt concentrations for structural integrity. Both in media containing high magnesium concentrations and in suspensions containing NaCl, salt concentrations of at least 15% were needed to maintain the rod shape of the cells. At lower concentrations the cells were spherical, and at salt concentrations less than 5% lysis occurred. At least 1 M NaCl was required for growth (in the presence of 0.8 M $MgCl_2$). The optimal NaCl concentration range was 1.5 to 2.5 M at 35°C. The magnesium concentrations which resulted in optimal growth were extremely high; optimal growth was observed in media containing 0.6 to 1 M $MgCl_2$ in the presence of 2.1 M NaCl, and in the presence of magnesium concentrations less than 0.2 M growth was poor (Fig. 2). The optimum temperature for growth was 40°C (in medium containing 2.1 M NaCl and 0.8 M $MgCl_2$). At 45°C growth was slow, and at temperatures above 50°C no growth occurred. The optimum pH for growth was 6 to 7. Growth was not observed at pH values below 5.5 and above 8. The minimal doubling time measured under optimal growth conditions was 5.5 h.

Biochemical and physiological characterization. The cells were red because of the presence of carotenoid pigments. Methanol-acetone extracts produced the characteristic absorption spectrum of bacterioruberins, with peaks at 494 and 528 nm and a shoulder at 430 nm. The purple color of bacteriorhodopsin was never observed, not even in cultures incubated under reduced oxygen tensions in the light. Formation of poly-β-hydroxybutyrate was not observed in cells grown in standard medium or in medium supplemented with 1 g of sodium acetate per liter.

Strain DS2807^T was obligately aerobic and exhibited positive oxidase and catalase reactions. Nitrate was reduced to nitrite in aerobic cultures, but anaerobic growth was not observed in the presence of nitrate, nor did the organism grow anaerobically in the presence of arginine. *Halobacterium halobium* (*Halobacterium salinarium*) ferments arginine (11), but this property is not common among the halophilic archaea. Anaerobic growth on arginine has been observed only in members of the *Halobacterium halobium-Halobacterium salinarium* group and in the alkaliphilic organism *Natronobacterium pharaonis* (35).

Isolate DS2807^T did not hydrolyze gelatin, and indole was not formed in standard growth medium or in medium supplemented with 0.1 or 0.5 g of L-tryptophan per liter. Hydrolysis of Tween 80 could not be tested as this compound inhibited growth.

Growth on single carbon sources was never observed. In media in which the yeast extract and Casamino Acids concentrations were reduced to 0.25 g/liter each and starch was omitted, glucose, maltose, sucrose, galactose, xylose, trehalose, starch, and glycerol stimulated growth and acid was produced. Growth was also stimulated by DL-lactate. Starch was hydrolyzed; we did not determine whether the enzyme responsible for this is an amylase or, as is the case in *Halobacterium sodomense*, an amyloglucosidase (2, 30). Acid was not produced from mannose, fructose, ribose, lactose, arabinose, mannitol, and sorbitol. Growth was not stimulated by acetate, citrate, propionate, succinate, glycine, L-alanine, and L-glutamate. Strain DS2807^T was susceptible to anisomycin, novobiocin, bacitracin, deoxycholate, and taurocholate (all at a concentration of 25 μg/ml), as well as to vibriostatic agent 0/129 (2,4-diamino-

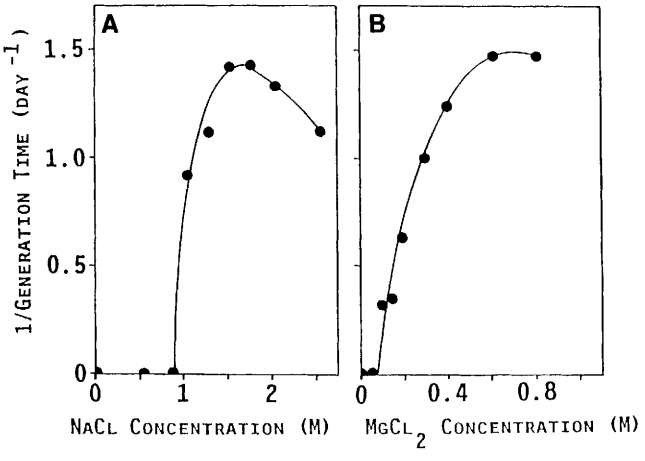

FIG. 2. Effects of NaCl (A) and $MgCl_2$ (B) concentrations on the growth rate of strain DS2807^T. The media used to determine the effects of NaCl and $MgCl_2$ contained 0.8 M $MgCl_2$ and 2.1 M NaCl, respectively, as well as 0.1% yeast extract, 0.1% Casamino Acids, and 0.2% starch as organic nutrients. The cultures were incubated with shaking at 35°C.

