

Central Washington University
ScholarWorks@CWU

All Faculty Scholarship for the College of the Sciences

College of the Sciences

1-2007

Sulfur-Oxidizing Bacteria in Soap Lake (Washington State), a Meromictic, Haloalkaline Lake with an Unprecedented High Sulfide Content


Dimitry Y. Sorokin
Russian Academy of Sciences

Mirjam Foti
Delft University of Technology

Holly C. Pinkart
Central Washington University

Gerard Muyzer
Delft University of Technology

Follow this and additional works at: <https://digitalcommons.cwu.edu/cotsfac>

 Part of the [Bacteriology Commons](#), [Environmental Microbiology and Microbial Ecology Commons](#), [Environmental Sciences Commons](#), and the [Fresh Water Studies Commons](#)

Recommended Citation

Sorokin, D. Y., Foti, M., Pinkart, H. C., & Muyzer, G. (2006). Sulfur-Oxidizing Bacteria in Soap Lake (Washington State), a Meromictic, Haloalkaline Lake with an Unprecedented High Sulfide Content. *Applied and Environmental Microbiology*, 73(2), 451–455. <https://doi.org/10.1128/aem.02087-06>

This Article is brought to you for free and open access by the College of the Sciences at ScholarWorks@CWU. It has been accepted for inclusion in All Faculty Scholarship for the College of the Sciences by an authorized administrator of ScholarWorks@CWU. For more information, please contact scholarworks@cwu.edu.

Sulfur-Oxidizing Bacteria in Soap Lake (Washington State), a Meromictic, Haloalkaline Lake with an Unprecedented High Sulfide Content[∇]

Dimitry Y. Sorokin,^{1,2*} Mirjam Foti,¹ Holly C. Pinkart,³ and Gerard Muyzer¹

Department of Biotechnology, Delft University of Technology, 2628 BC Delft, The Netherlands¹; Winogradsky Institute of Microbiology, Russian Academy of Sciences, 117811 Moscow, Russia²; and Department of Biological Sciences, Central Washington University, Ellensburg, Washington 98926³

Received 4 September 2006/Accepted 7 November 2006

Culture-dependent and -independent techniques were used to study the diversity of chemolithoautotrophic sulfur-oxidizing bacteria in Soap Lake (Washington State), a meromictic, haloalkaline lake containing an unprecedentedly high sulfide concentration in the anoxic monimolimnion. Both approaches revealed the dominance of bacteria belonging to the genus *Thioalkalimicrobium*, which are common inhabitants of soda lakes. A dense population of *Thioalkalimicrobium* (up to 10⁷ cells/ml) was found at the chemocline, which is characterized by a steep oxygen-sulfide gradient. Twelve *Thioalkalimicrobium* strains exhibiting three different phenotypes were isolated in pure culture from various locations in Soap Lake. The isolates fell into two groups according to 16S rRNA gene sequence analysis. One of the groups was closely related to *T. cyclicum*, which was isolated from Mono Lake (California), a transiently meromictic, haloalkaline lake. The second group, consisting of four isolates, was phylogenetically and phenotypically distinct from known *Thioalkalimicrobium* species and unique to Soap Lake. It represented a new species, for which we suggest the name *Thioalkalimicrobium microaerophilum* sp. nov.

Soap Lake, located in central Washington State, is the terminal lake at the end of a chain of lakes characterized by increasing salinity and alkalinity. It contains high concentrations of sodium carbonate and sulfate, resulting in a high alkalinity and pH (around 10), typical for soda lakes. However, two features distinguish this lake from many other soda lakes: (i) its sharp stratification into two layers with different features and (ii) an unprecedentedly high sulfide concentration in the anaerobic layer. The bottom layer of the lake, termed the monimolimnion, is hypersaline (140 g liter⁻¹), cold (6 to 8°C), and highly sulfidic, with anaerobic waters containing up to 200 mM sulfide, the highest concentration ever recorded in natural water. In contrast, the top layer, termed the mixolimnion, is brackish (around 15 g liter⁻¹) and aerobic. It is separated from the monimolimnion by a chemocline with oxygen concentrations changing from saturation to zero (1, 7, 13). Despite the large difference in density between the two layers, sulfide diffuses into the aerobic layer and, therefore, the role of sulfide-oxidizing bacteria (SOB) must be particularly important in this unusual lake.

