Desulfonatronovibrio hydrogenovorans gen. nov., sp. nov., an Alkaliphilic, Sulfate-Reducing Bacterium

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A new alkaliphilic, sulfate-reducing bacterium, strain Z-7935^T (T = type strain), was isolated from a soda-depositing lake, Lake Magadi in Kenya. This organism is a motile vibrio which utilizes only hydrogen and formate as electron donors and sulfate, sulfite, and thiosulfate, but not sulfur, as electron acceptors. Thiosulfate is dismutated. Strain Z-7935^T is an obligately sodium-dependent alkaliphile which grows in sodium carbonate medium and does not grow at pH 7; the maximum pH for growth is more than pH 10, and the optimum pH is 9.5 to 9.7. The optimum NaCl concentration for growth is 3% (wt/vol). The optimum temperature for growth is 37°C. The G+C content of the DNA is 48.6 mol%. 16S ribosomal DNA sequence analysis revealed that strain Z-7935^T represents a new lineage with genus status in the delta subclass of the Proteobacteria. The name Desulfonatronovibrio hydrogenovorans gen. nov., sp. nov. is proposed for this organism; the type strain of D. hydrogenovorans is strain Z-7935 (= DSM 9292).

The development of alkalinity is often ascribed to sulfate-reducing bacteria, as suggested by Abd-el-Malek and Rizk for the Wadi-el-Natrun (1). Sulfate-reducing bacteria are thought to be responsible for the disappearance of sulfate in Lake Magadi. Direct evidence of sulfate reduction is found in the black muds interbedded with trona ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$), which indicate that H_2S was formed and sulfate was depleted in the groundwater and that subsequent binding of sulfide by iron occurred in trachitic lava rocks in Lake Magadi (2). However, as pointed out by Tindall (17), this theory has not been supported by the successful isolation of alkaliphilic sulfate reducers which can grow at pH values greater than 9 from soda lakes, in which the pH is often greater than 10. Nevertheless, it has been shown for a group of Central Asian soda lakes with pH values greater than 9 that in these alkaliphilic environments, in which the salinity is as high as 15%, sulfidogenesis constitutes a dominant hydrogen sink, competing with acetogenesis but not with methanogenesis, which occurs via the methylotrophic pathway with the utilization of methanol and/or methylamines (26). Thus, the search for and isolation of alkaliphilic, sulfate-reducing bacteria is important for our understanding of (i) the bacterial diversity which employs bioenergetics that are currently unknown or considered impossible, (ii) the role of these bacteria in community metabolism as the main electron sink for hydrogen-producing primary anaerobes, and (iii) the geochemical processes that are influenced by producers of extreme alkalinity in such environments. The processes for which causative agent(s) have yet to be isolated remain unelucidated from a microbiological point of view.

Isolation of alkaliphilic, hydrogen-consuming, sulfate-reducing bacteria from the alkaliphilic anaerobic community in Lake Magadi was first reported in 1994 (27). Here, we describe the isolation from Lake Magadi of an alkaliphilic, sulfate-reducing bacterium which grows in an extremely alkaline environment. The phylogenetic affiliation of this organism with representatives of the sulfate-reducing bacteria is described.

MATERIALS AND METHODS

Sampling. A sample was collected from sediments of an alkaline lake, Lake Magadi (East African Rift, Kenya). The sample was taken during the dry season of 1991 from a trench dug into the trona; it was assumed that the liquor at the sample site came from a depth of 8 to 10 ft under the surface of the trona and was enriched with NaCl. Subsamples of the mud were taken anaerobically, and these subsamples were transported to Moscow at the ambient temperature; in the laboratory they were stored at 4°C.

