

## *Desulfohalobium retbaense* gen. nov., sp. nov., a Halophilic Sulfate-Reducing Bacterium from Sediments of a Hypersaline Lake in Senegal

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Sulfate-reducing bacterial strain HR<sub>100</sub> was isolated from sediments of Retba Lake, a pink hypersaline lake in Senegal. The cells were motile, nonsporulating, and rod shaped with polar flagella and incompletely oxidized a limited range of substrates to acetate and CO<sub>2</sub>. Acetate and vitamins were required for growth and could be replaced by Biotrypcase or yeast extract. Sulfate, sulfite, thiosulfate, and elemental sulfur were used as electron acceptors and were reduced to H<sub>2</sub>S. Growth occurred at pH values ranging from 5.5 to 8.0. The optimum temperature for growth was 37 to 40°C. NaCl and MgCl<sub>2</sub> were required for growth; the optimum NaCl concentration was near 10%. The guanine-plus-cytosine content of the DNA was 57.1 ± 0.2 mol%. On the basis of the morphological and physiological properties of this strain, we propose that it should be classified in a new genus, *Desulfohalobium*, which includes a single species, *Desulfohalobium retbaense*. The type strain is strain DSM 5692.

Although oxygen availability in hypersaline environments is low because of poor solubility, most studies of life in these ecosystems have been restricted to the aerobic microflora. A few strict anaerobes growing under high salt concentrations were recently described among the fermentative bacteria (22, 25, 26, 32, 42) and methylotrophic methanogens (15, 16, 29, 40, 44).

Significant biological sulfate reduction has been reported to occur in various hypersaline ecosystems, such as the Dead Sea (19, 23) and the Great Salt Lake (41). However, none of the sulfate-reducing bacteria involved have been described. The existence of a salt-tolerating strain whose growth was inhibited by 17% NaCl was reported by Trüper (37). Cord-Ruwisch et al. (3) investigated pure mesophilic and moderately thermophilic cultures of sulfate reducers isolated from an oil field which grew at NaCl concentrations up to 19 and 27%, respectively. However, no further characterization of these strains has been performed.

In this paper we describe the isolation of a halophilic hydrogenotrophic sulfate-reducing bacterium from sediments of Retba Lake, a pink hypersaline lake in Senegal, West Africa. The morphological and physiological characteristics of this strain, strain HR<sub>100</sub>, are consistent with classification in a new genus of sulfate reducers. The name *Desulfohalobium retbaense* gen. nov., sp. nov. is proposed.

### MATERIALS AND METHODS

**Origin of strain HR<sub>100</sub>.** Strain HR<sub>100</sub> was isolated from Retba Lake surface sediments that were sampled in July 1988 at a depth of 1.50 m. The in situ temperature was about 32°C. The total salt concentration was 340 g/liter, and the pH was 7.15. Retba Lake is a hypersaline African lake (area, about 4 km<sup>2</sup>) that is located near Dakar 100 m from the Atlantic Ocean. Its level is about 4 m below sea level.

**Culture medium.** Strain HR<sub>100</sub> was cultured on medium containing (per liter) 1.0 g of NH<sub>4</sub>Cl, 0.3 g of K<sub>2</sub>HPO<sub>4</sub>, 20.0

g of MgCl<sub>2</sub> · 6H<sub>2</sub>O, 2.0 g of CaCl<sub>2</sub>, 4.0 g of KCl, 0.3 g of KH<sub>2</sub>PO<sub>4</sub>, 3.0 g of Na<sub>2</sub>SO<sub>4</sub>, 100.0 g of NaCl, 1.0 g of sodium acetate, 0.001 g of resazurin, 1 ml of a trace element solution (10), and 1 ml of a sodium selenite solution (30). The medium was 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 by using the Hungate anaerobic technique (9). The serum bottles were outgassed with 80% N<sub>2</sub>-20% CO<sub>2</sub> and sterilized for 40 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> (sterile, anaerobic solutions), as well as 0.2 ml of a vitamin solution (30) and 0.1 ml of a 0.2% sodium dithionite solution (filter-sterilized solutions), were injected into the bottles before inoculation. In the case of roll tubes, 2% agar (Difco Laboratories, Detroit, Mich.) was added to the medium; otherwise, 5-ml portions of medium were distributed into Hungate tubes as described above. A gas mixture containing 80% H<sub>2</sub> and 20% CO<sub>2</sub> was added at a pressure of 200 kPa after the medium was sterilized.

**Isolation.** Pure cultures were obtained by repeated application of the agar shake dilution method in anaerobic Hungate tubes as described previously (21). Purity was tested on a complex sulfate-free medium containing 0.1% Biotrypcase (Biomérieux, Craponne, France), 0.1% yeast extract (Difco), and 20 mM glucose, as well as on a complex rich NaCl-free medium.

**Analytical techniques.** Volatile fatty acids and alcohols were analyzed as described previously (4). H<sub>2</sub>S was determined photometrically as colloidal CuS after reaction with a mixture containing 50 mM HCl and 5 mM CuSO<sub>4</sub> (2). Bacterial growth was quantified by measuring optical density at 580 nm.

