***Thioalkalimicrobium cyclicum* sp. nov. and *Thioalkalivibrio jannaschii* sp. nov., novel species of haloalkaliphilic, obligately chemolithoautotrophic sulfur-oxidizing bacteria from hypersaline alkaline Mono Lake (California)**

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Two strains of haloalkaliphilic, obligately autotrophic, sulfur-oxidizing bacteria were isolated from the oxygen-sulfide interface water layer of stratified alkaline and saline Mono Lake, California, USA. Strain ALM 1^T was a dominant species in enrichment on moderate-saline, carbonate-buffered medium (0.6 M total Na⁺, pH 10) with thiosulfate as an energy source and nitrate as a nitrogen source. Cells of ALM 1^T are open ring-shaped and are non-motile. It has a high growth rate and activity of thiosulfate and sulfide oxidation and very low sulfur-oxidizing activity. Genetic comparison and phylogenetic analysis suggested that ALM 1^T (= DSM 14477^T = JCM 11371^T) represents a new species of the genus *Thioalkalimicrobium* in the γ -Proteobacteria, for which the name *Thioalkalimicrobium cyclicum* sp. nov. is proposed. Another Mono Lake isolate, strain ALM 2^T, dominated in enrichment on a medium containing 2 M total Na⁺ (pH 10). It is a motile vibrio which tolerates up to 4 M Na⁺ and produces a membrane-bound yellow pigment. Phylogenetic analysis placed ALM 2^T as a member of genus *Thioalkalivibrio* in the γ -Proteobacteria, although its DNA hybridization with the representative strains of this genus was only about 30%. On the basis of genetic and phenotypic properties, strain ALM 2^T (= DSM 14478^T = JCM 11372^T) is proposed as *Thioalkalivibrio jannaschii* sp. nov.

Keywords: haloalkaliphilic sulfur-oxidizers, soda lakes, Mono Lake, *Thioalkalimicrobium*, *Thioalkalivibrio*

INTRODUCTION

Colourless sulfur-oxidizing bacteria represent an important part of microbial community in natural and some industrial habitats. Until recently, known sulfur-oxidizing bacteria included neutrophilic and acidophilic species with possible pH range for stable growth between 2 and 8 (Kelly, 1989; Kuenen *et al.*, 1992). Natural environments with high alkalinity and stable

alkaline pH are represented by saline soda lakes, and recent intensive microbiological investigation of these habitats has revealed the presence of alkaliphilic bacteria belonging to various physiological groups (Jones *et al.*, 1998; Zavarzin *et al.*, 1999). In particular, it became clear that there exists a third, haloalkaliphilic, group of sulfur-oxidizing bacteria, which includes both heterotrophic (Sorokin *et al.*, 1996) and obligately autotrophic (Sorokin *et al.*, 2000b) representatives. Overall, more than 80 strains of alkaliphilic, obligately autotrophic, sulfur-oxidizing strains have been isolated and characterized (Sorokin *et al.*, 2001a) from the volcanic soda lakes in Kenya and from the shallow soda lakes in dry steppes of the south-east

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The GenBank accession numbers for the 16S rDNA sequence of strains ALM 1^T and ALM 2^T are AF329082 and AF329083, respectively.

