Short communication

Anaerobic oxidation of methane in differences types of geological structures at Lake Baikal

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ABSTRACT. Although in sediments of Lake Baikal process of anaerobic oxidation methane was revealed by radioisotope techniques, by of the methane profile we know a little about the microorganisms carrying out this process. Here we studied diversity of archaeal communities in different types geological structures (methane seeps and mud volcano) on Lake Baikal with different composition of pore waters and the discharged fluid. In investigate were used different methods molecular biology including of high-throughput sequencing of gene 16S rRNA and PCR analysis different genes of AOM (*mcrA*). Molecular analysis did not reveal methane-oxidizing archaea ANME-1, -2, or -3, which are responsible for anaerobic oxidation of methane in marine sediments. Hydrogenotrophic methanogenic archaea representatives of the orders *Methanomicrobiales, Methanococcales*, as well as acetoclastic methanogens of the order *Methanosarsinales* were abundant in a mud volcano at Lake Baikal. In investigation sites we found divers *M. nitroreducens*-like archaea (ANME-2d subcluster) both in deep sediments of a methane seeps and in a mud volcanoes. Their closest homologues involved in process anaerobic oxidation of methane in different freshwater systems, bioreactors, paddy field soils.

Keywords: Lake Baikal, microbial community, anaerobic oxidation methane, Archaea, ANME-2d, *mcrA*

1. Introduction

Lake sediments are globally important carbon stores, but they are also important contributors of methane (CH_4) (Bastviken et al., 2004). Approximately 70% of atmospheric methane is of microbial origin. Anaerobic oxidation of methane (AOM) reduces methane emissions from marine ecosystems but we know little about AOM in freshwater reservoir. The most studied processes are sulfate-dependent methane oxidation via the reverse methanogenesis pathway performed by archaea of the ANME clusters in marine sediments (Knittel and Boetius, 2009). In recent years, there has been an active search for microbial agents that carry out AOM in freshwater ecosystems. Recent studies have shown that in oxygen-free soil layers and bottom sediments oxidation of methane provide anaerobic methanotrophic bacteria and/or archaea, which can use as electron acceptors nitrate, nitrite and metal oxides. This is members of the archaeal family *Methanoperedenaceae,* formerly known as ANME-2d subclusters, and representatives *Candidatus* Methylomirabilis oxyfera (known as NC10 bacteria) (Ettwig et al., 2010; Haroon et al., 2013).

Lake Baikal has unique characteristics and phenomena observed in its ecosystem and processes are noted in both freshwater and marine ecosystems. The unique characteristics of Lake Baikal include the presence in its sediments of gas hydrate (GH). Their formation is understandable, since as shown in the works of Namsaraev et al. (1995), Dagurova et al. (2004), Pimenov et al. (2014), methane generation is the main final process of destruction of organic matter in Baikal. And the low temperatures and deep sea of the lake ensure their formation and preservation. Another feature of the lake is the presence of autigenic carbonates in its deep sediments. These problems are related, since carbonates are confined to areas where there is discharge of hydrocarbon fluids (Krylov et al., 2008). On Lake Baikal describes more than 50 geological structures that differ in the composition of the discharged fluids (Khlystov et al., 2018). In the composition of the discharged fluid can presence ions of sulfate, nitrate, ammonium, acetate, iron, hydrocarbonate, increased concentrations of which are detected in the bottom sediments of the sites of Southern and Central Baikal (Zemskaya et al., 2015; Lomakina et al., 2020). Pore water of sediment Lake

