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ORIGINAL PAPER

Dethiobacter alkaliphilus gen. nov. sp. nov., and *Desulfurivibrio alkaliphilus* gen. nov. sp. nov.: two novel representatives of reductive sulfur cycle from soda lakes

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Abstract Anaerobic enrichments with H_2 as electron donor and thiosulfate/polysulfide as electron acceptor at pH 10 and 0.6 M total Na^+ yielded two non sulfate-reducing representatives of reductive sulfur cycle from soda lake sediments. Strain AHT 1 was isolated with thiosulfate as the electron acceptor from north–eastern Mongolian soda lakes and strain AHT 2—with polysulfide as the electron acceptor from Wadi al Natrun lakes in Egypt. Both isolates represented new phylogenetic lineages: AHT 1—within *Clostridiales* and AHT 2—within the *Deltaproteobacteria*. Both bacteria are obligate anaerobes with respiratory metabolism. Both grew chemolithoautotrophically with H_2 as the electron donor and can use thiosulfate, elemental sulfur and polysulfide as the electron acceptors. AHT 2 also used nitrate as acceptor, reducing it to ammonia. During thiosulfate reduction, AHT 1 excreted sulfite. *dsr*AB gene was not found in either strain. Both strains were moderate salt-tolerant (grow up to 2 M total Na^+) true alkaliphiles (grow between pH 8.5 and 10.3). On the basis of the phenotypic and phylogenetic data, strains AHT 1 and AHT 2 are proposed as new genera and species *Dethiobacter alkaliphilus* and *Desulfurivibrio alkaliphilus*, respectively.

Keywords Soda lakes · Haloalkaliphilic · Sulfur-reducing · Thiosulfate-reducing · Polysulfide

Introduction

Soda lakes are double extreme habitats with stable high pH and varying salt content from several g/L up to saturation. The main characteristic of soda lakes distinguishing them from other saline waters and non-saline alkaline habitats is their high alkalinity (buffering capacity) in solution due to a presence of free sodium carbonate/bicarbonate, which can reach molar concentrations. Such conditions selects for salt-tolerant/dependent alkaliphiles. Intensive microbiological survey of various soda lakes conducted within the last 15 years identified most of the functional-structural blocks in the haloalkaliphilic microbial community (Jones et al. 1998; Zavarzin et al. 1999; Sorokin and Kuenen 2005a). Sulfur cycle is very active even in hypersaline soda lakes. This might be due to several reasons, such as high productivity, high sulfate concentrations, high energy yield of sulfur-dependent dissimilatory metabolism. Our study of oxidative part of sulfur cycle in soda lakes resulted in isolation and characterization of more than a hundred strains of obligatory, facultative and heterotrophic sulfur-oxidizing haloalkaliphiles, belonging to the Gamma- and

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Alphaproteobacteria (Sorokin 2003; Sorokin and Kuenen 2005b; Sorokin et al. 2006). Among them, the genus *Thioalkalivibrio* is the most widespread and metabolically versatile. Less is known about the reductive part of the sulfur cycle in soda lakes. Until now, two culturable groups of low salt-tolerant alkaliphilic SRB belonging to the *Deltaproteobacteria* (order *Desulfovibrionales*) have been found in soda lakes. Both utilize acetate only as a carbon, but not energy source, and both can use H_2 as an electron donor (Zhilina et al. 1997, 2005; Pikuta et al. 1998, 2003). Culture-independent study of the SRB populations in soda lakes so far have been limited to two studies. In Mono Lake in California 16S rDNA targeting successfully identified representatives of several groups of *Deltaproteobacterial* SRB but also a novel lineage of possible sulfur reducers (Scholten et al. 2005). Similar study on sediments from soda lakes in Kulunda Steppe in Russia targeting functional *dsr*AB gene (encoding key enzyme dissimilatory sulfite reductase) demonstrated a presence of two major groups of Deltaproteobacterial SRB: one in *Desulfovibrionales* and several lineages within the family *Desulfobacteriaceae* (Foti et al. 2007). This pointed to a bounty of players within the reductive sulfur cycle yet untouched by cultivation.

Apart from sulfate, many SRB can also use thiosulfate as the electron acceptor, while elemental sulfur is mostly utilized by non sulfate-reducing Prokaryotes, including narrowly specialized sulfur respirers such as representatives of the *Desulfuromonadales* and more versatile anaerobes, such as *Sulfurispirillum* and *Wolinella* (*Epsilonproteobacteria*). One of the interesting features of sulfur chemistry at alkaline pH is active formation of polysulfide (S_x^{2-}) from sulphide and elemental sulfur, which is stable above pH 9. Since polysulfide, in contrast to sulfide, is actively reacting with oxygen with formation of thiosulfate, these reduced sulfur species might be important players in the sulfur cycling in soda lakes, both in its oxidative as well as reductive branches.

Recently, two new species of non sulfate-reducing bacteria capable of growth at high pH have been isolated from anthropogenic sources. *Desulfitibacter alkalitolerans* from commune water heating plant can use sulfur, thiosulfate and sulfite as electron acceptors and belongs to the *Clostridia* (Nielsen et al. 2006). *Desulfurispirillum alkaliphilum* is a moderately alkaliphilic deep-lineage bacterium (*Chrysiogenetes*) from sulphide-removing bioreactor utilizing sulfur/polysulfide, nitrate and fumarate as electron acceptors (Sorokin et al. 2007).

