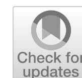
Extremophiles (2020) 24:657–672
https://doi.org/10.1007/s00792-020-01185-x

ORIGINAL PAPER



Methanogenesis in the Lake Elton saline aquatic system

Anna Kallistova[1] · Alexander Merkel[1] · Timur Kanapatskiy[1] · Yulia Boltyanskaya[1] · Ivan Tarnovetskii[1] · Anna Perevalova[1] · Vadim Kevbrin[1] · Olga Samylina[1] · Nikolai Pimenov[1]

Received: 22 March 2020 / Accepted: 8 June 2020 / Published online: 12 June 2020
© Springer Japan KK, part of Springer Nature 2020

Abstract
Cultivation and molecular approaches were used to study methanogenesis in saline aquatic system of the Lake Elton (southern Russia), the largest hypersaline lake in Europe. The potential rates of hydrogenotrophic, acetoclastic, methylotrophic and methyl-reducing methanogenesis and diversity of the growth-enriched for by adding electron donors methanogenic communities were studied in the sediment slurry incubations at salinity range from 7 to 275 g/L. The most active pathway detected at all salinities was methylotrophic with a dominance of *Methanohalobium* and *Methanohalophilus* genera, at salt saturation and moderately halophilic *Methanolobus* and *Methanococcoides* at lower salinity. The absence of methane production from acetate, formate and H_2/CO_2 under hypersaline conditions was most probably associated with the energy constraints. The contribution of hydrogenotrophic, acetoclastic, and methyl-reducing methanogens to the community increases with a decrease in salinity. Temperature might play an important regulatory function in hypersaline habitats; i.e. methylotrophic methanogens and hydrogenotrophic sulfate-reducing bacteria (SRB) outcompeting methyl-reducing methanogens under mesophilic conditions, and vice versa under thermophilic conditions. An active methane production together with negligible methane oxidation makes hypersaline environments a potential source of methane emission.

Keywords Salinity · Methanogenesis · Archaea · Lake Elton · Saline rivers

Introduction

Four pathways of anaerobic methanogenesis are known to date: hydrogenotrophic (by using H_2/CO_2, formate and CO as substrates), acetoclastic (acetate), methylotrophic (C_1-methylated compounds), and methyl-reducing (C_1-methylated compounds as acceptors + H_2 or formate as donors) (see details in Kallistova et al. 2017 and references therein). Hydrogenotrophic and acetoclastic methanogens compete for substrates with sulfate-reducing bacteria (SRB) in saline environments with high sulfate availability. SRB have higher growth rates and greater affinity to such competitive substrates as formate, acetate and H_2 than methanogens. In this case, an intense methane production occurs due to a spatial separation of methanogenesis and sulfate reduction (a zone of methanogenesis is often located in sediments below a zone of sulfate reduction) and/or by using the non-competitive C_1-methylated substrates by methanogens, e.g. methanol (MeOH), methylated amines, dimethyl sulfide. Methylamines and dimethyl sulfide mostly derive from compatible solutes (glycine betaine and dimethylsulfoniopropionate) accumulated by most halophiles (McGenity 2010; McGenity and Sorokin 2018 and references therein). Plants are the main terrestrial source of MeOH, which is produced as by-product of pectin demethylation during cell wall synthesis, and emitted through stomata during transpiration (Fall and Benson 1996). MeOH is also formed by various pectinolytic bacteria during decomposition of plant residues (Schink and Zeikus 1980). Phytoplankton (cyanobacteria, heterokont diatoms, coccolithophores, cryptophytes etc.) is the main source of MeOH in marine ecosystems (Mincer and Aicher 2016).

The cell's energy costs for the synthesis of organic osmolytes are high in hypersaline environments, and energy constraint is therefore an important regulatory factor. The energy constraint corresponds to the energy yield on the methanogenic substrates that do enable growth at high salt

Communicated by A. Driessen.

✉ Anna Kallistova
kallistoanna@mail.ru

[1] Research Center of Biotechnology, Winogradsky Institute of Microbiology, Russian Academy of Sciences, Leninsky Prospect, 33, Build. 2, 119071 Moscow, Russian Federation

concentrations taking into account the cost of osmolyte biosynthesis (Oren 1999). Analysis of free energy changes in various methanogenic reactions allowed Oren to conclude that methylotrophic pathway is superior to the hydrogenotrophic and acetoclastic ones in hypersaline ecosystems. Experimental data confirm that it is precisely methylotrophic methanogens that are most halotolerant (Oren 1999). The importance of methylotrophic methanogenesis can be illustrated by analyzing the upper limit in salinity, at which methanogenic growth with a certain substrate is possible. This limit is 270 g/L NaCl for methylotrophic methanogenesis and about half of that for hydrogenotrophic one. Acetoclastic methanogenesis is particularly vulnerable to high salt and does not occur above 40 g/L (Oren 1999; McGenity 2010; Andrei et al. 2012). However, acetate might still be converted to methane indirectly via syntrophy at salinity of up to 3 M total Na^+ as Na-carbonates in soda lakes (Sorokin et al. 2016a). These values should not be considered the upper limit for activity, however, they show the relative importance of each substrate at different salinity levels. Methylotrophic methanogens usually dominate in hypersaline conditions, including salt-saturated brines, although only two genera (*Methanohalobium* and *Methanosalsum*) from the order *Methanosarcinales* are known to be able to do it (Oren 1999; McGenity 2010; Andrei et al. 2012).

A majority of cultivated halophilic or highly halotolerant methanogens belong to the family *Methanosarcinaceae*. In this family, representatives of the genera *Methanohalobium, Methanohalophilus*, and *Methanosalsum* are confined to hypersaline habitats (Zhilina 1983, 2001; Zhilina and Zavarzin 1987; Mathrani et al. 1988; Boone 2001; Boone and Baker 2001; Sorokin et al. 2015; McGenity and Sorokin 2018). They all are classical methylotrophs. Highly salt-tolerant hydrogenotrophic methanogens of the family *Methanocalculaceae* are described as well (Zhilina et al. 2013; Oren 2014a; Sorokin et al. 2015). These two groups of methanogens also dominate in sediments of hypersaline soda lakes. The *Methanocalculus* group dominates at moderate salinity with formate or H_2 as a donor, while methylotrophs of the genus *Methanosalsum* take precedence at high salinity (Sorokin et al. 2015). However, neither truly halophilic nor highly salt-resistant acetoclastic methanogens have been isolated until now (Oren 2014b). New unique representatives of the class *Methanonatronarchaeia*, performing methyl-reducing methanogenesis, exhibit an unprecedented degree of halophilia that exceeds those of typical methanogenic inhabitants of hypersaline ecosystems, with *Methanohalobium evestigatum* as the only exception. Unlike all previously known methanogens, *Methanonatronarchaeia* most probably accumulate K^+ in the cell and lack the genes encoding the biosynthesis and import of any known organic osmolytes. Such a strategy is more beneficial for anaerobes carrying out processes with a low energy yield and allows them to exist in conditions of extremely high salt concentrations (Sorokin et al. 2017a, 2018; McGenity and Sorokin 2018). A discovery of *Methanonatronarchaeia* casts doubt on previously established principles of methanogenesis, namely the dominance of the classical methylotrophic pathway in hypersaline conditions. It has been shown that methanogenesis under methyl-reducing and moderately thermophilic conditions (50–60 °C) rapidly out-competed methylotrophic process in sediments of hypersaline soda and salt lakes (Sorokin et al. 2017a; McGenity and Sorokin 2018).

Thus, an extensive empirical and theoretical literature on the process of methanogenesis in saline (from weakly to extremely saline) ecosystems has been collected to date. From these data, it follows that competitive relationships between methanogens and SRB and mechanisms of osmoadaptation are among the most important factors affecting methanogenesis in saline habitats. The main patterns of methanogenesis under various salinities were defined based on the physiological and biochemical characteristics of halotolerant and halophilic methanogens isolated from geographically unrelated hydrologically and hydrochemically different habitats.

In contrast to previous studies, the influence of salinity on methanogenesis was investigated in this work in hydrologically unified aquatic system consisting of the Lake Elton and inflowing saline rivers with a naturally existing salinity gradient. Lake Elton is a unique and the largest hypersaline lake in Europe. Its brines are saturated with sodium/magnesium chloride with total salinity of above 200 g/L. Seven rivers flow into the lake. The dominance of saliferous and carbonate sedimentary rocks, solonetz and solonchak soils in the catchments area determines the level of salinity in the rivers, which increases from the rivers' headstream to the estuary. Estuary areas form shallow basins with low water flow rates and elevated salinity due to admixture of the lake's brine (see details in Kanapatskiy et al. 2018 and references therein). Several previous studies of the Lake Elton area were addressed to ecological and hydrobiological features of planktonic and benthic communities, determination of the rates of microbial processes (oxygenic and anoxygenic CO_2 assimilation, total heterotrophic activity, sulfate reduction and hydrogenotrophic methanogenesis) as well as phylogenetic diversity of microorganisms in cyanobacterial mats and river sediments (Kanapatskiy et al. 2018). Extremely halophilic and moderately thermophilic methyl-reducing methanogens belonging to '*Candidatus* Methanohalarchaeum thermophilum' were recently enriched from the sediments of the Lake Elton (Sorokin et al. 2017a, 2018).

The aim of the present work was to study the effect of salinity on the dynamics of methane formation and diversity of methanogenic communities in samples with salinity level from 7 to 275 g/L collected from the lake body and from the inflowing brackish rivers. The potential activity incubations

of sediment slurries were conducted with addition of substrates specifically inducing hydrogenotrophic, acetoclastic, methylotrophic and methyl-reducing methanogenesis.

Materials and methods

Sampling and field measurements

Near bottom brines and sediments (0–5 cm) were collected in May 2015 at shallow sites of Lake Elton (GR, 49°11′25″ N 46°39′58″ E), estuaries of Khara (X8, 49°11′59″ N 46°39′57″ E) and Soljanka (C2, 49°10′41″ N 46°35′38″ E) rivers, and midstream of Lantsug river (L1, 49°13′35″ N 46°37′14″ E) (Fig. 1). A maximal depth of the water layer in sampling points was 20 cm (L1), minimal of 10 cm (X8). The in situ temperatures were measured by an electronic thermometer (Isolab, Germany). The pH and Eh were determined with a pH 3210/Set 4 field pH-meter (WTW, Germany). The total salinity (S), sulfate and methane concentrations, and organic carbon content (C_{opr}) were determined as described previously (Kanapatskiy et al. 2018).

