

## *Haloicola saccharolytica* subsp. *senegalensis* subsp. nov., Isolated from the Sediments of a Hypersaline Lake, and Emended Description of *Haloicola saccharolytica*

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A new halophilic chemoorganotrophic bacterium (strain H150<sup>T</sup> [T = type strain]) related to *Haloicola saccharolytica* was isolated from the hypersaline sediments of Retba Lake in Senegal. This organism was a rod-shaped, motile, non-spore-forming, gram-negative obligate anaerobe that grew optimally in the presence of 10% NaCl and at 40°C. The DNA base composition was 31.7 ± 0.3 mol% guanine plus cytosine. The fermentation products from glucose were acetate, H<sub>2</sub>, and CO<sub>2</sub>. The fermentable substrates included cellobiose, fructose, glucose, maltose, lactose, glycerol, mannitol, mannose, ribose, raffinose, and sucrose. Penicillin G, cephalosporin C, novobiocin, vancomycin, and chloramphenicol inhibited growth. As determined by DNA-DNA hybridization, strain H150<sup>T</sup> was 71% related to *H. saccharolytica*, with  $\Delta T_m$  value of 6.0°C. However, strain H150<sup>T</sup> exhibited marked phenotypic differences, particularly in the range of substrates used, when it was compared with the type species of the genus *Haloicola*. For this isolate we propose the name *Haloicola saccharolytica* subsp. *senegalensis* subsp. nov.; strain H150 (= DSM 7379) is the type strain of this taxon.

Although most studies of hypersaline environments have been limited to aerobic organisms, these ecosystems are also inhabited by a wide range of anaerobic microorganisms, including photosynthetic, methanogenic, fermentative, and sulfate-reducing bacteria (14). In the past few years, there has been an increased interest in searching for new strains of anaerobic bacteria in these environments in order to understand the mechanisms involved in osmotic regulation and enzymatic activities at high salt concentrations.

Only the following 11 fermentative, strictly anaerobic, moderately halophilic bacteria have been described to date (12): *Haloanaerobium praevalens*, from bottom sediments of the Great Salt Lake in Utah (24); *Halobacteroides halobius* (17), *Sporohalobacter lortetii*, and *Sporohalobacter marismortui* (16), from sediments of the Dead Sea; *Halobacteroides acetoethylicus*, from deep subsurface gas-bearing sandstones and brine waters associated with an injection water filter on an offshore oil rig in the Gulf of Mexico (19); *Haloanaerobacter chitinovorans*, from a solar saltern in southern California (10); *Halobacteroides lacunaris*, from hypersaline Lake Chokrak (Kerch Peninsula) (25); *Haloicola saccharolytica* (27), *Acetohalobium arabaticum* (26), and *Halocella cellulolytica* (21), from Lake Sivash; and *Haloferoxigena orenii*, from sediment from a Tunisian hypersaline lake (chott El-Guettar) (4).

A 16S rRNA oligonucleotide cataloging analysis of most of these species showed that they are related to each other and are different in some ways from members of other subgroups belonging to the eubacterial kingdom. A new family, the *Haloanaerobiaceae*, has been described by Oren et al. (15).

All bacteria belonging to the family *Haloanaerobiaceae* produce acetate from carbohydrates. The homoacetogenesis

process occurs only in *A. arabaticum* (26) and *Haloicola saccharolytica* (27), but *Haloicola saccharolytica* is able to oxidize carbohydrates, while *A. arabaticum* is not. *Halobacteroides lacunaris*, *Halobacteroides acetoethylicus*, and *Halobacteroides halobius* produce ethanol in addition to acetate, while *S. lortetii*, *S. marismortui*, and *Haloanaerobium praevalens* produce diverse volatile fatty acids. The metabolic products obtained from cellulose oxidation by *Halocella cellulolytica* include ethanol, acetate, and lactate (21).

In this paper we describe the isolation from the sediments of a hypersaline lake of a moderately halophilic chemoorganotrophic bacterium (strain H150<sup>T</sup> [T = type strain]). Acetate is the only fatty acid produced by this organism during glucose fermentation. The results of DNA relatedness, physiological, and metabolic studies justified assignment of this strain to a new subspecies of the genus *Haloicola*.

