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INVESTIGATION OF MICROBIAL COMMUNITIES OF SUBSURFACE SEDIMENT LAYERS OF THE SHELF AND DEEP-SEA ZONES OF THE BLACK SEA USING OXFORD NANOPORE SEQUENCING

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Bacterial communities occurring in subsurface bottom sediment layers from the deep basin and shelf zone of the Black Sea were analyzed. The aim was to show that these sediments, formed under different bottom-water conditions (reduced environment characterized by hydrogen sulfide at a depth of 2,030 m *vs.* oxidized environment with oxygenated seawater at 49 m), serve as habitats for distinct microbial communities. DNA was extracted from various sediment layers; the procedure was followed by PCR amplification and Oxford Nanopore sequencing. The analysis revealed a total of 28 classified bacterial phylotypes: 24 recorded in both zones, and 1 being specific to the deep sediment of the hydrogen sulfide zone. In total, 335 representatives were identified from 171 families. Among those, 37 families were found to be specific to the shelf sediment of the oxygen zone of the Black Sea, while 46 families were exclusive to the deep sediment of the hydrogen sulfide zone. Moreover, 88 families were identified as overlapping between two zones. These findings indicate that the two zones of the Black Sea indeed harbor unique microbial communities, with greater diversity observed on the shelf. The results also highlight the potential for research on roles of these communities in biogeochemical cycles.

Keywords: Black Sea, core of bottom sediment, DNA concentration, Oxford Nanopore sequencing, prokaryote microbiome, subsurface bottom sediment, shelf oxygen-containing zone, deep-sea hydrogen sulfide zone

The Black Sea is the world’s largest naturally anoxic water body. The presence of a hydrogen sulfide zone in the Black Sea mostly determines specificity of its biodiversity, since deep waters (on average, deeper than 100–150 m) do not contain oxygen, but contain hydrogen sulfide. In the reduced hydrogen sulfide zone, there are no conditions for development of oxybionts.

The vertical structure of the Black Sea is shown in Fig. 1. As known, about 7.5 thousand years ago, due to a rise in the level of the World Ocean, the Bosporus Strait was formed, which connected the Black Sea (practically a freshwater body at that time) with the World Ocean *via* seas of the Mediterranean basin. Salinization of the Black Sea began due to the influx of salty Mediterranean waters, which led to density stratification of the sea, impeded vertical water exchange, and limited oxygen influx into deep waters. Due to these processes, 5–7 thousand years ago, a hydrogen sulfide zone began to form in the Black Sea: in its deep basin, in near-bottom layers. The boundary of the hydrogen sulfide zone gradually rose, and about 3 thousand years ago, it stabilized approximately within the modern boundaries of the hydrogen sulfide reduction zone [Kondratev, Vidnichuk, 2018, 2020] (Fig. 1).

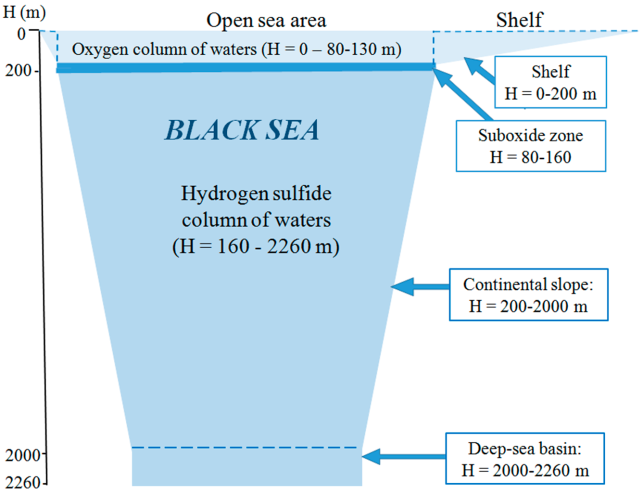

Fig. 1. Scheme of some characteristics of the Black Sea vertical structure (H is the depth)

Рис. 1. Схема некоторых характеристик вертикальной структуры Чёрного моря (H — глубина)

Spots for core sampling within the present study were chosen, taking into account the time when bottom sediment layers were formed, namely, the period of recent global hydrochemical and hydrological changes in the Black Sea. One core was sampled in the deep-sea hydrogen sulfide zone, and another one, on the shelf, in the oxygen zone (see Fig. 1).

Microorganisms are known to be less dependent on the oxygen content in the environment. Many types of bacteria can live in oxygen-free environments. The Black Sea bottom sediments in the deep-sea hydrogen sulfide zone have been studied to determine the presence of both anaerobic and aerobic organisms. In the Black Sea sediments, more than 80% of the total sequences retrieved belonged to *Bacteroidota* and *Pseudomonadota* (41 and 42%, respectively), and this slightly differs from the global composition of coastal sediments by a higher fraction of *Bacteroidota* [Zinger et al., 2011]. At the class level, the most abundant bacterial groups were *Deltaproteobacteria* and *Flavobacteriia*. These increased in sequence abundance toward anoxic conditions, especially a group of sulfate-reducing bacteria of the families *Desulfobacteraceae* and *Desulfobulbaceae* (*Deltaproteobacteria*) [Jessen et al., 2017].

