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Halo(natrono)archaea from hypersaline lakes can utilise sulfoxides other than DMSO as electron acceptors for anaerobic respiration

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The 16S rRNA gene sequences obtained in this study were deposited in the GenBank under the accession numbers MT928301- MT928305.

Summary

Dimethylsulfoxide (DMSO) has long been known to support anaerobic respiration in a few species of basically aerobic extremely halophilic euryarchaea living in hypersaline lakes. Recently, it has also been shown to be utilized as an additional electron acceptor in basically anaerobic sulfur-reducing haloarchaea. Here we investigated whether haloarchaea would be capable of anaerobic respiration with other two sulfoxides, methionine sulfoxide (MSO) and tetramethylene sulfoxide (TMSO). For this, anaerobic enrichment cultures were inoculated with sediments from hypersaline salt and soda lakes in southwestern Siberia and southern Russia. Positive enrichments were obtained for both MSO and TMSO enrichments with yeast extract but not with formate or acetate as the electron donor. Two pure cultures obtained from salt lakes, either with MSO or TMSO, were obligate anaerobes closely related to sulfur-reducing *Halanaeroarchaeum sulfurireducens,* although the type strain of this genus was unable to utilize any sulfoxides. Two pure cultures isolated from soda lakes were facultatively anaerobic alkaliphilic haloarchaea using O_2, sulfur and sulfoxides as the electron acceptors. One isolate was identical to the previously described sulfur-reducing *Natrarchaeobaculum sulfurireducens*, while another isolate, enriched at lower alkalinity, is forming a new species in the genus *Halobiforma*. Since all isolates enriched with either MSO or TMSO were able to respire all three sulfoxides including DMSO and the corresponding activities were cross-induced, it suggest that a single enzyme of the DMSO-reductase family with a broad substrate specificity is responsible for various sulfoxide-dependent respiration in haloarchaea.

Key words: haloarchaea, hypersaline lakes, methionine sulfoxide, tetramethylene sulfoxide, anaerobic respiration

Introduction

Extremely halophilic euryarchaea have long been considered as predominantly aerobic heterotrophs with a few exceptions of facultative anaerobic species capable of fermentative growth by fermentation of arginine or sugars, or anaerobic respiration with nitrate, fumarate, dimethyl sulfoxide (DMSO) or trimethylamine *N*-oxide (TMAO) as terminal electron acceptors (Antunes et al., 2008; Oren, 1991; 2006; Oren and Trüper, 1990; Werner et al., 2014). In particular, the anaerobic growth with DMSO as acceptor had been demostrated for members of the *Halobacteriaceae* family, including genera *Halobacterium*, *Haloarcula* and *Haloferax* (Oren, 1991). But it must be realized, that the majority of haloarchaea isolated in pure culture came from aerobic brines, whereby they represent a dominant part of the prokaryotic community (Banciu et al., 2012; Oren 2013). Recent investigation of a possible role of haloarchaea in dissimilatory sulfur cycle revealed a presence of functional groups of haloarchaea in anaerobic sediment of hypersaline lakes capable of anaerobic respiration of elemental sulfur, thiosulfate and DMSO. The obligately anaerobic neutrophilic sulfur-respiring haloarchaea from salt lakes include two genera: the genus *Halanaeroarchaeum* utilizing acetate and pyruvate as the electron donors and elemental sulfur as the electron acceptor (Sorokin et al., 2016 a,b) and the genus *Halodesulfurarchaeum*, growing by sulfur/thiosulfate-dependent respiration with H_2 or formate as the electron donors (Sorokin et al., 2017). In soda lakes sulfur-respiring alkaliphilic haloarchaea were represented by two genera of facultative anaerobes: a dominant natronophilic genus *Natrarchaeobaculum* (first described as *Natronolimnobius sulfurireducens*), which members uses fatty acids and formate as the electron donors and a moderately alkaliphilic genus *Halalkaliarchaeum* with a more restricted range of substrates limited to formate and pyruvate (Sorokin et al., 2018; 2019; 2020). In addition to sulfur and thiosulfate, members of the genera *Halodesulfurarchaeum* and *Natrarchaeobaculum* can also grow with DMSO as acceptor and the genomes of the type

78 strains contain an operon encoding DmsABC - dissimilatory DMSO reductase (Sorokin et al.,
79 2017; 2018). The midpoint redox potential of DMSO at pH 7 is estimated at +160 mV, which
80 makes it a much more favorable electron acceptor for anaerobic respiration than sulfur or
81 thiosulfate (Wood, 1981).

82 Apart from DMSO, which is forming by DMS oxygenation in the atmosphere and
83 from delignification of cellulose in paper industry (Lovelock et al., 1975), there is another
84 biologically important sulfoxide - methionine sulfoxide (MSO) forming from methionine as a
85 product of oxidative stress response. The cytoplasmically formed peptide-MSO can be
86 regenerated back to methionine by two types of oxidoreductases. The type 1 includes
87 cytoplasmic thiol-dependent enzymes methionine sulfoxide reductase MsrA, MsrB and fRMsr
88 are widely present in all domains of life. They have a catalytic cysteine or selenocysteine in
89 the active site and can reduce peptide-MSO (MsrA and MsrB), free MSO (MsrB and fRMsr)
90 and DMSO. The type 2 consists of the molybdopterin superfamily oxidoreductases, including
91 dimethylsulfoxide reductase DmsA and biotin sulfoxide reductase BisC, whose side activities
92 include MSO-reductase (Boschi-Muller and Branlant 2014; Ezrati et al., 2005; Maupine-
93 Furlow 2018; Tarrago and Gladyshev, 2012; Weisbach et al., 2002).