**Cell fractionation and isolation of the sulfite reductase.** The cells (6 g) from a 10-liter culture in lactate-sulfate medium were suspended in 20 ml of 10 mM Tris hydrochloride buffer (pH 7.6), and the soluble protein extract was obtained as described previously (20). Cell extracts were examined for the presence of cytochrome and desulfovibrin by using a Philips model PU8820 recording spectrophotometer. Protein

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contents were estimated by using the method of Lowry et al. (14).

The bisulfite reductase of strain HR<sub>100</sub> was purified from the soluble protein extract in four purification steps by using a previously described procedure (8) with the modifications described below. After dialysis of the soluble protein extract, which had a high salt content, the suspension (25 ml) was applied to a DEAE-cellulose (type DE 52; Whatman) column (2 by 5 cm) previously equilibrated with 20 mM Tris hydrochloride (pH 7.6). The adsorbed proteins were eluted by using a discontinuous gradient of 100 to 500 mM Tris buffer (pH 7.6). The bisulfite reductase-containing fraction was eluted with 340 mM Tris hydrochloride and was further purified by filtration on Ultrogel AcA 34 (IBF) and chromatography on hydroxyapatite (HTP Bio-Rad) and DEAE-cellulose. At this stage, the protein was judged to be pure on the basis of both polyacrylamide gel analysis results and its absorption spectrum.

**Lipid analysis.** Lipid extraction, fatty acid purification, and quantification by capillary gas chromatography were performed as previously described (38). 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.

The origin of the thin-layer chromatography plate was scraped. The ether lipids were eluted with CHCl<sub>3</sub>-methanol (1/2, vol/vol) and dried under a stream of nitrogen. Glycerol ethers were then formed by acidic hydrolysis of this fraction and were extracted by using hexane and water. The presence of glycerol ether was determined by using thin-layer chromatography.

Fatty acid methyl esters were identified and the position of methyl branching and the degree of unsaturation were checked by gas chromatography-mass spectrometry. Monounsaturations positions and geometry were chemically determined by using dimethyl disulfide derivatization (7).

**Fatty acid nomenclature.** Fatty acids were designated as total number of carbon atoms:number of double bonds, with the position of the double bond closest to the aliphatic ( $\omega$ ) end of the molecule and the geometry (*c*, *cis*; *t*, *trans*) indicated. The prefixes *iso*, *anteiso*, and *branched* refer to *iso*, *anteiso*, and methyl branching of unconfirmed positions, respectively.

**DNA base composition.** DNA was isolated by chromatography on hydroxyapatite (1). The guanine-plus-cytosine content was determined by using high-performance liquid chromatography; non-methylated  $\lambda$  virus DNA was used for calibration (18).

**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% (wt/vol) glutaraldehyde and 0.05% (wt/vol) ruthenium red. After the samples were washed in cacodylate buffer containing ruthenium red, they were postfixed 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% (wt/vol) uranyl acetate in 50% (wt/vol) and then with lead citrate. Micrographs were taken with a JEOL model 1200CX electron microscope.

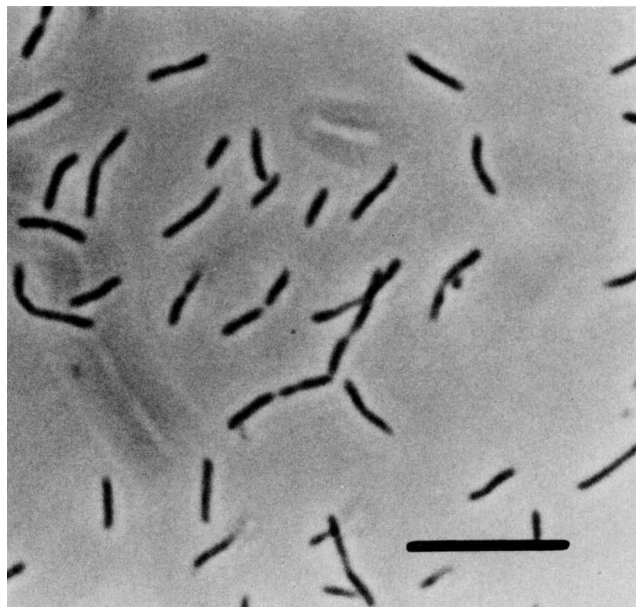


FIG. 1. Phase-contrast photomicrograph of strain HR<sub>100</sub>. Bar = 10  $\mu$ m.

## RESULTS

**Enrichment and isolation.** The enriched culture medium contained 50% water from the lake, sulfate, H<sub>2</sub> as the sole energy source, and 1 g of acetate per liter as a carbon source. After 2 months of incubation at 35°C, significant quantities of H<sub>2</sub>S were produced. The first transfer was carried out into defined media (see Materials and Methods) containing various NaCl concentrations (5, 10, 15, and 20%). Microscopically, the same organisms were observed in media containing NaCl at concentrations up to 20%. These organisms were small, motile, nonsporulated, rod-shaped bacteria. Organisms were isolated from bottles containing 5 and 10% NaCl. After several transfers into sterile medium under a hydrogen atmosphere, the enrichment cultures were diluted in agar roll tubes. Black, lens-shaped colonies developed after 1 month of incubation at 35°C. Colonies were successively picked and rediluted into agar dilutions series in order to obtain pure cultures. The two strains isolated (strains HR<sub>50</sub> and HR<sub>100</sub>) were inoculated onto a complex medium incubated at 35 and 55°C to control purity. The two strains closely resembled each other. Strain HR<sub>100</sub> was chosen for further characterization.

