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The research of relationship between microbiological factors with the distribution of anomalous methane fields and the presence of gas hydrate deposits using the example of two water areas in the northern part of Japan Sea

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Abstract. Our research deals with the possibilities of using microorganisms as bioindicators for methane ecosystems in areas with gas hydrates. We have conducted a study of the biodiversity of microorganisms and determined the physiological and biochemical properties of bacterial strains that can oxidize hydrocarbons isolated from the bottom sediments in the northern part of the Japan Sea for two areas: one with detected gas hydrates (Area 1) and one without the presence of gas hydrates (Area 2). Complex gas-geochemical, geological, and microbiological studies have been conducted in the waters of the northern Japan Sea, including the southern part of the Tatar Strait and the northern slope of Primorsky Krai. Obtained materials were gotten from marine expeditions: RV "Akademik Oparin" 54 (OP54) in September–October 2017 and RV "Akademik M.A. Lavrentyev" 81 (LV81) in May 2018. We used cultivation techniques to discover that members of the Nocardiaceae family from the Actinomycota phylum were associated with areas where gas hydrates had been detected. We found that bacteria isolated from these areas were able to ferment a wider variety of carbohydrate substrates than those obtained from non-gas hydrate areas. A positive correlation was observed between the ability of these bacteria to break down carboxylic acids and their absence from gas hydrate-rich environments.

Keywords: methane, gas hydrates, hydrocarbon degradation, bottom sediments, physiological and biochemical properties, Japan Sea, Tatar Strait

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Introduction

The issue of gas hydrate accumulation in the oceans is a long-term concern. Climate change has a global scale that affects all of humanity, with greenhouse gas emissions being the main cause of climate warming. As one of the largest organic carbon sources on the Earth, gas hydrates release large amounts of methane when decomposing that can have a significant impact on the marine environment (Collet, 2009).

Biogenic and thermogenic gases are two main sources of gas hydrate formation (Whiticar, 1999). Both types of methane hydrates can have a significant impact on the ecological characteristics of marine sediments, but research into microbial diversity in thermogenic hydrate-bearing sediments is limited. Biogenic methane is produced by microbial communities through anaerobic respiration, while thermogenic gases form as a result of the thermocatalytic breakdown of organic compounds at high temperatures (Schoell, 1988; Wuebbles, Hayhoe, 2002).

Marine sediments provide a unique habitat for a diverse range of microorganisms. These microorganisms that inhabit bottom sediments have a clear structure, which depends on factors such as depth, oxygen availability, and the amount of organic matter available (Walsh, 2016).

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The identification of the relationship between the structure and distribution of microorganisms and methane in gas hydrates within marine sediments has attracted the attention of scientists worldwide. Extensive studies on microbial diversity and activity have been conducted in sediments containing gas hydrates, including in the Gulf of Mexico (Mills et al., 2005), the Nankai Trench, West Pacific Ocean (Katayama et al., 2016, 2022), and the eastern part of Japan Sea (Yanagawa et al., 2014), the Andaman Sea (Briggs et al., 2012), the Tsushima Basin (Japan Sea) (Lee et al., 2013; Ryu et al., 2013; Cho et al., 2017), and the Arctic regions near Svalbard (Carrier et al., 2020), Shenhu region (South China Sea) (Jiao et al., 2015; Cui et al., 2019, 2020). Some studies have also compared microbial communities in marine sediments with and without gas hydrates (Yanagawa et al., 2014; Jiao et al., 2015; Cui et al., 2020; Liu et al., 2022).

While many parts of the World Ocean meet research devoted the distribution of microorganisms that break down hydrocarbons from areas around gas vent areas in order to understand their role in the circulation of substances and the possibility of using them as bioindicators, there is very little research in the Far Eastern seas of the Russian Federation, specifically in Japan Sea.

The marginal seas of the Pacific Ocean represent a unique morphostructural features of the Pacific mobile belt, which are particularly well developed in its western region. The Okhotsk Sea and Japan Sea are important components of the western Pacific basin system and are sensitive to global environmental changes.

The study of these seas is of a particular interest, not only from the perspective of modern active geological processes and the potential for oil and gas, but also in terms of understanding gas and geochemical parameters within the seas and in transit zones between a land and a shelf.

The most active degassing of the lithosphere occurs within the Hokkaido-Sakhalin folded system, in the area of the Sakhalin Island shelf and slope (Syrbu et al., 2022; Syrbu et al., 2024) and is of a great interest due to the genesis and ecological significance of the natural gases contained in sedimentary basins, underwater gas hydrates, geothermal systems, mud volcanoes, gas-saturated groundwater and marine sediments. This study of gas hydrate provinces on the Sakhalin shelf and slopes is relevant in light of ongoing global changes.

Cultured bacteria isolated from the water and bottom sediments of the Japan Sea have been presented in studies from different years. The strain *Oceanisphaera litoralis* gen. nov., sp. nov., has been isolated and described. Additionally, four new species of *Psychrobacter* have been described as well (Romanenko et al., 2004).

Hydrocarbon-oxidizing microorganisms have been the subject of intensive research in the Far Eastern seas, due to both oil pollution of surface waters and their potential use in bioremediation processes.

The strains of petroleum-oxidizing microorganisms isolated from the coastal waters of the Japan Sea (Zolotoy Rog Bay and Nakhodka Bay) and the Sea of Okhotsk (Anniva Gulf) have been described, and the minimum inhibitory concentration of oil for these strains has been determined (Buzoleva et al., 2008).

Joint studies have been conducted to investigate the taxonomic diversity of cultivated hydrocarbon-oxidizing bacteria cultivated in the Japan Sea, and to assess their ability to oxidize oil using isolated isolates (Bogatyrenko et al., 2021).

Studies have also been conducted in the coastal waters off the southern coast of Sakhalin Island. During these studies, 67 different strains of microorganisms were isolated and collected. These microorganisms have a high ability to break down basic hydrocarbons, such as alkanes and cycloalkanes, as well as aromatic compounds. This makes them useful for the bioremediation of marine environments (Repina (Smirnova), 2009).

The aim of our study is to investigate the diversity, physiological, and biochemical characteristics of hydrocarbon-oxidizing microorganisms that were cultivated from the upper layer of the sediment in the northern part of the Japan Sea, both in gas hydrate-bearing and non-hydrate areas. By comparing the distribution of anomalous methane gas-geochemical fields with the microorganisms present in the bottom sediments, it may be possible to consider these microorganisms as geomicrobiological indicators. The microbial community in these areas is unique, with a number of taxonomical and physiological features that are associated with the presence or absence of gas hydrates at shallow depths.

Research area

The Sakhalin Island and its surrounding area are located within the active Pacific oceanic island arc margin and represent a folded region from the Cenozoic era. This region has a sub-meridian orientation and is separated from neighboring structures by large faults, such as the Tatarsky and East-Sakhalin faults. Young marginal troughs also separate this area from adjacent structures. Extended sub-meridional segments can be found within this folded area. The largest of these segments, Central Sakhalin, extends from Hokkaido Island to the northern tip of Sakhalin Island. The style of dislocation of formations from different ages is influenced not only by accretionary processes, but also by shear movements along the meridional fault system. These shear movements contribute significantly to the overall structure of the region.

The formation and accumulation of hydrocarbons primarily occurs in sedimentary basins, which are regions of prolonged immersion within the Earth's crust. During this process, the sedimentary layer is heated by rising heat flows, which stimulate the formation of gas. Areas with natural gas emissions are typically located within thick sedimentary layers (more than 2 kilometers in thickness) that contain various accumulations of hydrocarbons, such as oil and gas deposits, as well as gas hydrates and gas-rich sediments.