Siberia and Mongolia. In general, these bacteria differ from the well-known neutrophilic species by their ability to grow optimally at pH > 9 and up to 10.5–10.6 in media strongly buffered by a sodium bicarbonate/carbonate mixture. All these bacteria have been assigned into two new genera *Thioalkalimicrobium* and *Thioalkalivibrio* in the γ -*Proteobacteria* (Sorokin *et al.*, 2001a). The two genera differ from each other in many aspects of the growth kinetics, metabolic activity and genetics. In general, the genus *Thioalkalimicrobium* includes fast-growing species with high activity of thiosulfate and sulfide oxidation but relatively low salt tolerance. Most of the strains were obtained from the low-mineralized steppe soda lakes. The genus is a member of the *Thiomicrospira* cluster. In contrast, the genus *Thioalkalivibrio* is represented by the slow-growing but more salt-tolerant organisms isolated mostly from the highly concentrated Kenyan soda lakes. Some of the isolates were even capable of growth in saturated soda brines. The genus *Thioalkalivibrio* is related to sulfur purple bacteria of the genus *Ectothiorhodospira* (Trüper & Schlegel, 1964). This genus also includes several strains capable of growth with thiocyanate as electron donor and nitrogen source (Sorokin *et al.*, 2001b) which form two new *Thioalkalivibrio* species (Sorokin *et al.*, 2002). In this paper we describe two new species of halo-alkaliphilic, obligately chemolithoautotrophic, sulfur-oxidizing bacteria isolated from the stratified, alkaline and saline Mono Lake in California, USA.

METHODS

Sampling. Water samples were collected in July 1999 from the sulfide-oxygen interface layer (depth 19–25 m) at the deepest point (48 m) of Mono Lake, California, using 5 l sampling bottles. The mean pH and total salinity values of the Mono Lake water were 9.8 and 80 g l⁻¹, respectively. The HS⁻ concentration decreased upwards from several hundred micromolar in the anaerobic layer to several micromolar in the upper part of the interface layer. The samples were brought into a laboratory within 5 h of collection and kept at 4 °C until use.

Media and growth conditions. Enrichment for and cultivation of aerobic alkaliphilic sulfur bacteria was performed using a mineral medium strongly buffered by NaHCO₃/Na₂CO₃ (0.6–4 M total Na⁺) at pH 10–10.1, as described previously (Sorokin *et al.*, 2001a). Thiosulfate (40–80 mM) served as the energy source and nitrate (5 mM as KNO₃) as the nitrogen source. Solid alkaline media with final salt concentrations 0.6 and 2 M total Na⁺ were prepared by 1:1 mixing of 4% agar and double-strength mineral base at 50 °C. Enrichments for denitrifying sulfur bacteria were performed in 100 ml serum bottles with butyl rubber stoppers filled with 50 ml of alkaline base with 20 mM thiosulfate and 20 mM nitrate. Anaerobiosis was achieved by five cycles of evacuation-flushing with argon.

Activity tests. The activity tests were performed with washed cells obtained from batch cultures grown at pH 10 with thiosulfate. Cells were harvested by centrifugation, washed twice with soda buffer and resuspended in the same buffer at 20 mg protein ml⁻¹ and kept on ice. This concentrated suspension was diluted 100–1000 times immediately before

experiments. The pH influence on the activity of thiosulfate oxidation was tested with an oxygen electrode as described previously (Sorokin *et al.*, 2001a) using 0.1 M HEPES + 0.6 M NaCl for the pH range 6–8 and NaHCO₃/Na₂CO₃ buffer for higher pH values. All buffers contained 50 mM KCl. The influence of salt concentration on the activity of thiosulfate oxidation was investigated using soda buffer, pH 10, with total Na⁺ concentration from 0.3 to 4 M.

Total DNA analysis. The isolation of the DNA and subsequent determination of the G + C content of the DNA and the DNA–DNA hybridization were performed according to standard procedures (Marmur, 1961; De Ley *et al.*, 1970).

Amplification and sequencing of 16S rRNA genes. For amplification and sequencing of 16S rRNA genes, the DNA was obtained by standard phenol/chloroform extraction. The 16S rRNA genes were selectively amplified using primers 5'-AGAGTTTGATCCTGGCTCAG-3' (forward) and 5'-TACGGTTACCT-TGTTACGACTT-3' (reverse). PCR products were cloned, and transformed using InvitroGene kit. Sequencing had been done by MWG Biotech, Inc (High Point North Carolina) with Licor machine using custom-designed primers to sequence samples to an accuracy of > 99%. A combination of both available sequencing reactions which include dye primer on the Licor Long Read IR 4200 sequencers and dye terminator on the ABI3700 capillary sequencers were used. Dye primer chemistry provided data read lengths from 750 to over 1100 bases, while the dye-terminator reaction was used to confirm base calls and provide gap closure with read lengths ranging from 500 to 750 bases. Nearly complete 16S rDNA gene sequences were obtained for the Mono Lake isolates ALM 1^T and ALM 2^T (1450–1470 nucleotides).