94 It had been showed previously that free MSO can be utilized by bacteria, along with
95 DMSO, for anaerobic respiration and that the reaction is most likely catalyzed by the DMSO-
96 reductase DmsABC, in particular in *E. coli* (Bilous and Weiner, 1985; Meganathan and
97 Schrementi, 1987) and in an identified marine bacterium strain DL-1 (might be a
98 *Campylobacter* species, judging from the combined phenotypic properties) (Zhinder and
99 Brock, 1978). The bacterium was also able to grow anaerobically with another, more exotic
100 compound - tetramethylene sulfoxide (TMSO) which was reduced to tetrahydrothiophene
101 (THT), a saturated analogue of thiophene commonly present in sulfurous oils
102 (**Supplementary Fig. S1**). Tetrahydrothiophene is a component of a plethora of volatile

103 organic sulfur compounds produced in the bulbs of *Allium* plants (Nohara et al., 2014).
104 However, no archaeal species have ever been reported to respire sulfoxides other than DMSO.
105 Here we show that haloarchaea from hypersaline lakes can use MSO and TMSO,
106 along with DMSO, for anaerobic respiration, most probably via a DMSO reductase with a
107 broad substrate specificity.
108
109 ## Methods
110
111 *Samples*
112 Two composite samples of anaerobic sulfidic sediments (5-15 cm deep) from hypersaline salt
113 and soda lakes in southwestern Siberia (Kulunda Steppe, Altai region, July 2016, ambient
114 temperature 30 ^{o}C) and south Russia (October 2017, ambient temperature 8 ^{o}C). The salinity
115 and the pH of brines of salt lakes in Kulunda Steppe (three lakes, N51^{o}42'/ E79^{o}42'- E79^{o}46')
116 were 200-240 g/l and 7.5-8.0, respectively and in the south Russia (lakes Elton and
117 Baskunchak,) - 350 g/l and 6.6. The
118 salinity and the pH in four sampled soda lakes of Kulunda Steppe (N51^{o}39'- N51^{o}40'/E79^{o}48'-
119 E79^{o}54') were 100-400 g/l and 10.1-11.0, with total carbonate alkalinity reaching up to 4 M.
120 Top 15 cm sediment samples were taken by a corer with 2 cm internal diameter, the top 5 cm
121 layer was cut off and the remaining sample was extruded into a 300 ml Schott bottle, covered
122 with 50 ml of the near bottom brines and closed without air bubbles. The mix inoculum for
123 each lake types was prepared by mixing equal parts of the sediment slurries from different
124 lakes, followed by a low speed centrifugation (2,000 rpm for 10 sec) to remove course
125 particles and mostly resulting in a colloidal fraction enriched in microbial cells.
126
127 *Enrichment and cultivation conditions*

Two basic media containing 4 M total Na^+ were used for enrichment and cultivation of anaerobic haloarchaea: the neutral base containing 4 M NaCl and buffered at pH 7 with 50 mM potassium phosphates and the alkaline base containing 4 M total Na^+ as a mixture of sodium carbonate/bicarbonate at pH 10. The neutral base was used as it is for cultivation of neutrophic haloarchaea. For alkaliphilic haloarchaea from soda lakes, the neutral and soda bases were mixed in various proportions to produce pH range from 8.8 to 10.5. Details of preparation of full anaerobic media can be found in Sorokin *et al*. (2016; 2017; 2018). Sulfoxides (Sigma-Aldrich, chemical grade) were added at 5 (DMSO and TMSO) -10 (MSO) mM final concentrations from 1 M stock solutions sterilized by filtration through the 0.2 μm syringe filters Millex-HV (PVDF). H_2 (0.5 atm overpressure on the top of argon atmosphere), formate (50 mM), acetate, pyruvate, butyrate (5 mM) and yeast extract (1 g/l) were used as the electron donors. In case of H_2 and formate, 0.1 g/l of yeast extract was added as the C-source. The incubation temperature was 37^{o}C. Positive development in the enrichments, followed by serial dilutions in liquid media was followed qualitatively by increase in the culture turbidity (in case of MSO) or by the appearance of characteristic smell of TMS in case of TMSO.

For solid media, 12 ml portions of liquid media supplemented with electron donor-acceptor were dispensed into 15 ml Hungate tubes and the incoculum was serially diluted in these tubes up to 10^{-7}. The tubes were subjected to three cycles of evacuation-flushing with argon and preheated on a water bath at 50 ^{o}C and 3 ml of 4% melted agar was added to each tube and poured into Petri dishes to prepare serial dilutions in soft agar. After cooling, the plates were placed into 3.5 anaerobic jars (Oxoid), the jars were made anoxic with 3 cycles of evacuation-argon flushing and incubated at 37^{o}C until appearance of visible colonies. Single colonies were picked from the agar with sterile Pasteur capillaries into a 0.2 ml of anoxic

media and the mix was injected into 10 ml ready to use anoxic medium in 12 ml serum bottles closed with butyl rubber stoppers.

For cell suspension experiments, the cultures were grown in 500 ml volumes in 550 ml bottles closed with butyl rubber stoppes with argon gas phase with periodic hand shacking. The growth was monitored by regular OD_{600} measurements, and at the end of exponential growth phase the cells were collected by centrifugation at 7,300 g for 30 min at 4 ^{o}C, washed twice with 10 ml of chilled 4 M NaCl and resuspended either in 4 M NaCl buffered at pH 7 by 50 mM K-P buffer (for neutrophilic haloarchaea) or in 3:1 mixture of the neutral:alkaline base media (see above) at pH 9.5 (both buffers were kept at 22 ^{o}C). 2 ml concentrated cell suspensions were placed into 7 ml serum bottles, supplemented with an electron donor/acceptor, closed with butyl rubber stoppers and made anoxic by 3 cycles of evacuation-argon flushing. The bottles were incubated statically at 37 ^{o}C for 48 h. The incubation was terminated by placing the bottles into a freezer.

Identification of isolates

The newly isolated halo(natrono)archaea utilizing sulfoxides as the electron acceptor were identified by 16S rRNA gene sequencing. The genomic DNA was extracted from cells obtained from 2 ml cultures using alkaline SDS lysis at 60^{o}C and purified with the Wizard Preps Kit (Promega, USA). The nearly complete 16S rRNA gene was amplified with the primer pair arch8f-1492r. The consensus sequences were obtained in BioEdit and blasted in NCBI against cultured haloarchaeal database.

Chemical analyses in pure culture experiments

Cell growth was monitored by OD_{600} and cell protein in the cell suspension experiments was quantified by the Lowry method after hydrolysis in 1 M NaOH at 100 ^{o}C for 5 min. Sulfide

and polysulfide sulfane in sulfur-reducing cultures was precipitated in 10% Zn acetate and analyzed by the methylene blue method (Trüper and Pfennig, 1964).

