Haloanaerobium alcophilum sp. nov., an Anaerobic Moderate Halophile from the Sediments of Great Salt Lake, Utah

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A strictly anaerobic, moderately halophilic, gram-negative, rod-shaped bacterium was isolated from Great Salt Lake, Utah, sediments and designated GSLST (T = type strain). Strain GSLST grew optimally at pH 6.7 to 7.0 but had a very broad pH range for growth (pH 5.8 to 10.0). The optimum temperature for growth was 37°C, and no growth occurred at 15 or 55°C. The optimum salt concentration for growth was 10%. Strain GSLST required yeast extract and Trypticase peptone to ferment carbohydrates, pyruvate, and glycine betaine. Strain GSLST was resistant to penicillin, n-cycloserine, tetracycline, and streptomycin. The G+C content of this isolate was 31 mol%. The fermentation products from glucose utilization were acetate, butyrate, lactate, CO2, and H2, and in addition strain GSLST fermented glycine betaine to acetate and trimethylamine. All of these traits distinguish this organism from all previously described halophilic anaerobes. The 16S rRNA gene sequence of strain GSLST was found to be similar to, but also significantly different from, the 16S rRNA sequences of Haloanaerobium salsugo and Haloanaerobium praevalens. Therefore, strain GSLST (= DSM 8275T) is described as a new species, Haloanaerobium alcophilum.

There have been few studies of halophilic anaerobes and the ability of these organisms to break down organic matter in saline environments. The ability to grow in the presence of various concentrations of salt distinguishes the following three groups of halophiles (11): (i) the haloduric halophiles (optimum salt concentration, 2 to 5%), (ii) the moderate halophiles (optimum salt concentration, 5 to 20%), and (iii) the extreme halophiles (optimum salt concentration, 20 to 30%). Members of all three groups have been reported to inhabit anaerobic saline habitats (34).

Eight halophilic, carbohydrate-fermenting, anaerobic genera containing 13 species and belonging to the domain Bacteria have been described previously (18). Seven of these species have been classified as members of the family Halanaerobaceae on the basis of their 16S rRNA oligonucleotide sequences (20, 21). All isolates of these species ferment carbohydrates, and some use starch (20, 36), glycerol (20), and CO2 (21). Halobacteroides saccharolytica (23, 30, 37, 38), Halobacteroides salinae (2, 40), and Halobacteroides arsenicalum (39) exhibit a homoacetogenic pathway of glucose metabolism.

We isolated a moderately halophilic, fermentative strain from sediments collected from underneath a salt layer in Great Salt Lake, Utah. In this paper we describe this strain as a new species of the genus Haloanaerobium, Haloanaerobium alcophilum.

MATERIALS AND METHODS

Origin of samples. Samples were collected from the north arm of the Great Salt Lake in Utah. The sampling site was located 3 km offshore at the midpoint of a line drawn between the Little Valley Shelter and the Cub Island Shelter. Grab samples were obtained from brine at a depth of 7 m after the salt crust was broken with an orange peel sampler. The samples were collected under anaerobic conditions in serum bottles, transported to a laboratory at the ambient temperature, and stored at 4°C until they were used.

