Isolation and Characterization of *Halothermothrix orenii* gen. nov., sp. nov., a Halophilic, Thermophilic, Fermentative, Strictly Anaerobic Bacterium

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The occurrence of thermophilic, halophilic anaerobic bacteria in the sediment of a Tunisian salted lake was tested in samples collected at 20-cm intervals down to a depth of 1.20 m. A long rod, present only in the 40- to 60-cm layer, was isolated at 60°C in a medium containing 100 g of NaCl per liter and designated strain H168. This strain produced acetate, ethanol, H_2, and CO_2 from glucose metabolism. Fructose, xylose, ribose, cellobiose, and starch were also oxidized. The optimum temperature for growth was 60°C. No growth was obtained at 42 or 70°C. Strain H168 grew optimally in NaCl concentrations ranging from 50 to 100 g per liter, with the upper and lower limits of growth around 200 and 40 g per liter, respectively. The G+C ratio of the DNA was 39.6 mol%. Although halophilic, moderately thermophilic bacteria have been characterized among anaerobes, particularly within methanogens, strain H168 is the first true thermophilic (growing above 60°C) halophilic anaerobic bacterium described so far. The phylogeny, physiology, morphology, lipid content, and high G+C content of strain H168 are sufficiently different from those of genera belonging to the family *Haloanaerobiaceae* to justify the definition of a new genus.

Extremophiles is a word coined to define microbes which live in extreme environments and includes thermophilic, halophilic, and alkalophilic members. All extremophiles are currently found among the domains *Bacteria* and *Archaea* (28, 32). Intensive research has been conducted on extremophilic microbes, with particular emphasis being placed on thermophilic microbes as this group of organisms has great commercial potential for thermostable enzymes (9, 14, 33, 34). Though a wide range of thermophilic physiological groups of bacteria is known to exist in the domain *Bacteria*, e.g., acidophiles, neutrophiles, aerobes, and anaerobes, very little is known about halophilic thermophiles. Indeed, the halophilic thermophilic bacteria studied so far are only slightly halophilic (haloduric) according to the scheme of Larsen (4, 5, 10, 16) as the optimum NaCl requirement for growth is less than 5%. In the same domain, members of the family *Haloanaerobiaceae* are moderate halophiles (22, 40) but are not able to grow up to or above 60°C (17). The halophiles found in the domain *Archaea* are extreme halophiles but are not true thermophiles. Therefore, no true halophilic and thermophilic bacteria have been described so far.

We report in this paper the first moderate halophilic thermophile (most rapid growth between 5 and 17.5% NaCl) which ferments carbohydrates to acetate, ethanol, H_2, and CO_2.

(Part of this work was presented at the Annual Meeting of the American Society of Microbiology, Atlanta, Ga., 16 to 20 May 1993 [1]).

MATERIALS AND METHODS

Origin of strain. The occurrence of thermophilic halophilic anaerobic bacteria in the sediment of a Tunisian hypersaline lake (Chott El Guettar) was tested in samples collected at 20-cm intervals down to 1.20 m. Strain H168 was present only in the 40- to 60-cm layer. It was isolated at 60°C in a medium containing 100 g of NaCl per liter (the culture medium is described immediately below).

Culture medium. Enrichment medium contained the following (in grams per liter): NH_4Cl, 1.0; KH_2PO_4, 0.3; K_2HPO_4, 0.3; $MgCl_2 \cdot 6H_2O$, 2.0; $CaCl_2 \cdot 2H_2O$, 0.2; KCl, 4.0; $CH_3COONa \cdot 3H_2O$, 1.0; glucose, 10.0; NaCl, 100; bio-Trypcase (bioMérieux), 3.0; yeast extract (Difco), 3.0; resazurin (0.1% [wt/vol]), 1 ml; trace element solution (7), 1 ml.