Previous work has demonstrated the presence of obligately chemolithoautotrophic SOB in soda lakes, capable of growth at extremely high pH values and variable salinity (11). The genus *Thioalkalimicrobium* was found mostly in hyposaline lakes of south Siberia and northeastern Mongolia and dominated the enrichments at low-salt conditions (i.e., below 1 M of total sodium). In contrast, the genus *Thioalkalivibrio* was dom-

inant in hypersaline soda lakes and was the only representative of chemolithoautotrophs known so far capable of growing in saturated soda brines (4 M of total sodium). Use of culture-independent molecular methods allowed the detection of *Thioalkalivibrio* in soda lake sediments, while the presence of the *Thioalkalimicrobium* population was obviously below the detection limit (M. Foti and D. Sorokin, unpublished results).

Here we describe the diversity of SOB in Soap Lake, a haloalkaline, meromictic lake with an unusually high sulfide concentration. We used both culture-dependent and -independent techniques to study the SOB present in this lake and found evidence for members belonging to the genera *Thioalkalimicrobium* and *Thioalkalivibrio*. A group of microaerophilic isolates affiliated with *Thioalkalimicrobium* belongs to a new species, for which we suggest the name *Thioalkalimicrobium microaerophilum*.

MATERIALS AND METHODS

Samples. Soap Lake is permanently stratified into two layers, an upper layer (mixolimnion) and lower layer (monimolimnion), separated by a chemocline at 20.5 m (Fig. 1). Five samples were obtained from Soap Lake (Washington State) in fall 2004, including samples from the mixolimnion, the chemocline, and the monimolimnion; from the deep sediment; and from the mixolimnion sediment at the fringe of the lake (Table 1). Part of the water samples was used for molecular analysis, for sulfide analysis, and for cultivation.

Cultivation of sulfur-oxidizing bacteria. Enrichments and cultivation of haloalkaliphilic SOB were performed using a mineral medium buffered with sodium carbonate-bicarbonate mixture (0.6 and 4 M total Na⁺) at pH 10 to 10.1 as described previously (12). Thiosulfate (20 to 40 mM) served as the energy source and NH₄Cl (4 mM) as the nitrogen source. The medium was supplemented with 1 ml liter⁻¹ of trace metal solution (6) and 1 mM MgCl₂. Microaerophilic cultivation was performed in 100-ml serum bottles with 10 ml medium in which the air was replaced with argon, containing 1 to 5% (vol/vol) O₂. Solid alkaline media with final salt concentrations of 0.6 and 2 M of total Na⁺ were prepared by 1:1 mixing of 4% (wt/vol) agar and double-strength mineral

* Corresponding author. Mailing address: Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-let Octyabrya 7/2, 117811 Moscow, Russia. Phone: (7095)1350109. Fax: (7095)1356530. E-mail: soroc@inmi.host.ru.

[∇] Published ahead of print on 17 November 2006.

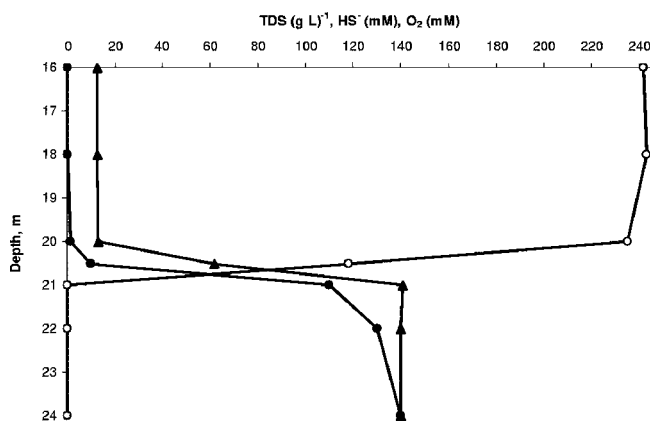


FIG. 1. Depth profiles of dissolved oxygen (open circles), total dissolved solids (TDS; filled triangles), and sulfide (filled circles).

medium at 50°C. The plates were incubated in closed jars in microaerophilic (2% oxygen) conditions. Enrichments for denitrifying SOB were performed in 100-ml serum bottles filled with 50 ml of the alkaline base media and sealed with butyl rubber stoppers. The medium was supplemented either with 20 mM thiosulfate–20 mM nitrate, 20 mM thiosulfate–50 mM N₂O, or 5 mM HS⁻–20 mM nitrate. Anaerobic conditions were achieved by five cycles of evacuation and flushing with argon. The cultivation was performed at 22 (enrichments) and 28°C (pure cultures).

