Haloanaerobium lacusroseus sp. nov., an Extremely Halophilic Fermentative Bacterium from the Sediments of a Hypersaline Lake

J.-L. CAYOL,¹ B. OLLIVIER,^{1*} B. K. C. PATEL,^{1,2} E. AGERON,³ P. A. D. GRIMONT,³
G. PRENSIER,⁴ AND J.-L. GARCIA¹

Laboratoire de Microbiologie ORSTOM, Université de Provence, 13331 Marseille Cedex 3,¹ Unité des
Entérobactéries, INSERM U199, Institut Pasteur, 75724 Paris Cedex 15,³ and Laboratoire de
Microbiologie, Université Blaise Pascal, 63177 Aubière Cedex,⁴ France, and Faculty of
Science and Technology, Griffith University, Brisbane, Queensland 4111, Australia²

A new extremely halophilic chemoorganotrophic bacterium (strain H200^T [T = type strain]) was isolated from the hypersaline sediments of Retba Lake in Senegal. This organism was a sluggishly motile, rod-shaped, non-spore-forming, gram-negative, obligate anaerobe that grew optimally at 40°C in the presence of 180 to 200 g of NaCl per liter. The DNA base composition was 32 mol% guanine plus cytosine. The fermentation products from glucose were ethanol, acetate, H₂, and CO₂. Yeast extract was required for growth. The fermentable substrates included D-fructose, galactose, D-xylose, cellobiose, lactose, maltose, sucrose, starch, D-mannitol, glycerol, and Casamino Acids. On the basis of the results of a 16S rRNA sequence analysis, strain H200^T was found to be related to *Haloanaerobium* species. The 16S rRNA sequence of strain H200^T differed from the sequences of the three previously described *Haloanaerobium* species, and strain H200^T also differed from these organisms in its NaCl range for growth (60 to 340 g/liter); strain H200^T grew in the presence of the highest NaCl concentration recorded for any halophilic anaerobic organism, including the three previously described *Haloanaerobium* species. We propose that strain H200^T (= DSM 10165) belongs to a new *Haloanaerobium* species, *Haloanaerobium lacusroseus*.

Scientific interest in extremophilic anaerobic microorganisms has recently increased because of the possible biotechnological use of enzymes and molecules from such organisms (17). Among the extremophilic anaerobic bacteria, less attention has been paid to extreme halophiles (organisms that grow most rapidly in the presence of 200 to 300 g of NaCl per liter) than to hyperthermophiles.

Hypersaline ecosystems (inland lakes, marine salterns) exhibit great variability in ionic composition, total salt concentration, and pH and are inhabited by aerobic and anaerobic microfloras (19). The strictly anaerobic halophiles that have been isolated from hypersaline ecosystems include sulfate-reducing bacteria, methanogenic bacteria, phototrophic bacteria, and fermentative bacteria (19, 21). Of the 14 species of fermentative halophilic anaerobes belonging to the family *Haloanaerobiaceae* that have been described (21, 23), only the following 3 are extreme halophiles that exhibit optimum growth in the presence of approximately 200 g of NaCl per liter but do not at more than 300 g of NaCl per liter: *Halobacteroides lacunaris*, an organism obtained from hypersaline Lake Chokrak on the Kerch Peninsula (33); *Haloanaerobacter chitinovorans*, an organism obtained from a southern California solar saltern (15); and *Acetohalobium arabaticum*, an organism obtained from Lake Sivash (34).

As very little attention has been paid to the extremely halophilic fermentative anaerobic bacteria, we initiated work to isolate such bacteria from hypersaline environments. In this paper we describe the isolation from the sediment of a hyper-

saline lake of an extremely halophilic chemoorganotrophic anaerobic bacterium, strain H200^T (T = type strain), which grows in the presence of 340 g of NaCl per liter. The results of 16S rRNA and DNA relatedness, physiological, and metabolic studies justified assignment of this strain to a new species of the genus *Haloanaerobium*.

MATERIALS AND METHODS

Strain origin. Strain H200^T was isolated from the sediment of hypersaline Retba Lake near Dakar, Senegal; this lake is located 100 m from the Atlantic Ocean. Sediment samples were collected from a depth of 1.50 m. The in situ temperature of the sediment was about 32°C, and the total salt concentration in the water was 340 g/liter. The pH was 7.15.

The following five type strains of haloanaerobic bacteria were used for DNA relatedness studies: *Haloicola saccharolytica* Z-7787 (= DSM 6643), *Haloicola saccharolytica* subsp. *senegalensis* H150 (= DSM 7379), *Halobacteroides acetethylicus* EIGI (= DSM 3532), *Halobacteroides lacunaris* Z-7888 (= DSM 6640), and *Haloanaerobium praevalens* GSL (= DSM 2228).

