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Halapricum desulfuricans sp. nov., carbohydrate-utilizing, sulfur-respiring haloarchaea from hypersaline lakes

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ABSTRACT

Nine pure cultures of neutrophilic haloaloarchaea capable of anaerobic growth by carbohydrate-dependent sulfur respiration were isolated from hypersaline lakes in southwestern Siberia and southern Russia. According to phylogenomic analysis the isolates were closely related to each other and formed a new species within the genus *Halapricum* (family *Haloarculaceae*). They have three types of catabolism: fermentative, resulting in H_2 formation; anaerobic respiration using sulfur compounds as *e*-acceptors and aerobic respiration. Apart from elemental sulfur, all isolates can also use three different sulfoxides as acceptors and the type strain also grows with thiosulfate, reducing it partially to sulfide and sulfite. All strains utilized sugars and glycerol as the *e*-donors and C source for anaerobic growth and some can also grow with alpha-glucans, such as starch and dextrins. The major respiratory menaquinones are MK-8:8 and MK-8:7, but 5–19% consists of "thermoplasmata" quinones (MMK-8:8 and MMK-8:7), whose occurrence in haloarchaea is unprecedented. On the basis of their unique physiological properties and results of phylogenomic analysis, the isolates are suggested to be classified into a novel species *Halapricum desulfuricans* sp. nov. (type strain HSR12-2^T = JCM 34032^T = UNIQEM U1001^T).

Introduction

Our previous research into anaerobic haloarchaea growing by sulfur respiration resulted in the discovery of two functional groups in this novel ecotype of extremely halophilic archaea, including obligately anaerobic acetate- and formate-utilizing genera *Halanaeroarchaeum* and *Halodesulfurarchaeum* [1–5]. Such haloarchaea seem to specialize on the final anaerobic mineralization of fermentation products – a metabolism not previously known among multiple pure cultures of predominantly aerobic haloarchaea, suggesting that extremely halophilic euryarchaea may play an active role in anoxic sulfidic sediments of hypersaline systems, such as athalassic salt lakes and thalassic evaporative salt crystallizers. However, to be self-sufficient, this new functional system of sulfur-respiring haloarchaea lacked one metabolic group, namely primary anaerobes, which would decompose carbohydrates and provide electron donors for the aforementioned subgroups. Such organisms have recently been discovered and their metabolism characterized [6]. They are facultatively anaerobic, in contrast to the two previously characterized groups of obligate anaerobes, fermenting sugars and glycerol in the absence of electron acceptors with formation of H_2. In the presence of sulfur or sulfoxides, they switch to anaerobic respiration and can also grow by aerobic respiration at microoxic conditions [6]. Here we provide a formal taxonomic description of these organisms as *Halapricum desulfuricans* sp. nov.

Materials and methods

Enrichment and cultivation conditions

The sources of the inocula were subsurface (3–30 cm deep) anaerobic sulfidic sediments from Russian hypersaline salt lakes in Kulunda Steppe (Altai region) and Volgograd region (Table 1).

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Table 1
Sulfur-respiring haloarchaea from hypersaline salt lakes utilizing sugars and glycerol as *e*-donor.

Strain	Enrichment and isolation conditions				Growth	
	Isolated from	Isolated with	pH	T, °C	electron donors*	electron acceptors
HSR12-1	Kulunda Steppe (Altai, Russia)	glucose + S_8	7.5	30	gl, fr, raf, gal**, mal, suc, trh, mlb, glc	S_8, MSO, DMSO, TMSO, O_2
HSR12-2^T	(51°39′ N/79°48′ E)		7.5	30		S_8, $S_2O_3^{2-}$, MSO, DMSO, TMSO, O_2
HSR-T1			7.5	46		S_8, DMSO, O_2
HSR-T2			7.5	46		
HSR-Kgl		glycerol + S_8	7.5	37	gl, fr, raf, mal, suc, trh, glc	S_8, MSO, DMSO, TMSO, O_2
HSR-Bgl	Baskunchak (south Russia) (49°10′ N/46°39′ E)		6.8	37		
HSR-Kst	Kulunda Steppe (Altai, Russia)	starch + S_8	7.5	37	starch, pullulan, dextrin, glycogen, cyclodextrin;	
HSR-Bst	Baskunchak (south Russia)		6.8	37	gl, mal, suc, glc	
HSR-Est	Elton (south Russia) (48°14′ N/ 46°35′ E)		6.8	37		

*gl, glucose; fr, fructose, raf, raffinose; man, mannose; gal, galactose; mal, maltose; suc, sucrose; trh, trehalose; mlb, melibiose; mlz, melezitose; glc, glycerol. **galactose was used only by HSR12-2. ***microaerophilic in all strains. Sulfoxides: MSO – methionine sulfoxide, DMSO – dimethylsulfoxide, TMSO – tetramethylene sulfoxide.

The samples were taken by a stratometer corer tube with 25 mm internal diameter into sterile 100 ml Schott bottles and filled to the top with the near-bottom brines. After transportation, part of the brine was removed and the head space was flushed with argon. For the inoculation, the 1:1 mixture of brine-sediment was homogenized by vortexing and subjected to a brief low speed centrifugation to remove a coarse sandy fraction which left a fine colloidal fraction containing most of the cells. The enrichment and isolation procedures, the medium composition, cultivation conditions and analyses of growth and sulfide/polysulfide formation have been described previously [5]. In short, 4 M NaCl base medium was supplemented with (g l^{-1}): g l^{-1} of HEPES 5, K_2HPO_4 2.5; NH_4Cl 0.5, KCl 5 and adjusted to pH 7 with 1 M NaOH. After sterilization, the medium was supplemented with trace metal and vitamin solutions, 1 mM $MgSO_4$ and 40 mM of $NaHCO_3$ (from 1 M filter-sterilized stock solution) to balance acidification by fermentation products. Sulfur was added at approximately 5 g l^{-1} directly into incubation vials, thiosulfate was used at 10 mM and sulfoxides – at 5–10 mM. The medium was reduced with 0.5 mM sulfide and the cultivation was done in serum bottles varying in volumes from 12 to 115 ml after creating anoxic conditions by evacuation-argon flushing. Microoxic cultivation was performed in 115 ml serum bottles with 20 ml medium at 2% O_2 in the gas phase. The isolation of pure cultures was achieved by several rounds of serial dilution to extinction in anaerobic conditions and the purity was confirmed by microscopy and by 16S rRNA gene and genome sequencing

Pure culture characterization

Cell morphology was examined by using phase contrast microscopy (Zeiss Axioplane Imaging 2, Germany) and two types of electron microscopy. For the flagella detection, the paraformaldehyde-fixed cells in 4 M NaCl were positively stained with 1% uranyl acetate and the salts were removed with a brief emersion into demineralized water. For thin section electron microscopy, the cells, resuspended in 4 M NaCl, were mixed 1:1 with 2% OsO_4, incubated at 4 °C for 1 week, embedded into agar, dehydrated in alcohol series and finally in acetone, incorporated into Epon resin and thin sectioned on ultramicrotome. The sections were contrasted in 1% (w/w) uranyl acetate and lead citrate solutions.