Media and growth conditions. Selective alkaline medium II (27) containing (per liter) 15 g of $NaHCO_3$, 10 g of Na_2CO_3, 10 g of NaCl, 1 g of NH_4Cl, 0.2 g of KCl, 0.2 g of K_2HPO_4, 3 g of Na_2SO_4, 0.5 g of $Na_2S \cdot 9H_2O$, 10 ml of a vitamin solution (24), 1 ml of a trace element solution (21), 0.5 g of yeast extract, and H_2 or formate as the substrate was used for enrichment and isolation. A pure culture was maintained in an optimized medium formulated after the study of the physiology of the new isolate was completed and was cultivated under strictly anaerobic conditions in rubber membrane-sealed glass vessels with H_2 in the gas phase. The optimized medium contained (per liter) 3.5 g of Na_2CO_3, 24 g of $NaHCO_3$ (added after cooling), 20 g of NaCl, 5 g of Na_2SO_4, 0.2 g of K_2HPO_4, 0.1 g of $MgCl_2 \cdot 7H_2O$, 0.5 g of NH_4Cl, 0.2 g of KCl, 0.12 g of sodium acetate or 0.5 g of yeast extract, 10 ml of a vitamin solution (24), 1 ml of a trace element solution (21), and 0.5 g of $Na_2S \cdot 9H_2O$ or thioglycolate (final pH 9.5). Cultures were incubated at 37°C. The substrates (electron donors) used were H_2 in the gas phase and sodium formate (5 g/liter, with N_2 in gas phase). Electron acceptors were added at the following concentrations: $Na_2SO_4{}^{2-}$, 30 mM; $Na_2SO_3{}^{2-}$, 5 mM; $Na_2S_2O_3{}^{2-}$, 10 mM; $NaNO_3{}^-$, 10 mM; sodium fumarate, 10 mM; S^0, 2 g/liter; and dimethyl sulfoxide, 2 ml/liter. Twenty-milliliter portions of the medium were distributed into 100-ml rubber-stoppered screw-cap flasks by using a standard strictly anaerobic technique. In the case of roll tube cultivation, 2% (wt/vol) agar (Difco Laboratories, Detroit, Mich.) was added to 4-ml portions of carbonate-free medium. The carbonate solution was injected into Hungate tubes after sterilization. NaCl requirements were studied with optimized medium in which the Na_2CO_3 and $NaHCO_3$ were replaced by 5 g of K_2CO_3 per liter and the Na_2S was replaced by K_2S. Growth, detected as visible turbidity, was measured by determining the optical density at 600 nm in Hungate tubes with a spectrophotometer (Spekol, Jena, Germany) equipped with a type ER tube adapter. Microscopic counts were also used to determine growth. The pH limits for growth and sulfidogenesis were determined in the carbonate medium containing 10 g of Na_2CO_3 per liter and 15 g of $NaHCO_3$ per liter; the pH was adjusted to the appropriate value by adding 6 N NaOH or 6 N HCl to an anaerobic flask with a built-in pH electrode under a stream of N_2. After the dispensed medium had been autoclaved and the pH had been verified, the flasks were inoculated with a 1% (vol/vol) inoculum and incubated for 9 days at 37°C. The growth response was measured by determining sulfide formation and turbidity. Sulfidogenesis at different pH values was measured by monitoring H_2S formation from radiolabelled sulfate at 37°C during incubation for 24 h (5, 20). All of the chemicals used in this study were obtained from Russian suppliers.

Analytical procedures. The presence of desulfoviridin was checked fluorometrically by using a spectrofluorimeter (Hitachi, Tokyo, Japan) (10). H_2S was quantified by the methylene blue reaction and colorimetric detection (18). Sulfate consumption (or formation of sulfate from $Na_2S_2O_3$) was measured by a

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nephelometric reaction with $BaCl_2$ (4). Hydrogen consumption was monitored by measuring pressure and/or by gas chromatography with a type 5A molecular sieve column. Lipids were extracted from cell biomass that was dried in a stream of helium and then under a vacuum. To 30 mg of dry biomass, 200 μl of a 5.4 N solution of anhydrous HCl in methanol was added, and the mixture was heated at 70°C for 2 h. The methyl esters of fatty acids and aldehyde derivatives obtained were extracted twice with 100 μl of hexane. The extract was dried and silylated in 20 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide for 15 min at 65°C. A 1-μl portion of the reaction mixture was analyzed with a model HP-5985B gas chromatography-mass spectrometry system (Hewlett-Packard, Palo Alto, Calif.) equipped with a capillary column (25 by 0.25 mm) consisting of fused quartz containing an Ultra-1 nonpolar methylsilicone phase. The temperature profile included a 2-min isotherm at 150°C and subsequent programmed temperature increases (the temperature was increased at a rate of 5°C/min to 250°C and then at a rate of 10°C/min to 300°C). Data processing was carried out with an HP-1000 computer by using the standard programs of the gas chromatography-mass spectrometry system (Hewlett-Packard).

Microscopy. The morphology of cultures was observed with an anoptral Zetopan microscope (Reicherdt, Vienna, Austria). Negative staining of whole cells by phosphotungstic acid and fixation for preparation of thin sections were carried out as described previously (28). Microscopy was performed with a model JEM-100C electron microscope (JEOL, Tokyo, Japan).

G+C content. The guanine-plus-cytosine (G+C) content of the genomic DNA was determined by a thermal denaturation method (9). *Escherichia coli* K-12 DNA was used as the standard.

16S rDNA sequence determination and data analysis. Genomic DNA was extracted and the gene coding for 16S rRNA (16S rDNA) was amplified as described previously (12). Purified PCR products were directly sequenced by using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.). Sequence reaction mixtures were electrophoresed with an Applied Biosystems model 373A DNA sequencer. The 16S rDNA sequence of strain Z-7935^T (T = type strain) was manually aligned with sequences of representatives of the delta subclass of the *Proteobacteria*. Pairwise evolutionary distances were computed by using the correction of Jukes and Cantor (6). The neighbor-joining method was used to reconstruct a phylogenetic tree from the distance matrices (14).

