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Metabolic versatility of haloalkaliphilic bacteria from soda lakes belonging to the *Alkalispirillum–Alkalilimnicola* group

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Abstract Four new isolates were obtained from denitrifying enrichments with various electron donors using sediment samples from hypersaline soda lakes. Based on 16S rRNA gene analysis and DNA-DNA hybridization results, they were all identified as members of the Gammaproteobacteria closely associated with the *Alkalispirillum–Alkalilimnicola* group. Two isolates were obtained from samples enriched with nitrate as electron acceptor and H_2 or polysulfide as electron donors, and another two strains were obtained with N_2O as the electron acceptor and sulfide or acetate as electron donors. All four new isolates, together with the type strains of the genera *Alkalispirillum* and *Alkalilimnicola* originally described as obligate aerobes, were capable of anaerobic growth with acetate using either nitrate or N_2O as electron acceptors. Their denitrification pathway, however, was disrupted at the level of nitrite. RuBisCO form I gene was detected and sequenced in the new isolates and in *Alkalilimnicola halodurans* but not in *Alkalispirillum mobile*. These data, together with the evidence of Oremland et al. (Appl Environ Microbiol 68:4795–4802, 2002) on the potential of *Alkalilimnicola* sp. MLHE-1 for autotrophic growth with arsenite as electron donor and nitrate as electron acceptor, demonstrate much higher metabolic diversity of this specific group of haloalkaliphilic Gammaproteobacteria than was originally anticipated.

Keywords *Alkalispirillum* · *Alkalilimnicola* · Soda lakes · Haloalkaliphilic denitrification

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Introduction

Soda lakes represent a unique habitat with high pH and variable (up to saturation) salt content, with a substantial proportion of alkaline carbonates among the soluble salts. The microbial diversity in soda lakes has received much attention with respect to the adaptation of life to haloalkaline conditions (Grant et al. 1990; Zavarzin and Zhilina 2000; Humayoun et al. 2003; Rees et al. 2004) and for possible biotechnological application of the extracellular enzymes of haloalkaliphilic bacteria (Horikoshi 2004).

Despite the doubly extreme conditions in alkaline and hypersaline soda lakes, most of the structural-functional groups present in normal microbial communities have been identified also there, except that they are almost entirely populated with prokaryotes represented by specific, haloalkali-philic or -tolerant species (Jones et al. 1998; Zavarzin et al. 1999). In general, the most well-studied groups in haloalkaliphilic communities are primary producers (cyanobacteria and purple sulfur bacteria) and anaerobes responsible for the degradation of complex organic compounds to simple final products, such as volatile fatty acids, methane, H_2, sulfide and ammonia. The latter are mostly represented by low GC Gram-positive bacteria. There is limited information on methanogenic and sulfate-reducing populations in soda lakes (Zavarzin et al. 1999; Zavarzin and Zhilina 2000). Recently, bacteria responsible for the oxidation of reduced inorganic compounds, such as methane, hydrogen, sulfide and ammonia, produced by the haloalkaliphilic anaerobes in soda lake sediments, have also been

identified (Sorokin and Kuenen 2005). However, some of the thermodynamically feasible pathways still lack culturable haloalkalphilic representatives. In particular, it is not clear, what kind of bacteria might be responsible for the oxidation of hydrogen and sulfide/polysulfide (instead of thiosulfate) under denitrifying conditions in soda lakes, as well as which group might benefit from the use of N_2O (instead of *Halomonas*-dominated nitrate/nitrite reduction) during heterotrophic denitrification. This paper describes four new haloalkaliphilic Gammaproteobacteria, members of the *Alkalispirillum–Alkalilimnicola* group, isolated from various soda lakes and capable of the above mentioned types of catabolic reactions.

Material and methods

Samples

Sediment samples from moderately and hypersaline alkaline lakes from three different locations have been used for the denitrifying enrichments: from the Kenyan lake Magadi (total salts 240 g l^{-1}, pH 10.5, carbonate alkalinity 1.2 M); two samples from North-Eastern Mongolia: a mixed sample from two hypersaline lakes (total salts 200–390 g l^{-1}, pH 9.5–10.3, carbonate alkalinity 0.8–1.0 M) and a sample from saline lake Gurvany-Nur (total salts 50 g l^{-1}, pH 10.2, carbonate alkalinity 0.3 M) (Sorokin et al. 2004); a mixed sample from eight Wadi Natrun lakes in Egypt (total salts 220–360 g l^{-1}, pH 9.2–10.2, carbonate alkalinity 0.13–0.75 M).