Previous investigations on microbiomes of bottom sediments in the hydrogen sulfide zone revealed the presence of viable spores and oxybionts. Genera of germinated unicellular algae, fungi, and bacteria were identified in sediment surface layers (0–1.5 and 1.5–5.0 cm). Thus, it was shown that bottom sediments in the hydrogen sulfide zone serve as a bank of viable spores of oxybionts [Zaitsev et al., 2008].

Earlier, the microbiome of a wider range of deep-sea bottom sediments was analyzed from the point of the environmental effect on its biological diversity. Changes in the microbial composition in various geological periods were studied in connection with both history of the sea formation [More et al., 2019] and role of microorganisms in the bottom sediment composition. Also, the participation of microorganisms in the biogeochemical cycles of individual substances was analyzed [Coolen, 2011; Coolen et al., 2009, 2013]. It was found that the decomposition of organic matter occurred with the participation of representatives of the classes *Deltaproteobacteria* and *Dehalococcoidia* [Suominen et al., 2021].

The ratio between *Flavobacteriia*, *Gammaproteobacteria*, and *Deltaproteobacteria*, involved in the decomposition of particulate organic matter, changed with reducing oxygenation, and it took longer for microbial community of the hypoxic zone to decompose similar amounts of deposited matter [Jessen et al., 2017]. *Desulfobacteraceae* were also abundant in sulfate-poor, methanogenic areas of the Black Sea sediment [Leloup et al., 2007]. *Crenarchaeota* and the bacterial candidate division JS-1, as well as the classes *Anaerolineae* and *Caldilineae* of the phylum *Chloroflexota*, were highly abundant in anoxic sediments [Schippers et al., 2012].

Bacterial community structure definitely differed in hypoxic and oxic zones. Conditions on the shelf are favorable for aerobic forms of microorganisms. The Black Sea shelf features a noticeable presence of methane. On the shelf, methane concentrations drop during a relatively short ascent from the seafloor to the sea surface. Previously published data clearly demonstrate that *Bacteria* mediate methane oxidation in oxygenated water [Durisch-Kaiser et al., 2005]. An increased abundance of *Archaea* in the oxygen zone was a surprise, since no members of this domain were known that could contribute to methane conversion rate under oxygen conditions [Durisch-Kaiser et al., 2005].

Microbial communities of the oxygen zone resembled the ones of standard marine (*e. g.*, Mediterranean) photic zones, with dominance of *Cyanobacteria* (*Synechococcus*, with conspicuously absent *Prochlorococcus*) and photoheterotrophs [Cabello-Yeves et al., 2021].

At the same time, microbes developed an adaptive metabolism, which included physiological adaptation to oxic-anoxic growth [Shah et al., 2019]. One of the most abundant microbes found in these zones belonged to *Candidatus* ‘Marinimicrobia,’ which thrived both in oxic and anoxic waters [Bertagnolli et al., 2017; Hawley et al., 2017]. In general, the investigation on microbial communities in bottom sediments, including subsurface deep layers, of various seas is of high scientific and practical relevance for both revealing microbial diversity and understanding the role of microbes in functioning of marine ecosystems [Badmadashiev et al., 2023; Bertagnolli et al., 2017; Leloup et al., 2007; Merkel et al., 2017; Roussel et al., 2008; Stroeva et al., 2023; *etc.*].

The aim of this work was:

1) to investigate the abundance and structure of microbial communities in different subsurface layers of deep and shallow bottom sediments in the Black Sea;
2) to study the microbial diversity in the hydrogen sulfide zone of the bathyal *vs.* shallow-depth bottom sediments in the oxygen zone on the shelf, thereby expanding our understanding of the Black Sea ecosystem as a meromictic one.

MATERIAL AND METHODS

Research areas and material. Samples were taken as whole cores with geological tubes for sampling of bottom sediments (TG-2) in the hydrogen sulfide zone in the western deep basin of the Black Sea and on the shelf during the 110th cruise of the RV “Professor Vodyanitsky” in October 2019 (Fig. 2). The deep-sea station, st. 1 (43°19.6632′ N, 32°10.1171′ E), was at a depth of 2,030 m. The shelf station, st. 2 (44°24.7851′ N, 33°41.7321′ E), was at a depth of 49 m (see Fig. 2). The Black Sea is known as an internal semi-enclosed continental sea due to its geographical location. This is a meromictic reservoir, as its deep waters contain hydrogen sulfide. A 200-cm core was taken at st. 1, and another core, a 140-cm one, was taken at st. 2 (Fig. 2).