### MATERIALS AND METHODS

**Strain origin.** Strain H150<sup>T</sup> 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 obtained in July 1988 at 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<sup>-1</sup>. The pH was 7.15.

The following five type strains of haloanaerobic bacteria were used for DNA relatedness studies: *Haloicola saccharolytica* Z-7787 (= DSM 6645), *S. lortetii* MD-2 (= ATCC 35059), *S. marismortui* DY-1 (= ATCC 35420), *Halobacteroides acetoethylicus* EIGI (= DSM 3532), and *Haloanaerobium praevalens* GSL (= DSM 2228).

**Culture medium.** Strain H150<sup>T</sup> was isolated on medium containing (per liter) 1.0 g of NH<sub>4</sub>Cl, 0.3 g of KH<sub>2</sub>PO<sub>4</sub>, 20.0 g of MgCl<sub>2</sub> · 6H<sub>2</sub>O, 2.0 g of CaCl<sub>2</sub> · 2H<sub>2</sub>O, 4.0 g of KCl, 1.0 g of CH<sub>3</sub>COONa · 3H<sub>2</sub>O, 10.0 g of glucose, 150 g of NaCl, 3.0 g of bio-Trypticase (bioMérieux), 3.0 g of yeast extract (Difco), 1

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ml of a 0.1% (wt/vol) resazurin solution, 1 ml of a trace element solution (9), and 50 ml of the mineral solution of Balch et al. (1).

The medium was adjusted to pH 7 with 10 M KOH, boiled under a stream of O<sub>2</sub>-free N<sub>2</sub>, and cooled to room temperature. Portions (20 ml) of medium were distributed into 60-ml serum bottles that were stoppered with butyl rubber stoppers according to the Hungate anaerobic technique (8). The serum bottles were outgassed with N<sub>2</sub>-CO<sub>2</sub> (80:20) and sterilized for 45 min at 110°C. After autoclaving, 0.2 ml of 2% Na<sub>2</sub>S · 9H<sub>2</sub>O and 1 ml of 10% NaHCO<sub>3</sub> (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 MgCl<sub>2</sub> · 6H<sub>2</sub>O (2.0 g liter<sup>-1</sup>) and CaCl<sub>2</sub> · 2H<sub>2</sub>O (0.2 g liter<sup>-1</sup>) were used.

**Isolation procedure.** Samples of the sediment were used to prepare enrichment cultures for halophilic sulfate reducers (13) at four NaCl concentrations (50, 100, 150, 200 g liter<sup>-1</sup>). Another set of cultures was prepared to isolate glucose-oxidizing microorganisms from the enrichment cultures at the same concentrations of NaCl. After several transfers, the enrichment cultures were diluted in roll tube media. Two or three colonies from each enrichment culture were picked and rediluted in an agar dilutions series. This process 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% H<sub>3</sub>PO<sub>4</sub> on Chromosorb WAW) operated at 150°C; nitrogen was used as the carrier gas. A flame ionization detector and a Delsi integrator were used. The amount of hydrogen was determined with a Girdel 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 with diluted samples by high-performance liquid chromatography (HPLC), using an Analprep 93 pump (Touzart et Matignon, Vitry sur Seine, France) and an ORH 801 column (Interaction Chemicals, Mountain View, Calif.); the flow rate was 0.6 ml min<sup>-1</sup>, the volume of the injection loop was 20 µl, the column temperature was 35°C, and the detector was a differential refractometer (Knauer, Berlin, Germany). Bacterial growth was quantified with a Shimadzu model UV 160A spectrophotometer by measuring the increase in turbidity at 660 nm in anaerobic Hungate tubes. All experiments were duplicated.