Cultivation

Autotrophic enrichments with nitrate as the electron acceptor were performed at low salt content (0.6 M total Na^+) on mineral medium containing (g l^{-1}): Na_2CO_3, 23; $NaHCO_3$, 7; NaCl, 6; K_2HPO_4, 1; KNO_3, 1. For heterotrophic enrichments with acetate and N_2O, hypersaline medium (4 M total Na^+) with the following composition was used (g l^{-1}): Na_2CO_3, 93; $NaHCO_3$, 17; NaCl, 120; K_2HPO_4. After sterilization, 5 mM NH_4Cl was added as nitrogen source instead of KNO_3. For autotrophic enrichment with sulfide (3 mM) as electron donor and N_2O as the electron acceptor, a medium containing (g l^{-1}): Na_2CO_3, 59; $NaHCO_3$, 20; NaCl, 21; K_2HPO_4, 0.2 was used. After sterilization, pH was 10.0–10.15 in both media, and both media were supplemented with 1 ml l^{-1} of trace element solution (Pfennig and Lippert 1966); 1 mM $MgSO_4$, 0.5 mM sodium thiosulfate, and 50 μg l^{-1} of vitamin B_{12}. In addition, enrichments with N_2O, contained 10–20 mM sodium acetate, 50 mg l^{-1} of yeast extract and, in some cases, 2 mM Na_2S. An amount of 50 ml portions of the medium were dispensed into 100 ml serum bottles inoculated with 0.5 ml sediments and made anaerobic by vigorous vacuum degassing/flushing with argon. H_2 was used at overpressure of 0.5 bar after removal of argon. In the case of N_2O, the flasks were evacuated and the atmosphere was replaced by pure N_2O gas without overpressure (2 mmol/flask). Polysulfide (S_6^{2-}) was synthesized as described previously (Sorokin et al. 2001) and added by syringe to a concentration of 2 mM sulfane after establishing the gas phase.

Isolation of pure cultures

Enrichment cultures were stabilized by several 1:100 transfers into the same media and then the cultures were serially diluted in 20 ml Hungate tubes with 10 ml medium. The highest dilutions showing growth were plated onto solid media prepared by 1:1 mixing of sterile 4% agarose and liquid media at 50°C. The plates were incubated in closed 3.5 l jars for anaerobic cultivation (Oxoid) with an oxygen-consuming catalyzer (Anaerogen, Oxoid). The atmosphere in the jar was the same as in the enrichment flasks. Plates with polysulfide were prepared and inoculated in an anaerobic box.

Analyses

Sulfur and nitrogen compounds and cell protein were analyzed as described previously (Sorokin et al. 2001). Nitrate- and nitrite reductase activity was tested by the whole cell approach using washed cells grown anaerobically with acetate and nitrate. The cells were resuspended in the mineral growth medium lacking all nutrients and trace metals, made anaerobic and incubated with either 5 mM nitrate or 2 mM nitrite in presence of 5 mM acetate with periodic test for nitrite production/consumption. Oxygen consumption by washed cells in the presence of various reduced sulfur compounds was measured with an oxygen electrode as described previously (Banciu et al. 2004). Cells for electron microscopy were fixed with glutaraldehyde (3% final, v/v) and stained either positively with 1% uranyl acetate or negatively with 1% phosphotungstic acid (w/v).

Isolation of DNA, subsequent determination of the G + C content of the DNA and DNA-DNA hybridization were performed by thermal denaturation/reassociation technique, according to Marmur (1961) and De Ley et al. (1970). DNA for PCR amplification and sequencing of 16S rRNA genes was purified by standard phenol-chloroform extraction. The 16S rRNA genes were selectively amplified using primers 5′-AGAGTTTGATCCTGGCTCAG-3′ (forward) and 5′-TACGGTTACCTTGTT-ACGACTT-3′ (reverse). PCR products were purified from low-melting agarose using the Wizard PCR-Prep kit (Promega, USA) according to the manufacturer's instruction. Almost complete sequences (1,400–1,450 nucleotides) were obtained using

the Promega Silver Sequencing kit (Promega) with minor modifications of the manufacturer's instructions. Amplification and sequencing of *cbbL* genes was performed according to Spiridonova et al. (2004). Phylogenetic analysis based on nucleotide sequences of 16S rRNA genes and *cbbL* inferred amino acid sequences were performed with treeing algorithms in the TREECON (van de Peer and De Wachter 1994) software packages. Pairwise evolutionary distances (expressed as estimated changes per 100 nucleotides) were computed by using the Jukes and Cantor method. A resulting phylogenetic tree was constructed by the neighbour-joining method. Bootstrap analysis (1,000 replications) was used to validate the reproducibility of the branching pattern of the trees.