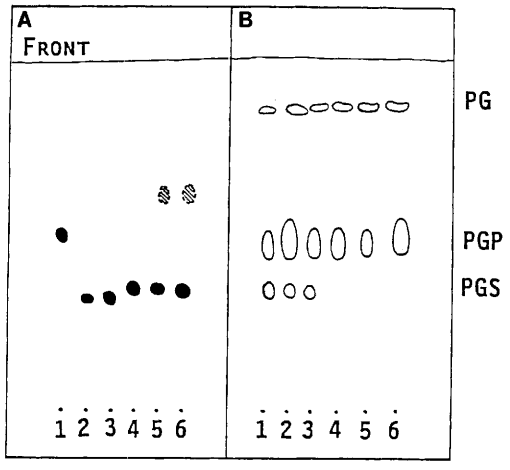

FIG. 3. Thin-layer chromatogram of polar lipids extracted from strain DS2807^T (lane 4), *Haloarcula marismortui* ATCC 43049^T (lane 1), *Halobacterium saccharovorum* ATCC 29252^T (lane 2), *Halobacterium sodomense* ATCC 33755^T (lane 3), *Haloferax volcanii* ATCC 29605^T (lane 5), and *Haloferax denitrificans* ATCC 35960^T (lane 6). The silica gel plate was developed once with chloroform-methanol-acetic acid-water (85:22.5:10:4, by volume) and was stained for glycolipids (A) or for phospholipids (B). The black spots indicate the position of the dominant glycolipid; the dashed spots are additional sugar-positive spots.

6,7-diisopropylpteridine phosphate) (10 and 50 μg/ml). Penicillin G, ampicillin, kanamycin, chloramphenicol, streptomycin sulfate, neomycin, and cycloheximide (all at a concentration of 25 μg/ml) did not inhibit growth.

Lipid analyses. Thin-layer chromatography of lipid extracts of strain DS2807^T revealed that this organism contained three polar lipids, the glycerol diether analogs of phosphatidylglycerol, phosphatidylglycerophosphate, and a single glycolipid (Fig. 3). The glycolipid exhibited a chromatographic behavior identical to the behavior of the major glycolipid of *Haloferax* species, both in one-dimensional thin-layer chromatography and in two-dimensional thin-layer chromatography, and was distinct from the glycolipids of *Halobacterium sodomense* and *Halobacterium saccharovorum*. The glycerol diether analog of phosphatidylglycerosulfate, which is present in all halophilic archaea except *Haloferax* species, was not detected.

DNA base composition. The G+C content of the DNA of strain DS2807^T was 70 mol%.

Phylogeny. Our phylogenetic tree, which was constructed by comparing the strain DS2807^T 16S rRNA sequence with the 16S rRNA sequences of other halophilic archaea, showed that strain DS2807^T occupies a position that is intermediate between the position of *Halobacterium saccharovorum* and related species and the position of the genus *Haloferax* (Fig. 4). The DS2807^T 16S rRNA gene exhibited 89.0 to 89.2 and 88.8 to 89.4% sequence similarity with representatives of the genus *Haloferax* and with representatives of the *Halobacterium saccharovorum* group, respectively, and the strain DS2807^T sequence was equidistant from the other halobacterial sequences included for comparison (Table 2).

DISCUSSION

Halophilic strain DS2807^T was isolated from a dense bloom of red archaea that developed in the Dead Sea in 1992 (34, 37). Because of the similarity of the polar lipids of this isolate and the polar lipids extracted from the biomass collected from the Dead Sea at the time that strain DS2807^T was isolated, the new isolate may represent the dominant type of halophilic archaea in the bloom (37).