The pH of the medium was adjusted to 7.0 with 10 M KOH, boiled under a stream of O_2-free N_2, and cooled to room temperature. Portions (20 ml) of medium were distributed into 60-ml serum bottles that were closed with butyl rubber stoppers according to the Hungate anaerobic technique (6). The serum bottles were gassed with N_2-CO_2 (80%-20%) and sterilized for 45 min at 110°C. After sterilization, 0.2 ml of 2% $Na_2S \cdot 9H_2O$, 1 ml of 10% $NaHCO_3$ (sterile, anaerobic solutions), and 0.1 ml of a 0.2% sodium dithionite solution (filter-sterilized solution) were injected into the bottles. For preparing roll tubes, 2% agar (Difco) was added to the medium, and 5-ml portions of medium were distributed into Hungate tubes as described above.

Depending on the experiments, various concentrations of yeast extract and bio-Trypcase were used. These concentrations will be mentioned when necessary.

Isolation procedure. Pure cultures were obtained by repeated application of the agar shake dilution method in anaerobic Hungate tubes as described previously (18). Purity was checked by lack of growth in a complex rich NaCl-free medium at mesophilic and thermophilic temperatures.

Analytical techniques. Volatile fatty acids and alcohols were measured with a Delsi series 30 gas chromatograph, using an SP 1000 column with 1% H_3PO_4 on Chromosorb WAW operating at 150°C with nitrogen as carrier gas. A flame ionization detector and a Delsi integrator were used.

Hydrogen and carbon dioxide were analyzed with a Girdel

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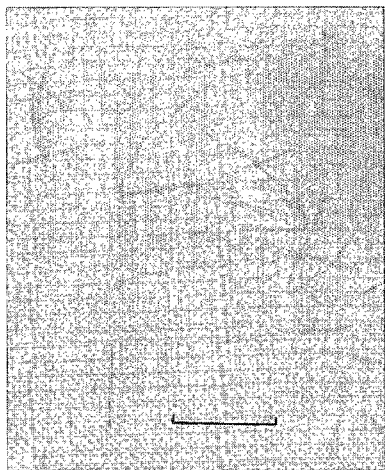
FIG. 1. Phase-contrast photomicrograph of strain H168. Bar = 10 μm.

FIG. 2. Electron micrograph of fine section of strain H168. (a) Cytoplasmic deformation. Bar = 0.2 μm. (b) Typical gram-negative cell wall. Bar = 0.3 μm. (c) Irregularities in the diameter of cells. Bar = 0.2 μm.

series 30 gas chromatograph equipped with a thermal conductivity detector. The column was filled with Carbosphere SS 60/80 mesh.

Glucose, lactate, and formate were assayed on diluted samples by high-performance liquid chromatography with an Analprep 93 pump (Touzart et Matignon, Vitry sur Seine, France) and an ORH 801 column (Interaction Chemicals, Inc., Mountain View, Calif.). The flow rate was 0.6 ml min^{-1}; the volume of the injection loop was 20 μl; the column temperature was 35°C; the detector was a differential refractometer (Knauer, Berlin, Germany). Bacterial growth was quantified with a Shimadzu UV 160A spectrophotometer by measuring the increase in turbidity at 660 nm in the anaerobic Hungate tubes. All experiments were duplicated.

Lipid analysis. Lipid extraction, fatty acid purification, and quantification by capillary gas chromatography were performed as previously described (29). Briefly, a modified Bligh-Dyer chloroform-methanol lipid extraction method was used. The total extractable lipids were fractionated by silicic-column chromatography. The fatty acid esters linked to the phospholipids were methylated by mild alkaline methanolysis, and the resulting fatty acid methyl esters were purified by thin-layer chromatography before gas chromatography analysis.

Lipid nomenclature. A shorthand nomenclature in the form of numbers separated by a colon was used. The number before the colon indicated the carbon chain length, and the number after the colon corresponded to the number of double bonds. The prefixes "i" and "a" referred to iso- and anteiso-, respectively. If necessary, the geometry of the double bonds was indicated by *cis* and *trans*.

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 ruthenium red (0.05%), they were fixed in 1% (wt/vol) OsO$_4$ 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. Micrographs were taken with a JEOL 1200CX electron microscope.