**Morphology.** Strain HR<sub>100</sub> cells were straight to slightly curved, motile, and rod shaped (Fig. 1) with one or rarely two polar flagella (Fig. 2). The cells were 0.7 to 0.9  $\mu$ m in diameter, and the cells were 1 to 3  $\mu$ m long when H<sub>2</sub> was used as the energy source. In medium containing lactate, the cells were longer (up to 20  $\mu$ m long). They occurred both singly and in pairs and were gram negative. The results of the Gram reaction were confirmed by electron microscopy, which showed the multilayered cell wall (Fig. 3) typical of gram-negative bacteria. Spores were never observed. No growth was obtained after pasteurization of the isolate at 80°C for 20 min.

**Growth conditions.** Strain HR<sub>100</sub> grew in a mineral medium supplemented with vitamins and acetate as the carbon source. In the absence of acetate and vitamins, growth occurred on H<sub>2</sub> when yeast extract or Biotrypcase was

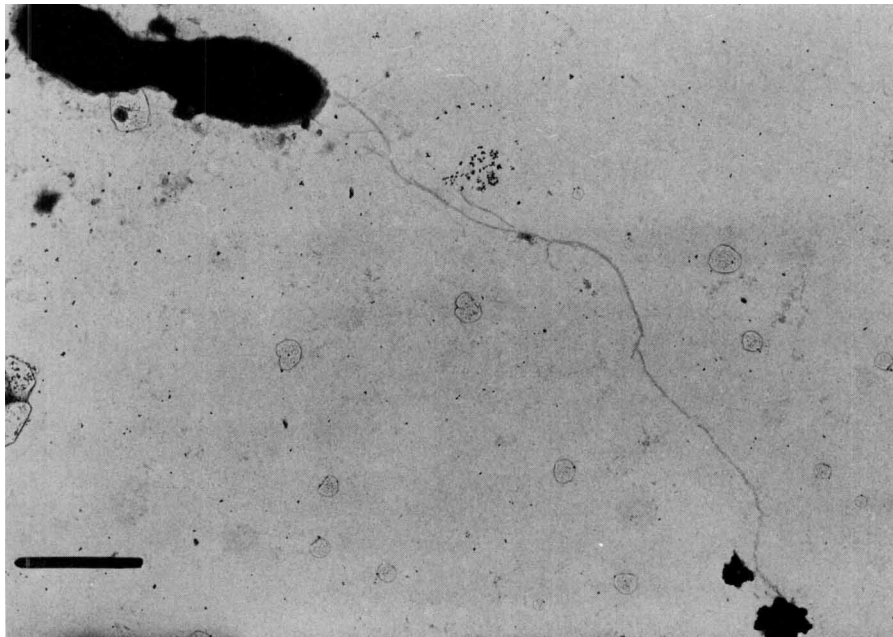


FIG. 2. Transmission electron micrograph of strain HR<sub>100</sub>, showing the polar flagella. Bar = 0.5  $\mu\text{m}$ .

added. The doubling time of the isolate cultivated on lactate-containing medium in the presence of 1 g of Biotrypcase per liter, 1 g of yeast extract per liter, and 100 g of NaCl per liter was about 5 h. Strain HR<sub>100</sub> required NaCl (Fig. 4) and MgCl<sub>2</sub> (Fig. 5) for growth. Growth occurred in the presence of NaCl concentrations up to 24%; optimum growth occurred at an NaCl concentration near 10%. The presence of 20% NaCl in the medium greatly diminished the activity of strain HR<sub>100</sub> (Fig. 4). The optimum pH for growth was 6.5 to 7.0. Growth was detected between pH 5.5 and 8.0. Strain HR<sub>100</sub> grew optimally at temperatures near 40°C (37 to 40°C) (Fig. 6); 45°C was the upper temperature limit for growth. The first replication from an inoculum incubated at 37°C grew at temperatures up to 52°C; however, after several replications at the same temperature, the strain no longer grew at temperatures between 45 and 52°C.

**Metabolic properties.** Nutritional studies were performed at 35°C. Strain HR<sub>100</sub> grew on H<sub>2</sub> when we used vitamins, acetate as the carbon source, and sulfate as the electron acceptor. In the absence of sulfate, the isolate incompletely oxidized ethanol, pyruvate, and lactate to acetate and presumably CO<sub>2</sub> (Table 1). Fumarate, malate, and succinate were not used. In the absence of sulfate, slight growth occurred on pyruvate. Elemental sulfur, sulfate, thiosulfate, and sulfite were used as electron acceptors.