In this paper two novel sulfidogenic bacteria from soda lakes are described. They belong to the *Clostridia* and the *Deltaproteobacteria*, respectively, and represent moderate salt-tolerant non sulfate-reducing alkaliphiles.

Methods

Samples

Surface sediment samples (0–10 cm) were obtained from soda lakes in north–eastern Mongolia, Kenya and Wadi Natrun in Egypt. 8–12 samples from individual lakes in each region were combined into a single pool. The pH of the brines varied from 9.2 to 10.6, total salt concentration from 20 to 400 g l^{-1} and total soluble alkalinity from 0.05 to 2 M. More information about the sites can be found in Sorokin et al. (2004a); Taher (1999) and Duckworth et al. (1996).

Cultivation

Anaerobic enrichment and routine cultivation was performed at 30°C on a mineral medium containing sodium carbonate/bicarbonate buffer (0.5 M total Na^+, pH 10), 0.1 M NaCl, and 0.5 g l^{-1} of K_2HPO_4. After sterilization, the medium was supplemented with 4 mM NH_4Cl, 1 mM $MgCl_2 \times 6H_2O$, and 1 ml l^{-1} of trace metal solution (Pfenning and Lippert 1966). For the enrichments and isolation, H_2 (100% gas phase) was used as energy source and either polysulfide (20 mM zero-valent sulfur) or thiosulfate (20 mM) as the electron acceptors. In case of thiousulfate, the medium was supplemented with 0.5 mM of sulfide. Other electron acceptors used were KNO_3 (10 mM), KNO_2, Na_2SO_3, sodium selenate and selenite, sodium arsenate, DMSO (5 mM each), sodium fumarate (20 mM; alone or with 2 mM acetate as carbon source), and freshly prepared ferrihydrite (50 mM). Elemental sulfur was sterilized as 50% suspension (w/v) at 110°C for 60 min and was used at final concentration 2 g l^{-1}. Polysulfide (S_5^{2-}) was prepared by autoclaving 0.2 M sulfide with excess of elemental sulfur. Different electron donors were tested at concentration 10 mM. Growth at microoxic conditions was tested with an oxygen concentration in the gas phase of 2%. Routine anaerobic cultivation was performed in 100 ml serum bottles with 40 (H_2 as the electron donor) to 80 ml medium. Solid alkaline media with a final salt concentration of 0.5 M Na^+ was prepared by 1:1 mixing of 4% (w/v) agar (Noble, Difco) and 1 M Na^+ reduced anaerobic mineral medium at 50°C. The plates were immediately placed in closed jars under the stream of argon with an oxygen-scavenging catalyzer (Oxoid). A brief exposure to air during inoculation did not result in substantial polysulfide oxidation as was evident from remaining yellow coloration of the control plates. The inoculated plates were incubated further in the jars under the H_2 atmosphere for up to 1 month before visible colonies appeared.

The pH dependence was examined at Na^+ content of 0.6 M, using the following filter-sterilized mineral media: for pH 6–8, 0.1 M HEPES and NaCl; for pH 8–11, a mixture of sodium bicarbonate/sodium carbonate containing 0.1 M NaCl. Growth resulted in a shift of initial pH values, especially in highly alkaline region. Therefore, final pH values were taken to indicate suitable range for growth. To study the influence of salt concentration on growth, mineral bases containing 0.1 and 4.0 M of total Na^+ were mixed in different proportions.

Analytical procedures

Chemical analysis of sulfur (sulfide, sulfur, thiosulfate and sulfite) and nitrogen (nitrite and ammonium) compounds, and cell protein were performed as described previously (Sorokin et al. 2001, 2004b). Sulfane atom of polysulfide was analyzed in the same way as free sulfide, i.e. after precipitation as ZnS. Zero-valent sulfur in polysulfide was analyzed in the same way as free sulfur (i.e. by cyanolysis of acetone extract) after decomposition of polysulfide molecules by acid treatment.

Phase contrast microphotographs were obtained with a Zeiss Axioplan Imaging 2 microscope (Göttingen, Germany). For electron microscopy of total cells they were resuspended in 0.5 M NaCl, fixed with glutaraldehyde (final 3% v/v) and positively contrasted with 1% (w/v) uranyl acetate. For thin sectioning, the cells were fixed in 1% (w/v) OsO_4 + 0.5 M NaCl for 3 h at room temperature, washed and stained overnight with 1% (w/v) uranyl acetate, dehydrated in ethanol series and embedded in Epon resin. Cellular fatty acids were extracted with a mixture of methanol–chloroform and analyzed by GC-MS according to Zhilina et al. (1997).