DNA base composition. The moles percent G+C of the DNA was determined by DSM, Braunschweig, Germany. The DNA was isolated and purified by chromatography on hy-
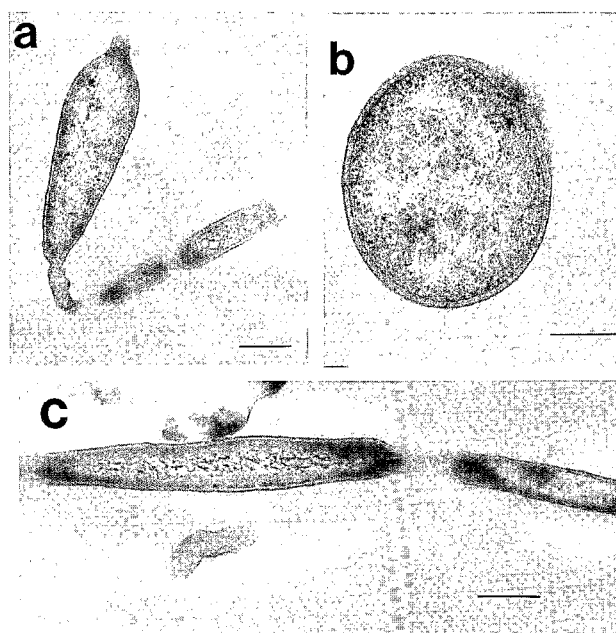
droxyapatite. The G+C content was determined by using high-performance liquid chromatography according to the method of Mesbah et al. (15). Nonmethylated lambda DNA (Sigma) served as the standard.

Phylogenetic analysis. The 16S rRNA gene was amplified from whole cells. For this, 1 ml of the culture was centrifuged and resuspended in 50 μl of sterile distilled water. A 4-μl aliquot was removed and added to a tube containing 10 μl of 10× *Taq* buffer (500 mM KCl, 100 mM Tris-HCl [pH 8.8], 15 mM MgCl$_2$, 1% Triton X-100). Fifty moles of a purified primer (designed against the conserved regions of the eubacterial 16S rRNA gene) pair was used: 5' ccgaattcgtcgacaacAGAGTTT GATCCTGGCTCAG 3' (positions 8 to 27 forward, *Escherichia coli* numbering) and 5' cccgggatccaagcttAAGGAGGT GATCCAGCC 3' (positions 1542 to 1526 reverse, *E. coli* numbering), where lowercase letters indicate the polylinker and the underlined regions indicate restriction enzyme sites of *Sal*I and *Bam*HI, respectively, which are useful in cloning. Portions (0.2 mM each) of dATP, dGTP, dCTP, and dTTP were added, and the mixture was made to a total of 98 μl with sterile distilled water and overlaid with 90 μl of light mineral oil (Sigma). The samples were placed in a Corbett thermal cycler, the cells were lysed, the released DNA was denatured at 95°C for 15 min and cooled to 50°C, and then 2 U of *Taq* DNA polymerase (Promega) was added. The samples were returned to the heating block of the thermal cycler where they were cycled through 32 cycles at 55°C for 1 min, 72°C for 4 min, and 95°C for 1 min, followed by a final cycle at 72°C for 20 min to ensure that all PCR products were completely extended.

The amplified product was purified, cloned, and sequenced essentially as described previously (13, 26). The sequence was aligned to various members of the domain *Bacteria* with the sequencing editor ae2, developed for the Ribosomal Database Project (20). The 16S rRNA sequences used for comparison were extracted from the ribosomal data base of Olsen et al. (20) (version 2), and the sequence for *Acidaminococcus fer-*

TABLE 1. 16S rRNA sequence matrix for various gram-positive bacteria and *H. orenii*[a]