Origin of strain SLP. Methanohalophilus mahii SLP (= OCM 68) was obtained from the Oregon Collection of Methanogens, Beaver. Isolation and culture techniques. The modified anaerobic technique of Hungate (8, 15) was used throughout this study. The basic medium used to produce axenic cultures contained (per liter) 17 g of MgSO4·7H2O, 100 g of NaCl, 50 ml of mineral solution A (6 g of K2HPO4 per liter of distilled water), 50 ml of mineral solution B (which contained (per liter of distilled water) 6 g of KH2PO4, 6 g of (NH4)2SO4, 12 g of NaCl, 2.6 g of MgSO4·7H2O, 25 g of NH4Cl, 0.28 g of CuCl2·2H2O, and 0.28 g of K2HPO4), 10 ml of a trace mineral solution (which contained (per liter of distilled water) 0.5 g of MnSO4·H2O, 0.1 g of FeSO4·7H2O, 0.1 g of CoCl2·6H2O, 0.1 g of ZnSO4·7H2O, 0.01 g of CuSO4·5H2O, 0.01 g of AlK(SO4)2·12H2O, 0.01 g of H3BO3, 0.01 g of Na2MoO4·2H2O, 0.03 g of NiCl2·6H2O, 0.26 g of NaSeO2·5H2O, and 1.5 g of nitritotriacetic acid; the components were dissolved with KOH and adjusted to pH 6.5), and the final pH was 7.0. 10 ml of a vitamin solution (which contained (per liter of distilled water) 2 mg of biotin, 2 mg of folic acid, 10 mg of pyridoxine hydrochloride, 5 mg of thiamine hydrochloride, 5 mg of riboflavin, 5 mg of nicotinamide, 5 mg of calcium pantothenate, 0.1 mg of vitamin B12, 5 mg of p-aminobenzoic acid, and 5 mg of lipoic acid), and 1 ml of 0.1% resazurin. After boiling under a stream of oxygen-free N2-CO2 (70:30), 4.1 g of NaHCO3 and 0.5 g of cysteine were added. The following components were added to the mineral medium after stock solutions were autoclaved at 120°C for 20 min (final concentrations): yeast extract (Difco Laboratories, Detroit, Mich.) (10 g/liter), Trypsin (Becton Dickinson, Cockeysville, Md.) (10 g/liter), Na2S·9H2O (0.25 g/liter). The medium was dispensed into Hungate tubes and flasks (17) under a stream of N2-CO2 (70:30). To prepare solid media for plates and roll tubes, Bacto Agar (Difco) was added to the medium to a final concentration of 2.25%.

Dilutions of each sediment sample were streaked directly onto plates in an
were transferred subsequently five more times every 3 days. Further purification using a finely drawn Pasteur pipette and were introduced into liquid dilution was determined from changes in optical density when cells were grown on a medium that contained the following four organic buffers: Waipo’s acetate buffer (pH 5.2), MES [2-(N-morpho- 
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no)ethanesulfonic acid], Bis-Tris propane [1,3-bis(tris(hydroxymethyl) 

methylamino)propane], and Sorenson’s glycine buffer (pH 5.2) at a final concentra-

tion of 10 mM. The pH values after addition ranged from pH 5.0 to 11.0.

Fermentation of carbon substrates (final concentration, 0.5%) was investi-

gated in triplicate by using the basic medium containing 1 g of yeast extract per liter and 1 g of Trypticase per liter. Each generation time was compared with the growth rate in a control containing 1 g of yeast extract per liter of Trypticase per liter but no carbohydrate. The criterion used for positive substrate utilization was a maximum growth rate of at least 0.2 h⁻¹. The generation time was determined in optical assays of cell growth when cells were grown on a medium containing 0.5% glucose under optimal conditions. Protothecand growth was tested in glucose medium that lacked yeast extract and Trypticase.

The Gram reaction was determined with heat-fixed liquid cultures stained with Diffco kit reagents. Antibiotic susceptibility was determined in liquid medium by using the following antibiotics at concentrations ranging from 100 to 1,000 μg/ml: d-cycloserine, penicillin G, streptomycin, and tetracycline. Whether oxygen could be used as an electron acceptor was determined on solid medium; whether fumarate (10 mM) or nitrate (10 mM) could be used as an electron acceptor was determined with glucose; and whether sulfate (10 mM) could be used as an electron acceptor was determined by using lactate in liquid medium under N₂,CO₂. Growth on H₂,CO₂ (80:20) was examined in liquid medium in order to test for homocysteogenesis.

