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

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A new halophilic chemoorganotrophic bacterium (strain H150^T [T = type strain]) related to *Haloincola* 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 \pm 0.3 mol% guanine plus cytosine. The fermentation products from glucose were acetate, H₂, and CO₂. 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^T was 71% related to *H. saccharolytica*, with ΔT_m value of 6.0°C. However, strain H150^T exhibited marked phenotypic differences, particularly in the range of substrates used, when it was compared with the type species of the genus *Haloincola*. For this isolate we propose the name *Haloincola* 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); *Haloincola saccharolytica* (27), *Acetohalobium arabaticum* (26), and *Halocella cellulolytica* (21), from Lake Sivash; and *Halothermothrix 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 *Haloincola* saccharolytica (27), but *Haloincola* 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 $H150^{T}$ [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 *Haloincola*.

MATERIALS AND METHODS

Strain origin. Strain H150^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 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⁻¹. The pH was 7.15.

The following five type strains of haloanaerobic bacteria were used for DNA relatedness studies: *Haloincola 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 $H150^{T}$ was isolated on medium containing (per liter) 1.0 g of NH_4Cl , 0.3 g of KH_2PO_4 , 20.0 g of $MgCl_2 \cdot 6H_2O$, 2.0 g of $CaCl_2 \cdot 2H_2O$, 4.0 g of KCl, 1.0 g of $CH_3COONa \cdot 3H_2O$, 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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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 according to the Hungate anaerobic technique (8). The serum bottles were outgassed with N_2 -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 $MgCl_2 \cdot 6H_2O$ (2.0 g liter⁻¹) and $CaCl_2 \cdot 2H_2O$ (0.2 g liter⁻¹) 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⁻¹). 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 ($1\% H_3PO_4$ 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⁻¹, the volume of the injection loop was 20 μ l, 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_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 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_m) was determined as described by Crosa et al. (5). The difference between the T_m of a homoduplex and the T_m of a heteroduplex (ΔT_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 $H150^{T}$) were significantly different from the metabolic properties of the two other isolates. Isolate $H150^{T}$ 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^T cells were straight rods that were 0.4 to 0.6 by 2 to 5 μ m 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 $C_{16:1\Delta9c}$ predominating. Cyclopropane fatty acid was also present as cyclo- $C_{17:0}$.

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



FIG. 1. Phase-contrast photomicrograph of strain H150^T. Bar = 10 $\mu m.$

 μ g ml⁻¹ and was partially inhibited by chloramphenicol at a concentration of 200 μ g ml⁻¹.

Growth and metabolic properties. The optimum temperature for growth of strain $H150^{T}$ 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 $H150^{T}$ required NaCl but not MgCl₂. 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^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.

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), H₂, and CO₂. H₂ was not used as an electron donor to reduce CO₂ to acetate. No ethanol was detected.

DNA base composition. The average DNA base composition of strain $H150^{T}$, based on the results of three HPLC determinations, was $31.7 \pm 0.3 \text{ mol}\%$ G+C.

DNA relatedness. The levels of DNA relatedness between strain H150^T and five other type strains are shown in Table 2. *Haloincola saccharolytica* Z-7787^T was 71% related to strain H150^T (ΔT_m value, 6.0°C).



FIG. 2. Electron micrograph of negatively stained strain $H150^{T}$ showing peritrichous flagellation. Bar = 1 μ m.



FIG. 3. (A) Electron micrograph of an ultrathin section of strain H150^T 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 μ m. (B) Enlargement of part of panel A showing the thin peptidoglycan layer in mean position. Bar = 0.1 μ m.

TABLE 1. Fatty acids and hydroxy fatty acids in membrane phospholipids of strain $\rm H150^{T}$ and *Haloincola saccharolytica* Z-7787^T