Immediately after sampling at the stations, both bottom sediment cores (11 cm in diameter) were split into sub-samples 5 cm in length. Sample aliquots were obtained aseptically from cores with sterile syringes. In this study, DNA concentrations at st. 1 were analyzed in the samples from subsurface sediment layers of 20–25, 45–50, 70–75, 95–100, and 170–175 cm. DNA concentrations at st. 2, on the shelf, were studied in the samples of sediment layers from depths of 20–25, 40–45, 65–70, and 125–130 cm.

Polymerase chain reactions (PCR) in layers of 20–25, 40–45, and 70–75 cm at st. 1 and in layers of 20–25, 45–50, and 65–70 cm at st. 2 were performed. Designations used in sampling and analysis are explained in Table 1.

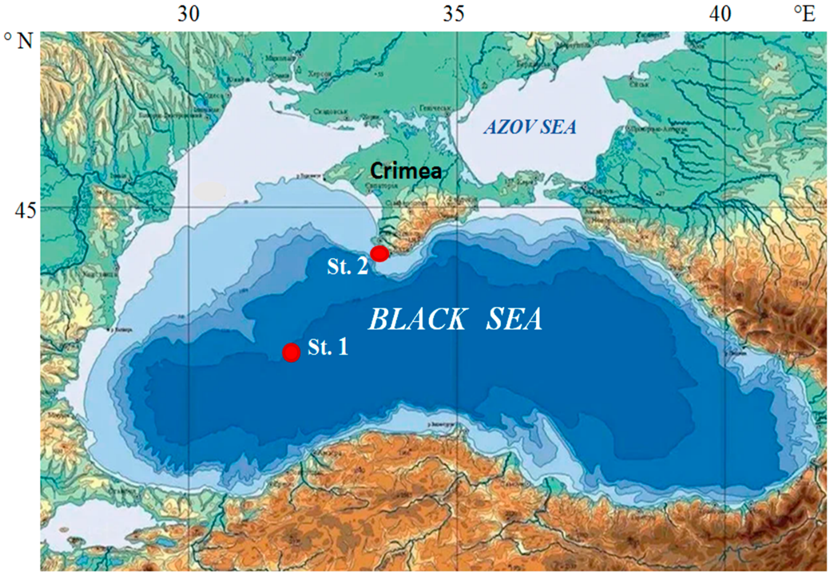

Fig. 2. Sampling sites in the Black Sea: st. 1 in deep-sea western basin and st. 2 on the shelf near the Crimean Peninsula

Рис. 2. Места́ отбора проб в Чёрном море — ст. 1 в глубоководной западной котловине и ст. 2 на шельфе у Крымского полуострова

Table 1. Description of analyzed samples of subsurface layers of the Black Sea bottom sediments (YBP, years before present)

Таблица 1. Описание исследованных образцов слоёв подповерхностных донных отложений Чёрного моря (YBP — лет тому назад)

Characteristic	Deep-sea basin, st. 1, H = 2,030 m						Shelf area, st. 2, H = 49 m				
Depth of the sediment layer, cm	0–5	20–25	45–50	70–75	95–100	170–175	0–5	20–25	45–50	65–70	125–130
Period of formation of the sediment layer (YBP)	467–800	1738–2025	3219–3521	4632–4910	5963–6225	9558–9796	18–37	112–137	224–280	447–488	946–988
Date of formation of the middle of the sediment layer (calendar year)	1385	125	1336 BC	2752 BC	4075 BC	8125 BC	1991	1895	1757	1559	1052

Seven samples were used for sequencing (see Table 2). One sample (bar1) served as the control (sham DNA extraction, negative PCR, and Nanopore reagents) despite its absence on a stained gel. The other six samples were as follows: bar2, bar3, and bar4 (st. 2, depth of 49 m; 20–25, 40–45, and 65–70 cm, respectively), and bar5, bar6, and bar7 (st. 1, depth of 2,030 m; 20–25, 40–45, and 70–75 cm). A total of 89,950 reads were performed; 11% of the reads represented the most abundant operational taxonomic units (hereinafter OTUs) which were taken into account.

The samples were stored intact, anaerobically, at −80 °C before being aseptically sub-sampled in a laminar airflow cabinet with a sterile stainless-steel corer (2 cm in diameter) and analyzed in a laboratory.

Dating of bottom sediments. All samples from the sediment core and all obtained layers were tested for radioactivity, in particular for the content of man-made cesium-137. The measurements were performed on a gamma-spectrometric complex with a semiconductor detector (RADEK, Russia).

The layers were dated according to literature data based on results of radioisotope dating using carbon-14 [Coolen et al., 2009] with corrections for the total carbon content in layers of bottom sediments and with correction for cesium-137 at the sediment surface, 0–7 cm.