**Pigments.** A soluble extract of strain HR<sub>100</sub> exhibited the characteristic absorption bands of cytochrome *c*<sub>3</sub>, with maxima at 418.5, 522.5, and 552 nm, when it was reduced with sodium dithionite. An oxidized extract had an absorption maximum at 408.5 nm corresponding to the Soret peak. The cytochrome was not reduced by sodium ascorbate, which indicated that it had a low midpoint redox potential. The extract was also examined for the presence of desulfoviridin, the dissimilatory sulfite reductase present in most species of the genus *Desulfovibrio* (6). The absorption spectrum (no absorption band at 628 nm) and the results of a fluorescence test on cell suspensions (31) showed that strain HR<sub>100</sub> was

devoid of desulfoviridin; however, the spectrum had two other broad absorption bands at about 540 and 580 nm (Fig. 7).

As other types of dissimilatory sulfite reductases have been isolated from sulfate-reducing bacteria (8, 12, 36), the bisulfite reductase of this halophilic microorganism was purified in order to identify this enzyme. Disc electrophoresis of the purified protein (100  $\mu\text{g}$ ) revealed the presence of two closely migrating reddish brown bands which appeared to be the only proteins in the preparation after it was stained with Coomassie blue. The protein bands in the gels directly catalyzed the sulfite-dependent oxidation of reduced methyl viologen (8).

The absorption spectrum of the oxidized bisulfite reductase isolated from strain HR<sub>100</sub> had major peaks at 544, 396, and 279 nm and a shoulder at about 580 nm. In addition, the spectrum had two weak absorption bands at around 700 and 730 nm. The ratio of *A*<sub>544</sub> to *A*<sub>279</sub> was 0.14. This absorption spectrum is similar in most respects to that of desulfurubidin, the dissimilatory bisulfite reductase isolated previously from *Desulfovibrio baculatus* (Norway strain) (12). However, the spectrum of strain HR<sub>100</sub> bisulfite reductase differed slightly from that of the homologous protein of *Desulfovibrio baculatus* in that the former had an additional weak absorption band at 700 nm and a more distinguishable shoulder in the 580-nm region.

**Lipid analysis.** The fatty acid distribution in the membrane phospholipids is shown in Table 2. Branched saturated fatty acids accounted for 30% of the total fatty acids, with branched iso-C<sub>15:0</sub> acid predominating. The following mono-saturated fatty acids were also present: C<sub>16:1</sub> $\omega$ 9, C<sub>16:1</sub> $\omega$ 7, C<sub>18:1</sub> $\omega$ 9, C<sub>18:1</sub> $\omega$ 7c, and C<sub>17:1</sub> $\omega$ 8 fatty acids. The fatty acid profile also contained branched-chain components, such as iso-C<sub>17:1</sub> $\omega$ 7c (4%) and branched C<sub>18:1</sub> $\omega$ 6.

**DNA base composition.** The average DNA base composition of strain HR<sub>100</sub> was 57.1  $\pm$  0.2 mol% guanine plus cytosine.