Culture medium. Strain H200^T was isolated on medium containing (per liter) 1.0 g of NH₄Cl, 0.3 g of K₂HPO₄, 0.3 g of KH₂PO₄, 20.0 g of MgCl₂ · 6H₂O, 2.0 g of CaCl₂ · 2H₂O, 4.0 g of KCl, 1.0 g of CH₃COONa · 3H₂O, 10.0 g of glucose, 200 g of NaCl, 1.0 g of bio-Trypticase (bioMérieux), 1.0 g of yeast extract (Difco), 0.001 g of resazurin, and 1 ml of a trace element solution (11). The medium was adjusted to pH 7 with 10 M KOH, boiled under a stream of O₂-free N₂, and cooled to room temperature. Portions (20 ml) of medium were distributed into 60-ml serum bottles that were stoppered with butyl rubber stoppers by using the Hungate anaerobic technique (10). The serum bottles were gassed with N₂-CO₂ (80:20) and sterilized for 45 min at 110°C. After autoclaving, 0.2 ml of 2% Na₂S · 9H₂O and 1 ml of 10% NaHCO₃ (both sterile, anaerobic solutions), as well as 0.1 ml of a filter-sterilized 0.2% sodium dithionite solution, were injected into each bottle. The final pH was 7.0. Roll tubes were prepared by adding 2% agar (Difco) to the medium. In some experiments, 5-ml portions of medium were distributed into Hungate tubes as described above. To avoid precipitation in the medium, lower concentrations of MgCl₂ · 6H₂O (2.0 g/liter) and CaCl₂ · 2H₂O (0.2 g/liter) were used.

Isolation procedure. Sediment samples were inoculated into growth medium that contained 200 g of NaCl per liter to initiate enrichment cultures. After several transfers, the enrichment cultures were serially diluted by using the roll tube technique. Two colonies were picked, and the process of serial dilution in

* Corresponding author. Mailing address: Laboratoire de Microbiologie ORSTOM, Université de Provence, 3 Place Victor Hugo, 13331 Marseille Cedex 3, France. Phone: (33) 91.10.64.80. Fax: (33) 91.10.64.81. Electronic mail address: rogerpa@loma.orstom.fr.

FIG. 1. Phase-contrast photomicrograph of strain H200^T. Bar = 10 μ m.

roll tubes was repeated in order to purify the cultures. Purity was checked by microscopic examination after growth on a complex, rich medium with or without NaCl.

Analytical techniques. The presence of volatile fatty acids and alcohols was detected with a Delsi series 30 chromatograph by using an SP-1000 column (1% H₃PO₄ on Chromosorb WAW) operated at 150°C and nitrogen as the carrier gas. A flame ionization detector and a Delsi integrator were used. Hydrogen was quantified with a Girdel series 30 gas chromatograph equipped with a thermal conductivity detector. The column was filled with Carboxsphere SS (60/80 mesh).

Glucose, lactate, and formate were quantified by using a high-performance liquid chromatograph (HPLC) equipped with an Analprep 93 pump (Touzart et Matignon, Vitry sur Seine, France) and a type ORH 801 column (Interaction Chemicals, Mountain View, Calif.); 0.05 N H₂SO₄ was used as the solvent at a flow rate of 0.6 ml/min, the volume of the injection loop was 20 μ l, the column temperature was 35°C, and the detector used was a differential refractometer (Knauer, Berlin, Germany).

Bacterial growth was measured with a Shimadzu model UV 160A spectrophotometer at 660 nm by inserting anaerobic Hungate tubes directly into the chamber of the cuvette holder. All experiments were performed in duplicate.

Electron microscopy. Cells were negatively stained with 4% (wt/vol) uranyl acetate in distilled water. Cells from an exponentially growing culture were fixed for 1 h in 0.07 M sodium cacodylate buffer (pH 7.3) containing 1.2% glutaraldehyde and 0.05% ruthenium red. After the samples were washed in cacodylate buffer containing 0.05% ruthenium red, they were fixed in 1% (wt/vol) OsO₄ in 0.07 M cacodylate buffer. The samples were embedded in Epon, and ultrathin sections were stained with 2% uranyl acetate in 50% ethanol and then with lead citrate (27). Micrographs were taken with a JEOL model 1200CX electron microscope.

DNA base composition. DNA was isolated and purified by chromatography on hydroxyapatite. The guanine-plus-cytosine (G+C) content of DNA was determined by HPLC by using the method of Meshbah et al. (18). Nonmethylated lambda DNA (Sigma) was used as the standard.

