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TAXONOMIC DESCRIPTION

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Natronolimnobius sulfurireducens sp. nov. and *Halalkaliarchaeum desulfuricum* gen. nov., sp. nov., the first sulfur-respiring alkaliphilic haloarchaea from hypersaline alkaline lakes

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Abstract

Eight pure cultures of alkaliphilic haloaloarchaea capable of growth by dissimilatory sulfur reduction (previously only shown for neutrophilic haloarchaea) were isolated from hypersaline alkaline lakes in different geographic locations. These anaerobic enrichments, inoculated with sediments and brines, used formate, butyrate and peptone as electron donors and elemental sulfur as an electron acceptor 4 M total Na^+ and at pH 9–10. According to 16S rRNA gene sequencing, the isolates fell into two distinct groups. A major group, comprising seven obligate alkaliphilic isolates from highly alkaline soda lakes, represents a new species-level branch within the genus *Natronolimnobius* (order *Natrialbales*), while a single moderately alkaliphilic isolate from the less alkaline Searles Lake forms a novel genus-level lineage within the order *Haloferacales*. The cells of the isolates are either flat rods or coccoid. They are facultative anaerobes using formate or H_2 (in the presence of acetate or yeast extract as carbon source), C_4–C_9 fatty acids or peptone (the major group) as electron donors and either sulfur or DMSO (the major group) as electron acceptors. Aerobic growth is only possible with organic acids and peptone–yeast extract. All isolates are extreme halophiles, growing optimally at 4 M total Na^+. On the basis of their unique physiological properties and distinct phylogeny, we propose that the seven isolates from the soda lakes are placed into a novel species, *Natronolimnobius sulfurireducens* sp. nov. (type strain AArc1^T=JCM 30663^T=UNIQEM U932^T), and the Searles Lake isolate, AArc-Sl^T, into a new genus and species *Halalkaliarchaeum desulfuricum* (=JCM 30664^T=UNIQEM U999^T).

Our recent discovery of two groups of obligately anaerobic haloarchaea that grow by elemental sulfur respiration either with acetate or formate/H_2 as electron donors [1–5] demonstrated that the widely accepted view of extremely halophilic euryarchaeota as mostly aerobic heterotrophs is far from complete. Previously, we have seen evidence of a similar possible metabolism in hypersaline soda lakes, where alkaliphilic haloarchaea (also known as natronoarchaea) thrive. This evidence arose from measurements of the potential sufidogenic activity in anaerobic sediment slurries from soda lakes in south-western Siberia. We saw an unexpected tendency for formate-dependent elemental sulfur-reduction rates to increase at salt-saturating conditions, in contrast to the usual sharp decrease in many other microbial activities at salinity above 2 M total Na^+ [6, 7]. This indicated that extreme halo(natrono)philes, possibly natronoarchaea, were responsible for this process. Indeed, further microbiological work resulted in the successful enrichment and isolation in pure culture of eight strains of natronoarchaea capable of anaerobic sulfur respiration, which were more versatile than previously described obligate anaerobic sulfur-reducing neutrophilic haloarchaea. The most significant differences were the ability of the natronoarchaeal isolates to: (1) produce much more reduced sulfur anaerobically (taking advantage of the chemical stability of polysulfide); (2) grow aerobically, as well as by sulfur respiration; and (3) utilize a broader range of electron donors for anaerobic respiration [8]. Extensive physiological, genomic and proteomic studies uncovered the key metabolic network involved in sulfur respiration of natronoarchaea, including the presence of polysulfide reductases, membrane-bound hydrogenase and

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The 16S rRNA gene sequences of the AArc strains described here have been deposited in the GenBank under the numbers KY612363–KY612370. The full genome sequences of strains AArc1^T, AArc-Mg and AArc-Sl^T are deposited in GenBank under the numbers CP024047, CP027033 and CP025066, respectively.
Five supplementary figures and two supplementary tables are available with the online version of this article.

formate dehydrogenase, and a machinery for fatty acid oxidation [8].

Here we provide a formal taxonomic description of the natronarchaeal sulfur reducers forming two new taxa, *Natronolimnobius sulfurireducens* sp. nov. and *Halalkaliarchaeum desulfuricum* gen. nov., sp. nov.

The sources of the inocula were surface (5–20 cm deep) anaerobic sulfidic sediments from hypersaline alkaline lakes (Table 1). The enrichment and isolation procedures, the medium composition, cultivation conditions and analyses of growth and sulfide/polysulfide formation have been described previously [8]. Briefly, we used two media, both containing 4 M total Na^+, one based on NaCl with pH 7 and the other based on a mixture of sodium carbonate–bicarbonate buffer at pH 10. These were mixed in various proportions after sterilization to create different ratios of carbonates and chloride effecting the total alkalinity (alkaline buffering capacity) and the final pH (from pH 8.5 to 10.5). The primary anaerobic enrichments were done with three electron donors, formate (50 mM), butyrate (10 mM) and peptone, which had shown an increase in sulfur reduction at salt-saturating conditions during previous sediment incubation experiments [6–8]. Use of acetate and propionate as electron donors failed to produce a visible increase in sulfide during several attempts at the initial enrichment stage. In contrast, the use of butyrate, formate and peptone resulted in active sulfidogenic, sediment-free enrichment cultures after three rounds of 1 : 100 transfers. Subsequently, after several rounds of serial dilutions to extinction, eight pure cultures of natronarchaeal sulfur reducers were produced, designated as AArc (except for strain AHT32; Table 1). It must be noted, that while using butyrate, the archaeal component (typically recognizable by its flattish cells) was already dominant in the primary enrichments, whereas the situation in formate- and peptone-supplied cultures was more complex. In some of those cultures a bacterial component was actively proliferating and outcompeting the natronarchaea in the primary enrichments. In both cases it was identified as a member of *Halanaerobiales*: in formate cultures it was closely related to *Natroniella sulfidigena*, previously shown as an active formate-utilizing sulfur reducer in soda-saturated conditions [9], while in the peptone enrichment it was a novel genus-level *Halanaerobiales* lineage, most closely related to '*Halanaerobium hydrogeniformans*'. Therefore, it was necessary to use antibiotics (a mixture of streptomycin, kanamicin and vancomycin, 100 mg l^{-1} each) to suppress the bacterial component in the later stages of enrichments, in order to purify the sulfur-reducing natronoarchaea using further serial dilutions to extinction technique. The purity of the isolates was verified by microscopy and 16S rRNA gene sequencing.

The cell morphology of AArc isolates varied, depending on growth conditions. Cells, grown anaerobically with formate and sulfur, were mostly thin flat rods and occasionally motile (Fig. 1a, c). When those cells, devoid of inclusions, were exposed to oxygen, globes of elemental sulfur started to form inside the cells, indicating intracellular accumulation (most probably in the 'pseudoperiplasm') of polysulfide

Table 1. Key chemical characteristics of hypersaline alkaline lakes as a source of isolation of sulfur-reducing natronoarchaea

Lake	Area	Brine parameters			Electron donor in enrichment	Strain
		Total salinity (g l^{-1})	pH	Soluble carbonate alkalinity (M)		
Bitter-1	Kulunda Steppe, Altai, Russia (2010–2012)	330	10.3	4.0	Peptone*	**AArc-P**
Trona crystallizer		380	9.6	3.1	Formate	**AArc1^T**
Tanatar-1		400	11.0	5.0		
Mixed sample from the three lakes		330–400	9.6–11.0	3.1–5.0	Butyrate	**AHT32**
Hotontyn Shar–Burdiin	North-east Mongolia (1999)	220–360	9.2–9.9	0.9–1.2	Butyrate	**AArc-Mg**
Badaina†	Badain-Jaran Desert, Inner Mongolia (2013)	495	9.7	1.4	Formate*	**AArc-Bj**
Hamra, Fazda, Beidah, Ruzita, Zugm, Khadra, Umm-Risha, Gaar	Wadi al Natrum, Egypt (2000)	200–360	9.1–9.9	0.1–0.9	Butyrate	**AArc-Wn**
Owens	California, USA (2008)	180	9.7	1.0	Butyrate	**AArc-Ow**
Searles‡	California, USA (2005)	350	9.8	0.2	Formate*	**AArc-Sl^T**

*In the presence of antibiotics.