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in this study are available from EMBL under accession numbers X99234 to X99237.

RESULTS

Enrichment and isolation. The sediment sample used in this study had an oily black appearance, and a white mineral film formed on the surface. The purple bacteria usually found in the lagoons of Lake Magadi were absent at the sampling site. At the moment of sampling, the pH of the water was 10.2 and the temperature was 50°C. Samples of the sediment from Lake Magadi were incubated in 100-ml screw-cap bottles containing selective alkaline medium II (pH 9.7) with H_2 as the substrate; after several weeks of incubation, dominant vibrioid bacteria were observed. A pure culture was isolated by the serial dilution method in liquid selective alkaline medium II containing 0.5 g of yeast extract per liter and H_2 as the substrates. Colonies of the sulfate reducer were obtained in roll tubes with sodium formate as the substrate. The colonies were yellowish, translucent, lens shaped, and less than 0.2 mm in diameter. The purity of the culture was indicated by the absence of growth on glucose-peptone sulfate-free medium containing excessive quantities of yeast extract. The absence of growth in media containing various substrates utilized by sulfate-reducing bacteria provided additional proof of purity. Several single colonies were isolated. One of these colonies, designated strain Z-7935^T, was chosen for further characterization.

Morphology. Strain Z-7935^T is a highly motile vibrio with a polar flagellum and filamentous appendages (Fig. 1). Motility was observed in young cultures but drastically diminished during 1 year of subcultivation. At pH 10 short spirilla were observed. The cells, which occurred singly or in pairs, were 0.5 μm in diameter and 1.5 to 2 μm long. Multiplication was by binary fission with the formation of two sister cells (Fig. 1b). The cell wall had a typical gram-negative structure in ultrathin sections, and the periplasmic space was well-developed (Fig. 2). Spores were never observed.
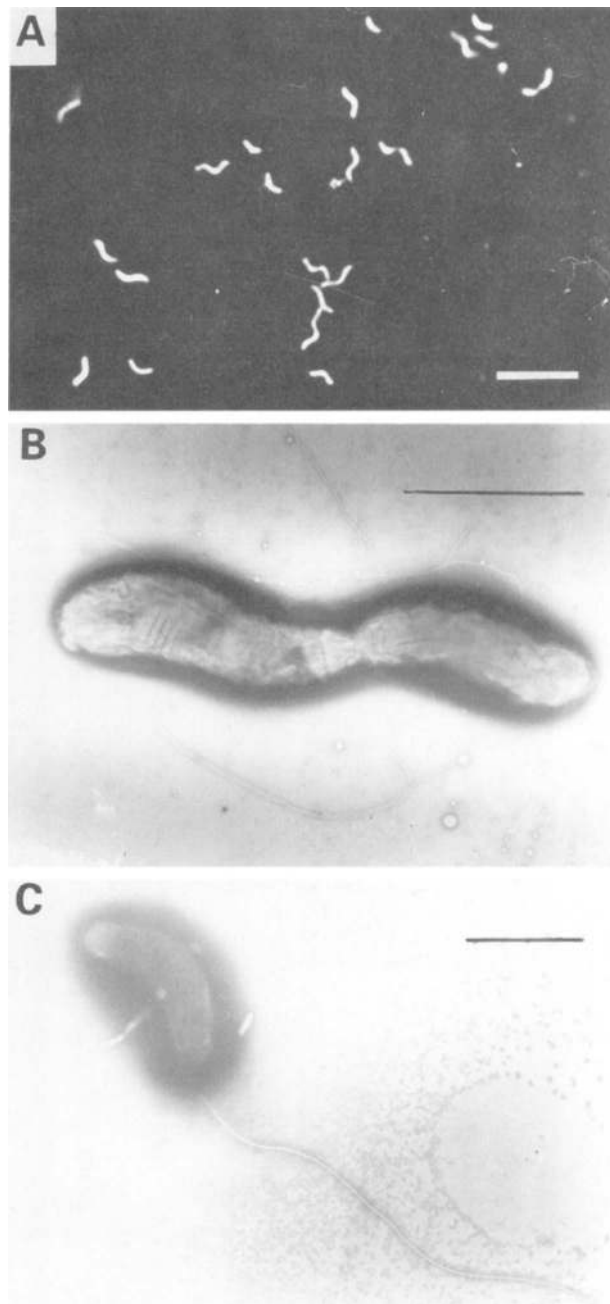

FIG. 1. Morphology of strain Z-7935^T. (A) Cells as viewed with the anoptral microscope. Bar = 10 μm. (B) Negatively stained dividing cells. Note the surface and appendages. Bar = 1 μm. (C) Negatively stained cell with monopolar flagellum. Bar = 1 μm.

