УДК 579.6+ 578

O.E. Bobrova[1], J.B. Kristoffersen[2], V.O. Ivanytsia[1]

[1]Odesa National Mechnykov University, 2, Dvoryanska str., 65082, Odesa, Ukraine,
e-mail: o.bobrova@onu.edu.ua
[2]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research,
Gournes 71500, 71003 Heraklion, Greece

METAGENOME 16S RRNA GENE ANALYSIS OF THE BLACK SEA MICROBIAL DIVERSITY IN THE REGION OF THE ZMIINIY ISLAND

The aim *of the study was to determine the marine microbial biodiversity of the Zmiiniy island coastal seawater with the help of metagenomic analysis.* ***Methods****. The dual-indexing primers were used to amplify v4 region of the 16S rRNA gene. Sequencing was performed on Illumina MiSeq platform. Nucleotide sequences were analyzed by SILVAngs and QIIME pipelines.* ***Results.*** *As the result of metagenomics 16S rRNA gene analysis there were detected about 3200 Operational Taxonomic Units. The main revealed phyla among domain Bacteria were: Proteobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria, Verrucomicrobiota, Planctomycetes, Tenericutes, Fusobacteria, Firmicutes and candidate divisions of uncultivated prokaryotic representatives: SR1, BD1-5, BHI80-139, OP3, OD1, WS3, NPL-UPA2, SHA-109, SM2F11and TM6. As a result of taxonomy analysis lots of bacterial families and genera were identified. The most abundant genera among studied samples were: Marivita, Loktanella, Paracoccus, Pseudoalteromonas, Vibrio, Coraliomargarita, Acholeplasma, Pseudomonas, Phaeobacter, Neptunomonas, Allivibrio, Oceaniserpentilla, Oceanospirillum, Robiginitalea, Acinetobacter, Haliea.* ***Conclusion.*** *The metagenomic 16S rRNA gene analysis allowed evaluating of rich taxonomic prokaryotes diversity in the Zmiiniy island area of the Black Sea surface water. Lots of them were either poorly investigated or uncultivable.*

K e y w o r d s : the Black Sea, the Zmiiniy island, seawater, the 16S rRNA gene analysis, taxonomic diversity, metagenomics, marine microbial diversity, candidate divisions, new generation sequencing technologies.

Microorganisms play an essential role in marine ecosystems [3]. Different global metagenomics investigations held in open Ocean and coastal waters [5, 14, 20] helped to estimate prokaryotic microorganisms distribution in space [19, 21], their taxonomic diversity [17] and role in biogeochemical processes [10]. Based on it there were observed dominant taxa distribution of microbial communities in water habitats: Proteobacteria, Actinobacteria, Flavobacteria, Bacteroidetes [11].

The Zmiiniy island is situated on the shelf in north-western part of the Black Sea at approximate distance of 37 km from the Kiliya Danube estuary. The island's coastal waters marine ecosystem is highly influenced by the Danube river flow which brings nutrients and allochtonic microorganisms. That leads to forming of the unique microbiota composition in this area of the sea.

In the last half of XX century, microbiological investigations gave first understanding about taxonomic composition of the Black Sea coastal and Deepwater regions living microorganisms [2, 3]. Due to the military activity on the island the studies of the Black Sea biological diversity in this area were not held till 2002. The following demilitarization studies made by Odessa National University microbiologists were fragmentary and allowed to determine different heterotrophic bacteria, to investigate separate groups of microorganisms and the influence of the environment on it [1, 4].

The investigations made with the help of classical isolation and pure cultures analysis methods do not allow to evaluate marine microbiota taxonomic composition. It's considered that only 1% of existing prokaryotes are capable of cultivation on nutrient media [12]. It is suggested that there are at least 38 known taxonomic groups of uncultivated prokaryotic representatives, referred to as candidate divisions or candidate phyla [13].