On the basis of its polar lipid composition, strain DS2807^T was found to be most closely related to the genus *Haloferax*. We detected a single glycolipid that had a chromatographic behavior identical to the chromatographic behavior of the major *Haloferax* glycolipid, S-DGD-1 {1-*O*-[α-D-mannose-(6'-SO_4^-)-(1'→2')-α-D-glucose]-2,3-di-*O*-phytanyl-*sn*-glycerol} (18, 40–42). This glycolipid differs structurally from the sulfated diglycosyl diether lipids of *Halobacterium sodomense* and related organisms (43, 44) (Fig. 3). The lack of phosphatidylglycerosulfate is also a diagnostic characteristic of the genus *Haloferax* (40–42).

On the phylogenetic tree, which was based on the results of a 16S rRNA nucleotide sequence comparison, strain DS2807^T did not cluster with the genus *Haloferax*, but appeared on a separate branch that was about equally removed from the

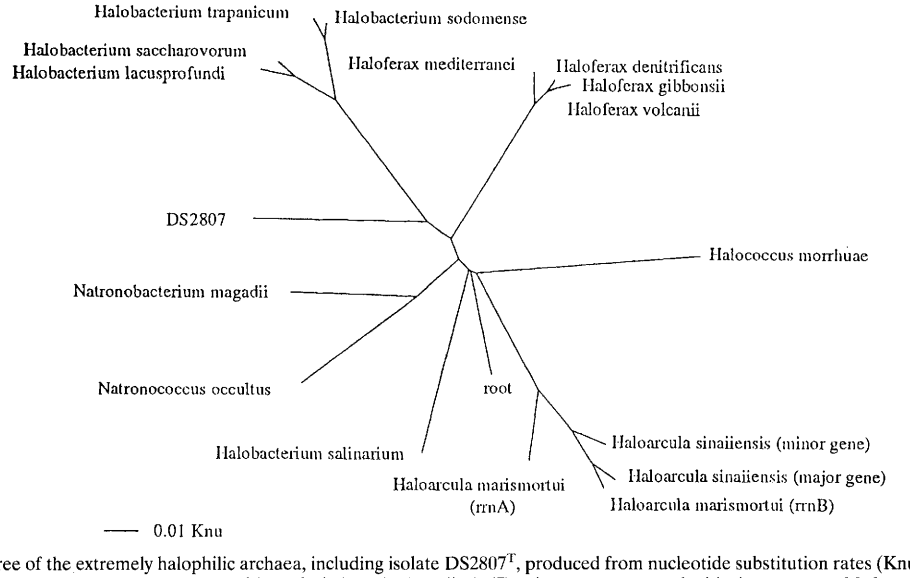

FIG. 4. Phylogenetic tree of the extremely halophilic archaea, including isolate DS2807^T, produced from nucleotide substitution rates (Knu values) derived by using the Jukes-Cantor model (14) and the least-squares algorithm of Fitch and Margoliash (7). The tree was rooted with the outgroup *Methanospirillum hungatei*.

TABLE 2. Similarity matrix for halobacteria based on 16S rRNA gene sequences[a]