Growth on glycine betaine was tested by using a coculture of strain GSLST and M. halili, a halophilic, methylotrophic methanogen isolated from Great Salt Lake (25). These experiments were performed in serum bottles containing the basic medium supplemented with 1% glycine betaine, 0.1% yeast extract, 0.1% Trypticase, and 10% NaCl and inoculated with 1.5 ml of an exponentially grow-

ing culture of each organism. Methane evolution was monitored by gas chroma-

tography as described below.

Analytical techniques. Growth rates were determined by measuring the optical densities of cultures at 560 μm with a Junior model 35 spectrophotometer (Perkin-Elmer, Oak Brook, Ill.). Volatile fatty acids and carboxylic acids from the fermentation of glucose and glycine betaine cocultures in the supernatants of the cultures were analyzed by gas chromatography as described previously (5) and by high-performance liquid chromatography (HPLC) (Shimadzu Corp., Kyoto, Japan), using a reverse- phase C₈ column (4 mm by 30 cm; Spherisorb C₈, Fischer, Montluçon, France) and a UV spectrophotometric detector (model SPD6A; Shimadzu Corp.) set at 225 nm. The mobile phase consisted of 20% acetonitrile in 0.01 N hydrochloric acid. H₂S contents were determined photometrically as previously described (5). The H₂,CO₂ and CH₄ in the gas phase above the growth medium were analyzed by gas chromatography as described previously (5).

Electron microscopy. Electron micrographs were obtained by using a JEOL model JEM-1200 EX transmission electron microscope and negative staining with 3% (wt/vol) sodium phosphotungstate.

DNA base composition. DNA was isolated by the technique of Ticho et al. (28). The guanine-plus-cytosine (G+C) content of the DNA was determined at the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany, by using the methods described by Motomura et al. (16) and Tamaoka and Komagata (33).

16S rRNA sequence studies. Semipurified DNA was extracted and used to amplify the 16S rRNA gene by the following protocol. Cultures (2 ml) were centrifuged, and the pellets were resuspended in 50 μl of lysozyme buffer (50 mM Tris-HCl [pH 7.2], 50 mM EDTA, 3% sodium dodecyl sulfate) and placed in microcentrifuge tubes. Each suspension was microwaved at 600 W for 30 s by using 15 s of heat on and 5 s of heat off four times while the lids were open. Then 350 μl of 2x PCR mix was added to each tube, and the lids were closed. The prepara-

tions were incubated at 95°C for 15 min, and 400 μl of phenol-chloroform (1:1) was added to each suspension. Each preparation was centrifuged at 13,000 × g for 15 min, and then the top aqueous phase (approximately 200 μl) was removed; we were careful to avoid any material from the interface when we did this. Then 10 μl of isopropanol and 5 μl of 5 M sodium acetate (pH 5.8) added were to the aqueous phase, and the preparation was mixed. The suspension was centrifuged at 13,000 × g, and the pellet washed with cold 80% ethanol, placed in a desiccator to evaporate the residual ethanol, suspended in 30 μl of sterile distilled water, and stored at −20°C until it was used.

Amplification of the 16S rRNA gene from the DNA and purification of the amplified product was performed as described previously (14, 29). The purified PCR product was sequenced directly with an ABI automated DNA sequencer by using a Prins dye terminator cycle sequencing kit (Applied Biosystems, Ltd., Foster City, Calif.), and the protocols recommended by the manufacturer. The primers used for sequencing have been described previously (29).

The 16S rRNA sequence obtained from the sequencing data and the 16S rRNA sequences of various members of the domain Bacteria obtained from the Ribosomal Database Project (version 4.0) were aligned by using sequence editor ac2 (12). Positions of sequence and alignment uncertainty were omitted from the analysis, and pairwise evolutionary distances based on 1,079 unambiguous nucleo-


tides were computed by using the method of Jukes and Cantor (9). Dendo-

grams were constructed from evolutionary distances by using the neighbor-

joining method, a transversion analysis was performed by using the program DNAPARS, and tree topology was examined by using 100 bootstrapped data sets by running the script file dboot. For dboot we used the following sequence of events during the analysis: SEQBOOT, DNADIST, FITCH, and CONSENSE. All programs are available as part of the PHYLIP package (6). Programs available in the Molecular Evolutionary Genetic Analysis (MEGA) package, version 1.1 (10), were also used in the analysis. All of the programs except MEGA were run on a Sun Sparc workstation; MEGA was run on a 386 Toshiba model T3100SX laptop computer.