For the cell suspension activity tests strain HSR12-1 was grown in 200 ml volume anaerobically with glucose and three different sulfoxides – dimethylsulfoxide (DMSO), methionine sulfoxide (MSO) and tetramethylene sulfoxide (TMSO). The cells were collected by centrifugation, washed and resuspended in mineral medium containing 4 M NaCl and 50 mM K-phosphate buffer at pH 7 at cell protein concentration of 0.12–0.15 mg ml^{-1}. 2 ml of the cell suspensions were incubated at 37 °C for 48 h anoxically in 9 ml serum bottles with 5 mM glucose or under H_2 atmosphere with 5 mM of each sulfoxides.

Analyses

Sulfide formation was measured with methylene blue method after fixation with 10% Zn acetate [7] and products of sulfoxide reduction were analyzed as described previously either by HPLC in liquid (methionine) or by the GC in the gas phase (dimethyl sulfide and tetrahydrothiophene) [8]. Catalase and oxidase activity were tested with 3% (v/v) H_2O_2 and 0.1% *N,N,N,N* tetramethyl-p-phenylenediamine hydrochloride, respectively, using cell-free extract from cells of HSR12-1 and HSR12-2^T grown microaerophilically. The protease, esterase and lipase activities were tested in microaerobic cultures of the type strains HSR12-2^T grown on plates with casein/gelatin (after flooding with 10% TCA) and emulsified tributyrin/olive oil (turbidity clearance), respectively. Antibiotic sensitivity of HSR12-2^T was tested aerobically in liquid medium at pH 7 with glucose as substrate.

The intact polar lipids (IPLs) and respiratory quinones were ultrasonically extracted twice for 10 min from freeze-dried biomass in methanol, dichloromethane (DCM) and phosphate buffer (2:1:0.8, v:v:v). The extracts were phase-separated by adding additional DCM and buffer to a final solvent ratio of 1:1:0.9 (v:v:v) The organic phase containing the IPLs was collected and the aqueous phase re-extracted twice with DCM. All steps of the extraction were then repeated, but with a solvent mixture of methanol, DCM and trichloroacetic acid pH 2–3 (2:1:0.8, v:v:v). Finally, the combined extract was dried under a stream of N_2 gas [9]. Before analysis, the extracts were redissolved in MeOH:DCM (9:1, v:v) and filtered through 0.45 μm cellulose syringe filters (4 mm diameter; Grace Alltech, Deerfield, IL, United States). Analysis of extracts was carried out using an Ultra High-Pressure Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS) according to the reversed phase method with modifications of [9,10]. Identification was carried out by comparison of accurate masses and mass spectral fragmentation with published data for IPLs [11] and for quinones [12].

Genomic and phylogenomic analyses

The genomes of four representative isolates, HSR12-1, HSR12-2^T, HSR-Bgl and HSR-Est, were sequenced, and analyzed as described previously [6]. The genomes of all four strains were assembled as a single circular chromosome. Isolates HSR-Bgl and HSR-Est also contained a plasmid. The genome size was ca. 3.0 Mbp. The details of genome statistics are given in the Supplementary Table S1. The G + C % was calculated from the four whole genome sequences.

For phylogenetic reconstructions, 122 archaeal single copy conservative marker genes were used according to the Genome Taxonomy Data Base [13] as well as 16S rRNA gene. The trees were built using the IQ-TREE 2 program [14] with fast model selection via ModelFinder [15] and ultrafast bootstrap approximation [16] as well as approximate likelihood-ratio test for branches [17]. Whole genome comparison was conducted by using three different methods: Average Nucleotide Identity (ANIb and ANIm), using JSpeciesWS web server; Average Amino acid Identity (AAI) by the AAI calculator online of Kostas lab (http://enve-omics.ce.gatech.edu/aai/index) and DDH by the Genome-to-Genome Distance Calculator 2.1 online tool (http://ggdc.dsmz.de/ggdc.php) [18,19].

Results and discussion

Enrichment and isolation of pure cultures

The primary anaerobic sulfur-reducing enrichments were performed with three *e*-donors (2 mM) including glucose, soluble starch and glycerol. Microbial growth, dominated by archaea (judged from the lack of inhibition by a mixture of kanamycin/streptomycin and vancomicin, 100 mg l^{-1} each), and sulfide formation (up to 6 mM) was observed with all three *e*-donors, and these efforts resulted eventually in nine pure cultures after several rounds of dilution to extinction (Table 1). Colony formation was not observed with sulfur as the electron acceptor, probably because of its practical insolubility at neutral pH. The isolates had mostly spheric nonmotile cells of variable size often containing refractive storage granules, most probably polyhydroxyalkanoates (as confirmed by Nile-Blue staining and inferred from genome analysis) (Fig. 1a-b); [6]. Thin section electron microscopy showed that the cells are not actually round but, rather, angular coccoids with a thin *S*-layer type of cell wall covered with EPS and often containing several large intracellular PHA-like granules (Fig. 1c-d). It is noteworthy that, in contrast to the previously described genera of obligate anaerobic sulfur-reducing haloarchaea, whose biomass was black, the cells of sulfur/thiosulfate and, especially, sulfoxide-reducing carbohydrate-utilizing isolates were red, suggesting production of carotenoids under strictly anoxic conditions.

Chemotaxonomy

In the two analyzed neutrophilic isolates HSR12-1 and HSR12-2^T and in the reference strain *H. salinum* [21] the core lipids were represented by archaeol (AR; C_{20}-C_{20}) and extended archaeol (Ext-AR; C_{20}-C_{25}) in roughly equal proportion (Supplementary Table S2), as is usual for haloarchaea. In all three strains the majority of IPLs had a PGP-Me or PG head groups. Furthermore, two glycolipids were also detected, with one or two hexose moieties.