Results

Strain AHN 1

Anaerobic autotrophic enrichments at 0.6 M Na^+, pH 10, with nitrate as the electron acceptor and H_2 as the electron donor resulted in almost complete reduction of 20 mM nitrate to nitrite within 10 days with sediments from lakes in Mongolia, Egypt and Lake Magadi in Kenya. Nitrite reduction and gas formation was either extremely slow (Mongolia) or not observed at all. The most active and stable nitrate-reducing culture from the Lake Magadi was further purified, after serial dilution, using solid medium. Large brownish colonies, transferred back to the liquid medium, were able to grow anaerobically with hydrogen and nitrate. However, autotrophic growth was less vigorous than in the enrichment culture ($\mu_{max} = 0.04\ h^{-1}$) Addition of 2–5 mM acetate as a carbon source, as well as yeast extract, significantly enhanced the growth rate and growth yield. The isolate was designated AHN 1. The cells of AHN 1 were fat rods (Fig. 1d), motile with 1–2 subpolar flagella (Fig. 1f). Cells grown in presence of acetate accumulated large amounts of PHB-like granules and it was virtually impossible to grow the culture back under autotrophic conditions, although DNA-DNA hybridization demonstrated 100% homology between the autotrophic and heterotrophic lineages. AHN 1 did not grow autotrophically with hydrogen under aerobic conditions at oxygen concentrations of 1–20% in the gas phase or with nitrogen oxides other than nitrate. Heterotrophic growth was possible under fully aerobic conditions and anaerobically with nitrate or N_2O as electron acceptors, but not with nitrite. The isolate required reduced sulfur (thiosulfate) and vitamins during both aerobic and anaerobic growth. During anaerobic growth with acetate and nitrate, sulfide was oxidized to elemental sulfur. Tests for salt requirement showed low salt tolerance for autotrophic growth (0.5–1 M Na^+), and extreme tolerance for heterotrophic growth (up to 4 M Na^+ with an optimum at 2 M).

Strains ALPs 2 and Z-7008

Anaerobic enrichments with nitrate as the electron acceptor and polysulfide as electron donor at 0.6 M Na^+, pH 10, resulted in rapid disappearance of the yellow color of polysulfide followed by elemental sulfur production. After several consecutive additions of polysulfide, about 90% of added nitrate (20 mM) was converted to nitrite. Afterwards no further polysulfide oxidation occurred. Hence, it might be concluded that the selected population was capable of incomplete denitrification with oxidation of flanking sulfane atoms of polysulfide to sulfur. Plating of the most active culture, obtained from the Mongolian soda lake sediments, onto the polysulfide-nitrate solid medium resulted in development of a single sulfur-forming colony containing short motile vibrios (Fig. 1b). The strain was designated ALPs 2. It was able to grow lithoautotrophically by oxidizing sulfane atoms of polysulfide or HS^- to elemental sulfur with nitrate as electron acceptor. The latter was reduced exclusively to nitrite. Nitrite and N_2O did not support anaerobic growth with polysulfide. Elemental sulfur and thiosulfate were not oxidized. ALPs 2 did not grow with hydrogen, either aerobically or in presence of nitrate. Acetate and yeast extract stimulated anaerobic growth with reduced sulfur compounds. Heterotrophic growth with acetate was possible with oxygen, nitrate and N_2O as electron acceptors, but only in presence of at least 0.1 mM of reduced sulfur, supplied as sulfide or thiosulfate. The latter indicated demand of reduced sulfur for assimilation.