Fatty acid	% of total fatty acids in:				
methyl ester	Strain H150 ^T	Strain Z-7787 ^{T2}			
Fatty acids	<u> </u>				
C _{12:0}	0.4	ND^b			
C _{13:0}	0.9	ND			
$C_{14:1\Delta5c}$	0.2	ND			
C _{14:1Δ7c}	2.6	ND			
$C_{14:1\Delta9c}$	1.9	2.4			
$C_{14:1\Delta 11}$	ND	1.8			
C _{14:0}	20.9	6.7			
C15:1A7c	3.3	ND			
$C_{15:1\Delta9c}$	8.9	3.3			
C _{15:1A11}	ND	10.3			
C _{15:0}	7.2	3.3			
C _{16:145c}	7.3	ND			
$C_{16:1\Delta9c}$	23.4	32.9			
C _{16:1A11c}	7.7	12.1			
$C_{16:0}$	11.1	15.3			
C _{17:1A9c}	1.2	2.0			
$C_{17:1\Delta11}$	ND	6.0			
Cyclo C _{17:0}	2.2	ND			
C _{17:0}	0.2	0.7			
$C_{18:1}$	ND	2.0			
$C_{18:1\Delta9c}$	0.3	ND			
$C_{18:1\Delta11c}$	0.2	ND			
$C_{18:1\Delta 13c}$	0.1	ND			
C _{18:0}	0.1	1.2			
Hydroxy fatty acids					
3OH-C _{10:0}	7.2	12.1			
br $3OH-C_{11:0}^{c}$	2.5	ND			
3OH-C _{11:1}	- ND	8.0			
3OH-C _{11:0}	10.4	12.3			
$3OH-C_{12:1}^{d}$	17.4	48.0			
3OH-C _{12:0}	50.0	16.2			
br 3OH-C _{13:0}	2.4	ND			
3OH-C _{13:1}	ND	2.1			
3OH-C _{13:0}	2.8	1.3			
3OH-C _{14:0}	4.6	ND			
3OH-C16:0	2.7	ND			

^a Data from reference 27.

^b ND, not detected.

c br, branched.

^d Double bond position not identified.

DISCUSSION

The characteristics of isolate H150^T indicated that it was a new, strictly halophilic, anaerobic, rod-shaped organism that used carbohydrates as energy sources. Strain H150^T 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^T was affiliated with the two anaerobic, halophilic, homoacetogenic, fermentative species that have been described previously, A. arabaticum and Haloincola saccharolytica. However, Haloincola saccharolytica and strain $H150^{T}$ can be easily distinguished from A. arabaticum by the ability to utilize sugars; A. arabaticum does not utilize sugars, but Haloincola saccharolytica does. Furthermore, as determined by DNA-DNA hybridization, Haloincola saccharolytica and strain H150^T 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 $H150^{T}$ and related bacteria

Source of unlabelled DNA	% Relatedness to ³ H-labelled DNA from strain H150 ^T
Strain H150 ^T Haloincola saccharolytica Z-7787 ^T Sporohalobacter marismortui DY-1 ^T Sporohalobacter lortetii MD-2 ^T	$ \begin{array}{r} 100 \ (0.0)^a \\ 71 \ (6.0) \\ 1 \\ 1 \end{array} $
Halobacteroides acetoethylicus EIGI ^T Haloanaerobium praevalens GSL ^T	11 9

" The values in parentheses are ΔT_m values (in degrees Celsius).

lobacter (Table 2). Strain H150^T differs from *Haloincola saccharolytica* in oxidizing raffinose (Table 3); furthermore, no growth occurs on media containing L-xylose, galactose, and gluconate. In contrast to strain H150^T, *Haloincola 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^T). Strain H150^T and *Haloincola saccharolytica* both contain straight-chain C_{16} fatty acids as the main membrane components. However, strain H150^T differs from *Haloincola saccharolytica* in the distribution of other fatty acids. Indeed, saturated $C_{12:0}$ and $C_{13:0}$ have not been found in *Haloincola saccharolytica*. Differences also occur in the positions of the double bonds in C_{14} , C_{15} , and C_{16} fatty acids. The unsaturated hydroxy fatty acid 3OH- $C_{12:1}$ is not common, and additional investigations will be needed to determine its biosynthetic pathway.

As observed by Zhilina et al. (27), in hypersaline ecosystems *Haloincola saccharolytica* and probably strain $H150^{T}$ 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 $H150^{T}$) 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^T and *Haloincola 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^T are different from *Haloincola saccharolytica* properties, we propose that strain H150^T is a member of a subspecies of *Haloincola saccharolytica*, *Haloincola saccharolytica* subsp. *senegalensis*. This proposal automatically creates *Haloincola saccharolytica*.