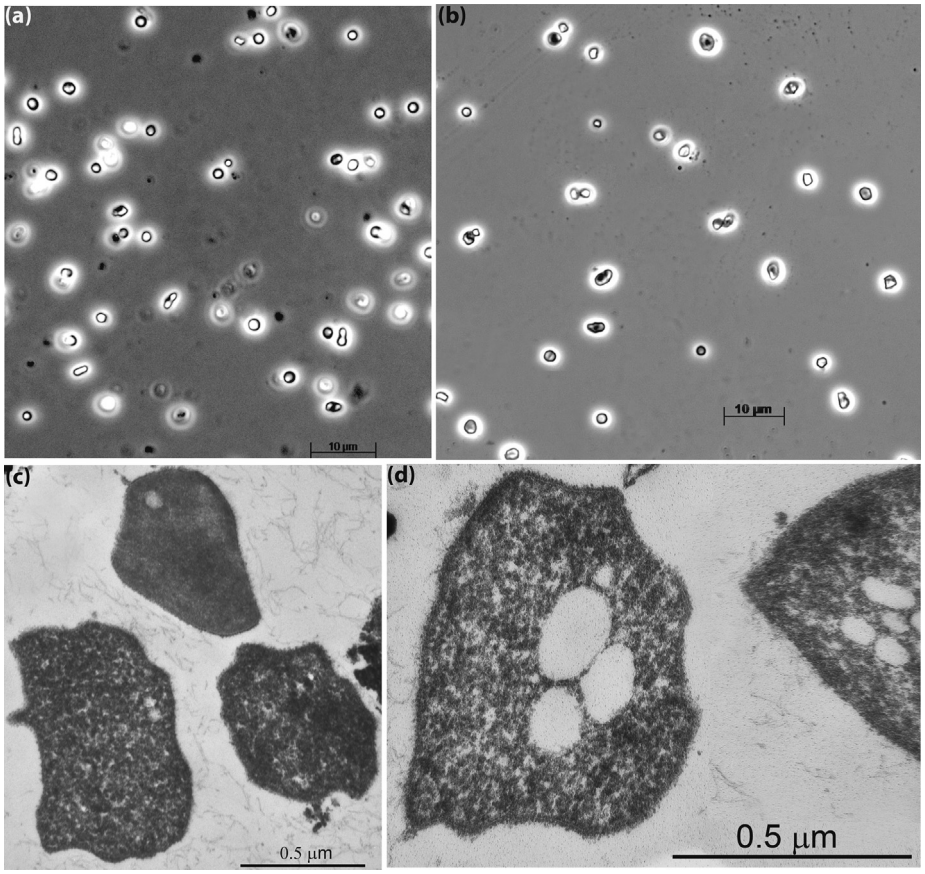

Fig. 1. Cell morphology of carbohydrate-utilizing sulfur-respiring haloarchaea growing anaerobically with sulfur as electron acceptor at 4 M NaCl, pH 7 (a-b), phase contrast microscopy of HSR12-1 and HSR12-2^T cells, respectively. (c-d), electron microphotographs of thin sections of HSR12-2^T cells showing the cell angularity, a thin monolayer cell wall covered with extracellular matrix and intracellular PHA-like storage granules.

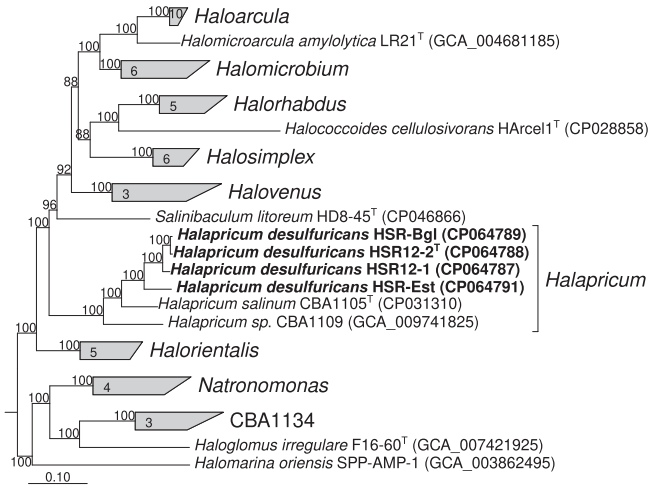

Fig. 2. Phylogenomic placement of carbohydrate-utilizing sulfur-reducing haloarchaea *Halapricum desulfuricans* within the *Haloarculaceae* family based on concatenated partial amino acid sequences of 122 archaeal single copy conserved marker proteins with taxonomic designations according to the Genome Taxonomy Data Base. Bootstrap consensus tree is shown with values above 85% placed at the nodes. Bar, 0.1 changes per position.

Strain HSR12-1 also contained trace levels of AR and Ext-AR with no polar head group, or with just a phosphate moiety. These are possibly precursors or break down products of the IPLs. In general, H. salinum profile was more similar to HSR12-2^T than to HSR12-1.

The dominant respiratory lipoquinone present in HSR12-1 and HSR12-2^T was MK-8:7 (one of the most common in haloarchaea along with MK-8:8 [12]. Surprisingly, however, apart from the MKs, two MMK species, MMK-8:8 and MMK-8:7, were also present as minor (5–19%) components in novel isolates HSR12-1 and HSR12-2^T (but not in the reference strain *H. salinum*) (Supplementary Table S3). These methylmenaquinones are also called "thermoplasmaquinones" (TPQs) because they were first found in the thermoacidiphilic euryarchaeal genus *Thermoplasma,* specifically when its members were grown anaerobically [20]. MMKs have, to the best of our knowledge, never been reported previously in any cultured haloarchaeal species.

Phylogenetic and genomic analyses

The previous 16S rRNA gene-based phylogenetic analysis showed that the nine carbohydrate-utilizing isolates from salt lakes clustered together and all had two highly dissimilar 16S rRNA genes (91.4–92.7% sequence identity to each other) [6], with one of them related to the genus *Halapricum* [21–22] and the other to the genus *Halosimplex* [23] (Fig. S1). Both these genera are classified in the family *Haloarculaceae*. To clarify the phylogeny of the novel isolates, an extended phylogenomic analysis based on 122 archaeal conserved single-copy protein markers was performed using the four sequenced genomes of the HSR isolates. The result showed that the novel haloarchaea belonged to the genus *Halapricum*, despite the significant difference in their *rrn* structure, forming a distinct novel species lineage (Fig. 2). The genus, apparently, includes one more yet uncharacterized species represented by *Halapricum* sp. CBA1109. This conclusion was also apparent from calculations of the whole genome comparison indexes, ANI, AAI and DDH (Supplementary Table S4). These calculations as well as

Table 2
Comparative properties of carbohydrate-utilizing sulfur-reducing haloarchaea with the type species of the nearest phylogenetically related genus *Halapricum salinum* [21,22].