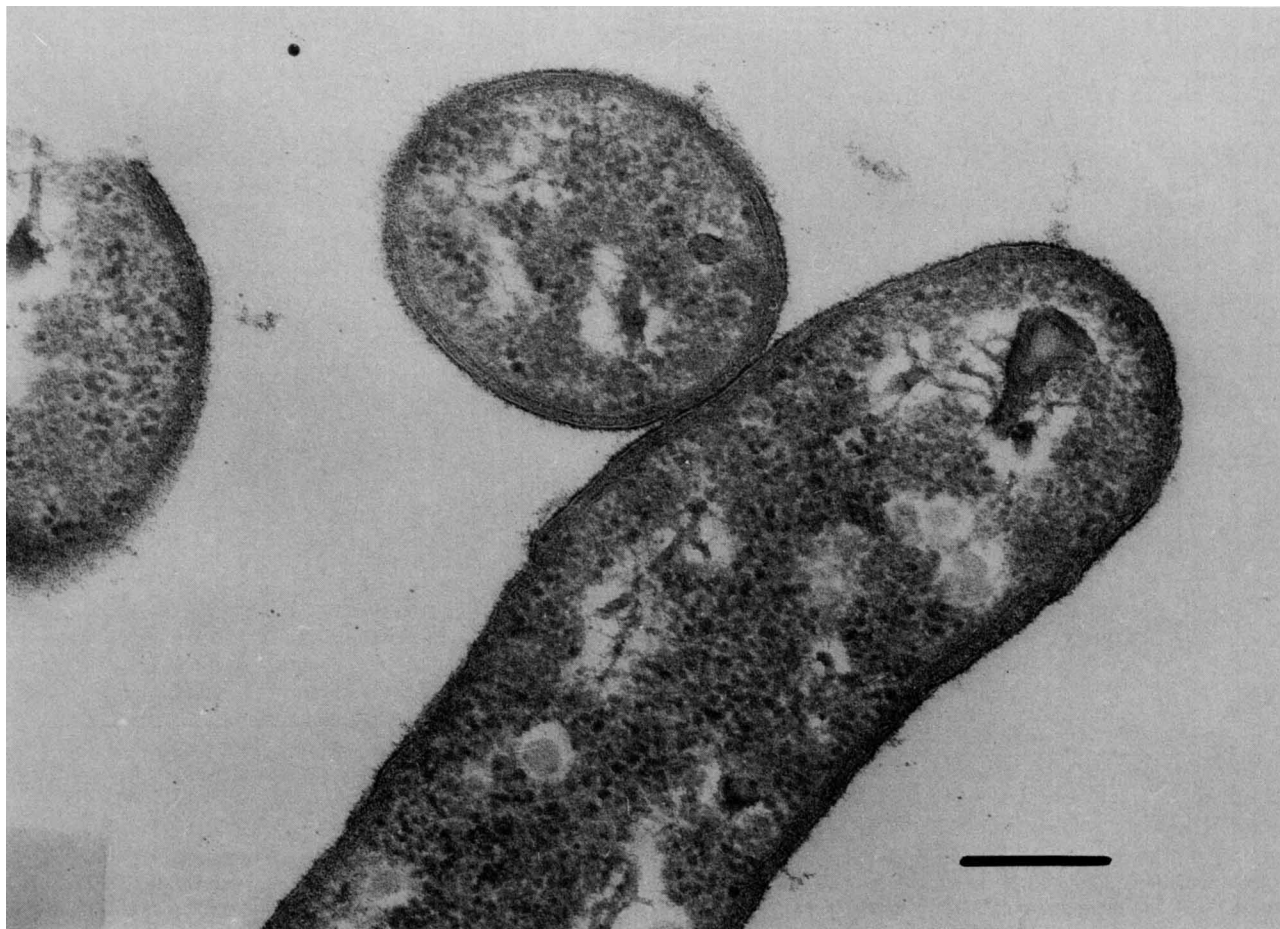


FIG. 3. Electron micrograph of fine sections of strain HR<sub>100</sub>, showing the multilayered cell wall. Bar = 0.2  $\mu\text{m}$ .

### DISCUSSION

Retba Lake is a hypersaline African lake (total salt concentration, 340 g/liter) near Dakar, Senegal. Preliminary work revealed the existence of methanogenic, cellulolytic, and sulfate-reducing bacteria in its sediments (17). Biological sulfate reduction has been observed in similar ecosystems (19, 41), and a limited range of substrates have been found to be involved in this process (24, 41). Strain HR<sub>100</sub>, which was isolated from the sediment of Retba Lake, incompletely oxidizes a few substrates, including ethanol and pyruvate, to acetate and CO<sub>2</sub>. It uses lactate; in contrast, the addition of this organic compound did not enhance the sulfate-reducing activity in sediments from the Dead Sea (24), which suggests that there are sulfate-reducing bacteria with different metabolic activities in halophilic environments.

Strain HR<sub>100</sub> is a moderately halophilic, sulfate-reducing bacterium which has to grow in the presence of salt concentrations that are greater than its optimum salt concentration in hypersaline Retba Lake, since salt concentrations greater than 18% reduce its growth. High salt concentrations have also been reported to decrease metabolic activity toward H<sub>2</sub> and volatile fatty acids, as indicated by the significant amounts of these compounds present in sediments (11, 41). The latter results indicate that the reduction of sulfate in hypersaline ecosystems may be due to the activity of moderately halophilic strains, such as strain HR<sub>100</sub>.

Our isolate is the first hydrogenotrophic anaerobe that has been reported to grow in the presence of NaCl concentrations greater than 10%. At such NaCl concentrations, no methanogenic bacteria were isolated by using H<sub>2</sub>, and there was no evidence that H<sub>2</sub> might be used to reduce CO<sub>2</sub> (24). These results suggest that in hypersaline ecosystems there is probably no competition for H<sub>2</sub> between sulfate reducers and methanogens, as described in other environments (5, 13), and that SO<sub>4</sub><sup>2-</sup> rather than CO<sub>2</sub> is probably a high-potential electron acceptor. In the case of strain HR<sub>100</sub>, the measured growth rate shows that active H<sub>2</sub> transfer might occur via sulfate reduction when the NaCl concentration in the medium is 10 to 18%.

No archaeobacterial isoprenyl glycerether lipids were detected in strain HR<sub>100</sub>, which indicates that this isolate is an eubacterium and not an archaeobacterium (39). Since saline environments might be similar to biota which occurred early in the evolution of the Earth, it will be interesting to compare this strain phylogenetically with the sulfate-reducing species described previously, particularly the newly described bacterium *Archaeoglobus fulgidus* (43).

The fatty acid profile contains components that are associated with sulfate-reducing bacterial species. Iso-C<sub>17:1 $\omega$ 7c</sub> fatty acid is present in significant amounts in the genus *Desulfovibrio* and has been recognized as a good biomarker of these microorganisms in marine sediments (28, 35), and