DNA relatedness. DNA was extracted and purified as described elsewhere (4). The procedures used for in vitro labelling of DNA with tritium-labelled nucleotides and for hybridization experiments (S1 nuclease-trichloroacetic acid procedure) have been described previously (9). The temperature at which 50% of the reassociated DNA became hydrolyzable by the S1 nuclease (T_m) was determined as described by Crosa et al. (7); the difference between the T_m of a homoduplex and the T_m of a heteroduplex provided an estimate of the level of divergence between two DNAs (3).

16S rRNA sequence studies. Semipurified DNA was extracted for amplification of the 16S rRNA gene by using the following protocol. Cultures (20 ml) were centrifuged, and the resulting pellets were resuspended in 50 μ l of lysis 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 by using 15 s with the heat on and 5 s with the heat off four times while the lids were open. Then an additional 350 μ l of lysis buffer was added, the lid was closed, the

preparation was incubated at 80°C for 15 min, the suspension was mixed with 400 μ l of phenol-chloroform (1:1), and the preparation was centrifuged at 13,000 $\times$ g for 15 min. The top aqueous phase (approximately 200 μ l) was removed; we were careful to avoid material from the interface when we did this. Then 10 μ l of isopropanol and 5 μ l of 5 M sodium acetate (pH 5.8) were added to the aqueous phase. The suspension was centrifuged at 13,000 $\times$ g, and the resulting pellet was washed with 80% cold ethanol, placed in a desiccator to evaporate the residual ethanol, resuspended in 50 μ l 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 were performed as described previously (16, 25). The purified PCR product was sequenced directly with an ABI automated DNA sequencer by using a Prism dideoxy terminator cycle sequencing kit as recommended by the manufacturer (Applied Biosystems, Ltd., Foster City, Calif.). The primers used for sequencing have been described previously (25).

The 16S rRNA sequence obtained from the sequencing data and 16S rRNA sequences of various members of domain *Bacteria* obtained from the rRNA Database Project (version 4.0) were aligned by using the sequence editor ae2 (14). Positions at which the sequence and/or alignment was uncertain were omitted from the analysis, and pairwise evolutionary distances for 1,097 unambiguous nucleotides were computed by using the method of Jukes and Cantor (12). Dendrograms were constructed from evolutionary distances by using the FITCH method, and tree topology was reexamined by using 100 bootstrapped data sets by running the script file dboot. dboot used the following sequence of events during the analysis: SEQBOOT, DNADIST, FITCH, and CONSENSE. All programs are available as part of the PHYLIP package (8). Programs available in the Molecular Evolutionary Genetic (MEGA) Analysis Package, version 1 (13), were also used in the analysis. All programs except the MEGA programs were run on a Sun Sparc workstation; the MEGA programs were run on a Toshiba model T3100SX 386 laptop computer.

Nucleotide sequence accession number. The strain H200^T 16S rRNA nucleotide sequence has been deposited in the GenBank database under accession number L39767.

RESULTS

Isolation. Two pure cultures were obtained. A culture designated strain H200^T was characterized further; the colonies of this organism were yellow, flat, and circular, had smooth edges, and were 0.5 to 1 mm in diameter (depending on the age).

Cellular properties. Strain H200^T cells were straight rods that were 0.4 to 0.6 by 2 to 3 μ m and occurred singly or in pairs; however, the cells became filamentous and aggregated in old cultures (Fig. 1). The cells were motile by means of peritri-

FIG. 2. (a) Electron micrograph of negatively stained cells of strain H200^T showing peritrichous flagella. Bar = 5 μm . (b) Ultrathin section of strain H200^T cells showing the cell wall ultrastructure. Bar = 0.25 μm . (c) Transverse section of strain H200^T cells. Bar = 0.5 μm .

FIG. 2—Continued.

chous flagella (Fig. 2a). Thin sections revealed a typical gram-negative cell envelope profile and a multilayer cell wall (Fig. 2b and c).

Growth and metabolic properties. The optimum temperature for growth of strain H200^T was about 40°C, and growth occurred at temperatures between 20 and 50°C (Fig. 3a). The optimum pH was 7. Strain H200^T required NaCl but not MgCl₂. Optimum growth occurred in the presence of NaCl concentrations around 200 g/liter, and growth occurred in the presence of NaCl concentrations between 60 and 340 g/liter (saturation) (Fig. 3b). Magnesium was tolerated up to a concentration of 120 g/liter (Fig. 3c). A minimum doubling time of 2.4 h was observed when the isolate was cultivated on a glucose-containing medium in the presence of 1 g of bio-Trypticase per liter, 1 g of yeast extract per liter, and 200 g of NaCl per liter. Strain H200^T was found to be an obligate anaerobe. Spores were not observed, and no growth was obtained after pasteurization at 90°C for 20 min.