Nucleotide sequence accession number. The 16S rRNA sequence of strain GSLST determined in this study has been deposited in the EMBL database under accession number X81850.

RESULTS

Morphology. Colonies in roll tubes and agar plates were round with entire edges, smooth, convex, opaque, and yellowish. The colony diameter was 3 mm after 1 week. The cells of strain GSLST were straight rods that ranged in size from 3.3 to 5 by 0.8 μm (Fig. 1) and were motile in young cultures by means of peritrichous flagella (Fig. 2). The cells occurred singly, in pairs, and rarely in chains. Strain GSLST was gram negative. Spores were not observed under the conditions we tested.

Growth and physiology. Strain GSLST was a strictly anaerobic, chemooorganotrophic bacterium. Optimal growth occurred in the presence of 10% NaCl. This organism was an obligate halophile and could not grow without NaCl in the
medium. No growth occurred in the presence of NaCl concentrations of more than 25% (Fig. 3a). The growth rate increased when glycine buffer was added, but there was no change in the optimum NaCl concentration (Fig. 3a). The optimum temperature for growth was 37°C; no growth was observed at 55 or at 15°C (Fig. 3b). Optimal growth occurred at pH 6.7 to 7.0, and the pH range for growth was broad (pH 5.8 to 10.0) (Fig. 3c). The doubling time of strain GSLST in the presence of glucose, yeast extract, and Trypticase under optimal conditions was 3.3 h. Growth did not occur in mineral medium when glucose was the sole carbon and energy source.

The following substrates were fermented: fructose, glucose, maltose, mannose, sucrose, N-acetylglucosamine, pyruvate, glycine betaine, and yeast extract. Slight growth was observed on raffinose. The following compounds were not fermented: arabinose, cellobiose, galactose, lactose, rhamnose, ribose, trehalose, xylose, glycogen, starch, adonitol, dulcitol, erythritol, glycerol, inositol, mannitol, methanol, sorbitol, formate, acetate, butyrate, propionate, citrate, lactate, oxalate, succinate, tartrate, methylamine, trimethylamine, glycine, Casamino Acids, cellulose, chitin, xylan, and peptin.

The products of glucose fermentation by strain GSLST were acetate, lactate, butyrate, CO₂, and H₂. Hydrogen accounted for 0.26 and 0.09 mmol of the metabolites obtained from fermentation of 0.32 mmol of glucose and 0.52 mmol of pyruvate, respectively. Fumarate, nitrate, and sulfate were not used as electron acceptors. No growth was observed on H₂-CO₂.

Degradation of glycine betaine by the coculture of strain GSLST and *M. malii* produced acetate and methane (Fig. 4). The amount of methane produced from 1.7 mmol of glycine betaine was 0.86 mmol.

Strain GSLST exhibited strong antibiotic resistance; growth was inhibited by 200 μg of penicillin G per ml, 400 μg of D-cycloserine per ml, 400 μg of tetracycline per ml, and 1,000 μg of streptomycin per ml.