†Provided by Zorigto Namsaraev.

‡Provided by Ronald Oremland.

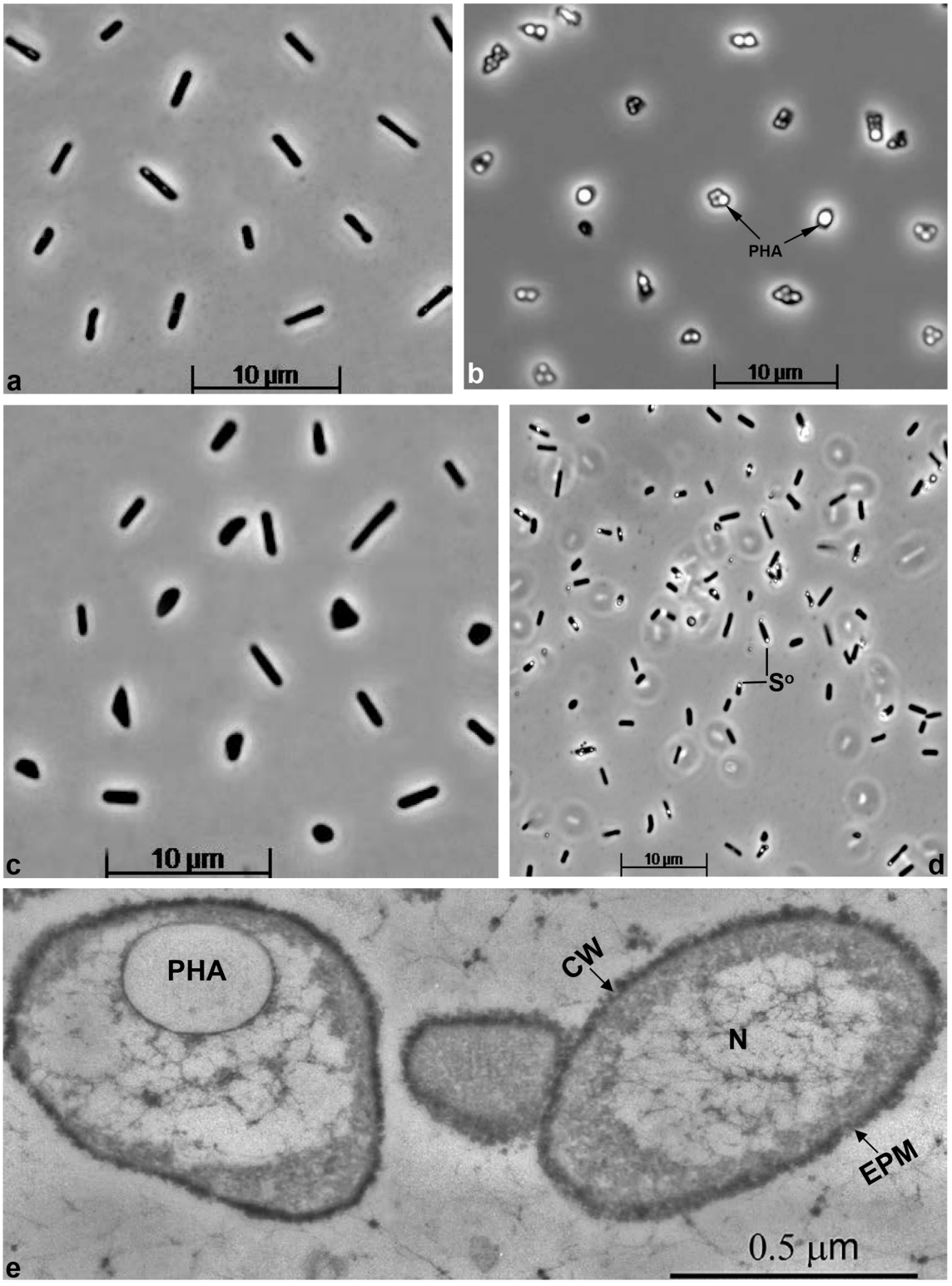


Fig. 1. Cell morphology of sulfur-respiring natronoarchaea growing anaerobically with sulfur as an electron acceptor at 4 M Na^+, pH 10 (AArc1^T) or pH 9.3 (AArc-Sl^T). (a)–(d) Phase contrast microscopy. (a) and (d) Cells of strain AArc1^T grown anaerobically with formate (a) and later on exposure to air, showing elemental sulfur (S^o) formation from internally accumulated polysulfide (d). (c) Cells of AArcel-Sl^T, grown with formate. (e) Thin section electron microscopy of a cells of strain AArc1^T from a culture grown with butyrate. CW, cell wall; EPM, extracellular polymer matrix; N, nucleoid; PHA, PHA granule.

during anaerobic sulfur-reducing growth (Fig. 1d). A similar phenomenon has been observed previously with cells of haloalkaliphilic sulfur-respiring bacterium *Desulfurispirillum alkaliphilum* [10]. Cells of the soda lake AArc strains grown with butyrate, either anaerobically or aerobically, were mostly coccoid with large refractive inclusions identified as polyhydroxyalkanoates (PHA) by Nile-blue staining (Fig. 1b, e). This is corroborated by the presence of a PHA synthetase operon *phaCE* in the genomes of AArc1^T and AArc-Mg, belonging to archaeal type IIIA (Fig. S1, available in the online version of this article). On the other hand, the cells of strain AArc-Sl^T, which did not use fatty acids for growth, lacked the PHA inclusions and the PHA synthetase genes in the genome. Cells of four out of other six AArc isolates from the soda lake group were similar in morphology to the type strain AArc1^T, while strains AArc-Bj and AArc-Wn were also coccoid during growth on formate and sulfur.

For thin sectioning, the cell pellets were fixed in 1 % (w/v) OsO_4 containing 3.0 M NaCl for 48 h at room temperature, washed, stained overnight with 1 % (w/v) uranyl acetate, dehydrated in an increasing ethanol series, and embedded in Epon resin. The sections were stained with 1 % (w/v) lead citrate. The AArc1^T cells grown anaerobically with butyrate and sulfur exhibited a large nucleoid, many large PHA granules and a thin monolayer cell wall covered with an extracellular matrix (Fig. 1e). Cells of all AArc isolates rapidly lysed at a salinity below 1–1.5 M total Na^+.

For membrane core lipids, freeze-dried biomass was hydrolysed with 5 % HCl/MeOH followed by extraction with dichloromethane and drying over Na_2SO_4. The core membrane lipid analysis was performed by using HPLC-atmospheric pressure chemical ionization/mass spectrometry (HPLC-APCI/MS) on an Agilent 1100/Hewlett Packard 1100 MSD instrument equipped with automatic injector and two Prevail Cyano columns in series (150×2.1 mm; 3 µm) with a starting eluent of hexane:propanol (99.5 : 0.5, v:v) and a flow rate of 0.2 ml min^{-1}. After 5 min, a linear gradient to 1.8 % propanol was applied up to 45 min. The injection volume was 10 µl [11].