Yeast extract was required for growth and could also be used as a sole energy source. The fermentable substrates included D-fructose, galactose, D-glucose, glycerol, D-mannose, D-ribose, D-xylose, cellobiose, lactose, maltose, sucrose, starch, D-mannitol, and Casamino Acids. No growth was observed with the following compounds: L-rhamnose, L-sorbose, arabinose, melibiose, cellulose, adonitol, ethanol, ethylene glycol, methanol, formate, acetate, propionate, butyrate, fumarate, DL-lactate, malate, methylamines, and bio-Trypticase. The products of glucose fermentation were ethanol, acetate, H₂, and presumably CO₂.

Growth of strain H200^T was completely inhibited by penicillin G at a concentration of 33 U/ml and by vancomycin or chloramphenicol at a concentration of 100 µg/ml.

DNA base composition. The average DNA base composition

of strain H200^T, as determined by three HPLC analyses, was 32 mol% G+C.

DNA relatedness. The levels of DNA relatedness between strain H200^T and five other type strains are shown in Table 1.

16S rRNA sequence analysis. Using eight primers, we determined an almost-complete sequence consisting of 1,544 bases for the 16S rRNA gene of strain H200^T. The sequence of strain H200^T (positions 8 to 1,541; *Escherichia coli* numbering of Winker and Woese [31]) was aligned with the sequences of representatives of the various divisions of the domain *Bacteria*, and a phylogenetic analysis was performed. This analysis revealed that strain H200^T was a member of the low-G+C-content gram-positive division. Additional sequence alignments and a phylogenetic analysis performed with members of this division revealed that the closest relatives of strain H200^T were the *Haloanaerobium* species, including *Haloanaerobium praevalens*, *Haloanaerobium alcaliphilum*, and *Haloanaerobium salsugo* (average level of sequence similarity, 96%) (Table 2). A dendrogram which was generated by the FITCH method (8) from a Jukes-Cantor similarity matrix (12) (Table 2) and shows this relationship is presented in Fig. 4. A bootstrap analysis indicated that the relationship was robust (96%).

DISCUSSION

Strain H200^T is a rod-shaped, carbohydrate-fermenting, non-spore-forming, extremely halophilic anaerobe which grows in the presence of 50 to 340 g of NaCl per liter. The morphology, physiology, and genetic properties of this organism are consistent with assignment to the family *Haloanaerobiaceae*. This family now includes seven genera and 14 species and, with the exception of *Haloferox* (6), consists exclusively of

FIG. 3. (a) Effect of temperature on the growth of strain H200^T. (b) Effect of NaCl concentration on the growth of strain H200^T. (c) Effect of MgCl₂ concentration on the growth of strain H200^T.

mesophilic species. Strain H200^T is not a member of the genus *Halotheothrix*. Strain H200^T can be easily differentiated from members of other genera, including the genera *Sporohalobacter* (21), *Haloanaerobacter* (15), *Halocella* (28), and *Haloicola* (5, 35), on the basis of its phenotypic and phylogenetic characteristics. Phenotypically, strain H200^T differs from *Sporohalobacter* species (21) in its cell morphology, salt tolerance, pattern of glucose fermentation, and lack of sporulation. Strain H200^T can be differentiated from *Haloanaerobacter chitinovorans* (15) on the basis of its metabolic properties (production of ethanol) and cell size. In contrast to *Haloicola saccharolytica* (5, 35), strain H200^T produces ethanol and has a broader salt tolerance and optimum salt concentration range. *Halobacteroides* species (24, 26, 33), *Haloanaerobium* species (2, 30, 32), and strain H200^T all produce ethanol, acetate, CO₂, and H₂ as end products during glucose fermentation. However, oligonucleotide catalog data have shown that the genera *Halobacteroides* and *Haloanaerobium* are two distinct taxa (23).

Our phylogenetic analysis based on 16S rRNA sequences revealed that strain H200^T is a member of the genus *Haloanaerobium*. Strain H200^T can also be differentiated from *Halobacteroides lacunaris* by its ability to use glycerol, lactose, and

TABLE 1. Levels of DNA relatedness between isolate H200^T and related bacteria

Source of unlabelled DNA	% Relatedness with ³ H-labelled DNA from strain H200 ^T
Strain H200 ^T	100
<i>Haloanaerobium praevalens</i> GSL ^T	8
<i>Halobacteroides acetoethylicus</i> EIGI ^T	11
<i>Halobacteroides lacunaris</i> Z-7888 ^T	3
<i>Haloicola saccharolytica</i> Z-7787 ^T	11
<i>Haloicola saccharolytica</i> H150 ^T	15

xylose (33). DNA-DNA hybridization of the genome of strain H200^T with the genomes of *Halobacteroides lacunaris* and *Halobacteroides acetoethylicus* also revealed a distant relationship (Table 1).