Sulfate reduction measurements

Sulfate reducing activity in upper sediments of studied sites GR, X8, C2, and L1 was determined by radioisotope method. A 3-mL sediment sample was placed into a cut-off 5-mL plastic syringe and sealed with a gas-tight butyl rubber stopper. Labeled substrate, $Na_2{}^{35}SO_4$ (10 µCi per sample, specific activity of 38.8–59.2 TBq/mmol), of 0.2 mL was added through the stopper. The samples were incubated for 48 h (C2 and L1) and for 72 h (X8 and GR) in the dark at an in situ temperature, and then fixed with 0.5 mL of 2 M KOH. The samples fixed with KOH and stored at 4 °C for

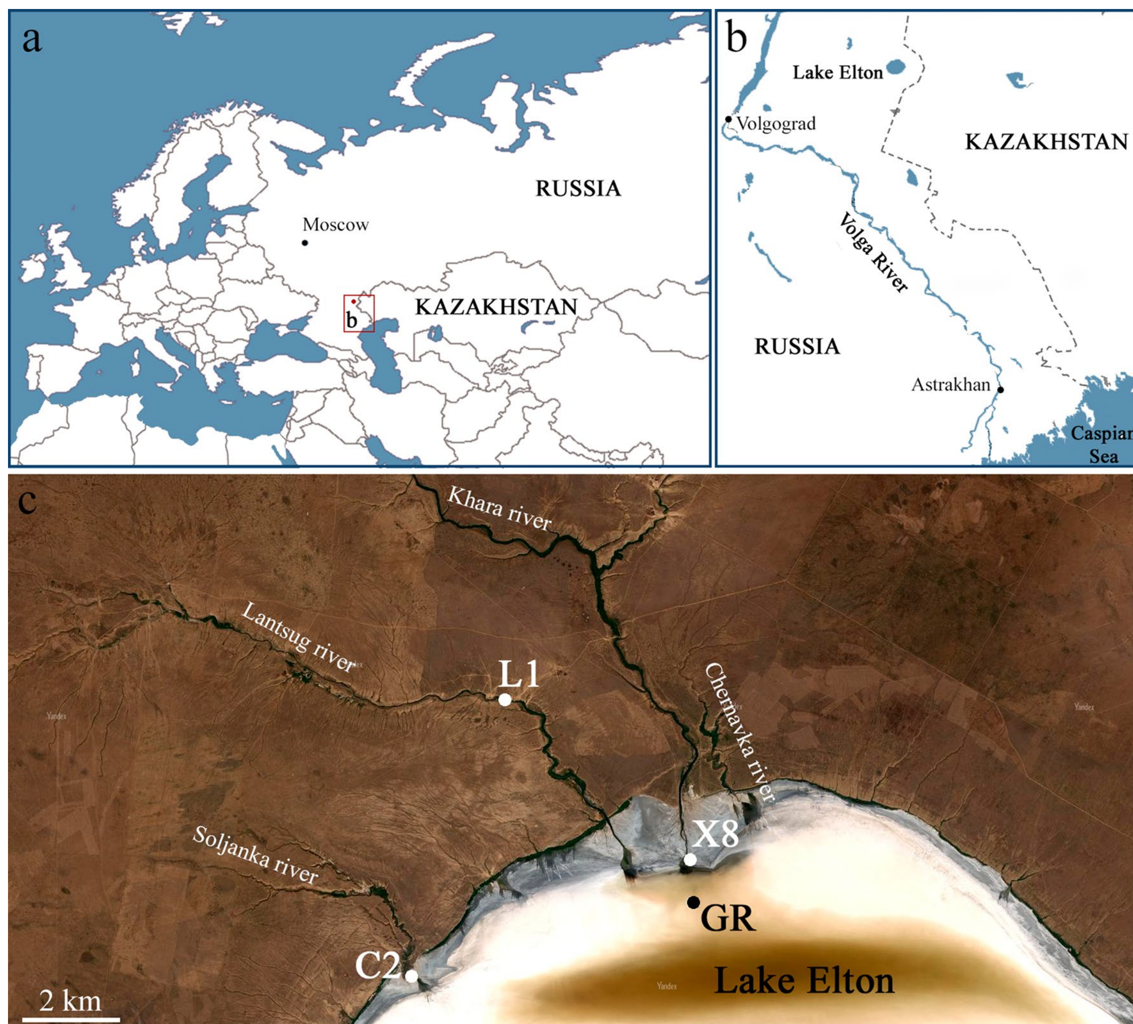


Fig. 1 Lake Elton location in Eurasia (**a**), in Volgograd region, Russia (**b**), and sampling sites (**c**): Lake Elton (GR) 49°11′25″ N 46°39′58″ E; Khara river (X8) 49°11′59″ N 46°39′57″ E; Soljanka river (C2) 49°10′41″ N 46°35′38″ E; Lantsug river (L1) 49°13′35″ N 46°37′14″ E

2 h before addition of the labeled substrate served as the controls (Kanapatskiy et al. 2018). The total sulfate reduction was determined by the measurement of radioactive sulfur incorporated in dissolved hydrogen sulfide, pyrite, other sulfide minerals, elemental and organic sulfur as described in Lein et al. (2002). The ^{35}S radioactivity was measured in a Packard TRI-Carb TR scintillation counter (USA). The rate of sulfate reduction (I) is normally calculated using the Eq. (1):

$$I = \frac{rC}{RT}, \tag{1}$$

where r is the difference between radioactivity of the reduced sulfur compounds formed in experimental sample and control, C is the concentration of $S\text{-}SO_4^{2-}$ in pore water, R is the total radioactivity of the added $^{35}S\text{-}SO_4^{2-}$, and T is the incubation time (Lein et al. 2002). We suppose however that this equation is inadequate for certain habitats like Lake Elton (GR site) with very high concentration of substrate (SO_4^{2-}). In the Lake Elton where all microbial processes are supposed to be low due to the extreme conditions, sulfate is accumulated up to molar concentrations and preserved in brine. Low microbial sulfate reducing activity results in measurement of small r values, which, if further multiplied with high C, result in unprecedentedly high rates of sulfate reduction. For this reason a sulfate reduction rate constant (k) was used instead of I to characterise the sulfate reducing activity. The I is now expressed as $k \times C$ (Kulp et al. 2006), where k is r/RT.

Potential methanogenic activity measurements

The dynamics of methane formation with different methanogenic substrates was studied in sediment slurries formed by mixing sediments with the near bottom brines at 1:2 ratio (*v*/*v*). The slurries were purged with argon and distributed under argon stream into glass bottles resulting in the liquid to gas ratio of 1:4 (*v*/*v*), closed with thick rubber stoppers (Bellco Glass, USA) and sealed with aluminum caps. No reducing agents were added. For samples from each studied site, a series of parallel incubations was prepared with an addition of methanogenic substrates, i.e. acetate, formate, MeOH, trimethylamine (TMA) in a final concentration of 5 mM each, and H_2/CO_2 in a final concentration of 5/1.25%. Slurries were also incubated with mixtures of MeOH + formate + H_2 and TMA + formate + H_2 in concentrations of 5 mM + 5 mM + 5%, respectively for the methyl-reducing methanogenesis. The substrates were added into closed vials by syringes and the incubations were carried out at 30 °C. Methanogenic activity was monitored by the increase in methane concentration in the gas phase using Crystal 5000.1 gas chromatograph (Chromatec, Russia) equipped with a flame ionization detector and a 2 m column filled with HayeSep N 80/100 mesh. The temperature of the injector was 50 °C, the temperature of the column was 50 °C, and the flow rate of the carrier gas argon was 25 mL/min. The average potential rate of methanogenesis was estimated as the amount of methane produced during the time period started from a beginning of the experiment, and terminated when the methane concentration in the gas phase ceased to increase. All results are presented as average values for two biological replicates.

Molecular analysis

Taxonomic composition of methanogenic communities was determined in incubation slurries amended with methylated compounds, MeOH and TMA ± (formate + H_2), after the methane production was terminated. DNA from slurries was isolated using FastDNA Spin Kit for Soil according to the instruction manual (MP Biomedicals, USA). The libraries were prepared using polymerase chain reaction with universal primers to the V4 region of 16S rRNA gene in accordance with the previously described technique (Fadrosh et al. 2014). The following primer systems were used: 515F (5′-GTGBCAGCMGCCGCGGTAA-3′) (Hugerth et al. 2014) and Pro-mod-805R 5′-GACTACNVGGGTMTCT AATCC-3′ (Merkel et al. 2019). According to SILVA (www.arb-silva.de), this primer set covers 86% of 16S rRNA gene sequence diversity of the *Archaea* and 84.3% of the *Bacteria*. In addition, the V4 region is well suited for assessing the ratio between *Bacteria* and *Archaea* because it has a very conservative length, unlike some other regions. Sequencing was carried out on a MiSeq system (Illumina, USA) using the reagent kit, which can read 250 bp from each end. All the sequencing data are deposited in NCBI BioProject PRJNA612308.

Data analysis

All incubation experiments were done in duplicate, and the results present average values. The sequences obtained were analysed as follows: cutting 16S rRNA gene primers was performed using Cutadapt 2.6 script (Martin 2011) using discard-untrimmed option. Merging forward and reverse reads and demultiplexing was performed using the corresponding scripts of QIIME software version 1.9.0 (Caporaso et al. 2010): join_paired_ends.py and demultiplex_fasta.py, respectively. Data was passed through a filter with a minimum base quality 20 and a minimum merged reads length of 200 bp. Chimera reads were checked using the identify_chimeric_seqs.py script with the USEARCH algorithm version 6.1544 (Edgar 2010) and Silva 123 reference reads database 16S rRNA (Quast et al. 2013). All sequence reads were processed by the NGS analysis pipeline of the SILVA rRNA

gene database project (SILVAngs 1.3) (Quast et al. 2013) using default settings: SINA was used for the alignment of sequences (Pruesse et al. 2012), CD-HIT for the clustering of sequences (Li and Godzik 2006) and BLAST for the classification of sequences (Camacho et al. 2009). A 98% similarity threshold was used for creating OTUs; 93% was the minimal similarity to the closest relative that was used for classification. Other reads were assigned as "No Relative".