**Lipid analysis.** Lipid extraction, fatty acid purification, and quantification by capillary gas chromatography were performed as described previously (13, 23). The methanol-water residue of a Bligh-Dyer extraction preparation was dried under a vacuum and then rediluted with 1 N HCl. After the supernatant was refluxed at 100°C for 5 h and cooled, it was transferred to a separatory funnel. The lipids were extracted with several washes of chloroform. The chloroform phase was allowed to dry under a vacuum, and the samples were esterified with a methanol-dichloromethane-HCl mixture for 1 h at 100°C. After addition of dichloromethane and water and thorough mixing, the dichloromethane layer was recovered, evaporated in a stream of nitrogen, and spotted onto a thin-layer chromatography plate. After ascending chromatography in a solvent consisting of hexane and diethyl ether (1:1,

vol/vol), the hydroxy fatty acid bands were scrapped from the plate and eluted from the silica gel with chloroform-methanol (1:1, vol/vol) into screw-cap glass test tubes.

The hydroxy esters were then converted to their corresponding trimethylsilyl esters by using *N,O*-bis(trimethylsilyl)trifluoroacetamide, dissolved in hexane, and analyzed by gas chromatography and gas chromatography-mass spectrometry.

The fatty acid nomenclature used has been described previously (13). The prefix OH indicates that there is a hydroxyl group at the position indicated from the carboxyl end.

**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) OsO<sub>4</sub> 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 model 1200CX electron microscope.

**DNA base composition.** DNA was isolated and purified by chromatography on hydroxyapatite. The guanine-plus-cytosine (G+C) content was determined by HPLC, using the method of Meshbah et al. (11). Nonmethylated lambda DNA (Sigma) was used as the standard.

**DNA relatedness.** DNA was extracted and purified as described elsewhere (3). The exact 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 (7). The temperature at which 50% of the reassociated DNA became hydrolyzable by the S1 nuclease (*T<sub>m</sub>*) was determined as described by Crosa et al. (5). The difference between the *T<sub>m</sub>* of a homoduplex and the *T<sub>m</sub>* of a heteroduplex ( $\Delta T_m$ ) provided an estimate of the divergence between two DNAs (2).

## RESULTS

**Isolation.** Three strains that grew in the presence of 100, 150, and 200 g of NaCl per liter were isolated. The metabolic properties of the isolate obtained from medium containing 150 g of NaCl per liter (strain H150<sup>T</sup>) were significantly different from the metabolic properties of the two other isolates. Isolate H150<sup>T</sup> was characterized further. Single 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 H150<sup>T</sup> cells were straight rods that were 0.4 to 0.6 by 2 to 5 µm and occurred singly or in pairs (Fig. 1); these cells were motile by means of peritrichous flagella (Fig. 2). Thin sections revealed a typical gram-negative cell envelope profile and a multilayer cell wall (Fig. 3).

The distribution fatty acid in the membrane phospholipids is shown in Table 1. No branched fatty acids were found. Monounsaturated fatty acids accounted for 57% of the total fatty acids, with C<sub>16:1Δ9c</sub> predominating. Cyclopropane fatty acid was also present as cyclo-C<sub>17:0</sub>.

The hydroxy fatty acid profile revealed that a high level of 3OH-C<sub>12:0</sub> was present (Table 1). 3OH-C<sub>10:0</sub>, 3OH-C<sub>12:0</sub>, and 3OH-C<sub>14:0</sub> are known constituents of lipopolysaccharides in gram-negative bacteria. C<sub>14:0</sub> was the major saturated fatty acid present in strain H150<sup>T</sup>. No archaeobacterial isopranyl glycerol ether lipids or other hydrophobic residues were detected. Growth of strain H150<sup>T</sup> was completely inhibited by penicillin G at a concentration of 33 U ml<sup>-1</sup> and by cephalosporin C, novobiocin, or vancomycin at a concentration of 100