The core lipids of strain AArc-Mg (from the soda lake group) consisted of two major DGE (dialkyl glycerol ether) components, common for haloarchaea, archaeol (C_{20}–C_{20}) and extended archaeol (C_{20}–C_{25}), in nearly equal proportion (44 and 56 %, respectively). In contrast, strain AArc-Sl^T, isolated from Searles Lake, archaeol was much more dominant (88 %), while another type of extended archaeol (C_{25}–C_{20}), less common in haloarchaea, was present at a relatively low amount (7 %). Both strains contained unsaturated forms of both archaeol and extended archaeol as minor components. Parallel analysis of two type strains of the genus *Natronolimnobius innermongolicus* JCM 12255^T and *Natronolimnobius baerhuensis* JCM 12253^T [12, 13] (obtained from the JCM culture collection) using the same methodology showed a domination of archaeol and C_{20}–C_{25} extended archaeol in both. In the former they were at a 1 : 2 ratio, while in the latter both forms were present in equal proportion.

The intact polar lipids (IPLs) were extracted from freeze-dried biomass ultrasonicated in a mixture of methanol, dichloromethane and phosphate buffer (2 : 1 : 0.8, v/v) and an additional re-extraction with pure dichloromethane. The final preparations were redissolved in hexane:2-propanol: water (72 : 27 : 1, v/v/v) and analysed by HPLC-ion trap mass spectrometry (HPLC-ITMS) using the Agilent 1200 series HPLC (Agilent) coupled to an LTQ XL linear ion trap with the Ion Max source with an electrospray ionization probe (Thermo Scientific) and a LiChrospher diol column (250×2.1 mm, 5 µm particles; Alltech), as described in Damsté *et al.*[14].

Both analysed AArc type strains exhibited a similar composition of IPLs, typically found in haloarchaea, dominated by phosphatidylglycerolphosphate methyl ester (PGP-Me), with lower levels of phosphatidylglycerol (PG) and minor amounts of phosphatidylglycerophosphate (PGP). These phospholipids were present with both archaeol and extended archaeol cores (Fig. S2). Glyco- and sulfo-lipids were not detected. In the two type strains of the genus *Natronolimnobius* mentioned above and analysed in parallel, PGP-Me and PG were also the dominant IPLs, consistent with the original description. In addition, a novel species of cardiolipin was detected in both *Natronolimnobius* type strains, phosphatidylglycerolphosphate glycerophosphate (PGPGP) (Fig. S3). This lipid has recently been discovered in three strains of natronoarchaea from hypersaline soda lakes [15], including two novel genera belonging to the family *Natrialbaceae*, chitinolytic '*Natrarchaeobius*' [16] and cellulolytic *Natronobiforma* [17]. There is a high probability that this novel type of polar lipid might be specific for alkaliphilic haloarchaea since, most probably, it was also detected earlier in *Natronococcus occultus* but described as an unknown glycolipid [18].

Respiratory quinones were extracted from cell pellets of strains AArc1^T and AArc-Sl^T, grown at pH 10 and 9.3, respectively, under aerobic conditions by three consecutive extractions with cold acetone. The combined extracts were concentrated by evaporation and the quinone fraction was separated from carotenoids by TLC (Sorbofil) in hexane-diethyl ether (85 : 15). The obtained quinone bands (Rf=0.52–0.6) were recovered by extraction with CCl_4–CH_3OH (1 : 1) and subjected to mass spectroscopy with chemical ionization at atmospheric pressure using quadrupol mass spectrometer Finnigan LCQ Advantage MAX [19]. The analysis identified two variations of dominant menaquinone: MK-8 : 0 in AArc1^T and MK-8(H2) in AArc-Sl^T (Fig. S4). Both are commonly found in haloarchaea [20]. Parallel analysis of the two reference strains from the genus *Nln. innermongolicus* JCM 12255^T and *Nln. baerhuensis* 12253^T (which were not analysed previously) showed a presence of MK-8 : 0 and MK-8(H2) at the ratio of 7 : 3 in both species.

The genomes of three representative species, AArc1^T, AArc-Mg and AArc-Sl^T, were sequenced using the MiSeq Illumina platform as described previously [8]. All three genomes were assembled as a single circular chromosome. The genome size was 3.3–3.8 Mbp. In addition, AArc-Mg has one plasmid and AArc1^{T}has two. Further genome statistical information is provided in Table S1.

The 16S rRNA gene sequences of the AArc strains were aligned with those of related species in the orders *Natrialbales* and *Haloferacales* [21] by using the SILVA Incremental Aligner [22]. The phylogenetic neighbours and pairwise sequence similarities were determined using EzTaxon-e [23]. Additional analyses were based on two other sets of conservative archaeal molecular markers using only the genome-sequenced type strains AArc1^T and AArc-Sl^T together with available genome-sequenced members of the orders *Natrialbales* and *Haloferacales*. For this, the complete genomes of 46 haloarchaea were obtained from the NCBI genome database (www.ncbi.nlm.nih.gov/sites/genome). The DNA-directed RNA polymerase beta subunit tree (*rpoB*) was reconstructed in the same manner, with concatenated two beta subunits in the following order: subunit B″ (*rpoB2*) and subunit B′ (*rpoB1*). The resulting nucleotide sequences were aligned by using the CLUSTAL_W plug-in included with the Geneious 7.1 software (Biomatters) employing the IUB cost matrix. These alignments were used for reconstruction of the maximum-likelihood phylogenetic trees by using the PhyML program version 3.0 [24] with the JC69 (Jukes–Cantor) substitution model and 100 bootstrap replicates. The trees were reconstructed in MEGA6 [25] with 100–1000 randomly selected bootstrap replicates. For extended phylogenomic reconstruction, 122 archaeal conservative marker genes were taken from the Genome Taxonomy Data Base [26]. These marker genes were identified in studied genomes using Prodigal version 2.6.3 [27], concatenated and then aligned using MAFFT version 7.427 [28]. Alignment was automatically trimmed using trimAl 1.2rev59 [29]. The phylogenetic tree was built using PhyML 3.0 [24] and the approximate likelihood-ratio test for branches [30]. Substitution model for phylogenetic reconstruction was automatically selected by using the SMS algorithm [31].

The 16S rRNA gene-based phylogenetic analyses revealed that the eight sulfur-reducing AArc strains form two different clusters: a major group 1 included seven closely related (>99 % sequence identity) isolates from soda lakes; and a minor group 2 consisted of a single Searles Lake isolate AArc-Sl^T. The soda lake group 1 strains form a novel distant species-level branch within the genus *Natronolimnobius* in the order *Natrialbales* (maximum sequence identity to its three described species is between 95 and 96 %), while strain AArc-Sl^T represents a novel genus-level lineage within the family *Halorubraceae*, order *Haloferacales*, with a maximum sequence identity of 92 % to its published species (Fig. 2a). The results of *rpoB* genes analysis (traditionally used as an alternative single-copy conservative marker for haloarchaea; Fig. S2) and of extended phylogenomic analysis based on 122 conservative archaeal protein markers (Fig. 2b) were in line with the 16S rRNA gene-based phylogeny showing that the two type AArc strains represented robust independent lineages in two different orders of the class *Halobacteria* (Fig. 2b, c).

The whole-genome comparison was conducted by using three different methods: average nucleotide identity (ANI), performed for both ANIb (ANI with BLAST) and ANIm (ANI with Mummer) methods by using the JSpeciesWS web server (http://jspecies.ribohost.com/jspeciesws/#analyse); the Genome-to-Genome Distance Calculator 2.1 online tool (http://ggdc.dsmz.de/ggdc.php), based on DNA–DNA hybridization (DDH); and average amino acid identity (AAI), performed by using the online AAI calculator from Kostas lab (http://enve-omics.ce.gatech.edu/aai/index) [32–34].

