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Discovery of extremely halophilic, methyl-reducing euryarchaea provides insights into the evolutionary origin of methanogenesis

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Supplementary Tables 1 and 2,

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Supplementary Tables (provided as separate Excel files):

Table 3. Comparative genomic analysis based on arCOG assignments. The Table provides complete list of arCOGs present in AMET1 and HMET1 and two groups of interest: halobacteriales and methanogenic archaea. arCOGs shown on the Figure 6 are mapped. Ribosomal proteins used for phylogeny reconstruction are mapped. Archaeal core genes are mapped. Gene sets used for multidimensional scaling analysis are mapped. Results of proteomic analysis are shown. Genes known to be involved in central metabolic pathways are shown together with indicated of the step of the respective pathway.

Table 4. Reconstruction of gene gain and loss.

Table 5. Isoelectric point calculation data.

Table 6. Proteomics analysis for AMET1. Identified proteins are sorted by emPAI index.

Table 7. Proteomics analysis for HMET1. Identified proteins are sorted by emPAI index.

Supplementary Data 1-4 includes original trees in Newick format (provided below)

Supplementary Data 3. Phylogenetic tree of McrA subunit (FastTree method) in Newick.

The maximum-likelihood phylogenetic tree of the McrA proteins was built using the FastTree program as described in the Materials and Methods section. Sequence set and underlying multiple alignment, which can be provided by a request, are the same as on the Supplementary Fig. 9. The tree (below) is provided in Newick format, could be open in MEGA or in any other tree visualization software. To open the file copy the text below the dotted line to a text file with “tre” extension. MEGA (or other tree visualization software) should open the file automatically.

Supplementary Data 4. Phylogenetic tree of McrA subunit (RAxML method) in Newick.

The maximum-likelihood phylogenetic tree of the McrA proteins was built using the RAxML program as described in the Materials and Methods section. Sequence set and underlying multiple alignment, which can be provided by a request, are the same as on the Supplementary Fig. 9. The tree (below) is provided in Newick format, could be open in MEGA or in any other tree visualization software. To open the file copy the text below the dotted line to a text file with “tre” extension. MEGA (or other tree visualization software) should open the file automatically.

Supplementary Table 1. Key chemical characteristics of investigated hypersaline lakes

Lake	Location		Sampling year	Brines			Sediments	
	Area	Coordinates		Total salts (g/l)	pH	Soluble carbonate alkalinity (M)	HS ⁻ +FeS mM	CH ₄ μM
Hypersaline salt lakes								
Cock Salt Lake	Kulunda Steppe (Altai, Russia)	N52°16'/ E79°52'	2015	280-320	7.5-7.7	-		
Lake Lomovoe		N51°42'/ E79°42'		300-340	7.9-8.1	-		
Hummocky Lake		N51°42'/ E79°56'		300-350	8.0-8.1	-		
Lake Elton	South Russia	N49°10'/ E46°39'	2013	320	6.7	-	nd	nd
Lake Baskunchak		N48°14'/ E46°35'		360	6.2	-	nd	nd
Eupatorian solar saltern	Crimea (Russia)	N45°11'/ E33°19'	2015	220	7.2	-	nd	nd
Hypersaline alkaline and soda lakes								
Tanatar-1	Kulunda Steppe (Altai, Russia)	N51°39'/ E79°48'	2013-	380	10.25	3.4		
Bitter-1		N51°40'/ E79°54'	2015	120-220	9.6-10.3	1.2-3.8		
Trona crystallizer		N51°39'/ E79°46'		350-400	9.6-10.2	3.8-5.2		
Cock Soda lake		N52°06'/ E79°09'		50-190	9.6-10.1	0.5-0.65	0.6-15	0.7-20
Stamp Lake		N51°41'/ E79°46'		240-320	9.1	0.15		
Hamra, Fazda, Beidah, Gaar, Umm-Risha, Zugm, Ruzita, Khadra	Wadi al Natrun (Libyan Desert, Egypt)	N30°24'/E30°18'	2000	200-360	9.1-9.9	0.1-0.9	nd	nd
Searles Lake	California (USA)	N35°44'/W117°20'	2005	350	9.8	0.2	nd	nd

Supplementary Table 2.