Taxon	% Sequence similarity to:											
	Escherichia coli	*Halothermothrix orenii*	*Haloanaerobium praevalens*	*Sporohalobacter lortetii*	*Sporomusa paucivorans*	*Megasphaera elsdenii*	*Heliobacterium chlorum*	*Acidaminococcus fermentans*	*Clostridium thermosaccharolyticum*	*Clostridium thermoaceticum*	*Syntrophospora bryantii*	*Syntrophomonas wolfei*
Escherichia coli												
Halothermothrix orenii	74.2											
Haloanaerobium praevalens	74.9	80.0										
Sporohalobacter lortetii	73.4	78.6	81.6									
Sporomusa paucivorans	73.8	73.2	77.5	77.1								
Megasphaera elsdenii	76.8	74.0	78.5	78.6	83.5							
Heliobacterium chlorum	79.3	78.3	78.6	77.9	81.0	81.3						
Acidaminococcus fermentans	73.1	73.6	76.6	78.6	87.0	82.8	79.8					
Clostridium thermosaccharolyticum	76.2	75.4	79.4	79.0	77.8	80.5	81.0	77.8				
Clostridium thermoaceticum	77.7	77.4	81.0	80.7	81.3	82.8	83.9	80.3	85.6			
Syntrophospora bryantii	76.5	75.4	76.5	78.3	81.6	79.4	82.4	80.5	80.4	93.7		
Syntrophomonas wolfei	71.9	71.3	72.8	74.3	77.3	75.2	76.3	77.7	76.1	79.7	87.4	

[a] Sequences used in the analysis are from the Ribosomal Database Project (version 2 [20]), and an additional 16S rRNA sequence of *A. fermentans* was obtained from GenBank (accession no. X65935). The sequence of the 16S rRNA gene of *H. orenii* has been deposited in GenBank (accession number L22016). A total of 1,106 unambiguous nucleotides were used for analysis by the Jukes and Cantor method (8) as modified by Olsen (19).

mentans (accession no. X65935) was extracted from GenBank (release 76). Positions of sequence and alignment uncertainties were omitted from the analysis, and 1,106 nucleotides were used in the analysis.

Pairwise evolutionary distances were computed from percent similarities by the distance correction method of Jukes and Cantor (8) described by Olsen (19). Phylogenetic trees of topology were constructed from evolutionary distances by using the algorithm of De Soete (2). PHYLIP (version 3.51c) was used to reexamine tree topology (3). Distance similarity matrices were computed by the Jukes and Cantor method (8) from multiple data sets (100 sets) produced with the SEQ BOOT program. The 100 distance matrices were computed as evolutionary distances by using the program FITCH, and the program CONSENSE was used to generate a consensus tree.

Nucleotide sequence accession number. The sequence of the amplified and cloned 16S rRNA gene has been deposited in GenBank under the accession number L22016.

RESULTS

Enrichment and isolation. A sediment suspension from each 20-cm layer from Chott El Guettar was inoculated into enrichment medium containing glucose and 100 g of NaCl per liter and incubated at 55°C. When growth was observed, the bacterial enrichments were further subcultured and incubated at 60°C. Although bacterial growth was observed with suspensions of the different layers at 55°C, growth was observed only with the 40- to 60-cm layer enrichment at 60°C. This sample was serially diluted in agar roll tubes, and several identical morphological colonies from the highest positive dilution were picked up with a sterile Pasteur pipet and rediluted into another agar dilution series. This process was repeated until pure cultures were obtained. Strain H168 was chosen for further characterization.

Colony and morphological characteristics. Colonies were yellow, flat, and circular with diameters ranging from 0.5 to 1.0 mm, depending on the incubation time.

Cells were long, flexible rods occurring mainly singly (10 to 20 by 0.4 to 0.6 μm) (Fig. 1) and presenting a cytoplasmic concentration with deformation of the membrane (Fig. 2a). The cell wall was typical of gram-negative bacteria (Fig. 2b). Irregularities in the diameter of cells were observed (Fig. 2c). Strain H168 had peritrichous flagella (data not shown).