16S rDNA sequence analysis. The sequences were aligned manually with sequences obtained from the database of small-subunit rRNAs in EMBL. The sequences were compared with those of the members of the *Proteobacteria*. Regions that were not sequenced in one or more reference organism were omitted from the analyses. Pairwise evolutionary distances (expressed as estimated changes per 100 nucleotides) were computed by using the Jukes & Cantor method. A resulting phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with bootstrap analysis of 100 trees using programs of the TREECON package (Van de Peer & De Wachter, 1994). Bootstrap analysis (100 replications) was used to validate the reproducibility of the branching pattern of the trees.

Electron microscopy. For total preparations, cells were washed and resuspended in 0.5–1 M neutral NaCl solution, pre-fixed (2 h) and then fixed (10 h) at 4 °C in 0.1% and 2.5% (v/v, final) glutaraldehyde solutions, respectively, containing the same amount NaCl, and then positively stained with 1% (w/v) uranyl acetate. For ultrathin sectioning cells after fixation were postfixated in 1% (w/v) OsO₄ solution containing 0.6–2 M NaCl for 10 h at 4 °C. Then the cells were washed, dehydrated and embedded into the resin. Thin sections were stained with 1% solutions (w/v) of uranyl acetate and lead citrate.

Chemical analysis. Thiosulfate in batch cultures and in experiments with washed cells was determined by the iodimetric titration after acidification of the samples by acetic acid to pH 5. Sulfide was measured colorimetrically (Trüper & Schlegel, 1964) after precipitation with zinc acetate (1%, w/v, final). Elemental sulfur was extracted from the cell pellets with acetone overnight and was assayed by a cyanolytic procedure (Sörbo, 1957). The biomass

protein was determined by the Lowry method, interfering sulfur compounds being removed either by several washings of the cell pellet with 0.6 M NaCl (thiosulfate) or by a double acetone extraction (elemental sulfur). The pigment from the cells of strain ALM 2 was extracted with a methanol/acetone (1:1) mixture. Its absorption spectrum was recorded on the UV-Visible diode-array Hewlett Packard HP 8453 spectrophotometer.

RESULTS AND DISCUSSION

Enrichment and isolation of pure cultures

Two different positive enrichment cultures from the interface water layer of Mono Lake were obtained using alkaline mineral medium with thiosulfate and different salt content. In low-salt enrichment (0.6 M Na⁺) a phenotype with coccoid, non-motile cells was seen. Tentative enumeration showed 10³–10⁴ cells ml⁻¹. In high-salt enrichment (2.5 M Na⁺) slightly curved motile rods dominated, and the cultures turned yellowish after complete consumption of thiosulfate and intermediately produced elemental sulfur. Plating of the cultures onto solid media with corresponding salt concentrations resulted in isolation of two different pure cultures of aerobic sulfur-oxidizing bacteria, strains ALM 1^T (0.6 M Na⁺) and ALM 2^T (2 M Na⁺).

Morphology

The colonies of strain ALM 1^T were up to 3 mm in diameter, reddish, transparent, without sulfur deposition. Under the light microscope the cells looked like tiny, non-motile, irregular spheres, often aggregated. The electron microscopy demonstrated that the cells of ALM 1^T were shaped as the open rings (Fig. 1a, b) with a diameter 0.5–0.8 µm and cell width 0.3–0.4 µm. Many cells contained multiple carboxysome-like structures (Fig. 1a). The colonies of strain ALM 2^T were up to 4 mm in diameter, yellowish, with sulfur deposition. ALM 2^T cells are curved rods, 0.3–0.4 × 1–2 µm, motile by a single polar flagellum (Fig. 2a). Cells often contained intracellular sulfur globules (Fig. 2b, c) apparently encapsulated via invagination of the cell membrane (Fig. 2d). The biomass of ALM 2^T was yellow-coloured due to membrane-associated pigment. Methanol/acetone extract of the cell membranes had absorption maxima at 405, 430 (main) and 450 nm. The specific pigment content in the biomass increased about twofold when salt content of the growth medium increased from 0.6 to 2–2.5 M total Na⁺, remaining at constant level upon further increase of salinity to 3–4 M Na⁺.