For deeper understanding of the processes, occurring in marine ecosystem, it is important to know more about biological diversity of prokaryotic populations that inhabit these waters. Therefore the aim of this research is to determine the microbial diversity of the Zmiiniy island coastal waters with the help of the metagenomic 16S RNA gene analysis by total DNA extraction and pyrosequencing with following bioinformatics analysis.

Materials and methods

Sampling of marine water for the analysis of microbial diversity took place in July 2014 in the region of the Zmiiniy island at the depth of 1 meter from the surface in three sterile glass bottles in a volume750 ml each. Three replicate samples marked as Zm1, Zm2, Zm3 were collected at the same location with coordinates 45.257426, 30.203378 (Fig. 1). The samples were stored at – 4 °C till further processing in the laboratory. Water was filtered through 0.22 μm membrane filters (Sartorius) for separation of microorganisms.

Isolation of the nucleic acids from microorganisms collected by filtration was performed with the Power Water DNA isolation kit (MO BIO Laboratories). The DNA extraction procedure was performed as described by the manufacturer's instructions. The size of extracted DNA was evaluated by electrophoresis on a 0.8% agarose gel.

The primer design followed Kozich et al. [15], who modified the single-index method by Caporaso et al. [7] to a more efficient dual index approach. The Caporaso et al. primers are used in the Earth Microbiome project. Each PCR primer (Table 1) consists of the appropriate Illumina adapter, an 8-nt index sequence, a 10-nt pad sequence, a 2-nt linker, and the 16S pDNA V4 variable region specific primer pair

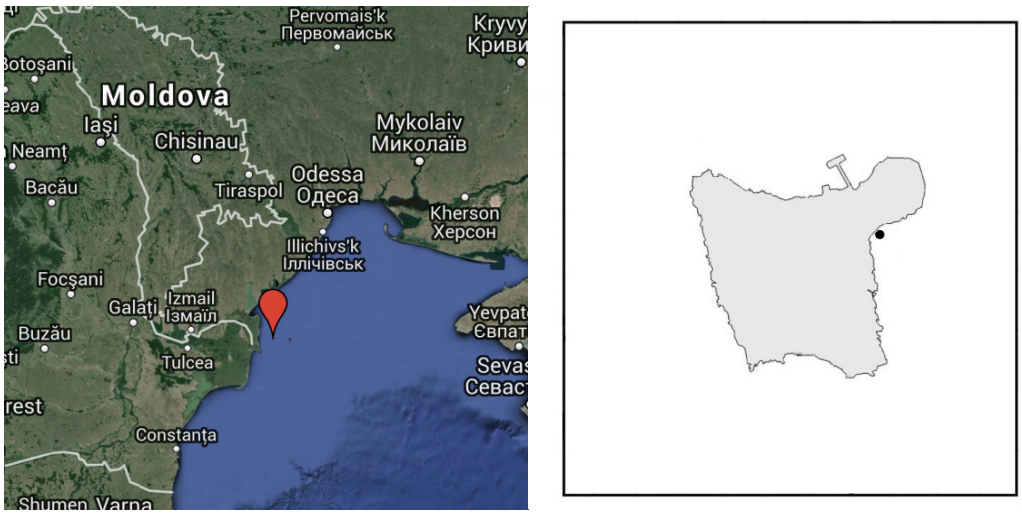

Fig. 1. Map-scheme of Zmiiniy island with marked sampling site

515f, 806r. That composed the insert fragment of about 253 bp. The complete amplified product by those primers was around 387 bp.

PCR reactions were performed using KAPA HiFi HotStart PCR kits (Kapa Biosystems). Each PCR reaction contained 0.2 M Trehalose, 5 µl Fidelity buffer, 0.75 µl KAPA dNTP mix, 0.3 µM of the forward and reverse primers, 0.5 units KAPA HiFi polymerase, about 25 ng template DNA, and PCR grade water until 25 µl. Thermal cycling conditions were: 95 °C for 3 min followed by 27 cycles of 98 °C for 20 s, 61 °C for 10 s, and 72 °C for 15 s. A final extension step was performed at 72 °C for 5 min.