Growth conditions. Strain H168 was an obligate anaerobe and a moderate halophile, with yeast extract being required for growth. It grew optimally at NaCl concentrations ranging from 50 to 100 g per liter; the upper and lower limits of growth were around 200 and 40 g of NaCl per liter. Strain H168 did not require $MgCl_2$ but tolerated it up to 0.7 M when 100 g of NaCl per liter was present in the medium. The optimal growth temperature was 60°C; growth occurred between 45 and 68°C but not at 70°C. No growth was observed above pH 8.2 or below pH 5.5, with an optimal range between pH 6.5 and 7.0. The following compounds served as energy sources: arabinose, cellobiose, fructose, galactose, glucose, mannose, melibiose, ribose, starch, and xylose. No growth was observed on lactose, maltose, rhamnose, sucrose, sorbose, cellulose, formate, acetate, butyrate, propionate, fumarate, lactate, malate, succinate, adonitol, dulcitol, glycerol, mannitol, Casamino Acids, bio-Trypcase, and trimethylamine. Products of glucose fermentation were ethanol, acetate, H_2, and CO_2.

G+C content. The average DNA base composition of strain H168 was 39.6 mol% G+C.

Phylogeny. Using 12 primers, a nearly complete sequence consisting of 1,482 bases (96%) of the amplified and cloned 16S rRNA gene of strain H168 was determined. Some 60 bases from the 5′ end could not be determined since there was a *Sal*I restriction site at position 68 (*E. coli* numbering according to Winkler and Woese [30]) and therefore these bases had been
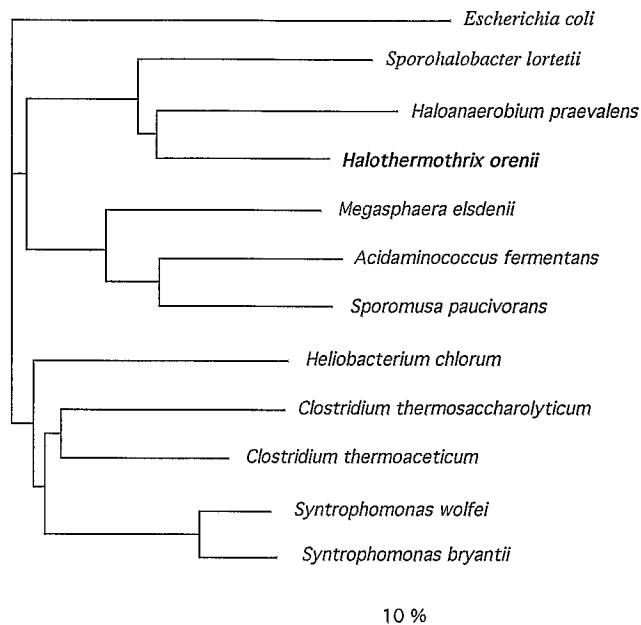
FIG. 3. An unrooted phylogenetic tree showing the relationship of *H. orenii* to other members of the gram-positive branch. The tree was derived from the sequence similarity matrix of Jukes and Cantor as implemented by Olsen (19) (Table 1) by the method of de Soete (2).
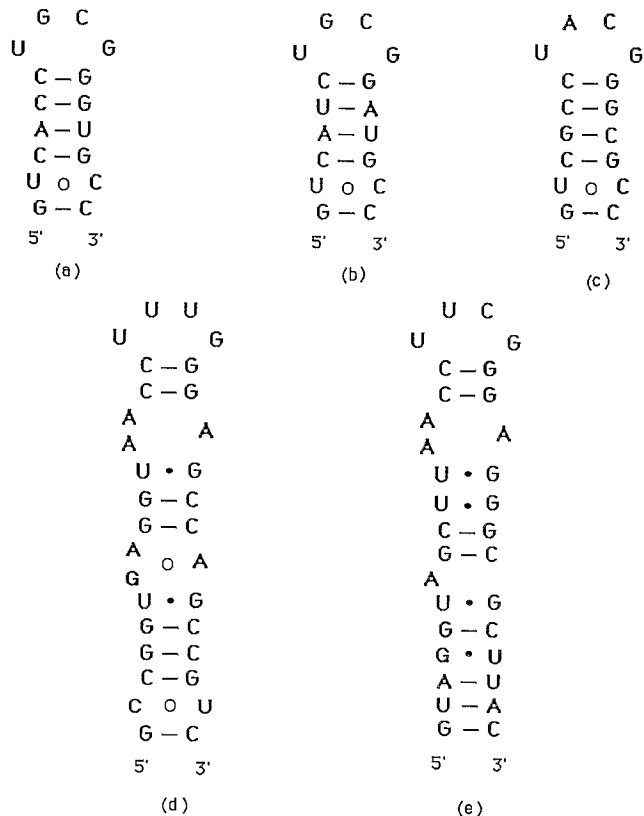

