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Methylotrophic methanogens in bottom sediments of Lake Baikal

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ABSTRACT. Recently many novel lineages of methanogens being suggested via metagenomics approaches. Most of these lineages potentially capable of methanogenesis from C_1-methylated compounds, a pathway that had previously received comparably little attention. Analysis of the 16S rRNA gene diversity showed that in the bottom sediments of Lake Baikal methylotrophic methanogens as well as H_2-depended methylotrophic methanogens are represented. However, under cultivation conditions, the addition of methanol stimulated methane production only in the case of microbial communities from upper silts. Among the methylotrophic methanogens in enrichment cultures, representatives of the genera *Methanosarcina* and *Methanomethylovorans* were identified.

Keywords: methanogenesis, methanol, *Archa*ea, Lake Baikal, bottom sediments, cultivation

1. Introduction

Despite the fact that the phylogeny, physiological and biochemical properties of methanogens are well understood, a number of important discoveries have recently been made. The possibility of methane formation has been declared for representatives of deep phylogenetic lineages both within the phylum *Euryarchaeota* (order *Methanomassiliicoccales*, classes Candidatus “Methanofastidiosa” and *Methanonatronarchaeia*) and outside it (phylums Ca. “Verstraetearchaeota” and *Bathyarchaeota*) (Borrel et al., 2011; Evans et al., 2015; Lang et al., 2015; Nobu et al., 2016; Vanwonterghem et al., 2016; Sorokin et al., 2017). It is noteworthy that all open microorganisms are capable of producing methane from C_1-methylated compounds. Despite the discoveries made, the global contribution of methylated compounds to the total production of methane, in comparison with two other main types of methanogenic substrates – H_2/CO_2 and acetate, is estimated as insignificant. However, it is believed that the contribution of a substrate such as methanol to methane production in low temperature bog and lake ecotopes is greatly underestimated (Jiang et al., 2010).

Earlier, based on the analysis of the 16S rRNA gene diversity, it was shown that the bottom sediments of Lake Baikal are inhabited by microorganisms of the orders *Methanosarcinales*, *Methanomassiliicoccales* (*Thermoplasmata*) and phylum *Bathyarchaeota*, potentially capable of forming methane from methylated substrates, but the process of methylotrophic methanogenesis has not been studied (Chernitsyna et al., 2016; Lomakina et al., 2018). Methylated compounds can be produced during the destruction of pectin and cellulose, which are part of the cell walls of algae and plants, and be a product of methane oxidation. For this reason, we evaluated the possibility of methane formation from methanol during anaerobic cultivation of microbial communities from Lake Baikal bottom sediments in areas with oil- and gas-bearing fluids discharge, as well as the in waste disposal areas of the Baikal Pulp and Paper Mill (BPPM) and the confluence of the Goloustnaya River, which has a swampy mouth. In addition, we estimated the taxonomic diversity of methyl coenzyme M-reductase (McrA) genes in methylotrophic enrichment cultures.

2. Results and discussion

The methanogenesis on a medium with methanol (1% v / v) was recorded only in the case of microbial communities from upper silts of all stations studied (0-7 cm), which may be related to their greater adaptability to increased concentrations of this substrate. In samples from the mud volcano (m/w) “Malenkiy” methane generation was also detected in the culture from a depth of 30 cm (Fig. 1). Communities from the lower layers did not show methanogenic activity upon addition of methanol. The concentrations of methane formed on the medium with CH_3OH varied from 0.36 to 27.01

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mmol L^{-1} and were maximum in samples from the area of the mouth of the Goloustnaya River.