Results and discussion

Site characterisation

Methanogenesis was studied in samples taken at four shallow sites (Fig. 1), which differed in the level of in situ salinity from hyper (GR, X8) to moderately saline (C2, L1) (Table 1). In rivers, water warmed during a daytime up to 33 °C, and the temperature of the upper layer of sediments varied from 20 to 31 °C. The temperature of the sediments in the Lake Elton under the salt crust of 6 cm thick was lower than in the rivers, 15–17 °C; brine warmed up to 40 °C. The bottom water in the moderately saline rivers (samples C2 and L1) was slightly oxic with a redox range from 9 to 92 mV, respectively, and the sediments starting from the uppermost layer were strongly reduced (− 350 mV). Hypersaline sediments of GR and X8 sites were anoxic as well (Table 1). The strongly reduced conditions in sediments are favorable for anaerobic processes, including sulfate reduction and methanogenesis.

Sulfate reduction

Since sulfate reduction affects methanogenesis in saline environments, we estimated the sulfate reduction rate constant, *k*, in sediment samples by radioisotope method. The lowest *k* value, 0.0000054 1/h, was revealed in GR sediments with the sediment salinity of 310 g/L and corresponded to the lowest microbial sulfate reducing activity in situ. The highest *k* value, 0.0018 1/h, was observed in C2 sediments with the salinity of 27 g/L (Table 1).

Sulfate reducing potential of studied sites was confirmed by detecting of 16S rRNA sequences affiliated with SRB in all growth-enriched slurries. It has to be noted that molecular analysis was conducted after the methane production was terminated in incubation slurries amended with C_1-methylated compounds. The SRB diversity in hypersaline samples (GR, X8) differed from that in moderately saline (C2, L1). In hypersaline GR and X8 samples, the relative contribution of SRB sequences to the total anaerobic microbial community was maximal (up to 48% of all 16S rRNA sequences), while the SRB diversity at genus level was oppositely the lowest. Sequences affiliated with members of the genus *Desulfovermiculus* (order *Desulfovibrionales*) dominated in GR slurries. The genus *Desulfovermiculus* consists of a single valid species, *D. halophilus* DSM 18,834, which is mesophilic (range of 25–47 °C, an optimum at 37 °C), chemolithoautotrophic, moderately halophilic (range of 30–230 g/L, an optimum at 80–100 g/L NaCl) SRB capable of autotrophic growth in the presence of sulfate on H_2/CO_2 or formate without other electron donors (Belyakova et al. 2006). The relative abundance of *Desulfovermiculus* sequences was lower in X8 samples, and the contribution of the sequences belonging to the order *Desulfobacterales*

Table 1 Characteristics of the near bottom water and upper sediments (0–5 cm) of the Lake Elton and inflowing rivers

Parameter		Lake Elton (GR)	River Khara (X8)	River Soljanka (C2)	River Lantsug (L1)
S[a] (g/L)	Water	240	140	26	7
	Sediments	310	81	27	7
pH	Water	7.0	7.2	7.4	8.0
	Sediments	6.3	7.4	7.45	7.75
Eh (mV)	Water	nd[b]	nd	+ 9	+ 92
	Sediments	− 410	− 400	− 350	− 300
CH_4 (μM)	Water	nd	nd	68	1
	Sediments	73	957	530	163
SO_4^{2-} (mM)	Sediments	2107	68	4.5	28.6
k^c (1/h)	Sediments	0.0000054	0.00027	0.0018	0.0008
C_{org} (%)	Sediments	0.4	2.4	1.1	1.2

[a]S means total salinity

[b]nd is not determined

[c]*k* is sulfate reduction rate constant

increased (12–22% of total 16S rRNA gene sequences) compared to GR samples. Among those, the sequences affiliated with SRB genera *Desulfosalsimonas*, *Desulfococcus*, *Desulfotignum*, and unculturable SRB were detected. For comparison, the contribution of SRB sequences affiliated with the order *Desulfobacterales* was only 0.2–4% in GR samples.

In moderately saline C2 and L1 samples, the contribution of above listed SRB sequences was less than 0.2% for each genus, while overall diversity of other SRB increased. The SRB community in C2 slurries (salinity of 26.5 g/L) included sequences affiliated with members of the orders *Desulfovibrionales* (genera *Desulfovibrio*, *Desulfomicrobium*), *Desulfobacterales* (genera *Desulfocapsa*, *Desulfofustis*, *Desulfobacterium*, *Desulfatiglans,* SEEP-SRB1) as well as unculturable SRB. However, their contribution was rather low, < 1% of the total number of 16S rRNA gene sequences. The exception was SRB of the genus *Desulfomicrobium*, whose sequences relative abundance reached 4%. Sequences affiliated with sulfur-reducing bacteria of the genera *Desulfurivibrio, Desulfuromonas*, and *Desulfuromusa* were also detected. The diversity of SRB in L1 slurries (salinity of 7 g/L) was similar to those in C2, but their contribution to the microbial community exceeded 1%. Sequences related to representatives of the genus *Desulfobulbus* (order *Desulfobacterales*) with a share of 6% were detected in L1 samples in addition to the above listed SRB. Along with *Desulfobulbus*, SRB sequences belonging to the genera *Desulfovibrio* and *Desulfomicrobium* dominated in L1 slurries. Despite a lower sulfate reduction rate constant determined in L1 sediments in situ compare with C2 sediments (0.0008 vs 0.0018 1/h), the relative contribution of SRB sequences to the total microbial community was higher in L1 substrate-enriched slurries than in C2, while the SRB diversity at genus level was similar.

Thus, sulfate-reducing activity detected in sediments in situ together with the abundant (in hypersaline GR and X8 samples) and diverse (in moderately saline C2 and L1 samples) SRB community in substrate-enriched slurries could led to a competition of SRB with methanogens for common substrates (acetate, formate and H_2). Nevertheless, the upper sediment layers were saturated with methane (73–957 µM) (Table 1). It indicates that an intense methanogenesis takes place in these layers and/ or in the below ones, of which methane diffused upward, or methanogenesis proceeds via a non-competitive pathway.

Dynamics of methane formation from methane inducing substrates

Methane formation from different methanogenic substrates varied depending on the site salinity (Fig. 2). As often found, methane production on acetate, formate, and H_2/CO_2 was not detected in hypersaline conditions (GR, X8 samples). This is due to energy constraints for the use of these substrates, so that only methylotrophic or methyl-reducing pathways of methanogenesis can work under extremely high salinities (Oren 1999; Sorokin et al. 2017a). Differences in the dynamics of methane production from methylated compounds were revealed for hypersaline GR and X8 samples (Fig. 2a, b). In both cases, a rather long lag phase was observed, which was shorter at lower salinity. The average potential rates of methane production in TMA and MeOH amended GR slurries (salinity of 275 g/L) differed by more than two times (Table 2), therewith MeOH was a more preferable substrate compared to TMA. The latter can be seen from the Fig. 2a: the methane production in MeOH ± (formate + H_2) amended slurries has been already terminated, while the lag phase has still lasted in parallel slurries with TMA ± (formate + H_2) addition. Such effect may occur due to possible TMA toxicity.

Under moderately saline conditions (C2 and L1 samples), methane formation occurred on all substrates tested. This indicates a higher metabolic, and, therefore, phylogenetic (since the genera of methanogens differentiate well depending on the substrate used) diversity of methanogens in moderately saline sites compared with hypersaline ones. The main differences were observed when competitive substrates were added (i.e. H_2/CO_2, formate and acetate). The rates of methane production from acetate, formate and H_2/CO_2 were higher in C2 than in L1 slurries (Table 2, Fig. 2c, d), and the relative contribution of SRB in C2 slurries were oppositely lower than in L1. The competition between SRB and methanogens must be, therefore, less prominent in C2 slurries, so that the conditions for methanogenesis seems to be more favorable in the Soljanka River (C2) compared with the Lantsug River (L1), despite of its higher salinity (26.5 vs 7 g/L). The results of incubation experiments are, however, not very consistent with the sulfate-reducing activity measurements in situ. The latter showed higher sulfate reduction rate constant, *k*, in C2 sediments compared with L1 (Table 1). It has to be noted that the incubation experiments do not always reflect in situ conditions of the resident microbial communities, but show the ones that were growth-enriched for by adding electron donors.

Methyl-reducing methanogenesis can still be important at low to moderate salinity range of 7–110.5 g/L, since the potential rates of methane formation in all slurries (except for L1) were higher, when formate and H_2 were added to methylated compounds. In the most saline GR slurry (275 g/L), methyl-reducing methanogenesis was apparently absent, because the amount of produced methane and the process rates were either too low, when formate and H_2 were added to methylated compounds, or similar to those with an addition of methylated compounds only.