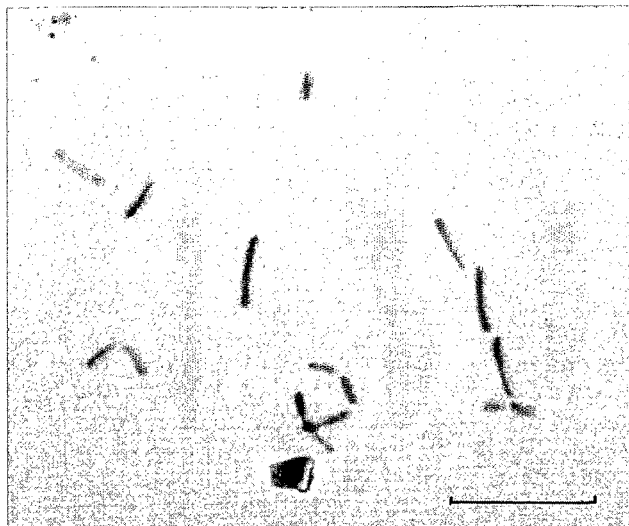


FIG. 1. Phase-contrast photomicrograph of strain H150<sup>T</sup>. Bar = 10  $\mu\text{m}$ .

$\mu\text{g ml}^{-1}$  and was partially inhibited by chloramphenicol at a concentration of 200  $\mu\text{g ml}^{-1}$ .

**Growth and metabolic properties.** The optimum temperature for growth of strain H150<sup>T</sup> was about 40°C, and growth occurred at temperatures between 25 and 45°C. The optimum pH was 7; no growth was detected below pH 6.3 and above pH

8.7. Strain H150<sup>T</sup> required NaCl but not MgCl<sub>2</sub>. Optimum growth occurred at NaCl concentrations between 7.5 and 12.5%. A minimum of 5% NaCl was required for growth; growth inhibition occurred at NaCl concentrations above 25%. Magnesium was tolerated up to a concentration of 1 M. A minimum doubling time of 4.2 h was determined for the isolate cultivated on glucose-containing medium in the presence of 1 g of bio-Trypticase per liter, 1 g of yeast extract per liter, and 150 g of NaCl per liter. Strain H150<sup>T</sup> was found to be an obligate anaerobe. Spores were not observed, and no growth was obtained after pasteurization at 90°C for 20 min.

The following compounds could be used as energy sources: cellobiose, fructose, glucose, maltose, lactose, glycerol, mannitol, mannose, ribose, raffinose, and sucrose. No growth was observed with the following compounds: acetate, butyrate, ethanol, formate, ethylene glycol, methanol, trimethylamine, propionate, adonitol, fumarate, galactose, gluconate, malate, arabinose, pectin, lactate, cellulose, Casamino Acids, rhamnose, xylose, and sorbose. The products of glucose fermentation were acetate (approximately 2 mol/mol of glucose degraded), H<sub>2</sub>, and CO<sub>2</sub>. H<sub>2</sub> was not used as an electron donor to reduce CO<sub>2</sub> to acetate. No ethanol was detected.

**DNA base composition.** The average DNA base composition of strain H150<sup>T</sup>, based on the results of three HPLC determinations, was 31.7  $\pm$  0.3 mol% G+C.

**DNA relatedness.** The levels of DNA relatedness between strain H150<sup>T</sup> and five other type strains are shown in Table 2. *Haloicola saccharolytica* Z-7787<sup>T</sup> was 71% related to strain H150<sup>T</sup> ( $\Delta T_m$  value, 6.0°C).



FIG. 2. Electron micrograph of negatively stained strain H150<sup>T</sup> showing peritrichous flagellation. Bar = 1  $\mu\text{m}$ .