These analyses also confirmed the separate species position of the main group 1 AArc isolates in the genus *Natronolimnobius*. The ANI (ANIb and ANIm) values within the genus *Natronolimnobius* ranged from 75.8 to 85.4 %, with the highest ANIm value being between strain AArc1^T and *Natronolimnobius aegyptiacus* DSM 23470^T. The genome-to-genome distances (GGDC values) ranged from 22.2 to 24.1 %. Finally, the AAI values presented a similar trend to the ANIb/ANIm values, because it ranged from 71.2 to 75.4 %, with the highest value between AArc1^T and DSM 23470^T (Table S2). The values were robustly below the threshold values (ANI, AAI of 94–95 % and GGDC of 70 % cut-offs) frequently used to separate prokaryotic species, strongly supporting that the main AArc group 1 represented a distinct novel species in the genus *Natronolimnobius*, different from the other three species, which are obligate aerobic heterotrophs [12, 35, 36].

Indeed, the tests of type strains of *Nln. innermongolicus* 12255^T and *Nln. baerhuensis* 12253^T for the ability to grow anaerobically with various electron donors and either sulfur or DMSO as the electron acceptors yielded negative results. We also did not find the determinant for sulfur/DMSO-reducing potential (*psr*/*dms* genes) in the published genome of *Nln. aegyptiacus* DSM 23470^T. On the other hand, the unclassified isolate 93dLM4 from Little Lake Magadi in Kenya, closely related to the AArc group 1 according to the 16S rRNA gene sequence identity, was able to grow by sulfur respiration with formate (but not with butyrate) as an electron donor and yeast extract or pyruvate as a carbon source and, hence, can be included as an additional member of the soda lake sulfur-reducing group 1. Furthermore, we tested *Natronobacterium gregoryi* DSM 3393^T, a related member of the family *Natrialbaceae*, the genome of which contains the necessary genetic determinants for sulfur reduction (*psr* and hydrogenase operons) [8], for the ability to grow anaerobically by sulfur respiration and it was positive with H_2 as an electron donor, but not with formate or butyrate.

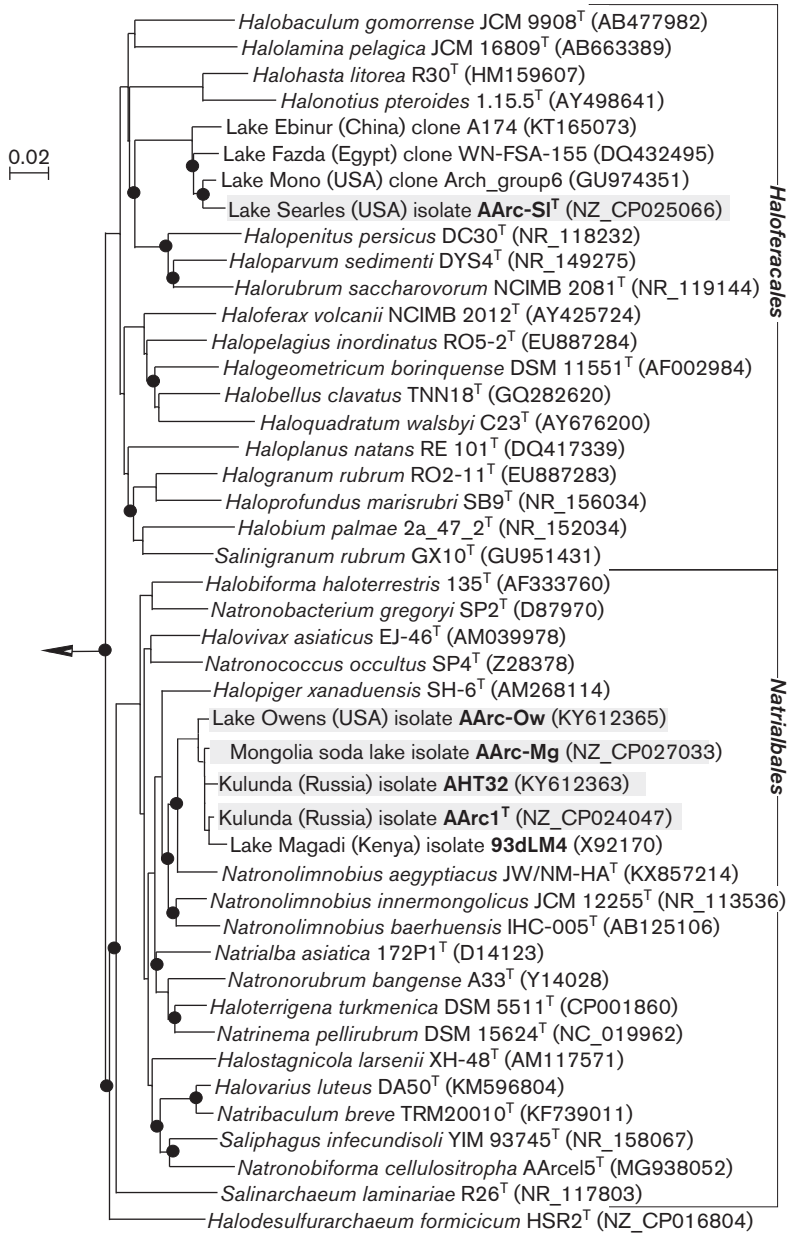
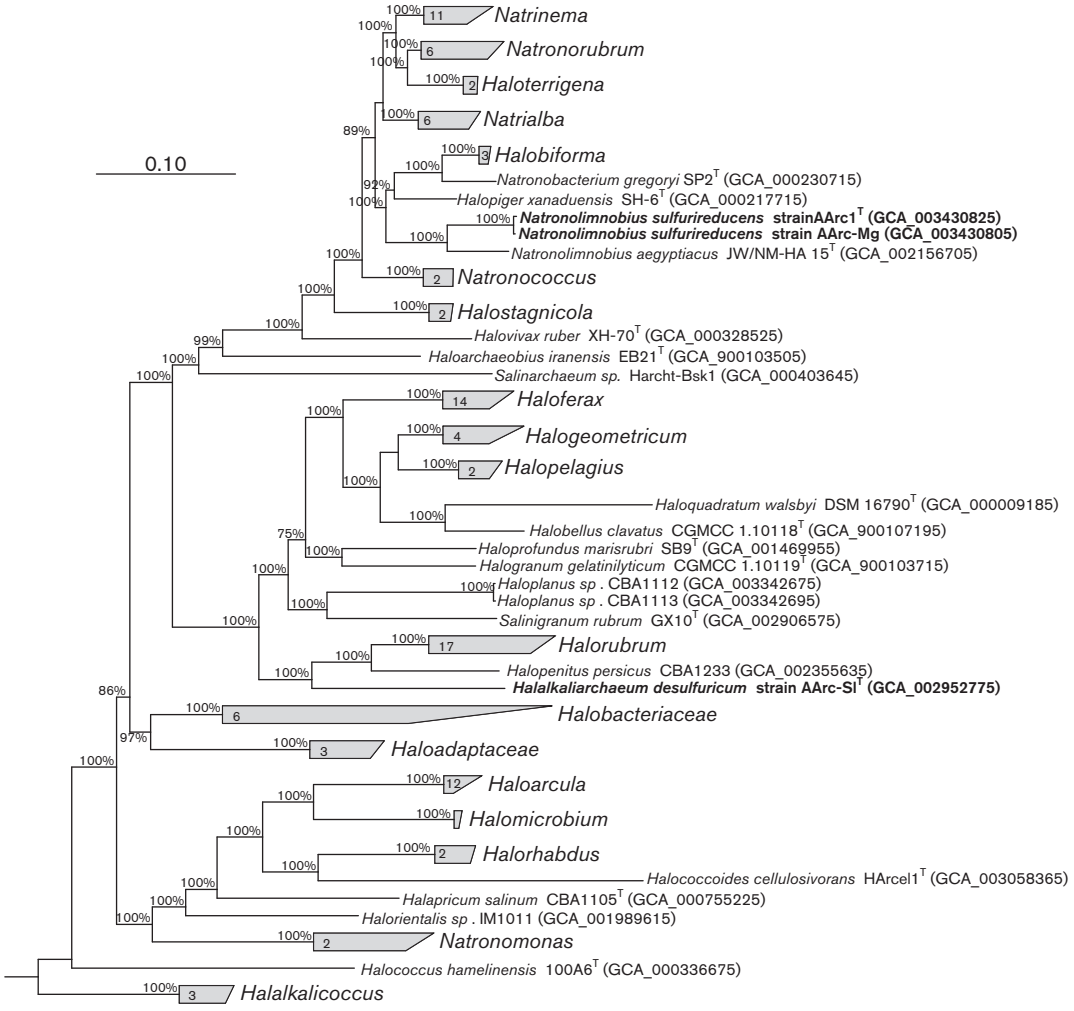