Taxon	Evolutionary distance or % similarity[b]																	
	Methanospirillum hungatei DSM 864	*Natronococcus occultus* NCIMB 2192	*Natronobacterium magadii* NCIMB 2190	*Halobacterium salinarium* DSM 671	*Haloferax volcanii* ATCC 29605	*Haloferax mediterranei* ATCC 33500	*Haloferax gibbonsii* ATCC 33959	*Haloferax denitrificans* ATCC 35960	*Halococcus morrhuae* ATCC 17082	*Haloarcula marismortui* Ginzburg strain 11C8	*Haloarcula marismortui* Ginzburg strain 111110	"*Haloarcula sinaiiensis*" ATCC 33800 (major gene)	"*Haloarcula sinaiiensis*" ATCC 33800 (minor gene)	*Halobacterium sodomense* ATCC 33755	*Halobacterium trapanicum* NRC 34021	*Halobacterium saccharovorum* NCIMB 2081	*Halobacterium lacusprofundi*; ACAM 34	*Halobaculum gomorrense* DS2807[T]
Methanospirillum hungatei DSM 864		77.9	77.2	79.1	79.0	79.2	78.9	78.9	78.2	79.0	78.2	78.0	78.5	77.2	76.9	77.3	77.6	78.8
Natronococcus occultus NCIMB 2192	0.2625		92.5	89.8	88.9	89.1	88.5	88.3	88.0	87.6	87.2	86.6	87.5	87.4	87.2	88.0	87.6	88.1
Natronobacterium magadii NCIMB 2190	0.2722	0.0795		89.9	89.8	89.8	89.8	89.3	88.6	89.4	88.0	87.6	88.3	86.6	86.2	87.5	87.5	88.0
Halobacterium salinarium DSM 671	0.2443	0.1095	0.1087		88.0	87.7	87.8	87.7	88.6	90.1	87.6	87.1	88.3	87.4	86.9	87.9	87.4	88.8
Haloferax volcanii ATCC 29605	0.2470	0.1206	0.1095	0.1313		98.4	99.0	99.4	88.3	88.2	87.3	87.1	87.8	88.4	88.2	88.5	88.5	89.2
Haloferax mediterranei ATCC 33500	0.2441	0.1182	0.1095	0.1345	0.0165		97.9	98.2	87.9	88.2	87.6	87.3	88.0	87.7	87.5	87.8	87.9	89.2
Haloferax gibbonsii ATCC 33959	0.2484	0.1245	0.1093	0.1337	0.0096	0.0208		99.0	88.0	87.7	87.0	86.8	87.5	87.9	87.8	88.1	88.1	89.0
Haloferax denitrificans ATCC 35960	0.2480	0.1269	0.1155	0.1343	0.0062	0.0186	0.0103		87.9	87.8	87.0	86.8	87.6	88.0	87.8	88.2	88.3	89.0
Halococcus morrhuae ATCC 17082	0.2578	0.1310	0.1233	0.1242	0.1273	0.1322	0.1304	0.1319		89.2	87.0	86.7	87.8	85.4	85.5	85.5	85.5	87.6
Haloarcula marismortui Ginzburg strain 11C8	0.2460	0.1352	0.1144	0.1066	0.1288	0.1280	0.1344	0.1327	0.1168		95.0	93.9	95.8	85.7	85.9	86.0	85.6	88.5
Haloarcula marismortui Ginzburg strain 111110	0.2575	0.1402	0.1305	0.1354	0.1386	0.1361	0.1426	0.1425	0.1427	0.0520		98.4	96.7	86.1	86.2	86.0	85.8	87.2
"*Haloarcula sinaiiensis*" ATCC 33800 (major gene)	0.2605	0.1474	0.1358	0.1417	0.1415	0.1390	0.1454	0.1453	0.1465	0.0640	0.0165		97.3	85.7	85.8	85.5	85.4	86.9
"*Haloarcula sinaiiensis*" ATCC 33800 (minor gene)	0.2532	0.1367	0.1270	0.1272	0.1335	0.1310	0.1365	0.1356	0.1328	0.0436	0.0335	0.0278		85.7	86.0	85.5	85.3	88.0
Halobacterium sodomense ATCC 33755	0.2720	0.1379	0.1474	0.1374	0.1266	0.1348	0.1315	0.1313	0.1624	0.1582	0.1540	0.1588	0.1582		98.8	95.8	95.4	89.4
Halobacterium trapanicum NRC 34021	0.2763	0.1401	0.1523	0.1438	0.1280	0.1362	0.1329	0.1327	0.1613	0.1563	0.1521	0.1578	0.1554	0.0118		95.7	95.1	89.0
Halobacterium saccharovorum NCIMB 2081	0.2708	0.1312	0.1365	0.1316	0.1249	0.1331	0.1298	0.1280	0.1607	0.1548	0.1548	0.1606	0.1617	0.0427	0.0447		98.3	89.1
Halobacterium lacusprofundi ACAM 34	0.2656	0.1357	0.1371	0.1379	0.1247	0.1320	0.1296	0.1277	0.1610	0.1594	0.1569	0.1626	0.1637	0.0470	0.0506	0.0168		88.8
Halobaculum gomorrense DS2807[T]	0.2491	0.1295	0.1309	0.1218	0.1162	0.1170	0.1193	0.1191	0.1352	0.1245	0.1401	0.1440	0.1309	0.1141	0.1187	0.1173	0.1210	