A necessary condition for the degassing of hydrocarbons from these sites is typically the presence of fracture systems (Hovland, 1994). Additional features that can contribute to this process include folded dislocations and increased seismic activity. All of these active degassing conditions can be found on the shelf and slope areas of Sakhalin Island.

There are three Cenozoic basins on Sakhalin Island and the adjacent shelf. These basins have different characteristics in terms of sedimentation and oil and gas content. They are called North Sakhalin, West Sakhalin, and South Sakhalin basins. These correspond to the namesake oil and gas regions. The research area under discussion is located within the West Sakhalin oil and gas region, which corresponds to a large Late Cretaceous-Cenozoic depression between the anticline uplifts of Sakhalin Island and the East Sikhote-Alin volcanic belt that forms the edge of the Asian continent.

Geological and geophysical data indicate that favorable conditions for methane emissions currently exist in the studied areas (Syrbu et al., 2022). One of the most favorable areas for underwater methane emissions in the Japan Sea is the southwestern shelf and slope of Sakhalin Island.

The research areas of the 54th cruise of RV "Akademik Oparin" and the 81st cruise of RV "Akademik M.A. Lavrentyev" cover the southern part of the Tatar Strait and the northern slope of Primorsky Krai (Figure 1).

The Tatar Trough is a large, 1,200-kilometer-long and 60–300-kilometer-wide depression that formed as a result of rifting that began in the Middle and Late Oligocene periods and lasted until the end of the Miocene (Kharakhinov, 2010). It is framed by the mountainous structures of Sikhote-Alin and Western Sakhalin from the west and east, respectively.

The Trough is filled with Mesozoic and Cenozoic sedimentary and volcanic-sedimentary rocks, and the Cenozoic deposits in the Western Sakhalin mountains have a steep slope towards the west. These deposits are largely disrupted by discharges and upward displacements, with movements along the faults ranging from tens to hundreds of meters or even several kilometers.

Volcanoes that were active 5–10 million years ago are associated with the fault zone, and deep-seated faults that dissect the Earth's crust can be clearly seen. High levels of heat flow as well as magmatic and seismic activity indicate ongoing tectonic activity in the area. The Tatar Strait fault is the northern extension of a spreading center located in the deep-sea depression of the Japan Sea. It includes three sedimentary basins: the North Tatar, South Tatar, and Isikari-West Sakhalin, formed by Cenozoic terrigenous and, to a lesser extent, volcanogenic deposits up to 8,000 meters thick. These basins are the North Tatar, South Tatar, and Isikari-West Sakhalin basins (Nechayuk, 2017).

The Tatar Strait has been extensively studied from the perspective of oil and gas geology (Kharakhinov, 2010). Seismic surveys have revealed numerous features that formed as a result of gas migration into the sedimentary layer. During the investigation of the gas-geochemical properties of the bottom water column of the Tatar Strait, several significant findings were made (Obzhirov, 1993). These findings indicate the presence of anomalous methane fields with concentrations up to 45 nmol/L, which is consistent with the potential for oil and gas resources.

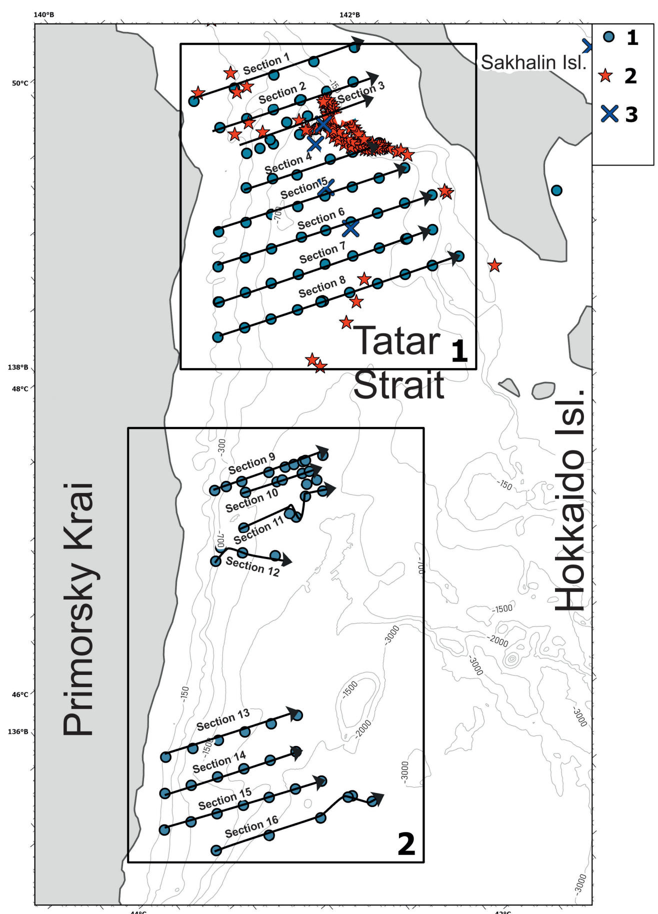

Figure 1. Sampling scheme for gas-geochemical studies. The study area is conditionally divided into two areas: 1 – gas hydrate-bearing, and 2 – non-gas hydrate. Within these areas, there are three types of sampling stations: 1 – bottom sediment sampling, 2 – gas seeps, and 3 – gas hydrates (Jin et al., 2013; Shoji et al., 2014; Minami et al., 2016).

During the international project SSGH Project II – The Sakhalin Slope Gas Hydrate Project II it was discovered that the upper portion of the sedimentary layer, primarily in the eastern region of the South Tatar Trough, exhibits a widespread occurrence of numerous gas vents. Typically, these gas vents and manifestations of gas hydrates are associated with gas seeps. These gas seeps are located at sea depths ranging from approximately 100 to 300 meters, with isolated instances detected up to depths of 600 meters (Figure 1).

In the area of these structures, studies have been conducted on the distribution of hydrocarbon gases, the carbon isotope composition of methane and ethane, and the concentrations of hydrogen and helium. These studies have revealed the predominance of catagenetic gases in gas hydrate accumulations (Shakirov et al., 2016).

Gas hydrates were discovered within the southwestern slope of Sakhalin Island in areas of various seismic anomalies, which were mapped using continuous seismic profiling (Jin et al., 2013; Shoji et al., 2014; Minami et al., 2016). For the first time, gas hydrates in the northern part of the Japan Sea (the Tatar Strait) were found on cruise 59 of the RV "Akademik M.A. Lavrentyev" in 2012 on the southwestern upper slope of Sakhalin Island. Subsequent studies conducted from 2013 to 2015 significantly expanded the area where gas hydrates are present.

Gas hydrates are present in the form of thin lenses, interlayers, and isometric inclusions within sedimentary rocks. They are located at depths of 1.6 to 2.4 kilometers below the seafloor. At the same time, acoustic seeps, indicating the release of gas from sedimentary layers, were recorded at a depth of approximately 300 meters. However, the potential for further discoveries is high, as there is a possibility to find gas hydrate accumulations at greater depths of 500 to 600 meters. Analysis of the geological structure in the area indicates that gas hydrates are closely associated with landslide and turbidity sedimentary layers common to the South Tatar sedimentary basin. These layers, formed by underwater landslides and turbidity flows, have increased porosity and permeability, creating favorable conditions for methane migration and accumulation.

The gas hydrate-bearing sediments in this area consist of layers up to one meter thick. These layers are mainly composed of thermogenic methane, with an average concentration of approximately –43‰ (Syrbu et al., 2022). A potential source of this thermogenic methane could be deep-lying gas- and coal-bearing strata, as well as gases from underlying sediments. Almost all sediment samples collected from the bottom of the research area were characterized by a high level of gas saturation. They also contained a significant number of carbonate nodules and showed signs of severe fracturing (Jin et al., 2013; Shoji et al., 2014; Minami et al., 2016). These features indicate a complex geological history for the area, as well as ongoing geochemical processes occurring within the sedimentary layers.