Extremely halophilic and moderately thermophilic mixotrophic methanogens isolated from hypersaline lakes at 4 M total Na⁺

Strain	Lake	Area	Enrichment conditions			Growth parameters		% similarity to AMET1		Culture collection deposition
			Substrate	pH	T, °C	T _{max} , °C (activity)	Dependence on	16S rRNA gene	<i>mcrA</i>	
AMET1	Mix 5 soda lakes	Kulunda Steppe (Altai, Russia) 2013-2015	MeOH+formate	9.6	48	55	FeS	100	100	DSM 28684 NBRC 110805 UNIQEM 982
AMET3	Tanatar-1				48	62		99.6	99	UNIQEM 985
AMET4	Picturesque Lake				48	50		99.5	99	UNIQEM 986
AMET5	Mix 6 soda lakes		TMA +formate		48	50	sediments	99.5	99	UNIQEM 987
AMET6-2	Tanatar-1		MeOH+formate		60	65		98.4	98.2	UNIQEM 988
AMET7	Soda crystallizer				55	62		99.5	100	UNIQEM 989
AMET8	Mix 6 soda lakes				30	55		99.6	99	
AMET9	Soda crystallizer				43	60		100	99	
AMET10	Stamp Lake		9.2	54	62	99.6		99		
AMET2	Mix from 8 hypersaline alkaline lakes	Wadi al Natrun (Egypt, 2000)	MeOH+formate	9.6	60	62		99.5	100	UNIQEM 984
AMET-SI	Searles Lake	California			48	50		98.2		UNIQEM 1004
HMET1 (mixed culture)	Mix from 4 salt lakes	Kulunda Steppe 2014	TMA+H ₂	7.0	48	55	sediments	90	76	
HMET-EI (mixed culture)	Lake Elton	Southa Russia 2015	MeOH+formate		54	55		90	78	
HMET-Eu (mixed culture)	Salt crystallizer	Crimea (Russia) 2015			55	55		90	78

Supplementary Figure 1 Influence of temperature on potential methanogenic activity of anoxic sediment-brine slurries from 3 different hypersaline systems: (a) - hypersaline soda lake Tanatar-1 (Kulunda Steppe, Altai, Russia); (b) - mixed sediments from 4 hypersaline salt lakes (Kulunda Steppe, Altai, Russia); (c) - sea salt evaporative concentrator (Eupatoria, Crimea, Russia). The error bars are SD from biological replicates (n=2). VCH₄ is the specific rate of methane formation.

Supplementary Figure 2 McrA-DGGE phylogenetic results of sediment incubation experiments at high temperature. Kul-013 - a reference sequence found previously in Kulunda soda lakes in 2013; Elt-Eu – sequences from high temperature enrichments on Me+formate from the salt lake Elton (south Russia) and the sea solar saltern in Eupatoria (Crimea).

AMET1 – colonies

AMET1: biofilm formation
in hydrothermalite and its
disturbance by CH₄ bubbles

strain AMET 2

strain AMET-SI

strain AMET5

strain AMET6-2

strain AMET7

strain AMET8

HMET-Elt

HMET-Eu

Supplementary Figure 3

Morphology of methyl-reducing
methanogens from hypersaline
lakes. The images are typical of 5
technical replicates of single cultures.

a.

b.

Supplementary Figure 4 Elements of membrane-bound respiratory chain of strain AMET1. (a) Dithionite-reduced minus air-oxidized difference cytochrome spectrum of cell-free extract (2 mg protein/ml) prepared from cells of strain AMET1 grown with sterile sediments at 4 M total Na⁺, pH 9.8, 48°C and with MeOH+formate. The γ -peak at 423 nm and the α -peak at 557 nm belong to reduced cytochrome b. (b) Chemical ionization mass spectra of the quinone-like compounds extracted from cells of strain AMET1 and separated in 3 fraction. Fraction 2 contained too little material and was not analyzed. The results represent data from single sample analysis.

Supplementary Figure 5 Growth dynamics at 50°C (a) and temperature profile of methanogenic activity of washed cells (b) of HMET1 with TMA +H₂ at 4 M NaCl. No growth and no activity were observed with mono-substrates (i.e. TMA, MeOH, H₂ or formate alone). Results of single experiment.

Halobacteria

SA1

Supplementary Figure 6 16S-rRNA gene-based phylogenetic tree showing position of the uncultured SA1 group, including the methyl-reducing methanogens from hypersaline lakes within the Euryarchaeota. The tree was reconstructed using the ML algorithm (RAxML in ARBv5.2), 1214 positions used with a SSUref: archaea position variability filter. The nodes indicate the bootstrap values (250 rounds) above 90%.