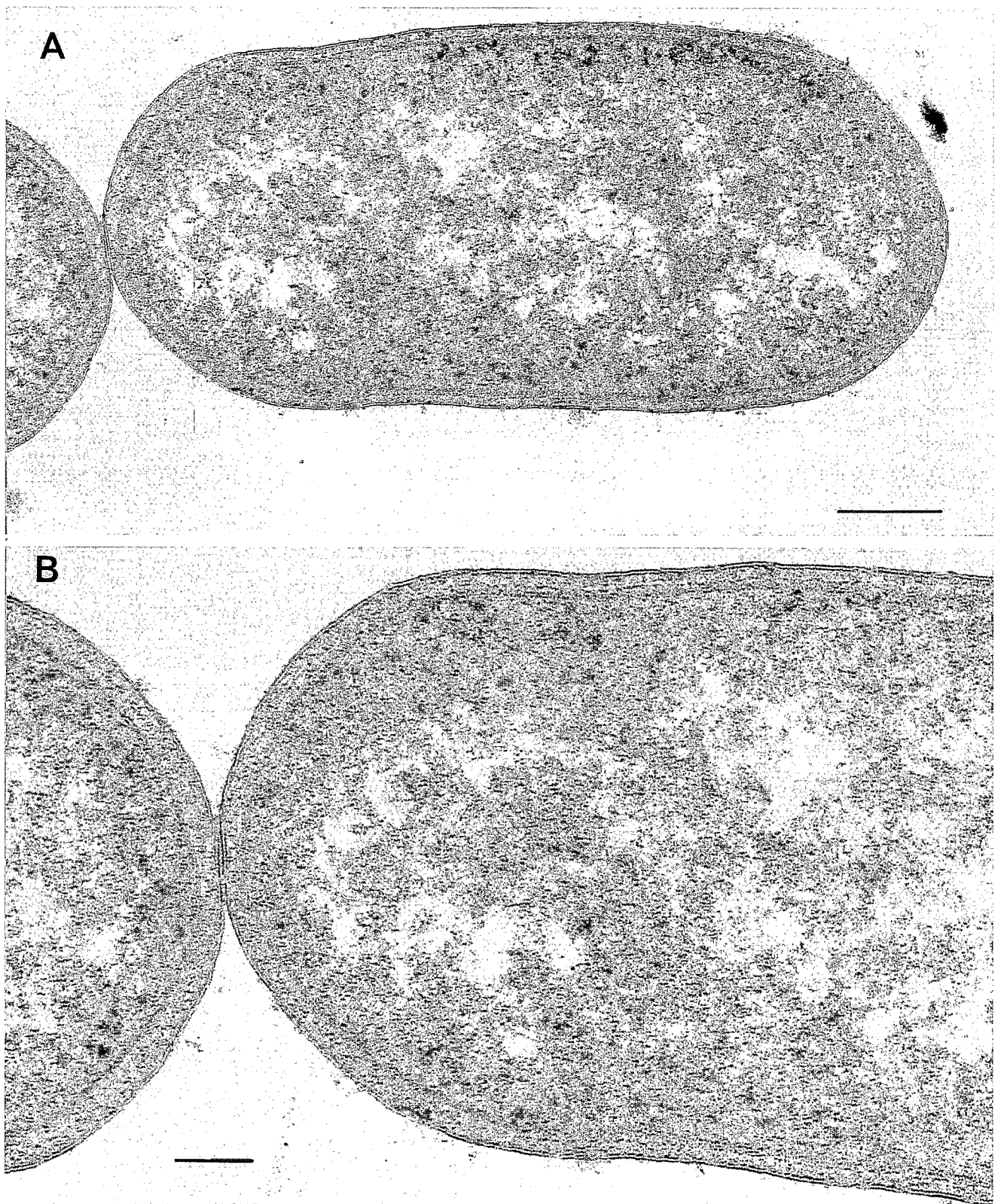


FIG. 3. (A) Electron micrograph of an ultrathin section of strain H150<sup>T</sup> showing the cell wall structure. Note the large interwall space (width, 40 nm), which was larger (100 nm) at the end of the cell. Bar = 0.2  $\mu\text{m}$ . (B) Enlargement of part of panel A showing the thin peptidoglycan layer in mean position. Bar = 0.1  $\mu\text{m}$ .