Chemical and other analyses. Chemical analysis of sulfur compounds (i.e., sulfide, thiosulfate, and sulfur) and cell protein and respiration tests were performed as described previously (12). The dissolved oxygen in-lake measurements were performed with an oxygen probe associated with a Hydrolab (Hach Environmental, Loveland, CO). Phase-contrast microphotographs were obtained with a Zeiss Axioplan Imaging 2 microscope (Göttingen, Germany). For electron microscopy, the cells were fixed with glutaraldehyde (final concentration, 3% [vol/vol]) and positively contrasted with 1% (wt/vol) uranyl acetate. The isolation of the DNA from pure cultures was performed according to Marmur (4). Determination of the G+C content of the DNA and DNA-DNA hybridization were performed by the thermal denaturation/reassociation technique (2).

DNA extraction and PCR amplification of 16S rRNA genes. Ten-milliliter water samples, 2-cm³ sediment samples, and 1-ml cultures were used for DNA extraction after centrifugation and washing of the pellet with 0.5 M NaCl. Genomic DNA was extracted from the cells using the UltraClean soil DNA extraction kit (Mo Bio Laboratories, West Carlsbad, CA) by following the manufacturer's instructions.

The partial 16S rRNA gene was amplified using bacterial primers 341F-GC and 907R and a touchdown protocol (8). The nearly complete 16S rRNA gene was obtained from pure cultures using bacterial primers GM3F and GM4R (5).

DGGE of PCR products. Denaturing gradient gel electrophoresis (DGGE) was performed as described by Schäfer and Muyzer (8) with minor modifications. The PCR products were separated on polyacrylamide gel with a linear gradient of 35 to 70% urea and formamide and run at a constant voltage of 100 V for 16 h. Subsequently, the gels were photographed with the Gel Doc 2000 system (Bio-Rad, Hercules, CA) after 30 min of ethidium bromide staining and 30 min of rising in Milli-Q water. Bands of interest were excised, reamplified, and checked for purity in a second DGGE. PCR products were then purified using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and sequenced.

Phylogenetic analysis. The sequences obtained in this study were first compared to sequences stored in GenBank using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/BLAST>). Subsequently, the sequences were imported into the ARB software program (3), aligned, and added to a phylogenetic tree using the QUICK_ADD_TO_EXISTING_TREE tool. The alignment was further corrected by eye, and a tree was calculated using the neighbor-joining algorithm with Felsenstein correction.

Nucleotide sequence accession numbers. All sequences obtained in this work were deposited in GenBank under the accession numbers DQ900619 to DQ900627.

TABLE 1. Samples from Soap Lake

Sample no.	Sample	Depth (m)	Salinity (g liter ⁻¹)	O ₂ (mg liter ⁻¹)	HS ⁻ concn (mM)
SL1	Mixolimnion, water	10	14	9.2	0
SL2	Mixolimnion, sediment	NA ^b	NA	0 ^a	5.04 ^a
SL3	Chemocline, water	20	20	0.1	1.99
SL4	Monimolimnion, water	23	140	0	175
SL5	Monimolimnion, sediment	25	NA	0	143.5 ^a

^a In the pore water.

^b NA, not applicable.

RESULTS AND DISCUSSION

Enrichment and enumeration of haloalkaliphilic SOB. Results of a first screening demonstrated the presence of an active population of aerobic, low-salt-tolerant, alkaliphilic, lithoautotrophic SOB in the different samples from Soap Lake, able to grow at 0.6 M of total Na⁺ and pH 10 (Table 2). The most abundant populations were isolated from the chemocline samples. Smaller populations were detected in the anaerobic, high-sulfide samples from the monimolimnion. This was likely a result of sedimentation of the SOB developing in the oxygen-containing layers of the lake, since no growth of anaerobic (denitrifying) SOB was observed in the enrichments. In this sampling period, there was no evidence of a mass development of anaerobic phototrophic sulfur bacteria, commonly present below the chemoclines of meromictic lakes. This was not unexpected since the chemocline does not experience any light penetration (13). Based on these observations, it seems likely that the aerobic chemotrophic SOB are responsible for most of the biological oxidation of sulfide and other reduced sulfur compounds in the lake.