**DNA base composition.** The G+C content of strain GSLST DNA as determined in triplicate by HPLC was 31 mol%.

**16S rRNA sequence analysis.** Using eight primers, we determined nearly the complete sequence of the 16S rRNA gene of isolate GSLST. This sequence (positions 8 to 1443; *Escherichia coli* numbering of Winker and Woese [35]) was aligned with other sequences, and a phylogenetic analysis was performed with representatives of the various phyla of the domain *Bacteria*. This analysis revealed that isolate GSLST was a member of the low-G+C-content Gram-positive phylum. Additional sequence alignments and phylogenetic analysis performed with members of this phylum revealed that the closest relatives of isolate GSLST were *Halonaerobium* species and that *Halonaerobium praevalens* was more closely related (level of similarity, 98.1%) than *Halonaerobium salsugoides* (level of simil-
Illegible text
TABLE 1. Dissimilarity matrix derived from a comparison of the 16S rRNA sequences of Haloanaerobium alcaliphilum and other gram-positive bacteria*

<table>
<thead>
<tr>
<th>Species</th>
<th>Halobacterium chlorum</th>
<th>Halobacterium salgado</th>
<th>Aerocaldococcus fermentans</th>
<th>Aerocaldococcus thermooxidans</th>
<th>Clostridium thermoaceticum</th>
<th>Clostridium thermoacetoxidans</th>
<th>Clostridium thermosaccharolyticum</th>
<th>Clostridium tertioventri</th>
<th>Halobacterium chlorum</th>
<th>Halobacterium salgado</th>
<th>Aerocaldococcus fermentans</th>
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</thead>
<tbody>
<tr>
<td>Haloanaerobium praevales</td>
<td>15.2</td>
<td>13.6</td>
<td>16.4</td>
<td>16.7</td>
<td>22.5</td>
<td>22.2</td>
<td>21.0</td>
<td>20.1</td>
<td>22.1</td>
<td>23.8</td>
<td>23.6</td>
</tr>
<tr>
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<td></td>
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<td>16.4</td>
<td>16.7</td>
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<td>20.1</td>
<td>22.1</td>
<td>23.8</td>
<td>23.6</td>
</tr>
<tr>
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<td>18.8</td>
<td>18.9</td>
<td>16.4</td>
<td>21.9</td>
<td>23.8</td>
<td>22.5</td>
<td>21.9</td>
<td>23.8</td>
<td>24.2</td>
<td>24.3</td>
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<tr>
<td>Haloanaerobium alcaliphilum</td>
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<td>22.1</td>
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<td>19.0</td>
<td>18.4</td>
<td>20.9</td>
<td>20.8</td>
<td>20.8</td>
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<td>24.2</td>
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<td>16.2</td>
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<tr>
<td>Acidaminococcus fermentans</td>
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<td>23.9</td>
<td>23.1</td>
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<tr>
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<td>26.1</td>
<td>20.9</td>
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<td>24.8</td>
<td>21.4</td>
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<td>18.1</td>
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<tr>
<td>Peptococcus niger</td>
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<td>23.5</td>
<td>24.1</td>
<td>23.0</td>
<td>25.0</td>
<td>22.4</td>
<td>20.1</td>
<td>19.0</td>
<td>21.3</td>
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<td>16.6</td>
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<td>29.6</td>
<td>29.0</td>
<td>27.4</td>
<td>31.1</td>
<td>25.1</td>
<td>25.1</td>
<td>27.3</td>
<td>25.5</td>
<td>25.0</td>
<td>26.6</td>
</tr>
</tbody>
</table>

* The sequences used in the analysis were obtained from the Ribosomal Database Project, version 4.0 (12). Only 1,079 unambiguous nucleotide positions were used in the analysis.

ganism found in this habitat. Strain GSLS\(^7\) differs markedly from all previously described isolates by its formation of large amount of hydrogen during glucose fermentation. According to Phelps and Zeikus (27), H\(_2\) accumulates in sediments of Great Salt Lake. Perhaps strain GSLS\(^7\) plays a role in hydrogen production in these sediments.

Morphological and physiological characteristics further distinguish strain GSLS\(^7\) from other fermentative halophiles (Table 2). A. arabaticum and strain GSLS\(^7\) are the only alkaline-tolerant isolates that have been described so far, and only strain GSLS\(^7\) grows at pH values greater than 8.5. Within the genus Haloanaerobium, strain GSLS\(^7\) is the only motile isolate.