TABLE 1. Fatty acids and hydroxy fatty acids in membrane phospholipids of strain H150<sup>T</sup> and *Haloicola saccharolytica* Z-7787<sup>T</sup>

Fatty acid methyl ester	% of total fatty acids in:	
	Strain H150 <sup>T</sup>	Strain Z-7787 <sup>Ta</sup>
<b>Fatty acids</b>		
C <sub>12:0</sub>	0.4	ND <sup>b</sup>
C <sub>13:0</sub>	0.9	ND
C <sub>14:1Δ5c</sub>	0.2	ND
C <sub>14:1Δ7c</sub>	2.6	ND
C <sub>14:1Δ9c</sub>	1.9	2.4
C <sub>14:1Δ11</sub>	ND	1.8
C <sub>14:0</sub>	20.9	6.7
C <sub>15:1Δ7c</sub>	3.3	ND
C <sub>15:1Δ9c</sub>	8.9	3.3
C <sub>15:1Δ11</sub>	ND	10.3
C <sub>15:0</sub>	7.2	3.3
C <sub>16:1Δ5c</sub>	7.3	ND
C <sub>16:1Δ9c</sub>	23.4	32.9
C <sub>16:1Δ11c</sub>	7.7	12.1
C <sub>16:0</sub>	11.1	15.3
C <sub>17:1Δ9c</sub>	1.2	2.0
C <sub>17:1Δ11</sub>	ND	6.0
Cyclo C <sub>17:0</sub>	2.2	ND
C <sub>17:0</sub>	0.2	0.7
C <sub>18:1</sub>	ND	2.0
C <sub>18:1Δ9c</sub>	0.3	ND
C <sub>18:1Δ11c</sub>	0.2	ND
C <sub>18:1Δ13c</sub>	0.1	ND
C <sub>18:0</sub>	0.1	1.2
<b>Hydroxy fatty acids</b>		
3OH-C <sub>10:0</sub>	7.2	12.1
br 3OH-C <sub>11:0</sub> <sup>c</sup>	2.5	ND
3OH-C <sub>11:1</sub>	ND	8.0
3OH-C <sub>11:0</sub>	10.4	12.3
3OH-C <sub>12:1</sub> <sup>d</sup>	17.4	48.0
3OH-C <sub>12:0</sub>	50.0	16.2
br 3OH-C <sub>13:0</sub>	2.4	ND
3OH-C <sub>13:1</sub>	ND	2.1
3OH-C <sub>13:0</sub>	2.8	1.3
3OH-C <sub>14:0</sub>	4.6	ND
3OH-C <sub>16:0</sub>	2.7	ND

<sup>a</sup> Data from reference 27.<sup>b</sup> ND, not detected.<sup>c</sup> br, branched.<sup>d</sup> Double bond position not identified.

## DISCUSSION

The characteristics of isolate H150<sup>T</sup> indicated that it was a new, strictly halophilic, anaerobic, rod-shaped organism that used carbohydrates as energy sources. Strain H150<sup>T</sup> was a moderate halophile which grew in the presence of NaCl concentrations between 0.8 and 4.3 M. Its morphology, physiology, and genetic properties were consistent with assignment to the family *Haloanaerobiaceae*. The only fatty acid produced from glucose fermentation was acetate. Therefore, we concluded that strain H150<sup>T</sup> was affiliated with the two anaerobic, halophilic, homoacetogenic, fermentative species that have been described previously, *A. arabaticum* and *Haloicola saccharolytica*. However, *Haloicola saccharolytica* and strain H150<sup>T</sup> can be easily distinguished from *A. arabaticum* by the ability to utilize sugars; *A. arabaticum* does not utilize sugars, but *Haloicola saccharolytica* does. Furthermore, as determined by DNA-DNA hybridization, *Haloicola saccharolytica* and strain H150<sup>T</sup> are more closely related to each other than to the other members of the family *Haloanaerobiaceae*, including the genera *Halobacteroides*, *Haloanaerobium*, and *Sporoha-*

TABLE 2. Levels of DNA relatedness between isolate H150<sup>T</sup> and related bacteria

Source of unlabelled DNA	% Relatedness to <sup>3</sup> H-labelled DNA from strain H150 <sup>T</sup>
Strain H150 <sup>T</sup> .....	100 (0.0) <sup>a</sup>
<i>Haloicola saccharolytica</i> Z-7787 <sup>T</sup> .....	71 (6.0)
<i>Sporohalobacter marismortui</i> DY-1 <sup>T</sup> .....	1
<i>Sporohalobacter lortetii</i> MD-2 <sup>T</sup> .....	1
<i>Halobacteroides acetoehtylicus</i> EIGI <sup>T</sup> .....	11
<i>Haloanaerobium praevalens</i> GSL <sup>T</sup> .....	9

<sup>a</sup> The values in parentheses are Δ*T*<sub>m</sub> values (in degrees Celsius).