Anaerobic enrichments at high salt content with sulfide as the electron donor and N_2O as the electron acceptor were obtained with the Wadi Natrun mixed sample and with the sample from Mongolian lake Gurvany-Nur. The latter was more active, and stable and further work resulted in the isolation of fat vibrios with a single polar flagellum designated strain Z-7008 (Fig. 1a, e). The culture developed in two phases: first sulfide was converted to polysulfide, most likely from the abiotic reaction between elemental sulfur (a true product of biological oxidation) and the remaining sulfide. As soon as the free sulfide was consumed, polysulfide began to disappear with accumulation of elemental sulfur as the final product. N_2O was reduced to dinitrogen gas. The same sequence was observed in presence of nitrate instead of N_2O as the electron acceptor with elemental sulfur and nitrite as final products. Thiosulfate was not oxidized either aerobically or under denitrifying conditions. Addition of acetate stimulated both the biomass formation and sulfide oxidation. Maximum sulfide oxidation rate by washed cells grown anaerobically in presence of sulfide/polysulfide was 120 nmol (mg protein min)$^{-1}$. The isolates also grew well heterotrophically with acetate as substrate and oxygen, nitrate and N_2O (but not nitrite) as the electron acceptors. Under aerobic conditions with acetate as the substrate, both isolates grew over a wide salinity range 0.05–4 M Na^+, with an optimum around 1 M.

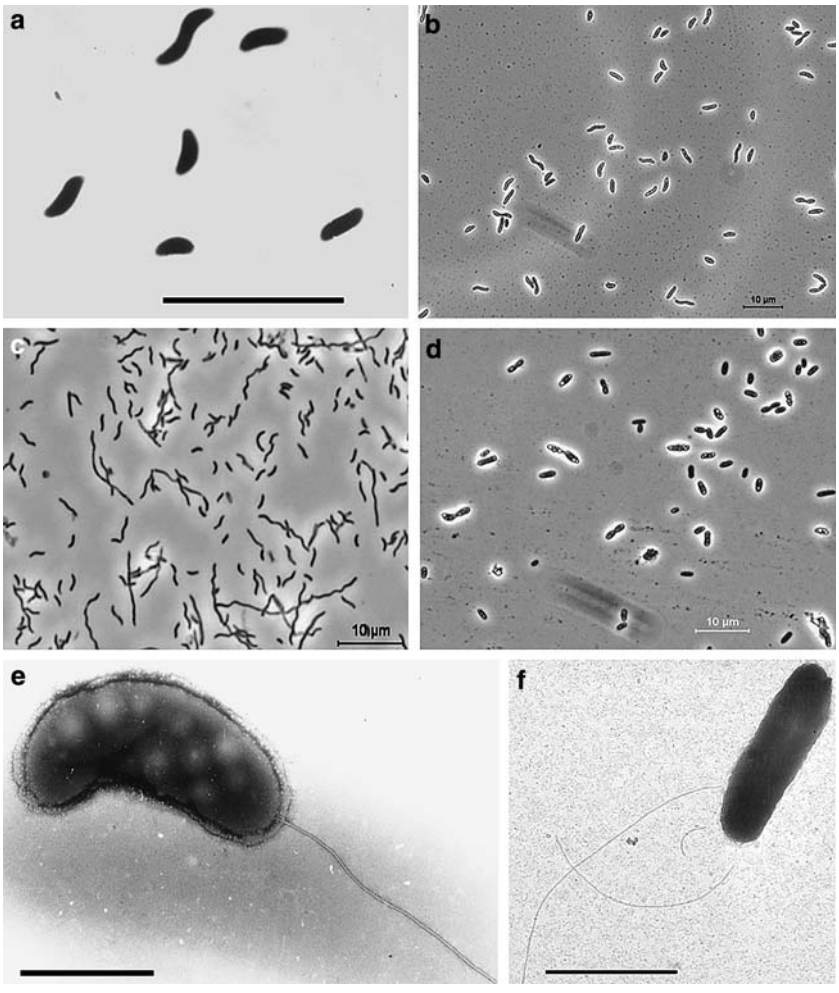

Fig. 1 Cell morphology of the new isolates. **a**–**d** Phase contrast micrographs, bar = 10 µm; **e**–**f** Electron microphotographs of whole cells, bar = 1 m. **a** and **e** str. Z 7008; **b** str. ALPs 2; **c** str. AGDZ; **d** and **f** str. AHN 1