Fig. 2. Phylogeny of the sulfur-reducing AArc strains. (a) The 16S rRNA gene-based maximum-likelihood phylogenetic tree showing positions of the sulfur-reducing AArc strains (evidenced in bold) within the orders *Natrialbales* and *Haloferacales*. All positions with less than 95 % site coverage were eliminated. In total, 1359 positions were used in the alignment of 42 sequences [except for the partial sequences AArc-Bj, AArc-P and AArc-Wn, highly identical (>99 %) to AArc1^T]. Filled circles at the nodes indicate the bootstrap values >70 % (1000 bootstrap iterations). *Methanohalophilus halophilus* DSM 3094^T(FN870068) was used as the outgroup. Branch lengths (see scale) correspond to the number of substitutions per site with corrections, associated with the model (GTR, G+I, four categories). (b) Phylogenomic placement of sulfur-reducing natronoarchaea based on concatenated partial amino acid sequences of the 122 archaeal conservative proteins. The trees were built using the PhyML 3.0 program and the approximate likelihood-ratio test for branches. Bootstrap values above 70 % are shown at the nodes. Bar, 0.10 changes per position.

Fig. 2. (cont.)



The natronarchaeal isolates were clearly different from the previously described obligate anaerobic sulfur-reducing haloarchaea (genera *Halanaeroarchaeum* and *Halodesulfurarchaeum*) [1–5]) in respect to their key physiology. All AArc strains strains were able to grow aerobically, after gradual adaptation to increasing O_2 concentrations, with the cells acquiring the intense pink-orange colour usual for aerobic haloarchaea. Hence, they can be classified as facultative anaerobes. Another important difference is the broader range of electron donors/carbon sources utilized. Similar to *Halodesulfurarchaeum* [4, 5], the AArc strains were able to use formate or H_2 as electron donors in the presence of low concentrations of yeast extract or acetate as the carbon source during anaerobic growth with sulfur. But, at these conditions, the AArc isolates formed much higher concentrations of reduced sulfur (HS^-+sulfan of polysulfides S_x^{2-}) in comparison to their neutrophilic counterparts, resulting in solubilization of up to 100 mM sulfur in the form of polysulfides with an average formula of $S_{5.5}^{-2}$. However, H_2 and formate can only be used as electron donors anaerobically for sulfur or DMSO respiration, but not aerobically, while aerobic growth was possible in presence of acetate, pyruvate or butyrate. The latter, as well as longer fatty acids up to C_9, also served as energy and carbon sources for anaerobic growth with sulfur for the group 1 isolates (except for strain AArc-Bj and AArc-Wn and AArc-Ow, which can only grow up to C_6), but in this case the amount of polysulfide produced was much less than with formate or H_2. Finally, the group 1 isolates from soda lakes also utilized some other organic compounds as substrate both for aerobic and anaerobic growth, including peptone (all seven strains), lactate and glycerol (AArc1^T, AHT32 and AArc-Mg). In contrast, a single strain, AArc-Sl^T, of group 2 had a much more restricted range of organic substrate for growth, using only pyruvate in addition to H_2/formate for anaerobic growth with sulfur

and pyruvate or peptone–yeast extract for aerobic growth. Sugars were not utilized either aerobically or during sulfur respiration. Fermentative growth with either sugars or peptone was not observed. Of the tested electron acceptors with formate or butyrate as electron donors (sulfur, DMSO, thiosulfate, sulfite, nitrate, nitrite, arsenate, arsenite, selenate, ferric citrate, fumarate), only the first two could be used by the soda lake group 1, while strain AArc-Sl^T grew only with sulfur. The genomes of strains AArc1^T, AArc-Mg and AArc-Sl^T showed a presence of catalase and several types of quinol oxidases (but not cytochrome *c* oxidases). Consistent with these results, the catalase test (colony assay with 3 % H_2O_2) was positive for the type strains, while the oxidase test (colony assay with *N,N,N,N*-tetramethyl-p-phenylendiamine hydrochloride) was only weakly positive. Other standard activity tests were performed as recommended according to Oren *et al.* [37]. Protease, esterase and lipase activities were tested in aerobically grown colonies on plates with casein/gelatin (after flooding with 10 % TCA) and emulsified tributyrin/olive oil (turbidity clearance), respectively, in strains AArc1^T and AArc-Sl^T. Both strains were negative in all three tests. Of the tested inorganic N-sources (nitrate, nitrite, urea and ammonium) with pyruvate as carbon and energy source, only the latter supported aerobic growth of the type strains. Indole formation from tryptophan (Kovac's reagent test, [38]) showed negative results for strains AArc1^T and AArc-Sl^T. Antibiotic sensitivity was tested aerobically in liquid medium at pH 9.5 for AArc1^T (butyrate as substrate) and pH 9 for AArc-Sl^T (pyruvate as substrate). Both were sensitive to rifampicin and chloramphenicol above 50 mg l^{-1}, but resistant to streptomycin, ampicillin, kanamicin, vancomycin and gentamicin at 100 mg l^{-1}.

All isolates belonged to the extreme halophiles, with optimal growth occurring at 4 M total Na^+ and in the range from 2.5 to 5 M. However, there was a clear difference in the Cl^- demand and the pH profiles between the major group of soda lake isolates and strain AArc-Sl^T (detailed results were presented by Sorokin *et al.* [8]). In general, the soda lake group 1 is truly natronophilic, capable of growth in saturated sodium carbonate brines with Cl^- concentrations as low as 0.1 M and the pH up to 10.5 (with the pH values monitored during growth). In contrast, strain AArc-Sl^T was highly Cl^- dependent, demanding at least 2 M NaCl for growth and it was moderate alkaliphilic, with the pH maximum between 9.0 and 9.3. This difference corresponds well to the difference in their respective habitats. Interestingly, the temperature maximum for aerobic growth was also pH dependent in both groups reaching highest values at the lowest pH possible for growth: 50 °C for AArc-Sl^T at pH 8 and 48 °C for the soda lakes at pH 9 (tested for AArc1^T, AArc-Wn and AArc-P). Such temperature–pH interdependence might be explained by the fact that proteins become unstable at a combination of high pH–high temperature. Results of phenotypic comparisons of the soda lake group 1 with their nearest aerobic relatives from the genus *Natronolimnobius* are given in Table 2, while the properties of the deep-lineage strain AArc-Sl^T are compared with the members of the family *Halorubraceae* in Table 3. The main difference of the soda lake AArc strains from their three related species of the genus *Natronolimnobius* [12, 35, 36] is their ability to grow anaerobically by sulfur respiration. In other phenotypic properties, in particular alkalitolerance and substrate profile for aerobic growth, they are more similar to *Nln. innermongolicus*. Furthermore, there is a difference in both core archaeal lipids and polar lipids between the AArc strains and *Nln. aegyptiacus* from one side and *Nnl. innermongolicus/Nnl. baerhuensis* from another side.