Property	*Halapricum desulfuricans* (9 isolates)	*Halapricum salinum* JCM 19729^T
Cell morphology	nonmotile coccoids	rods, nonmotile
Pigmentation	red (aerobic); pink (anaerobic)	pink
PHA accumulation	+ (with fatty acids)	-*
Aerobic growth	+	+
Anaerobic growth by:		
sugar fermentation	+	-*
sulfur respiration	+	w+*
thiosulfate respiration	+	-*
sulfoxide respiration	MSO, DMSO, TMSO	w + DMSO*
Number of Psr operons in genomes	3	1
e-donors for anaerobic growth	sugars, glycerol, starch (v), H_2 (v; w)	glucose*
Substrates for aerobic growth	sugars, starch (v), glycerol	glucose, sucrose, mannose, maltose, glutamate
Amylase	+	-
Esterase/lipase	- (tributyrin/olive oil)	- (Tweens 20, 40, 80)
Protease	- (casein; gelatin)	- (gelatin)
Catalase/oxidase	-/w+	-/+
Indole from tryptophane	-	-
Salinity range (opt.)		
M Na^+	3.0–5.0 (4.0)	2.5–5.0 (3.2–4.6)
pH range (opt.)	6.5–7.8 (7.0–7.2)	7.0–8.0 (7.0–7.2)
Temperature max (°C)	50	45
Core lipids	C_{20}-C_{20}, C_{20}-C_{25} DGE	C_{20}-C_{20}, C_{20}-C_{25} DGE
Intact membrane polar lipids:		
phospholipids	PG, PGP-Me	PG, PGP-Me
glycolipids	MG, DG	MG, DG
Respiratory Lipoquinones	MK-8:7; MK-8:8; (major) TPQ-8:7; TPQ-8:8 (minor)	MK-8:7; MK-8:8
DNA G + C (%)	63.7–64.6 (genomes, 4 strains)	63.7 (genome)

NR, not reported; (v) – variable property in different strains; w (weak); Psr – polysulfide reductase; *determined in this study. Lipids: (PG) phosphatidylglycerol, phosphatidylglycerophosphate (PGP), (PGP-Me) phosphatidyl-glycerophosphate methyl ester, monoglycosyl ether (MG), diglycosyl (DG), (DGE) – dialkyl glycerol ether; (TPQ) Thermoplasmataquinon

phylogenomic analysis also suggested that among the four genome-analyzed HSR strains, HSR-Est might represent a separate species. However, we could not sufficiently differentiate this strain phenotypically from the other neutrophilic isolates and, therefore, suggest to classify all HSR isolates in a single species.

Metabolic properties

The key physiological property of all isolates is their ability to utilize sugars and glycerol as the energy and carbon source during sulfidogenic growth in presence of elemental sulfur and sulfoxides as the electron acceptor [6]. To the best of our knowledge, anaerobic growth with glycerol as an electron donor has not been previously demonstrated in any pure haloarchaeal culture, and anaerobic growth by sugar fermentation has been reported for only a few known species of extremely halophilic members of *Halobacteria*.

The HSR isolates were able to grow anaerobically by fermentation (i.e. without external electron acceptor) producing H_2 as one of the major products, while in the presence of sulfur, the formation of H_2 drastically decreased in favor of H_2S accompanied by a significant increase in the biomass yield [6]. Such behavior is commonly observed in fermentative sulfur-reducing archaea using sulfur reductase-hydrogenase for cytoplasmic dump of excessive electrons to prevent H_2 accumulation [24], but the HSR isolates used a different mechanism, based on the respiratory membrane-bound PsrABC system [6]. Moreover, external H_2 also served as a direct electron donor for sulfur reduction in HSR strains pregrown on glucose or glycerol due to the presence of a membrane-bound Ni,Fe hydrogenase type Ia [6]. In contrast, formate was not utilized for sulfur respiration in the novel sulfur-reducing isolates. The potential ability for elemental sulfur respiration has already been proposed previously for *Halapricum salinum* due to the presence of a putative PsrABC-encoding operon in its genome [6]. Therefore, the type species, *H. salinum,* was tested for the *in vivo* capability to grow anaerobically with glucose and sulfur or DMSO as acceptors. Although the results can be considered as positive, only 3 mM sulfide and trace amount of DMS were produced in a month incubation, which is much lower than in the HSR strains. Apart from elemental sulfur, the type strain HSR12-2^T was able to use thiosulfate as an *e*-acceptor partially reducing it to sulfide and sulfite. This property has previously been detected only in members of the sulfur-respiring genus *Halodesulfurarchaeum* [4,5]. Finally, the novel isolates, in addition to DMSO, which until recently was the only sulfoxide known to serve as the *e*-acceptor for haloarchaea, also used methionine sulfoxide (MSO) and tetramethylene sulfoxide (TMSO) for anaerobic growth, converting them into methionine and tetrahydrothiophene, respectively (Supplementary Fig. S2). Such potential has recently also been demonstrated in other sulfur-reducing genera of halo(natrono)archaea, including *Halanaeroarchaeum, Natrarchaeobaculum* and *Halobiforma* [8].

Concerning the utilized spectrum of carbohydrates as the *e*-donor/C source, apart from a limited number of mono- and disugars and glycerol, three strains enriched with starch were able to grow anaerobically with soluble starch and some other homologous alpha-glucans (Table 1). This is a first example of the polysaccharide-dependent sulfur respiration in haloarchaea. This potential is confirmed by the presence of multiple gene copies encoding the extracellular alpha-amylase of the GH13 family in the starch-utilizing strains HSR-Est (Supplementary Table S1).

The four sequenced genomes of HSR strains do not encode catalase/peroxidase proteins but, instead, contain several genes for peroxiredoxins (cysteine-containing H_2O_2-reactive proteins involved in oxidative stress response) and a superoxide dismutase. Testing confirmed the absence of a catalase reaction and also showed a weak-positive result for oxidase. This probably reflects the fact that it was difficult to adapt HSR strains to grow aerobically from sulfidogenic cultures.

Table 3
Halapricum desulfuricum: protologue.