Table 1

Primers used in this study

Name	Adapter	Index	Primer pad	Linker	16S primer
SB502 (F)	AATGATACGGCGA CCACCGAGATCTACAC	CGTTACTA	TATGGTAATT	GT	GTGCCAGCMGCC GCGGTAA
SB504 (F)	AATGATACGGCGACCA CCGAGATCTACAC	TACGAGAC	TATGGTAATT	GT	GTGCCAGCMGCC GCGGTAA
SB505 (F)	AATGATACGGCGAC CACCGAGATCTACAC	ACGTCTCG	TATGGTAATT	GT	GTGCCAGCMGCC GCGGTAA
SB501(F)	CAAGCAGAAGACG GCATACGAGAT	CTACTATA	TATGGTAATT	GT	GTGCCAGCMGCC GCGGTAA
SB704(R)	CAAGCAGAAGACG GCATACGAGAT	CATAGAGA	AGTCAGTCAG	CC	GGACTACHVGGG TWTCTAAT

(F) – Forward primer, (R) – Reverse primer

The PCR products were purified with AMPure XP magnetic beads (Beckman Coulter). The amount of DNA in purified reactions was estimated by fluorimeter using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies) and a plate fluorometer (QuantiFluor). Then the equimolar mix was made from the purified products in order to get approximately equal contribution from each sample. There were also included an empty extraction blank (negative control probe) from the Power Water DNA isolation kit as a negative control. The final pool of obtained 16S rDNA amplicon library was quantified with a KAPA Universal qPCR kit (Kapa Biosystems).

Custom Read1, Read2 and Index1 primers [7] were used for sequencing run. Read1 primer consisted of same as pad+linker+16S (F), Read2 – pad+linker+16S (R), Index1 – Reverse Compliment of Read2 primer. The library was diluted to a final concentration of five pM before loading.

Sequencing was performed on an Illumina MiSeq using v2 reagents kit (MiSeq Reagent kit v2) with 250 cycles for Read1, 8 cycles each for index F and index R reads, and 250 cycles for Read2.

The unprocessed raw sequences after run were analyzed with the help of two bioinformatics pipelines, QIIME (Quantative Insights into Microbial Ecology) [6] and SILVAngs [18]. The pair end reads were quality controlled in FastQC v. 0.11.2 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The PEAR tool [24] was used for merging the overlapping pair end data and generating of assembled read files. The merged reads were converted to fasta format.

First step of bioinformatic analysis of obtained nucleotide sequences was made in SILVAngs pipeline. For the second analysis all the manipulations were performed in the Bio-linux 7 operating system. After quality control reads were manually trimmed from contamination and quality controlled again. The obtained sequences were used as input for the bioinformatics pipeline QIIME, version 1.8.0 [6]. For visualization of the results MEGAN (version 5_7_0) analyzer was applied.

Results and discussion

As the result of sequencing run there were produced 80206 raw sequences belonged to the 3 studied marine water samples replicas Zm1, Zm2, Zm3 and to the one negative control. Demultiplexing was done automatically by the MiSeq Reporter software at the completion of the sequencing run. All the reads were assigned to their sample according to the unique index sequences for each sample. The sequences were quality controlled in FastQC (Babraham Bioinformatics project), trimmed from adapters, primers and highly repeated regions, and quality controlled again. Finally, there were 79748 sequences remained and were used as the input for bioinformatics analysis.

After SILVAngs analysis there were classified 99.11% of all sequences using reference Silva database, and only 0.82% of sequences were identified as “No relative”.

For the QIIME workflow we used the *de novo* picking strategy. Based on sequence identity the trimmed sequences were clustered in Operational Taxonomic Units (OTUs), which in traditional taxonomy represent groups of organisms defined by intrinsic phenotypic similarity [16]. The level of threshold was set at 97%

of sequence similarity. Most part of sequences (99.8%) were classified in QIIME workflow and 0.2% sequences had no relative. Comparing to SILVAngs analysis the use of QIIME allowed classifying the OTUs up to genus level.