Methionine was measured using LC-MS (Agilent 6420 LC-MS/MS) with a selective electrospray triple quad LC-MS/MS MRM transition. A SeQuant ZIC-cHILIC column (100 mm × 2.1 mm, 3 µm pore size, Merck, the Netherlands) equipped with a guard column (SeQuant ZIC-cHILIC Guard kit, Merck, the Netherlands) were used for the analyte separation at 40 °C. Mobile phases A (0.1% formic acid) and B (acetonitrile with 0.1% formic acid) were applied at a constant ratio of 31:69 with a flow rate of 0.25 mL min^{-1}. The injection volume was 3 µL. Before liquid analysis, samples were filtrated over a 0.45 µm membrane syringe filter (HPF Millex, Merck, the Netherlands) and then diluted with Milli-Q water. DMS and THT in the gas phase were quantified by GC with a flame photometric detector (GC-FPD) (Kiragosyan et al., 2020). To recalculate the concentration of these volatile compounds the liquid phase, the dimensionless gas-to-liquid partition coefficient (K_c) had to be recalculated. K_c is defined as the ratio of the concentration of a compound in the gas phase and the concentration in the liquid phase at equilibrium. For this four vials with the total volume of around 240 mL were filled with 50 mL of the experimental buffer (4 M Na^+ buffer, pH = 7). The vials were closed with stoppers made of blue butyl rubber resistant to sulfur compounds. Each vial contained one sulfur compound at a concentration of 0.1 mM. Vials were left for 24 hours at 23 °C to ensure the equilibrium between the gas and liquid phase. Next, the headspace of each vial was analyzed using GC-FPD. Knowing the volume of the gas and liquid phase, the total concentration of the sulfur compound, and its concentration in the headspace, it was possible to determine K_c for each compound. The resulting K_c for DMS and tetrahydrothiophene were 0.163 and 0.032, respectively.

Results and discussion

Enrichment and isolation of MSO and TMSO-respiring halo(natrono)archaea

Enrichment cultures either with 50 mM formate/H_2 (+100 mg/l yeast extract as carbon source), 5 mM acetate or butyrate or 1 g/l yeast extract as electron donors and 5 mM MSO or TMSO as acceptors showed significant increase in cell density only in the presence of yeast extract after 3-4 weeks of incubation. Growth in all enrichments intensified by increasing yeast extract concentration, while omitting other *e*-donors (H_2, formate, acetate, butyrate) had no influence. Therefore, further cultivation efforts were focused only on the cultures whereby yeast extract served as both *e*-donor and C-source. In those cultures, stable growth was observed in several 1:100 transfers, and in the sediment-free subcultures formation of methionine (MT) from MSO and THT from TMSO was detected. Serial dilutions, however, did not result in single phenotypes and the final isolation of pure cultures was achieved in soft agar plates incubated in anaerobic jars under argon atmosphere.

Finally, two pure cultures of neutrophilic haloarchaea utilizing yeast extract as the electron donor and C-source for anaerobic growth were isolated from salt lakes, one on MSO (strain HMSO) and another on TMSO (strain HTMSO) as the electron acceptors. Strain HMSO has small coccoid cells, while HTMSO cells are small flat rods, both nonmotile (**Fig. 1 a-b**). In case of soda lakes, only the MSO/yeast extract enrichments were stable in transfers and resulted in isolation of two pure cultures of alkaliphilic haloarchaea: strain AMSO1 was isolated at pH 10 and strain AMSO2 - at pH 9.3. The AMSO1 cells were flat motile rods and the AMSO2 cells were highly polymorphic, from flat rods and discs to coccoids, mostly nonmotile (**Fig. 1 c-d**). In an enrichment with TMSO, archaea (even in presence of antibiotics) were apparently inhibited and outcompeted with a rod-shaped bacterium isolated in pure culture and identified as a known extremely salt-tolerant member of *Bacillales*, *Salisediminibacter halotolerance* (99.6% 16S rRNA gene sequence identity to the type strain) (**Fig. 1 e**).

Identification of sulfoxide-utilizing haloarchaea (**Table 1**)

The salt lake isolates HMSO and HTMSO were identified as members of the genus *Halanaeroarchaeum*, which, so far, includes a single species *Haa. sulfurireducens* (Sorokin et al., 2016 b). HMSO has 98.9 % and HTMSO - 99.2 % of the 16S rRNA gene sequence identity to the type strain *Haa. sulfurireducens* HSR2, and the two isolates were 99.2 % similar to each other. Such close relation actually came as a surprise, since *Haa. sulfurireducens* is unable to use even DMSO as acceptor and also can not use yeast extract as donor for anaerobic sulfur respiration (Sorokin et al, 2016 a,b). Moreover, neither HMSO nor HTMSO grew anaerobically with acetate + elemental sulfur - a main physiological trait of the genus *Halanaeroarchaeum*, and only HMSO used sulfur in combination with pyruvate for anaerobic growth. All these differences call for an emendation of the genus *Halanaeroarchaeum* diagnosis. Furthermore, it is also clear that despite the high level of 16S rRNA gene identity to *H. sulfurireducens* the two novel sulfoxide isolates are fundamentally different from the type species and might represent another species in the genus *Halanaeroarcheum*.

The two alkaliphilic MSO-respiring haloarchaea from soda lakes were identified as members of two different genera: AMSO1, enriched at more alkaline conditions, was practically identical in its 16S rRNA gene sequence (2 nt difference in 1,428) to the type strain AArc1 of *Natrarchaeobaculum sulfuriredicens* (former *Natronolimnobius sulfurireducens*), a dominant cultured group of facultatively anaerobic sulfur-respiring natronoarchaea in hypersaline soda lake sediments (Sorokin et al., 2017; 2018; 2019). Therefore, we also checked the type strain AArc1 of *N. sulfurireducens* for its ability to grow anaerobically with MSO and TMSO with formate as the *e*-donor, which was the most active *e*-donor for both elemental sulfur and DMSO respiration in this genus as shown in the original

work. However, similar to AMSO1, the result was negative. On the other hand, AArc1 grew with all three sulfoxides in presence of yeast extract as the e-donor/C source.

The second isolate, strain AMSO2, probably represents a new species in the genus *Halobiforma* with the maximum 16S rRNA gene sequence identity of 98.6% to its type species *H. haloterrestris*, an alkalitolerant haloarchaeon from a hypersaline soil (Xu and Oren, 2016). We have already suggested previously on the basis of genomic content that members of this genus, in particular *H. nitratireducens*, might potentially be capable of anaerobic sulfur respiration (Sorokin et al., 2017).