[a] *Methanospirillum hungatei* was used as the outgroup.
[b] The values on the lower left are evolutionary distances (14), and the values on the upper right are percentages of similarity.

genus *Haloferax* and the group formed by *Halobacterium saccharovorum*, *Halobacterium sodomense*, *Halobacterium trapanicum*, and *Halobacterium lacusprofundi*. The hypothesis that the new isolate may not be related to the genus *Haloferax* is supported by the morphological and physiological characteristics of this organism (Table 3). Strain DS2807^T does not exhibit the typical pleomorphic flattened shape of *Haloferax* species. However, when this strain was first isolated, it was pleomorphic, and it acquired its rod shape after it was subcultured. Strain DS2807^T did not require a high divalent cation concentration to retain its rod shape, and strain DS2807^T cells did not turn into spheroplasts in the absence of high magnesium and calcium concentrations, a behavior characteristic of *Haloferax volcanii* (3, 31). In addition, strain DS2807^T was not able to grow in defined media containing a single carbon source, one of the characteristics of *Haloferax* species.

Strain DS2807^T resembled *Halobacterium sodomense* and its relatives in many properties (Table 3). For example, very high magnesium concentrations (0.6 to 1.0 M) were required for optimal growth, a characteristic also exhibited by *Halobacterium sodomense* (29); thus, both of these organisms are adapted to the extremely high magnesium concentrations (around 1.8 M) found in the Dead Sea (31, 32). In addition, the growth medium recommended for *Halobacterium sodomense* resulted in good growth of strain DS2807^T, which was not able to grow in most of the other media recommended for halophilic archaea. This was due in part to the high divalent cation concentrations required and also to the high concentrations of organic nutrients commonly used in the other media. Yeast extract and Casamino Acids at a concentration of 0.5% were inhibitory. The nature of the growth-inhibiting substance is not known. It has been shown previously that certain brands of peptone may contain bile acids in concentrations high enough to cause lysis of halophilic archaea. Addition of starch to the media was found to relieve this effect to a certain extent (33). Peptone was not added to the media used in this study, but growth inhibition still occurred despite the presence of starch. Isolate DS2807^T produced acids from certain sugars, a property shared with members of the *Halobacterium saccharovorum-Halobacterium sodomense* group. The G+C content of strain DS2807^T (70 mol%) is also in the range of values reported for *Halobacterium saccharovorum* and *Halobacterium sodomense* (68 to 72 mol%) (29, 40) and is much higher than the values determined for representatives of the genus *Haloferax* (59 to 67 mol%) (9, 40, 42). Thus, the intermediate position of strain DS2807^T between the *Halobacterium saccharovorum* group and the genus *Haloferax*, as suggested by the 16S rRNA sequence data, was confirmed by its physiological characteristics.

16S rRNA sequence distances in the range from 0.1 to 0.15 are considered sufficient for distinguishing genera and defining families (4). Since the 16S rRNA distances between strain DS2807^T and previously described halophiles were greater than 0.114, the creation of a new genus appears to be justified, and so we propose the name *Halobaculum gomorrense* for this taxon. Strain DS2807^T is the only known representative of the new genus *Halobaculum*, but we expect that additional strains of *Halobaculum gomorrense* and other species of the genus will be isolated and characterized in the future.

Halobaculum gomorrense DS2807^T has been deposited in the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, as strain DSM 9297^T.

Description of *Halobaculum* gen. nov. *Halobaculum* Oren, Gurevich, Gemmell, and Teske (Ha.lo.ba'cu.lum. Gr. masc. n. *halos*, salt; L. neut. n. *baculum*, stick; M.L. neut. n. *Halobaculum*, salt stick). Gram-negative rods. Colonies are small, round,

TABLE 3. Characteristics that differentiate strain DS2807^T from *Haloferax* and *Halobacterium* species[a]