Phylogeny

Phylogenetic analysis of nearly complete 16S rDNA gene sequences of strains ALM 1^T and ALM 2^T demonstrated their close relationship to two recently described genera of alkaliphilic sulfur bacteria in the *γ-Proteobacteria*. Strain ALM 1^T is a member of the genus *Thioalkalimicrobium* with a high level of se-

Fig. 1. Morphology of alkaliphilic sulfur bacterium strain ALM 1^T from Mono Lake (electron photomicrographs of thin sections; bars, 0.5 µm) grown with thiosulfate at pH 10 and 0.6 M Na⁺. C, carboxysome-like structures.

quence similarity to type strains AL 3^T and AL 7^T (96.8–98.5%). Strain ALM 2^T is more distantly related but definitely belongs to a phylogenetic cluster of the genus *Thioalkalivibrio* (92.7–96.6% sequence similarity) (Fig. 3).

Genetic analysis

The results of total DNA analysis of the Mono Lake isolates are given in Table 1. The G+C content in DNA of both strains fitted well into the range typical

Fig. 2. Morphology of alkaliphilic sulfur bacterium strain ALM 2^T from the Mono Lake (electron photomicrographs) grown with thiosulfate at pH 10 and 2 M Na⁺. (a) Total preparation (bar, 1 µm); (b)–(d), thin sections (bar, 1 µm). S, Intracellular sulfur globules; M, circular membrane invaginations.

for the corresponding genera of previously described haloalkaliphilic sulfur bacteria. Comparison of total DNA of strain ALM 1^T and the type strains of the genus *Thioalkalimicrobium* confirmed the results of phylogenetic analysis, in that this strain, despite its peculiar morphology, is a member of the genus *Thioalkalimicrobium*. It had relatively high hybridization to rod-shaped type strain *Thioalkalimicrobium aerophilum* AL 3^T but it was still less than is usual between strains of the same species (< 70%; Stackebrandt & Goebel, 1994). In contrast, strain ALM 2^T showed a close phenotypic similarity to the members of genus *Thioalkalivibrio*, in particular with the yellow coloured, extremely salt-tolerant subgroup, but displayed a very low level of DNA hybridization (< 32%) with the representative strains of *Thioalkalivibrio*. Nevertheless, with clear phenotypic and phylogenetic indications, strain ALM 2^T should be considered as a member of the genus *Thioalkalivibrio*.

Physiological properties

Both Mono Lake isolates belong to obligately chemolithoautotrophic sulfur-oxidizing bacteria. Growth with hydrogen as electron donor and denitrification (thiosulfate + nitrate, nitrite or N₂O) were not ob-

served. Like other sulfur-oxidizing strains isolated previously from various soda lakes, the Mono Lake isolates were obligately alkaliphilic. Growth in batch culture with thiosulfate at 0.6 M Na⁺ salt content was possible within the pH range 7.5–10.5. The thiosulfate-dependent O₂ consumption by washed cells of strain ALM 1^T and ALM 2^T was observed within the pH range 6.5–11 and 7.0–11.2, respectively, with an optimum at pH 9.5 for both organisms (Fig. 4). The salt tolerance of strain ALM 1^T was in the moderate range while ALM 2^T belongs to an extremely tolerant type, being able to function within a very broad salinity range from 0.4 to 4 M total Na⁺ (Fig. 5). The difference between the strains was more evident with growing cultures (measured by the rate of thiosulfate consumption, Fig. 5a) as compared to activity of the resting cells (Fig. 5b). Strain ALM 1^T never formed sulfur as an intermediate during growth with thiosulfate, while prolific sulfur production was observed in ALM 2^T cultures at salt concentration less than 2 M total Na⁺.