Genetic and phylogenetic analysis

The isolation of the DNA and determination of the G + C content of the DNA was performed according to Marmur (1961) and Marmur and Doty (1962). Genomic DNA was extracted from the cells using the UltraClean Soil DNA Extraction Kit (MolBio Laboratories, USA), following the manufacturer's instructions. The nearly complete 16S rRNA gene was obtained from pure cultures using bacterial primers GM3f (5′-AGAGTTTGATCCTGGCTCAG-3′) and GM4r (5′TACGGTTAC-CTTGTTACGACTT-3′). To attempt to amplify the *dsr*AB gene, a mix of primers according to Zverlow et al. (2005) was used. The 16S rRNA gene PCR products were purified from low-melting agarose using the Wizard PCR-Prep kit (Promega, USA). Sequencing was performed using Big Dye Terminator v.3.1 sequencing reaction kit on an ABI 3730 DNA automatic sequencer (Applied Biosystems, Inc., USA). The sequences were aligned with those from GenBank using CLUSTALW. The 16S rDNA sequences were first compared to sequences stored in the GenBank database using BLAST search tool. Phylogenetic trees were reconstructed with four different algorithms using the TREECONW software package (van de Peer and de Wachter 1994). Pairwise evolutionary distances (expressed as estimated changes per 100 nucleotides) were computed by using Jukes and Cantor (1969) method. A resulting phylogenetic tree was constructed by the neighbour-joining method.

Results and discussion

Enrichment and isolation of pure cultures

Positive enrichment cultures with H_2 as the electron donor and polysulfide as the electron acceptor were obtained from all three sites after 4 weeks of incubation. It did not result in a visible growth but polysulfide sulfur was completely reduced to sulfide. In further stabilizing positive transfers only Wadi Natrun culture consistently reduced polysulfide and after serial dilution it was plated onto polysulfide alkaline agar. Several colony types were observed which formed a halo of decolorized polysulfide pointing to the presence of an active organism. However, only the dominant reddish colonies resulted in stable growth in liquid medium. The isolate obtained from these colonies was designated AHT 2. Among the anaerobic enrichments with H_2 and thiosulfate only Mongolian culture actively produced sulfide. The dominant colonies obtained on thiosulfate alkaline agar from the final dilution were black due to FeS accumulation and their transfer back into the liquid medium yielded strain AHT 1.

Cell morphology

Cells of strain AHT 1 are rods of variable length, motile with peritrichous flagella and with Gram-positive type of cell wall. Forms terminal ellipsoid thermostable endospores in swollen sporangia (Fig. 1a, c, e). AHT 2 is a nonmotile (phase contrast examination of live cultures and electron microscopy) vibrio with Gram-negative cell wall (Fig. 1b, d).

Phylogenetic analysis

Phylogenetic analysis based on nearly complete sequence of the 16S rRNA gene indicated that both isolates represent novel bacterial lineages. AHT 1 is a member of the family *Syntrophomonadaceae* with only a distant relationship to its culturable representatives. A highest match among the uncultured Gram-positive bacteria was a clone from Mono