Growth physiology of the sulfoxide-utilizing haloarchaea

Growth tests with various sulfoxides demonstrated that all four isolates were able to grow anaerobically with DMSO, MSO, and TMSO as the electron acceptors and yeast extract as the electron donor/C source forming DMS, MT and THT as products, respectively. The highest cell density and sulfoxide reduction activity was achieved with MSO, while with DMSO and especially with TMSO, the growth was already inhibited at an early stage by accumulation of toxic volatile products DMS and THT. That was obvious from the growth stimulation after removal of the products by three cycles of vacuum boiling/argon flushing (**Fig. 2**). On the other hand, calculated specific biomass yield had an opposite trend, i.e. TMSO>DMSO>MSO (Supplementary Table S1). This might be related to the difference in redox potentials, but since the values for MSO and TMSO are not available in the literature, it remains unclear.

Testing other donors/acceptors (**Table 1**) revealed that strain HMSO and both alkaliphilic AMSO isolates can grow anaerobically with sulfur in presence of yeast extract and pyruvate, but the growth yield and sulfide(polysulfide) formation were much weaker at these conditions in comparison with the closely related type species on acetate (for *Haa. sulfurireducen*) or formate (*Nab. sulfurireducens*), respectively. Most active reduction of

277 sulfur (but still with a very low growth yield) for all sulfur-respiring isolates was observed
278 with H_2 as the electron donor. On the other hand, the reduction of sulfoxides was most active
279 with yeast extract. This is not an easily explainable fact. One of the possibility is that growth
280 by sulfoxide respiration not only depends on the reducing power of an electron donor, but,
281 perhaps on the additional detoxifying/chaperoning effect of the complex amino acid substrate,
282 like yeast extract.

283 The two neutrophilic strains were obligate anaerobes, similar to the type species *Haa.*
284 *sulfurireducens*, while the soda lake isolates grew well under aerobic conditions after a
285 gradual adaptation from anaerobiosis via microaerophily (2% O_2 in the gas phase). The ability
286 of *Halobiforma* strain AMSO2 to grow anaerobically with sulfur and sulfoxides is the first
287 demonstration of such potential in the genus *Halobiforma* and suggests a necessity for
288 reevaluation of its species physiology. Moreover, to our knowledge, *Halobiforma* AMSO2 is
289 a first cultured representative of this genus isolated from soda lakes.

290

291 *Cross induction of the various sulfoxide-reducing activity in anaerobic haloarchaea*

292 As has been mentioned above, there are evidences that a single reductase from the Dms
293 family might be responsible for anaerobic respiration with various sulfoxides in bacteria. The
294 case of haloarchaea seems to be similar, judging from the growth experiments. To confirm
295 this, the cross induction tests were performed with resting cells of three isolates grown
296 anaerobically with three different sulfoxides. First interesting (and surprising) fact was that
297 the biomass color of neutrophilic strains HMSO and HTMSO grown on sulfoxides was from
298 pale to dark pink, indicating that the cells contained bacterioruberins (Supplementary **Fig. S2**)
299 that were lacking in sulfur-reducing cultures of the type species *Haa. sulfurireducens*. Next,
300 the results demonstrated that the cells of sulfur-reducing strains (HTMSO and AMSO1)
301 grown on a given sulfoxide possessed reducing activity with other two sulfoxides, but not

sulfur-reducing activity (**Table 2**). This indicated that all three sulfoxides might be substrates for a DmsABC enzyme with a broad substrate specificity, while the sulfur/polysulfide reductase (PsrABC) is a separate enzyme, as also follows from the previous genomic analysis of the closely related species of sulfur-respiring haloarchaea (Sorokin et al., 2016 a; 2018). Furthermore, we now have evidences that another group of neutrophilic sulfur-reducing haloarchaea related to the genus *Halapricum* is also capable of anaerobic growth with all three sulfoxides (unpublished data). However, it must be stressed here that a focused enzymatic work would be necessary targeting substrate specificity of the DMSO-reductase in sulfoxide-respiring haloarchaea.

Concluding, here we demonstrated for the first time that haloarchaea from hypersaline lakes have the potential for anaerobic respiration with two more sulfoxide species in addition to the previously known DMSO, and that, most probably, all three sulfoxides are reduced by the same type of enzyme responsible for the DMSO respiration. A second important finding is the ability for sulfur respiration in the genus *Halobiforma*. And, lastly, the results on the neutrophilic sulfoxide-respiring haloarchaea indicated that the diagnosis of genus *Halanaeroarchaeum* needs emendation.

Emended description of the genus *Halanaeroarchaeum* Sorokin *et al*., 2016 b

In addition to the key metabolic properties described previously for the type species (Sorokin et al., 2016 a, b), some of the newly discovered closely related strains can grow anaerobically using DMSO, methionine sulfoxide and tetramethylene sulfoxide as the electron acceptors and yeast extract as the electron donor/carbon source, but can not use acetate as the electron donor for sulfur respiration. In contrast to the type species, the sulfoxide-respiring members produce carotenoids.

Conflict of interest: The authors declare that they have no conflict of interest.

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References

Antunes A, Taborda M, Huber R, Moissl C, Nobre MF, da Costa MS (2008) *Halorhabdus tiamatea* sp. nov., a non-pigmented extremely halophilic archaeon from a deep-sea, hypersaline anoxic basin of the Red Sea, and emended description of the genus *Halorhabdus*. Int J Syst Evol Microbiol 58:215-220.

Andrei AS, Banciu HL, Oren A\. (2012)\. Living with salt: metabolic and phylogenetic diversity of archaea inhabiting saline ecosystems. FEMS Microbiol Lett 330:1–9.

Bilous PT, Weiner JH (1985) Dimethyl sulfoxide reductase activity by anaerobically grown *Escherichia coli* HB101. J Bacteriol 162:1151-1155.

Boschi-Muller S, Branlant G (2014) Methionine sulfoxide reductase: Chemistry, substrate binding, recycling process and oxidase activity. Bioorg Chem 57: 222-230.