The respiratory profiles of both strains (Table 2) are consistent with the patterns of representatives of the genera *Thioalkalimicrobium* and *Thioalkalivibrio*. As is typical for members of the genus *Thioalkalimicrobium*

Fig. 3. Phylogenetic tree showing the relationships of the Mono Lake strains of alkaliphilic sulfur-oxidizing bacteria in the γ -Proteobacteria. The numbers on the branches refer to bootstrap values; only values above 90 % are shown. The bar represents 5 % sequence divergence.

Table 1. DNA–DNA hybridization between Mono Lake isolates and members of genera *Thioalkalimicrobium* and *Thioalkalivibrio*

Thioalkalimicrobium: group 1 – *Thioalkalimicrobium aerophilum* AL 3T; group 2 – *Thioalkalimicrobium sibiricum* AL 7T. *Thioalkalivibrio*: group 1 – yellow-coloured, extremely salt-tolerant strains from Mongolian, Kenyan and Egyptian soda lakes; group 2 – low-salt tolerant, colourless strains from Kenyan and Siberian soda lakes. Non-specific background values for DNA hybridization (*Escherichia coli*) was not higher than 5 %.

Strain	DNA G + C content (mol %)	DNA–DNA hybridization (%)			
		<i>Thioalkalimicrobium</i>		<i>Thioalkalivibrio</i>	
		Group 1	Group 2	Group 1 (8 strains)	Group 2 (6 strains)
ALM 1 ^T	49.6	60	30	ND	ND
ALM 2 ^T	63.7	ND	ND	25–32	15–20

ND, No data.

(Sorokin *et al.*, 2001a), strain ALM 1^T oxidized thiosulfate and sulfide, less actively polysulfide and tetrathionate and had no activity with elemental sulfur. Strain ALM 2^T oxidized all five sulfur compounds with more or less equal but relatively low activity, which is a characteristic of the members of genus *Thioalkalivibrio* (Sorokin *et al.*, 2001a). Addition of sulfite did not stimulate oxygen consumption in the cell suspensions of the new isolates.

Mono Lake belongs to a comparatively rare type of stratified alkaline and highly saline lakes. In this lake, a hypersaline, anaerobic, sulfide-containing water body is overlaid by less saline, aerobic waters. The result is a rather stable oxic/anoxic interface that has been shown to act as an active aerobic chemolithotrophic ‘filter’ for reduced inorganic compounds, such as ammonia, methane and sulfide (Joye *et al.*, 1999; Ward *et al.*, 2000). Given the high sulfate

Fig. 4. Influence of pH on the activity of thiosulfate-dependent oxygen consumption by washed cells of the Mono Lake isolates ALM 1^T (●) and ALM 2^T (○). All buffers contained 0.6 M total Na⁺. pH 6.5–8, HEPES + NaCl; pH 8–11.2, NaHCO₃/Na₂CO₃. Means from the two independent experiments.

Fig. 5. Influence of salt concentration (NaHCO₃/Na₂CO₃/NaCl) on growth and activity of thiosulfate oxidation by washed cells of the Mono Lake isolates at pH 10. (a) Amount of thiosulfate consumed during 24 and 47 h growth in batch cultures of the strains ALM 1^T and ALM 2^T, respectively; (b) activity of thiosulfate-dependent respiration. ●, ALM 1^T; ○, ALM 2^T. Means from the two independent experiments.

concentration (130 mM) (Oremland & Miller, 1993), the presence of sulfur-oxidizing bacteria in the redox interface of Mono Lake was suspected as well. Our investigation demonstrated that at least two different

Table 2. Respiration rate of washed cells

ALM 1^T and ALM 2^T were grown with thiosulfate at pH 10 and 0.6 M Na⁺. Values are given as μmol O₂ (mg protein)⁻¹ min⁻¹ (means of 2–3 independent measurements); endogenous rates were subtracted.