Phylogenetically, strain GSLS\(^7\) is related to Haloanaerobium praevales (level of similarity, 98.1%) and Haloanaerobium sal-sugo (level of similarity, 94.4%), but is different enough to warrant designation of a new Haloanaerobium species. Strain GSLS\(^7\) also differs markedly in its G+C content (31 mol%) from Haloanaerobium praevales (27 mol%) and Haloanaerobium salugo (34 mol%); thus, the G+C content data support the proposal that strain GSLS\(^7\) belongs to a new Haloanaerobium species.

We propose that strain GSLS is the type strain of a new species of the genus Haloanaerobium, Haloanaerobium alcaliphilum.

Description of Haloanaerobium alcaliphilum. Haloanaerobium alcaliphilum (al.ca.li'phi.lum. N. L. n. alcali, from Arabic al, end, and gally, soda ash; Gr. adj. philm, loving; N. L. adj. alcaliphilum, liking alkaline media). Cells are straight rods that are 3.3 to 5 by 0.8 \(\mu m\), are motile by means of peritrichous flagella, and occur singly, in pairs, or rarely in chains. Gram negative. Spores are not formed. Colonies (diameter, up to 3 mm) are round with entire edges, smooth, convex, opaque, and yellowish. Moderately halophilic. Optimum NaCl concentration for growth, 10%; range of NaCl concentrations for growth, 2.5 to 25%. Optimum temperature for growth, 37°C; growth occurs at 25 to 50°C. Alkalitolerant. pH range, 5.8 to 10.0; optimum pH, 6.7 to 7.0. Growth factors found in yeast extract are required for growth. Cells are resistant to penicillin G (200 \(\mu g/ml\)), D-cycloserine (400 \(\mu g/ml\)), tetracycline (400 \(\mu g/ml\)), and streptomycin (1,000 \(\mu g/ml\)).

Strictly anaerobic. Chemoorganotrophic. Fumarate, nitrate, and sulfate are not reduced. No growth occurs with H\(_2\)-CO\(_2\). Ferments fructose, glucose, mallose, mannose, sucrose, N-acetylglucosamine, pyruvate, glycine betaine, and yeast extract. End products of glucose fermentation are acetate, butyrate, lactate, H\(_2\), and CO\(_2\). Acetate and trimethylamine are produced from glycine betaine.