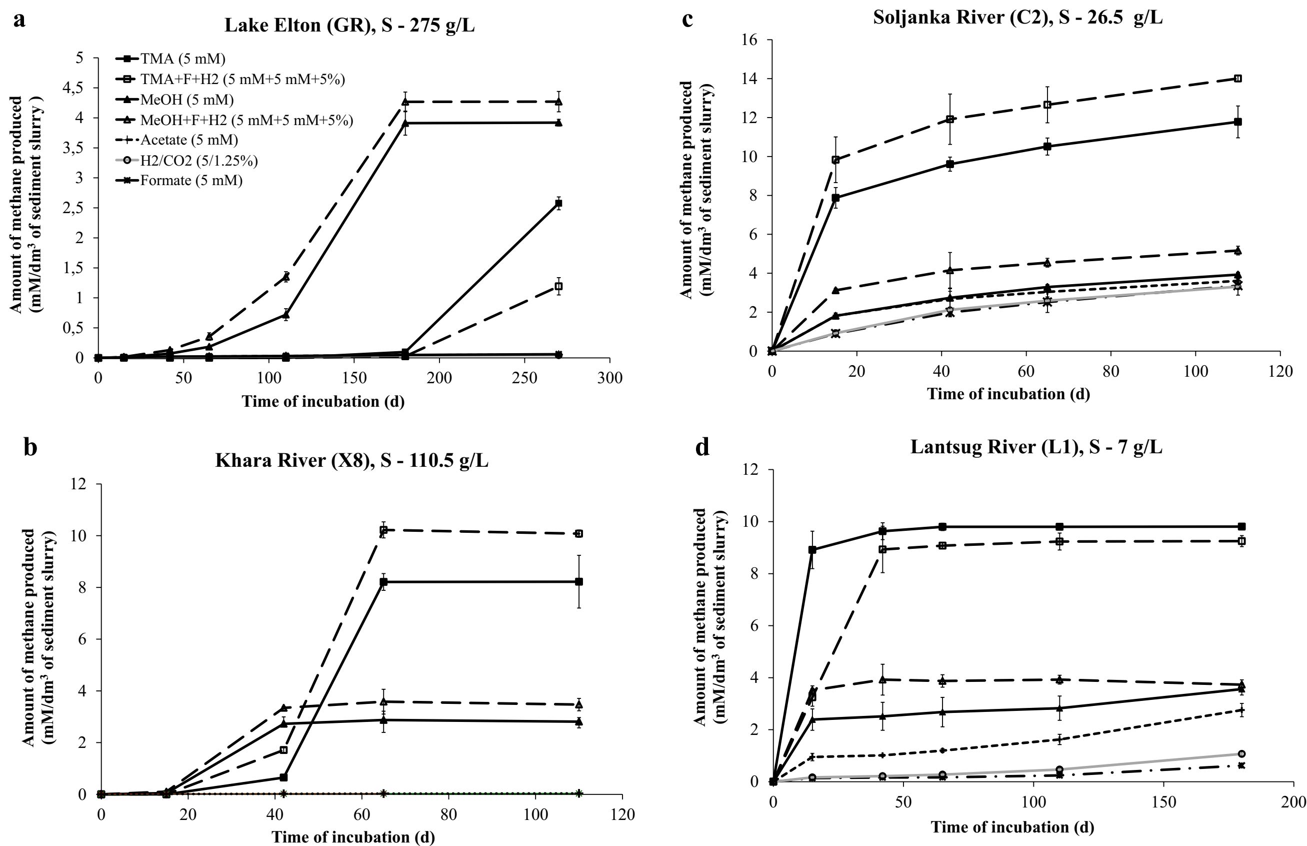


Fig. 2 Methane formation by sediment slurries amended with different methanogenic substrates. Samples were taken by the Lake Elton (**a**) and inflowing rivers Khara (**b**), Soljanka (**c**), and Lantsug (**d**). The substrate concentration is given in parentheses. The symbols indicate averages for two replicates, and the error bars indicate standard deviations. F means formate, S means salinity

Table 2 An average potential rates of methane formation (μmol CH_4/dm³ of sediment slurry/d)

Substrate	GR (275)	X8 (110.5)	C2 (26.5)	L1 (7)
TMA	9.5	126.3	228.7	229.3
TMA + formate + H_2	4.4	157.3	283.7	212.6
MeOH	21.7	65.0	65.2	59.9
MeOH + formate + H_2	23.7	79.7	98.9	93.4
Acetate	0	0	63.7	15.3
H_2/CO_2	0	0	50.5	5.9
Formate	0	0	47.3	3.5

An average salinity of sediment slurries in g/L is given in parentheses. The rates were calculated at day 42 of incubation for C2 and L1 samples with all substrates added; the rates for X8 sample amended with TMA ± (formate + H_2) were calculated at day 42 of incubation, and MeOH ± (formate + H_2) at day 65 of incubation; the rates for GR samples amended with MeOH ± (formate + H_2) were calculated at day 180 of incubation, and with TMA ± (formate + H_2)—at day 270

Overall archaeal diversity

The C_1-methylated compounds were the universal substrates for methanogenesis in the salinity range of 7–275 g/L. The composition of the anaerobic microbial community at different salinities was therefore studied in the sediment slurries with an addition of C_1-methylated compounds ± (formate + H_2) after the methanogenesis was terminated. Relative abundances of sequences affiliated with *Archaea* and *Bacteria* in studied slurries are shown in Table 3. A contribution of archaeal sequences was higher than bacterial only in GR slurry amended with TMA. In all other slurries, *Bacteria* predominated over *Archaea* (73.6–97.7% vs 2–25.7% of total number of 16S rRNA sequences). High proportion (3.6–5.5%) of sequences attributed to unknown microorganisms was revealed in GR (MeOH + formate + H2) and in all X8 slurries. The abundance of bacterial sequences in moderately saline C2 and L1 slurries was the higher the more diverse substrate was added.

Overall archaeal diversity is shown on Fig. 3. *Euryarchaeota* represented a major part of archaeal population in most of the samples. *Woesearchaeia* (superphylum DPANN)

Table 3 Relative abundance of bacterial and archaeal sequences in slurries amended with C_1-methylated compounds

Sample	Substrate	Relative sequence abundance (% of total number of 16S rRNA sequences)			Ratio of *Archaea* to *Bacteria*
		Bacteria	*Archaea*	No relative	
GR	TMA	45.7	54.2	0.1	1: 0.8
	TMA + formate + H_2	95.7	4.2	0.1	1: 23
	MeOH	73.6	25.7	0.7	1: 3
	MeOH + formate + H_2	83.8	11.5	4.7	1: 8
X8	TMA	89.0	7.4	3.6	1: 12
	TMA + formate + H_2	87.0	7.5	5.5	1: 12
	MeOH + formate + H_2	91.0	5.4	3.6	1: 17
C2	TMA	78.6	21.2	0.2	1: 4
	TMA + formate + H_2	96.6	2.7	0.7	1: 36
	MeOH + formate + H_2	92.5	6.8	0.7	1: 14
L1	TMA	82.8	16.6	0.6	1: 5
	TMA + formate + H_2	97.7	2.0	0.3	1: 49
	MeOH + formate + H_2	96.5	3.0	0.5	1: 32

No Relative corresponds to all sequences with similarity to any of known taxon for less than 93%

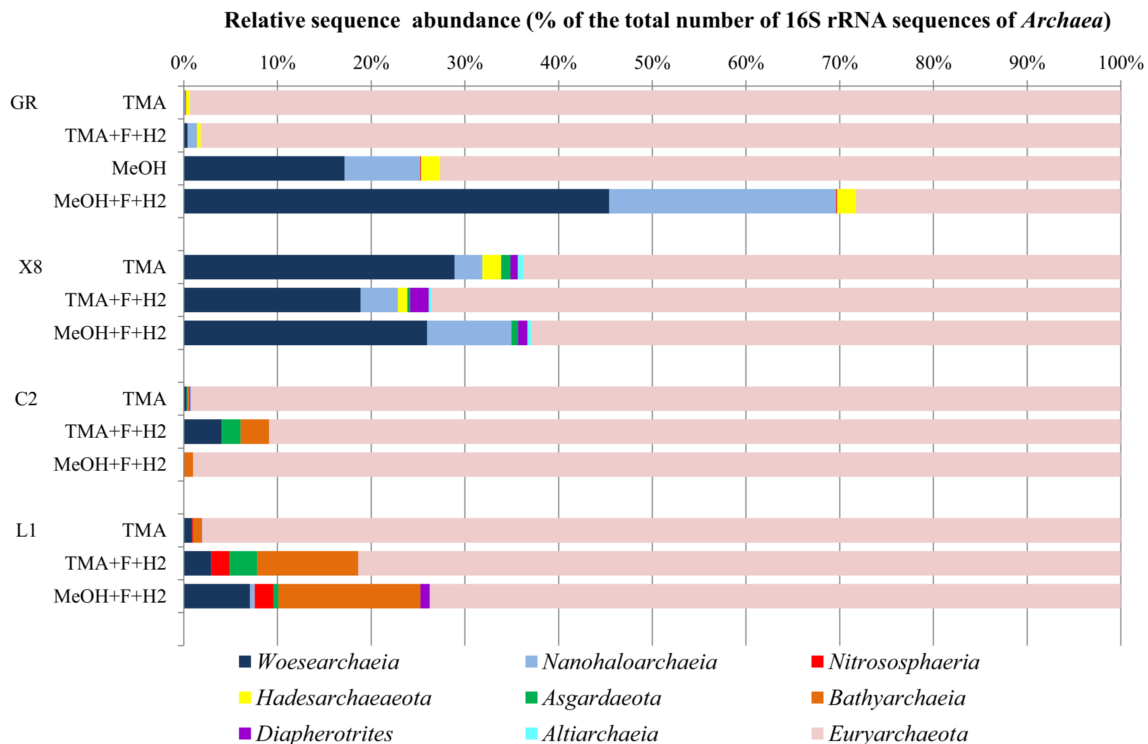


Fig. 3 Overall diversity of *Archaea* in sediment slurries from the Lake Elton (GR), and rivers Khara (X8), Soljanka (C2), and Lantsug (L1) incubated with an addition of C_1-methylated compounds. F means formate

were the second abundant archaeal phylum. Superphylum DPANN includes archaea with small cell size and reduced genomes (Rinke et al. 2013; Castelle et al. 2015). Analysis of the genomes of *Woesearchaeota, Pacearchaeota, Aenigmarchaeota, Diapherotrites* showed that these archaea can be either symbionts or parasites, or free-living organisms capable of saccharolytic or fermentative metabolism (Youssef et al. 2015; Castelle et al. 2015; Spang et al. 2017). In our case, the contribution of sequences affiliated with members of the phylum *Woesearchaeia* in some samples exceeded 20% of archaeal sequences (1% of the total number of 16S rRNA sequences), and it reached 45% of archaeal

sequences (5% of the total number of 16S rRNA sequences) in the GR slurry (MeOH + formate + H_2). The reason for varying the proportion of these microorganisms is not yet fully understood. Most likely, they are not related to methanogenesis, but probably have a potential for hydrogenotrophic acetogenesis. Sequences affiliated with members of the phylum '*Nanohaloarchaea*' were abundant (up to 24% of archaeal sequences, 3% of the total number of 16S rRNA sequences) in hypersaline GR and X8 slurries. Initially, these ultrasmall *Archaea* were supposed to be free-living organisms possessing aerobic, heterotrophic lifestyle or could have fermentative metabolism under microaerophilic conditions (Narasingarao et al. 2012). Recently, however, it was discovered that members of the globally distributed '*Nanohaloarchaea*' lineage have evolved as symbionts (Hamm et al. 2019). Different haloarchaea were shown to be their hosts (Hamm et al. 2019; La Cono et al. 2019).