Substrate	Respiration rate	
	ALM 1 ^T	ALM 2 ^T
Thiosulfate (S ₂ O ₃ ²⁻)	2.50	0.60
Sulfide (HS ⁻)	3.04	0.70
Polysulfide (S ₆ ²⁻)	1.80	0.66
Sulfur (S ₈)	0.00	0.52
Tetrathionate (S ₄ O ₆ ²⁻)	0.42	0.33

cultivable types of obligately chemolithoautotrophic, aerobic, haloalkaliphilic sulfur bacteria inhabit this lake. One type (represented by strain ALM 1^T), enriched at moderate salt concentration, probably corresponds to a population adapted to the surface aerobic waters of the Mono Lake mixolimnion. The other type (represented by strain ALM 2^T), enriched at extremely high salt concentrations, functions optimally at 1–3 M Na⁺, which encompasses the salt content of the monimolimnion (1.4 M Na⁺) but also would allow such bacteria to survive a local increase of salinity. Such conditions may occur in the shallow evaporative pools temporarily separated from the main lake water body.

Unlike many other freshwater and saline stratified shallow lakes (where sufficient light penetrates to a sulfide-containing layer), in Mono Lake the anaerobic sulfide-oxidizing phototrophs do not build up a substantial population (V. Gorlenko, unpublished). The reasons are not quite clear. It could be presence of unusually high concentrations of toxic oxyanions, such as AsO₄³⁻ and SeO₃²⁻ (Stolz & Oremland, 1999) or light interception by the abundant mixolimnion population of the unicellular algae '*Picocystis salinarum*', which was recently discovered in the Mono Lake ecosystem (<http://www.monobasinresearch.org/research/microbial>). Whatever the reason, in the absence of active population of anaerobic phototrophs it might be speculated that the aerobic, haloalkaliphilic, sulfur-oxidizing bacteria play a key role in the oxidation of sulfide coming up from the anaerobic layer in Mono Lake.

On the basis of physiological and genetic properties, the Mono Lake isolates were clearly related to two different groups of previously described haloalkaliphilic sulfur-oxidizing bacteria isolated from the Kenyan and Siberian soda lakes (Sorokin *et al.*, 2001a). Strain ALM 1^T, being physiologically very similar to representatives of the genus *Thioalkalimicrobium*, differs from the known species of this genus by its morphology. Phylogenetically strain ALM 1^T is closely related to members of the genus *Thioalkalimicrobium*

but since its DNA similarity with described species was less than 70%, this strain is proposed as a new species, *Thioalkalimicrobium cyclicum*. Strain ALM 2^T, despite its very low DNA hybridization with the representative strains, phenotypically clearly resembles the extremely salt-tolerant members of the genus *Thioalkalivibrio*. In view of the results of phylogenetic analysis, this isolate should be affiliated with the genus *Thioalkalivibrio* and is proposed as a new species *Thioalkalivibrio jannaschii*. We have also found this type of sulfur-oxidizing double extremophiles dominating in highly saline alkaline lakes in Mongolia, Kenya and Wadi an Natrun in Egypt (Sorokin *et al.*, 2001a; our unpublished results). It is interesting to mention that these sulfur-oxidizing bacteria probably represent the only type of aerobic chemolithoautotrophs capable to grow in saturated alkaline brines. Other types of alkaliphilic chemolithoautotrophs (methane-, hydrogen-, ammonia- and nitrite-oxidizing), although being detected in and isolated from the extremely saline soda lakes, are able to grow only at salinity below 1 M Na⁺ (Ward *et al.*, 2000; Sorokin *et al.*, 1998, 2000a, c, 2001c).

Description of *Thioalkalimicrobium cyclicum* sp. nov.

Thioalkalimicrobium cyclicum (cyc.li'cum Gr. n. *cyclos* circle; M.L. n. *cyclicum* circle-like).

