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 C₁-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 – H₂ 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 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 CH₃OH 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 *Methanobacterium* 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 CO₂ 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.

The absence in enrichment cultures of molecular markers of H₂-dependent methylotrophic methanogens of the *Methanomassiliicoccales*, *Verstraetaarchaeota*, *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.
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References