Metabolic properties. Strain Z-7935^T is very restricted with respect to the range of electron donors utilized. A total of 28 substrates were tested as electron donors, and only H_2 and formate were utilized. No growth occurred on the following compounds (each at a concentration of 5 g/liter): acetate, propionate, butyrate, pyruvate, lactate, malate, fumarate, succinate, methanol, ethanol, glycerol, glycine, cysteine, cystine, serine, alanine, glutamate, aspartate, Casamino Acids, yeast extract, choline, betaine, glucose, fructose, rhamnose, and mannose. Sulfate, sulfite, and thiosulfate were utilized as elec-
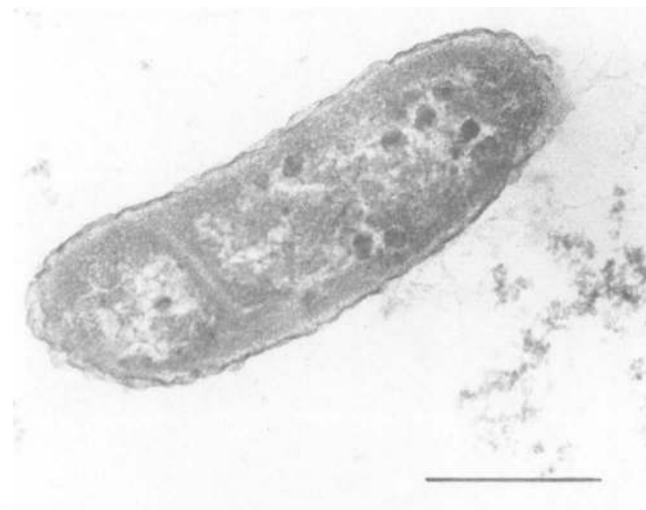
FIG. 2. Ultrastructure of strain Z-7935^T. Bar = 0.5 μm.

TABLE 1. Fatty acids in the cells of strain Z-7935^T and *Desulfohalobium retbaense* HR$_{100}$

Fatty acid	% of total fatty acids in:	
	Strain Z-7935^T	*Desulfohalobium retbaense* HR$_{100}$[a]
$C_{13:0}$	0	0.11
iso-$C_{14:0}$	1.9	1.68 ± 0.1
$C_{14:0}$	5.6	0.95
iso-$C_{15:0}$	3.7	15.28 ± 2.3
anteiso-$C_{15:0}$	3.9	3.6 ± 0.3
$C_{15:1}$ ω-6	0	0.23
$C_{15:0}$	2.6	3.93 ± 0.4
iso-$C_{16:0}$	4.4	5.34 ± 0.5
$C_{16:1}$ ω-9	0	0.58
$C_{16:1}$ ω-7	2.7	4.10 ± 0.7
$C_{16:0}$	32.8	13.33 ± 2.8
iso-$C_{17:1}$ ω-7c	0	3.88 ± 0.1
iso-$C_{17:0}$	1.1	1.9 ± 0.4
anteiso-$C_{17:0}$	2.1	2.24 ± 1.1
$C_{17:1}$ ω-8	0	8.05 ± 1.5
$C_{17:1}$ ω-6	0	0.71 ± 0.6
$C_{17:0}$	5.9	3.33 ± 0.8
Branched $C_{18:1}$ ω-6	0	0.36
$C_{18:1}$ ω-9	3.4	0
$C_{18:1}$ ω-7c	0	6.53 ± 2.2
$C_{18:1}$ ω-5c	0	0.23
$C_{18:1}$ ω-11	5.9	3.86 ± 1.4
$C_{18:0}$	18.2	15.97 ± 5.2
cyclo $C_{19:0}$	0	0.43
$C_{19:1}$	1.0	0
$C_{20:0}$	1.6	0

[a] Data from reference 8.

tron acceptors. Formation of sulfide was observed in the presence of dimethyl sulfoxide; however, there was no growth in subcultures. Elemental sulfur inhibited growth. Fumarate and nitrate did not support growth. Strain Z-7935^T was strictly anaerobic, and its growth was inhibited by air. Thiosulfate was decomposed; equimolar quantities of sulfate and H_2S were formed from 10 mmol of thiosulfate, indicating that the organism had the ability to dismutate.