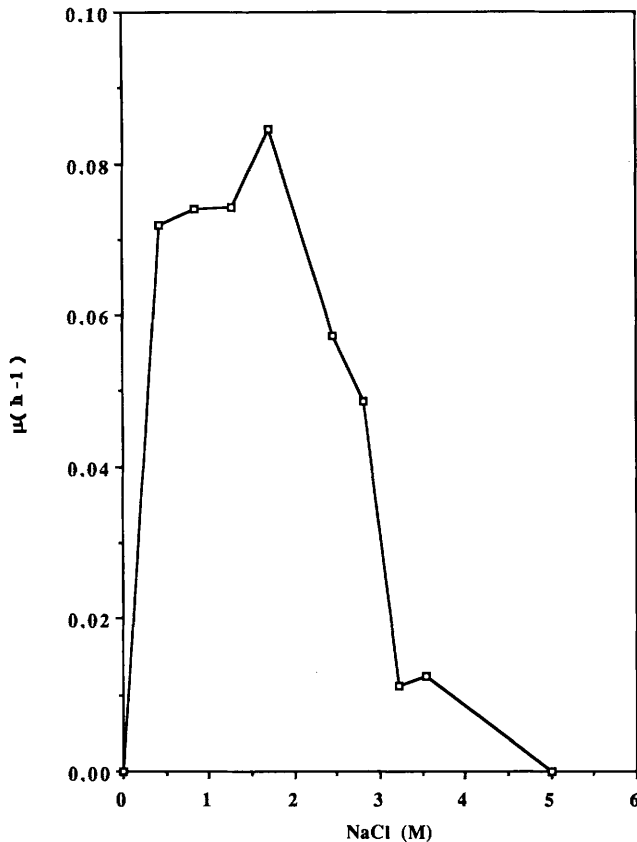


FIG. 4. Effect of NaCl concentration on the growth rate of strain HR<sub>100</sub> cultivated on lactate-containing medium. Cultures were incubated at 35°C. The medium contained 5 g of yeast extract per liter and 5 g of Biotrypcase per liter. The initial pH was 6.4.

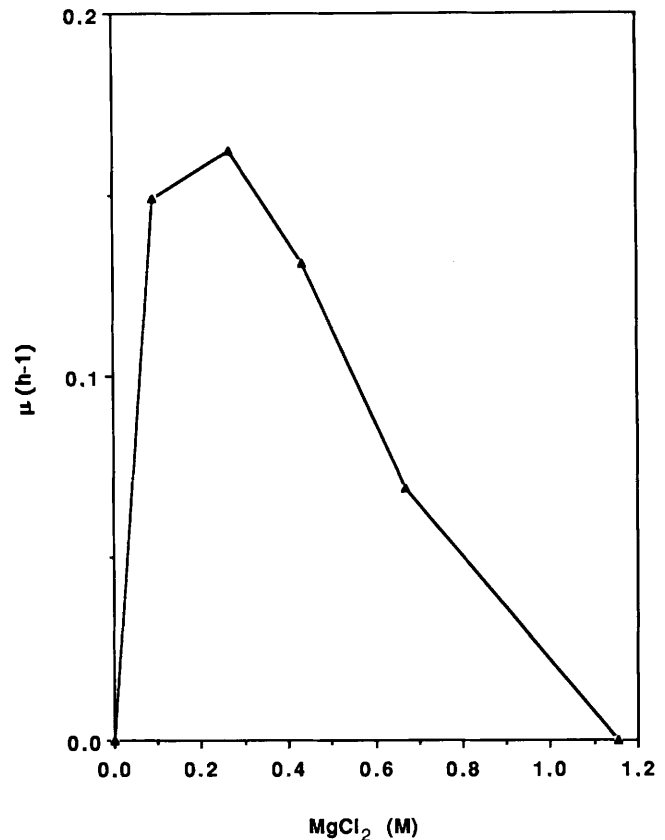


FIG. 5. Effect of MgCl<sub>2</sub> concentration on the growth rate of strain HR<sub>100</sub> cultivated on lactate-containing medium. Cultures were incubated at 35°C. The medium contained 100 g of NaCl per liter, 1 g of yeast extract per liter, and 1 g of Biotrypcase per liter.

C<sub>17:1</sub>ω8 fatty acid has been suggested for use as a biomarker of the genus *Desulfobulbus* (27). However, further analyses will be required in order to determine the influence of both nutritional and external parameters on the fatty acid distribution in the membrane of strain HR<sub>100</sub>.

Strain HR<sub>100</sub> clearly belongs to the group of sulfate-reducing bacteria which incompletely oxidize organic substrates (30). It differs from *Desulfomonas pigra* in morphology and from species of the genus *Desulfotomaculum* in its lack of spores. In contrast to *Desulfobulbus* species, strain HR<sub>100</sub> does not oxidize propionate, and it cannot belong to the genus *Thermodesulfobacterium* because of its growth temperature range.

Recent studies of *Desulfovibrio thermophilus* and *Desulfovibrio baculatus* have shown that these two organisms belong to the genera *Thermodesulfobacterium* (34) and *Desulfomicrobium* (33), respectively, and all of the incomplete oxidizers belonging to *Desulfovibrio* species are vibriolike forms containing only desulfovibrin (6). The cells of strain HR<sub>100</sub> are mainly straight rods and contain desulforubidin. This halophilic strain cannot be affiliated with the genus *Desulfomicrobium* (33), which is characterized by small cells that are 1.3 μm long. Furthermore, our isolate differs from *Desulfomicrobium baculatus* in that it readily oxidizes H<sub>2</sub>, uses ethanol, and does not oxidize malate, and it differs from *Desulfomicrobium apsheronum* in that it does not use fumarate. No requirements for NaCl and MgCl<sub>2</sub> have been reported for *Desulfomicrobium* species. The recent halo-

philic sulfate reducer isolated by P. Caumette (1a) has a vibrioid form and gave a positive desulfovibrin test.