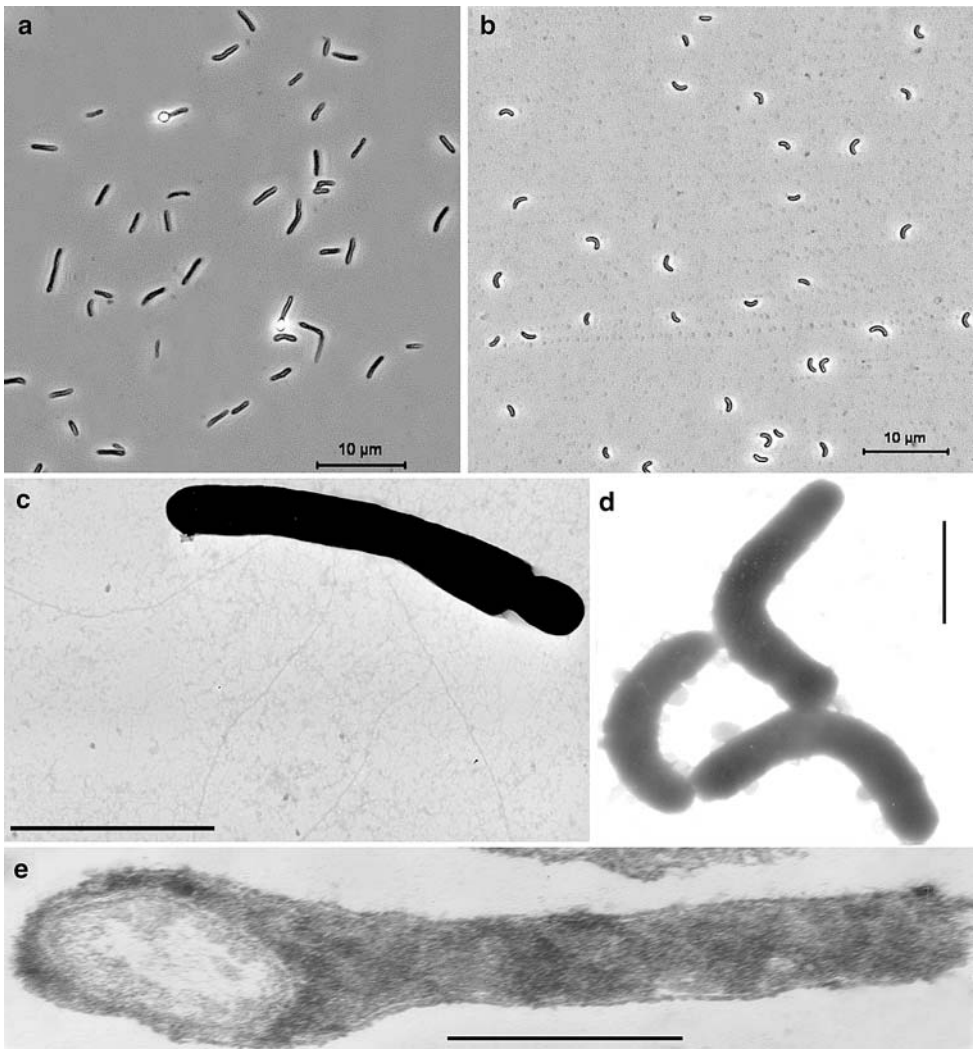

Fig. 1 Cell morphology of strain AHT 1 (**a, c, e**) and AHT 2 (**b, d**). **a**, **b** Phase-contrast microphotographs, **c, d** total electron microphotographs of positively stained cell, *bar* 1 µm, **e** electron microphotograph of thin section, *bar* 1 µm

Lake with 95% sequence identity to AHT 1 (Fig. 2a). In the same cluster is recently described "Candidatus Contubernalis alkalaceticum"—a clostridium from soda lake, oxidizing acetate in syntrophic association with alkaliphilic SRB (Zhilina et al. 2005). AHT 2 is a new member of the *Deltaproteobacteria* with arsenate-respiring haloalkaliphilic strain MLMS-1 from Mono Lake (Hoeft et al. 2004) as the closest culturable relative (94% sequence similarity). Strain MLMS-1 is unique in its capacity to grow lithoautotrophically with sulfide as electron donor and arsenate as acceptor, but, similar to AHT 2, can not use sulfate as acceptor. Together with the other sequences of uncultured bacteria from Mono Lake they formed a separate group loosely associated with the family *Desulfobulbaceae* (Fig. 2b).

Cellular fatty acid analysis

Three dominant species (16:1w5, 16:0 and 18:1w7) were identified among the fatty acid of membrane lipids in strain AHT 2 comprising more than 75% of the total. In AHT 1 the distribution among the different species was more even but with an obvious domination of saturated species, such as 14:0, 16:0, 18:0 and 20:0 (Supplementary Table).

Cytochromes

The cell mass of both strains was pinkish when separated from the black FeS colloids by low-speed centrifugation. In the cell-free extracts a presence of cytochromes *b* and *c*-types have been detected in dithionite-reduced minus air-oxidized difference spectra with alpha peaks at 559–561 and 553–554 nm, respectively (data not shown).

Metabolic properties and growth characteristics

The basic metabolic properties of the novel isolates are summarized in Table 1. Both isolates are characterized by anaerobic respiratory metabolism and the ability to grow chemolithoautotrophically with H_2 as the electron donor.