In contrast to the low-salt medium, enrichments in saturated soda brine at 4 M Na⁺ demonstrated much smaller populations, indicating a minor role of these bacteria at the time of sampling.

Isolation and identification of pure cultures. Enrichments at low salt (0.6 M Na⁺) yielded 12 pure cultures of obligately chemolithoautotrophic SOB with three different morphotypes (Table 2). Seven strains with morphotype A were obtained

TABLE 2. Isolation of halotolerant alkaliphilic SOB from Soap Lake

Sample no.	O ₂ concn (%)	Maximal positive dilution	Morphotype present in dilutions	Pure culture(s)
SL1	20	3	A (very small and short motile rods)	ASL9
SL2	5	7	A C (large motile vibrio with empty patches)	ASL5, ASL7 ASL8-2
SL3	5	7	A C (dominating) B (motile curved rod)	ASL4-1, ASL4-2, ASL4-3 ASL6, ASL8-1 ASL3
SL4	2	5	A	ASL1
SL5	2	5	C	ASL2

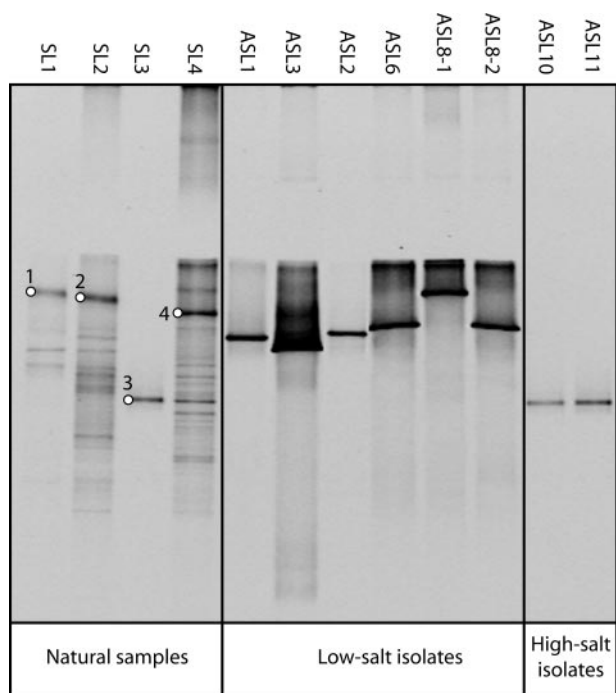


FIG. 2. DGGE analysis of 16S rRNA gene fragments amplified from water samples (SL) and bacterial isolates (ASL) of Soap Lake. SL1, chemocline; SL2, mixolimnion, sediment pore water; SL3, monolimnion, water; SL4, monolimnion, sediment pore water. Sequenced bands that were used for phylogenetic analysis (see Fig. 3) are indicated by white circles and numbers.

from all samples except the deep sediments. They were short motile rods. Longer, motile, and slightly curved rods of morphotype B were represented by a single strain from the chemocline. Our main target for the isolation, however, was morphotype C, since the cells of this type were seen in water samples of the chemocline and, after concentration, in samples of the monolimnion. From four pure cultures of this type, three were long, bent, motile rods at pH 10, turning to fat vibrios at pH 8 to 9. The isolate ASL8-1 was similar in morphology, but the cells were significantly smaller.

DGGE analysis of PCR-amplified 16S rRNA gene fragments from pure cultures and samples of different layers of Soap Lake (Fig. 2) demonstrated that (i) the genetic diversity in Soap Lake was higher in the sediments than in the water column, at least during the sampling period, and (ii) dominant DNA fragments from the DGGE analysis detected in the chemocline, mixolimnion, and monolimnion sediments were related to low-salt SOB isolates, while the PCR product obtained from the monolimnion water layer was related to high-salt SOB isolates.