Based on these observations, it can be inferred that the South Tatar sedimentary basin may serve as an active source for methane emissions from its bottom sediments.

Materials and methods

Gas-geochemical studies were conducted in the northern part of the Japan Sea, the southern part of the Tatar Strait, and the northern slope of Primorsky Krai, as shown in Figure 1. The materials used for the study include data from the RV "Akademik Oparin" cruise 54 (OP54) in September–October 2017 and the RV "Akademik M.A. Lavrentyev" cruise 81 (LV81) in May 2018.

Geographically, the work area is divided into two regions: gas hydrate (Area 1), which includes 8 sections located from the continental shelf to the shelf of Sakhalin Island, and the non-gas hydrate (Area 2), which consists of 8 additional sections on the continental slope of Primorsky Krai near the northern part of the Central Basin of the Japan Sea (Figure 1).

A total of 519 sediment samples were collected for gas-geochemical analysis. The samples were taken from sediment cores to measure gas content after 20–30 cm. Gas was extracted from bottom sediments using the "HeadSpace" equilibrium method and analyzed using a Chromatek-Cristall-9000 gas chromatograph with a sensitivity of 10^{-5}%. The ionization and thermal conductivity sensors were provided by the Chromatek Design Bureau CJSC, located in Yoshkar-Ola, Russia.

Bottom sediments were collected using a hydrostatic sampling device with a diameter of 138 millimeters and a length of 575 centimeters. Two-piece (cut lengthwise into two pieces and tightly sealed) plastic tubes with a smaller diameter of 125 millimeters were inserted into the device to quickly extract sediment from the sample. After lifting the device onto the vessel, the plastic tube containing the sediment was transferred to a laboratory, where it was cut into two pieces for further processing according to a standard procedure – photographing, description of the sediment, and selection for various types of analysis.

The sediments were collected using 10 mL syringes with cut-off spouts into 68 mL vials filled with saturated NaCl solution, with the addition of a preservative (0.5 mL chlorhexidine bigluconate 0.05%). When measuring methane, helium was used as the gas phase. It was injected into the flasks using a Tedlar Bag Dual Valve (USA) gas bag with two valves. Helium was injected through one valve of the bag equipped with a needle, while the aqueous solution was extracted through another valve with a syringe. The samples were vigorously shaken for at least 4 hours using an LS 110 mixer (Russia) before the gas was introduced into the chromatograph.

The concentrations of dissolved methane in seawater were determined using equilibrium phase analysis, based on solubility constants, according to the method described by (Yamamoto et al., 1976), as modified by (Wiessenburg, Guinasso, 1979).

Research on Microbial Biodiversity

The upper layer of restored bottom sediments from the northern part of the Japan Sea was used to investigate the biodiversity of microorganisms in the bottom sediments associated with gas emissions. This layer was collected during cruises OP54 on the RV "Akademik Oparin" in 2017 and LV81 on the RV "Akademik M.A. Lavrentyev" in 2018. Samples from 23 different stations were analyzed (Figure 2), each one has varying level of gas hydrate potential and presence of underwater methane vents.

Samples for microbiological analysis were collected using sterile plastic syringes with cutting spouts. The samples were taken from a depth of 5 to 30 centimeters and stored at –30 degrees Celsius prior to analysis.

The studied bottom sediments, from the surface of the seabed to a depth of 30 centimeters, were characterized by mainly siltstone and pelite differences, with a restored, homogeneous structure that ranged in color from dark olive to gray-green, often with inclusions of hydrotroilite (dark layers of iron sulfides in the form of a hydrogel).

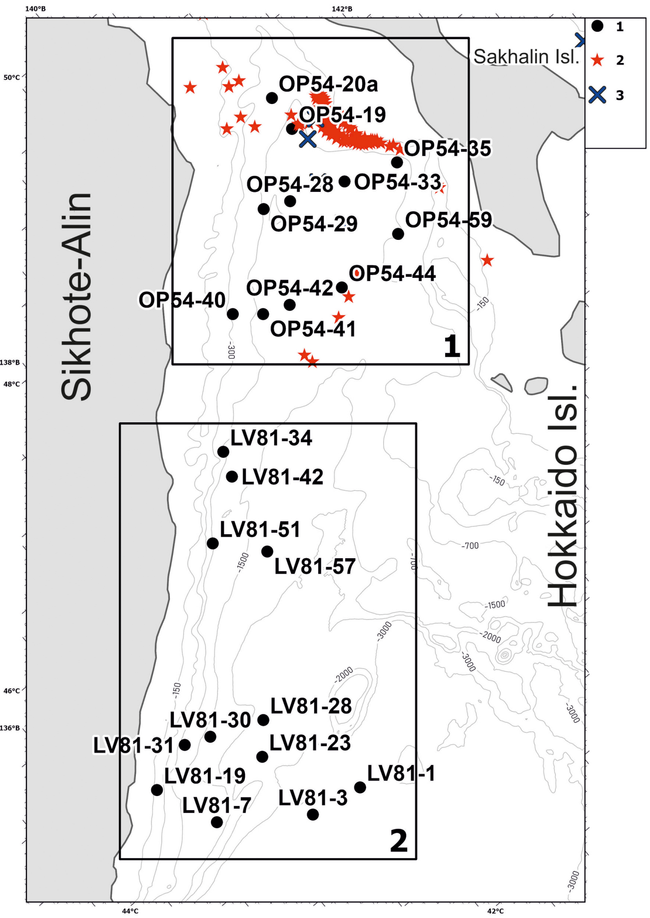

Figure 2. Sampling chart for the study of microbial biodiversity. The study area is conditionally divided into two areas: 1 – gas hydrate-bearing and 2 – non-gas hydrate. Within these areas, there are three types of sampling stations: 1) bottom sediment sampling stations for the study of microbial biodiversity, 2) gas seeps, 3) gas hydrates (Jin et al., 2013; Shoji et al., 2014; Minami et al., 2016).

Isolation and identification of bacteria

We used marine mineral medium and Voroshilova-Dianova medium, both modified with a salt content of 35% to create storage cultures of hydrocarbon-oxidizing microorganisms. Then we added 2% sterile ESPOgrade oil to the media as a carbon source.

A collection of 55 strains of pure cultures capable of oxidizing hydrocarbons was isolated from cumulative cultures using the agar plate method. The morphology of the pure cultures was examined using an Axiostar PLUS light microscope (Carl Zeiss, Germany) equipped with phase contrast. The physiological and biochemical characteristics of the isolated strains were studied using commonly accepted methods (Matsui, 2003; Labinskaya et al., 2005; Netrusov et al., 2006). Biomass of the cultures was collected on a medium for marine microorganisms. The isolation of chromosomal DNA of pure cultures was carried out by the modified Marmur method (Marmur, 1961). The identification was carried out by sequencing a highly conservative 16S rDNA site using the Sanger method using the BigDye v3.1 kit on the ABI 3130xl genetic analyzer (ThermoFisher Scientific, USA) at the company "Syntol" LLC (Moscow). Universal bacterial primers 11F (5' – AGTTTGATCATGGCTCAG – 3') and 1100R (5' – GGGTTGCGCTCGTTG – 3') were used for sequencing (Seki et al., 2015). The obtained nucleotide sequences from the 16S rRNA gene (800 bp in length) were aligned using Bioedit Version 7.0.4 and ClustalW, and the presence of chimeras using the Pintal 1.1 program (Ashelford et al., 2005). Phylogenetic analysis was performed by searching for homologous sequences in the International Data Bank (GenBank) using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The obtained sequences were identified up to individual operational taxonomic units (OTU) at a similarity level of 98%.