DNA extraction and quantification. DNA was extracted from sediment samples (~ 500 mg) by one of the best methods for deep-sea sediments: the FastDNA Spin Kit for Soil with modifications [Webster et al., 2003] while leaving PCR inhibitors bound to DNA and following the recommended protocol (MP Biomedicals, LLC, the USA). The technique was found to be reliable and consistent in terms of DNA yield and the ability to generate PCR products. Therefore, in the given study, we used FastDNA Spin Kit for Soil as recommended by the manufacturer. Briefly, a sample was soaked with sodium phosphate buffer and MT buffer solutes, and homogenized with Q125 Sonicator (Qsonica, LLC, the USA). Then, a sample was centrifuged to get rid of the pellet debris. The supernatant was transferred to a clean 2-mL microcentrifuge tube, where PBS (phosphate-buffered saline, a buffer solution, pH ~ 7.4) was added and mixed 10 times. A sample was re-centrifuged to precipitate the pellet. Then, supernatant was transferred to a 15-mL tube, and a binding matrix solution was added. A tube was rotated for 2 min and placed in a rack for 3 min. Subsequently, 500 µL of supernatant was discarded, and remaining 600 µL of DNA solution was transferred to a SPIN filter tube. Then, 500 µL of SEWS-M solution was added, and a tube was rotated for 1 min. After SPIN filter air-drying for 5 min at room temperature, 50–100 µL of DES elution solution was added. After centrifugation, DNA in the catch tube finally was ready to use for DNA analyses. It was shown, that DNA contained PCR/DNA polymerase inhibitors. Therefore, for subsequent PCR runs, DNA samples were diluted 10–20 times with molecular biology grade water.

To visualize DNA, the agarose (1% weight/volume) gel electrophoresis was used with DNA stained with 0.5 $\mu g \cdot mL^{-1}$ ethidium bromide. DNA concentrations were measured involving NanoDrop Microvolume Spectrophotometer (Thermo Fisher Scientific, the USA).

PCR amplification. Microbial community composition may vary significantly because of differences in sequencing method, including choice of variable region of 16S rRNA gene. We used the v3–v4 region that has proven itself in the study of extremophiles [Merkel et al., 2017] and marine ecosystems [Ghate et al., 2021]. The extracted DNA was amplified by PCR using v3–v4 region-specific bacterial 16S rRNA gene-targeted degenerate primers Merk-341F and Merk-805R [Merkel et al., 2017] for 37 cycles involving FastStart polymerase (Roche, the USA) at annealing temperature of +53 °C. The obtained sharp signals (about 485 base pairs, bp) were Nanopore-sequenced.

Oxford Nanopore sequencing. The MinION device equipped with Flow Cell R9.4 was used for Nanopore sequencing. The sequencing run was operated by MinKNOW software (Oxford Nanopore, the UK). The sequencing was performed with libraries prepared for 16S rRNA gene v3–v4 region amplicons (about 485 bp). The corresponding kits were implemented: to repair amplicon ends (NEBNext Ultra II End Repair/dA-tailing Module reagents E7546), ligate barcodes (Native Barcoding Expansion 1–12 EXP-NBD104), and then sequencing adapters (Adapter Mix II AMII). All these and further steps (loading the libraries in a flow cell, *etc.*) followed instructions provided by Oxford Nanopore Technologies. The sequencing was run for 72 h. Obtained Fast5 files were basecalled under the “fast basecalling” option with trimming barcodes using MinKNOW software. The resulting FastQ files were classified with GenBank entries [Benson et al., 2013] and EPI2ME software (Oxford Nanopore) [EPI2ME, 2008–2024].

Bioinformatics analysis. At the first step, data quality was monitored using FASTQC software [Babraham Bioinformatics, 2019], and then data cleaning of low-quality reads was performed applying Trimmomatic [2021]. To confirm the success of the previous step, the quality of the reads was re-checked using FASTQC. At the second step, the reads were classified with the software Kraken 2 [DerrickWood/kraken 2, 2023; Wood et al., 2019].

Two different approaches were implemented to classify the reads. The first one involved the FastQ 16S program of the software package EPI2ME [Oxford Nanopore Technology, 2008–2024]. This approach allows classifying the reads using daily updated GenBank entries. Upon classification, the similarity threshold was settled at 88%. Most reads remained unclassified, likely because of their low similarity to GenBank classified entries, and they were excluded. The second approach involved WIPM program in the same software package. It uses a specialized database (that of Oxford Nanopore Technologies) containing only classified entries, which is far from the updated GenBank. The two approaches produced consistent results presented as a list of OTUs.