Growth conditions. Like most hydrogen-consuming, sulfate-reducing bacteria, strain Z-7935^T needs organic compounds for anabolic reactions, and so its type of nutrition can be described as lithoheterotrophic. Growth dependence on yeast extract was observed (Fig. 3). However, yeast extract could be partially replaced by vitamins and acetate as a carbon source. A sodium acetate concentration of 0.16 g/liter was sufficient for optimal growth. The limits for growth and sulfidogenesis were determined with various pH values and salt contents. In growth experiments sulfidogenesis occurred without a lag phase at pH values between 9.5 and 9.7; this range of pH values was optimal, based on the rate of sulfide formation, and growth occurred at pH 8.0 to 10.2. In short-time experiments with radioactive sulfate, sulfidogenesis increased linearly to a maximal value at pH 9.5 and rapidly decreased to one-tenth the maximal value at pH 10. The pH of the medium did not change
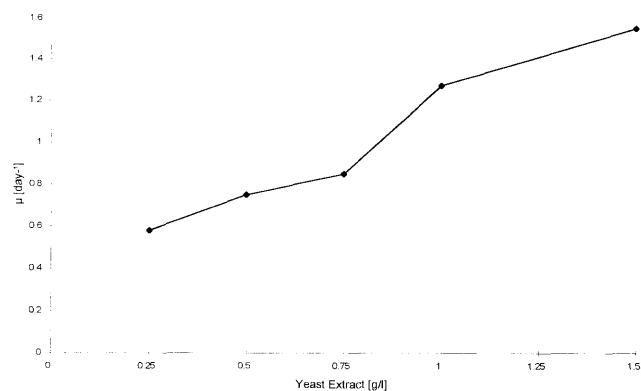

FIG. 3. Effect of yeast extract concentration on the specific growth rate (μ) of strain Z-7935^T.

during growth. At pH values higher than 10 there was a decrease in the rate of sulfidogenesis, while at pH 11.5 to 12 formation of H_2S did not occur. Strain Z-7935^T is an obligate alkaliphile which does not grow at pH 7; carbonate ions are required for growth. When Na_2CO_3-$NaHCO_3$ was replaced by NaCl and the pH values were maintained with 50 mM serine buffer (pK_a, 9.4), no growth occurred. Dependence on sodium was studied in media containing 5 g of K_2CO_3 per liter and increasing concentrations of NaCl. There was no growth or sulfidogenesis without sodium ions. Maximal sulfidogenesis was observed in the presence of 3% (wt/vol) NaCl, and at higher NaCl concentrations sulfidogenesis decreased. Strain Z-7935^T is a weak halophile that is obligately dependent on sodium ions. Dependence on chloride ions was not detected. Strain Z-7935^T is a mesophile. The optimal temperature for growth was 37°C, while at 40°C growth stopped after a fast start. At 45 and 15°C there was no growth, and at 22 or 26°C growth began after a lag phase. Desulfoviridin, a pigment common to *Desulfovibrio* species, was not found in strain Z-7935^T.

Lipid analysis. The fatty acid distribution in the cells of strain Z-7935^T is shown in Table 1. Saturated fatty acids accounted for 66.7% of the total fatty acids, with $C_{16:0}$ and $C_{18:0}$ acids predominating. Branched saturated fatty acids accounted for only 11% of the total fatty acids. The following monounsaturated fatty acids, which accounted for 12% of the total fatty acids, were also present: $C_{16:1}$, $C_{18:1}$ ω-9, $C_{18:1}$ ω-11, and $C_{19:1}$ fatty acids. The absence in strain Z-7935^T of isoheptadecenoic acid (iso $C_{17:1}$) and the presence of cis-vaccenic acid ($C_{18:1}$ ω-11) are notable.

DNA base composition. The G+C content of the DNA of strain Z-7935^T is 48.6 mol%.
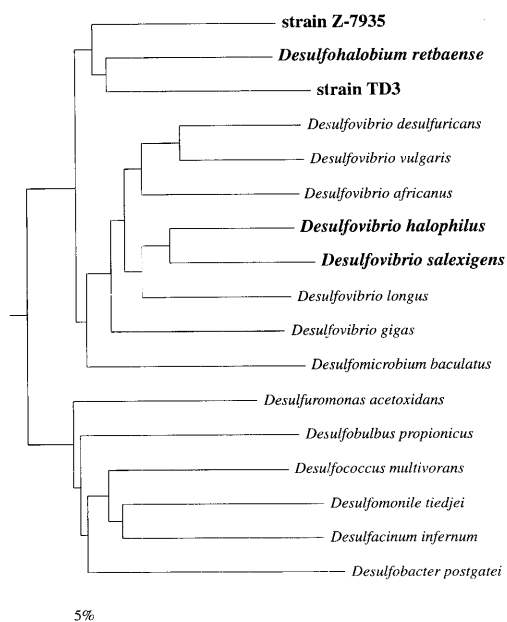
FIG. 4. Phylogenetic dendrogram showing the position of alkaliphilic, sulfate-reducing bacterial strain Z-7935^T within the radiation of sulfate-reducing bacteria belonging to the delta subclass of the *Proteobacteria*. The position of the root was determined by including *Escherichia coli* as an outgroup organism. Scale bar = 5 inferred substitutions per 100 nucleotides.