Characteristic	Strain DS2807^T	*Haloferax volcanii*	*Haloferax mediterranei*	*Haloferax gibbonsii*	*Haloferax denitrificans*	*Halobacterium sodomense*	*Halobacterium trapanicum*	*Halobacterium saccharovorum*	*Halobacterium lacusprofundi*
Cell morphology	Rod	Pleomorphic	Pleomorphic	Pleomorphic	Pleomorphic	Rod	Pleomorphic	Rod	Rod
Motility	±	±	±	+	−	+	−	+	±
Gas vacuoles	−	−	+	−	−	−	−	−	−
Bacteriorhodopsin	−	−	−	−	−	+	ND[b]	−	−
Lipids									
S-DGD-1[c]	+	+	+	+	+	−	−	−	−
Phosphatidylglycerosulfate	−	−	−	−	−	+	+	+	+
Optimum Mg^{2+} concentration is >0.5 M	+	−	−	−	−	+	−	−	−
Indole produced from tryptophan	−	+	+	+	+	−	−	ND	−
Nitrite produced from nitrate	+	+	+	±	+	+	+	+	+
Gas produced from nitrate	−	−	+	−	+	−	+	−	±
Acid produced from:									
Mannose	−	ND	ND	+	−	−	ND	+	−
Galactose	+	+	ND	+	+	−	ND	+	ND
Fructose	−	+	+	+	+	+	+	+	ND
Lactose	−	+	+	ND	−	−	ND	+	−
Arabinose	−	ND	+	+	ND	−	ND	+	ND
Starch hydrolysis	+	−	+	−	−	+	−	−	−
G+C content (mol%)	70	63–66	59–62	62–64	64	68	64	69–72	65–66

[a] The organisms used for comparison in the growth experiments with strain DS2807^T were *Haloferax volcanii*, *Haloferax denitrificans*, and *Halobacterium sodomense*. Data were obtained from references 9, 13, 27, 29, 38, 40, and 42.

[b] ND, not determined.

[c] S-DGD-1 is the major sulfated diglycosyl diether lipid present in *Haloferax* species.

convex, entire, and translucent. Pigmented red because of carotenoids. Oxidase and catalase positive.

Chemoorganotrophic and aerobic. Halophilic, requiring at least 1 M NaCl for growth.

The polar lipids are glycerol diether analogs of phosphatidylglycerol, phosphatidylglycerophosphate, and a single glycolipid (S-DGD-1). Phosphatidylglycerosulfate is absent.

Growth does not occur on single carbon sources. Certain carbohydrates stimulate growth with acid production.

The G+C content of the type species is 70 mol%.

The type species is *Halobaculum gomorrense*.

Description of *Halobaculum gomorrense* sp. nov. *Halobaculum gomorrense* (go.mor.rense'. M.L. neut. adj. *gomorrense*, pertaining to Gomorra, a biblical city near the Dead Sea). Rods are 5 to 10 by 0.5 to 1 μm. Motile cells occur occasionally. Gas vacuoles are not present.

Chemoorganotrophic and aerobic. Yeast extract and Casamino Acids at low concentrations are good sources of organic nutrients. No growth occurs anaerobically with nitrate or with arginine.

Requires at least 1 M NaCl for growth (in the presence of 0.8 M $MgCl_2$). The optimal NaCl concentration range is 1.5 to 2.5 M at 35 to 40°C; the optimal $MgCl_2$ concentration range is 0.6 to 1 M (in the presence of 2.1 M NaCl). The optimum temperature is 40°C (in medium containing 2.1 M NaCl and 0.8 M $MgCl_2$).

Pigmented red because of carotenoids. Purple membrane is not produced.

Nitrate is reduced to nitrite.

No indole is produced from tryptophan.

Susceptible to novobiocin, bacitracin, anisomycin, vibriostatic agent 0/129, taurocholate, and deoxycholate. Not susceptible to penicillin G, ampicillin, kanamycin, chloramphenicol, streptomycin sulfate, neomycin, and cycloheximide.

Glucose, maltose, sucrose, galactose, xylose, trehalose, starch, and glycerol stimulate growth with acid production. Starch is hydrolyzed. No acid is produced from mannose, fructose, ribose, lactose, arabinose, mannitol, and sorbitol. Growth is stimulated by DL-lactate. Growth is not stimulated by acetate, citrate, propionate, succinate, glycine, L-alanine, and L-glutamate.

Isolated from the Dead Sea.

The G+C content of the type strain is 70 mol% (as determined by HPLC).

The type strain is DSM 9297 (= DS2807).

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