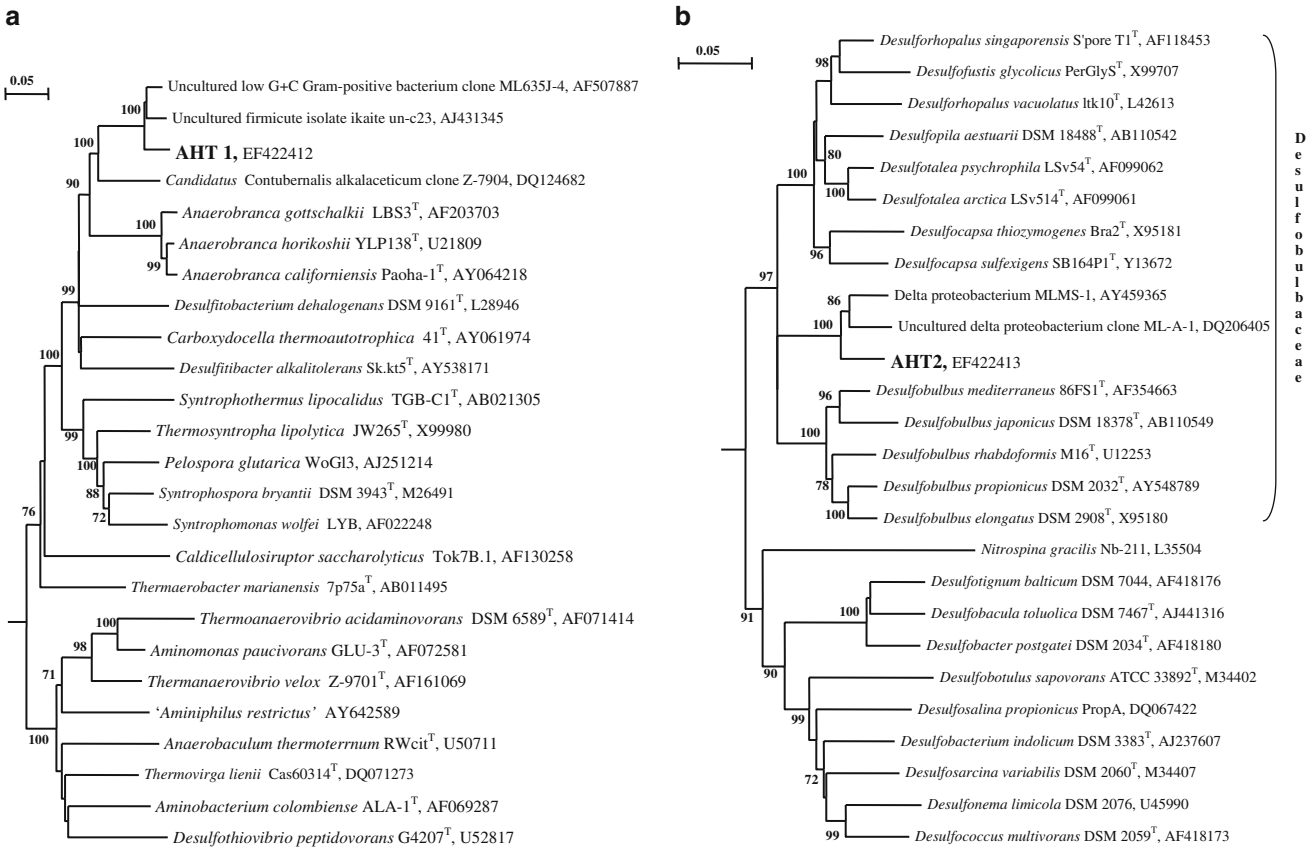

Fig. 2 Phylogenetic position based on 16S rDNA sequence analysis of strain AHT 1 within the family *Syntrophomonadaceae* (**a**) and AHT 2 within the Deltaproteobacteria (**b**). The trees were constructed using maximum likelihood method and filter. The *scale bar* represents 10 nucleotide changes per 100 nucleotides. The percentage of bootstraps was derived from 1000 resampling using neighbour-joining algorithm, only values greater than 70 are given

Both can utilize elemental sulfur/polysulfide and thiosulfate as electron acceptor, but AHT 1 preferred thiosulfate, while AHT 2 preferred sulfur/polysufide. Furthermore, AHT 2 utilized thiosulfate only when H_2 was present as the electron donor. In both strains growth with insoluble elemental sulfur resulted in accumulation of intermediate yellow–orange polysulfide in a narrow layer of liquid medium adjacent to the layer of sulfur (Supplementary Figure). Polysulfide was formed in abiotic reaction between sulfide (final product of sulfur reduction) and elemental sulfur and was a stable and abundant intermediate at high pH, in contrast to the cultures of neutrophilic sulfur reducers, where it immediately decomposes into sulfur and sulfide. The latter demands a means to stabilize polysulfide in cultures of neutrophilic sulfur reducers, such as polysulfide-binding protein (Sud) described in *Wolinella* (Klimmek et al. 1998), while in alkaliphiles such elements in sulfur-reducing system might not be necessary. Apart from mentioned sulfur species, AHT 2 was also capable of growth by dissimilatory reduction of nitrate to ammonia. However, the reduction was never complete, since intermediate nitrite accumulation inhibited growth even at limited nitrate supply (5 mM) in contrast to previously described alkaliphilic sulfur reducer *Desulfurispirillum alkaliphilum* (Sorokin et al. 2007). In addition to the electron donors commonly utilized by sulfidogens, such as short chain fatty acids and alcohols, strain AHT 1 appears to be able to dissimilate glucose and fructose in presence of thiosulfate as acceptor, but not without. The ability to utilize thiosulfate as electron acceptor with H_2 as electron donor has been shown in some members of the *Thermoanaerobiaceae*, although this group basically consists of fermentative clostridia (Fardeau et al. 1994). In contrast to them, AHT 1 seems to move further into the direction of secondary sulfidogenic anaerobes. Other examples of such specialization in clostridia are represented by recently described alkalitolerant *Desulfitibacter alkalitolerans* from communal heating system (Nielsen et al. 2006) and halo-alkaliphilic thermophile *Natrananaerobius thermophilus* from alkaline Wadi Natrun lakes (Mesbach et al. 2007). In contrast to AHT 2, however, strain AHT 1 was not able to use acetate and butyrate as electron donor, since there was no growth with these compounds without H_2. Addition of 2–5 mM acetate to H_2-growing cultures of AHT 1

substantially increased the biomass yield but not sulfide formation, indicating utilization only as a carbon source. AHT 2 on its basic properties is very similar to acetate-oxidizing SRB except its inability to reduce sulfate. The anaerobic growth dynamics and product formation at optimal conditions are shown on Fig. 3. Estimated maximal specific growth rates for AHT 1 (H_2 + acetate/thiosulfate) and AHT 2 (butyrate/sulfur) were 0.015 and 0.04 h^{-1}, respectively. Accumulation of sulfite was noticed in thiosulfate-respiring cultures of AHT 1 but not AHT 2. Accumulation of sulfide in the medium was in most of the cases parallel to biomass growth.