Phylogenetic analysis. An almost complete 16S rDNA sequence comprising 1,503 nucleotides was determined for strain Z-7935^T. A comparative analysis of this 16S rDNA sequence with the sequences available for representatives of the major bacterial groups indicated that strain Z-7935^T is a member of the delta subclass of the *Proteobacteria*. In order to complete the data set for the halophilic members of the delta subclass of the *Proteobacteria*, the 16S rDNA sequences of *Desulfohalo-*

bium retbaense DSM 5692^T and *Desulfovibrio halophilus* DSM 5663^T were determined and included in the phylogenetic analysis. The phylogenetic dendrogram in Fig. 4 shows the position of strain Z-7935^T within the radiation of the members of the delta subclass of the *Proteobacteria* for which 16S rDNA sequence data are available. Strain Z-7935^T represents a distinct lineage, clustering with *Desulfohalobium retbaense* and strain TD3 (13). The highest levels of 16S rDNA sequence similarity were the levels of similarity between strain Z-7935^T and *Desulfohalobium retbaense* (88.7%) and between strains Z-7935^T and TD3 (86.6%) (Table 2).

DISCUSSION

The role of hydrogen-consuming, sulfate-reducing bacteria in the alkaliphilic community investigated in this study is interesting from an ecological and physiological viewpoint. The type of nutrition displayed by strain Z-7935^T is typical of lithoheterotrophic, sulfate-reducing bacteria that utilize hydrogen or formate for the reduction of oxidized sulfur compounds in the catabolic reaction and acetate in the anabolic pathway (16). Strain Z-7935^T differs from other sulfate-reducing bacteria by its unusually narrow range of electron donors utilized. Strain Z-7935^T is a lithoheterotrophic, sodium-dependent alkaliphile which may be responsible for the consumption of hydrogen in the alkaliphilic community from which it was isolated. This organism is clearly a member of an alkaliphilic anaerobic community in which primary anaerobes (e.g., alkaliphilic spirochetes [27, 28]) produce hydrogen and acetate.

The geographical distribution of alkaliphilic sulfate reducers in continental soda lakes was revealed by the study of anaerobic communities in the lakes of Central Asia. Physiologically similar pure cultures were isolated from four alkaline lakes in Tuva, and these cultures were found to belong to the same species and to have DNA-DNA homology values of more than 90% (7). It was demonstrated that in Asian lakes, which represent a range of pH and salinity combinations and harbor different microbial communities, as in "natural elective cul-

TABLE 2. 16S rDNA similarity values for strain Z-7935^T and related taxa

Organism	Strain Z-7935^T	*Desulfohalobium retbaense*	Strain TD3	*Desulfomicrobium baculatus*	*Desulfovibrio vulgaris*	*Desulfovibrio desulfuricans*	*Desulfovibrio halophilus*	*Desulfovibrio salexigens*	*Desulfovibrio africanus*	*Desulfovibrio gigas*	*Desulfovibrio longus*	*Desulfomonile tiedjei*	*Desulfacinum infernum*	*Desulfococcus multivorans*	*Desulfobulbus propionicus*	*Desulfobacter postgatei*	*Desulfuromonas acetoxidans*
Desulfohalobium retbaense	88.7																
Strain TD3	86.6	88.0															
Desulfomicrobium baculatus	86.5	85.7	85.1														
Desulfovibrio vulgaris	86.0	85.9	85.8	85.9													
Desulfovibrio desulfuricans	86.3	85.9	85.0	87.4	91.9												
Desulfovibrio halophilus	86.5	86.7	85.8	86.5	88.3	87.8											
Desulfovibrio salexigens	87.1	87.1	85.9	85.5	88.4	87.4	91.0										
Desulfovibrio africanus	86.5	85.6	84.7	87.1	90.3	89.0	88.6	87.8									
Desulfovibrio gigas	87.1	88.1	86.5	85.5	87.8	89.0	87.6	87.0	89.1								
Desulfovibrio longus	86.3	86.6	84.9	86.1	89.1	89.2	90.2	89.2	89.5	88.7							
Desulfomonile tiedjei	82.9	83.3	82.5	81.7	81.4	82.4	83.7	81.5	82.7	82.6	84.1						
Desulfacimum infernum	83.2	84.4	84.1	82.5	82.4	82.7	82.3	82.0	83.5	83.5	82.9	87.2					
Desulfococcus multivorans	83.9	84.3	83.2	84.4	83.5	84.1	85.1	83.0	83.4	83.4	84.0	87.9	86.5				
Desulfobulbus propionicus	84.0	84.0	84.2	83.0	83.9	83.5	82.9	82.1	82.5	83.4	83.5	85.8	86.4	85.2			
Desulfobacter postgatei	83.0	82.8	80.7	83.4	81.5	83.0	81.7	82.0	82.3	83.1	82.7	84.0	83.5	87.0	85.3		
Desulfuromonas acetoxidans	85.3	85.3	83.4	83.6	84.1	84.5	84.6	84.4	84.4	85.3	84.5	87.6	86.4	86.1	87.1	86.4	
Escherichia coli	80.1	80.3	80.0	79.5	79.8	79.8	80.6	79.5	80.5	79.4	79.2	80.0	78.1	81.2	80.8	78.2	80.9