Analysis of the diversity of McrA gene sequences in enrichment cultures revealed methanogenic archaea of the *Methanomicrobia* and *Methanobacteria* classes (Fig. 2). Methylotrophic methanogens of the genus *Methanosarcina* were identified in samples from the “Posolskaya Bank” methane seep, m/v “Khoboy” and “Malenkiy” (6 cm), and the “Gorevoy Utes” oil-methane seep. In cultures from the Goloustnaya River mouth, the presence of methanol-utilizing methanogens of the genus *Methanomethylovorans* was confirmed. At the same time, all analyzed sequences in the samples from the BPPM areas and m/v “Malenkiy” (3-4 and 30 cm) had homology only with McrA gene of the genera *Methanobacterium* and *Methanoregula*, most of the cultivated representatives of which form methane as a result of CO_2 reduction. However, the methane concentrations in these cultures significantly exceeded those in the control samples exposed without additional substrates (Fig. 1). Perhaps some representatives of these taxa in Lake Baikal are capable of using methylated substrates, or methane formation during cultivation could occur due to interspecies transfer of hydrogen between anaerobic methylotrophic microorganisms and hydrogenotrophic methanogens.

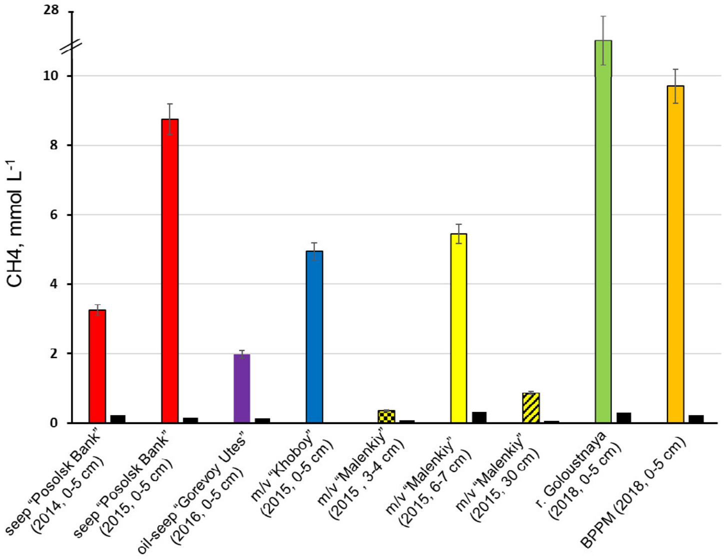

Fig.1. The concentration of methane generated during the cultivation of communities of bottom sediments of Lake Baikal with methanol (colored columns) and without additional substrates (black columns).

The absence in enrichment cultures of molecular markers of H_2-dependent methylotrophic methanogens of the *Methanomassiliicoccales*, *Verstraetearchaeota*, *Bathyarchaeota* groups can be explained by inappropriate cultivation conditions or impaired syntrophic interactions with other anaerobes. However, the presence of these taxa in the native communities of the sediments of Lake Baikal may indicate their important role in the metabolism of methylated compounds. Thus, further research is required to clarify the features of their metabolism and ecology.