FIG. 4. Secondary structure of halophilic anaerobes, showing the common shortened structure of a part of helix 47 consisting of 16 nucleotides in *H. orenii* (a), *Halobacteroides praevalens* (b), and *S. lortetii* (c) compared with those of other gram-positive members which consist of more than 32 nucleotides; the structure of *M. elsdenii* (d) is shown as an example. The corresponding region of helix 47 in *E. coli* (positions 1435 to 1466) (e) is shown for comparison.

restriction enzyme digested prior to the cloning process. The sequence had 62 signatures, which indicated that it was a member of the domain *Bacteria* (30). Additional signatures indicated that it was a member of the gram-positive line of descent (31). The sequence of strain H168 was therefore aligned and compared to the 16S rRNAs of various characterized gram-positive bacteria (Table 1). Several phylogenetic trees were generated by changing the composition of reference organisms. All the trees generated were comparable, and a tree generated by using the algorithm of De Soete (2) is shown in Fig. 3. The strain H168 sequence was more closely related to those of two halophilic anaerobic bacteria whose 16S rRNA sequences have been determined, viz., *Haloanaerobium praevalens* and *Sporohalobacter lortetii* (80 and 81.6%, respectively). A signature region of 22 nucleotides (positions 821 to 842) with the sequence GGAUACUAGGUGUUGGRGGUUC, which differentiates the three halophilic anaerobes from all other gram-positive bacteria, has been identified. In addition, a unique shortened secondary structural variant in part of helix 47 (positions 1435 to 1466) which is common to all three halophilic anaerobes but not present in any of the other gram-positive bacteria has been identified (Fig. 4).

Bootstrap analysis has indicated that the relationship of strain H168 to *Haloanaerobium praevalens* is not robust (76%), but the relationship of strain H168 and *Haloanaerobium praevalens* to *S. lortetii* is very stable (100%). 16S rRNA sequence analysis of more halophilic anaerobes may assist in stabilizing the branch.

Lipid analysis. The phospholipid fatty acid profile is listed in Table 2. This profile is characterized by the absence of unsaturated fatty acids. Branched chain saturates account for 75% of the total fatty acids, with i15:0 fatty acid predominating.

DISCUSSION

Research on hypersaline ecosystems has led to the description of the family *Haloanaerobiaceae* (22, 23), which now includes six genera and nine species (12, 21, 24, 25, 27, 35–37, 39, 40) (Table 3). The family consists of exclusively mesophilic

TABLE 2. Fatty acid profile of strain H168

Fatty acid	% of fatty acids
13:0	2.29
i14:0	1.54
14:0	7.96
i15:0	54.3
a15:0	9.79
15:0	1.57
i16:0	2.68
16:0	9.94
i17:0	4.99
a17:0	2.34
17:0	0.35
i18:0	0.20
18:0	1.22
Total	99.07
Branched	75.64