Fig. 2. illustrates alpha diversity (within sample diversity), made in QIIME workflow for studied samples.

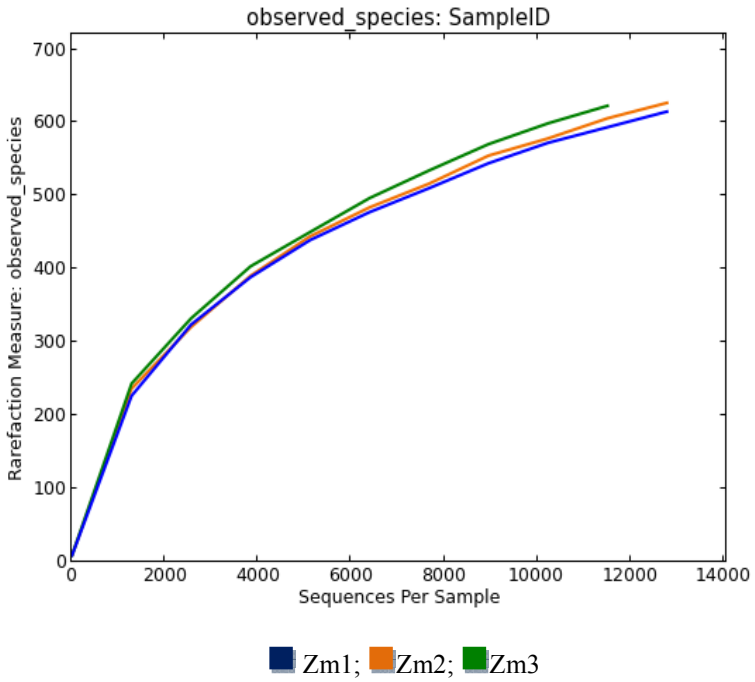

Fig. 2. Analysis of alpha rarecurves at the 97% similarity level. The curves demonstrate the number of observed OTUs as a function from samples sequences

Both SILVAngs and QIIME workflows identified 10 main phyla among the domain *Bacteria*: *Proteobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria, Verrucomicrobiota, Planctomycetes, Tenericutes, SR1, Fusobacteria* and *Firmicutes* (Fig. 3, 4).

Graphical representation of the sequence frequency in the studied water samples, showing major detected classes within the *Bacteria* domain. The color of the symbol represents the relative frequency of the taxonomic path within the sample. The size of the symbol represents the number of the OTUs at deeper phylogenetic levels within the taxonomic path. The shape of the symbol represents the number of sequences in the specific taxonomic path.

At the same level some important differences in taxonomic assignments between two bioinformatic analyses were found. The SILVAngs data points at the presence of candidate divisions such as: BD1-5, BHI80-139, OP3, OD1, WS3, NPL-UPA2, SHA-109, SM2F11 and TM6 (Fig. 3). All these divisions were formed on the base of investigations of the 16S rRNA gene data derived from metagenomics studies