Strain AGDZ

Heterotrophic anaerobic enrichments under hypersaline conditions (4 M Na^+, pH 10) with acetate and N_2O gave the most active development with the mixed sample from the Wadi Natrun lakes in Egypt. It resulted in the isolation of strain AGDZ, which had spiral cells of variable length, from short vibrios to long spirillae (Fig. 1c), motile with a single polar flagellum. Apart of N_2O, the isolate grew aerobically and anaerobically with nitrate and acetate. It required a reduced sulfur source and vitamins for anaerobic growth. Similar to strains Z-7008 and ALPs 2, AGDZ was able to oxidize sulfide and polysulfide to elemental sulfur during anaerobic heterotrophic growth with nitrate, but it did not grow with sulfide or polysulfide without an organic electron donor. Furthermore, high sulfide or polysulfide concentrations that could be tolerated by Z-7008 and ALPs 2 completely inhibited N_2O-dependent denitrification in AGDZ. Similar to the other isolates, strain AGDZ was not able to grow anaerobically with nitrite. AGDZ is a moderate halophile, growing over a salt range of 0.3–4 M Na^+ with an optimum at 0.5–0.75 M for aerobic growth ($\mu_{max} = 0.35\ h^{-1}$) and 1 M for anaerobic growth with N_2O ($\mu_{max} = 0.15\ h^{-1}$).

Identification of the isolates

Phylogenetic analysis demonstrated close affiliation of the new isolates with the *Alkalispirillum–Alkalilimnicola* cluster in the family *Ectothiorhodospiraceae* of the Gammaproteobacteria (Fig. 2a). Both *Alkalispirillum mobile* and *Alkalilimnicola halodurans* are high salt-tolerant haloalkaliphiles isolated from soda lake environments in close association with their phototrophic relatives (Rijkenberg et al. 2001; Yakimov et al. 2001). Of the four new isolates, hydrogen-utilizing strain AHN 1 clustered with *Al. halodurans* (99.5% sequence similarity), while the other three clustered with *As. mobile* (98.8–99.9% of sequence similarities). Arsenite-oxidizing, nitrate-reducing alkaliphile strain MLHE-1, isolated from alkaline, hypersaline Mono Lake (Oremland et al. 2002), is a member of the genus *Alkalilimnicola* (98.2% sequence similarity). DNA-DNA hybridization results (Table 1) furthermore indicated that: (a) *Al. halodurans* and *As. mobile* represent either distantly related species of the same genus or two separate, but closely related genera; (b) close relation of the new sulfide/polysulfide-utilizing denitrifying strains Z-7008 and ALPs 2 and the heterotrophic N_2O-reducing isolate AGDZ to each other and with *Al. mobile* on the subspecies level. Results