Phylogenetically, strain H200^T is closely related to the three members of the genus *Haloanaerobium*. However, the sequence of the 16S rRNA gene of H200^T differs significantly (>3%) from the 16S rRNA gene sequences of the previously described members of the genus *Haloanaerobium*, which means that a new species should be described (1, 29). Dissimilarity between strain H200^T and its closest phylogenetic relative, *Haloanaerobium praevalens* (96.8%), was revealed by low DNA-DNA hybridization values (Table 1).

Phenotypically, strain H200^T can be differentiated from all three previously described *Haloanaerobium* species by its tolerance of higher NaCl concentrations during growth and by the fact that it uses glycerol and starch. In contrast to *Haloanaerobium praevalens*, strain H200^T uses cellobiose, galactose, lactose, sucrose, and xylose (Table 3). Finally, strain H200^T also differs from *Haloanaerobium salsugo* in its use of mannitol and from *Haloanaerobium alcaliphilum* in its use of cellobiose, galactose, mannitol, lactose, D-ribose, and D-xylose.

We have isolated several halophiles from the hypersaline Lake Retba sediments, including *Desulfohalobium retbaense* (20), *Haloicola saccharolytica* subsp. *senegalensis* (5), and strain H200^T (this study). Both *Desulfohalobium retbaense* and *Haloicola saccharolytica* are moderate halophiles and are restricted to areas where the salt concentrations are relatively low. Hence, these organisms probably play a minor role in the

FIG. 4. Dendrogram showing the position of *Haloanaerobium lacusroseus* H200^T among representatives of members of the genus *Haloanaerobium* and related genera. The dendrogram was derived from the dissimilarity matrix shown in Table 2.

TABLE 2. Sequence similarity matrix obtained by using the distance correction method of Jukes and Cantor^a

Species	% Sequence similarity to:														
	<i>Haloanaerobium lacusroseus</i>	<i>Haloanaerobium praevalens</i>	<i>Haloanaerobium alcaliphilum</i>	<i>Haloanaerobium salsugo</i>	<i>Sporohalobacter lortetii</i>	<i>Halothermothrix orenii</i>	<i>Sporomusa paucivorans</i>	<i>Megasphaera elsdenii</i>	<i>Hellobacterium chlorum</i>	<i>Acidaminococcus fermentans</i>	<i>Clostridium thermosaccharolyticum</i>	<i>Clostridium thermoaceticum</i>	<i>Syntrophospora bryantii</i>	<i>Syntrophomonas wolfei</i>	<i>Peptococcus niger</i>
<i>Haloanaerobium praevalens</i>	96.8														
<i>Haloanaerobium alcaliphilum</i>	95.9	98.1													
<i>Haloanaerobium salsugo</i>	95.4	95.8	94.5												
<i>Sporohalobacter lortetii</i>	83.5	84.0	83.0	83.9											
<i>Halothermothrix orenii</i>	86.1	86.5	85.2	87.2	85.9										
<i>Sporomusa paucivorans</i>	79.4	79.6	78.4	79.9	80.2	80.9									
<i>Megasphaera elsdenii</i>	78.8	79.9	78.5	79.9	80.5	81.1	85.7								
<i>Hellobacterium chlorum</i>	78.4	78.8	77.7	80.2	80.0	82.0	83.4	82.0							
<i>Acidaminococcus fermentans</i>	79.0	79.5	78.0	79.9	80.2	81.9	88.2	85.7	82.1						
<i>Clostridium thermosaccharolyticum</i>	80.1	80.5	79.6	81.7	81.8	82.8	80.7	82.5	83.9	81.5					
<i>Clostridium thermoaceticum</i>	81.8	82.3	81.0	81.9	83.6	83.5	83.5	83.8	84.7	82.5	87.2				
<i>Syntrophospora bryantii</i>	79.5	79.6	78.3	79.9	82.0	82.7	82.1	81.8	84.4	81.5	84.5	86.8			
<i>Syntrophomonas wolfei</i>	79.9	80.3	79.0	80.4	80.7	81.8	82.0	82.3	84.3	82.0	84.2	87.1	94.6		
<i>Peptococcus niger</i>	79.9	80.5	79.4	80.0	80.4	81.6	81.0	82.8	83.6	82.0	82.4	85.4	84.0	84.6	
<i>Escherichia coli</i>	75.6	76.5	75.5	76.8	75.3	77.5	77.6	79.1	78.5	76.3	78.9	79.2	80.0	79.9	77.8

^a See reference 12. The sequences used in the analysis were obtained from the Ribosomal Database Project, version 4.0 (14). Only 1,097 unambiguous nucleotides were used in the analysis.