TABLE 3. Characteristics of the species of the family *Haloanaerobiaceae*

Species (reference[s])	Morphology	Size (μm)	Gram stain	Spores	Motility	% NaCl Range	% NaCl Optimum	Temp (°C) range	pH range
Halothermothrix orenii	Rods	0.4–0.6 by 10–20	Negative	−	+	4–20	5–10	42–70	5.5–8.2
Halobacteroides halobius MD-1 (25)	Flexible rods	0.5 by 10–20	Negative	+	+	8–17	9–14	37–42	ND
Halobacteroides halobius Z-7287 (36)	Flexible rods	0.3–0.5 by 2–20	Negative	+[b]	+	8–30	9–15	30–47	ND
Halobacteroides acetoethylicus (27)	Rods	0.4–0.7 by 1–1.6	Negative	−	+	6–20	10	15–45	5.4–8.0
Halobacteroides lacunaris (37)	Flexible rods	0.7–1 by 0.5–6	Negative	−	+	5–30	15–18	25–52	6.0–8.0
Haloanaerobium praevalens (35)	Rods	0.5 by 1.5	Negative	−	−	2–30	12.5	15–50	6.0–8.0
Haloanaerobacter chitinovorans (12)	Rods	0.5 by 1.4–8	Negative	−	+	3–30	12–18	25–50	ND
Sporohalobacter lortetii (21, 24)	Rods	0.5–0.6 by 2.5–10	Negative	+	+	4–15	8–9	25–52	ND
Sporohalobacter marismortui (24)	Rods	0.6 by 3–13	Negative	+	+	3–18	3–12	25–50	ND
Acetohalobium arabaticum (39)	Curved rods	0.7–1 by 2–5	Negative	+[b]	+	10–25	15–18	ND–47	5.6–8.4
Haloincola saccharolytica (40)	Rods	0.5–0.7 by 1–1.5	Negative	−	+	3–30	10	15–47	6.0–8.0

[a] ND, not determined.
[b] Oligospores.
[c] ±, poorly used.
[d] In rich medium, sugar is poorly used.
[e] From betaine degradation.

bacteria with the exception of *Halobacteroides lacunaris*, a moderately thermophilic bacterium with an upper temperature limit of 52°C (37). Our report on strain H168, isolated from a hypersaline Tunisian lake (Chott El Guettar), extends the temperature limit of halophilic anaerobic bacteria to 68°C for the first time. Similarly, the optimum temperature for growth of strain H168 is 60°C, higher than that of any moderate halophilic microbe reported to date, including that of the extremely halophilic thermophilic archaean *Methanohalobium evestigatum* (38).

The phospholipid fatty acid profile of strain H168 contains a preponderance of branched saturated fatty acids (75.6%) of which i- and a15:0 and 16:0 dominate. This is a trait commonly found among genuine thermophilic bacteria because of growth at high temperatures. Strain H168 is a thermophile, but its obligate salt requirement and lower optimum growth temperature (60°C) indicate that it is unrelated to other thermophilic bacteria, e.g., *Thermoanaerobacter* spp. (11). Similarly, the lack of lactate production from glucose by strain H168 also rules out any similarity with other thermophilic bacteria, as all of the latter produce lactate.

Strain H168 is a rod-shaped anaerobic and halophilic bacterium and therefore resembles members of the family *Haloanaerobiaceae*. However, there are considerable biochemical and physiological differences between strain H168 and validly published and approved members of the family *Haloanaerobiaceae* (Table 3). This relationship is further strengthened by phylogenetic analysis of 16S rRNA. The presence of a common stretch of sequence at positions 821 to 842 and of a common shortened secondary structural variant in part of helix 47 at positions 1435 to 1466 also supports this analysis. However, strain H168 produces acetate and ethanol as the major end products of glucose fermentation and hence resembles *Halobacteroides* species. Because data on the phylogenetic positions of the members of *Halobacteroides* are not available for comparison, it could be argued that it is a species of *Halobacteroides*. However, numerous differences in the physiological and biochemical characteristics do not support this contention. The main difference is the higher G+C content of strain H168 (39.6%) than of *Halobacteroides* species (30.7 to 32.4%) along with the higher growth temperature and nutritional profiles. Strain H168 differed from *Halobacteroides*

halobius in using ribose, xylose, and cellobiose but not oxidizing maltose and sucrose. In contrast to *Halobacteroides acetoethylicus*, strain H168 oxidized galactose and starch but not sucrose or lactose. Finally, *Halobacteroides lacunaris* uses a significantly different range of substrates, including maltose, mannitol, sucrose, and sorbose.

On the basis of the evidence presented in this paper, we propose to place strain H168 as the type strain of a new genus *Halothermothrix* gen. nov., in the species *Halothermothrix orenii* sp. nov.