Parameter	Species: *Halapricum desulfuricans* sp. nov.
Author	Dimitry Y. Sorokin
Species name	*Desulfuricans*
Genus name	*Halapricum*
Specific epithet	*Desulfuricans*
Species status (SPST)	sp. nov.
Etymology	de.sul.fu'ri.cans. L. pref. *de-* from; L. neut. n. *sulfur* sulfur; N.L. neut. adj. *desulfuricans* reducing sulfur
Description of the new taxon	The cells are nonmotile angular coccoids highly variable in size from 0.8 to 3 μm, depending on the growth conditions often accumulating PHA-like storage granules. The cell wall consists of a thin monolayer covered with an extracellar matrix. The cells lyze in hypotonic solutions below 1 M NaCl. Red carotenoids are produced both during aerobic and anaerobic growth. The core membrane diether lipids are composed of C_{20}-C_{20} DGE (archaeol) and C_{20}-C_{25} DGE (extended archaeol) with 0–4 double bonds. The polar lipid head groups include phosphatidylglycerolphosphate methyl ester (PGP-Me), phosphatidylglycerol (PG) and mono- and diglycosyl ether glycolipids. The dominant respiratory quinone is MK-8:7 with the MK-8:8 second in abundance. Furthermore methylmenaquinones MMK-8:7 and MMK-8:8 (“thermoplasma quinones”) also present in lesser proportions. Facultatively anaerobic. Anaerobic growth is possible by fermentation of carbohydrates with the formation of acetate, lactate and H_2. Also grow by anaerobic respiration with sulfur compounds as acceptors, including elemental sulfur and sulfoxides (DMSO, MSO and TMSO) and some strains can also perform 2-electron reduction of thiosulfate to sulfide and sulfite. In the presence of electron acceptors, the H_2 formation decreases with a concomitant biomass yield increase. Aerobic growth occurs at microoxic conditions. The utilized substrates include hexoses (glucose, fructose, mannose, raffinose, trehalose, maltose, sucrose), starch, pullulane, dextrin and cyclodextrin (three out of the nine isolates) and glycerol. Ammonium and yeast extract are utilized as the N-source. Oxidase is weakly positive, catalase is negative. Maximum growth temperature is 50 °C. Extremely halophilic with a range of total Na^+ for growth from 3 to 5 M (optimum at 4 M) and neutraphilic, with a pH range for growth from 6.5 to 7.8 (optimum at 7.0–7.2). The G + C content of the DNA is 63.7–64.6 % (four genomes). Habitat – anaerobic sediments of hypersaline salt lakes and salterns. The type strain (HSR12-2^T = JCM 34032^T = UNIQEM U1001^T) was isolated sediments of hypersaline salt lakes in Kulunda Steppe (Altai, Russia). The species also includes other eight closely related strains isolated from various hypersaline salt lakes in Russia. The four genomes of strains belonging to this species (with two of them containing a plasmid) are deposited in the GenBank under accession numbers CP064787-CP064792.

(*continued on next page*)

Authors	Dimitry Y. Sorokin, Michail M. Yakimov, Enzo Messina, Alexander Y. Merkel, Michel Koenen,Nicole J. Bale, Jaap S. Sinninghe Damsté
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Journal	Systematic and Applied Microbiology
Corresponding author	Dimitry Y. Sorokin
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Designation of the type strain	HSR12-2
Strain collection numbers	JCM 34032; UNIQEM U1001
16S rRNA gene accession number	Genomes
Genome accession numbers	CP064787-CP064792
Genome status	Complete
G + C, %	63.7–64.6 (genomes of 4 strains)
Country of origin	Russian Federation
Region of origin	Altai region, Volgograd rtegion, Crimea
Date of isolation	2015–11-15
Source of isolation	Surface sediments from hypersaline salt lakes
Sampling dates	2013–07-07; 2015–09-05
Geographic location	*S-W* Siberia, Kulunda Steppe; southern Russia
Latitude	51°39′ N; 49°10′ N; 48°14′ N
Longtitude	79°48′ E; 46°39′ E; 46°35′ E
Depth	0.1 m
Temperature of the sample	8–20 °C
pH of the sample	6.6–7.8
Salinity of the sample	22–36%
Number of strains in study	9
Source of isolation of non-type strains	Hypersaline lakes in Russia
Growth medium, incubation conditions	4 M total NaCl, pH 7; incubation – 37 °C; sulfur as e-acceptor; sugars, glycerol or starch as substrates; anaerobic
Conditions of preservation	Deep freezing in 15% glycerol (v/v)
Gram stain	Negative
Cell shape	Pleomorphic coccoids
Cell size	0.8–3 μm in diameter
Motility (MOTY)	Nonmotile
Sporulation	None
Colony morphology	Not forming
Temperature range for growth	nd
Lowest temperature for growth	nd
Highest temperature for growth	50
Optimal temperature for growth	37–40
Lowest pH for growth	6.5
Highest pH for growth	7.8
Optimum pH for growth	7–7.2
pH category	Neutrophilic
Lowest NaCl concentration for growth	3.0 M
Highest NaCl concentration for growth	5.0 M
Optimum salt concentration for growth	4.0 M
Other salts important for growth	KCl
Salinity category	Extremely halophilic
Relation to oxygen	Facultative aerobe
O_2 conditions for strain testing	Microoxic
Carbon source used (class)	Carbohydrates
Specific compounds	Sugars or glycerol as energy and C source + sulfur ore sulfoxides as *e*-acceptors
Nitrogen source	Ammonium
Terminal electron acceptor	O_2, sulfur, sulfoxides, thiosulfate (type strain)
Energy metabolism	Chemoorganotrophic
Phospholipids	Core membrane lipids are C_{20}-C_{20} DGE (archaeol) and C_{20}-C_{25} DGE (extended archaeol). Polar head groups are phosphatidylglycerophosphate methylester (PGP-Me) and phosphatidylglycerol (PG)
Respiratory lipoquinones	MK-8:7; MK-8:8, MMK8:7, MMK8:8
Glycolipids (GLYC)	Monoglycosyl ether (MGE) and diglycosyl diether (DGDE)
Habitat (HABT)	Hypersaline lakes
Extraordinary features (EXTR)	Anaerobic sulfur respiration with carbohydrates as *e*-donor

The protease, esterase and lipase activities in microaerobic cultures of HSR12-2^T grown on plates were negative. Ammonium and yeast extract (but not nitrate or urea) can serve as the N-source in cultures grown anaerobically with glucose. Indole formation from tryptophan (Kovac's reagent test) showed negative results for HSR12-2^T. Antibiotic sensitivity was tested aerobically in liquid medium for HSR12-2^T grown with glucose. It was sensitive to rifampicin and chloramphenicol above 25 mg l^{-1}, but resistant to streptomycin, ampicillin, kanamycin, vancomycin and gentamicin up to 100 mg l^{-1}.

All isolates belonged to low Mg-requiring extreme halophiles, with optimal growth occurring at 4 M total Na^+ and the range from 3 to 5 M (tested aerobically from 1 to 5 M). The pH profiling in aerobic cultures at 4 M total Na^+ showed that HSR12-2^T is a typical neutrophile with the pH range for growth from 6.5 to 7.8 and an optimum at 7–7.2 (buffer system based on 50 mM HEPES/potassium phosphate/$NaHCO_3$; tested range from pH 6 to 8.5). The temperature range for aerobic growth of HSR12-2^T was from 20 to 50 °C with an optimum at 37–40 °C.