Cells are mostly in a form of open ring with a diameter 0.5–0.8 and cell width 0.3–0.4 µm. Many cells contained multiple carboxysome-like structures. Obligately chemolithotrophic, alkaliphilic and moderately halophilic. Grows up to 1.5 M Na⁺. Oxidizes sulfide, thiosulfate, polysulfide and tetrathionate. G + C content in DNA is 49.6 mol% (*T_m*). Isolated from the O₂-sulfide interface layer of Mono Lake (California, USA). Other properties as for the genus. The type strain is ALM 1^T (= DSM 14477^T = JCM 11371^T).

Emended description of genus *Thioalkalimicrobium*

The description of the genus *Thioalkalimicrobium* (Sorokin *et al.*, 2001a) should be emended by the following:

Cells vary in shape from straight rods with sharp edges to spirilla, 0.3–0.5 × 0.8–1.5 µm, motile by means of 1–3 polar flagella at one end. Also includes strains with non-motile, circular and coccoid cells.

Description of *Thioalkalivibrio jannaschii* sp. nov.

Thioalkalivibrio jannaschii (jann.asch'i.i. N.L. gen. n. *jannaschii* in honour of German microbiologist Holger Jannasch).

Cells are curved rods, 0.3–0.4 × 1–2 µm, motile by a single polar flagellum. Cells often contain intracellular sulfur globes when grown with thiosulfate at low salinity. Produces yellow, membrane-associated pigment during growth at high salinity. The methanol/acetone extract of the cell membranes has absorption

maxima at 405, 430 (main) and 450 nm. Obligately chemolithoautotrophic, haloalkaliphilic bacterium. Tolerates up to 4 M Na⁺. Oxidizes sulfide, thiosulfate, polysulfide, sulfur and tetrathionate. G + C content in DNA is 63.7 mol% (*T_m*). Isolated from the O₂-sulfide interface layer of alkaline Mono Lake (California). Other properties as for the genus. The type strain is ALM 2^T (= DSM 14478^T = JCM 11372^T).

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REFERENCES

- De Ley, J., Caffon, H. & Reinaerts, A. (1970). The quantitative measurements of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–140.
- Jones, B. E., Grant, W. D., Duckworth, A. W. & Owenson, G. G. (1998). Microbial diversity of soda lakes. *Extremophiles* **2**, 191–200.
- Joye, S. B., Conell, T. L., Miller, L. G., Oremland, R. S. & Jellison, R. S. (1999). Oxidation of ammonia and methane in an alkaline, saline lake. *Limnol Oceanogr* **44**, 178–188.
- Kelly, D. P. (1989). Physiology and biochemistry of unicellular sulfur bacteria. In *Autotrophic Bacteria*, pp. 193–217. Edited by H. G. Schlegel & B. Bowien. Berlin: Springer.
- Kuenen, J. G., Robertson, L. A. & Tuovinen, O. H. (1992). The genera *Thiobacillus*, *Thiomicrospira* and *Thiosphaera*. In *The Prokaryotes*, 2nd edn, vol. 3, pp. 2638–2657. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. Schleifer. Berlin: Springer.
- Marmur, J. (1961). A procedure for isolation of DNA from microorganisms. *J Mol Biol* **3**, 208–214.
- Oremland, R. S. & Miller, L. G. (1993). Biochemistry of natural gases in three alkaline, permanently stratified (meromictic) lakes. In *The Future of Energy Gases* (USGS professional paper 1570), pp. 439–452. Edited by D. Howell. Reston, VA: USGS.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for constructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sörbo, B. (1957). A colorimetric determination of thiosulfate. *Biochim Biophys Acta* **23**, 412–416.
- Sorokin, D. Y., Lysenko, A. M. & Mityushina, L. L. (1996). Isolation and characterization of alkaliphilic heterotrophic bacteria capable of oxidation of inorganic sulfur compounds to tetrathionate. *Microbiology* (English translation of *Mikrobiologiya*) **65**, 326–338.
- Sorokin, D. Y., Muyzer, G., Brinkhoff, T., Kuenen, J. G. & Jetten, M. (1998). Isolation and characterization of a novel facultative alkaliphilic *Nitrobacter* species, *N. alkalicus* sp. nov. *Arch Microbiol* **170**, 345–352.
- Sorokin, D. Yu., Jones, B. E. & Kuenen, J. G. (2000a). A novel, obligately methylotrophic, methane-oxidizing *Methylomicrobium* sp. from highly alkaline environment. *Extremophiles* **4**, 145–155.
- Sorokin, D. Y., Robertson, L. A. & Kuenen, J. G. (2000b). Isolation and characterization of obligately chemolithoautotrophic alkaliphilic sulfur-oxidizing bacteria. *Antonie Leeuwenhoek* **77**, 251–260.
- Sorokin, D. Yu., Tourova, T. P. & Kuenen, J. G. (2000c). A novel facultatively autotrophic hydrogen- and sulfur-oxidizing bacterium from alkaline environment. *Extremophiles* **4**, 237–245.