Short-term experiments with washed cells (Table 2) demonstrated that both strains grown with thiosulfate as acceptor are capable of thiosulfate and sulfur reduction, while the cells grown with sulfur/polysulfide only reduced sulfur. This is an indication of involvement of different enzymes in thiosulfate and sulfur reduction. It is known, for example, that the dissimilatory thiosulfate reductase (*Phs*) in enteric bacteria can catalyze both thiosulfate and sulfur reduction, while polysulfide/sulfur reductase (*Psr*)—only polysulfide (Hinsley and Bercks 2002). Same enzyme types might be involved in sulfur dissimilation in novel alkaliphiles. However, in contrast to strain AHT 1, AHT 2 cells grown with thiosulfate also exhibited sulfite-reductase activity, albeit weak in comparison with the thiosulfate/sulfur-reducing potential. The cells of strain AHT 1 grown with thiosulfate, similar to growing culture, released sulfite into the supernatant during thiosulfate reduction. Together with the absence of sulfite-reducing activity this suggests a different mechanism for overall thiosulfate reduction in AHT 1. On the other hand, our attempts to find *dsrAB* gene (coding for the dissimilatory sulfite reductase), failed for both strains. In the case of AHT 1, no amplification was observed at all, while AHT 2 gave a product of the right size but its sequence was not from the *dsrAB* family. Clearly, an enzyme reducing sulfite in AHT 2 is different from the ordinary DSR. It might be speculated, that this could be due to unspecific activity of *ccNIR*—cytochrome *c* dissimilatory nitrite reductase. This enzyme, fulfilling 6e-reduction of nitrite to ammonia, must be present in AHT 2 since it can grow anaerobically with nitrate with ammonia as a final product. The ability to reduce sulfite is known for *ccNIR* from sulfate-reducing bacteria (Stach et al. 2000).

Influence of pH and salt on growth and sulfidogenic activity

Both growing cultures and washed cells exhibited optimal performance within the alkaline pH range with optimum for growth at pH 9.5 and for the activity at pH 10 (Figs. 3, 4, 5). In both strains growth was not observed already at pH below 8.5, thus qualifying them as obligate alkaliphiles. On the other hand, maximum pH limit for growth (10.3) indicated moderate growth tolerance for high pH, despite the fact that sulfidogenesis remained active until pH 11.

Table 1 Metabolic properties of the novel haloalkaliphilic sulfidogenes

Property	AHT 1	AHT 2
Autotrophic growth with H_2	+	+
Electron acceptors	S_8/S_x^{2-}, thiosulfate	S_8/S_x^{2-}, thiosulfate, nitrate
Electron donors/C source	Lactate, ethanol, pyruvate, propanol, fumarate, glucose, fructose, glycerol	Formate, ethanol, pyruvate, lactate, fumarate, glycerol butyrate, isobutyrate, malate, succinate, yeast extract
Utilization of acetate	C-source	C-source and e-donor

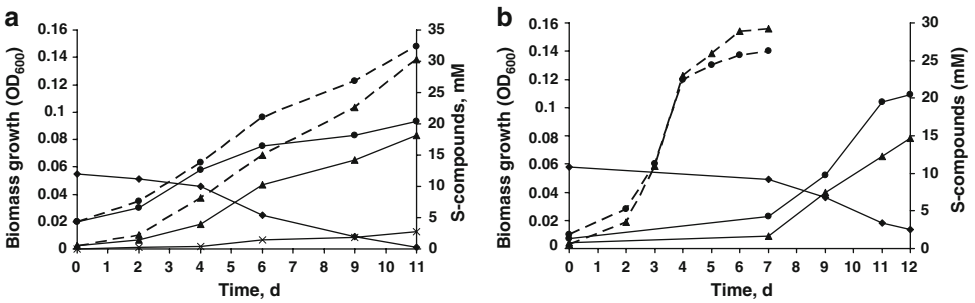

Fig. 3 Growth and dynamics of sulfur compounds in batch cultures of strain AHT 1 (**a**) and AHT 2 (**b**) at pH 10 and 0.6 M total Na^+. AHT 1 was grown with H_2/acetate. AHT 2 was grown either with H_2/acetate when thiosulfate served as the electron acceptor or with butyrate when sulfur was the electron acceptor. *Solid lines* growth with thiosulfate, *dashed lines* growth with sulfur, *circles* biomass, *triangles* sulfide/sulfane, *diamonds* thiosulfate, *X* sulfite

The same trend was observed in aerobic chemolithoautotrophs from soda lakes (Sorokin and Kuenen 2005a, b). According to their salt tolerance (Figs. 3, 4), the novel isolates belong to moderately salt-tolerant alkaliphiles.

In conclusion, strains AHT 1 and AHT 2 represent further examples of non sulfate-reducing sulfur/thiosulfate-reducing haloalkaliphiles from soda lakes, together with a recently described *Geaoalkalibacter ferrihydriticus*—an iron-reducing alkaliphile also capable of sulfur reduction (Zavarzina et al. 2006). Bacteria respiring thiosulfate and sulfur/polysulfide might have selective advantage against sulfate-reducing sulfidogens in anaerobic soda lake sediments. First of all, reduction of sulfur/polysulfide and thiosulfate, in contrast to sulfate, do not demand energy-consuming activation. Secondly, in soda lakes, thiosulfate and polysulfide could be particularly important intermediates of the sulfur cycle. Polysulfide (S_x^{2-}) is a linear polymeric sulfur species, forming in a spontaneous reaction of elemental sulfur with sulfide, stable at anoxic alkaline conditions. Thiosulfate is a product of spontaneous oxidation of polysulfide at alkaline conditions. Active polysulfide/thiosulfate formation was recently documented in a lab-scale sulfide-oxidizing bioreactor operating under extremely haloalkaline and oxygen-limited conditions (van de Bosch et al. 2007).