The G+C content is 31 mol% (as determined by HPLC). Type strain GSLS (= DSM 8275) was isolated from the north arm of Great Salt Lake in Utah.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Morphology</th>
<th>Cell size (µm)</th>
<th>Gram reaction</th>
<th>Motility</th>
<th>NaCl concn (%)</th>
<th>Optimum NaCl concn (%)</th>
<th>Optimum Temp (°C)</th>
<th>Optimum Temp (°C)</th>
<th>pH range</th>
<th>Optimum pH</th>
<th>Doubling time (h)</th>
<th>Habitat</th>
<th>G+C content (mol%)</th>
<th>End products from glucose fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Halobacteroides halobius</em></td>
<td>Rods</td>
<td>0.4-0.7 × 1-1.6</td>
<td>Negative</td>
<td>+</td>
<td>6.0-20.0</td>
<td>10.0</td>
<td>15-45</td>
<td>30-35</td>
<td>5.4-8.0</td>
<td>6.3-7.4</td>
<td>7.8-9.5</td>
<td>Gulf of Mexico</td>
<td></td>
<td>Acetate, ethanol, H₂, CO₂</td>
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<tr>
<td><em>Halobacteroides acetethylicus</em></td>
<td>Rods</td>
<td>0.7-1 × 0.5-6</td>
<td>Negative</td>
<td>+</td>
<td>5.0-30.0</td>
<td>15.0-18.0</td>
<td>25-52</td>
<td>35-40</td>
<td>6.0-8.0</td>
<td>6.5-7.0</td>
<td>2.9-4.5</td>
<td>Lake Chokrak</td>
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<td>Acetate, ethanol, H₂, CO₂</td>
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<tr>
<td><em>Halobacteroides lacunaris</em></td>
<td>Rods</td>
<td>0.5 × 1.5</td>
<td>Negative</td>
<td>−</td>
<td>2.0-30.0</td>
<td>12.5</td>
<td>15-45</td>
<td>37</td>
<td>6.0-9.0</td>
<td>7.0-7.4</td>
<td>4</td>
<td>Great Salt Lake</td>
<td></td>
<td>Acetate, butyrate, propionate, H₂, CO₂</td>
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<tr>
<td><em>Halanaerobium praevalem</em></td>
<td>Rods</td>
<td>0.3-0.4 × 2.6-4</td>
<td>Negative</td>
<td>−</td>
<td>6-24</td>
<td>9</td>
<td>22-51</td>
<td>40</td>
<td>5.6-8.0</td>
<td>6.1</td>
<td>9</td>
<td>Oil well, Oklahoma</td>
<td></td>
<td>Acetate, ethanol, H₂, CO₂</td>
</tr>
<tr>
<td><em>Halonaerobacter chitinovoran</em></td>
<td>Rods</td>
<td>0.5 × 1.4-8</td>
<td>Negative</td>
<td>+</td>
<td>3.0-30.0</td>
<td>12.0-18.0</td>
<td>25-55</td>
<td>37-45</td>
<td>7.0</td>
<td>2.5-2.9</td>
<td>3.3</td>
<td>Solar saltern</td>
<td></td>
<td>Acetate, isobutyrate, H₂, CO₂</td>
</tr>
<tr>
<td><em>Haloincola saccharolytica</em></td>
<td>Rods</td>
<td>0.5-0.7 × 1-1.5</td>
<td>Negative</td>
<td>+</td>
<td>3.0-30.0</td>
<td>10</td>
<td>15-47</td>
<td>37-40</td>
<td>6.0-8.0</td>
<td>7.5</td>
<td>3.9</td>
<td>Lake Sivash</td>
<td></td>
<td>Acetate</td>
</tr>
<tr>
<td><em>Halocella cellulolytica</em></td>
<td>Rods</td>
<td>0.4-0.6 × 3.8-12</td>
<td>Negative</td>
<td>+</td>
<td>ND</td>
<td>15</td>
<td>20-50</td>
<td>39</td>
<td>5.5-8.5</td>
<td>7.0</td>
<td>ND</td>
<td>Lake Sivash</td>
<td></td>
<td>Acetate, ethanol, lactate, H₂, CO₂</td>
</tr>
<tr>
<td><em>Acetohalobium arabaticum</em></td>
<td>Curved rods</td>
<td>0.7-1 × 2-5</td>
<td>Negative</td>
<td>+</td>
<td>10-25</td>
<td>15-18</td>
<td>ND-47</td>
<td>38-40</td>
<td>5.6-8.4</td>
<td>7.4-8.0</td>
<td>ND</td>
<td>Lake Sivash</td>
<td></td>
<td>Acetate, trimethylamine</td>
</tr>
<tr>
<td><em>Halothermohrix orenii</em></td>
<td>Rods</td>
<td>0.4-0.6 × 20</td>
<td>Negative</td>
<td>+</td>
<td>4-20</td>
<td>10</td>
<td>45-68</td>
<td>60</td>
<td>5.5-8.2</td>
<td>6.5-7.0</td>
<td>ND</td>
<td>Chott El Guettar</td>
<td></td>
<td>Acetate, ethanol, H₂, CO₂</td>
</tr>
</tbody>
</table>

* Data from reference 23 and 36.
+ ND, not determined.
* Data from reference 29.
# Data from reference 37.
| Data from reference 35.
/ Data from reference 1.
* Data from reference 13.
! Data from reference 39.
* Data from reference 30.
* Data from reference 38.
* Formed in the presence of glycine betaine.
+ Data from reference 2.
ACKNOWLEDGMENTS

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REFERENCES