TABLE 3. Differentiation of strain H200^T from other *Haloanaerobium* species

Characteristic	<i>Haloanaerobium praevalens</i> ^a	<i>Haloanaerobium salsugo</i> ^b	<i>Haloanaerobium alcaliphilum</i> ^c	Strain H200 ^T
Cell morphology	Rods	Rods	Rods	Rods
Cell size (μm)	0.5 × 1.5	0.3–0.4 × 2.6–4	0.8 × 3.3–5	0.5 × 2–3
Gram stain reaction	Negative	Negative	Negative	Negative
Motility	–	–	+	+
NaCl concn range (%)	2.0–30	6–24	2.5–25	7.5–34
Optimum NaCl concn (%)	12.5	9	10	18–20
Temp range (°C)	15–45	22–51	20–50	20–50
Optimum temp (°C)	37	40	37	40
pH range	6.0–9.0	5.6–8.0	5.8–10	ND ^d
Optimum pH	7.0–7.4	6.1	6.7–7.0	7.0
Doubling time (h)	4	9	3.3	2.4
Habitat	Great Salt Lake	Oil well, Oklahoma	Great Salt Lake	Retba Lake
G+C content (mol%)	27	34	31	32
Use of substrates ^e				
L-Arabinose	ND	+	–	–
Cellobiose	–	ND	–	+
Fructose	+	+	+	+
Galactose	–	+	–	+
D-Glucose	+	+	+	+
Glycerol	–	–	–	+
Lactose	–	+	–	+
D-Mannose	+	+	+	+
Mannitol	ND	–	–	+
Maltose	+	+	+	+
Melibiose	ND	+	ND	–
D-Ribose	ND	+	–	+
Rhamnose	ND	+	–	–
L-Sorbose	ND	+	ND	–
Starch	–	–	–	+
Sucrose	–	+	+	+
D-Xylose	–	+	–	+
End products from glucose fermentation	Acetate, butyrate, propionate, H ₂ , CO ₂	Acetate, ethanol, H ₂ , CO ₂	Acetate, lactate, butyrate, H ₂ , CO ₂	Ethanol, acetate, H ₂ , CO ₂

^a Data from reference 32.

^b Data from reference 2.

^c Data from reference 30.

^d ND, not determined.

^e +, supported growth; –, did not support growth.

overall organic matter decomposition in Lake Retba. In contrast, strain H200^T grows in the presence of a wide range of NaCl concentrations (up to saturation), indicating that it may play a more dominant role in the microbial fermentation processes in Lake Retba.

Glycerol is an important osmolyte synthesized by *Dunaliella salina* and is probably one of the principal nutrients used by natural communities of halophilic aerobic *Archaea* since it is subject to rapid turnover by these microorganisms (22). However some members of the *Haloanaerobiaceae*, including the isolates obtained from Lake Retba (5, 35; this study), ferment glycerol. We have found that glycerol oxidation by anaerobic halophiles can be markedly improved through interspecies hydrogen transfer when organisms are cocultured with a hydrogenotrophic sulfate-reducing bacterium. This indicates that glycerol might be a significant factor for the growth of anaerobes in hypersaline ecosystems (manuscript in preparation).

Because of phenotypic, phylogenetic, and DNA-DNA hybridization differences between strain H200^T and the other members of genus *Haloanaerobium*, we propose that strain H200^T is a member of a new species in the family *Haloanaerobiaceae*, *Haloanaerobium lacusroseus*.

Description of *Haloanaerobium lacusroseus* sp. nov. *Haloanaerobium lacusroseus* (la. cus. ro'se.us. N. L. n. lacus, lake; adj. roseus, rose colored; lacusroseus, Rose Colored Lake, another name for Retba Lake in Senegal). Cells are straight rods that are 0.4 to 0.6 by 2 to 3 µm and are sluggishly motile by means of peritrichous flagella. Gram negative. Extreme halophile and obligate anaerobe that grows fastest in the presence of NaCl concentrations around 200 g/liter. Grows in the presence of NaCl concentrations up to saturation. Magnesium concentrations up to 120 g/liter are tolerated. Growth occurs at temperatures ranging from 20 to 50°C, and optimum growth occurs at 40°C. The optimum pH is 7.0. Penicillin G (33 U/ml), vancomycin (100 µg/ml), and chloramphenicol (100 µg/ml) inhibit growth.