Ezraty B, Bos J, Barras F, Aussel L (2005) Methionine sulfoxide reduction and assimilation in *Escherichia coli*: new role for the biotin sulfoxide reductase BisC. J Bacteriol 187:231-237.

Kiragosyan K, Picard M, Sorokin DY, Dijkstra J, Klok JB, Roman P, Janssen AJ (2020) Effect of dimethyl disulfide on the sulfur formation and microbial community composition during the biological H_2S removal from sour gas streams. J Hazardeous Materials 386:121916.

Lovelock JE, Maggs RJ, Rasmussen RA (1972). Atmospheric dimethyl sulfide and the natural sulphur cycle. Nature 237:452-453.

Maupine-Furlow JA (2018) Methionine sulfoxide reductase of archaea. Antioxydants 7:124.

Meganathan R, Schrementi J (1987) Tetrahydrothiophene 1-Oxide as an electron acceptor for *Escherichia coli.* J Bacteriol 169:2862-2865.

Nohara T, Fujiwara Y Kudo R, Yamaguchi K, Ikeda T, Murakami K, Ono M, Kajimoto T, Takeya M. (2014) Isolation and characterization of new onionins A_2 and A_3 from *Allium cepa*, and of onionins A_1, A_2, and A_3 from *Allium fistulosum*. Chem Pharm Bull 62:1141-1143.

Oren A (2006) The order *Halobacteriales*. The Prokaryotes. A Handbook on the Biology of Bacteria, Vol 3, 3rd edn (Dworkin M, Falkow S, Rosenberg E, Schleifer KH and Stackebrandt E eds), pp. 113–164. Springer, New York.

Oren A (2013) Life at high salt concentrations, intracellular KCl concentrations, and acidic proteomes. Front Microbiol 4:315.

Oren A, Trüper HG (1990) Anaerobic growth of halophilic archaeobacteria by reduction of dimethylsulfoxide and trimethylamine N-oxide. FEMS Microbiol Lett 70:33-36.

Oren A. (1991). Anaerobic growth of archaeobacteria by reduction of fumarate. J Gen Microbiol 137: 1387-1390.

Pfennig N, Lippert KD (1966). Über das Vitamin B_{12} –bedürfnis phototropher Schwefelbacterien. Arch Microbiol 55:245-256.

Sorokin DY, Kublanov IV, Gavrilov SN, Rojo D, Roman P, Golyshin PN, Slepak VZ, Smedile F, Ferrer M, Messina E, La Cono V, Yakimov MM (2016 a) Elemental sulfur and acetate can support life of a novel strictly anaerobic haloarchaeon. The ISME J 10:240-252.

Sorokin DY, Kublanov IV, Yakimov M, Rijpstra WIC, Sinninghe Damsté JS (2016 b) *Halanaeroarchaeum sulfurireducens* gen. nov., sp. nov., a first obligately anaerobic sulfur-respiring haloarchaeon from hypersaline lakes. Int J Syst Evol Microbiol 66:2377-2381.

Sorokin DY, Kublanov IV, Yakimov M, Rijpstra WIC, Sinninghe Damsté JS (2016) *Halanaeroarchaeum sulfurireducens* gen. nov., sp. nov., a first obligately anaerobic sulfur-respiring haloarchaeon from hypersaline lakes. Int J Syst Evol Microbiol 66:2377-2381.

Sorokin DY, Messina E, Smedile F, Roman P, Sinninghe Damste JS, Ciordia S, del Carmen Mena M, Ferrer M, Golyshin PN, Kublanov IV, Samarov NI, Toshchakov SV, La Cono V, Yakimov MM

(2017) Discovery of the anaerobic lithoheterotrophic haloarchaea, ubiquitous in hypersaline habitats. The ISME J 11:1245–1260.

Sorokin DY, Messina E, La Cono V, Ferrer M, Ciordia S, del Carmen Mena M, Toshchakov SV, Golyshin PN, Yakimov MM (2018). Sulfur respiration in a group of facultatively anaerobic natronoarchaea ubiquitous in hypersaline soda lakes. Front Microbiol 9:2359.

Sorokin DY, Merkel AY, Messina E, Yakimov MM, Itoh T, Mesbah NM, Wiegel J, Oren A. (2020) Reclassification of the genus *Natronolimnobius*: proposal of two new genera, *Natronolimnohabitans* gen. nov. to accommodate *Natronolimnobius innermongolicus* and *Natrarchaeobaculum* gen. nov. to accommodate *Natronolimnobius aegyptiacus* and *Natronolimnobius sulfurireducens*. Int J Syst Evol Microbiol 70:3399-3405.

Sorokin DY, Yakimov MM, Messina E, Merkel AY, Bale NJ, Sinninghe Damste JS (2019) *Natronolimnobius sulfurireducens* sp. nov., and *Halalkaliarchaeum desulfuricum* gen. nov., sp. nov., the first sulfur-respiring alkaliphilic haloarchaea from hypersaline alkaline lakes. Int J Syst Evol Microbiol 69:2662-2673.

Tarrago L, Gladyshev V (2012) Recharging oxidative protein repair: catalysis by methionine sulfoxide reductases towards their amino acid, protein, and model substrates. Biochemistry (Moscow, English Translation) 77:1097-1117.

Weissbach H, Etienne F, Hoshi T, Heinemann SH, Lowther WT, Matthews B, St. John G, Nathan C, Brot N (2002) Peptide methionine sulfoxide reductase: structure, mechanism of action, and biological function. Arch Biochem Biophys 397:172-178.

Werner J, Ferrer M, Michel G, Mann AJ, Huang S, Juarez S, Ciordia S, Albar JP, Alcaide M, La Cono V, Yakimov MM, Antunes A, Taborda M, da Costa MS, Hai T, Glöckner FO, Golyshina OV, Golyshin PN, Teeling H, The MAMBA Consortium (2014) *Halorhabdus tiamatea*: proteogenomics and glycosidase activity measurements identify the first cultivated euryarchaeon from a deep-sea anoxic brine lake as potential polysaccharide degrader. Environ Microbiol 16:2525–2537.

Wood PM (1981) The redox potential for dimethyl sulphoxide reduction to dimethyl sulphide. Evaluation and biochemical implications. FEBS Lett 134:11-14.

Xu X-W, Oren A (2016) *Halobiforma*. In: Bergey's Manual of Systematics of Archaea and Bacteria, Bergey's Manual Trust. doi: 10.1002/9781118960608.gbm01336. John Wiley & Sons, Inc.