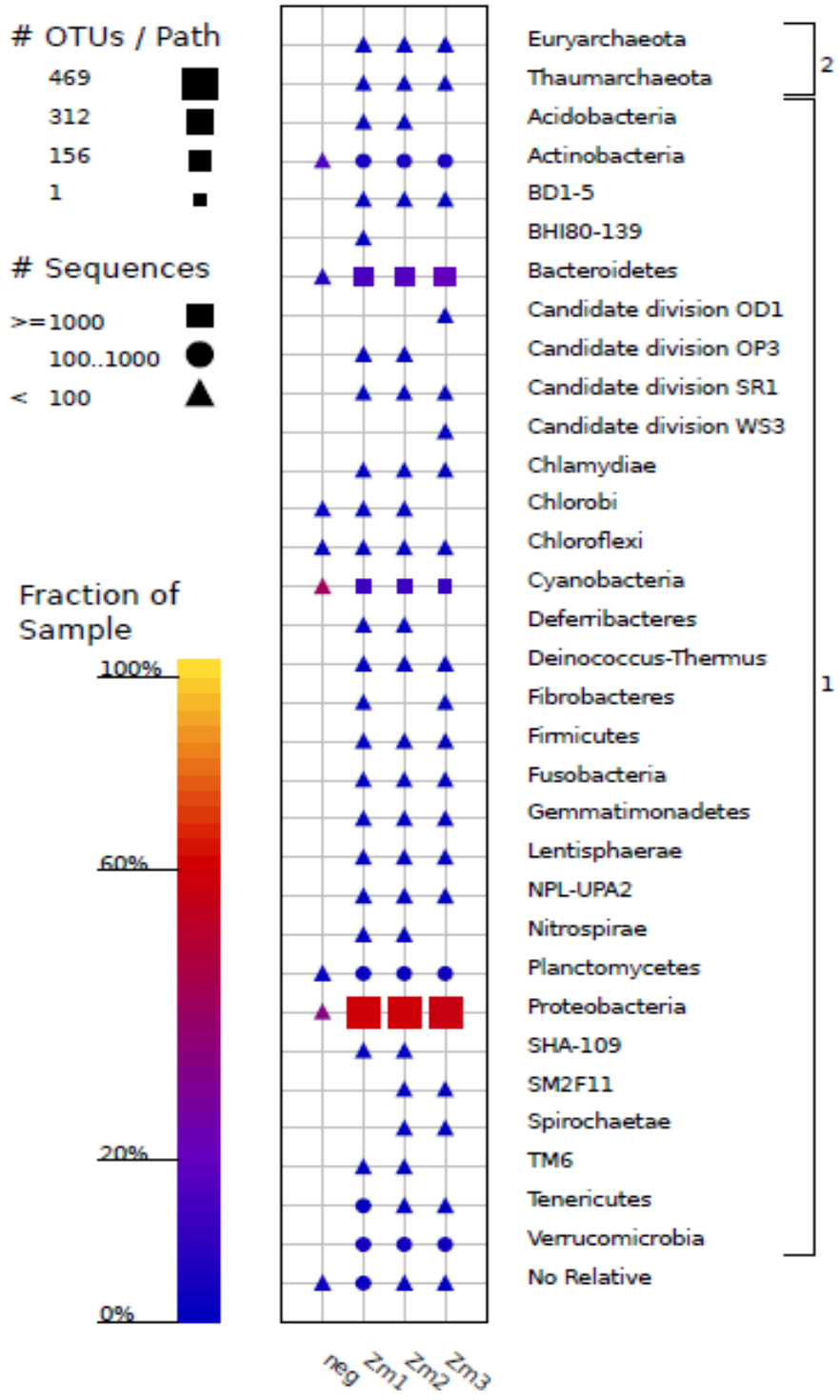

Fig. 3. Taxonomy fingerprint at the phylum level obtained in the SILVAngs pipeline.

of environmental samples. Most part of them was first described for water samples from Yellowstone National Park [9].

QIIME workflow gave opportunity to identify only representatives of SR1 candidate division. However, it has a big group marked as ‘Others”. It includes species, where little information is known or the information is absent in reference Greengenes database (http://greengenes.secondgenome.com). Otherwise, their representatives might be presented in small amounts. This can be explained that Silva is the database for the16S rRNA gene sequences from marine samples whereas Greengenes database contains information from other databases to produce a set of sequences for complete phylogenetic assay.