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Baikal are often rich in Fe and Mn (up to 240 and 95 μM, respectively), and in all deep basins of Lake Baikal the occurrence of cm-thick layers of Fe and Mn oxides buried within the reducing part of the sediments is well known (e.g. Granina et al., 2004; Och et al., 2012; Zemskaya et al., 2018). In deep sediments near GH, the rate of methane oxidation ranged from 35 to 273.2 μl $CH_4/(dm^3 day)$ (Dagurova et al., 2004), increasing at depths of 20-60 cm, which may indicate the functioning of the anaerobic community of microorganisms (Zemskaya et al., 2010). Process of AOM in deep sediments of Lake Baikal was revealed by radioisotope techniques, with the maximum that was usually close to the GH layers, as well as by of the methane profile (Zemskaya et al., 2010). Our investigation showed that in deep sediments of sites gas-oil containing fluids the process of AOM carried out by representatives ANME-2d subclusters and NC10 (Lomakina et al., 2018; 2020). ANME-2d archaea may play an important role in controlling methane emissions from nitrate-depleted and low-sulfate freshwater systems (Weber et al., 2017). Molecular analysis of Baikal sediments did not reveal methane-oxidizing archaea ANME-1, -2, or -3, which are responsible for anaerobic oxidation of methane in marine sediments (Lomakina et al., 2018; 2020). Sediment from associated with oil, GH, in addition to authigenic carbonates of Southern and Central Baikal, were collected and analyzed with 16S rRNA and *mcrA* gene phylogenetic data. These results establish baseline data for future AOM studies at Lake Baikal and elucidate metabolic pathways this process, as well as and electron acceptors.

2. Materials and methods

The materials for the study were sediments samples from expedition (2010, 2012, 2016, 2019) on board the research vessel (RV) "G.Yu. Vereshchagin". Samples of bottom sediments were collected from geomorphologically distinct sites: the mud volcano Malenky (~1371 water depth) Kedr (~600 m), the methane seep Posolsk Bank (PB, 400 m) and Krasny Yar (~730 m). Layers of methane hydrates occurred in the sediments of the mud volcano Malenky (130-131, 132-139 cm) and sediments of the methane seep Posolsk Bank (100 cm, 140 cm). In samples of sediments the mud volcano Kedr (127-130 cm, 125-127 cm, 150-152 cm) and Malenky (145 cm) detected authigenic carbonates. After lifting the core onto the RV board and cutting it into two lengthwise halves, the sediment samples were collected for chemical analysis of pore waters and DNA extraction. Chemical analysis of pore waters was carried out on board immediately after sampling. Sediments selected for DNA extraction were stored in liquid nitrogen until the laboratory analysis. For further analysis and investigation were selected samples containing GHs (Malenky 130-131 cm, 132-139 cm; Posolsk Bank 100 cm, 140 cm), authigenic carbonates (Malenky 140-146 cm, Kedr 127-130 cm, 125-127 cm, 150-152 cm) and oxidized channel in bottom sediment of mud volcano Krasny Yar (35-37 cm).

DNA was extracted according to phenol-chloroform alcohol method. PCR amplification with primers on the archaeal (V5–V7 region) 16S rRNA gene fragments were carries out.

Analysis of the similarity of the 16S rRNA gene sequences to the known GenBank sequences (http://www.ncbi.nlm.nih.gov/genbank/) was carried out using the BLAST software package. Corresponding phylogenetic trees were constructed by the NJ and Kimura two-parameter methods implemented in MEGA version 6.0.

3. Results and discussion

This study examined samples of deep sediments from different type's geological structures at Lake Baikal. Sediments of the investigated core from a methane seep Posolsk Bank were highly saturated with gas. Methane concentration increased with depth (up to 10 μM in surface sediments), reaching maximum values (up to 9000 μM) in the sedimentary strata where GHs were detected. Throughout the core, there were increased concentrations of CH_3COO^- ions up to 379 μM and Fe^{2+} up to 134 μM. Concentration ion of NO_3^- was 20 μM in surface sediments, and it was not detected in other pore waters, as well as concentrations of SO_4^{2-} ions had maximum values in the surface sediments (62 μM). Unlike the methane seep Posolsk Bank, methane concentrations in sediments of a methane seep Krasny Yar not exceeded 190 μM in the surface and 500 μM in depth sediments. Ions Fe^{2+}, Mn^{2+}, NH_4^+, NO_2^-, and CH_3COOH^- in pore waters were absent. Sulfate was detected in pore water only in the uppermost interval of sediments (0-50 cm) and at very low concentration up to 60 μm.