Comparative properties of the HSR isolates strains with the only known (type) species of the genus *Halapricum* in Table 2. The main difference of the new isolates from the related *Halapricum salinum* is their better adaptation for anaerobic growth, the ability to grow anaerobically with alpha-glucans and the presence of a second highly dissimilar *rrn* operon in the genome.

Overall, on the basis of distinct phenotypic and genomic features, the nine carbohydrate-utilizing sulfur-reducing haloarchaeal isolates from hypersaline salt lakes are suggested to be classified as a novel species in the genus *Halapricum*, i.e. *Halapricum desulfuricans sp. nov*. The species protologue is presented in Table 3.

Emended description of the genus Halapricum Song et al. 2014

In addition to the genus description given in the original publication by the Song et al. (2014) [21], both species of the genus are capable of anaerobic respiration using sugars as the *e*-donor and elemental sulfur or DMSO as the *e*-acceptor. Furthermore, one species can also ferment sugars, starch or glycerol. The major respiratory lipoquinones are MK-8:7 and MK-8:8. The major core lipids are C_{20}-C_{20} (archaeol) and C_{20}-C_{25} (extended archaeol) with the PGP-Me and PG as the dominant polar groups.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.syapm.2021.126249.

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Halapricum desulfuricans sp. nov., carbohydrate-utilizing sulfur-respiring haloarchaea from hypersaline lakes

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Supplementary Data

Table S1: Genome statistics of carbohydrate-utilizing sulfur-reducing haloarchaea.

Table S2. Intact polar lipids identified and their abundance (in percent of lpid peak area) in the carbohydrate-utilizing haloarchaea and the type species of the genus *Halapricum*: **1** - strain HSR12-1; **2** - strain HSR12- 2^T, **3** - *H. salinum*. Δ mmu = (measured mass – calculated mass) x 1000; AEC = assigned elemental composition; PGP-Me = phosphatidylglycerolphosphate methyl ester; PG = phosphatidylglycerol; MGE - glycosyl monoether; DGDE - diglycosyl diether; AR = archaeol (C_{20}-C_{20}); EXT-AR = extended archaeol (C_{20}-C_{25}); lyso = one alkyl chain is absent; uns = unsaturated; nd - not detectable

Table S3. Respiratory lipoquinones identified in three strains of the carbohydrate-utilizing sulfur-reducing haloarchaea. MMK - methylmenaquinones ("thermoplasma" quinones) **Table S4**: Full genome comparison indexes of carbohydrate-utilizing sulfur-reducing haloarchaea and their closest related genera.

Fig. S1. Phylogenetic position of carbohydrate-utilizing sulfur-reducing haloarchaea *Halapricum desulfuricum* within the family *Haloarculaceae* based on the 16S rRNA gene sequence comparison. Bootstrap consensus tree is shown with values above 50% placed at the nodes. Bar, 0.1 changes per position.

Figure S2. Induction of sulfur- and sulfoxide-reducing activities in washed cells of carbohydrate-utilizing sulfur-reducing haloarchaeon HSR12-1 grown anaerobically with glucose and various electron acceptors. The activity was tested with either glucose or H_2 as the *e*-donors and four *e*-acceptors: sulfur, methionine sulfoxide (MSO), dimethylsulfoxide (DMSO) and tetrathmethylene sulfoxide (TMSO). The cells were incubated in 4 M NaCl/50 mM K-P buffer (pH 7) at 37°C.

Supplementary **Table S1.**

Paprameter	**HSR12-2^{T}**	**HSR12-1**	**HSR-Bgl**	**HSR-Est**
Genome composition	1 chromosome	1 chromosome	1 chromosome 1 plasmid	1 chromosome 1 plasmid
Chromosome size	**2,893,909 bp**	**2,940,338 bp**	**2,940,228 bp**	**2,699,657 bp**
GC content	64.0%	64.7%	64.2%	63.6%
Total genes	3,078	3,109	3,021	2,755
rRNA genes (5S-16S-23S)	6 (2 operons) dissimilar	6 (2 operons) dissimilar	6 (2 operons) dissimilar	6 (2 operons) dissimilar
Protein-coding genes	3,024	3,055	2,968	2,701
CRISPR regions	2	2	2	-
Plasmid (size)	**-**	**-**	**137,790 bp**	**180,725 bp**
G+ C mol%	-	-	57.0	56.2
Protein-coding genes	-	-	149	158
Total number of glycosidase genes	21	17	20	26
GH13 family extracellular amylase genes	0	0	0	4

Table S2. Intact polar lipids identified and their abundance (in percent of lipid peak area) in carbohydrate-utilizing haloarchaea grown anaerobically with sulfur and glucose at 37°C and 4 M NaCl until late exponential growth phase : **1** - strain HSR12-1; **2** - strain HSR12-2^T ; **3** - *Halapricum salinum* JCM 19729^T

Polar headgroup	Core	$[M+NH_4]^+$	$[M+H]^+$	AEC	Δ mmu	Abundance (%)		
						1	**2**	**3**
PGP-Me	EXT-AR		971.7449	$C_{52}H_{109}O_{11}P_2$	0.9	25.4	32.1	34.1
	Uns-EXT-AR*		969.7286	$C_{52}H_{107}O_{11}P_2$	0.3	13.9	12.7	41.5
	AR		901.6658	$C_{47}H_{99}O_{11}P_2$	0.1	19.4	27.3	8.5
	Lyso-EXT-AR		691.4316	$C_{32}H_{69}O_{11}P_2$	0.6	0.0	0.1	0.3
	Lyso-AR		621.3532	$C_{27}H_{59}O_{11}P_2$	0.4	0.0	0.2	0.1
	Total					**59**	**72**	**85**
PG	EXT-AR		877.7623	$C_{51}H_{106}O_8P$	0.3	0.4	8.8	5.7
	Uns-EXT-AR*		875.7461	$C_{51}H_{104}O_8P$	0.2	0.2	4.7	2.8
	AR		807.6837	$C_{46}H_{96}O_8P$	0.0	0.0	9.5	0.7
	Lyso-AR		527.3704	$C_{26}H_{56}O_8P$	0.3	0.1	0.6	0.8
	Total					**1**	**24**	**10**
DG	EXT-AR	1064.8926		$C_{60}H_{122}NO_{13}$	1.5	6.9	0.7	0.5
	Uns-EXT-AR*	1062.8763		$C_{60}H_{120}NO_{13}$	0.9	6.0	0.5	0.6
	AR	994.8124		$C_{55}H_{112}NO_{13}$	0.4	5.1	1.2	0.1
	Uns(1)-AR	992.7982		$C_{55}H_{110}NO_{13}$	1.1	1.4	0.0	0.0
	Total					**19**	**2**	**1**
MG	EXT-AR	902.8386		$C_{54}H_{112}NO_8$	0.4	7.1	0.6	1.4
	Uns-EXT-AR*	900.8231		$C_{54}H_{110}NO_8$	0.5	3.9	0.4	2.3
	AR	832.7605		$C_{49}H_{102}NO_8$	0.5	6.9	0.7	0.7
	Total					**18**	**2**	**4**
PA	EXT-AR	803.7253		$C_{48}H_{100}O_6P$	0.1	1.5	nd	nd
	AR	733.6475		$C_{43}H_{90}O_6P$	0.5	1.8	nd	nd
	Total					**3**	**nd**	**nd**
	Sum AR					**33**	**39**	**10**
	Sum EXT-AR					**41**	**42**	**42**
	Sum uns-AR					**0**	**1**	**1**
	Sum uns-EXT-AR					**0**	**0**	**0**
	Sum lyso-AR					**1**	**0**	**0**
	Sum lyso-EXT-AR					**24**	**18**	**47**