Phylogenetic analysis of the sequences of the dominant DGGE bands from the lake samples and from the representative SOB isolates with different morphologies isolated at low-salt conditions identified them as members of the genus *Thioalkalimicrobium* (Fig. 3). More specifically, the dominant band sequences from the chemocline and the monolimnion sediments and the morphotype A and B isolates (Table 2) were closely related to *Thioalkalimicrobium cyclicum*, found previously in the chemocline water of the Mono Lake in California

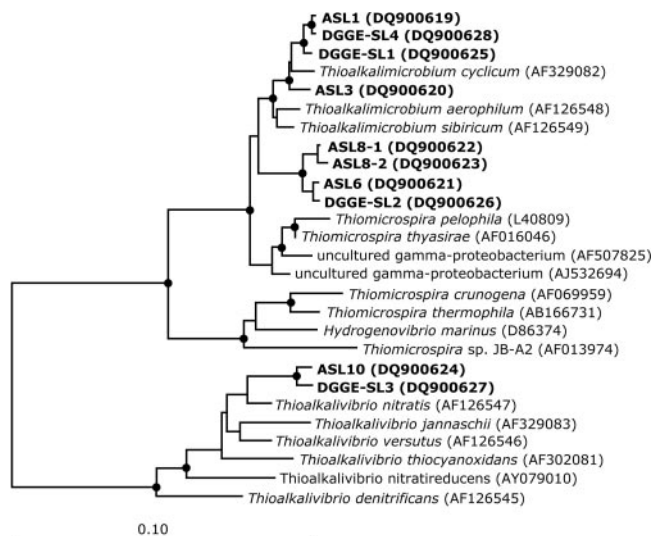


FIG. 3. Neighbor-joining tree based on 16S rRNA gene sequences, showing the phylogenetic affiliation of bacteria from Soap Lake. Names of the sequences determined in this study are in boldface. Sequences with DGGE-SL are obtained from DGGE bands as indicated in Fig. 1; sequences with ASL are from pure cultures. The sequence accession numbers are in parentheses. The nodes on the branches indicate bootstrap values above 90% from 1,000 replications. The bar indicates 10% sequence variation.

(10) and absent in soda lakes of central Asia (Siberia and Mongolia) and Africa (Kenya and Egypt) (11). It appears that this particular species of haloalkaliphilic SOB is endemic to the North American continent. The morphotype C isolates, together with the dominant sequence from the mixolimnion sediments, formed a separate cluster with a sequence difference of 3% or more with other members of the genus *Thioalkalimicrobium*. The results of DNA-DNA hybridization analysis of the low-salt-tolerant SOB isolates (Table 3) confirmed their phylogenetic identification, demonstrating the intraspecies relation of morphotypes A and B with *T. cyclicum* and the separate species position of the morphotype C isolates.

High-salt enrichments at 4 M Na⁺ resulted in the isolation of two morphologically similar strains from the chemocline and monolimnion water samples. Both strains contained a yel-

TABLE 3. Total DNA analysis of the haloalkaliphilic SOB isolates from Soap Lake

Strain	G+C content (mol%)	% DNA-DNA homology ^a with strain:			
		ASL1	ASL3	ASL8-2	ASL10
ASL1	50.0	100	75	23	
ASL3	49.9	75	100	35	
ASL2	49.3	30	21	85	
ASL6	49.8	32	26	72	
ASL8-2	49.5	23	34	100	
ASL8-1	49.5	ND ^b	ND	95	
<i>T. cyclicum</i> ALM 1	49.6	65	58	32	
<i>T. aerophilum</i> AL 3	49.5	43	50	48	
ASL10	65.5				100
<i>T. versutus</i> AL 2	63.7				80
<i>T. jannaschii</i> ALM 2	63.7				32

^a Average from two experiments; the deviation is ca. ±10%.

TABLE 4. Influence of pH on growth and respiration of strain ASL8-2

Buffering system (total Na ⁺ is 0.6 M)	pH		Growth rate (% of maximum ^a)	Rate of thiosulfate-dependent respiration (% of maximum ^b)
	Starting	Final (for growth)		
HEPES-NaCl	6.1		ND ^c	20
	7.0		ND	57
	8.0		ND	75
CO ₂ -NaHCO ₃ -NaCl	6.8	6.85	0	ND
	7.3	7.33	0	ND
NaHCO ₃ -NaCl	8.0	7.5	60	70
NaHCO ₃ -Na ₂ CO ₃ -NaCl	8.5	8.3	100	90
	9.07	8.90	90	100
	9.4	9.31	30	92
	10.0	9.75	10	80
	10.3		0	38
	10.5		0	10

^a Maximum growth rate was 0.32 h⁻¹.

^b Maximum respiration rate was 1.6 μmol O₂ mg protein⁻¹ min⁻¹.