Therefore, we propose that strain HR<sub>100</sub> should be placed in a new genus, *Desulfohalobium*, and new species, *Desulfohalobium retbaense*.

**Description of *Desulfohalobium* gen. nov.** *Desulfohalobium* (De.sul.fo.hal.o'bi.um. L. pref. *de*, from; L. n. *sulfur*, sulfur; Gr. n. *hals*, the sea, salt; Gr. n. *bios*, life; M. L. adj. *halobius*, living on salt; M. L. masc. n. *Desulfohalobium*, a sulfate-reducing salt-requiring rod-shaped bacterium).

Rod-shaped eubacteria with cells that are 0.7 to 0.9 by 1 to 3 μm, have rounded ends, and occur singly or in pairs when H<sub>2</sub> is the energy source. Longer cells up to 20 μm long occur in medium containing lactate. Motile by means of one or rarely two polar flagella. Spores are not formed. Gram negative.

Chemoorganotroph; respiratory metabolism; strictly anaerobic. Incompletely oxidizes organic substrates. Sulfate and other oxidized sulfur compounds serve as electron acceptors and are reduced to H<sub>2</sub>S.

NaCl and MgCl<sub>2</sub> are required for growth. Vitamins and acetate as a carbon source are necessary for growth on H<sub>2</sub> and can be replaced by adding Biotrypcase or yeast extract. The optimum temperature for growth is 37 to 40°C.

The cells contain desulforubidin.

**Description of *Desulfohalobium retbaense* sp. nov.** *Desulfohalobium retbaense* (ret.ba.en'se. N. L. adj. *retbaense*, pertaining to Retba Lake in Senegal). Straight to slightly

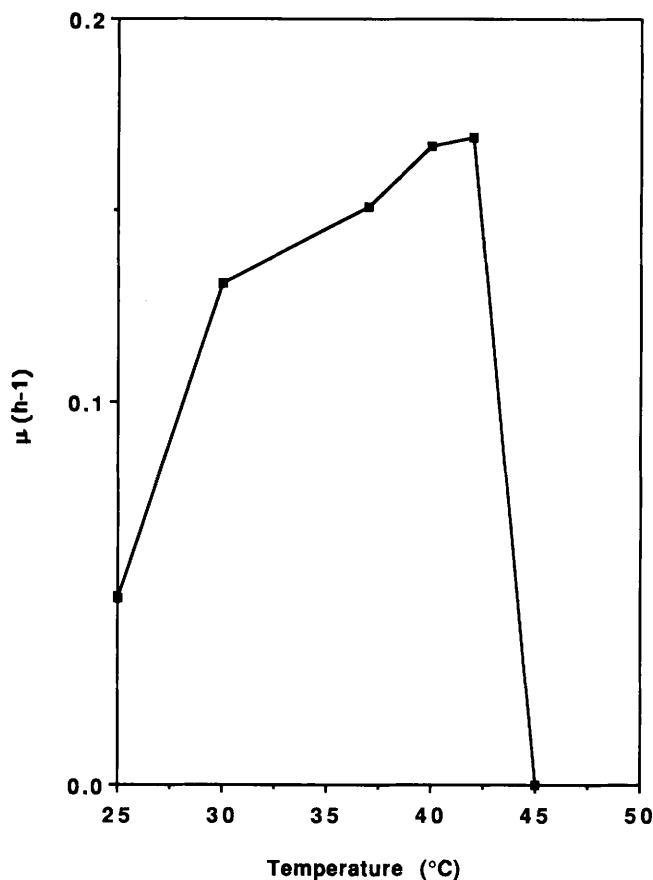


FIG. 6. Effect of temperature on the growth rate of strain HR<sub>100</sub> cultivated on lactate-containing medium supplemented with 100 g of NaCl per liter.

curved rods that are 0.7 to 0.9 by 1 to 3 μm, have rounded ends, and occur singly or in pairs. When the organism is grown on H<sub>2</sub> as an energy source, longer cells up to 20 μm long occur in lactate-containing medium. Motile by means of

TABLE 1. Substrates used as energy sources by strain HR<sub>100</sub> in the presence and absence of sulfate

| Substrate <sup>a</sup> | Use as an energy source                          |   |
|------------------------|--|---|
|                        | In the presence of SO <sub>4</sub> <sup>2-</sup> | In the absence of SO <sub>4</sub> <sup>2-</sup> |
| H <sub>2</sub>         | + <sup>b</sup>                                   | -   |
| Formate                | +  | -   |
| Ethanol                | +  | -   |
| Pyruvate               | +  | (+)   |
| Lactate                | +  | -   |

<sup>a</sup> Most substrates were tested at concentrations of 20 mM. Casamino Acids, yeast extract, and Biotrypcase were tested at concentrations of 2 g/liter. The growth medium contained 1 g of Biotrypcase per liter and 1 g of yeast extract per liter. We tested the following compounds which did not support sulfide production: acetate, propionate, butyrate, fumarate, malate, succinate, glycerol, glucose, fructose, choline, Casamino Acids, yeast extract, and Biotrypcase. Growth was measured by determining H<sub>2</sub>S production and absorbancy after 7 days at 35°C.