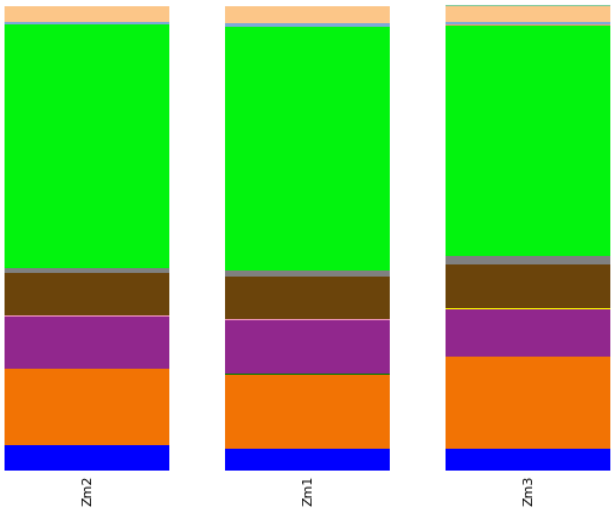

Fig. 4. Bar charts constructed in the QIIME workflow represent the taxonomic distribution of phylogenetic groups at the phylum level

Note: phyla name definitions see in Table 2.

Archaeal sequences were detected in small amounts (less than 100 sequences) by SILVAngs analysis. They belonged to two classes *Thaumarchaeota* and *Euryarchaeota* (Fig. 3).

In the result of the sequence run, there were obtained 272 raw sequences, belonging to negative control. The presence of sequences in the extraction blank may occur due to technical issues such as mixed clusters and tag jumping. These are limitations presently inherent to this sequencing technology.

As it is seen from Table 2 the most abundant phylum for the *Bacteria* domain was *Proteobacteria* (51.4% from all sequences). The most represented classes among *Proteobacteria* were: *Gammaproteobacteria* and *Alphaproteobacteria*. The members of these two classes were also dominating in the Sargasso Sea investigation [20] with the only difference that Alphaproteobacteria was the most abundant in Sargasso Sea region. The studied samples are characterized by relatively low representation of other *Proteobacteria* members such as *Betaproteobacteria*, *Epsilonproteobacteria* and *Deltaproteobacteria*.

Table 2

Quantity distribution of *Bacteria* domain representatives at the phylum level

Legend	Taxonomy	Total %	Zm1 %	Zm2 %	Zm3 %
	Bacteria; Proteobacteria	51.4	52.4	52.3	49.6
	Bacteria; Bacteroidetes	17.3	15.9	16.2	19.7
	Bacteria; Cyanobacteria	11.1	11.6	11.3	10.4
	Bacteria; Other	9.2	9.2	9.1	9.4
	Bacteria; Actinobacteria	5.1	4.9	5.7	4.8
	Bacteria; Verrucomicrobia	3.5	3.7	3.4	3.4
	Bacteria; Planctomycetes	1.4	1.3	1.2	1.8
	Bacteria; Tenericutes	0.6	0.8	0.5	0.6
	Bacteria; SR1	0.1	0.1	0.1	0.1
	Bacteria; Fusobacteria	0.06	0.1	0.1	0.0
	Bacteria; Firmicutes	0.03	0.0	0.1	0.0

The second sufficiently abundant phylum among domain *Bacteria* were the members of *Bacteroidetes* (17.3%). This taxonomy group includes non-spore forming Gram-negative anaerobic bacteria that are widely spread in the marine environment and sediments. Within this taxa, the most represented were members of two classes such as *Flavobacteria* and *Sphingobacteria*. The representatives of Flavobacteria were observed in similar investigation of Arctic and Antarctic regions where it was shown that most part of these bacteria inhabit surface water [11].

Cyanobacteria members known as typical marine inhabitants presented 11.1% of all sequences.

Actinobacteria members presented 5.1% from total diversity in the studied area. Among this phylum the most abundant were members of order *Actinomycetales* that are well known as antibiotics producing bacteria.

The Verrucomicrobia bacteria are detected in amount of 3.5% from total OTUs. This phylum is characterized by inhabitants of fresh water and soil environments. The most abundant orders among this phylum in studied samples were *Opitutales* and *Verrucomicrobiales*.

The other phylum *Planctomycetes* composed of 1.4 % of the bacterial diversity. These species are the typical colonizers of salt and fresh water reservoirs.