Strain AArc-Sl^T might not be unique in its capacity for anaerobic sulfur respiration among the members of the *Halorubraceae*. Based on the genomic content, two species from the genera *Halorubrum* and *Halapricum* may potentially be sulfur reducers [8]. However, this ability still needs to be confirmed by direct growth/activity experiments. Another property which sets strain AArc-Sl^T apart is its lipid composition including a presence of extended archaeol in the C_{25}–C_{20} conformation instead of much more common C_{20}–C_{25} conformation and a presence of PGP polar head group. Furthermore, most of the *Halorubraceae* members are neutraphilic and for those species where alkalitolerance has been reported neither the alkaline buffering capacity of the media used nor the absence of the final pH check would guarantee the adequacy of the reported results.

Overall, on the basis of distinct phenotypic and genetic features, we propose that the sulfur-reducing natronoarchaea from soda lakes are classified as a new species, *Natronolimnobius sulfurireducens* (with an emended description of the genus), and the Searles Lake isolate strain, AArc-Sl^T, as a new genus and species *Halalkaliarchaeum desulfuricum*.

DESCRIPTION OF *NATRONOLIMNOBIUS SULFURIREDUCENS* SP. NOV.

Natronolimnobius sulfurireducens (sul.fu.ri.re.du′cens L. n. *sulfur*, sulfur; L. part. adj. *reducens* leading back, reducing, N.L. part. adj. *sulfurireducens* reducing sulfur).

The cells are either flattened rods, occasionally motile or non-motile coccoids, 0.5–0.6×1–3.5 µm, depending on the growth conditions. The cell wall consists of a thin monolayer covered with an extracellar matrix. The cells lyse in hypotonic solutions below 1–1.5 M Na^+. Red pigments are produced during aerobic growth. The core membrane diether lipids are composed of C_{20}-C_{20} DGE (archaeol) and C_{20}-C_{25} DGE (extended archaeol) in equal proportion. The polar phospholipids include phosphatidylglycerolphosphate methyl ester, phosphatidylglycerol and phosphatidylglycerophosphate. The dominant respiratory quinone is MK-8 : 0. Facultatively anaerobic. Anaerobic respiratory growth is possible with either elemental sulfur or DMSO as electron acceptor and the following electron donors: H_2 and formate

Table 2. Comparative properties of sulfur-reducing natronoarchaea of group 1 with the nearest phylogenetic relatives in the genus *Natronolimnobius: Natronolimnobius aegyptiacus* DSM 23470^T [35]; *Natronolimnobius innermongolicus* JCM 12255^T and *Natronolimnobius baerhuensis* JCM 12253^T [12, 36].

NR, not reported; Psr, polysulfide reductase; PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerophosphate methylester, PGP, phosphatidylglycerophosphate; PGPGP, phosphatidylglycerolphosphate glycerophosphate; DGE, dialkyl glycerol ether.

Property	Soda lake group 1 (seven strains)	*Natronolimnobius aegyptiacus*	*Natronolimnobius innermongolicus*	*Natronolimnobius baerhuensis*
Cell morphology	Motile flat rods and coccoids	Rods, non-motile	Rods, non-motile	Pleomorphic, motile
Pigmentation	Pink (aerobic)	Yellow or pink	Red	Red
PHA accumulation	+ (With fatty acids)	NR*	–**	–**
Aerobic growth	+	+	+	+
Anaerobic growth	Respiratory with sulfur and DMSO as acceptor	–	–**	–**
Number of Psr operons in genomes	1	0	0	0
Electron donors for anaerobic growth	H_2, formate, pyruvate, lactate, glycerol, C_4-C_9 fatty acids, peptone	–	–	–
Substrates for aerobic growth	Acetate, pyruvate, lactate, glycerol, butyrate, peptone	Pyruvate, glucose, fructose, mannose, galactose, maltose, cellobiose raffinose	Acetate, propionate, pyruvate, butyrate**, lactate, malate, fumarate, citrate, glycerol, glucose, galactose, arabinose, raffinose, sorbitol	Acetate, fumarate, pyruvate, glucose, arabinose, fructose, mannose, galactose, rhamnose, xylose**, maltose, cellobiose**, raffinose, lactose, glycerol
Amilase	–	+	–	+**
Esterase/lipase	– (Tributyrin/olive oil)	– (Tween 80)	– (Tween 80)	+ (Tween 80)
Protease	– (Casein; gelatin)	– (Gelatin)	+ (Gelatin)	Gelatin (–)/casein (+)**
Catalase/oxidase	+/w	+/+	+/+	+/+
Indole from tryptophane	– (For the type strain)	–	+	+
Salinity range (optimum) (M Na^+)	3.0–5.0 (4.0)	2.5–5.0 (3.2–4.6)	2.5–4.5 (3.5)	1.6–4.2 (2.5–3.2)
pH range for growth (optimum)	7.0–10.0 (9.1–9.3)	7.5–10.5*** (9.0–9.5)	7.5–10.0 (9.5)	7.0–10.0 (9.0)
Maximum temperature for growth (°C) (optimum)	48 (40–43)	56 (52)	54 (45)	46 (37)
Core lipids	C_{20}–C_{20}, C_{20}–C_{25} DGE	C_{20}–C_{20} DGE	C_{20}–C_{20}, C_{20}–C_{25} DGE**	C_{20}–C_{20}, C_{20}–C_{25} DGE**
Intact membrane polar lipids	major: PGP-Me, PG minor: PGP	PGP-Me, PG	PGP-Me, PG	
Cardiolipins		NR	PGP**	
Respiratory quinones	MK-8 : 0	NR	PGPGP**	
DNA G+C (mol%; genome)	62.8–62.9	64.1	MK-8 :0 (70 %); MK-8(H2) (30 %)**	60.1

*PHA synthetase type IIIA operon *phaCE* is present in the genome.

**Determined in this study.

***The carbonate buffering capacity of the used medium is not enough to ensure stable pH above 10, the final pH was not measured.

Table 3. Comparative properties of sulfur-reducing natronoarchaeon AArc-Sl^T with the members of the family *Halorubraceae* [39]

PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerophosphate methylester; PGP, phosphatidylglycerophosphate; PGS, phosphatidylglycerol sulfate; PA,phosphatidic acid; S-DGD-1, sulfated mannosyl glucosyl diether; DGD-1, mannosyl glucosyl diether; S2-DGD, a bis-sulfated diglycosyl diether; Psr, polysulfide reductase; variable property; R, rare property among multiple species; NR, not reported; W, weak.