Accession number. The sequences of libraries prepared for 16S rRNA gene v3–v4 region amplicons obtained in this study were deposited in the NCBI database under submission ID: BioProject accession PRJNA1137415 (GenBank).

RESULTS AND DISCUSSION

Dating of layers in geological tube. The gamma-spectrometry data obtained showed as follows: in some surface layers, there was no cesium-137 in samples of bottom sediment core. This indicates the absence of a surface layer, 0–7 cm, since cesium-137 should be contained in this layer of bottom sediments as anthropogenic radionuclide both of Chernobyl and global origin. This is due to peculiarities of sediment core sampling with a geological tube, as evidenced by studies of [Coolen et al., 2009]. In the article, the figures illustrate the depth of the layers of bottom sediments, as they were in the geological tube. The sedimentation rate (hereinafter SR) for bottom sediments deeper than 8 cm was 0.15–0.21 mm·year^{-1} depending on the depth of the layer of bottom sediments, and the value decreased with sediment depth [Coolen et al., 2009, 2013; More et al., 2019].

The results on layer dating for bottom sediment sampled at st. 1 are shown in Table 1. As known, in the Holocene, which began 11.7 thousand years ago, the mean SR on the Black Sea shelf was about 1.2 mm·year^{-1} for naturally compacted sediment [Yesin, Kos'yan, 2006]. In the Anthropocene, which began in 1950, the SR on the shelf increased and amounted to 2.3–5.5 mm·year^{-1} [Egorov et al., 2013; Tereshchenko et al., 2018; Yesin, Kos'yan, 2006]. This is probably due to the intensification of anthropogenic activity, in particular on the coast. Economic development and urbanization resulted in an increase in land erosion and coast abrasion, and also in a rise in solid runoff from rivers and pipe drainage systems. The above data supported the dating of the sediment core sampled at st. 2 (Table 1).

DNA concentrations in subsurface bottom sediment. In Table 2, the results of the analysis are presented: DNA concentrations at st. 1 in samples from subsurface bottom sediment layers of 20–25, 45–50, 70–75, 95–100, and 170–175 cm.

Table 2. DNA concentrations in subsurface bottom sediment

Таблица 2. Концентрация ДНК в подповерхностных донных отложениях

Characteristic	Deep-sea basin, st. 1, H = 2,030 m						Shelf area, st. 2, H = 49 m				
Depth of the sediment layer, cm	0–5	20–25	45–50	70–75	95–100	170–175	0–5	20–25	45–50	65–70	125–130
DNA concentration, ng·μL^{-1}	–	78.0	*	*	62.0	44.0	–	65.0	*	55.0	43.0
Sample	–	bar5	bar6	bar7	–	–	–	bar2	bar3	bar4	–
Number of identified bacterial OTUs	–	129	190	110	–	–	–	162	140	147	–

Note: *, concentration was measured approximately by ethidium bromide staining after gel electrophoresis; –, no data.

Примечание: астериск (*) — концентрацию измеряли приблизительно с помощью окрашивания бромидом этидия после гель-электрофореза; прочерк (–) — данные отсутствуют.

For the control, DNA was extracted from local soil and pure water. This control was used in DNA quantification trial, while another control (negative PCR) was used in Nanopore sequencing.

Comparative analysis of microbial communities from different samples. Microbial communities of sediments sampled at st. 1 and st. 2 and the control sample were analyzed. Data in Table S1 (available as a supplementary file at https://marine-biology.ru/mbj/article/view/527) provide evidence for the fact that the biome of microorganisms from the shelf zone (bar2–bar4) features higher diversity at the genus level than that in the deep-sea zone (bar5–bar7). Notably, our research did not claim to compile a complete list of genera in the studied bottom sediment layers. At the same time, there is a significant part of the genera that were found in the bottom sediment samples from both stations, as well as in the control. Twenty-two OTUs for control and bottom sediment from both stations, in addition to 5 OTUs only from st. 1, and 6 OTUs only from st. 2 were common and were excluded. However, a considerable number of bacterial genera in microbiome of subsurface sediment layers in the hydrogen sulfide and oxygen zones differed within layers and also differed from the control. A total of 217 OTUs were recorded in three sediment layers from st. 1. Out of those, 135 OTUs were common to sediments from st. 2, and 87 OTUs were characteristic of the deep-sea bottom sediments only. For st. 2, 226 OTUs were identified. Out of those, 135 OTUs were common to bottom sediments from st. 1, and 91 OTUs were noted on the shelf at st. 2 alone (see Table S1 at https://marine-biology.ru/mbj/article/view/527).