Emendation of the species description of Haloincola saccharolytica Zhilina, Zavarzin, Bulygina, Kevbrin, Osipov, and Chumakov 1992. Haloincola 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 μ m. 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

Strain	Substrates used			Growth in the presence of:		Doubling	Major fatty acids	
	Gluconate	L-Xylose	Galactose	Raffinose	3% NaCl	30% NaCl	time (ii)	in memorane
H150 ^T	_		_	+			4.2	30H-C _{12:0} , C _{16:1} , C _{14:0} , C _{15:1}
Haloincola saccharolytica Z-7787 ^T	+	+	+	-	+	+	3	30H-C _{12:1} , C _{16:1} , C _{16:0} , C _{15:1}

TABLE 3. Characteristics that distinguish strain H150^T from Haloincola saccharolytica Z-7787^T

^a 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, H₂, and CO₂. The G+C content of the DNA is 31 to 32 mol%. Isolated from hypersaline lakes.

Description of Haloincola saccharolytica subsp. saccharolytica Zhilina, Zavarzin, Bulygina, Kevbrin, Osipov, and Chumakov 1992. Haloincola 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 μ m. 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_2 , and CO_2 . 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 Haloincola saccharolytica subsp. senegalensis subsp. nov. Haloincola 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 μ m 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_2 , and CO_2 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 Haloincola 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 Haloincola saccharolytica subsp. saccharolytica is 71%. The G+C content of the DNA is $31.7 \pm 0.3 \text{ mol}\%$ (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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REFERENCES

- Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe. 1979. Methanogens: reevaluation of a unique biological group. Microbiol. Rev. 43:260–296.
- 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, 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.
- Gilmour, D. 1990. Halotolerant and halophilic microorganisms, p. 147–178. *In C. Edwards* (ed.), Microbiology of extreme environments. McGraw-Hill Publishing Co., Oxford.
- 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.
- 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.
- Meshbah, M., U. Premachandran, and W. 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. Desulfohalobium 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. 1988. Anaerobic degradation of organic compounds at high salt concentrations. Antonie van Leeuwenhoek 54:267–277.
- Oren, A., B. J. Paster, and C. R. Woese. 1984. *Haloanaerobiaceae*: a new family of moderately halophilic obligatory anaerobic bacteria. Syst. Appl. Microbiol. 5:71–80.

- Oren, A., H. Pohla, and E. Stackebrandt. 1987. Transfer of *Clostridium lortetii* to a new genus *Sporohalobacter* gen. nov. as *Sporohalobacter lortetii* comb. nov., and description of *Sporohalobacter marismortui* sp. nov. Syst. Appl. Microbiol. 9:239-246.
- 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.
- Reed, R. H., L. J. Borowitzka, M. A. Mackay, J. A. Chudek, R. Foster, S. R. C. Warr, D. J. Moore, and W. D. P. Stewart. 1986. Organic solute accumulation in osmotically stressed cyanobacteria. FEMS Microbiol. Rev. 39:51-56.
- Rengpipat, S., S. E. Lowe, and J. G. Zeikus. 1988. Effect of extreme salt concentrations on the physiology and biochemistry of *Halobacteroides acetoethylicus*. J. Bacteriol. 170:3065–3071.
- Reynaud, P. A., and P. A. Roger. 1981. Variations saisonnières de la flore algale et de l'activité fixatrice d'azote dans un sol engorgé de dune. Rev. Ecol. Biol. Sol 18:9–27.
- Simankova, M. V., N. A. Chernych, G. A. Osipov, and G. A. Zavarzin. 1993. *Halocella cellulolytica* gen. nov., sp. nov., a new obligately anaerobic, halophilic, cellulolytic bacterium. Syst. Appl. Microbiol. 16:385–389.
- Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Trüper. 1987.

Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. Int. J. Syst. Bacteriol. **37**:463–464.

- White, D. C., W. M. Davis, J. S. Nickels, J. D. King, and R. J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia (Berlin) 40:51-62.
- 24. 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.
- Zhilina, T. N., L. V. Miroshnikova, G. A. Osipov, and G. A. Zavarzin. 1992. *Halobacteroides lacunaris* sp. nov., a new saccharolytic, anaerobic, extremely halophilic organism from the lagoon-like hypersaline Lake Chokrak. Mikrobiologiya (Engl. Tr.) 60: 495–503.
- 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.
- Zhilina, T. N., G. A. Zavarzin, E. S. Bulygina, V. V. Kevbrin, G. A. Osipov, and K. M. Chumakov. 1992. Ecology, physiology and taxonomy studies on a new taxon of *Haloanaerobiaceae*, *Haloincola saccharolytica* gen. nov., sp. nov. Syst. Appl. Microbiol. 15: 275–284.