TABLE 3. Comparison of the main characteristics of *Desulfonatronovibrio hydrogenovorans* Z-7935[T] and some other sulfate-reducing bacteria

Characteristic	*Desulfonatronovibrio hydrogenovorans* Z-7935[T]	*Desulfovibrio halophilus* SL 8903[a]	*Desulfovibrio salexigens*[b]	*Desulfohalobium retbaense* HR100[c]	Strain TD3[d]
Cell shape	Vibrio	Vibrio	Vibrio	Curved rod	Curved rod
Motility (monopolar)	+	+	+	+[e]	NR[f]
Cell size (μm)	0.5 × 1.5–2.0	0.6 × 2.5–5.0	0.5–1.0 × 3.5	0.7–0.9 × 1.0–3.0	0.8 × 2.0
pH range (optimal pH)	8.0–10.2 (9.5–9.7)	5.5–8.5 (6.5)	NR (7.0)	5.5–8.0 (6.5–7.0)	NR (6.8)
Salinity range (optimal salinity) (%)	1.0–12.0 (3.0)	3.0–18.0 (6.0–7.0)	0.5–12.0 (2.0–4.0)	1.0–24.0 (10.0)	NR (3.5)
Optimal temp (°C)	37	35	34–37	37–40	55–65
Yeast extract requirement	+	–	–	+	NR
G+C content of DNA (mol%)	48.6	60.7	46.0–49.0	57.1	NR
Utilization of electron donors (with SO_4^{2-})					
H_2 + CO_2	–	–	–	–	–
H_2 + acetate	+	+	+	+	–
Formate + acetate	+	+	+	+	–
Lactate	–	+	+	+	–
Pyruvate	–	+	+	+	NR
Malate	–	–	+	–	NR
Fumarate	–	–	–	–	NR
Acetate	–	–	–	–	NR
Propionate	–	–	–	–	NR
Ethanol	–	+	+	+	–
Glycerol	–	–	–	–	NR
Serine	–	+	NR	NR	NR
Fatty acids (C_{14}–C_{18})	ND[g]	NR	NR	NR	+
n-Alkanes (C_6–C_{16})	ND	NR	NR	NR	+
Fermentation of pyruvate (no SO_4^{2-})	–	–	–	+/–[h]	NR
Utilization of electron acceptors					
Sulfate	+	+	+	+	+
Sulfite	+	+	NR	+	NR
Thiosulfate	+	+	NR	+	NR
Sulfur	–	+	NR	+	NR

[a] Data from reference 3.
[b] Data from references 11 and 23.
[c] Data from reference 8.
[d] Data from reference 13.
[e] One or two flagella are present.
[f] NR, not reported.
[g] ND, not determined.
[h] +/–, substrates are used as donors, but growth is slow.

tures," hydrogen-consuming sulfate-reducing bacteria are dominant at levels of salinity up to 15% (26). Organisms such as strain Z-7935[T] represent the putative alkaliphilic sulfate reducers that may be responsible for the formation of alkaline conditions up to pH 10. The complete breakdown of organic matter by anaerobes requires the removal of hydrogen; this is achieved by conversion of the hydrogen to H_2S. The H_2S in turn is oxidized anaerobically by purple bacteria (e.g., in the alkaliphilic community in Lake Magadi by *Ectothiorhodospira* species) or aerobically by alkaliphilic thionic bacteria, as in Asian Lake Khadyn (15). In accordance with the assumption that athalassic soda lakes may harbor novel terrestrial communities (25), the new alkaliphilic isolate represents a distinct phylogenetic lineage whose closest relative is the moderately halophilic organism *Desulfohalobium retbaense*. A comparison of the characteristics of strain Z-7935[T] with those of halophilic, sulfate-reducing bacteria is shown in Table 3. Strain Z-7935[T] clearly represents a new taxon of sulfate-reducing bacteria, since it has a unique combination of characteristics and grows in a habitat from which pure cultures of sulfate-reducing bac-