A comparative analysis was also performed for the microbial genus composition only for samples from bottom sediments from various depths at both stations. The distribution showed that the control sample overlapped strongly in microbial composition with the sediment samples. Therefore, the microbial genera of the control samples were excluded from the list of genera considered when analyzing the composition of the microbial community in different layers of bottom sediments from the shelf and deep-sea zone. This is important in order not to obtain a falsely inflated positive relationship between the genus composition of samples from different sediment layers. A total of 49 common microbial OTUs was revealed for all three investigated sediment layers from st. 1, while 53 OTUs were recorded in three sediment layers from st. 2. In each sediment layer at st. 1, the number of original OTUs varied within 38–65, and at st. 2, 32–52.

Taxonomic affiliations of the identified bacteria. In the samples studied, 28 bacterial phyla were identified (see Table S2 at https://marine-biology.ru/mbj/article/view/527). Out of those, 24 phyla were present in both microbiota zones, and 1 phylum, *Deinococcota*, occurred in the hydrogen sulfide zone only, but 3 phyla were found in the shelf zone only. The main contribution to the microbiome of bottom sediments was made by seven phyla (listed in descending order): *Chloroflexota* – *Pseudomonadota* – *Bacillota* – *Actinomycetota* – *Thermodesulfobacteriota* – *Bacteroidota* – *Cyanobacteriota* (Fig. 3). The bar plot shows the most abundant OTUs.

The phylum *Chloroflexota* strongly dominated the data set comprising about 27% of all the reads. *Chloroflexota*, *Pseudomonadota*, and *Bacillota* amounted to 56% of the total reads in two zones. *Actinomycetota* was the fourth dominant phylum in the sediment samples, with 10.3% of the reads.

A complete list of the identified genera of bacteria and their count in bottom sediments layers are given in Table S1 (see https://marine-biology.ru/mbj/article/view/527). In total, representatives of 335 genera of bacteria were identified in the studied microbial communities in all six examined layers of bottom sediments.

The taxonomic structure of the identified OTUs of the domain *Bacteria* from sediment samples is presented in Fig. S1a–f (see a supplementary file at https://marine-biology.ru/mbj/article/view/527). At the family level, *Pseudomonadota*, *Bacillota*, and *Actinomycetota* made up 53% (see Fig. 3). The bar plot showed the most abundant OTUs.

The distribution of families across the two zones was compared (see Table S2 at https://marine-biology.ru/mbj/article/view/527). *Pseudomonadota*, *Bacillota*, and *Actinomycetota* made up 65% of the bacterial genera.

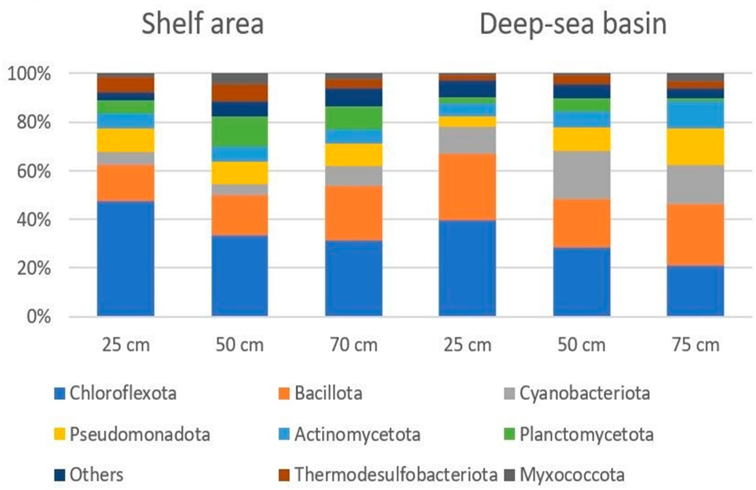

Fig. 3. Relative abundance of bacterial community in subsurface layers of the Black Sea bottom sediments at the phylum level

Рис. 3. Относительное обилие бактериального сообщества в подповерхностных слоях донных осадков Чёрного моря на уровне филума

There were some variations in the microbial composition among phyla in sediments. In both zones, the proportion of *Chloroflexota* dropped with depth. *Bacillota* and *Cyanobacteriota* were the dominant phyla in the deep-sea zone (24 and 16%, respectively), while *Chloroflexota* (27%) colonized the shelf sediments in large numbers (Fig. 3). The presence of *Cyanobacteriota* in the deep-sea zone seems unexpected. *Cyanobacteria* are capable of both oxygenic and sulfide-driven anoxygenic photosynthesis [Klatt et al., 2015], and this may explain their adaptation to fluctuating far-red light and sulfide concentrations.

In this work, sequences could be classified at the genus level, and distribution of families could be reconstructed. *Anaerolineaceae* was the predominant family in both zones (30% on the shelf and 24% in the deep-sea zone), significantly ahead of *Thermoanaerobacterales Family III* (2.4 and 7.0%, respectively), *Leptolyngbyaceae* (3.1 and 5.5%), *Anaerohalosphaeraceae* (5.6 and 1.5%), *Clostridiaceae* (3.1 and 4.2%), and *Azonexaceae* (0.7 and 3.2%) (Fig. 4). We registered 47 families in the deep-sea zone only and 55 families on the shelf only. As for the class level, *Syntrophorhabdia*, *Terriglobia*, *Epsilonproteobacteria*, *Cytophagia*, *Desulfarculia*, *Fibrobacteria*, and *Chthonomonadia* were found in the deep-sea zone alone.