Description of the genus *Halothermothrix* gen. nov. *Halothermothrix* (Ha.lo.ther.mo'thrix Gr. n. *hal*, salt; Gr. adj. *thermus*, heat; Gr. n. *thrix*, hair; M.L. fem. m. *Halothermothrix*, a thermophilic fermentative halophile). Rod-shaped eubacterium with cells that are 0.4 to 0.6 by 10 to 20 μm and occur mainly singly. Motile by peritrichous flagella. Spores are not formed. Gram negative. Chemoorganotrophic, strictly anaerobic, and obligately halophilic. The G+C content of the DNA is 39.6%. Does not possess unsaturated fatty acid in the lipid cell wall composition but contains a preponderance of saturated fatty acids, a reflection of its thermophilic nature. Phylogenetically, the genus is a member of the *Haloanaerobiaceae* family and is contained within the low-GC-containing subdivision of the gram-positive branch.

Description of *Halothermothrix orenii* sp. nov. *Halothermothrix orenii* (o.ren'i.i M.L. gen. n. *orenii*, of Oren, name in honor of Aharon Oren, who made important contributions to the knowledge of halophilic anaerobic bacteria). In addition to the characteristics given in the description above, *H. orenii* has the characteristics described below. Colonies are yellow, flat, and circular with 0.5- to 1-mm diameters. Chemoorganotrophic and strictly anaerobic. Oxidizes carbohydrates including arabinose, cellobiose, fructose, galactose, glucose, melibiose, mannose, starch, ribose, and xylose but not lactose, maltose, rhamnose, sucrose, sorbose, cellulose, formate, acetate, butyrate, propionate, fumarate, lactate, malate, succinate, adonitol, dulcitol, glycerol, mannitol, Casamino Acids, bio-Trypcase, or trimethylamine. The end products of fermentation are acetate, ethanol, H_2, and CO_2. NaCl and yeast extract are required for growth. The optimum NaCl concentration for growth is about 10%, with upper and lower limits of 20 and 4%, respectively. The pH range for growth is 5.5 to 8.2; the

TABLE 3—*Continued*

Doubling time (h)	Habitat	G+C (mol%)	Substrates used						End products from glucose
			Carbo-hydrates	Amino acids	N-Acetyl-glucos-amine	Starch	Chitin	Cellulose	
5.3	Chott El Guettar	39.6	+	−	ND^a	+	ND	−	Acetate, ethanol, H_2, CO_2
8	Dead Sea	30.7	+	−	−	+	ND	ND	Acetate, ethanol, H_2, CO_2
1–2	Dead Sea-Lake Sivash	30.7	+	−	−	+	ND	ND	Acetate, ethanol, H_2, CO_2
7.8	Oil well	32.0	+	−	+	−	−	ND	Acetate, ethanol, H_2, CO_2
2.9	Lake Chokrak	32.4	+	−	−	+	−	ND	Acetate, ethanol, H_2, CO_2
4	Great Salt Lake	27.0	+	+	+	−	−	ND	Acetate, butyrate, propionate, H_2, CO_2
2.5	Solar saltern	34.8	+	ND	+	±^{c}	+	−	Acetate, isobutyrate, H_2, CO_2
8	Dead Sea	31.5	+	ND	ND	+	ND	ND	Acetate^d, propionate, isobutyrate, butyrate, isovalerate, H_2
ND	Dead Sea	29.6	+	−	ND	+	−	ND	Acetate, ethanol, butyrate, formate, H_2, CO_2
ND	Lake Sivash	33.6	−	+	ND	ND	ND	ND	Acetate^e, trimethylamine^e
3.9	Lake Sivash	31.3	+	−	+	ND	−	ND	Acetate, H_2, CO_2

optimum pH is 6.5 to 7.0. Grows between 45 and 68°C, with no growth occurring at 70°C. The optimum temperature for growth is 60°C. Isolated from sediments of a Tunisian salted lake (Chott El Guettar) from a depth of 40 to 60 cm but not from any other depth (up to 1.2 m). The type strain is strain H168, deposited in the Oregon Collection of Methanogens (OCM 544).

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