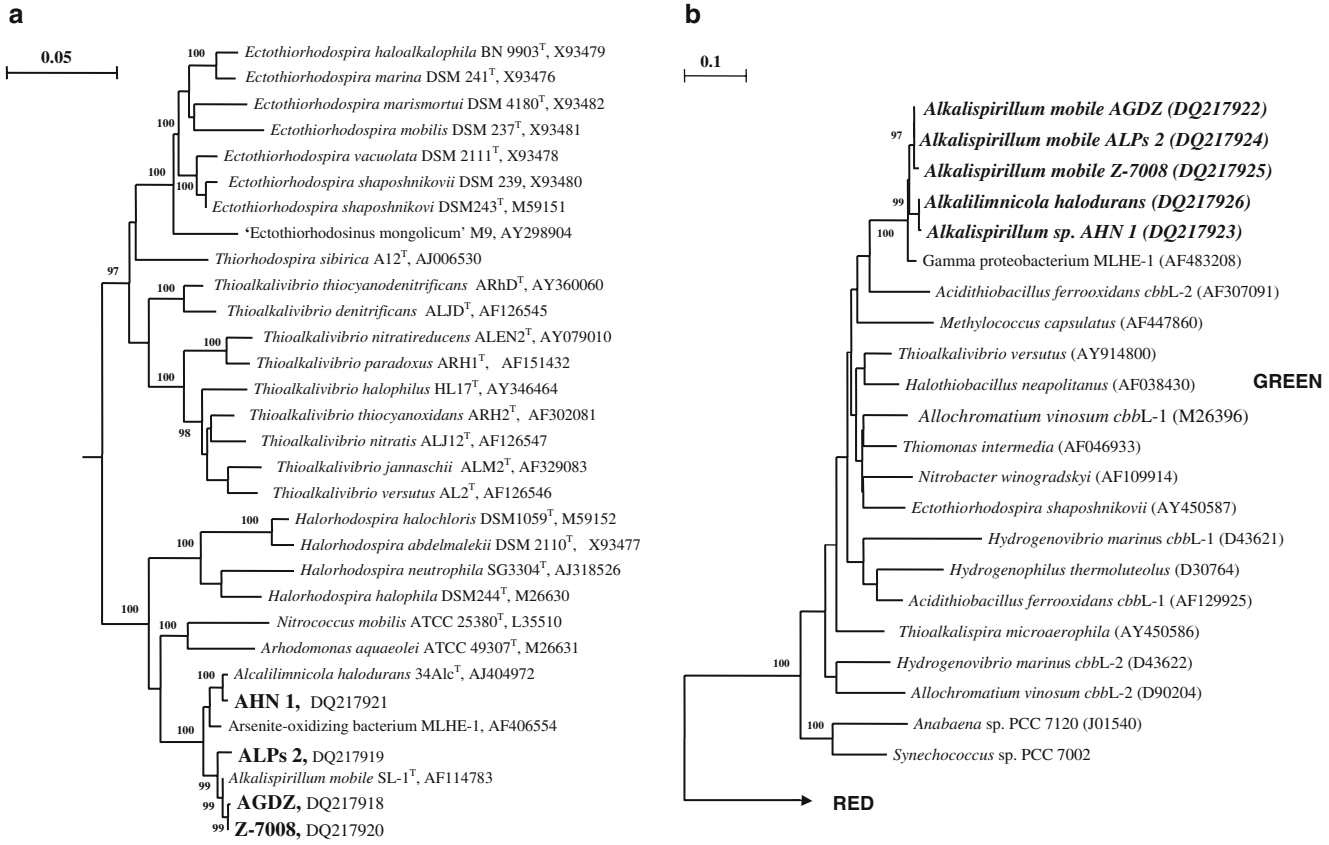

Fig. 2 Phylogenetic position of the new *Alkalispirillum–Alkalilimnicola* strains based on analysis of: **a** the 16S rRNA gene sequences; **b** inferred amino acid sequences encoded by *cbb*L genes. The sequences obtained in this study are shown in *bold*. Tree topography and evolutionary distances are given by the neighbor-joining method with Jukes-Cantor distances. *Numbers* at the nodes indicate percentage bootstrap support >95% for the clade (1,000 iterations)

Table 1 Results of DNA-DNA reassociation within the *Alkalispirillum–Alkalilimnicola* group

Strain	G+C content, mol %	DNA homology, %				
		As. mobile	*Al. halodurans*	Z-7008	ALPs 2	AGDZ
As. mobile	67.0					
Al. halodurans	65.8	31				
Z-7008	66.5	84	35			
ALPs 2	66.6	88	35	79		
AGDZ	66.6	80	33	81	91	
AHN 1	65.0	53	37	55	45	51

of DNA-DNA hybridization for hydrogen-utilizing strain AHN 1 are more difficult to interpret: in accordance with the phylogenetic data, it was distant from the other three isolates and more close related to the genus *Alkalilimnicola*, while the DNA reassociation data placed this bacterium in the genus *Alkalispirillum*, but as a distinct species.

Analysis of the RuBisCO genes

Assuming that some of the new isolates showed ability to grow autotrophically either with hydrogen or reduced sulfur compounds, and taking into consideration the data of Oremland et al. (2002) on the autotrophic growth of *Alkalilimnicola* strain MLHE-1, the presence of *cbb* genes, coding for RuBisCO, the key enzyme of the Calvin-Benson-Bassham cycle, was tested in the new isolates as well as in the type strains of *Alkalispirillum* and *Alkalilimnicola*. The *cbb*L gene, coding for a large subunit of the "green-like" type of form I RuBisCO, was detected in all strains except *Al. mobile*. The final *cbbL*-based phylogenetic tree (Fig. 2b) was consistent with the tree based on the alignment of 16S-rRNA gene sequences (compare with Fig. 2a), indicating monophyletic origin of the group and close relatedness (96.7–100% of amino acids identities) of its members.