<sup>b</sup> +, Substrate used as electron donor (turbidity increased); -, substrate not used as electron donor; (+), substrate used as electron donor but growth was slow.

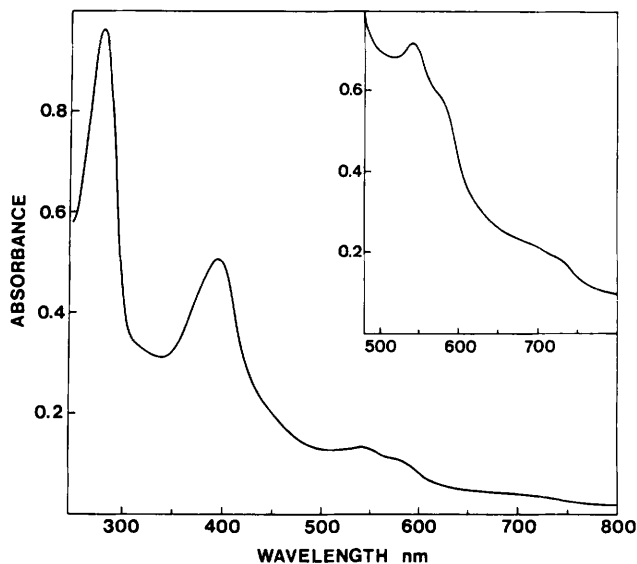


FIG. 7. Absorption spectrum of strain HR<sub>100</sub> bisulfite reductase. The spectrum of the bisulfite reductase (0.34 mg/ml) in 20 mM Tris hydrochloride buffer (pH 7.6) was recorded by using a cuvette with a 1-cm light path. The inset shows the spectrum from 480 to 800 nm in greater detail; this spectrum was obtained by using a concentrated sample of bisulfite reductase (1.89 mg/ml).

one or rarely two polar flagella. No spore formation. Gram negative.

Strictly anaerobic chemoorganotroph. Incompletely oxidizes lactate, ethanol, and pyruvate to acetate and CO<sub>2</sub>. Ferments pyruvate slowly. Does not use glycerol, fructose, glucose, fumarate, malate, succinate, choline, yeast extract, and Biotrypcase.

The optimum temperature for growth is 37 to 40°C. NaCl

TABLE 2. Fatty acid distribution in membrane phospholipids of strain HR<sub>100</sub>

| Fatty acids                  | % of total fatty acids |
|------------------------------|------------------------|
| C <sub>13:0</sub>            | 0.11                   |
| Iso-C <sub>14:0</sub>        | 1.68 ± 0.1             |
| C <sub>14:0</sub>            | 0.95                   |
| Iso-C <sub>15:0</sub>        | 15.28 ± 2.3            |
| Anteiso-C <sub>15:0</sub>    | 3.61 ± 0.3             |
| C <sub>15:1ω6</sub>          | 0.23                   |
| C <sub>15:0</sub>            | 3.93 ± 0.4             |
| Iso-C <sub>16:0</sub>        | 5.34 ± 0.5             |
| C <sub>16:1ω9</sub>          | 0.58                   |
| C <sub>16:1ω7</sub>          | 4.10 ± 0.7             |
| C <sub>16:0</sub>            | 13.33 ± 2.8            |
| Iso-C <sub>17:1ω7c</sub>     | 3.88 ± 0.1             |
| Iso-C <sub>17:0</sub>        | 1.90 ± 0.4             |
| Anteiso-C <sub>17:0</sub>    | 2.24 ± 1.1             |
| C <sub>17:1ω8</sub>          | 8.05 ± 1.5             |
| C <sub>17:1ω6</sub>          | 0.71 ± 0.6             |
| C <sub>17:0</sub>            | 3.33 ± 0.8             |
| Branched-C <sub>18:1ω6</sub> | 0.36                   |
| C <sub>18:1ω9</sub>          | 3.86 ± 1.4             |
| C <sub>18:1ω7c</sub>         | 6.53 ± 2.2             |
| C <sub>18:1ω5</sub>          | 0.23                   |
| C <sub>18:0</sub>            | 15.97 ± 5.2            |
| Cyclo-C <sub>19:0</sub>      | 0.43                   |
| Unidentified                 | 3.37                   |



and  $MgCl_2$  are required for growth. The optimum NaCl concentration is near 10%. Grows in media containing NaCl at concentrations up to 24%. The pH range for growth is 5.5 to 8.0; the optimum pH is 6.5 to 7.0.

Cells contain cytochrome  $c_3$  and desulforubidin.

The guanine-plus-cytosine content of the DNA is  $57.1 \pm 0.2$  mol%.

Isolated from sediments of a hypersaline African lake, Retba Lake, near Dakar, Senegal.

The type strain is strain DSM 5692.

#### ACKNOWLEDGMENTS

We are indebted to K. D. Jahnke, Deutsche Sammlung von Mikroorganismen, Braunschweig, Federal Republic of Germany, for determining the DNA base ratio. We thank J. L. Cayol and A. Jacq for technical assistance and J. Blanc for revising the English.

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