Sequences belonging to the phylum *Hadesarchaeaeota*, which probably have a potential to anaerobic CO and H_2 oxidation (or H_2 production) coupled to dissimilatory nitrite reduction (Baker et al. 2016), were detected only in hypersaline GR and X8 slurries, while sequences belonging to the phylum *Bathyarchaeia* (superphylum TACK) were detected only in moderately saline C2 and L1 slurries. The question of what metabolic features are inherent to *Bathyarchaeota* still remains open. An important finding was made by Evans and co-authors in 2015, which discovered Mcr-like genes in genomes of several (but not all) *Bathyarchaeota* metagenomes. This allowed the authors to conclude that *Bathyarchaeota* are able to carry out methyl-reducing methanogenesis (Evans et al. 2015). Prior this, all known methanogens have been described exclusively within the phylum *Euryarchaeota*. Recently, however, Evans and co-authors refuted themselves by expressing a new assumption that *Bathyarchaeota* are not involved in methane cycling (Evans et al. 2019). The remote Mcr homologues found in some *Bathyarchaeota* are also present in the '*Ca.* Syntrophoarchaeum', for which anaerobic oxidation of alkanes has been proven (Laso-Pérez et al. 2016). It became therefore clear, that *Bathyarchaeota* also belong to anaerobic alkanotrophs, using propane and butane rather than produce/oxidize methane (Evans et al. 2019). However, direct evidences (activity or cultivation) for the key type of metabolisms in *Batyarchaeota* are still lacking. Our results showed that the relative abundance of sequences affiliated with this group was higher at the combined presence of methylated compounds and formate/H_2 in comparison with only C_1-methylated compounds addition. This could be more consistent with the original version of Evans et al., but further analysis would be needed. It was also suggested that *Bathyarchaeota* could carry out homoacetogenesis, fermentation, grow heterotrophically on proteins, cellulose, chitin, aromatic compounds and fatty acids, and switch from heterotrophic to autotrophic growth (Spang et al. 2017 and references therein) but that remains highly speculative.

In addition to the archaea listed above, sequences affiliated with *Nitrososphaeria* (*Thaumarchaeota*, TACK group) and archaea of superphylum *Asgard* were detected in our slurries. The ability to aerobically oxidise ammonium to nitrite was shown for *Nitrososphaeria* (Stieglmeier et al. 2014). *Asgard* archaea are regarded as evolutionary predecessors of eukaryotes (Zaremba-Niedzwiedzka et al. 2017). Of these, representatives of the candidate phyla *Thorarchaeota* and *Lokiarchaeota* possibly carry out homoacetogenesis and/or fermentation (Spang et al. 2017). Comparative genomic inference suggests mixotrophic lifestyle for *Thorarchaeota* (Liu et al. 2018). It has to be noted that Lake Elton is a system opened for transfer of microorganisms from brackish soils surrounding the lake. These microorganisms might be conserved inside the brine, instead of be growing or active. Without observing difference in population dynamics between amended slurries and endogenous blanks, it cannot be said whether these populations are active or not.

Overall diversity of *Euryarchaeota*

Overall diversity of archaea within the phylum *Euryarchaeota* is shown on Fig. 4. Sequences affiliated with members of the order *Methanosarcinales* were detected in all samples, regardless of salinity and substrate. Sequences belonging to the haloarchaea of the class *Halobacteria* predominated under extreme conditions (GR, 275 g/L), and their proportion decreased with a decrease in salinity. In GR slurries, a half of the haloarchaeal sequences was affiliated with the members of the family *Halobacteriaceae*. Among those, sequences belonging to the genera *Halodesulfurarchaeum* and *Halanaeroarchaeum* were the most abundant. These haloarchaea are involved in anaerobic sulfur and carbon cycling in hypersaline environments. Members of the genus *Halodesulfurarchaeum* are anaerobic lithoheterotrophs growing with formate or H_2 as electron donors and sulfur compounds as electron acceptors (Sorokin et al. 2017b). Member of the genus *Halanaeroarchaeum* are anaerobic acetate-oxidizing and sulfur-reducing microorganisms (Sorokin et al. 2016b). A minor part of the family *Halobacteriaceae* was represented by sequences belonging to the genus *Halobacterium*. Most members of this genus are chemoorganotrophic aerobes, but many strains are capable of anaerobic fermentation, some grow photoheterotrophically or by anaerobic respiration in the presence of nitrate (Oren et al. 2009). Another half of haloarchaeal sequences detected in GR slurries was affiliated with different members of the families *Haloarculaceae* (genera *Halapricum, Haloarcula, Natronomonas, Halovenus, Halorhabdus*), *Halorubraceae* (genus *Halorubrum*), *Natrialbaceae* (genus *Salinarchaeum*), and *Haloferacaceae* (genus *Haloplanus*). These haloarchaea

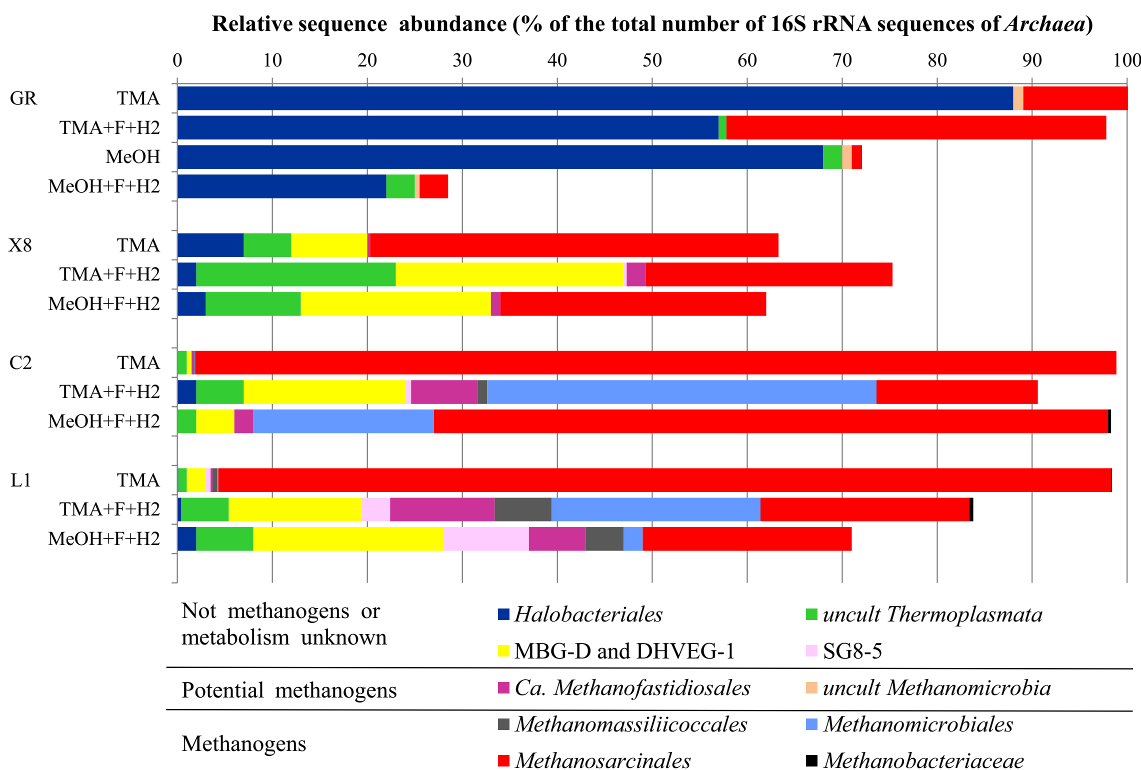


Fig. 4 Overall diversity of *Euryarchaeota* in sediment slurries from the Lake Elton (GR), and rivers Khara (X8), Soljanka (C2), and Lantsug (L1) incubated with an addition of C_1-methylated compounds. F means formate

are capable of aerobic chemoorganotrophic growth, fermentation, and anaerobic nitrate and sulfur reduction (Wainø et al. 2000; Oren et al. 2009).

Sequences belonging to archaea of Marine Benthic Group D (MBG-D) and DHVEG-1 detected at the salinity range of 7–110.5 g/L, i.e. attended everywhere except sample GR. Sequences attributed to uncultured *Thermoplasmata* made a significant contribution to archaeal community (up to 21% of the number of archaeal sequences, 2% of the total number of 16S rRNA sequences) in individual samples. Sequences belonging to members of the orders *Methanomassiliicoccales* (methyl-reducing methanogens), *Methanomicrobiales* (hydrogenotrophic methanogens mainly) and unclassified *Euryarchaeota* of SG8-5 (key metabolism is unknown) appeared only in moderately saline samples (C2 and L1) amended with C_1-methylated compounds with formate and H_2. The proportion of sequences affiliated with representatives of the class "*Ca.* Methanofastidiosa" (potential methyl-reducers) increased with a decrease in salinity in slurries with an addition of C_1-methylated compounds with formate and H_2. Thus, the methanogenic sequences were affiliated with members of such taxa as *Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriaceae,* and *Methanomassiliicoccales,* as well as unculturable *Methanomicrobia* and "*Ca*. Methanofastidiosa". The remaining *Euryarchaea* either did not participate in the methanogenesis (e.g. *Halobacteria*), or such ability has not yet been described for them (e.g. unculturable *Thermoplasmata*, MBG-D, SG8-5). For example, it was suggested that MBG-D archaea, one of the most frequently found archaeal lineages in sediments, are able to transport and assimilate peptides and produce acetate and ethanol via fermentation. The MBG-D genomes also include genes that might encode Wood–Ljundahl pathway and an incomplete dicarboxylate/4-hydroxybutyrate cycle. These findings suggested that MBG-D group is most probably narrowly specialized secondary anaerobes (Zhou et al. 2019). Genome of moderate halophilic, anaerobic, mesophilic unclassified *Euryarchaeota* SG8-5 from aquatic environment was sequenced, but metabolic properties are not yet annotated. Few sequences belonging to anaerobic methanotrophic *Archaea* of the ANME-3 cluster were detected in two samples, L1 (7 g/L) and X8 (110.5 g/L), amended with TMA.