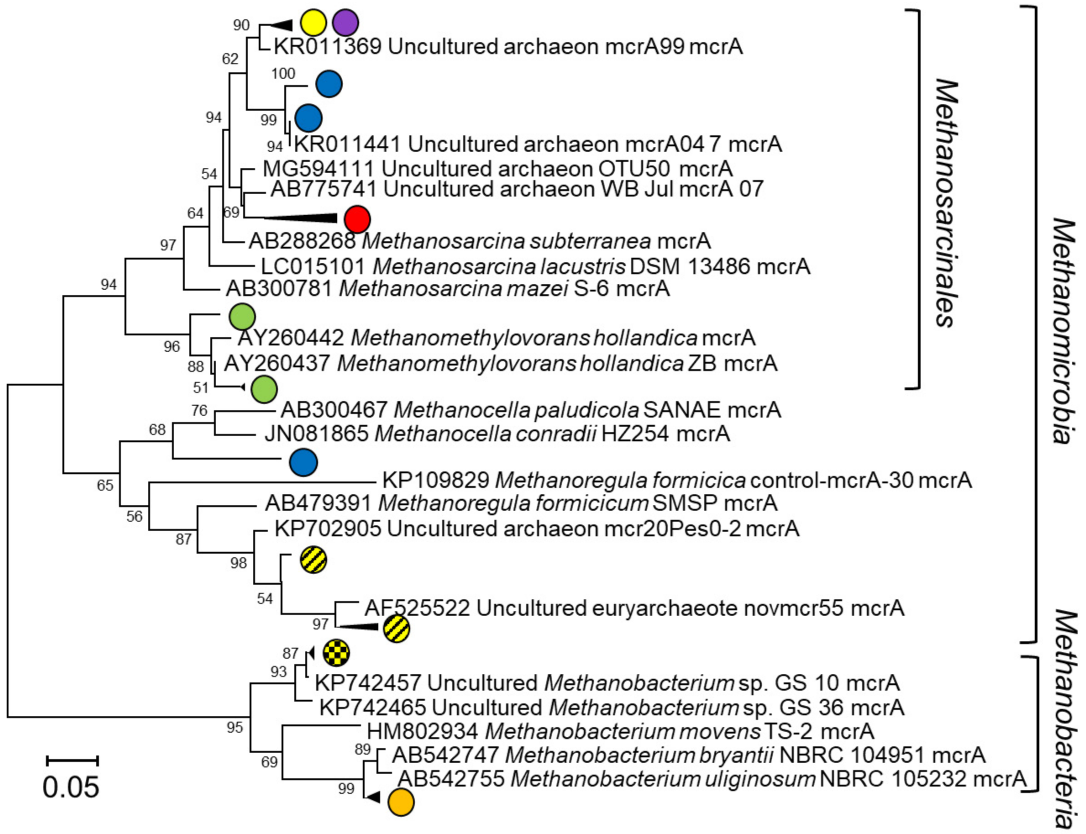

Fig.2. Phylogenetic tree of McrA gene sequences detected in microbial communities of enrichment cultures with methanol.

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References

Borrel G., Jézéquel D., Biderre-Petit C. et al. 2011. Production and consumption of methane in freshwater lake ecosystems. Research in Microbiology 162: 832-847. DOI: 10.1016/j.resmic.2011.06.004

Chernitsyna S.M., Khal'zov I.A., Khanaeva T.A. et al. 2016. Microbial community associated with *Thioploca* sp. sheaths in the area of the Posolsk Bank methane seep, Southern Baikal. Microbiology (Microbiologiya) 85: 562-569. DOI: 10.1134/s0026261716050027

Evans P.N., Parks D.H., Chadwick G.L. et al. 2015. Methane metabolism in the archaeal phylum *Bathyarchaeota* revealed by genome-centric metagenomics. Science 350: 434-438. DOI: 10.3410/f.725872683.793534800

Jiang H., Chen Y., Jiang P. et al. 2010. Methanotrophs: multifuncional bacteria with promising applications in environmental bioengineering. Biochemical Engineering Journal 49: 277-288. DOI: 10.1016/j.bej.2010.01.003

Lang K., Schuldes J., Klingl A. et al. 2015. New mode of energy metabolism in the seventh order of methanogens as revealed by comparative genome analysis of Candidatus "Methanoplasma termitum". Applied and Environmental Microbiology 81: 1338-1352. DOI: 10.1128/aem.03389-14

Lomakina A.V., Mamaeva E.V., Galachyants Y.P. et al. 2018. Diversity of *Archaea* in bottom sediments of the discharge areas with oil- and gas-bearing fluids in Lake Baikal. Geomicrobiology Journal 35: 50-63. DOI: 10.1080/01490451.2017.1315195

Nobu M.K., Narihiro T., Kuroda K. et al. 2016. Chasing the elusive *Euryarchaeota* class WSA2: genomes reveal a uniquely fastidious methyl-reducing methanogen. ISME Journal 10: 2478-2487. DOI: 10.1038/ismej.2016.33

Sorokin D.Y., Makarova K.S., Abbas B. et al. 2017. Discovery of extremely halophilic, methyl-reducing euryarchaea provides insights into the evolutionary origin of methanogenesis. Nature Microbiology 2. DOI: 10.1038/nmicrobiol.2017.81

Vanwonterghem I., Evans P.N., Parks D.H. et al. 2016. Methylotrophic methanogenesis discovered in the archaeal phylum *Verstraetearchaeota*. Nature Microbiology 1. DOI: 10.1038/nmicrobiol.2016.170