435 Zinder SH, Brock TD (1978) Dimethyl sulfoxide as an electron acceptor for anaerobic growth. Arch
436 Microbiol 116:35-40.

437
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439

440 **Table 1**. Characteristics of sulfoxide-utilizing haloarchaea from hypersaline lakes

Property	**HMSO**	**HTMSO**	**AMSO1**	**AMSO2**
Source	Salt lakes		Soda lakes	
Enriched with	MSO/ye	TMSO/ye	MSO/ye	
Colony morphology	Small, lens-shaped, pale orange		Small, lens-shaped, red	Large lens-packets, orange
Cell morphology	Small coccoids, nonmotile	Flat rods, nonmotile	Flat rods, motile	Flat, polymorphic, motile
Maximum growh pH	Neutrophilic		10.5	9.7
Sulfoxides as electron acceptor with ye as donor	DMSO, MSO and TMSO		DMSO, MSO and TMSO	
Additional *e*-acceptors*	Sulfur	none	O_2, sulfur	
e-donors for elemental sulfur reduction	Pyruvate, ye	none	Formate, H_2, lactate, pyruvate, C_4-C_6 VFA, ye	H_2, lactate, pyruvate, ye
Closest relative(s)	*Halanaeroarchaeum sulfurireducens*		*Natrarchaeobaculum sulfurireducens**	*Halobiforma* spp.
% 16S-rRNA gene identity	98.9	99.2	99.9	98.2-98.6

441 *former *Natronolimnobius sulfurireducens*
442 VFA- volatile fatty acids
443

444 **Table 2**. Cross-induction tests on sulfoxide-reducing activity with resting
445 cells of anaerobic haloarchaea pregrown with different sulfoxides as
446 *e*-acceptors and yeast extract as *e*-donor/C source.

Strain	Grown with:	Activity of sulfoxide reduction nmol/(mg protein min)			Activity ratio: MT:DMS:THT
		MSO	DMSO	TMSO	
HMSO	MSO	97	37	15	6.7: 2.6: 1
	DMSO	83	59	29.5	3.7: 2.6: 1
HTMSO	TMSO	450	268	100	4.5: 2.7: 1
	DMSO	108	63	25	4.2: 2.5: 1
AMSO1	MSO	750	511	355	2.1: 1.4: 1
	DMSO	680	650	290	2.3: 2.2: 1

447 Cells of neutrophilic haloarchaea were incubated in 4 M NaCl buffered at pH 7 with 50 mM
448 K-P buffer; cells of soda lake isolate AMSO1 were incubated in a buffer containing 4 M
449 total Na^+ containing 2 M Na^+ as carbonate buffer and 2 M NaCl at pH 10. The cells were
450 incubated statically at 37^{o}C for 48 h. Sulfoxides were added at 5 mM concentration and 1 g/l
451 of yeast extract was used as the electron donor. The sulfoxide-grown cells had no sulfur-reducing
452 activity. The data are from a single experiment.

453 **Legend to figures**

454

455 **Fig. 1**. Cell morphology of sulfoxide-respiring haloarchae (**a-d**) and a bacterial isolate (**e**)
456 from hypersaline lakes grown anaerobically with sulfoxides and yeast extract (phase contrast
457 microphotographs). (**a)** neutrophilic haloarchaeon HMSO; (**b)** neutrophilic haloarchaeon
458 HTMSO; (**c)** and **(d)**, alkaliphilic haloarchaea AMSO1 and AMSO2.

459

460 **Fig. 2**. Maximum growth yields and product formation during anaerobic growth of
461 haloarchaea with three different sulfoxides (10 mM DMSO and MSO and 5 mM of TMSO) as
462 *e*-acceptors and yeast extract (1 g/l) as *e*-donor/C source. **A**, neutrophilic strains HMSO and
463 TMSO; **B**, alkaliphilic strains AMSO1 (pH 10) and AMSO2 (pH 9.3). Incubation time for
464 HMSO and HTMSO: 100 h with MSO, 200 h with DMSO and 300 h with TMSO; for AMSO
465 strains: AMSO1 - 140 h, AMSO2 - 112 h. In case of DMSO and TMSO, the gas phase was
466 refreshed 2 times in the course of incubation to decrease DMS or THT toxicity. The data
467 represent average values from parallel duplicate cultures.