^c ND, not determined.

low pigment with an absorption spectrum typical for the high-salt-tolerant representatives of the genus *Thioalkalivibrio* (11). The PCR products obtained from these strains had a migration distance identical to that of the dominant band obtained from the monimolimnion water sample (Fig. 2, band 3). A band at the same position in the gel, although not so intense, was also visible in the profile obtained from the monimolimnion sediment sample (SL4).

Both phylogenetic (Fig. 2) and total DNA (Table 3) analyses of the two high-salt-tolerant SOB isolates confirmed their affiliation with the genus *Thioalkalivibrio* and in particular to the core group of this genus, most of which are extremely salt tolerant (11). It is interesting, however, that there was very low DNA homology between the Soap Lake *Thioalkalivibrio* isolates and *Thioalkalivibrio jannaschii* isolated from Mono Lake (10).

Some of the other bands from the DGGE profiles of the environmental samples have also been successfully identified. Since general diversity was not a subject of this work, we can only briefly mention that most of the sequences belonged to the *Clostridiales*, but with relatively low homology (usually less than 90%) to sequences deposited in the GenBank.

Influence of environmental parameters on growth and activity of the *Thioalkalimicrobium* isolates. Strains ASL1 and ASL8-2, representatives of the two different morphotypes and the genetic groups of the low-salt-tolerant SOB from Soap Lake, responded differently to increasing pH. While ASL1 had a pH profile for growth and respiratory activity typical for obligately alkaliphilic *Thioalkalimicrobium* species, with an optimum at 9.5 to 10 (data not shown), strain ASL8-2 grew and respired best at pH 8.5 to 9.0. However, no growth occurred at neutral pH values in either strain, similar to the known obligately alkaliphilic *Thioalkalimicrobium* species (Table 4). Therefore, strain ASL8-2 can be qualified as a moderate, but obligate, alkaliphile. The respiratory profile of strain ASL8-2 was typical for the representatives of the genus *Thioalkalimicrobium* (9, 12), with extremely high oxidation rates for thiosulfate and sulfide (1.6 and 2.4 μmol O₂ mg protein⁻¹ min⁻¹ at pH 10, respectively) and no activity with tetrathionate, elemental sulfur, and sulfite. In respect to its salt tolerance, both

strains had low salt tolerance, with a range for growth (at pH 9) from 0.2 to 1.2 M total Na⁺ (optimum at 0.4 to 0.5 M). In contrast to ASL1, ASL8-2 (and all other isolates of this type) was incapable of initiating growth at fully aerobic conditions. The best way to grow these bacteria was to start a culture in closed bottles with a gas phase containing 2% O₂. After one-half of the thiosulfate was consumed, and the biomass level exceeded 30 to 40 mg protein liter⁻¹, oxygen content in the gas phase could be increased up to normal atmospheric concentrations. However, active aeration on the rotary shaker inhibited growth and eventually caused cell lysis.

Despite extremely high sulfide concentrations in anaerobic parts of the lake, the relevance of extraordinary sulfide tolerance seems to be not of utmost importance for the obligately aerobic SOB, such as the *Thioalkalimicrobium* population, dominating in Soap Lake, in contrast to the obvious necessity of such an adaptation among anaerobic inhabitants of the lake. The aerobic SOB populations develop and are active only in the interface, i.e., within a sulfide-oxygen gradient, where sulfide concentration is low. Furthermore, our experiments with soda solutions at pH 10 demonstrated that sulfide at concentrations above 1 mM starts to react with oxygen spontaneously. Therefore, the role of SOB in sulfide oxidation in the lake is important at micromolar concentrations. Two other factors are certainly important at so high a sulfide content: (i) the ability to retain activity at short-term exposure to high sulfide, in case of temporary exposure to anaerobic high-sulfide waters, and (ii) extremely high sulfide-oxidizing capacity, which allows this particular group of haloalkaliphilic SOB to cope with high upward flux of sulfide. In a short-term respiratory experiment at pH 10, strain ASL8-2 showed only slightly higher tolerance to sulfide than the type strain, *Thioalkalimicrobium aerophilum* AL3, isolated from a low-sulfide soda lake (50 and 70% inhibition of sulfide respiration at 2 mM sulfide, respectively). In contrast, the tolerance of sulfide of anaerobic haloalkaliphiles can be extraordinarily high. For example, strains of a fermentative *Amphibacillus* sp. isolated from soda soils can grow at pH 10 in the presence of up to 100 mM sulfide (our unpublished data).

