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 MgCl₂. 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 Halofex; 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 Halofex 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 (≥10⁷ 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, Halofex, 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), Halofex volcanii (27), and Halocula 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 Halofex. 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 Halofex volcanii and Halofex mediterranei on both one-dimensional and two-dimensional thin-layer chromatograms (37). Moreover, phosphatidylglycerosulfate, a polar lipid present in all halophilic archaea except Halofex 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 DS2807T) 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 Halofex 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 DS2807T 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 33755T, Halobacterium saccharovorum ATCC 29252T, Halofex volcanii ATCC 29605T, Halofex mediterranei ATCC 33503T, Halofex denitrificans ATCC 35960T, and Haloarcula marismortui ATCC 43049T were used as reference strains in biochemical tests.

Media and growth conditions. The standard medium used for strain DS2807T and Halobacterium sodomense contained (per liter) 125 g of NaCl, 160 g of MgCl₂·6H₂O, 5.0 g of K₂SO₄, 0.1 g of CaCl₂·2H₂O, 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 35C 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...
reduction were performed by using standard procedures (8, 29). Carotenoid pigments were extracted in methanol-acetone (1:1, vol/vol), and the absorbance spectrum of the extract was determined with a Hewlett-Packard model 8452A diode array spectrophotometer. To test for induction of formation of bacterio-
rhodopsin, 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 108°C with concentrated sulf-
uric acid, and assessing the formation of crotonic acid on the basis of its
high-performance liquid chromatography (HPLC) as described by Mesbah et al.

After precipitation with 0.1 volume of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 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
(SuperTag; 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 Desulfovibrio 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.

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 ampli-
fied by PCR by using primers 4F-Archaea (5'-TCCGGTTGATCCTGCCGG-3';
comparing to Escherichia coli positions 4 to 21) and 1542R-Archaea/Bacteria
(3'-ACCTAGTTGAGGAAG-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
72°C. Each reaction mixture (total volume, 50 μl) contained 50 mM KCl, 10 mM
Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 0.01% (w/v) gelatin, 200 μM dGTP, 200
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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.
pH for growth was 6 to 7. Growth was not observed at pH 3. Growth was not observed at pH 3.

Haloarcula temperatures above 50°C no growth occurred. The optimum temperature range was 35°C. The magnesium concentrations which resulted in optimal growth were 0.6 to 1 M MgCl₂.

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 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 bacteriorubins, 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-beta-hydroxybutyrate was not observed in cells grown in standard medium or in medium supplemented with 1 g of sodium acetate per liter.

Strain DS2807 was obligately aerobic and exhibited positive oxidase and catalase reactions. Nitrates were 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 Halo-bacterium halobium-Halo bacterium salinarium group and in the alkaliphilic organism Natronobacterium pharaonis (35).

Isolate DS2807 did not hydrolize 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, xylitol, trehalose, starch, and glycerol stimulated growth and acid was produced. Growth was also stimulated by m-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 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-1,4-DNAM).

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.
A nylon column was used to separate the polar lipids. 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.

DNA base composition. The G+C content of the DNA of strain DS2807T was 70 mol%.

Phylogeny. Our phylogenetic tree, which was constructed by comparing the strain DS2807T 16s rRNA sequence with the 16s rRNA sequences of other halophilic archaea, showed that strain DS2807T occupies a position that is intermediate between the position of Halobacterium saccharovoror and related species and the position of the genus Haloferax (Fig. 4). The DS2807T 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 saccharovoror group, respectively, and the strain DS2807T sequence was equidistant from the other halobacterial sequences included for comparison (Table 2).

DISCUSSION

Halophilic strain DS2807T 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 DS2807T 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 DS2807T 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′-SO₄⁻)-(1→2′)-α-D-glucose]-2,3-di-O-phytanyl-s-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 DS2807T did not cluster with the genus Haloferax, but appeared on a separate branch that was about equally removed from the
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<th>Halobacterium gomorrense DS2807</th>
<th>Haloflexia marismortui NRC 3401</th>
<th>Haloflexia marismortui DSM 2881</th>
<th>Halobacterium sodomense ATCC 33755</th>
<th>Halobacterium sodomense ATCC 33380 (major gene)</th>
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**TABLE 2** Similarity matrix for halobacteria based on 16S rDNA gene sequences.
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 DS2807T 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 DS2807T did not require a high divalent cation concentration to retain its rod shape, and strain DS2807T 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 DS2807T was not able to grow in defined media containing a single carbon source, one of the characteristics of Haloferax species.

Strain DS2807T 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 DS2807T, 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 DS2807T produced acids from certain sugars, a property shared with members of the Halobacterium saccharovorum-Halobacterium sodomense group. The G+C content of strain DS2807T (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 DS2807T 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 DS2807T 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 DS2807T 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** DS2807T has been deposited in the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, as strain DSM 9297T.

**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. Halobacu-lum, salt stick). Gram-negative rods. Colonies are small, round,
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 phosphatidyleglycerol, phosphatidylglycerophosphate, and a single glycolipid (S-DGD-1). Phosphatidylglycerol 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.ren.se’. 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 0.6 to 1 M (in the presence of 2.1 M NaCl). The optimum NaCl concentration range is 1.5 to 1.8 M MgCl₂. The optimal NaCl 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 MgCl₂).

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 mannosse, fructose, ribose, lactose, arabinose, mannitol, and sorbitol. Growth is stimulated by NaCl. Growth is not stimulated by acetate, 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 (go.mor.ren.se’. M.L. neut. adj. gomorrense, pertaining to Gomorra, a biblical city near the Dead Sea).

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**References**