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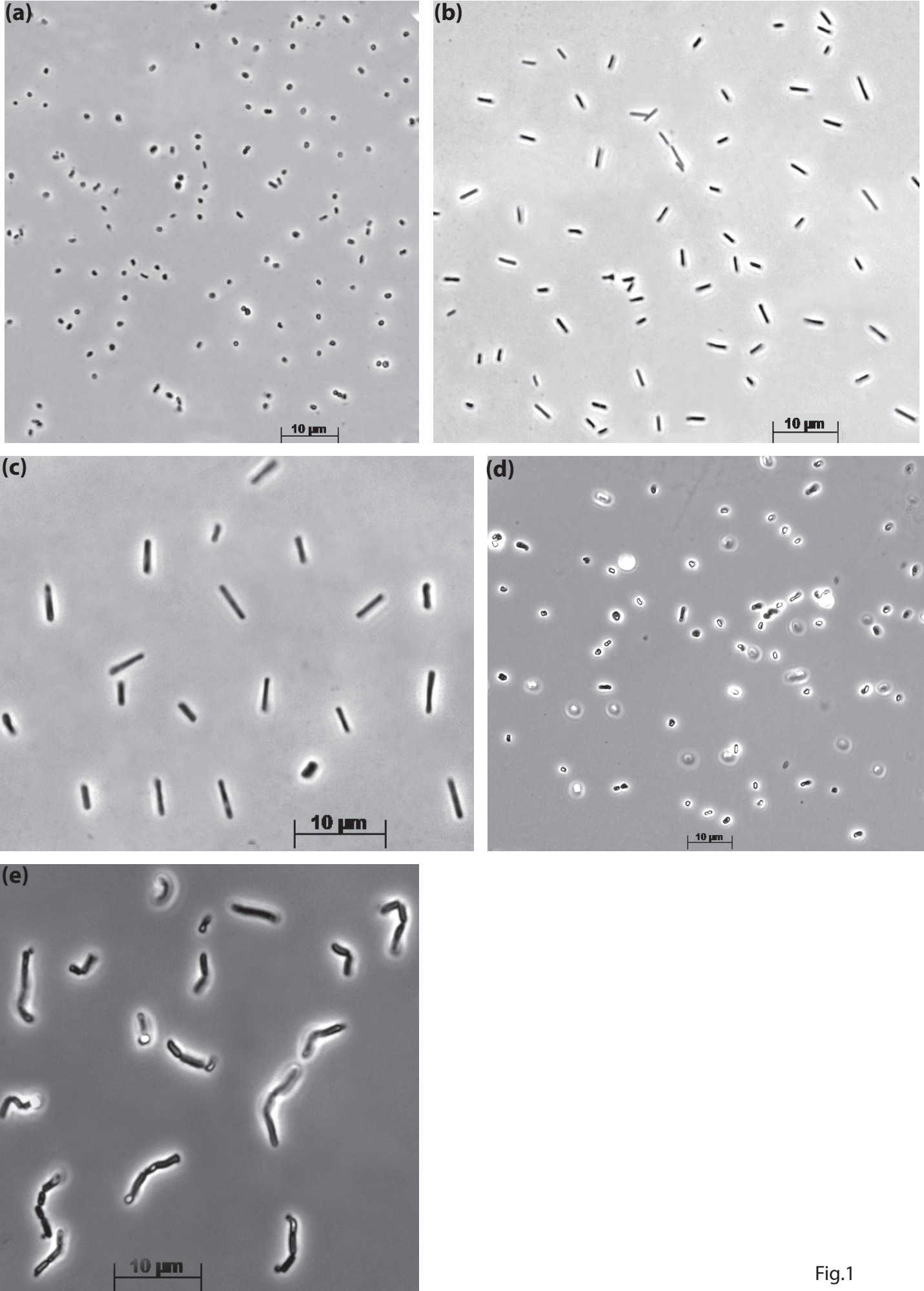


Fig.1

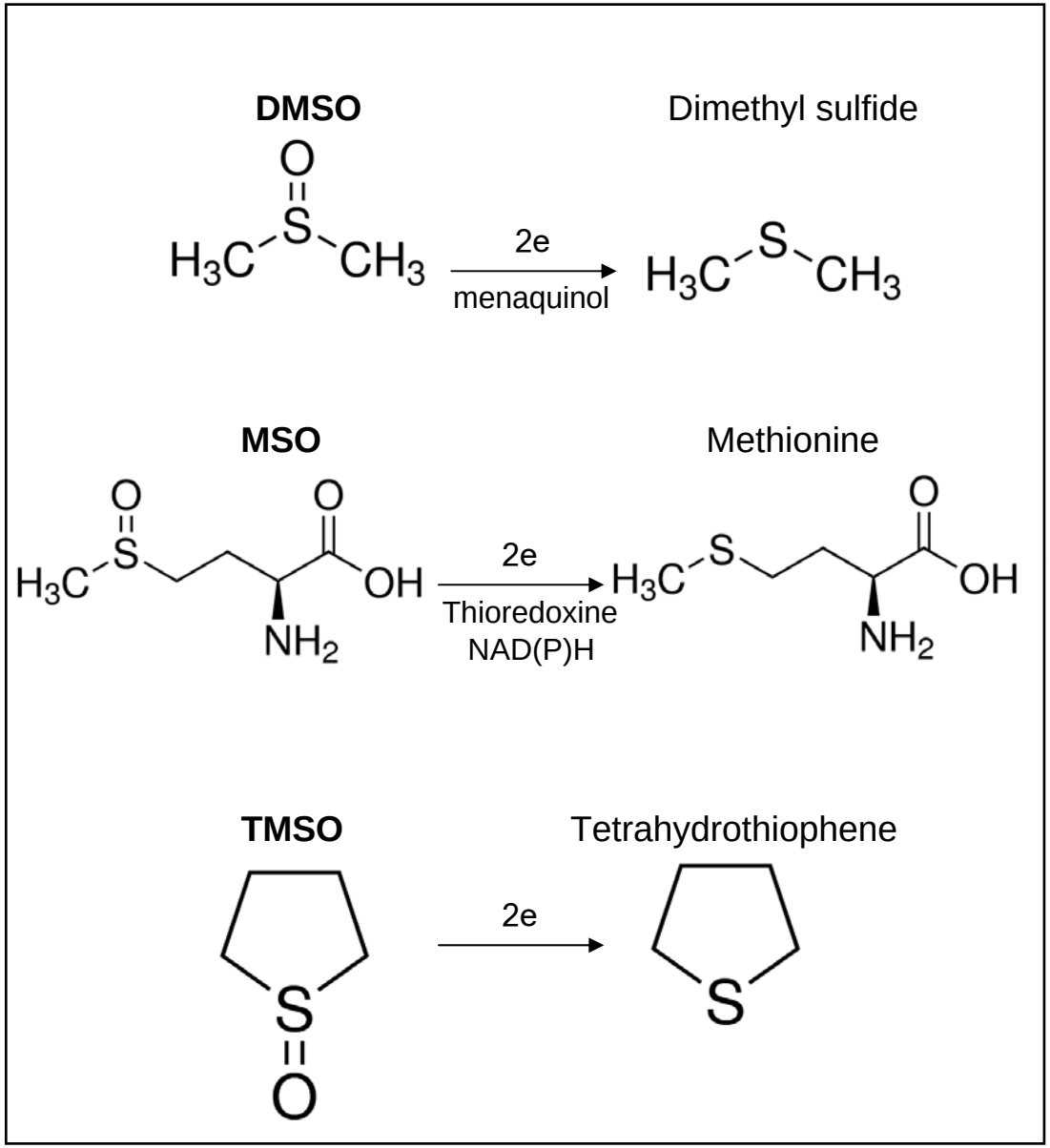


Fig. S1. Chrmical structures and reduction reactions of sulfoxides used in the work