Property	Strain AArc-Sl^T	*Halorubrum*	*Halonotius*	*Halopenitus*	*Halohasta*	*Halolamina*	*Halobaculum*	*Salinigranum*
Number of species		32	1	3	2	2	3	1
Cell morphology	Pleomorphic, non-motile	Rod, motility (V)	Motile flattened rods	Pleomorphic, motility (V)	Motile, rods	Pleomorphic motility (V)	Pleomorphic	Pleomorphic, motile
Pigmentation	Red-orange (aerobic)	Red-orange	Red	Cream or red	Red	Red	Red	Red
Anaerobic growth	Respiratory with sulfur	Fermentative (R) DMSO (R) nitrate (R)	–	– DMSO (V), nitrate (V)	–	–	Mostly – – mostly –	+, Arginine
Psr operons in the genome	1	1 (1 species)	1*	0	0	0	0	0
Substrates for aerobic growth	Pyruvate, peptone, yeast extract	Mostly sugars	Pyruvate, glucose, glycerol	Sugars (2 species) aminoacids (1 species)	Sugars and organic acids	Mostly aminoacids and pyruvate	Mostly sugars and few organic acids	Glucose, mannose, galactose, sucrose, lactose, glycerol, mannitol, sorbitol, acetate, pyruvate, lactate, malate, fumarate, citrate
Amilase	–	V	–	–	–	–	V	–
Protease	– (Casein)	(R)	NR	–	–	–	–	–
Catalase/oxidase	+/W	+/+	–/–	+/+	+/V	+/+	V/V	+/+
Indole formation	–	V	–	–	–	–	V	–
Salinity range (optimum) (M Na^+)	3.0–5.0 (3.5)	1.0–5.2 (>2.5)	2.5–5.1 (3.1–4.0)	0.9–5.0 (3.0–3.1)	2.1–4.8 (2.6–3.1)	1.4–5.4 (3.4–4.3)	1.0–5.1 (1.5–2.6)	1.4–4.8 (3.1)
pH range for growth (optimum)	6.5–9.5 (8.0–8.5)	Mostly neutrophilic; alkaliphilic (R)	5.5–8.5 (7.0–7.5)	6.0–9.5* (7.0–7.5)	5.5–9.0 (7.0–7.5)	5.5–9.5* (7.0–7.5)	5.5–9.5* (6.0–7.0)	5.0–9.0 (7.0)
Core lipids	C_{20}–C_{20}, C_{25}–C_{20}	C_{20}–C_{20}, C_{20}–C_{25}	NR	NR	NR	NR	C_{20}–C_{20} (1 species)	NR
Phospholipids	PGP-Me, PG; PGP	PGP-Me, PG	PGP-Me, PG	PGP-Me, PG	PA, PGP-Me, PG	PGP-Me, PG	PGP-Me, PG	PGP-Me, PG
Sulfolipids	–	PGS	–	PGS (V)	PGS	PGS	–	–
Glycolipids	–	S1-DGD	S-DGD-1	+, unidentified	S-DGD-1	S2-DGD, DGD-1	S-DGD-1	S-DGD-1, DGD-1
Respiratory quinones	MK-8(H2)	MK-8; MK-8(H2) (2 species)	NR	MK-8(H2) (2 species)	NR	NR	NR	MK-8; MK-8(H2)
DNA G+C (mol%)	63.1 (genome)	60.0–71.2	58.4–58.7	63.8–67.0	58.9–62.9	62.7–67.9	65.9–67.6	62.9
Habitat	Hypersaline alkaline Searles Lake (California)	Hypersaline habitats, neutral or alkaline	Salt crystallizer	Hypersaline sakes and crystalline salt	Hypersaline lake and aquaculture	Salt crystallizes and crystalline salt	Dead Sea and crystalline salt	Salt crystallizer

*In the metagenomically assembled genome of uncultured *Halonotius* J07YN4 (100 % of 16S rRNA gene identity to the type species).

**Final pH was not checked, the medium was not buffered properly.

(in presence of acetate or yeast extract as C source), C_4–C_9 fatty acids, pyruvate, lactate, glycerol and peptone. Aerobic growth occurs with acetate and the above-mentioned organic acids and peptone–yeast extract, but not with H_2/formate. Sugars are not utilized under any conditions. Ammonium is utilized as a N-source. Oxidase is weakly positive, catalase is positive. Optimum growth temperature is 40–43 °C (maximum is 48 °C at pH 9). Extremely halophilic with a range of total Na^+ for growth from 2.5 to 5 M (optimum at 3.5–4 M) and obligately alkaliphilic, with a pH range for growth from pH 8.5 to 9 to 10.5 (optimum at pH 9.5–10). The G+C content of the DNA is 62.8–62.9 mol% (two genomes). Habitat: hypersaline soda lakes. The type strain (AArc1^T=JCM 30663^T=UNIQEM U932^T) was isolated from anaerobic sediment sampled at a hypersaline soda lake in Kulunda Steppe (Altai, Russia). The species also includes other six closely related strains isolated from various soda lakes in Central Asia and Africa. The genome of the type strain consists of a chromosome and two plasmids with the GenBank accession numbers CP024047 and CP024045/CP024046, respectively.

EMENDED DESCRIPTION OF THE GENUS *NATRONOLIMNOBIUS* ITOH *ET AL.* 2005

The three previously described species of the genus, including *Nln. innermongolicus*, *Nln. baerhuensis* [12, 13, 36] and *Nln. aegyptiacus* [35], are obligately aerobic organoheterotrophs. This must be emended to include also a potential for other species to grow anaerobically with elemental sulfur or DMSO as electron acceptor, either organoheterotrophically with organic acids and peptone or lithoheterotrophically with H_2/formate as electron donor and yeast extract or acetate as carbon sources.

DESCRIPTION OF *HALALKALIARCHAEUM* GEN. NOV.

Halalkaliarchaeum [hal.al.ka.li.ar.chae′um Gr. n. *hals*, *halos* salt of the sea; N.L. n. *alkali* (from Arabic article *al*, the and Arabic n. *qaliy*, ashes of saltwort); N.L. neut. n. *archaeum* archaeon from Gr. adj. *archaios-ê-on* ancient; N.L. neut. n. *Halalkaliarchaeum* - haloalkaliphilic archaeon].

Moderately alkaliphilic and facultatively aerobic haloarchaea with the ability to grow anaerobically by sulfur-dependent respiration. Extremely halophilic with a high Cl^- requirement. The cells are irregularly shaped, flattened and non-motile. The dominant core lipids are $C_{20\text{-}20}$ and $C_{25\text{-}20}$ dialkylglycerol ethers, the dominant intact polar lipids are PGP-ME and PG. A member of the family *Halorubraceae*, order *Haloferacales*. The type species is *Halalkaliarchaeum desulfuricum*. Recommended three-letter abbreviation: *Hla*.

DESCRIPTION OF *HALALKALIARCHAEUM DESULFURICUM* SP. NOV.

Halalkaliarchaeum desulfuricum (de.sul.fu′ri.cum L. pref. *de-*, from; L. n. *sulfur*, sulfur; N.L. neut. adj. de*sulfuricum* reducing sulfur).

The cells are pleomorphic nonmotile flattened rods and coccoids, 0.5–0.6×1.5–2.5 µm, depending on the growth conditions. The cells lyse in hypotonic solutions below 2 M NaCl. Red-orange pigments are produced during aerobic growth. The core membrane diether lipids include three components: dominant C_{20}–C_{20} DGE (archaeol) and C_{25}–C_{20} DGE (extended archaeol), and unsaturated forms of both archaeol and extended archaeol as minor components. The polar phospholipids include phosphatidylglycerolphosphate methyl ester, phosphatidylglycerol and phosphatidylglycerophosphate. The dominant respiratory quinone is MK-8 (H2). Facultatively anaerobic. Anaerobic respiratory growth is possible with elemental sulfur as an electron acceptor and H_2 or formate as electron donors (in the presence of yeast extract as a carbon source). Aerobic growth was observed with pyruvate and peptone–yeast extract. Sugars are not utilized under any conditions. Ammonium is assimilated as a N-source. Oxidase is weak positive, catalase is positive. Optimum growth temperature is 43 °C (maximum is 50 °C at pH 8). Extremely halophilic with a range of total Na^+ for growth from 2.5 to 5 M (optimum at 4 M) and obligately (but moderately) alkaliphilic, with a pH range for growth from pH 7.8 to 9.5 (optimum is at pH 8.8–9.0). The G+C content of the DNA is 63.1 mol% (genome). The type strain (AArc-Sl^T=JCM 30664^T=UNIQEM U999^T) was isolated from anaerobic sediment sampled at the hypersaline alkaline Searles Lake in California (USA). The GenBank accession number of the genome sequence of strain AArc-Sl^T is CP025066.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References
1. Sorokin DY, Kublanov IV, Gavrilov SN, Rojo D, Roman P *et al.* Elemental sulfur and acetate can support life of a novel strictly anaerobic haloarchaeon. *Isme J* 2016;10:240–252.
2. Sorokin DY, Kublanov IV, Yakimov MM, Rijpstra WI, Sinninghe Damsté JS. *Halanaeroarchaeum sulfurireducens* gen. nov., sp. nov., the first obligately anaerobic sulfur-respiring haloarchaeon,

isolated from a hypersaline lake. *Int J Syst Evol Microbiol* 2016;66: 2377–2381.