*lobacter* (Table 2). Strain H150<sup>T</sup> differs from *Haloicola saccharolytica* in oxidizing raffinose (Table 3); furthermore, no growth occurs on media containing L-xylose, galactose, and gluconate. In contrast to strain H150<sup>T</sup>, *Haloicola saccharolytica* grows in the presence of 30% NaCl and has a faster doubling time (3 h, instead of the 4.2-h doubling time of strain H150<sup>T</sup>). Strain H150<sup>T</sup> and *Haloicola saccharolytica* both contain straight-chain C<sub>16</sub> fatty acids as the main membrane components. However, strain H150<sup>T</sup> differs from *Haloicola saccharolytica* in the distribution of other fatty acids. Indeed, saturated C<sub>12:0</sub> and C<sub>13:0</sub> have not been found in *Haloicola saccharolytica*. Furthermore, strain H150<sup>T</sup> is characterized by a higher level of C<sub>14:0</sub> than *Haloicola saccharolytica*. Differences also occur in the positions of the double bonds in C<sub>14</sub>, C<sub>15</sub>, and C<sub>16</sub> fatty acids. The unsaturated hydroxy fatty acid 3OH-C<sub>12:1</sub> is not common, and additional investigations will be needed to determine its biosynthetic pathway.

As observed by Zhilina et al. (27), in hypersaline ecosystems *Haloicola saccharolytica* and probably strain H150<sup>T</sup> might be involved in anaerobic oxidation of compatible solutes, such as glycerol, which is usually produced by *Dunaliella salina* (6). The fate of this organic compound in hypersaline environments is of interest, particularly with regard to electron transfer from glycerol to biological acceptors such as sulfate reducers. Oxidation of sucrose and trehalose by these organisms indicated that they might also be responsible for degrading other organic osmolytes since such sugars are found in the cytoplasm of halotolerant cyanobacteria (18) that are abundant on the shore of Retba Lake (the source of strain H150<sup>T</sup>) during the dry season (20). After being washed out from the shore by rain during the wet season, algae provide suitable substrates for these halophilic eubacteria.

The results of DNA-DNA hybridization experiments indicated that there is a close relationship between strain H150<sup>T</sup> and *Haloicola saccharolytica*. However, the values which we obtained are at the borderline of the range used to distinguish species (22). Since physiological and metabolic properties of strain H150<sup>T</sup> are different from *Haloicola saccharolytica* properties, we propose that strain H150<sup>T</sup> is a member of a subspecies of *Haloicola saccharolytica*, *Haloicola saccharolytica* subsp. *senegalensis*. This proposal automatically creates *Haloicola saccharolytica* subsp. *saccharolytica*.

**Emendation of the species description of *Haloicola saccharolytica* Zhilina, Zavarzin, Bulygina, Kevbrin, Osipov, and Chumakov 1992.** *Haloicola saccharolytica* (sac.cha.ro.ly'.ti.ca. Gr. n. *sacchar*, sugar; Gr. adj. *lyticus*, able to dissolve, decompose; M. L. adj. *saccharolytica*, digesting sugar). Short rods that are 0.4 to 0.7 by 1 to 5 μm. Cells occur singly or in pairs and are motile by means of peritrichous flagella. Colonies are raised, circular, 0.5 to 2 mm in diameter, and white with glossy

TABLE 3. Characteristics that distinguish strain H150<sup>T</sup> from *Haloicola saccharolytica* Z-7787<sup>T</sup>