Yeast extract is required for growth and can also be used as the sole energy source. The fermentable substrates include D-fructose, galactose, D-glucose, glycerol, D-mannose, D-ribose, D-xylose, cellobiose, lactose, maltose, sucrose, starch, D-mannitol, and Casamino Acids. Produces ethanol, acetate, H₂, and presumably CO₂ from glucose metabolism.

The G+C content of the DNA is 32 mol% (as determined by HPLC).

Type strain H200 has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as strain DSM 10165. Isolated from sediments of Retba Lake, a hypersaline lake in Senegal.

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REFERENCES

- Amann, R. I., C. Lin, R. Key, L. Montgomery, and D. A. Stahl. 1992. Diversity among *Fibrobacter* strains: towards a phylogenetic classification. *Syst. Appl. Microbiol.* **15**:23–31.
- Bhupathiraju, V. K., A. Oren, P. K. Sharma, R. S. Tanner, C. R. Woese, and M. J. McInerney. 1994. *Haloanaerobium salsugo* sp. nov., a moderately halophilic, anaerobic bacterium from a subterranean brine. *Int. J. Syst. Bacteriol.* **44**:565–572.
- Brenner, D. J. 1978. Characterization and clinical identification of *Enterobacteriaceae* by DNA hybridization. *Prog. Clin. Pathol.* **7**:71–117.
- Brenner, D. J., A. C. McWhorter, J. K. Leete-Knudson, and A. G. Steigerwalt. 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. *J. Clin. Microbiol.* **15**:1133–1140.
- Cayol, J.-L., B. Ollivier, A. Lawson Anani Soh, M.-L. Fardeau, E. Ageron, P. A. D. Grimont, G. Prensier, J. Guezennec, M. Magot, and J.-L. Garcia. 1994. *Haloicola saccharolytica* subsp. *senegalensis* subsp. nov., isolated from the sediments of a hypersaline lake, and emended description of *Haloicola saccharolytica*. *Int. J. Syst. Bacteriol.* **44**:805–811.
- Cayol, J.-L., B. Ollivier, B. K. C. Patel, G. Prensier, J. Guezennec, and J.-L. Garcia. 1994. Isolation and characterization of *Halothermothrix orenii* gen. nov., sp. nov., a halophilic, thermophilic, fermentative, strictly anaerobic bacterium. *Int. J. Syst. Bacteriol.* **44**:534–540.
- Crosa, J. H., D. J. Brenner, and S. Falkow. 1973. Use of a single-strand-specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *J. Bacteriol.* **115**:904–911.
- Felsenstein, J. 1993. PHYLIP (phylogenetic inference package), version 3.51c. Department of Genetics, University of Washington, Seattle.
- Grimont, P. A. D., M. Y. Popoff, F. Grimont, C. Coynault, and M. Lemelin. 1980. Reproducibility and correlation study of three deoxyribonucleic acid hybridization procedures. *Curr. Microbiol.* **4**:325–330.
- Hungate, R. E. 1969. A roll-tube method for the cultivation of strict anaerobes. *Methods Microbiol.* **3B**:117–132.
- Imhoff-Stuckle, D., and N. Pfennig. 1983. Isolation and characterization of a nicotinic acid-degrading sulfate-reducing bacterium, *Desulfococcus niacini* sp. nov. *Arch. Microbiol.* **136**:194–198.
- Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules, p. 21–32. In H. N. Munro (ed.), *Mammalian protein metabolism*. Academic Press, Inc., New York.
- Kumar, S., K. Tamura, and M. Nei. 1993. MEGA: molecular evolutionary genetic analysis, version 1.0. The Pennsylvania State University, University Park.
- Larsen, N., G. J. Olsen, B. L. Maidak, M. J. McCaughey, R. Overbeek, T. J. Macke, T. L. Marsh, and C. R. Woese. 1993. The Ribosomal Database Project. *Nucleic Acids Res.* **21**(Suppl.):3021–3023.
- Liaw, H., and R. A. Mah. 1992. Isolation and characterization of *Haloanaerobacter chitinovorans* gen. nov., sp. nov., a halophilic, anaerobic, chitinolytic bacterium from a solar saltern. *Appl. Environ. Microbiol.* **58**:260–266.
- Love, C. A., B. K. C. Patel, P. D. Nichols, and E. Stackebrandt. 1993. *Desulfotomaculum australicum* sp. nov., a thermophilic sulfate-reducing bacterium isolated from the Great Artesian Basin of Australia. *Syst. Appl. Microbiol.* **16**:244–251.
- Lowe, S. E., M. K. Jain, and J. G. Zeikus. 1993. Biology, ecology, and biotechnological applications of anaerobic bacteria adapted to environmental stresses in temperature, pH, salinity, or substrates. *Microbiol. Rev.* **57**:451–509.
- Meshbah, M., U. Premachandran, and W. B. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39**:159–167.
- Ollivier, B., P. Caumette, J.-L. Garcia, and R. A. Mah. 1994. Anaerobic bacteria from hypersaline environments. *Microbiol. Rev.* **58**:27–38.
- Ollivier, B., C. Hatchikian, G. Prensier, J. Guezennec, and J.-L. Garcia. 1991. *Desulfobacterium retbaense* gen. nov., sp. nov., a halophilic sulfate-reducing bacterium from sediments of a hypersaline lake in Senegal. *Int. J. Syst. Bacteriol.* **41**:74–81.
- Oren, A. 1992. The genera *Haloanaerobium*, *Halobacteroides*, and *Sporohalobacter*, p. 1893–1900. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), *The prokaryotes*, 2nd ed., vol. 2. Springer Verlag, New York.
- Oren, A. 1993. Availability, uptake and turnover of glycerol in hypersaline environments. *FEMS Microbiol. Ecol.* **12**:15–23.
- Oren, A., B. J. Paster, and C. R. Woese. 1984. *Haloanaerobiaceae*: a new family of moderately halophilic obligate anaerobic bacteria. *Syst. Appl. Microbiol.* **5**:71–80.
- Oren, A., W. G. Weisburg, M. Kessel, and C. R. Woese. 1984. *Halobacteroides halobius* gen. nov., sp. nov., a moderately halophilic anaerobic bacterium from the bottom sediments of the Dead Sea. *Syst. Appl. Microbiol.* **5**:58–70.
- Redburn, A. C., and B. K. C. Patel. 1993. Phylogenetic analysis of *Desulfotomaculum thermobenzoicum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. *FEMS Microbiol. Lett.* **113**:81–86.
- Rengpipat, S., T. A. Langworthy, and J. G. Zeikus. 1988. *Halobacteroides acetohalophilus* sp. nov., a new obligately anaerobic halophile isolated from deep surface hypersaline environments. *Syst. Appl. Microbiol.* **11**:28–35.
- Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**:208–212.
- Simankova, M. V., N. A. Chernych, G. A. Osipov, and G. A. Zavarzin. 1992. *Halocella cellulolytica* gen. nov. spec. nov., a new obligately anaerobic, halophilic, cellulolytic bacterium. *Syst. Appl. Microbiol.* **16**:385–389.
- Stackebrandt, E., and B. M. Goebel. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44**:846–849.
- Tsai, C. R., J.-L. Garcia, B. K. C. Patel, J.-L. Cayol, L. Baresi, and R. A. Mah. 1995. *Haloanaerobium alcaliphilum* sp. nov., an anaerobic moderate halophile from the sediments of Great Salt Lake. *Int. J. Syst. Bacteriol.* **45**:301–307.