Soap Lake harbors relatively dense populations of obligately chemolithoautotrophic haloalkaliphilic SOB which, similar to other soda lakes, are represented by the genera *Thioalkalimicrobium* and *Thioalkalivibrio*. The former is clearly dominant in the low-salinity, aerobic parts of the mixolimnion and seems to play an important role in the oxidation of sulfide diffusing across the chemocline from the extremely concentrated anaerobic brines of the monimolimnion. This is not surprising, owing to the remarkably high sulfide-oxidizing capacity typical for the haloalkaliphilic SOB of the genus *Thioalkalimicrobium* (11). The environmental importance of this particular group is confirmed by its detection as a dominant in bacterial populations of three out of four analyzed environmental samples from Soap Lake using PCR-DGGE.

One of the genotypes of *Thioalkalimicrobium* (represented by four isolates) detected in Soap Lake was not detected previously in any other soda lakes. In general, this group has much in common with the other *Thioalkalimicrobium* species. Particularly, it could not grow without reduced-sulfur compounds on purely organic medium (acetate and yeast extract) and could not utilize organic carbon instead of bicarbonate. Only

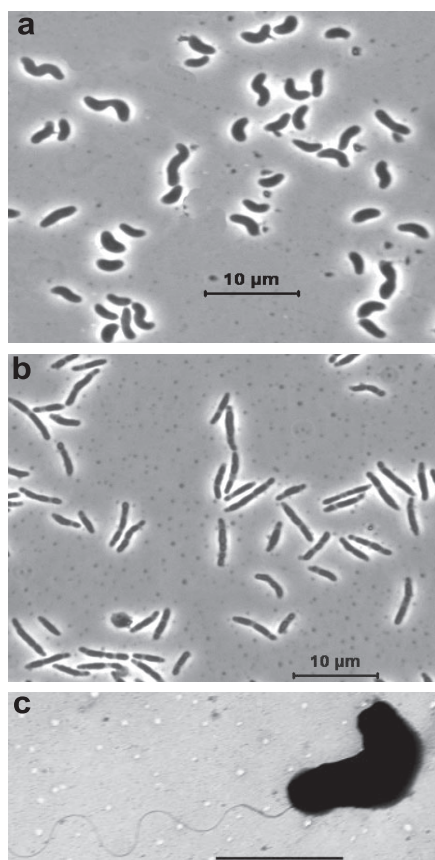


FIG. 4. Cell morphology of *Thioalkalimicrobium microaerophilum* sp. nov. strain ASL8-2^T. (a and b) Phase-contrast photographs of the cells grown at pH 8.5 (a) and at pH 10.0 (b); (c) electron-microscopic photograph of the cell, grown at pH 8.5. Scale bar, 1 µm.

reduced sulfur compounds, such as sulfide and thiosulfate, but not H₂ or formate, could be used as electron donors. On the other hand the group clearly differed from the known species in morphology, phylogeny, and some physiological properties and therefore is proposed to form a new species, *Thioalkalimicrobium microaerophilum*.

Description of *Thioalkalimicrobium microaerophilum* sp. nov. *Thioalkalimicrobium microaerophilum* (mic.ro.ae.ro.phi'lum. Gr. adj. *micro*s, small, little; Gr. masc. n. *aër*, gas; Gr. adj. *philum*, loving; M.L. n. *microaerophilum*, loving low-oxygen conditions). The cell morphology varies from thick vibrios, 1 to 2 µm by 2 to 5 µm, at optimal pH values (pH 8 to 9) to long rods, 0.6 to 0.8 µm by 3 to 8 µm, which are motile by means of a single polar flagellum (Fig. 4c). It is obligately chemolithoautotrophic and microaerophilic. It grows best in soda-buffered culture medium at pH 8.5 to 9. Thiosulfate or sulfide is used as an electron donor. It grows within a pH range from 8 to 10 and at a salt concentration equivalent to 0.2 to 1.2 M of total Na⁺. The temperature optimum for growth at pH 9 and

0.6 M Na⁺ is between 25 and 28°C. It oxidizes sulfide and thiosulfate to sulfate at high rates within a pH range of 6 to 10.5, with an optimum at 9 to 9.5. The G+C content in the DNA is 49.3 to 49.8 mol% (melting temperature). Other properties are similar to those described for the genus. It was isolated from the saline, alkaline Soap Lake (Grant County, Washington State). It is represented by four strains with a DNA-DNA homology level of more than 70%. The type strain is ASL8-2^T (DSM 17327, UNIQEM U242). The accession number of the 16S rRNA gene sequence is DQ900623.