Methyltransferases AMET1

Methyltransferases HMET1

Supplementary Figure 7 Methyltransferases in the genome of AMET1 and HMET1. mta, methanol-specific; mtm, methamine-specific; mtb, dimethylamine-specific; mtt, trimethylamine-specific; pyl, methyltransferase specific pyrrolysine synthesis. Shaded: highly expressed; shaded with dashed lines – moderately expressed.

Methanomicrobiales

Methanocellales

Methanosarcinales

Methanococcales 1

Methanococcales 2

Methanothermobacteriales 2

Methanopyrales

Methanothermobacteriales 1

ANME-1 group

Candidatus Methanofastidiosia

Methanonatronarchaeia

Methanomassiliococcales

Candidatus Verstraetearchaeota

Candidatus Bathyarchaeota

Supplementary Figure 9 Phylogeny of Methyl coenzyme M reductase alpha subunit (McrA). Branch support values calculated by PhyML are shown and for the clade containing AMET1 and HMET1 are colored red. The sequences are denoted by locus tag ID or genome partition ID, genome names and its code on the genbank FTP site. First part of the sequence name indicates the following: x- incomplete genome (colored gray), 0 – one McrA gene in complete genomes (colored black), 1 – first copy of McrA and 2 – second copy of McrA (both colored blue). The original tree is available in Supplementary data 2.

Methanomicrobiales

Methanocellales

Methanosarcinales

ANME1

Methanonatronarchaeia

Methanomassiliicoccales

Methanopyrales

Methanothermobacteriales

Methanococcales

Supplementary Figure 10 Phylogenetic tree of concatenated subunits of Mcr operon (FastTree method). The maximum-likelihood phylogenetic tree of the concatenated subunits of Methyl coenzyme M reductase complex (McrA, McrB, McrD and McrG) proteins was built using the FastTree program and includes 55 genomes and 1303 positions in the multiple alignment. Underlying multiple alignment can be provided by a request.

Supplementary Data 1. Original phylogenetic tree (PHYML method) of concatenated ribosomal proteins in Newick format schematically shown on the Figure 3A.

The maximum-likelihood phylogenetic tree of the concatenated ribosomal proteins was built using the PHYML program as described in the Materials and Methods section. Underlying multiple alignment can be provided by a request.

The tree (below) is provided in Newick format, could be open in MEGA or in any other tree visualization software. To open the file copy the text below the dotted line to a text file with “tre” extension. MEGA (or other tree visualization software) should open the file automatically.

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Supplementary Data 2. Original phylogenetic tree of McrA subunit (PHYML method) in Newick format shown on the Supplementary Figure 9.

The maximum-likelihood phylogenetic tree of the McrA proteins was built using the PHYML program as described in the Materials and Methods section. Underlying multiple alignment can be provided by a request.

The tree (below) is provided in Newick format, could be open in MEGA or in any other tree visualization software. To open the file copy the text below the dotted line to a text file with “tre” extension. MEGA (or other tree visualization software) should open the file automatically.

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|Methanotorris_igneus_Kol_5_GCA_000214415.1|1005029408:0.02604484,(((Metig_0445|Methano  
torris_igneus_Kol_5_GCA_000214415.1|1005028635:0.03087334,(Metvu_1194|Methanocaldococcus_vul  
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07)0.000000:9.81892455737642e-  
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Supplementary Data 3. Phylogenetic tree of McrA subunit (FastTree method) in Newick format.

The maximum-likelihood phylogenetic tree of the McrA proteins was built using the FastTree program as described in the Materials and Methods section. Sequence set and underlying multiple alignment, which can be provided by a request, are the same as on the Figure 3B.

The tree (below) is provided in Newick format, could be open in MEGA or in any other tree visualization software. To open the file copy the text below the dotted line to a text file with “tre” extension. MEGA (or other tree visualization software) should open the file automatically.

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Supplementary Data 4. Phylogenetic tree of McrA subunit (RAxML method) in Newick format.

The maximum-likelihood phylogenetic tree of the McrA proteins was built using the RAxML program as described in the Materials and Methods section. Sequence set and underlying multiple alignment, which can be provided by a request, are the same as on the Figure 3B.

The tree (below) is provided in Newick format, could be open in MEGA or in any other tree visualization software. To open the file copy the text below the dotted line to a text file with “tre” extension. MEGA (or other tree visualization software) should open the file automatically.

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