31. Winker, S., and C. R. Woese. 1991. A definition of the domains Archaea, Bacteria and Eucarya in terms of small subunit ribosomal RNA characteristics. *Syst. Appl. Microbiol.* **13**:161–165.
32. Zeikus, J. G., P. W. Hegge, T. E. Thompson, T. J. Phelps, and T. A. Langworthy. 1983. Isolation and description of *Haloanaerobium praevalens* gen. nov. and sp. nov., an obligately anaerobic halophile common to Great Salt Lake sediments. *Curr. Microbiol.* **9**:225–234.
33. Zhilina, T. N., L. V. Miroshnikova, G. A. Osipov, and G. A. Zavarzin. 1991. *Halobacteroides lacunaris* sp. nov., a new saccharolytic, anaerobic, extremely halophilic organism from the lagoon-like hypersaline Lake Chokrak. *Microbiology* (Engl. Transl. *Mikrobiologiya*) **60**:714–724.
34. Zhilina, T. N., and G. A. Zavarzin. 1990. A new extremely halophilic homoacetogen bacterium, *Acetohalobium arabaticum* gen. nov., sp. nov. *Dokl. Akad. Nauk SSSR* **311**:745–747. (In Russian.)
35. Zhilina, T. N., G. A. Zavarzin, E. S. Bulygina, V. V. Kevbrin, G. A. Osipov, and K. M. Chumakov. 1991. Ecology, physiology and taxonomy studies on a new taxon of *Haloanaerobiaceae*, *Haloincola saccharolytica* gen. nov., sp. nov. *Syst. Appl. Microbiol.* **15**:275–284.