teria have not been isolated. Morphologically, strain Z-7935[T] resembles typical desulfovibrios. This typical morphology is found in species of the genera *Desulfovibrio*, *Desulfobacter*, and *Desulfobacterium* (23). Desulfoviridin is not present in strain Z-7935[T], and the G+C content of this organism is 48.6 mol%, which is similar to the values reported for *Desulfovibrio salexigens* and *Desulfovibrio simplex*, which are phenotypically and phylogenetically distinct from strain Z-7935[T]. The halophilic species *Desulfobacter hydrogenophilus* can utilize H_2 in addition to acetate (22). The halophilic organism *Desulfohalobium retbaense* (8) could be considered a thalassic counterpart of alkaliphilic strain Z-7935[T]. The fatty acid profile of strain Z-7935[T] contains compounds that are typical of the membranes of members of the bacterial domain and are associated with sulfate-reducing bacterial species (8, 19). The lipid profile of sulfate-reducing bacteria is quite characteristic, and the profiles of strain Z-7935[T] and 72 sulfate-reducing bacteria, including previously studied *Desulfovibrio* species (19), were compared. The lipid profile of strain Z-7935[T] lacks iso-$C_{17:1}$ fatty acids, which are biomarkers of species of the genus *Desulfo-*

vibrio. On the basis of its fatty acid profile, strain Z-7935[T] cannot be affiliated with any species except *Desulfohalobium retbaense* and *Desulfomicrobium baculatus* [sic] (correlation coefficient, 0.23). On the basis of the phylogenetic analysis it can be concluded that strain Z-7935[T] is a member of a recently discovered new lineage within the delta branch of the *Proteobacteria* that comprises hydrocarbon-utilizing strain TD3 (13) and *Desulfohalobium retbaense* (Fig. 4 and Table 2). With its distinct branch point and 16S rDNA sequence difference of >11% compared with its nearest relative, as well as its distinct phenotypic characteristics (Table 3), strain Z-7935[T] clearly represents a new genus. We propose that the alkaliphilic, sulfate-reducing strain Z-7935[T] should be placed in a new genus and species, *Desulfonatronovibrio hydrogenovorans*; the name of this organism describes its ecophysiology.

Description of *Desulfonatronovibrio* gen. nov. *Desulfonatronovibrio* (De.sul.fo.nat.ro.no.vi' b.rio. M. L. pref. *de*, negative; M.L.n. *sulfo*, sulfate; M.L.n. *natron*, soda; M.L.n. *vibrio*, curved rod; M.L.n. *Desulfonatronovibrio*, sulfate-reducing curved rod from a soda environment). Alkaliphilic sulfate-reducing eubacterium. Cells are motile, asporogenous, gram-negative vibrios with polar flagella. Lithoheterotrophic, utilizing hydrogen for the reduction of sulfur compounds. Strictly anaerobic. Obligately dependent on sodium ions. Vitamins and acetate as a carbon source are necessary for growth on hydrogen or formate and can be replaced by yeast extract. Phylogenetically a member of the delta subclass of the *Proteobacteria*. The type species is *Desulfonatronovibrio hydrogenovorans*.

Description of *Desulfonatronovibrio hydrogenovorans* sp. nov. *Desulfonatronovibrio hydrogenovorans* (hy.dro.ge.no.vo'rans. M.L.n. *hydrogen*, hydrogen; M.L. part. *vorans*, utilizing; M.L. part. *hydrogenovorans*, hydrogen utilizing). Motile vibrio with one polar flagellum. Cells are 0.5 by 1.5 to 2 μm, occur singly or in pairs, and develop short spirilla under suboptimal conditions. Multiplication is by binary fission. Gram-negative cell wall structure. Strictly anaerobic and lithoheterotrophic. Utilizes only hydrogen and formate as electron donors and sulfate, sulfite, and thiosulfate as electron acceptors. Sulfur is not reduced. Sulfide is the only product of catabolism. Yeast extract and acetate are utilized for anabolism. Obligate alkaliphile which does not grow at pH 7; the maximum pH for growth is about 10.2, and the optimal pH for growth and sulfidogenesis in sodium carbonate medium is 9.5 to 9.7. Sodium ions are required for growth; no growth occurs in the presence of NaCl concentrations less than 1% (wt/vol) or more than 12% (wt/vol). Growth occurs if NaCl is replaced by equimolar amounts of Na_2CO_3 and $NaHCO_3$. Requires carbonate anion. The optimum temperature for growth is 37°C, and the temperature range for growth is 15 to 43°C. Slow growth occurs at 22 to 26°C after a long lag phase. The G+C content of the DNA is 48.6 mol% (as determined by the thermal denaturation method). Habitat: bottom deposits of alkaline athalassic soda lakes. The type strain is strain Z-7935, which was isolated from the sediments of an equatorial soda lake, Lake Magadi. This strain has been deposited in the Deutsche Sammlung von Mikroorganismen as strain DSM 9292[T].

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