The nucleotide sequences of fragments from the 16S rRNA gene in individual bacterial strains have been deposited in the GenBank database with the following accession numbers: MZ540778, MZ573198, MZ573186, MZ573209, MZ540874, MZ573210, MZ540892, MZ573212, MZ573229, MZ573232, MZ540913, MZ573237, MZ540941, MZ543973, MZ543976,

MZ544024, MZ595665, MZ544190, MZ544252, MZ544365, MZ573241, MZ595666, MZ573242, MZ595782, MZ573409, MZ573761, MZ562688, MZ562702, MZ562704, MZ596003, MZ562706, MZ574136, MZ577107, MZ577115, MZ577118, MZ577121, MZ577132, MZ577136, MZ577169, MZ569490, MZ569847, MZ569674, MZ573202, MZ569847, MZ569721, MZ569678, MZ573187, MZ569315, MZ577172, MZ577174, MZ577178, MZ577183, MT758443, MZ577213, MT758444.

The databases (Baldanova et al., 2022; Ponomareva et al., 2022) present the information on the bacteria we identified, as well as their physiological and biochemical properties and the degree of hydrocarbon degradation and biodegradation by strains.

Statistical processing of the results was conducted using Microsoft Excel 2010 and ArcGIS 10.4, with the Geostatistical Analyst module and the R programming language in RStudio 3.3.1 IDE (https://cran.r-project.org/bin/windows/base/old/3.1.1/). Pearson correlations were calculated among taxonomic groups to assess relationships between substrate utilization abilities and methane content in sediments, with a significance level set at $p < 0.05$.

Results

Gas-geochemical composition of bottom sediments

Figure 1 shows the location of Areas 1 and 2 where gas-geochemical studies of bottom sediments were conducted in the central and southern parts of the Tatar Strait and on the continental slope of the Japan Sea correspondently. Several interesting sediment cores were collected in the southern Tatar Strait, where sediments consist of sandy deposits and siltstone with a dark, almost black color, enriched with hydrotroilite. There is a faint odor of hydrogen sulfide or no odor at all. It is likely that hydrogen sulfide has been used to form hydrotroilite through diagenesis. Pelitic particles increase in concentration from siltstone towards the bottom.

Watersaturated sandy-silty sediment with water content is up to 30–40% dominates in Area 2 on the continental slope of the Japan Sea (Figure 1). These sediments turn into denser silty-pelitic deposits without organic matter interlayers with increasing of the layer depth. However, sections 10, 11 and 16 on the continental slope have significantly different sediment composition. These sections are dominated by pelitic deposits that are low in water content (up to 10–15%), with layers of organic matter within the pelites.

Signs of gas saturation were recorded in many sediment cores within the gas seeps in Area 1. The maximum concentrations of methane were up to 2.2×10^6 nmol/dm³ (OP54-37). Generally, methane concentrations are evenly distributed in both areas, with a gradual increase as the depth of the sediment layer increases (Figure 3). Methane was detected in all bottom sediment samples, ranging from 10 nmol/dm³ to 4.3×10^6 nmol/dm³. The absolute methane concentration maximum of 4.3×10^6 nmol/dm³ was recorded at a bottom sediment depth of 1.5 meters in the area 45.9°N, 138.2°E. The methane content in lower sediment layers was 3–4 orders of magnitude higher than in upper sediment layers (Figure 3). Studies in this area also found positive correlations between methane, ethane, and propane concentrations (Shakirov et al., 2023).

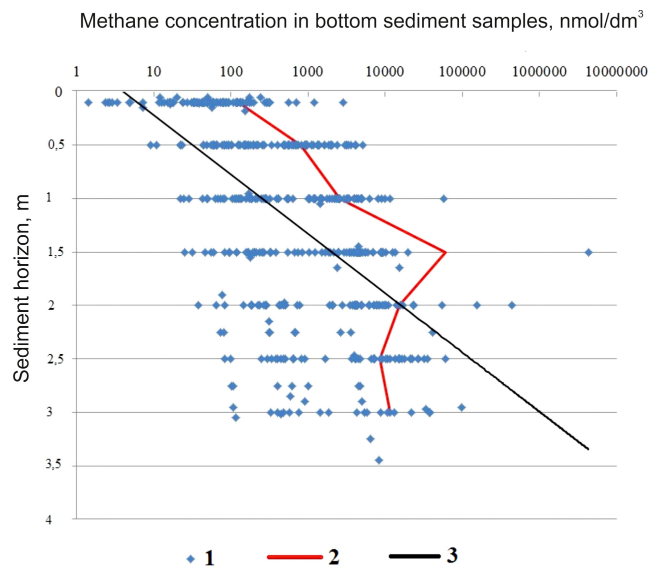

Figure 3. Methane concentration in bottom sediment samples at different horizons from cruise 54 on RV "Akademik Oparin". The scale is logarithmic. 1 – methane concentrations in sediments; 2 – the arithmetic mean of methane concentrations in sediment across horizons; 3 – the logarithmic trend line.

In the research area, there are two areas with abnormally high methane concentrations in the sediment. The first area is the gas hydrate region, located in the southern part of the Tatar Strait, where there are numerous gas seeps and evidence of gas hydrates. The second area is located on the continental slope of the Japan Sea and does not contain gas hydrates. The methane concentration in these two areas increases with depth (Figure 4a-d).

In the upper layer of sediment at a depth of 50 centimeters, several areas with increased concentrations of methane can be seen (with a maximum methane concentration dissolved in the sediment of 4150 nmol/dm³, with an average value for this layer of 802 nmol/dm³) (Figure 4b). These areas with increased methane concentration are found in the immediate vicinity of tectonically active fault zones.

At the 1.5-meter horizon, an area with elevated (more than 10^5 nmol/dm³) and abnormal (with an absolute maximum of 4.3×10^6 nmol/dm³) methane concentrations stands out off the coast of Primorsky Krai (Figure 4b).