Diversity of methanogens

Diversity of methanogens detected in this study is presented in Table 4. The hypersaline GR and X8 samples differed from the moderately saline ones (C2, L1) in the composition of methanogenic population. Sequences affiliated with

Table 4 Relative abundances (% from archaeal sequences) of methanogens in sediment slurries from Lake Elton (GR), and rivers Khara (X8), Soljanka (C2), and Lantsug (L1) incubated with an addition of C_1-methylated compounds as determined by high-throughput sequencing of 16S rRNA gene fragments

Sample	GR (275)				X8 (110.5)			C2 (26.5)			L1 (7)		
Taxon	TMA	TMA+F[a]+H_2	MeOH	MeOH+F+H_2	TMA	TMA+F+H_2	MeOH+F+H_2	TMA	TMA+F+H_2	MeOH+F+H_2	TMA	TMA+F+H_2	MeOH+F+H_2
Potential methyl-reducers:													
Methanosphaera	0	0	0	0	0	0	0	0	0	0	0	0.4	0
unc[b]. *Methanomassiliicoccales*	0	0	0	0	0	0	0	0.1	1	0	0.5	6	4
Ca. Methanofastidiosum	0	0	0	0	0	0	0	0.2	4	1	0.1	5	2
unc. *Methanofastidiosales*	0	0	0	0	0.3	2	1	0	3	0.9	0.09	6	4
Methylotrophs:													
Methanolobus	0	0	0	0	0	0	0	0	0	2	79	4	1
Methanomethylovorans	0	0	0	0	0	0	0	0	0	0	0	0	1
Methanosarcina	0	0	0	0	0	0	0	0	5	2	0	4	1
Methanohalobium	11	40	1	3	1	2	0.4	0	0	0	0	0	0.5
Methanohalophilus	0	0	0	0	41	24	28	0	0	0	0	0	0
Methanococcoides	0	0	0	0.2	0	0.3	0	97	8	63	14	0.4	0
Hydrogenotrophs:													
Methanocalculus	0.03	0	0	0	0	0	0	0	22	14	0	0.4	0
Methanofollis	0	0	0	0	0	0	0	0	0	0	0.09	8	2
Methanolinea	0	0	0	0	0	0	0	0	0.6	0	0	0	0.5
Methanobrevibacter	0	0	0.07	0	0	0	0	0	0	0	0	0	0
Methanospirillum	0	0	0	0	0	0	0	0	2	0.3	0	0	0
Methanogenium	0	0	0	0	0	0	0	0	3	0	0.05	1	0
Methanoplanus	0	0	0	0	0	0	0	0	0.6	0.9	0	0	0
Methanobacterium	0	0	0	0	0	0	0	0	0	0.3	0.09	0	0
Methanoculleus	0	0	0	0	0	0	0	0	0	0.3	0	0	0
unc. *Methanomicrobia*	0.1	0	1	0.5	0.3	0	0	0.07	13	3	0	12	0
Acetoclastic:													
Methanothrix	0	0.4	0	0	0	0	0	0	4	3	0.05	14	17

An average salinity of sediment slurries in g/L is given in parentheses

[a]F means formate, [b]unc. means uncultured

methylotrophic methanogens of the genus *Methanohalobium* dominated (up to 40% of archaeal sequences, 2% of the total number of 16S rRNA sequences) in the extremely saline conditions. Their contribution was significantly higher (11–40% of archaeal sequences) in the TMA ± (formate + H_2) supplementations than in the MeOH ± (formate + H_2) ones (1–3%). The genus *Methanohalobium* is represented by a single valid species, *M. evestigatum* Z-7303. This methylotrophic methanogen isolated from the Lake Sivash (Russia) is the most halophilic of all the methanogens described to date. A type strain grows in the range of 150–300 g/L NaCl, and has a growth optimum at 250 g/L NaCl. It is moderately

thermophilic organism growing in the temperature range of 35–60 °C with an optimum at 40–55 °C (Zhilina and Zavarzin 1987; Zhilina 2001). The incubation temperature in our experiments was 30 °C, i.e. was closer to the in situ temperature of sediments, but it was much lower than the optimal and even minimum growth temperature for this organism. Perhaps that was the reason why in our experiments such a low rate of methane formation on C_1-methylated compounds was observed for the GR samples. Although *M. evestigatum* prefers methylamines, it also grows with methanol, but slowly. Latter does not completely agree with our data on the dynamics of methane formation from these substrates, according to which MeOH was a more preferable substrate compared to TMA in the GR sample (Fig. 2a). Since only single sequences related to other methanogens, *Methanobrevibacter* and *Methanococcoides* of 0.07 and 0.2% of archaeal sequences, respectively, were detected in the slurries with an addition of MeOH, the question arises of who else could form methane from MeOH $\pm$ (formate + H_2) in extremely halophilic conditions. Potential methanogens can probably be found among uncultivated *Methanomicrobia* and *Thermoplasmata*. However, the relative contribution of sequences belonging to these archaea also did not exceed 3% of archaeal sequences (0.4% of the total number of 16S rRNA sequences). Sequences affiliated with *Methanohalobium* sp. were detected in X8 sample (110.5 g/L) as well, while representatives of another genus, *Methanohalophilus*, dominated there. It has to be noted that in GR (MeOH + formate + H2) and in all X8 slurries high proportion (3.6–5.5% of total number of 16S rRNA sequences) of sequences with low similarity to known microorganisms (referred in Table 3 as "No Relative") was revealed. These sequences might be also affiliated with yet unknown methanogens.

The sequences belonging to above-mentioned methanogens were not detected in moderately saline C2 and L1 samples (salinity of 7–26.5 g/L). The overall diversity of methanogens in moderately saline C2 and L1 samples was similar (Fig. 4). However, the relative contribution of sequences affiliated with various genera varied depending from salinity and type of substrate. For example, sequences belonging exclusively to members of methylotrophic genus *Methanococcoides* (97% of archaeal sequences, 21% of the total number of 16S rRNA sequences) were detected in C2 samples (26.5 g/L) amended with TMA. Sequences affiliated with methylotrophic *Methanolobus* spp. (79% of archaeal sequences, 13% of the total number of 16S rRNA sequences) prevailed on TMA in L1 samples (7 g/L), and the relative contribution of sequences related to members of the genus *Methanococcoides* was 14% of archaea (2% of the total number of 16S rRNA sequences). Diversity of methanogens in moderately saline C2 and L1 samples was higher after the incubations with the mixtures of C_1-methylated compounds with formate and H_2 than in methylotrophic conditions. In addition to *Methanococcoides*, sequences belonging to representatives of the genera *Methanolobus, Methanosarcina, Methanocalculus, Methanothrix, Methanogenium,* and *Methanospirillum* (contribution $\geq$ 1% of archaea) appeared in sample C2. Sequences affiliated with members of the genus *Methanocalculus* prevailed on TMA + formate + H_2, and with members of the genus *Methanococcoides* prevailed on MeOH + formate + H_2. It is known that genus *Methanocalculus*, with some exception, consists of halotolerant hydrogenotrophic methanogens (Sorokin et al. 2015). Members of the genera *Methanogenium* and *Methanospirillum* are hydrogenotrophic methanogens as well.

Sequences affiliated with acetoclastic methanogens of the genus *Methanothrix* were detected in moderately saline slurries amended with a mixture of C_1-methylated substrates with formate and H_2. Their contribution increased with a decrease in salinity from 3 to 4% of archaeal sequences (0.1–0.2% of the total number of 16S rRNA sequences) in C2 samples to 14–17% of archaeal sequences (0.3–0.5% of the total number of 16S rRNA sequences) in L1 samples. Since the diversity of methanogens was studied in the slurries without acetate addition, acetate might appear due to endogenous processes involving various fermentative and acetogenic microorganisms of the sediments. Among archaea, MBG-D organisms could be considered as possible candidates to acetogens, since potential in acetate formation was suggested for them based on genome analyses (Zhou et al. 2019). The relative contribution of MBG-D archaea in our samples also increased with a decrease in salinity in mixtures of C_1-methylated substrates + formate + H_2. The participation of bacterial part of the community in acetate formation could not be excluded as well. Apart from acetate, *Methanothrix* spp. are able to reduce CO_2 to CH_4 with electrons derived from electrogens (*Geobacter, Rhodoferax*) by means of direct interspecies electron transfer, DIET (Rotaru et al. 2014; Holmes et al. 2017; Yee and Rotaru, 2020). Only a few sequences belonging to members of the genera *Rhodoferax* and *Geobacter* were detected in moderately saline slurries and no one in hypersaline. The family *Geobacteraceae* was represented mostly by genera *Geoalkalibacter* (sequences affiliated with members of this genus were more abundant in moderately saline slurries) and *Geothermobacter* (sequences affiliated with members of this genus were more abundant in hypersaline slurries). Thus, the main difference between the methanogenic populations in the moderately saline samples L1 and C2 was the increase of relative abundance of sequences attributed to members of the genus *Methanothrix* in L1 samples compared to C2, and the predominance of sequences attributed to members of the genus *Methanolobus* over members of the genus *Methanococcoides*.

Overall, methylotrophic, hydrogenotrophic and acetoclastic methanogens and their activity were detectable

in growth-enriched sediments of the Lake Elton aquatic system. As for methyl-reducers, in contrast to Sorokin, who discovered extremely halophilic methyl-reducing '*Ca.* Methanohalarchaeum thermophilum' in sediments of the Lake Elton (Sorokin et al. 2017a, b, 2018), we failed in their detection in the same lake (GR samples). The absence of sequences related to methyl-reducers in the GR sample is consistent with the results of determination of the dynamics and rates of methane formation (Fig. 2, Table 2). It is possible that, a temperature plays an important regulatory function in an extremely halophilic methanogenic community. Extremely halophilic methyl-reducers outcompeted towards the classical methylotrophs only at moderately thermophilic conditions (Sorokin et al. 2017a, b, 2018). It has been shown that the '*Ca.* Methanohalarchaeum thermophilum' (HMET1) enrichment did not grow below 40 ºC. It grew optimally at 50 ºC with the upper limit for growth at 60 ºC (Sorokin et al. 2018). However, taking into account that *Methanohalobium* is also a moderate thermophile (growing in the temperature range of 35–60 °C with an optimum at 40–55 °C), the competition is probably decided by some other factors, such as a presence of external reductants or specific local ionic composition. Another possible explanation of our results is a consumption of H_2 and formate by hydrogenotrophic and mesophilic *Desulfovermiculus* sp. as we did not use of selective inhibitors of SRB. Sequences affiliated with members of this genus were abundant in GR slurries. Thus, the only TMA (MeOH) has left for methanogenesis. *Methanohalobium* sp. consumed TMA (MeOH) instead of HMET1 as latter obligatory depends on H_2/formate. So, we had a competition between SRB and methanogens for substrate, which seems to be important not only in moderately saline but also in extremely saline conditions. Such situation might appear also in situ, if to consider a simultaneous presence of HMET1, *Methanohalobium* sp. and *Desulfovermiculus* sp. in the sediments of Lake Elton. Then HMET1 could take an advantage in nature only in such conditions when it grows faster than both hydrogenotrophic and mesophilic SRB and methylotrophic methanogens, i.e. under the thermophilic conditions.