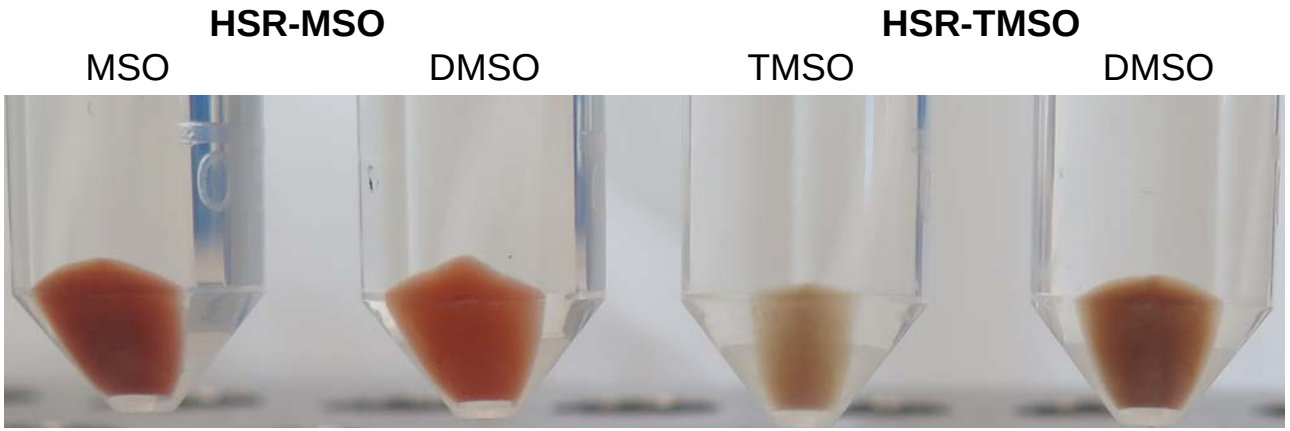
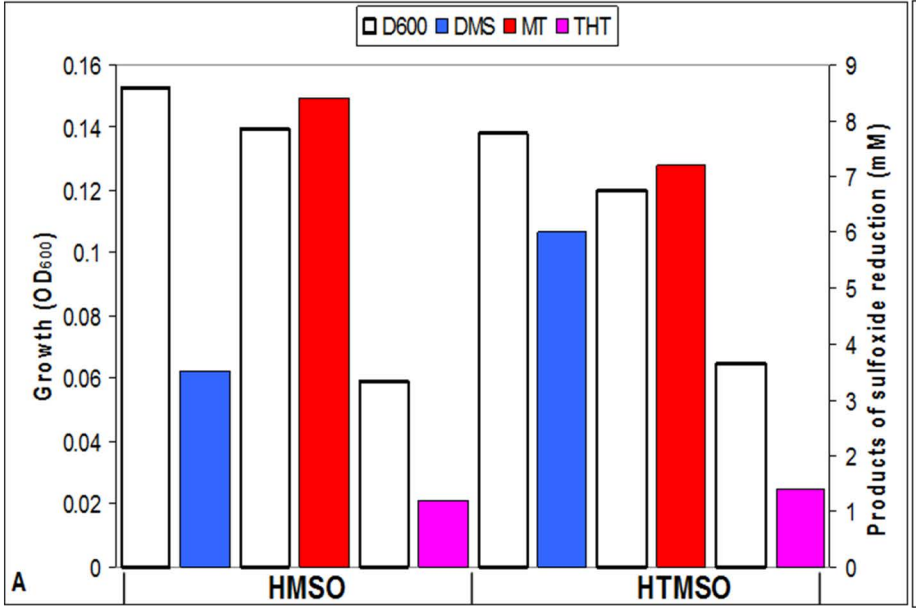
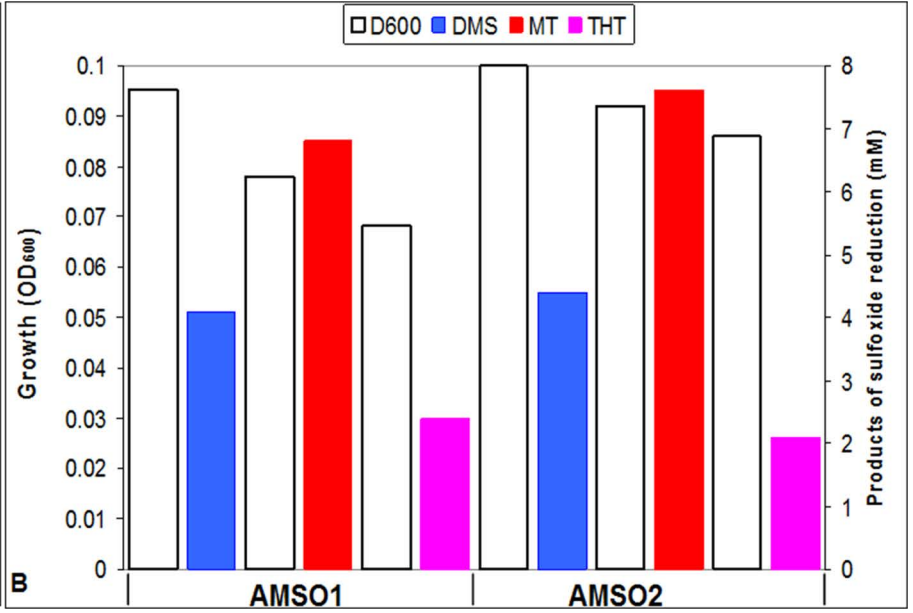


Fig. S2 Biomass color of neutrophilic haloarchaeal strains grown anaerobically with different sulfoxides

Supplementary Table S1. Specific biomass yield (mg cell protein/mM reduced sulfoxide) of haloarchaea grown anaerobically with three different sulfoxides as the electron acceptors and 1 g/l of yeast extract as the electron donor/C source

Sulfoxides	Neutrophilic isolates belonging to the genus *Halanaeroarchaeum*		Alkaliphilic isolates	
	HMSO	HTMSO	*Natranaerobaculum* sp. AMSO1	*Halobiforma* sp. AMSO2
MSO	8.3	8.3	5.7	6.1
DMSO	21.8	11.2	10.4	11.4
TMSO	24.6	23.2	14.2	20.5


D600
DMS
MT
THT
Growth (OD600)
Products of sulfoxide reduction (mM)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
9
8
7
6
5
4
3
2
1
0
A
HMSO
HTMSO


D600
DMS
MT
THT
Growth (OD600)
Products of sulfoxide reduction (mM)
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
8
7
6
5
4
3
2
1
0
B
AMSO1
AMSO2