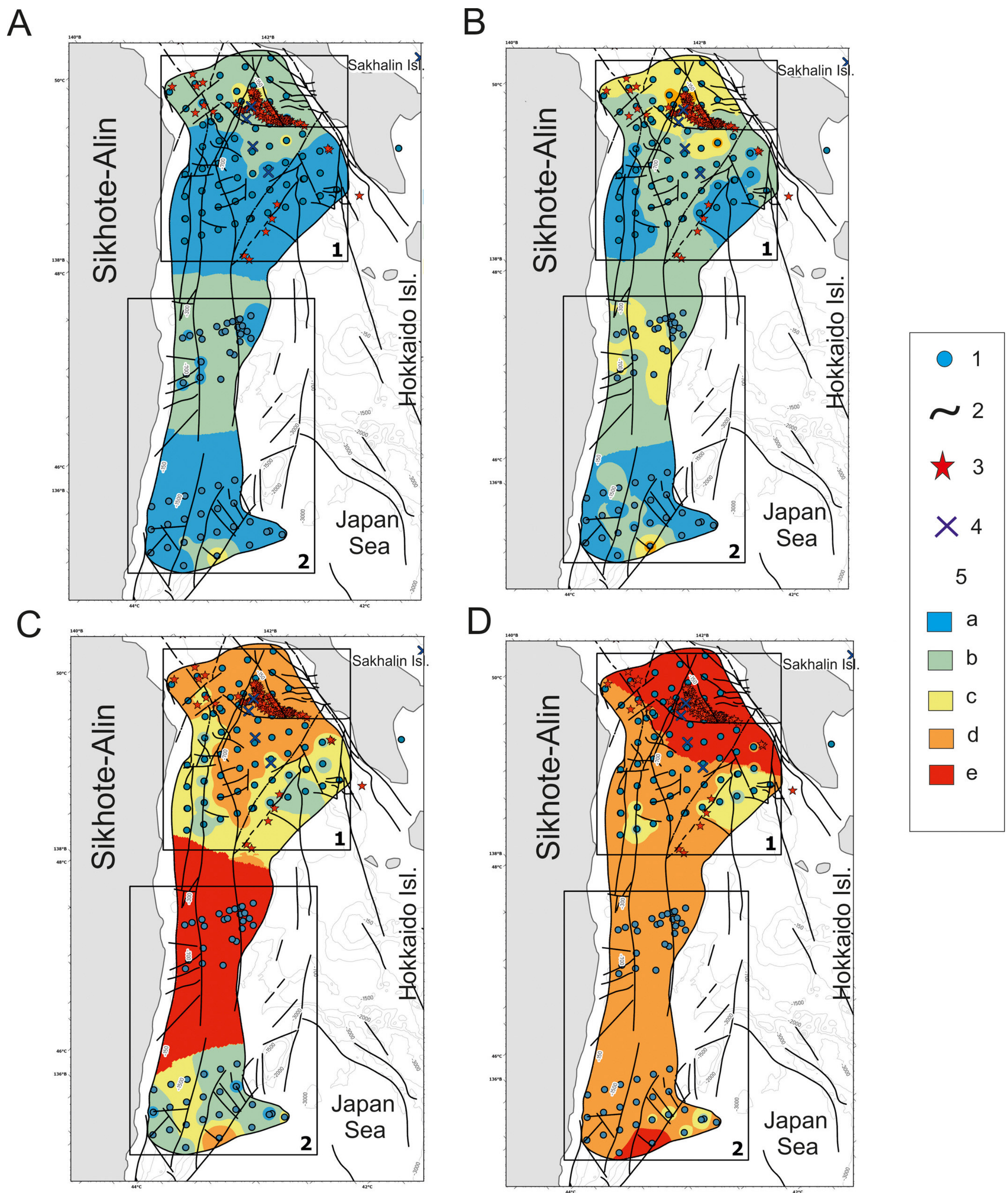

Figure 4. Diagram of the main faults (Shaposhnikov et al., 1994, 1995; Kharakhinov, 2010; Zharov et al., 2004) and distribution of methane concentration over sediment horizons with indication of bottom sediment sampling stations in cruise 54 on the RV "Akademik Oparin": A – distribution of methane concentration on the 0.3 m horizon; B – distribution of methane concentration at the horizon of 0.5 m; C – distribution of methane concentration at the 1.5 m horizon; D – distribution of methane concentration at 2.5 m. The areas are divided into 1– gas hydrate-bearing area; 2 – non-gas hydrate area. Symbols: 1 – bottom sediment sampling stations on cruise 54 of RV "Akademic Oparin"; 2 – main faults; 3 – gas seeps; 4 – gas hydrates (Jin et al., 2013; Shoji et al., 2014; Minami et al., 2016); 5 – methane concentrations: a – up to 250 nmol/dm³; b – 250–1000 nmol/dm³; c – 1000–2500 nmol/dm³; d – 2500–15000 nmol/dm³; e – more than 15000 nmol/dm³

The average methane concentration values for the 1.5 meter layer are 60.6 nmol/dm³.

Carbonate nodules were found in a pelagic sediment of presumably Pleistocene-Holocene age within the zone of abnormally high methane concentrations at depths of 748 meters and 932 meters, respectively, the depth ranged from 1.3 to 1.5 meters below the bottom surface (Yakimov et al., 2023). This zone also belongs to the continental slope of the Japan Sea. As part of cruise 81 on the RV "Akademik M.A. Lavrentyev", an ikaite was found in the same area for the first time in the zone of the methane anomaly associated with the fault zone (Shakirov et al., 2020).

Figure 4d shows that the all the resaerch area faces a high methane concentration in the sediment level of 250 cm. The average value of the methane concentration for the entire studied area is 8679 nmol/dm³. The maximum values of methane anomalies are 36×10³ nmol/dm³ for the Tatar Strait and 60×10³ nmol/dm³ in the northern part of the Central Basin.

Elevated methane concentrations were observed in all sediment layers at stations in the Krasnogorsk uplift area, at the intersection of tectonic faults in the northern part of the South Tatar sedimentary basin (Area 1 with gas hydrates), and in the north of the Terney trough on the continental slope (Area 2 with no detected gas hydrates).

The model for the South Tatar Strait by (Zhemchugova, 2013) proclaims that the direction of hydrocarbon migration is upward along the section, so when seismotectonic activity occurs, it results in microcracks forming, which in turn leads to the acceleration of gas-fluid migration, particularly of methane being the lightest hydrocarbon. Figure 5 gives the distribution of methane in the sediment layer in the gas hydrate region.

An absolute maximum of methane concentration of more than 2×10⁶ nmol/dm³ was detected at the OP54-37 station, in the area of the Sakhalin Island slope, at the 2.5 meter sediment core horizon (Figure 5). This area has numerous gas seeps and gas hydrate manifestations in the sediment, with layers up to 1 meter thick. Gas hydrates in this area are formed by a mixture of thermogenic and biogenic methane, with an average carbon isotopic