Discussion

The genera *Alkalispirillum* and *Alkalilimnicola* were originally described as non-phototrophic, aerobic, heterotrophic relatives of the *Ectothiorhodospira-Halorhodospira* group with a remarkable capacity for haloalkalitolerance (Rijkenberg et al. 2001; Yakimov et al. 2001). Although the possibility for anaerobic growth with nitrate was briefly mentioned for *Alkalilimnicola*, their denitrifying potential was never studied in detail, nor was the capability for lithoautotrophy.

Detailed investigation of As cycling in Mono Lake, California resulted in a discovery of a new type of metabolism–chemolithoautotrophic growth with arsenite as the electron donor and nitrate as the electron acceptor, during which arsenite was oxidized to arsenate with concomitant reduction of nitrate to nitrite (Oremland et al. 2002). The bacterium responsible for this reaction (strain MLHE-1) was isolated in pure culture and identified as a new member/species of the *Alkalispirillum–Alkalilimnicola* group. Furthermore, this bacterium was also able to grow lithoautotrophically with sulfide and hydrogen as substrates and nitrate as an electron acceptor, as well as heterotrophically with acetate under denitrifying and aerobic conditions. This work demonstrated remarkable metabolic versatility, not previously recognized in this group of haloalkaliphilic Gammaproteobacteria.

Our investigation of possible oxidation of various simple inorganic and organic electron donors (final products of anaerobic degradations) under denitrifying conditions in soda lake sediments also demonstrated that there is much more to the metabolic versatility of the *Alkalispirillum–Alkalilimnicola* group and suggested a greater role for thyis group in the transformation of inorganic compounds in soda lakes than was previously anticipated. Combining the information now available on six strains isolated under different conditions and from remote geographical locations, certain generalization can be made on common properties of the group (Table 2).

First of all, obligate alkaliphily and a very broad range of salt tolerance is common for all strains. The next common property is an incomplete denitrification pathway blocked at the level of nitrite reductase, e.g. nitrate can be reduced to nitrite and N_2O to N_2 but, nitrite did not support anaerobic growth with either inorganic or organic electron Donors. Furthermore nitrite reductase activity was not detectable in cell-free extracts obtained from the cells of four different strains (this study) with reduce methyl viologen as electron donor. It must be stressed that our previous investigation of complete denitrification under haloalkaline conditions, both in heterotrophic *Halomonas* spp. or obligate chemolithoautotrophs (genus *Thioalkalivibrio*) isolated from soda lakes, identified nitrite-reduction as a bottle-neck in the denitrification process, with increasing inhibition of nitrite reductase activity at increasing salinity (Sorokin et al. 2001; Sorokin 2003). The interesting question, then, is why representatives of the *Alkalispirillum–Alkalilimnicola* group outcompeted complete denitrifyers in certain enrichments of soda lake communities? With respect to denitrification under heterotrophic conditions (acetate), the only condition when alkalispirilla were selected over halomonads was combination of extreme salt content (4 M Na^+) and N_2O as electron acceptor. Use of nitrate, nitrite or low salt favored development of *Halomonas* (Sorokin 2003).

Table 2 Comparison of the physiological properties of different strains of the *Alkalispirillum–Alkalilimnicola* group

Property	*A. mobile*	*A. halodurans*	ALPs 2	Z-7008	AGDZ	AHN 1	MLEH-1[b]
Cell shape	Vibrio	Oval rod	Vibrio	Vibrio	Spirilla	oval rods	rods
Flagellation	Single, polar	Subpolar	Single, polar	Single, polar	Single, polar	subpolar	+
PHB accumulation on acetate	nd	+	+	+	–	+	nd
Utilization of sugars	–	+	–	–	–	–	nd
Anaerobic growth with:							
Acetate + NO_3^-/N_2O	+[a]	+[a]	+	+	+	+	+[c]
$H_2 + NO_3^-$	–	–	–	–	–	+	+
$HS^-/S_n^{2-} + NO_3^-$	–	–	+	+	–	–	+[d]
$AsO_3^- + NO_3^-$	nd	nd	nd	nd	nd	nd	+
Nitrite reduction	–	–	–	–	–	–	–
Aerobic oxidation of HS^-/S_n^{2-} to S^0	+[a]	+[a]	+	+	+	+	nd
Oxidation of thiosulfate	–[a]	–[a]	–	–	–	–	nd
Salt range (M Na^+) for heterotrophic growth	0–4.1	0–4.5	0.05–4.0	0.05–4.0	0.3–4.0	0.3–4.0	nd
Enrichment	Aerobic, heterotrophic	Aerobic, heterotrophic	$S_n^{2-} + NO_3^-$	$HS^- + N_2O$	Acetate + N_2O	$H_2 + NO_3^-$	$AsO_3^- + NO_3^-$
Isolated from	Summer Lake, Oregon, USA	Lake Natron, Tanzania	N.E. Mongolia, soda lakes	soda lake Gurvany-Nur n-e Mongolia	alkaline lakes Wadi Natrun, Egypt	soda Lake Magadi, Kenya	Mono Lake, California