Sequences affiliated with potential methyl-reducers '*Ca.* Methanofastidiosales' (WSA2 or Arc I class) (Nobu et al. 2016) were detected only at much lower salinity in the range of 7–110.5 g/L. Their contribution increased with a decrease of salinity and was higher in TMA + formate + H_2 amended samples than in MeOH + formate + H_2 ones. Sequences belonging to representatives of the order *Methanomassiliicocccales* were detected in moderately saline C2 and L1 samples, and their relative contribution also increased with a decrease in salinity. The ability to methyl-reduction has already been proven for representatives of this order (Dridi et al. 2012; Lang et al. 2015). Additionally, sequences related to methyl-reducing *Methanosphaera* sp. (Miller and Wolin 1985; Biavati et al. 1988) was detected in L1 sample.

Conclusions

The use of C_1-methylated compounds in the process of methanogenesis was a general characteristic of the Lake Elton saline system with the salinity range of 7–275 g/L. The absence of methane production from acetate, formate and H_2/CO_2 in hypersaline conditions was apparently associated with energy constraints. The effect of sulfate reduction on methanogenesis must be more prominent under moderately halophilic conditions (7–26.5 g/L) with use of competitive substrates. However a competition between hydrogenotrophic SRB and methyl-reducing methanogens for H_2 and formate might be important under hypersaline conditions as well. Lake Elton and inflowing rivers are inhabited by classical halophilic methylotrophic methanogens belonging to the genera *Methanohalobium, Methanohalophilus, Methanococcoides, Methanolobus* and *Methanosarcina*. Their salinity optimum for growth corresponds well to the in situ salinity levels. The abundance and activity of hydrogenotrophic (*Methanocalculus, Methanogenium, Methanospirillum*), acetoclastic (*Methanothrix*), and methyl-reducing (*Methanosphaera, Methanomassiliicoccales*, '*Ca.* Methanofastidiosales') methanogens to the methanogenic community increases with a decrease in salinity. Temperature can play an important regulatory function in an extremely halophilic methanogenic community, i.e. methylotrophic methanogens and hydrogenotrophic SRB outcompeting methyl-reducing methanogens under mesophilic conditions, and vice versa under thermophilic conditions. Active methane production together with negligible methane oxidation (in sediments of Lake Elton, anaerobic methane oxidation did not exceed 0.1 μmol/dm^3/day; ANME archaea were not found) makes hypersaline habitats a potential source of methane emission.

Acknowledgements The authors are very grateful to D. Sorokin (Winogradsky Institute of Microbiology) for his valuable comments and manuscript correction. Authors are grateful to E. Zakharova and I. Rusanov (Winogradsky Institute of Microbiology) for their help with radioisotope measurements. Biogeochemical and cultivation experiments were supported by the Ministry of Science and Higher Education of the Russian Federation (goszadanie). Molecular biological research was supported by the Russian Science Foundation (Grant Number 17-74-30025).

References

Andrei A-S, Banciu HL, Oren A (2012) Living with salt: metabolic and phylogenetic diversity of archaea inhabiting saline ecosystems. FEMS Microbiol Lett 330:1–9. https://doi.org/10.1111/j.1574-6968.2012.02526.x

Baker BJ, Saw JH, Lind AE, Lazar CS, Hinrichs K-U, Teske AP, Ettema TJG (2016) Genomic inference of the metabolism of cosmopolitan subsurface Archaea, *Hadesarchaea*. Nat Microbiol 1:16002. https://doi.org/10.1038/nmicrobiol.2016.2

Belyakova EV, Rozanova EP, Borzenkov IA, Tourova TP, Pusheva MA, Lysenko AM, Kolganova TV (2006) The new facultatively chemolithoautotrophic, moderately halophilic, sulfate-reducing bacterium *Desulfovermiculus halophilus* gen. nov., sp. nov., isolated from an oil field. Microbiology 75:161–171. https://doi.org/10.1134/S0026261706020093

Biavati B, Vast M, Ferry JG (1988) Isolation and characterization of "*Methanosphaera cuniculi*" sp. nov. Appl Environ Microbiol 54:768–771

Boone DR (2001) Genus IV. Methanohalophilus Paterek and Smith 1988, 122VP. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology. The Archaea and the deeply branching and phototrophic Bacteria, vol 1, 2nd edn. Springer, New York, pp 281–283

Boone DR, Baker CC (2001) Genus VI. Methanosalsum gen. nov. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology. The Archaea and the deeply branching and phototrophic Bacteria, vol 1, 2nd edn. Springer, New York, pp 287–289

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden T (2009) BLAST+: architecture and applications. BMC Bioinf 10:421. https://doi.org/10.1186/1471-2105-10-421

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Gonzalez Peña A, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Castelle CJ, Wrighton KC, Thomas BC, Hug LA, Brown CT, Wilkins MJ, Frischkorn KR, Tringe SG, Singh A, Markillie LM, Taylor RC, Williams KH, Banfield JF (2015) Genomic expansion of domain archaea highlights roles for organisms from new phyla in anaerobic carbon cycling. Curr Biol 25:690–701. https://doi.org/10.1016/j.cub.2015.01.014

Dridi B, Fardeau M-L, Ollivier B, Raoult D, Drancourt M (2012) *Methanomassiliicoccus luminyensis* gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces. Int J Syst Evol Microbiol 62:1902–1907. https://doi.org/10.1099/ijs.0.033712-0

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Evans PN, Parks DH, Chadwick GL, Robbins SJ, Orphan VJ, Golding SD, Tyson GW (2015) Methane metabolism in the archaeal phylum *Bathyarchaeota* revealed by genome-centric metagenomics. Science 350:434–438. https://doi.org/10.1126/science.aac774

Evans PN, Boyd JA, Leu AO, Woodcroft BJ, Parks DH, Hugenholtz P, Tyson GW (2019) An evolving view of methane metabolism in the Archaea. Nat Rev Microbiol 17:219–232. https://doi.org/10.1038/s41579-018-0136-7

Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, Ravel J (2014) An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome 2:6. https://doi.org/10.1186/2049-2618-2-6

Fall R, Benson A (1996) Leaf methanol - the simplest natural product from plants. Trends Plant Sci 1(9):296–301. https://doi.org/10.1016/S1360-1385(96)88175-0

Hamm J, Erdmann S, Eloe-Fadrosh EA, Angeloni A, Zhong L, Brownlee C, Williams TJ, Barton K, Carswell S, Smith MA, Brazendale S, Hancock AM, Allen MA, Raftery MJ, Cavicchioli R (2019) Unexpected host dependency of Antarctic Nanohaloarchaeota. Proc Natl Acad Sci USA 116:14661–14670. https://doi.org/10.1073/pnas.1905179116

Holmes DE, Shrestha PM, Walker DJF, Dang Y, Nevin KP, Woodard TL, Lovley DR (2017) Metatranscriptomic evidence for direct interspecies electron transfer between *Geobacter* and *Methanothrix* species in methanogenic rice paddy soils. Appl Environ Microbiol 83:e00223–e317. https://doi.org/10.1128/AEM.00223-17

Hugerth LW, Muller EE, Hu YO, Lebrun LA, Roume H, Lundin D, Wilmes P, Andersson AF (2014) Systematic design of 18S rRNA gene primers for determining eukaryotic diversity in microbial consortia. PLoS ONE 9:e95567. https://doi.org/10.1371/journal.pone.0095567

Kallistova AY, Merkel AY, Tarnovetskii IY, Pimenov NV (2017) Methane formation and oxidation by prokaryotes. Microbiology 86:671–691. https://doi.org/10.1134/S0026261717060091

Kanapatskiy TA, Samylina OS, Plotnikov AO, Selivanova EA, Khlopko YA, Kuznetsova AI, Rusanov II, Zakharova EE, Pimenov NV (2018) Microbial processes of organic matter production and decomposition in saline rivers of the Lake Elton area (Volgograd oblast, Russia). Microbiology 87:66–78. https://doi.org/10.1134/S0026261718010095

Kulp TR, Hoeft SE, Miller LG, Saltikov C, Murphy JN, Han S, Lanoil B, Oremland RS (2006) Dissimilatory arsenate and sulfate reduction in sediments of two hypersaline, arsenic-rich soda lakes: Mono and Searles Lakes, California. Appl Environ Microbiol 72:6514–6526. https://doi.org/10.1128/AEM.01066-06

La Cono V, Messina E, Rohde M, Arcadi E, Ciordia S, Crisafi F, Denaro R, Ferrer M, Giuliano L, Golyshin PN, Golyshina OV, Hallsworth JE, La Spada G, Mena MC, Shevchenko MA, Smedile F, Sorokin DY, Toshchakov SV, Mushegian A, Yakimov MM (2019) Differential polysaccharide utilization is the basis for a nanohaloarchaeon: haloarchaeon symbiosis. BioRxiv. https://doi.org/10.1101/794461

Lang K, Schuldes J, Klingl A, Poehlein A, Daniel R, Brune A (2015) New mode of energy metabolism in the seventh order of methanogens as revealed by comparative genome analysis of "*Candidatus* Methanoplasma termitum". Appl Environ Microbiol 81:1338–1352. https://doi.org/10.1128/AEM.03389-14

Laso-Pérez R, Wegener G, Knittel K, Widdel F, Harding KJ, Krukenberg V, Meier DV, Richter M, Tegetmeyer HE, Riedel D, Richnow HH, Adrian L, Reemtsma T, Lechtenfeld OJ, Musat F (2016) Thermophilic archaea activate butane via alkyl-coenzyme M formation. Nature 539:396–401. https://doi.org/10.1038/nature20152