ACKNOWLEDGMENTS

This work was supported by NWO-RFBR grant 47.011.2004.010, by the Program of the Russian Academy of Sciences "Molecular and Cell Biology," by the Dutch Foundation for Applied Research (STW, grant WBC 5939), and by a United States National Science Foundation Microbial Observatories program (MCB-0132158).

REFERENCES

1. Anderson, G. C. 1958. Seasonal characteristics of two saline lakes in Washington. *Limnol. Oceanogr.* **3**:51–68.
2. De Ley, J., H. Caffon, and A. Reinaerts. 1970. The quantitative measurements of hybridization DNA from renaturation rates. *Eur. J. Biochem.* **12**:133–140.
3. Ludwig, W., O. Strunk, R. Westram, L. Richter, H. Meier, Yadhukumar, A. Buchner, T. Lai, S. Steppi, G. Jobb, W. Forster, I. Brettske, S. Gerber, A. W. Ginhart, O. Gross, S. Grumann, S. Hermann, R. Jost, A. König, T. Liss, R. Lussmann, M. May, B. Nonhoff, B. Reichel, R. Strehlow, A. Stamatakis, N. Stuckmann, A. Vilbig, M. Lenke, T. Ludwig, A. Bode, and K. H. Schleifer. 2004. ARB: a software environment for sequence data. *Nucleic Acids Res.* **32**:1363–1371.
4. Marmur, J. 1961. A procedure for isolation of DNA from microorganisms. *J. Mol. Biol.* **3**:208–214.
5. Muyzer, G., A. Teske, C. O. Wirsen, and H. W. Jannasch. 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Arch. Microbiol.* **164**:165–172.
6. Pfennig, N., and K. D. Lippert. 1966. Über das Vitamin B₁₂-bedürfnis phototropher Schwefel bacterien. *Arch. Microbiol.* **55**:245–256.
7. Rice, C. A., M. L. Tuttle, and P. H. Briggs. 1988. Sulfur speciation, sulfur isotope, and elemental analyses of water-column, pore water, and sediment samples from Soap Lake, Washington. USGS Open File Report 88-22. U.S. Geological Survey, Denver, CO.
8. Schäfer, H., and G. Muyzer. 2001. Denaturing gradient gel electrophoresis in marine microbial ecology. *Methods Microbiol.* **30**:426–468.
9. Sorokin, D. Y., H. Banciu, M. van Loosdrecht, and J. G. Kuenen. 2003. Growth physiology and competitive interaction of obligately chemolithoautotrophic, haloalkaliphilic, sulfur-oxidizing bacteria from soda lakes. *Extremophiles* **7**:195–203.
10. Sorokin, D. Y., V. M. Gorlenko, T. P. Tourova, T. V. Kolganova, A. I. Tsapin, K. H. Neelson, and J. G. Kuenen. 2002. *Thioalkalimicrobium cyclicum* sp. nov. and *Thioalkalivibrio jannaschii* sp. nov., new species of alkaliphilic, obligately chemolithoautotrophic sulfur-oxidizing bacteria from a hypersaline alkaline Mono Lake (California). *Int. J. Syst. Evol. Microbiol.* **52**:913–920.
11. Sorokin, D. Y., and J. G. Kuenen. 2005. Haloalkaliphilic sulfur-oxidizing bacteria in soda lakes. *FEMS Microbiol. Rev.* **29**:685–702.
12. Sorokin, D. Y., A. M. Lysenko, L. L. Mityushina, T. P. Tourova, B. E. Jones, F. A. Rainey, L. A. Robertson, and J. G. Kuenen. 2001. *Thioalkalimicrobium sibiricum*, *Thioalkalimicrobium aerophilum* gen. nov., sp. nov., and *Thioalkalivibrio versutus*, *Thioalkalivibrio nitratis*, *Thioalkalivibrio denitrificans* gen. nov., sp. nov., new obligately alkaliphilic and obligately chemolithoautotrophic sulfur-oxidizing bacteria from soda lakes. *Int. J. Syst. Evol. Microbiol.* **51**:565–580.
13. Walker, K. F. 1975. The seasonal phytoplankton cycles of two saline cycles in central Washington. *Limnol. Oceanogr.* **20**:211–224.