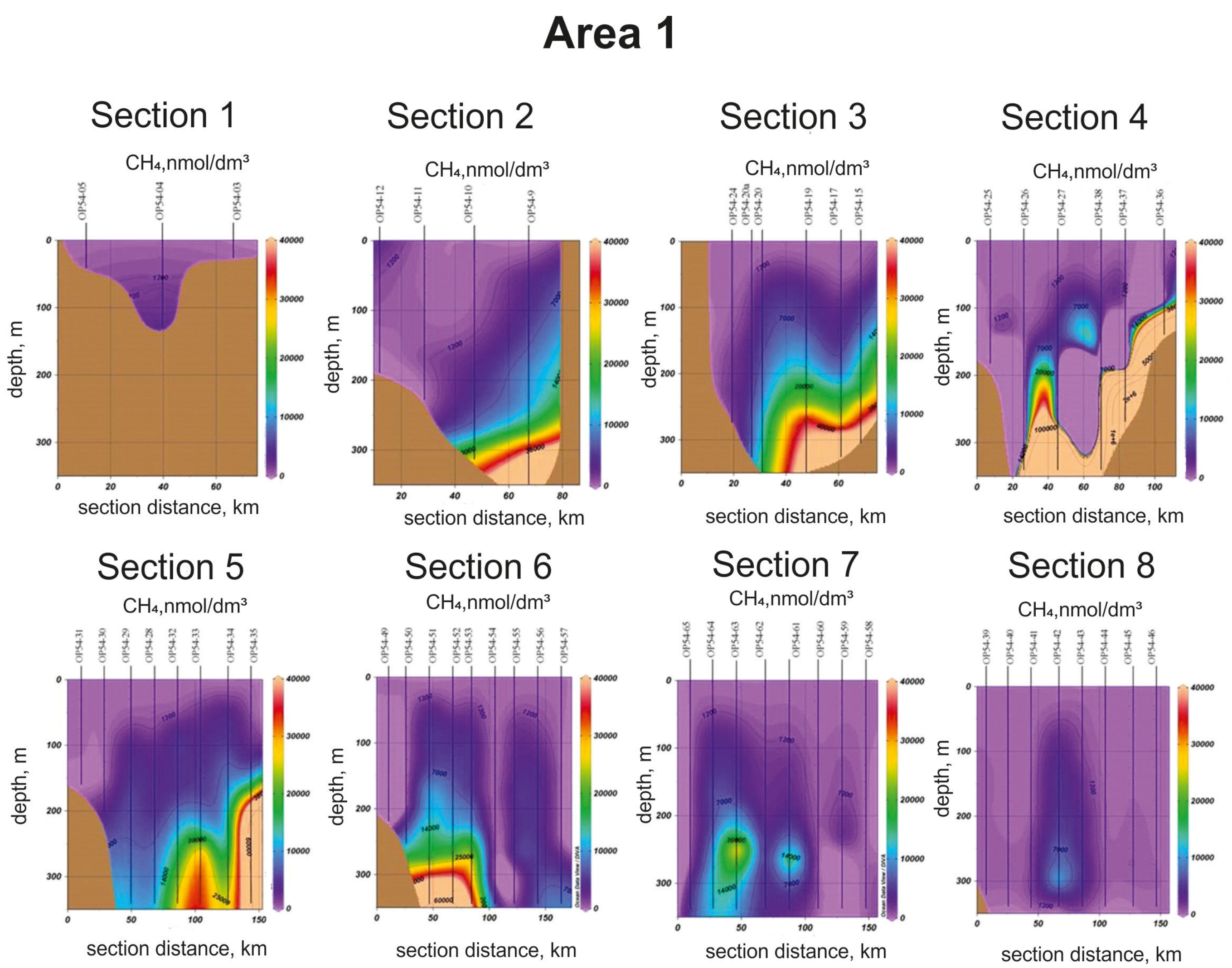

Figure 5. Distribution of methane concentrations in the sediment (nmol/dm³) in the gas hydrate Area 1, by section, showing the sampling stations from cruise 54 on RV "Akademik Oparin".

composition of 43‰ (Syrbu et al., 2022). The source of the thermogenic methane is likely to be gas- and coal-bearing strata, with a contribution from gases from underlying sediments (Shakirov et al., 2016). Generally, for Area 1, higher concentrations of methane are found in the lower layers of the sediment core (2–3 meters).

Figure 6 reflects the distribution of methane in the bottom sediment layer of the non-gas hydrate Area 2.

Study Area 2 has no gas hydrates detected. But is meets elevated methane concentrations found in the sediments at stations OP54-51, OP54-05 and OP54-07, with an absolute methane maximum of more than 2×10^6 nmol/dm^3 for a layer of 150 cm at station OP54-51 on the continental slope. A second area with methane concentrations of approximately 60×10^3 nmol/dm^3 was found at a depth of 2 to 3 m at section 16 near these stations OP54-05, OP54-07. In this area, carbonate nodules were discovered, which were later identified as pseudomorphic glendonites formed from ikaite (Yakimov et al., 2023). This suggests the presence of previously unknown extended zones of methane release along the continental slope of the Japan Sea, adjacent to Primorsky Krai, which are characterized by unique autogenic carbonate mineralization features.

The taxonomic diversity of hydrocarbon-oxidizing bacteria

A collection of 55 strains of pure cultures that can oxidize hydrocarbons was obtained from the recovered sediment layer at the bottom of the northern Japan Sea, both in gas hydrate and non-gas hydrate areas.

Based on the results of 16S rRNA sequencing, the strains of microorganisms from the bottom sediment were found to belong to the following phyla: Pseudomonadota (Gammaproteobacteria), Bacillota, and Actinomycetota.

The proportion of sequences that belonged to the phylum Pseudomonadota (Gammaproteobacteria) was 69%, with Bacillota accounting for 14.5% and Actinomycetota making up 16.5%. All of the studied strains were identified at the genus or species level.

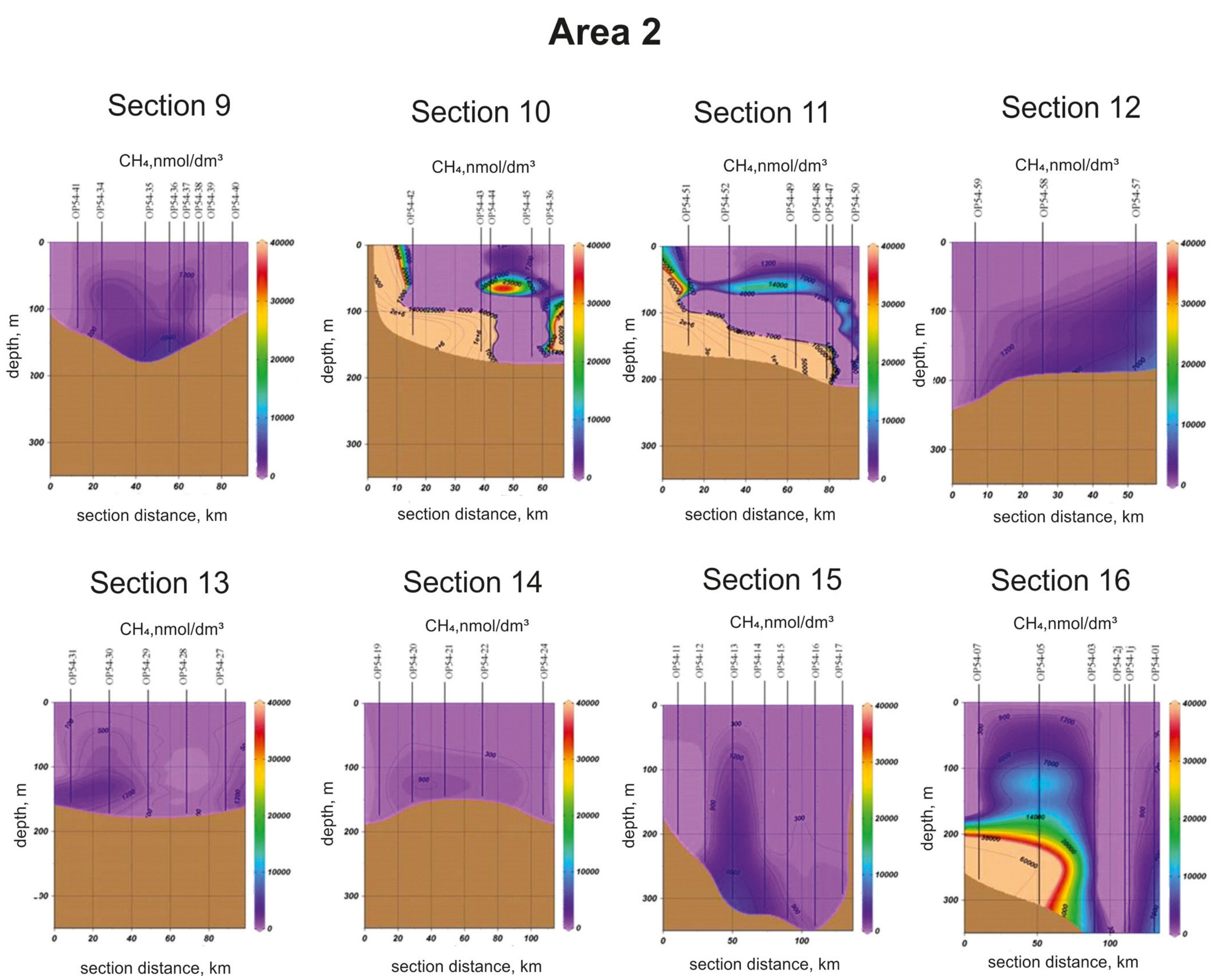

Figure 6. Distribution of methane concentrations in sediment (nmol/dm³) by sections in the non-gas hydrate Area 2, with indication of sampling stations from cruise 54 of RV "Akademik Oparin".

The results of comparing the nucleotide sequences obtained with the NCBI database using BLAST protocol indicate that the 16S rRNA sequences most similar to those obtained belong to microorganisms capable of biodegradation of hydrocarbons in water, sediment, soil, and other ecosystems.

Representatives of the genera *Pseudomonas* (14 strains), *Psychrobacter* (13), *Stenotrophomonas* (11), *Bacillus* (5), *Rhodococcus* (4) were dominant, while minor genera included *Micrococcus* (1), *Nesterenkonia* (1), *Brevibacillus* (1), *Promicromonospora* (1), *Peribacillus* (1), *Robertmurraya* (1), *Curtobacterium* (1), *Nocardioides* (1).