The novel isolates belong to the taxonomic groups in which sulfate-independent respiratory sulfidogenesis is well known, but both represent new lineages, according to the phylogenetic analysis. They are proposed to be assigned into new genera: AHT 1 as *Dethiobacter alkaliphilus* gen. nov. sp. nov., within the family *Syntrophomonadacea* (*Clostridiales*) and AHT 2 as *Desulfurivibrio alkaliphilus* gen. nov. sp. nov. in the *Deltaproteobacteria*.

Table 2 Sulfidogenic activity [μmol S^- (mg protein h)$^{-1}$] of washed cells grown with H_2 + acetate as electron donor/carbon source at pH 10 and 0.6 M total Na^+

Conditions	AHT 1		AHT 2	
	Grown: $S_2O_3^{2-}$	Grown: S_8	Grown: $S_2O_3^{2-}$	Grown: S_8
$H_2 + S_2O_3^{2-}$	2.1	0	6.9	0
$H_2 + SO_3^{2-}$	0	0	0.6	0
$H_2 + S_8$	0.5	1.5	3.2	6.0

Description of *Dethiobacter* gen. nov

Dethiobacter (De.thi.o.bac.'ter. L. pref. *de* from, off, away; Gr. n. *thios* sulfur; Gr. n.; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Dethiobacter* rod-shaped bacterium that reduces sulfur compounds).

Gram-positive, endospore-forming rods. Obligately anaerobic with respiratory metabolism. Use sulfur compounds, but not sulfate, as electron acceptor, and short-chain fatty acids and hydrogen as electron donors. Facultatively autotrophic. Obligately alkaliphilic and moderately salt-tolerant. Belongs to the family *Syntrophomonadaceae*

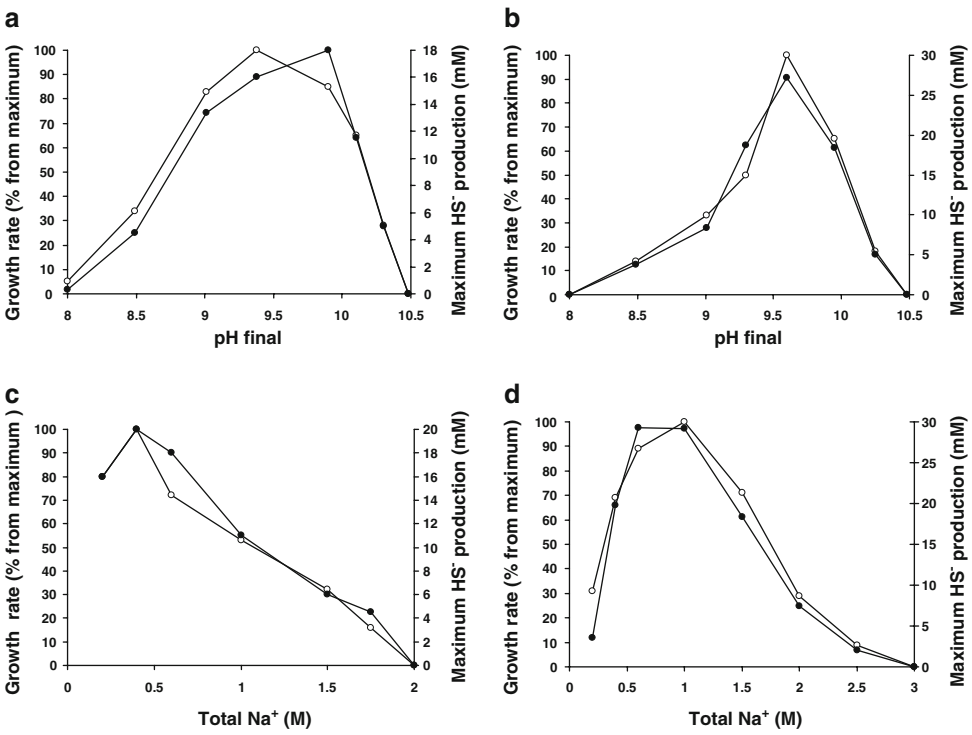

Fig. 4 Influence of pH (**a**, **b**) at 0.6 M Na^+ and soda at pH 10 (**c**, **d**) on growth and sulfide production. AHT 1 (**a**, **c**) was grown with H_2 + acetate + thiosulfate and AHT 2 (**b**, **d**) was grown with butyrate + sulfur. *Open circles* growth rate, *closed circles* formation of sulfide