3. Sorokin DY, Yakimov MM, Kublanov Iv OA. Halanaeroarchaeum. In: Whitman WB (editor). *Bergey's Manual of Systematic of Bacteria and Archaea*. Chichester: John Wiley & Sons, Ltd; 2017.
4. Sorokin DY, Messina E, Smedile F, Roman P, Damsté JSS *et al.* Discovery of anaerobic lithoheterotrophic haloarchaea, ubiquitous in hypersaline habitats. *Isme J* 2017;11:1245–1260.
5. Sorokin DY, Messina E. Halodesulfurarchaeum. In: Whitman WB (editor). *Bergey's Manual of Systematic of Bacteria and Archaea*. Chichester: John Wiley & Sons, Ltd; 2018.
6. Sorokin DY, Rusanov II, Pimenov NV, Tourova TP, Abbas B *et al.* Sulfidogenesis under extremely haloalkaline conditions in soda lakes of Kulunda Steppe (Altai, Russia). *FEMS Microbiol Ecol* 2010; 73:278–290.
7. Sorokin DY, Kuenen JG, Muyzer G. The microbial sulfur cycle in soda lakes. *Front Microb Physiol* 2011;2.
8. Sorokin DY, Messina E, La Cono V, Ferrer M, Ciordia S *et al.* Sulfur respiration in a group of facultatively anaerobic natronoarchaea ubiquitous in hypersaline soda lakes. *Front Microbiol* 2018;9.
9. Sorokin DY, Detkova EN, Muyzer G. Sulfur-dependent respiration under extremely haloalkaline conditions in soda lake 'acetogens' and the description of *Natroniella sulfidigena* sp. nov. *FEMS Microbiol Lett* 2011;319:88–95.
10. Sorokin DY, Foti M, Tindall BJ, Muyzer G. *Desulfurispirillum alkaliphilum* gen. nov. sp. nov., a novel obligately anaerobic sulfur- and dissimilatory nitrate-reducing bacterium from a full-scale sulfide-removing bioreactor. *Extremophiles* 2007;11:363–370.
11. Weijers JWH, Panoto E, van Bleijswijk J, Schouten S, Rijpstra WIC *et al.* Constraints on the biological source(s) of the orphan branched tetraether membrane lipids. *Geomicrobiol J* 2009;26: 402–414.
12. Itoh T, Yamaguchi T, Zhou P, Takashina T. *Natronolimnobius baerhuensis* gen. nov., sp. nov. and *Natronolimnobius innermongolicus* sp. nov., novel haloalkaliphilic archaea isolated from soda lakes in inner mongolia, China. *Extremophiles* 2005;9:111–116.
13. Validation list no. 105. *Int J Syst Evol Microbiol* 2005;55:1743–1745.
14. Damsté JS, Rijpstra WI, Hopmans EC, Weijers JW, Foesel BU *et al.* 13,16-Dimethyl octacosanedioic acid (iso-diabolic acid), a common membrane-spanning lipid of *Acidobacteria* subdivisions 1 and 3. *Appl Environ Microbiol* 2011;77:4147–4154.
15. Bale NJ, Sorokin DY, Hopmans EC, Koenen M, Rijpstra WIC *et al.* New insights into the polar lipid composition of extremely halo (alkali)philic euryarchaea from hypersaline lakes. *Front Microbiol* 2019;10:377.
16. Sorokin DY, Elcheninov AG, Toshchakov SV, Bale NJ, Sinninghe Damsté JS *et al.* *Natrarchaeobius chitinivorans* gen. nov., sp. nov., and *Natrarchaeobius halalkaliphilus* sp. nov., alkaliphilic, chitin-utilizing haloarchaea from hypersaline alkaline lakes. *Syst Appl Microbiol* 2019;42:309–318.
17. Sorokin DY, Khijniak TV, Kostrikina NA, Elcheninov AG, Toshchakov SV *et al.* *Natronobiforma* cellulositropha gen. nov., sp. nov., a novel haloalkaliphilic member of the family *Natrialbaceae* (class *Halobacteria*) from hypersaline alkaline lakes. *Syst Appl Microbiol* 2018;41:355–362.
18. Angelini R, Corral P, Lopalco P, Ventosa A, Corcelli A. Novel ether lipid cardiolipins in archaeal membranes of extreme haloalkaliphiles. *Biochim Biophys Acta* 2012;1818:1365–1373.
19. Collins MD. Analysis of isoprenoid quinones. *Meth Microbiol* 1985; 18:329–363.
20. Elling FJ, Becker KW, Könneke M, Schröder JM, Kellermann MY *et al.* Respiratory quinones in *Archaea*: phylogenetic distribution and application as biomarkers in the marine environment. *Environ Microbiol* 2016;18:692–707.
21. Gupta RS, Naushad S, Baker S. Phylogenomic analyses and molecular signatures for the class *Halobacteria* and its two major clades: a proposal for division of the class *Halobacteria* into an emended order *Halobacteriales* and two new orders, *Haloferacales* ord. nov. and *Natrialbales* ord. nov., containing the novel families *Haloferacaceae* fam. nov. and *Natrialbaceae* fam. nov. *Int J Syst Evol Microbiol* 2015;65:1050–1069.
22. Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
23. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017; 67:1613–1617.
24. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W *et al.* New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 2010;59:307–321.
25. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
26. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A *et al.* A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat Biotechnol* 2018; 36:996–1004.
27. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW *et al.* Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 2010;11:119.
28. Nakamura T, Yamada KD, Tomii K, Katoh K. Parallelization of MAFFT for large-scale multiple sequence alignments. *Bioinformatics* 2018;34:2490–2492.
29. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 2009;25:1972–1973.
30. Anisimova M, Gascuel O. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Syst Biol* 2006;55:539–552.
31. Lefort V, Longueville JE, Gascuel O. SMS: smart model selection in PhyML. *Mol Biol Evol* 2017;34:2422–2424.
32. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2016;32: 929–931.
33. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
34. Rodriguez-R LM, Konstantinidis KT. Bypassing cultivation to identify bacterial species. *Microbe Magazine* 2014;9:111–118.
35. Zhao B, Hu Q, Guo X, Liao Z, Bowers KJ *et al.* *Natronolimnobius aegyptiacus* sp. nov., an extremely halophilic alkalithermophilic archaeon isolated from the athalassohaline Wadi An Natrun, Egypt. *Int J Syst Evol Microbiol* 2018;68:498–506.
36. Itoh T. Natronolimnobius. In: Whitman WB (editor). *Bergey's Manual of Systematic of Bacteria and Archaea*. Chichester: John Wiley & Sons, Ltd; 2016.
37. Oren A, Ventosa A, Grant WD. Proposed minimal standards for description of new taxa in the order *Halobacteriales*. *Int J Syst Bacteriol* 1997;47:233–238.
38. Holding AJ, Collee JG. Routine biochemical tests. *Meth Microbiol* 1971;6A:1–32.
39. Oren A. Halorubraceae. In: Whitman WB (editor). *Bergey's Manual of Systematic of Bacteria and Archaea*. Chichester: John Wiley & Sons, Ltd; 2017.
40. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR *et al.* Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.