[a]This work; [b]Oremland et al. 2002; [c]N_2O was not checked; [d]Final product is sulfate; *nd* Not determined

More surprising is the ability of alkalispirilla, basically heterotrophic bacteria, to outcompete chemoautotrophic thiotrophic alkaliphiles, such as *Thioalkalivibrio*, under autotrophic (HS^-/S_n^{2-}), denitrifying (NO_3^-, N_2O) conditions, despite their very slow growth ($\mu_{max} = 0.02\ h^{-1}$).The most probable explanation for this anomaly is high sulfide tolerance of alkalispirilla, allowing them to grow at starting sulfide/polysulfide concentrations as high as 5 mM. Recently, however, we were able to selectively enrich and isolate a nitrate-reducing *Thioalkalivibrio* strain using 2 mM sulfide as substrate under hypersaline conditions (3 M Na^+). The anaerobic growth of this bacterium was completely inhibited by sulfide at concentration above 3 mM. The capacity of alkalispirilla to oxidize sulfide/polysulfide (sulfane atomes) to elemental sulfur seems to be another common feature found in all the strains, although production of sulfate as the final product is mentioned for strain MLHE-1 (Oremland et al. 2002). We have found that the inability of certain bacteria to oxidize thiosulfate to sulfate often correlates with incomplete oxidation of sulfide to sulfur, which demands only a single enzyme in contrast to the thiosulfate-oxidizing enzyme complex needed for complete oxidation. This was true for the five alkalispirilla strains available to us in this study, evident both from analysis of the products and from the respiratory stoicheometry. Perhaps, sulfur metabolism in strain MLHE-1 needs more detailed investigation.

The ability to grow autotrophically with hydrogen or arsenite as electron donors under anaerobic conditions with nitrate as the electron acceptor seems to be a unique feature of the *Alkalispirillum–Alkalilimnicola* group among the soda lake microbial community. However, among our four strains isolated under different conditions, only a single isolate grew with hydrogen. Furthermore, it was virtually impossible to grow this organism under autotrophic conditions after prolong cultivation under heterotrophic conditions. This might indicate either tight genetic control or location of the genes responsible for hydrogenotrophy on a plasmid (that was lost during heterotrophic growth) in this group.

The potential of the *Alkalispirillum–Alkalilimnicola* to grow chemo-autotrophically based on the Calvin cycle is evident from the presence of the key enzyme RuBisCO. Additional evidence for the presence of this pathway in *Alkalispirillum–Alkalilimnicola* group has recently been obtained by culture-independent analysis of the RuBisCO genes in chemocline water of the Mono Lake (Giri et al. 2004). In pure cultures, however, this property is not easily manifested, especially in strains that originated from heterotrophic cultures. Most probably, this type of organisms derive benefit from mixotrophic (lithoheterotrophic) growth using inorganic compounds for energy generation and organic substrates as the carbon sources. For example, autotrophic growth of the strains ALPs 2 and Z 7008 on sulfide and polysulfide was generally very slow ($\mu = 0.01$–$0.02\ h^{-1}$) and addition of small amounts of acetate as a carbon source (1–2 mM) greatly enhanced growth rate and oxidation of sulfide/polysulfide.

Overall, the results of this more detailed investigation clearly demonstrated broad metabolic potential of the obligately haloalkaliphilic, Gammaproteobacteria belonging to the *Alkalispirillum–Alkalilimnicola* group and indicates their potential significance in several redox transformations in soda lakes.

Nucleotide sequence accession numbers

The GenBank accession numbers for the nucleotide sequences determined in this study are DQ217918–DQ217926.

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