37 strains were assigned to 9 different genera for the area with gas hydrate deposits. Also about a half of the strains – 18 were assigned to 8 different genera for the area without gas hydrates.

Representatives of the genus *Pseudomonas* were more prevalent in the area where gas hydrates were got (31%). *Psychrobacter* was more prevalent in areas without gas hydrates (28%).

The strains classified as *Psychrobacter* and *Stenotrophomonas* in the gas hydrate area accounted for 22% and 19%, respectively. Bacteria of the genus *Bacillus* were got from both areas, with a proportion of 17% in the area without gas hydrates and 8% in the gas hydrate area. In the abnormal gas field area, the proportion of *Pseudomonas* bacteria was 17%, while the share of *Stenotrophomonas* was 22%. Species such as *Pseudomonas brenneri*, *Stenotrophomonas maltophilia*, *Psychrobacter nivimaris*, and *Psychrobacter glacincola* were found in both gas hydrate and non-gas hydrate study areas.

Representatives of the genera *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, and *Psychrobacter* were found in both study areas. The stations closest to the gas hydrate accumulation sites are OP54-19 and OP54-35, as shown in Figure 2.

Methane, ethylene, ethanol, and propane were all detected at the OP54-19 station. This station has an increased methane content of 1,000 ppm (39222 nmol/dm^3), with a clear increase towards the lower part of the core.

From the samples collected at this station, five bacterial isolates were got, which were subsequently assigned to the following species: *Peribacillus simplex* (MZ573186), *Nesterenkonia lutea* (MZ573232), *Stenotrophomonas maltophilia* (MZ573242) and *Stenotrophomonas sp*. (MZ573761), *Rhodococcus erythropolis* (MZ573202).

Station OP54-35. The total concentration of hydrocarbon gases at this station exceeds the values observed at other stations three times, with methane reaching a concentration of 1687 ppm. Propylene was also detected in the sediment along the entire core length. Six bacteria isolates of *Psychrobacter celer* (MZ573209), *Pseudomonas* sp. (MZ544024), *Nocardioides dokdonensis* (MZ573241), *Psychrobacter maritimus* (MZ577169), *Pseudomonas brenneri* (MZ569847), *Stenotrophomonas maltophilia* (MZ569721), were also got from samples collected at this station and cultured in pure form.

All strains belonging to the Actinomycetota phylum were unique to the gas hydrate area. At the same time, representatives of the Nocardiaceae and Nocardioidaceae families, including 1 strain of Nocardioides sp., were only found in the gas hydrate area.

Physiological and biochemical properties of hydrocarbon-oxidizing strains

As a result of our research, we found that microorganisms from non-gas hydrate area consumed a lower variety of substrates than microorganisms got from bottom sediments from gas hydrate area. The biggest difference was seen in the use of lactate and succinate, with strains. Lactate was absorbed by microorganisms isolated in the gas hydrate area absorbing lactate twice as quickly as succinate (Figure 7).

Discussion

When considering two Areas, namely gas hydrate-bearing and non-gas hydrate, there are at least two areas that can be identified as having high-intensity methane emission: the slope of Sakhalin Island, in the area where gas hydrate deposits are located, and the continental slope of Primorsky Krai, where carbonate mineralization occurs. The highest concentrations of methane are found in the range of 250–300 cm in pelitic sediments, which contain organic matter, and concentrations decrease moving northwest along the sedimentary section.

The lithological composition and water-physical properties of bottom sediments are one of the main factors of hydrocarbon accumulation (Starobinets et al., 1993; Abrams, 2017). Filtration-diffusion and migration processes are often complicated by the influence of deep fluid dynamics and are accompanied by changes in the lithological and gas-geochemical composition of bottom sediments with the formation of anomalous gas-geochemical fields in the latter (Gresov, Yatsuk, 2021).

According to one hypothesis, the widespread presence of representatives of the Pseudomonadota and Actinomycetota phyla in oil and gas deposits may be explained by their "adaptive" metabolism, which allows them to adjust to changes in environmental conditions that occur during sediment diagenesis and fluid flow circulation (Ciobanu et al., 2014).

The Pearson Correlation Coefficient was calculated by taxonomic groups to assess the relationship between the ability to utilize substrate sources and the methane content in sediments. A negative correlation was found

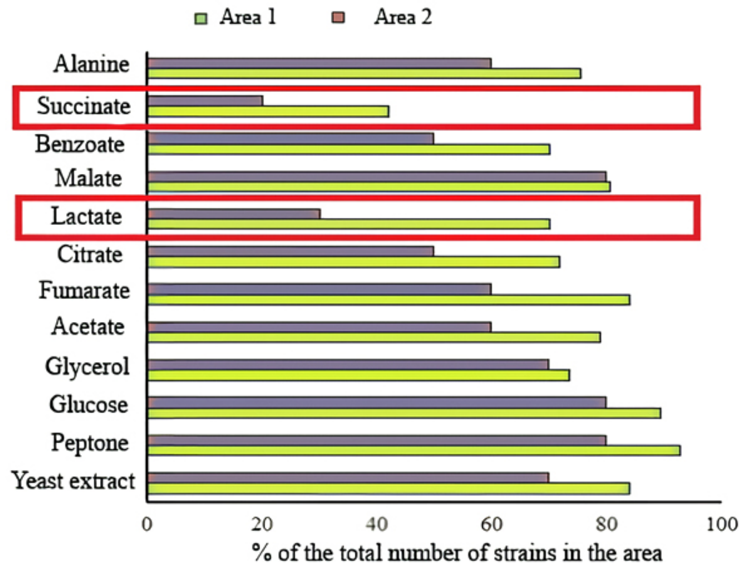

Figure 7. Substrate specificity of the studied hydrocarbon-oxidizing strains. Area 1 – gas hydrate area; Area 2 – non-gas hydrate area.

between the use of sugar strains as a source of carbon and energy with the values of methane in sediments. The correlation (R) values ranged from 0–0.2, at $p < 0.05$. Tween-esterase activity was detected in 5 strains from all got isolates. There was a positive correlation between the utilization of tween-80 with the maximum methane content (0.93), as well as tween-60 (the correlation value was 0.87), $p < 0.05$.

The use of amino acids by strains negatively correlates with the values of methane.

Figure 8 reflects a positive correlation between the ability to destroy carboxylic acids and the absence of gas hydrates. However, a positive correlation of lactate consumption with methane concentration was noted in the non-gas hydrate region. As shown earlier, the largest number of strains using lactate as a substrate were got from gas hydrate area.

Conclusions

The northern part of the Japan Sea is an area with some unique characteristics. Here, the shallowest gas hydrates have been discovered in the world's oceans. Thet were found at a depth of 322 meters and there is a possibility that they could be found even at shallower depths in the Tatar Strait. In the sedimentary layers of this region, gas hydrates lie close to the ocean floor and can be detected using direct methods at depths ranging from 5 to 6 m below the bottom surface.

The area of accumulation of gas hydrates is characterized by the presence of numerous gas seeps, indicating active gas-geochemical processes in the bottom environment. This area is part of the western gas and geochemical province of the Okhotsk Sea region. No gas hydrates have been found on the border between the southern part of the Tatar Strait and the Central Basin of the Japan Sea, nor on the continental slope. However, abnormal levels of methane concentration and promising structures for gas accumulation in the sediment column have been noted. It is possible that gas hydrates exist at great depths in sediment and do not impact the microbiome of the upper layer of bottom sediment.