Lein A, Pimenov N, Guillou C, Martin JM, Lancelot C, Rusanov I, Yusupov S, Miller Y, Ivanov M (2002) Seasonal dynamics of the sulfate reduction rate on the North-Western Black Sea shelf. Estuar Coast Shelf Sci 54:385–401. https://doi.org/10.1006/ecss.2000.0654

Li W, Godzik A (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659. https://doi.org/10.1093/bioinformatics/btl158

Liu Y, Zhou Z, Pan J, Baker BJ, Gu J-D, Li M (2018) Comparative genomic inference suggests mixotrophic lifestyle for *Thorarchaeota*. ISME J 12:1021–1031. https://doi.org/10.1038/s41396-018-0060-x

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet Journal 17:10–12. https://doi.org/10.14806/ej.17.1.200

Mathrani IM, Boone DR, Mah RA, Fox GE, Lau PP (1988) *Methanohalophilus zhilinae* sp. nov., an alkaliphilic, halophilic, methylotrophic methanogen. Int J Syst Bacteriol 38:139–142. https://doi.org/10.1099/00207713-38-2-139

McGenity TJ (2010) Methanogens and methanogenesis in hypersaline environments. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer-Verlag, Berlin, pp 665–680

McGenity TJ, Sorokin DY (2018) Methanogens and methanogenesis in hypersaline environments. In: Stams AJM, Sousa DZ (eds) Biogenesis of hydrocarbons, handbook of hydrocarbon and lipid microbiology. Springer International Publishing AG, Berlin, pp 1–27

Merkel AY, Tarnovetskii IY, Podosokorskaya OA, Toshchakov SV (2019) Analysis of 16S rRNA primer systems for profiling of thermophilic microbial communities. Microbiology 88:671–680. https://doi.org/10.1134/S0026261719060110

Miller TL, Wolin MJ (1985) *Methanosphaera stadtmaniae* gen. nov., sp. nov.: a species that forms methane by reducing methanol with hydrogen. Arch Microbiol 141:116–122. https://doi.org/10.1007/BF00423270

Mincer TJ, Aicher AC (2016) Methanol production by a broad phylogenetic array of marine phytoplankton. PLoS ONE 11:e0150820. https://doi.org/10.1371/journal.pone.0150820

Narasingarao P, Podell S, Ugalde JA, Brochier-Armanet C, Emerson JB, Brocks JJ, Heidelberg KB, Banfield JF, Allen EE (2012) *De novo* metagenomic assembly reveals abundant novel major lineage of *Archaea* in hypersaline microbial communities. ISME J 6:81–93. https://doi.org/10.1038/ismej.2011.78

Nobu MK, Narihiro T, Kuroda K, Mei R, Liu WT (2016) Chasing the elusive *Euryarchaeota* class WSA2: genomes reveal a uniquely fastidious methyl-reducing methanogen. ISME J 10:2478–2487. https://doi.org/10.1038/ismej.2016.33

Oren A (1999) Bioenergetic aspects of halophilism. Microbiol Mol Biol Rev 63:334–348

Oren A (2014a) The Family Methanocalculaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The Prokaryotes. Springer, Berlin, pp 195–199

Oren A (2014b) Taxonomy of halophilic Archaea: current status and future challenges. Extremophiles 18:825–834. https://doi.org/10.1007/s00792-014-0654-9

Oren A, Arahal DR, Ventosa A (2009) Emended descriptions of genera of the family *Halobacteriaceae*. Int J Syst Evol Microbiol 59:637–642. https://doi.org/10.1099/ijs.0.008904-0

Pruesse E, Peplies J, Glöckner FO (2012) SINA: accurate high throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823–1829. https://doi.org/10.1093/bioinformatics/bts252

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219

Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA, Hedlund BP, Tsiamis G, Sievert SM, Liu WT, Eisen JA, Hallam SJ, Kyrpides NC, Stepanauskas R, Rubin EM, Hugenholtz P, Woyke T (2013) Insights into the phylogeny and coding potential of microbial dark matter. Nature 499:431–437. https://doi.org/10.1038/nature12352

Rotaru A-E, Shrestha PM, Liu F, Shrestha M, Shrestha D, Embree M, Zengler K, Wardman C, Nevin KP, Lovley DR (2014) A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane. Energy Environ Sci 7:408–415. https://doi.org/10.1039/C3EE42189A

Schink B, Zeikus JG (1980) Microbial methanol formation: a major end product of pectin metabolism. Cur Microbiol 4:387–389. https://doi.org/10.1007/BF02605383

Sorokin DY, Abbas B, Merkel AY, Rijpstra WI, Damsté JS, Sukhacheva MV, van Loosdrecht MC (2015) *Methanosalsum natronophilum* sp. nov., and *Methanocalculus alkaliphilus* sp. nov., haloalkaliphilic methanogens from hypersaline soda lakes. Int J Syst Evol Microbiol 65:3739–3745. https://doi.org/10.1099/ijsem.0.000488

Sorokin DY, Abbas B, Geleijnse M, Kolganova TV, Kleerebezem R, van Loosdrecht MCM (2016a) Syntrophic associations from hypersaline soda lakesconverting organic acids and alcohols to methane atextremely haloalkaline conditions. Environ Microbiol 18:3189–3202. https://doi.org/10.1111/1462-2920.13448

Sorokin DY, Kublanov IV, Yakimov MM, Rijpstra WI, Sinninghe Damsté JS (2016b) *Halanaeroarchaeum sulfurireducens* gen. nov., sp. nov., the first obligately anaerobic sulfur-respiring haloarchaeon, isolated from a hypersaline lake. Int J Syst Evol Microbiol 66:2377–2381. https://doi.org/10.1099/ijsem.0.001041

Sorokin DY, Makarova KS, Abbas B, Ferrer M, Golyshin PN, Galinski EA, Ciordia S, Mena MC, Merkel AY, Wolf YI, van Loosdrecht MCM, Koonin EV (2017a) Discovery of extremely halophilic, methyl-reducing euryarchaea provides insights into the evolutionary origin of methanogenesis. Nat Microbiol 2:17081. https://doi.org/10.1038/nmicrobiol.2017.81

Sorokin DY, Messina E, Smedile F, Roman P, Sinninghe Damsté JS, Ciordia S, Mena MC, Ferrer M, Golyshin PN, Kublanov IV, Samarov NI, Toshchakov SV, La Cono V, Yakimov MM (2017b) Discovery of anaerobic lithoheterotrophic haloarchaea, ubiquitous in hypersaline habitats. ISME J 11:1245–1260. https://doi.org/10.1038/ismej.2016.203

Sorokin DY, Merkel AY, Abbas B, Makarova KS, Rijpstra WIC, Koenen M, Sinninghe Damsté JS, Galinski EA, Koonin EV, van Loosdrecht MCM (2018) *Methanonatronarchaeum thermophilum* gen. nov., sp. nov. and '*Candidatus* Methanohalarchaeum thermophilum', extremely halo(natrono)philic methyl-reducing methanogens from hypersaline lakes comprising a new euryarchaeal class *Methanonatronarchaeia* classis nov. Int J Syst Evol Microbiol 68:2199–2208. https://doi.org/10.1099/ijsem.0.002810

Spang A, Caceres EF, Ettema TJG (2017) Genomic exploration of the diversity, ecology, and evolution of the archaeal domain of life. Science 357:eaaf3883. https://doi.org/10.1126/science.aaf3883

Stieglmeier M, Klingl A, Alves RJ, Rittmann SK, Melcher M, Leisch N, Schleper C (2014) *Nitrososphaera viennensis* gen. nov., sp. nov., an aerobic and mesophilic, ammonia-oxidizing archaeon from soil and a member of the archaeal phylum *Thaumarchaeota*. Int J Syst Evol Microbiol 64:2738–2752. https://doi.org/10.1099/ijs.0.063172-0

Wainø M, Tindall BJ, Ingvorsen K (2000) *Halorhabdus utahensis* gen. nov., sp. nov., an aerobic, extremely halophilic member of the *Archaea* from Great Salt Lake. Utah Int J Syst Evol Microbiol 50:183–190. https://doi.org/10.1099/00207713-50-1-183

Yee MO, Rotaru AE (2020) Extracellular electron uptake in *Methanosarcinales* is independent of multiheme c-type cytochromes. Sci Rep 10:372. https://doi.org/10.1038/s41598-019-57206-z

Youssef NH, Rinke C, Stepanauskas R, Farag I, Woyke T, Elshahed MS (2015) Insights into the metabolism, lifestyle and putative evolutionary history of the novel archaeal phylum '*Diapherotrites*'. ISME J 9:447–460. https://doi.org/10.1038/ismej.2014.141

Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Bäckström D, Juzokaite L, Vancaester E, Seitz KW, Anantharaman K, Starnawski P, Kjeldsen KU, Stott MB, Nunoura T, Banfield JF, Schramm A, Baker BJ, Spang A, Ettema TJ (2017) Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature 541:353–358. https://doi.org/10.1038/nature21031

Zhilina TN (1983) New obligate halophilic methane-producing bacterium. Microbiology 52:290–297

Zhilina TN (2001) Genus III. Methanohalobium Zhilina and Zavarzin,1988, 136VP. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology. The Archaea and the deeply branching and phototrophic Bacteria, vol 1, 2nd edn. Springer, New York, pp 279–281

Zhilina TN, Zavarzin GA (1987) *Methanohalobium evestigatum* gen. nov., sp. nov., extremely halophilic methane-producing archaebacteria. Dokl Akad Nauk SSSR 293:464–468

Zhilina TN, Zavarzina DG, Kevbrin VV, Kolganova TV (2013) *Methanocalculus natronophilus* sp. nov., a new alkaliphilic hydrogenotrophic methanogenic archaeon from a soda lake, and proposal of the new family *Methanocalculaceae*. Microbiology 82:686–694. https://doi.org/10.1134/S0026261713060131

Zhou Z, Liu Y, Lloyd KG, Pan J, Yang Y, Gu J-D, Li M (2019) Genomic and transcriptomic insights into the ecology and metabolism of benthic archaeal cosmopolitan, *Thermoprofundales* (MBG-D archaea). ISME J 13:885–901. https://doi.org/10.1038/s41396-018-0321-8

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.