- Sorokin, D. Yu., Lysenko, A. M., Mityushina, L. L., Tourova, T. P., Jones, B. E., Rainey, F. A., Robertson, L. A. & Kuenen, J. G. (2001a).** *Thioalkalimicrobium aerophilum* gen. nov., sp. nov. and *Thioalkalimicrobium sibericum* sp. nov., and *Thioalkalivibrio versutus* gen. nov., sp. nov., *Thioalkalivibrio nitratis* sp. nov. and *Thioalkalivibrio denitrificans* sp. nov., novel obligately alkaliphilic and obligately chemolithoautotrophic sulfur-oxidizing bacteria from soda lakes. *Int J Syst Evol Microbiol* **51**, 565–580.
- Sorokin, D. Yu., Tourova, T. P., Lysenko, A. M. & Kuenen, J. G. (2001b).** Microbial thiocyanate utilization under highly alkaline conditions. *Appl Environ Microbiol* **67**, 528–538.
- Sorokin, D. Yu., Tourova, T. P., Schmid, M., Wagner, M., Koops, H.-P., Kuenen, J. G. & Jetten, M. (2001c).** Isolation and properties of obligately chemolithoautotrophic alkaliphilic ammonia-oxidizing bacteria from Mongolian soda lakes. *Arch Microbiol* **176**, 170–177.
- Sorokin, D. Yu., Tourova, T. P., Lysenko, A. M., Mityushina, L. L. & Kuenen, J. G. (2002).** *Thioalkalivibrio thiocyanoxidans* sp. nov. and *Thioalkalivibrio paradoxus* sp. nov., novel alkaliphilic, obligately autotrophic, sulfur-oxidizing bacteria capable of growth on thiocyanate, from soda lakes. *Int J Syst Evol Microbiol* **52**, 657–664.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Stolz, J. F. & Oremland, R. S. (1999).** Bacterial respiration of arsenic and selenium. *FEMS Microbiol Rev* **23**, 615–627.
- Trüper, H. G. & Schlegel, H. G. (1964).** Sulphur metabolism in *Thiorhodaceae*. Quantitative measurements on growing cells of *Chromatium okeanii*. *Antonie Leeuwenhoek* **30**, 225–237.
- Van de Peer, Y. & De Wachter, R. (1994).** TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* **10**, 569–570.
- Ward, B. B., Martino, D. P., Diaz, M. C. & Joye, S. B. (2000).** Analysis of ammonia-oxidizing bacteria from a hypersaline Mono Lake, California, on the basis of 16S rRNA sequences. *Appl Environ Microbiol* **66**, 2873–2881.
- Zavarzin, G. A., Zhilina, T. N. & Kevbrin, V. V. (1999).** The alkaliphilic microbial community and its functional diversity. *Microbiology* (English translation of *Mikrobiologiya*) **68**, 503–521.