Δ mmu = (measured mass – calculated mass) x 1000 as calculated for strain 1; AEC = assigned elemental composition; PGP-Me = phosphatidylglycerolphosphate methyl ester; PG = phosphatidylglycerol; PA = phosphatidic acid; MG = monoglycosyl; DG = diglycosyl; AR = archaeol (C_{20}-C_{20}); EXT-AR = extended archaeol (C_{20}-C_{25}); lyso = one alkyl chain is absent; uns = unsaturated. *Sum of species with 1-4 unsaturations, mass given for species with one unsaturation. nd = not detected.

Table S3. Menaquinones identified in carbohydrate-utilizing haloarchaea.
1 - strain HSR12-1; **2** - strain HSR12-2^T; **3** - *Halapricum salinum* JCM 19729^T

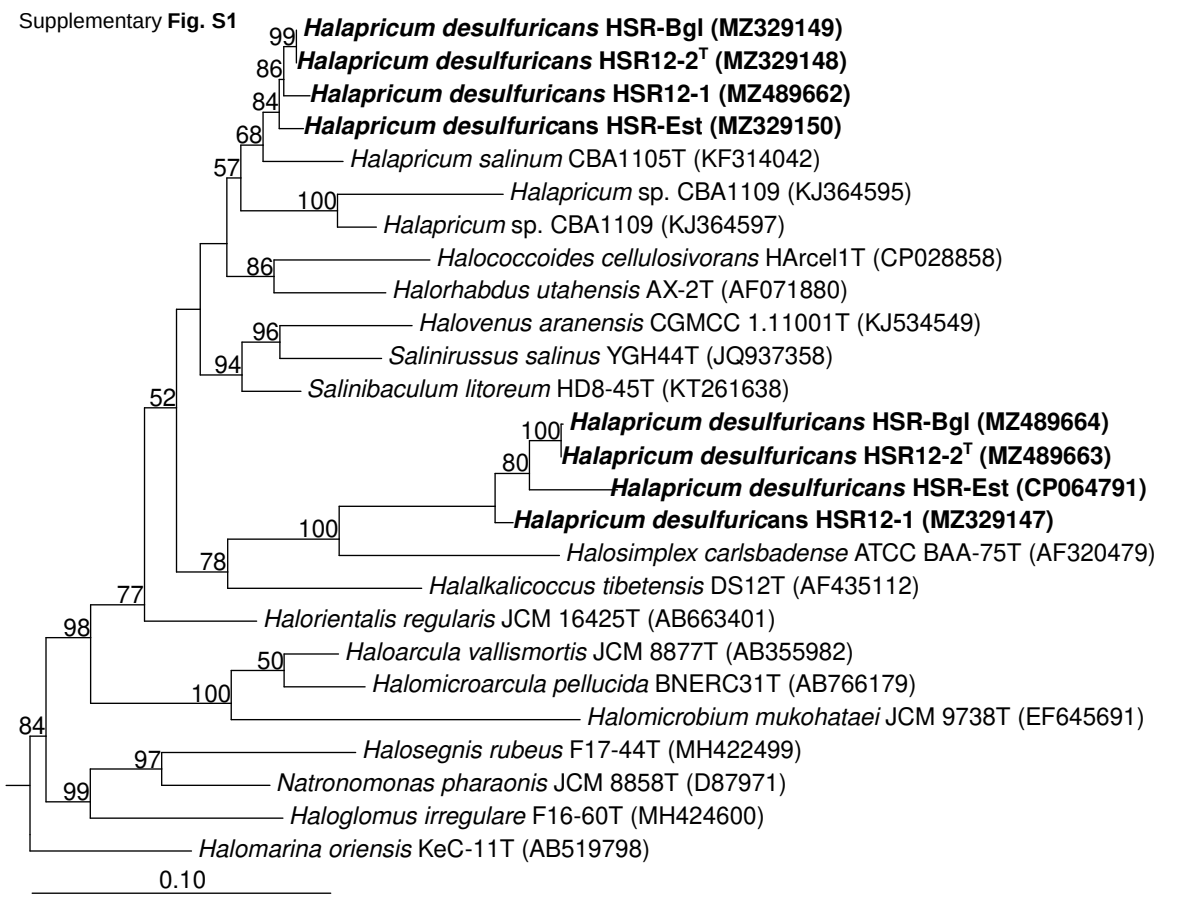
Core	**$[M+H]^+$**	**AEC**	**Δ mmu**	**Abundance (%)**		
				1	**2**	**3**
MK-8:8	717.5615	$C_{51}H_{73}O_2$	1.0	**36.1**	**16.7**	**12.9**
MK-8:7	719.5772	$C_{51}H_{75}O_2$	1.0	**55.5**	**61.4**	**68.0**
MK-7:7	649.4987	$C_{46}H_{65}O_2$	0.8	1.4	0.8	0.2
MK-7:6	651.5143	$C_{46}H_{67}O_2$	0.8	1.9	2.0	0.9
Total				**94.9**	**80.9**	**100**
MMK-8:8	731.5773	$C_{52}H_{75}O_2$	1.1	2.5	3.3	nd
MMK-8:7	733.5924	$C_{52}H_{77}O_2$	0.6	2.6	**15.8**	nd
Total				**5.1**	**19.1**	nd