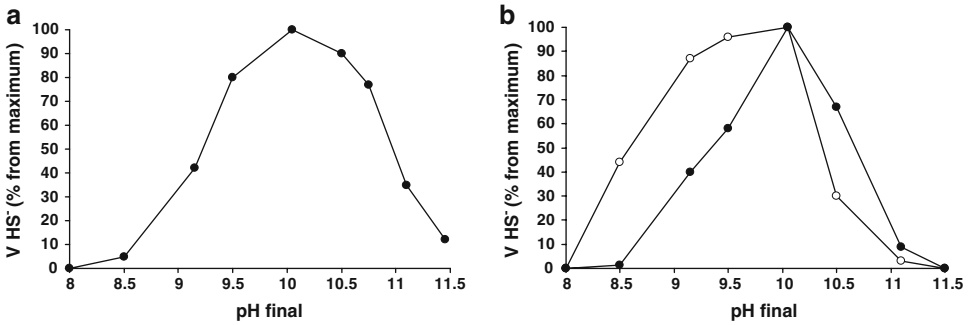

Fig. 5 Influence of pH at 0.6 M total Na^+ on the sulfidogenic activity of washed cells (VHS^-) of strains AHT 1 (**a**) and AHT 2 (**b**). AHT 1 was grown and the cells were tested with H_2+acetate + thiosulfate; AHT 2 was grown and the cells were tested with either H_2/acetate + thiosulfate (*open circles*) or with butyrate + sulfur (*closed circles*)

within the phylum *Clostridiales*. The type species is *Dt. alkaliphilus*. Habitat—soda lake sediments.

Description of *Dethiobacter alkaliphilus* sp. nov

alkaliphilus (al.ka.li.phi′lus M.L. n. *alkali* soda ash; Gr. adj. phylum *loving*; M.L. adj. *alkaliphilus* loving alkaline conditions)

Cells are Gram-positive terminal endospore-forming rods with swollen sporangia, 0.4–0.5 × 1.5–4 μm, motile by peritrichous flagella. Strictly anaerobic with respiratory metabolism. Use thiosulfate and elemental sulfur/polysulfide as electron acceptors. Sulfide is the final product and sulfite is an intermediate of thiosulfate reduction. Facultatively autotrophic (with H_2 as electron donor). Utilizes acetate as carbon source and lactate, pyruvate, ethanol, glycerol, glucose and fructose as both electron donor and carbon source. Obligately alkaliphilic with a pH range for growth between 8.5 and 10.3 and an optimum at pH 9.5 and moderately salt-tolerant with a total Na^+ range for growth from 0.2 to 1.8 M (optimum at 0.4 M). Mesophilic, with a maximum temperature for growth at 45°C. Cellular fatty acids are dominated by $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ and $C_{20:0}$. The G + C content of the genomic DNA is 46.5 ± 0.5 mol% (T_m). The type strain is AHT 1^T (DSM 19026^T = UNIQEM $U266^T$). Isolated from mixed sediments of north–eastern Mongolia soda lakes. The GenBank 16S rRNA gene sequence accession number is EF422412.

Description of *Desulfurivibrio* gen. nov

Desulfurivibrio (De.sul.fu.ri.vib.'.rio L. pref. *de* from, off, away; L. n. *sulfur* sulfur; M.L. masc. v*ibrio* that which vibrates; M.L. masc. n. *Desulfurivibrio* vibrio that reduces sulfur compounds).

Gram-negative bacterium with vibrio-shaped cells. Obligately anaerobic with respiratory metabolism. Utilizes sulfur compounds, but not sulfate, as electron acceptor, and short-chain fatty acids and hydrogen as electron donors. Obligately alkaliphilic and moderately salt-tolerant. Belongs to the *Deltaproteobacteria*. The type species is *Dsv. alkaliphilus*. Habitat—soda lake sediments.

Description of *Desulfurivibrio alkaliphilus* sp. nov

alkaliphilus (al.ka.li.phi′lus M.L. n. *alkali* soda ash; Gr. adj. phylum *loving*; M.L. adj. *alkaliphilus* loving alkaline conditions)

Cells are vibrio-shaped, 0.4–0.5 × 1.5–2.0 μm, non-motile. Strictly anaerobic with respiratory metabolism. Uses thiosulfate and elemental sulfur/polysulfide as electron acceptor with sulfide as the final reduced product and nitrate with ammonia as the final product. Facultatively autotrophic (with H_2 as electron donor). Utilizes formate, acetate, lactate, pyruvate, ethanol, butyrate, isobutyrate, malate, succinate and yeast extract as electron donors and carbon source. Obligately alkaliphilic with a pH range for growth between 8.5 and 10.3 and an optimum at pH 9.5 and moderately salt-tolerant with a total Na^+ range for growth from 0.2 to 2.5 M (optimum at 0.5–1.0 M). Mesophilic, with a maximum temperature for growth at 42°C. $C_{16:0}$, $C_{16:1w5}$ and $C_{18:1w7}$ are the dominant cellular fatty acids. The G + C content of the genomic DNA is 40.5 ± 0.5 mol% (T_m). The type strain is AHT 2^T (DSM 19089 = UNIQEM U267). Isolated from mixed sediments of Wadi Natrun haloalkaline lakes in Egypt. The GenBank 16S rRNA gene sequence accession number is EF422413.

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