Our experiments revealed that the microbiome of the gas hydrate area is characterized by a greater number of strains than the microbiome of the non-gas hydrate area. 37 strains classified in 9 genera were isolated in the area of gas hydrate deposits, and 18 strains classified in 8 genera in the area of absence of gas hydrates. Representatives of the genus *Pseudomonas* predominate in the area of gas hydrate detection, while *Psychrobacter* predominate in the non–gas hydrate area.

A greater number of bacterial isolates were isolated from samples of bottom sediments closest to the sites of gas hydrate accumulations at stations OP54-19 and OP54-35 than from samples from stations in the non-gas hydrate area. Representatives of the Nocardiaceae family of the Actinomycetota phylum were associated with the area where the gas hydrates were found.

A study of the physiological and biochemical properties of strains of hydrocarbon-oxidizing bacteria showed that isolates from the area of gas hydrate deposits had the ability to ferment a greater variety of carbohydrate substrates than cultures isolated from a non-gas hydrate area. When assessing the correlation of the physiological and biochemical properties of the studied bacteria with the methane content in the sediment samples, we found that the ability to digest sugars by strains did not correlate with the methane content; also, the presence of the enzyme esterase correlated weakly or not at all with the methane content. However, there is a correlation between the ability to destroy carboxylic acids and their derivatives and the

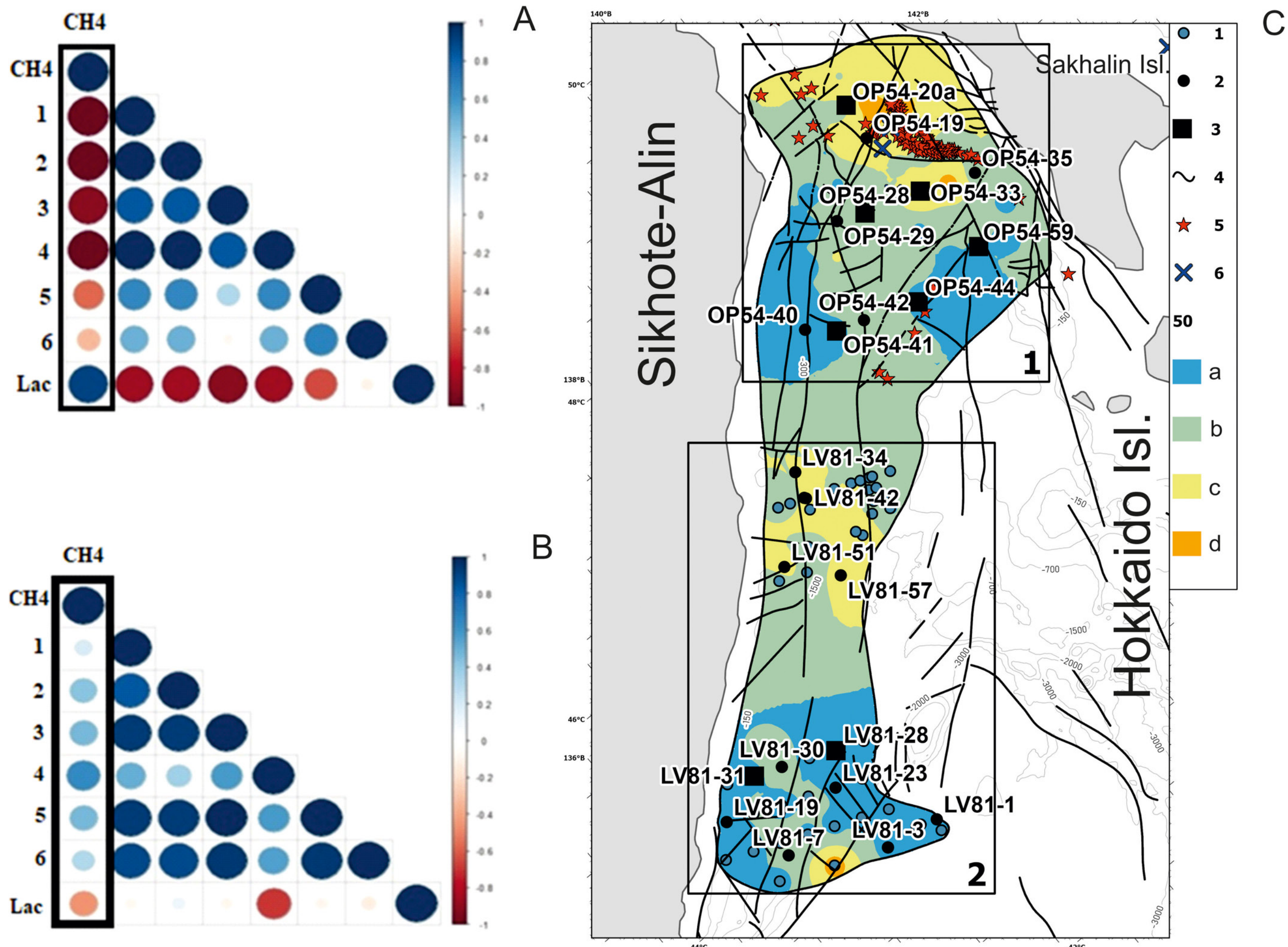

Figure 8. Correlation plots of the ability to utilize carboxylic acids with the methane content in bottom sediments: A – gas hydrate-bearing area; B – non–gas hydrate area. Symbols: 1 – palmitic acid, 2 – oxalic acid, 3 – succinic acid, 4 – citric acid, 5 – malonic acid, 6 – salicylic acid, 7 – Lac-lactic acid. B is a map of methane distribution: 1 – sampling stations; 2 – isolation stations for cultured bacteria; 3 – stations where the ability of strains to utilize lactate is noted; 4 – fractures; 5 – gas seeps; 6 – gas hydrates; "50" – concentrations of methane at a horizon of 50 cm in nmol/dm^3: a – up to 250; b – up to 1000; c – up to 2500; d – up to 15000.

absence of gas hydrates. The largest number of strains using lactate as a substrate and unable to utilize palmitate, succinate, oxalate and citrate were isolated from the non-gas hydrate region.

Thus, our research revealed that the microbial community in the gas hydrate and non-gas hydrate areas is unique and has specific taxonomic and physiological characteristics associated with the presence or absence of shallow-water gas hydrates.

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Исследование связи микробиологических факторов с распределением аномальных полей метана и наличием залежей газовых гидратов на примере двух акваторий северной части Японского моря

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Рассмотрены возможности использования микроорганизмов в качестве биоиндикаторов метановых экосистем в местах залегания газовых гидратов. Проведено исследование биоразнообразия микроорганизмов, и определены физиологические и биохимические свойства бактериальных штаммов, способных к окислению углеводородов, выделенных из донных отложений северной части Японского моря для двух районов: с обнаруженными газовыми гидратами (район 1) и без присутствия газовых гидратов (район 2). Комплексные газогеохимические, геологические и микробиологические исследования проведены на акватории северной части Японского моря – южной части Татарского пролива, и северного склона Приморского края. Использованы материалы морских экспедиций: НИС «Академик Опарин» № 54 (OP54, сентябрь – октябрь 2017 г.) и НИС «Академик М.А. Лаврентьев» № 81 (LV81, май 2018 г.). Используя методы культивирования, выявлено, что представители семейства Nocardiaceae типа Actinomycetota привязаны к местам обнаружения газовых гидратов. Показано, что бактерии, выделенные из района с обнаруженными газовыми гидратами, проявляли способность ферментировать более широкий спектр углеводных субстратов по сравнению с культурами, полученными из негазогидратного района. Отмечена положительная корреляция между способностью к деструкции карбоновых кислот и отсутствием газогидратов.

Ключевые слова: метан, газовые гидраты, деструкция углеводородов, донные отложения, физиолого-биохимические свойства, Японское море, Татарский пролив

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