In total, 191 genera were identified (see Table S1 at https://marine-biology.ru/mbj/article/view/527). Distribution of the most abundant 15 genera representing 54% of the reads in both zones is given in Table S3 (available as a supplementary file at https://marine-biology.ru/mbj/article/view/527).

This study also revealed presence of *Archaea* in both zones. Data are not submitted before confirmation involving the set of *Archaea* specific primers.

As shown in earlier investigations, the sediment mass accumulation rate, as well as the SR on the Black Sea shelf, is much higher than that in the deep-sea zone [Tereshchenko, Parkhomenko, 2021]. This is due to the fact that no more than 10% of lithogenic suspended matter reaches the deep-sea zone: it is mostly deposited on the shelf. Moreover, oxidation of suspended organic matter occurs in the upper oxygen zone of waters. Only about 5% of suspended organic matter reaches the hydrogen sulfide zone [Tereshchenko, Parkhomenko, 2021]. Consequently, the biogeographic conditions of the Black Sea, including its hydrological and hydrochemical characteristics, determine the fact that the SR values on the shelf are several times higher than in the deep sea. This results in the formation of a discrepancy in the age of layers at the same depth in sediments both on the shelf and in the deep-sea hydrogen sulfide zone (Table 1).

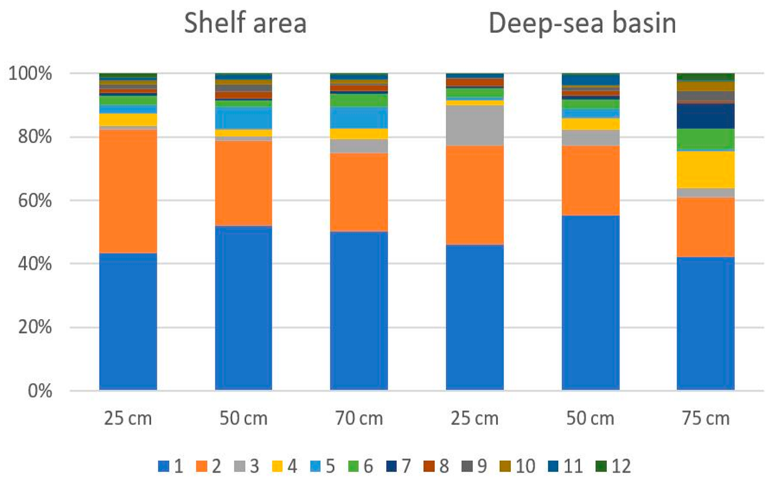

Fig. 4. Relative abundance of bacterial community members in the subsurface layers of the Black Sea bottom sediments at the family level: 1, the total contribution of 207 families; 2, *Anaerolineaceae*; 3, *Thermoanaerobacterales Family III*; 4, *Leptolyngbyaceae*; 5, *Anaerohalosphaeraceae*; 6, *Clostridiaceae*; 7, *Azonexaceae*; 8, *Caldilineacea*; 9, *Streptomycetaceae*; 10, *Kofleriaceae*; 11, *Dehalococcoidaceae*; 12, *Desulfocapsaceae*

Рис. 4. Относительное обилие членов бактериального сообщества в подповерхностных слоях донных осадков Чёрного моря на уровне семейства: 1 — общий вклад 207 семейств; 2 — *Anaerolineaceae*; 3 — *Thermoanaerobacterales Family III*; 4 — *Leptolyngbyaceae*; 5 — *Anaerohalosphaeraceae*; 6 — *Clostridiaceae*; 7 — *Azonexaceae*; 8 — *Caldilineacea*; 9 — *Streptomycetaceae*; 10 — *Kofleriaceae*; 11 — *Dehalococcoidaceae*; 12 — *Desulfocapsaceae*

To study the genus composition in bottom sediments, we carried out sampling that covered layers from the deep-sea zone, from a sediment depth of 70–75 cm and more, since, according to dating, these sediments were already formed under conditions of hydrogen sulfide water column.