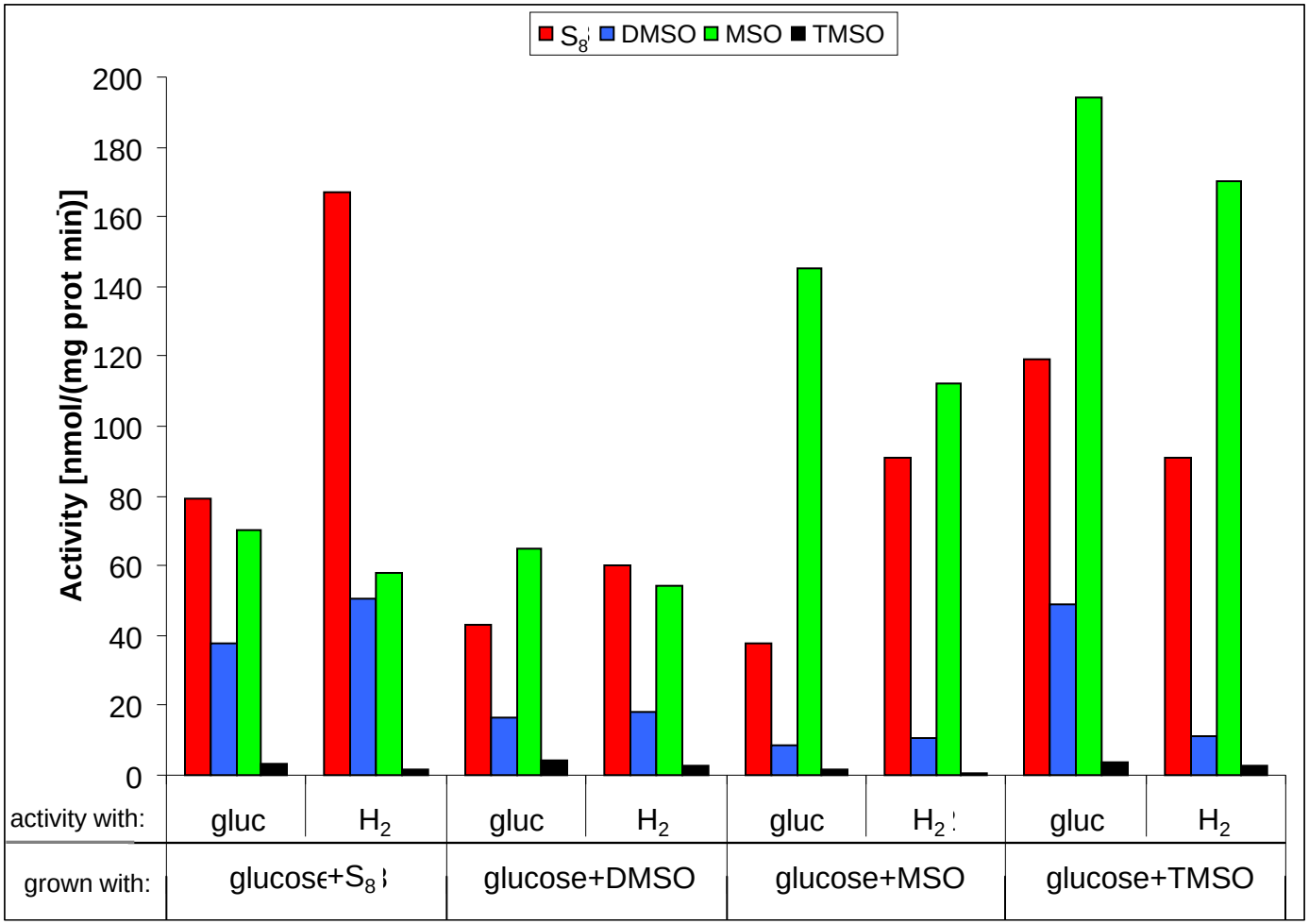
MMK - methylmenaquinones ("thermoplasma" quinones). Δ mmu = (measured mass – calculated mass) x 1000 as calculated for strain 1; AEC = assigned elemental composition. nd = not detected.

Table **S4**.

Index	**HSR-Bgl**	**HSR-Est**	**HSR12-1**	**HSR12-2^{T}**	CBA1105^{T}
ANIb					
HSR-Bgl	*	83.45 [58.29]	92.79 [72.59]	97.82 [82.67]	77.58 [48.65]
HSR-Est	83.57 [62.10]	*	83.57 [64.51]	83.74 [62.89]	77.45 [52.62]
HSR12-1	93.10 [71.99]	83.56 [60.13]	*	93.03 [71.62]	77.50 [48.56]
HSR12-2^{T}	98.01 [84.12]	83.71 [59.15]	92.98 [72.33]	*	77.69 [46.61]
Halapricum salinum CBA1105^{T}	77.37 [41.18]	77.08 [42.07]	77.50 [41.15]	77.54 [39.54]	*
ANIm					
HSR-Bgl	*	86.98 [51.42]	93.76 [77.64]	98.39 [86.11]	84.51 [24.40]
HSR-Est	86.96 [55.52]	*	87.12 [57.40]	87.18 [56.11]	84.36 [26.44]
HSR12-1	93.75 [77.49]	87.12 [52.66]	*	93.72 [76.83]	84.54 [24.81]
HSR12-2^{T}	98.36 [87.67]	87.19 [52.69]	93.72 [78.16]	*	84.54 [24.90]
Halapricum salinum CBA1105^{T}	84.50 [20.48]	84.37 [20.45]	84.54 [20.82]	84.54 [20.44]	*
AAI (Two-way) [SD] percentage					
HSR-Bgl	*	82.89 [12.86]	92.81 [10.85]	97.60 [7.99]	73.21 [15.16]
HSR-Est	82.89 [12.86]	*	83.34 [12.39]	83.35 [12.47]	73.50 [15.08]
HSR12-1	92.81 [10.85]	83.34 [12.39]	*	93.12 [9.95]	73.23 [15.09]
HSR12-2^{T}	97.60 [7.99]	83.35 [12.47]	93.12 [9.95]	*	73.28 [15.41]
Halapricum salinum CBA1105^{T}	73.21 [15.16]	73.50 [15.08]	73.23 [15.09]	73.28 [15.41]	*
GGDC (DDH formula 2)	[p(DDH)>70%]	[same species]			
HSR-Bgl	*	28.30	53.00	85.10 [93.79]	19.20
HSR-Est	28.30	*	28.50	28.80	22.40
HSR12-1	53.00	28.50	*	52.80	23.00
HSR12-2^{T}	85.10 [93.79]	28.80	52.80	*	22.90
Halapricum salinum CBA1105^{T}	19.20	22.40	23.00	22.90	*

Supplementary **Fig. S1**





Supplementary **Fig. S2**