Strain	Substrates used				Growth in the presence of:		Doubling time (h)	Major fatty acids in membrane
	Gluconate	L-Xylose	Galactose	Raffinose	3% NaCl	30% NaCl		
H150 <sup>T</sup>	—	—	—	+	—	—	4.2	3OH-C <sub>12:0</sub> , C <sub>16:1</sub> , C <sub>14:0</sub> , C <sub>15:1</sub>
<i>Haloicola saccharolytica</i> Z-7787 <sup>T</sup>	+	+	+	—	+	+	3	3OH-C <sub>12:1</sub> , C <sub>16:1</sub> , C <sub>16:0</sub> , C <sub>15:1</sub>

<sup>a</sup> Substrate utilization and growth comparison tests were performed in the same culture media (see Materials and Methods).

surfaces. Obligately halophilic. The optimum NaCl concentration for growth is 7.5 to 12.5%; the NaCl concentration range for growth is 3 to 30%. Mesophilic. The optimum growth temperature is 37 to 40°C; the temperature range for growth is 15 to 47°C. The pH range for growth is pH 6.0 to 8.5; the optimum pH is pH 7.0 to 7.5. Obligate anaerobe. Chemoorganotrophic. Ferments carbohydrates. The end products of sugar fermentation are acetate, H<sub>2</sub>, and CO<sub>2</sub>. The G+C content of the DNA is 31 to 32 mol%. Isolated from hypersaline lakes.

**Description of *Haloicola saccharolytica* subsp. *saccharolytica*** Zhilina, Zavarzin, Bulygina, Kevbrin, Osipov, and Chumakov 1992. *Haloicola saccharolytica* subsp. *saccharolytica* (sac.cha. ro.ly'ti.ca. Gr. n. *sacchar*, sugar; Gr. adj. *lyticus*, able to dissolve, decompose; M. L. adj. *saccharolytica*, digesting sugar). Short rods that are 0.5 to 0.7 by 1 to 1.5 µm. Cells occur singly or in pairs and are motile by means of a few peritrichous flagella. Colonies are raised, circular, 1 to 2 mm in diameter, and white with glossy surfaces. Obligately halophilic. The optimum NaCl concentration for growth is 10%; the NaCl concentration range for growth is 3 to 30%. Mesophilic. The optimum growth temperature is 37 to 40°C; the temperature range for growth is 15 to 47°C. The pH range for growth is pH 6.0 to 8.0; the optimum pH is pH 7.5. Obligate anaerobe. Chemoorganotrophic. Ferments carbohydrates, including sucrose, cellobiose, trehalose, maltose, lactose, melibiose, glucose, fructose, and ribose. The end products of fermentation are acetate, H<sub>2</sub>, and CO<sub>2</sub>. The G+C content of the DNA is 31.3 mol% (as determined by thermal denaturation). Isolated from a hypersaline lagoon of Lake Sivash with a cyanobacterial mat. The type strain is Z-7787 (= DSM 6645).

**Description of *Haloicola saccharolytica* subsp. *senegalensis*** subsp. nov. *Haloicola saccharolytica* subsp. *senegalensis* (se. ne. ga. len'sis. N. L. adj. *senegalensis*, from Senegal, West Africa). Cells are straight rods that are 0.4 to 0.6 by 2 to 5 µm and are motile by means of peritrichous flagella. Halophilic obligate anaerobe. Grows fastest at NaCl concentrations between 7.5 and 12.5% and is inhibited by NaCl concentrations over 25% and below 5%. Produces acetate, H<sub>2</sub>, and CO<sub>2</sub> from carbohydrate metabolism. The optimum growth temperature is 40°C; the temperature range for growth is 20 to 47°C. The pH range for growth is pH 6.3 to 8.7; the optimum pH is 7.0. Differs from *Haloicola saccharolytica* subsp. *saccharolytica* mainly by using raffinose but not L-xylose, galactose, and gluconate, by growing in the presence of a different range of NaCl concentrations, and by the fatty acid distribution in its membrane phospholipids. The level of DNA-DNA hybridization with *Haloicola saccharolytica* subsp. *saccharolytica* is 71%. The G+C content of the DNA is 31.7 ± 0.3 mol% (as determined by HPLC). Type strain H150 has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as strain DSM 7379; it was isolated from sediments of Retba Lake, a hypersaline lake in Senegal.

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