In deep sediments of a mud volcano Malenky concentration of methane exceeded 90 μM almost from the surface and showed uneven distribution with the maximum value at a depth of 140 cm (up to 1200 μM). The concentrations of SO_4^{2-} in pore waters of the investigated core reached 3781 μM in sediments that contained carbonate (140-146 cm); the minimum concentrations were recorded in GH layers (130-131 cm and 132-139 cm). Higher concentrations of HCO_3^- ions in comparison with the reference values (up to 1098 μM) are typical of all studied sediments with the maximum in GH layer of 130-131 cm (up to 4049 μM). Nitrate and acetate were not detected in the pore water (Lomakina et al., 2020).

The concentration of SO_4^{2-} in pore waters a mud volcano Kedr varied from 56 μM in surface sediments to 52 μM in the deep sediments. The concentrations of Fe^{2+} reached 3.5 μM, Mn^{2+} 11.4 μM. Nitrate ions presented in all investigation sediments with concentration 9.1 μM in surface layer and 6.6 μM in the deep sediments. Acetate was not detected in the pore water of Kedr. Maximum value of methane was fixed in deep sediments (to 1138 μM), in surface layer concentration methane not reached 9.8 μM.

The studied sites differed in composition of dominated archaeal communities. So, analysis of 16S rRNA gene libraries showed that in sediments of a mud

volcano Malenky the dominant phyla were sequences of *Bathyarchaeota* (12%, 54% in GH layers), *Euryarchaeota* (99% in carbonate layer; 27%, 35.7%), *Crenarchaeota* (17%, 25% in GH layers). Nucleotide sequences of phylum *Thaumarchaeota* dominated (up to 20% of all 16S rRNA reads) only one of GH layer (130-131 cm). In the composition of bottom sediments from a methane seep Posolsk Bank, *Euryarchaeota* (60%, 95% of all 16S rRNA reads) dominated. Representatives of *Bathyarchaeota* (27%), *Crenarchaeota* (10%) were presented only in layer 100 cm. In the oxidized channel *Euryarchaeota* (45%), *Bathyarchaeota* (28%), *Woesearchaeota* (14%), *Crenarchaeota* (6.5%), *Thaumarchaeota* (2.7%). Among archaea dominated phyla were *Bathyarchaeota* (38-76%), *Euryarchaeota* (5-33%) at all investigation sediments of a mud volcano Kedr. Archaea of phyla *Nanoarchaeota, Vestraetearchaeota* were detected in bottom layers of a mud volcano Kedr. Previously, nucleotide sequences of these phyla were not detected in other of the discharged fluids sites. In addition, representatives of phylum *Vestraetearchaeota* know as methilotrophic methanogens (Vanwonterghem et al., 2016).

In result of phylogenetic analysis members of the know archaea (ANME-1, -2 and -3 clusters) performed AOM were not found. Analysis nucleotide sequences of gene 16S rRNA showed the presence of ANME-2d subcluster. However, representatives of this subcluster were absent in GHs sediments of site Posolsk Bank, but abundant in carbonate layers of a mud volcanoes (9% in site Kedr, and 20% of all 16S rRNA sequences from site Malenky), GH sediments of a mud volcano Malenky, as well as in oxidized channel a methane seep Krasny Yar (to 3.5% of all 16S rRNA reads *Archaea*). The nucleotide sequences of ANME-2d obtained in this study were similar to the sequences from different environments, including Lake Ørn sediments, freshwater iron-enriched microbial mat, coal-rich sediments.

We tested all recovered DNA samples for *mcrA* genes of the ANME-2d subcluster. The analysis of the obtained translated amino acid sequences has confirmed the presence of *mcrA* genes of the ANME-2d in the communities. The analysed amino acid sequences of the bottom sediments investigated areas were similar to *mcrA* gene sequences of *M. nitroreducens* (ANME-2d). Sequences from sediments Lake Baikal were closest relatives from the microbial mat of Hydrate Ridge North, Pacific Ocean; bioreactor, in which AOM processes, occurred together with iron reduction.

4. Conclusion

Our studies showed that despite the different chemical composition of pore waters, as well as the composition of the dispersing fluids this does not affect the distribution of the dominant archaea phyla. In studied sites were found representatives of methane oxidizing archaea ANME-2d subcluster, but no marine ANME clusters. However, diversity methanogenic archaea is characterized by a significant variety of sequences than methanotropic archaea (ANME-2d).

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