The results obtained show that DNA concentration in subsurface bottom sediment of the Black Sea (40–70 cm) decreases with depth. We applied analytical methods of molecular biology to sequences, involving special software for the final analysis of extracted nucleic acid and identification of microbial taxa present, and received new data on the microbiome diversity in bottom sediments of subsurface layers on the shelf and in the deep-sea hydrogen sulfide zone. Our results show as follows: there is a sufficiently large percentage of the common genera in bottom sediments from the oxygen and hydrogen sulfide zones, but also there are significant differences in the genus composition of biomes of the two zones. This is probably due to both the different hydrochemical composition of waters during the formation of bottom sediments in these two zones and the specificity in conditions for the existence of microorganisms in deep layers of sediments. Moreover, a certain role is definitely played by the ability of microorganisms to carry out vital activities in more extreme and specific conditions compared to macrobiota of the Black Sea, the range of which is limited primarily to oxygen-containing habitats.

The analysis of taxonomic composition showed the presence of 28 prokaryotic phyla, with *Chloroflexota* dominating. *Anaerolineaceae* was the predominant family in marine anoxic sediments, the same as in [Schippers et al., 2012]. *Anaerolineaceae* is functionally linked to amino acid metabolism and biosynthesis of other secondary metabolites.

The metabolic abilities of *Chloroflexota* representatives, encompassing aerobic and anaerobic conditions, thermophilic adaptivity, oxygen-free photosynthesis, and the use of toxic compounds as electron acceptors, emphasize the stability of this phylum and its ecological significance. These diverse metabolic strategies, driven by the interaction of temperature, variation in oxygen concentration, and energy metabolism, illustrate the adaptations that allowed *Chloroflexota* to inhabit a wide range of ecological niches. Apparently, a high proportion of the class *Anaerolineae* is explained by the role of its representatives in the decomposition of complex organic compounds, which makes a noticeable contribution to the carbon cycle, including the anaerobic environment. *Anaerolineaceae* was the predominant family in marine sediments, functionally linked to amino acid metabolism and biosynthesis of other secondary metabolites. The most abundant in our study *Thermomarinilinea lacunofontalis* belonging to the class *Anaerolineae* is a thermophilic, obligatory anaerobic, and heterotrophic marine bacterium; it was isolated earlier in a Japanese shallow hydrothermal system [Nunoura et al., 2013].

Conclusion. In this study, new data were obtained on the bacterial community composition in the Black Sea sediment ecosystems in the oxygen zone on the shelf and hydrogen sulfide zone in deep-sea area, and additionally along a gradient of depth in the bottom sediment core. The phylum *Chloroflexota* dominated in both zones, with 27% in the marine ecosystem. Interestingly, 5% of families made up 51% of the total abundance.

This is the first report on the Oxford Nanopore sequencing studies of the bacterial community of bottom sediments where ecosystems of the shelf and deep-sea zone were compared. Further research is required to understand the bacterial community composition of the Black Sea ecosystem and to monitor occurring changes. These data lay the foundation for the analysis of microbiomes of the subsurface deep bottom sediment layers of the sea as a meromictic reservoir.

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ИССЛЕДОВАНИЕ МИКРОБНЫХ СООБЩЕСТВ В ПОДПОВЕРХНОСТНЫХ СЛОЯХ ДОННЫХ ОТЛОЖЕНИЙ ШЕЛЬФОВОЙ И ГЛУБОКОВОДНОЙ ЗОНЫ ЧЁРНОГО МОРЯ С ИСПОЛЬЗОВАНИЕМ СЕКВЕНИРОВАНИЯ OXFORD NANOPORE

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Проведён анализ бактериальных сообществ, присутствующих в подповерхностных слоях донных осадков глубоководной котловины и шельфовой зоны Чёрного моря. Целью исследования было продемонстрировать, что эти отложения, сформированные в разных условиях придонных вод (восстановительных — из-за наличия сероводорода в глубоководной части моря на глубине 2030 м, окислительных — в кислородсодержащих шельфовых морских водах на глубине 49 м), служат средой обитания для различных микробных сообществ. ДНК извлечена из разных слоёв осадков, затем амплифицирована с помощью ПЦР. Далее проведено секвенирование методом Oxford Nanopore. Анализ выявил в общей сложности 28 классифицированных бактериальных филотипов, из которых 24 присутствуют в обеих зонах, а 1 специфичен для глубоководной сероводородной зоны осадков. Впервые установлены 335 представителей из 171 семейства. Специфичными для шельфовых осадков кислородной зоны Чёрного моря оказались 37 семейств, а только в подповерхностных отложениях сероводородной глубоководной зоны обнаружены 46 семейств. Кроме того, 88 семейств встречались в обеих зонах. Результаты свидетельствуют о то́м, что две зоны Чёрного моря действительно содержат разные микробные сообщества с более разнообразной жизнью на шельфе, и указывают на потенциал будущих исследований роли этих сообществ в биогеохимических циклах.

Ключевые слова: Чёрное море, керн донных отложений, концентрация ДНК, секвенирование методом Oxford Nanopore, микробиом прокариотов, подповерхностные донные отложения, шельфовая кислородсодержащая зона, сероводородная глубоководная зона