The QIIME workflow allowed identification of studied samples' sequences up to genus level. During this analysis Megan analyzer tool was used for bright visualizations of the results. The Fig. 5 illustrates so called taxonomy clouds for Zm1, Zm2 and Zm3 samples. The size of the print reflects the bacterial composition of mentioned group representatives' in the samples.

As it is seen from Fig. 5, besides *Bacteroidetes* and *Actinobacter* (described above) members of the *Pseudoalteromonas* genus are sufficiently represented. These are non-spore forming, aerobic, Gram-negative marine bacteria.

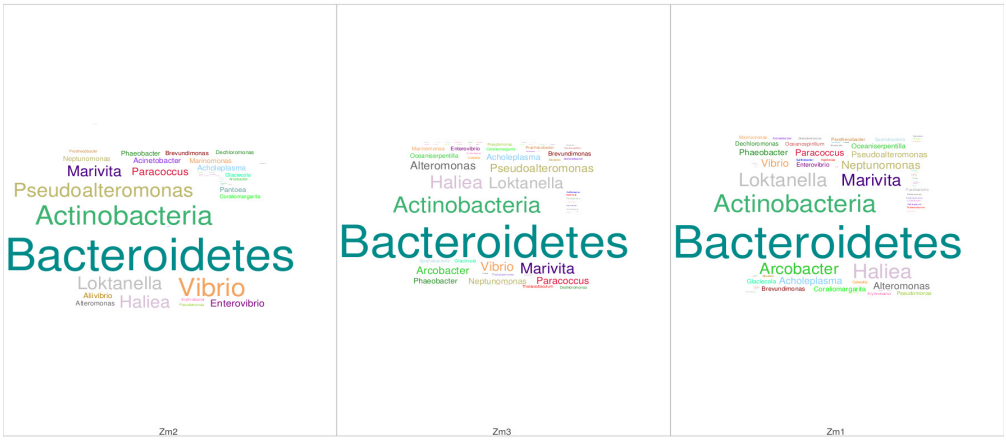

Fig. 5. Cloud charts represent the taxonomic diversity compositions among studied samples

The members of the *Vibrio* genus are also very abundant among studied samples and are typically found in seawater and are facultative anaerobes. Such genera as *Marivita, Loktanella* and *Paracoccus* are the representatives of *Rhodobacteraceae* family. Loktanella genus was previously observed in surface waters of Arctic and Antarctic polar regions [11].The members of this family were observed in the coastal waters of the Atlantic and the Pacific oceans, surface waters of the Sargasso Sea, the Arctic and the Antarctic seas [8, 11, 20]. *Rhodobacterales* are also known for its ability to photosynthesis.

Fig. 5 shows that there are lots of representatives of the *Haliea* genus in samples. In literature, it is little known about this genus. These are novel aerobic, Gram-negative bacteria previously isolated from the surface of coastal waters of the north-western Mediterranean Sea [22].

The members of *Acrobacter* genus noticed in Zm1 and Zm3 samples are Gram-negative, spiral-shaped bacteria in the *Campylobacteraceae* family. These species inhabit wide range of environmental niches.

Among other taxonomic genera abundant in studied samples were such representatives as: *Lutibacter, Robiginitales, Tenacibacilum, Winogradskyella, Lewinella, Bacillariophyta, Cryptomonadaceae, Rhodopirellula, Brevundimonas, Maricaulis, Phaeobacter, Erythrobacter, Prosthecobacter, Spartobacteria,*

Coraliomargarita, Acholeplasma, SR1, Enterovobrio, Aliivibrio, Pseudomonas, Oceanospirillum, Oceaniserpentilla, Neptunomonas, Marinomonas, Umboniibacter and others.

As a result of downstream QIIME analysis there was obtained a biom file (Biological Observation Matrix) with a table of all detected OTUs. This file was used for the visualization of phylogeny in MEGAN application. Fig. 6 shows a phylogenetic tree, which demonstrates the phylogenetic relatedness between all OTUs in studied samples. It is seen that the microbiota of the studied water is diverse and abundant

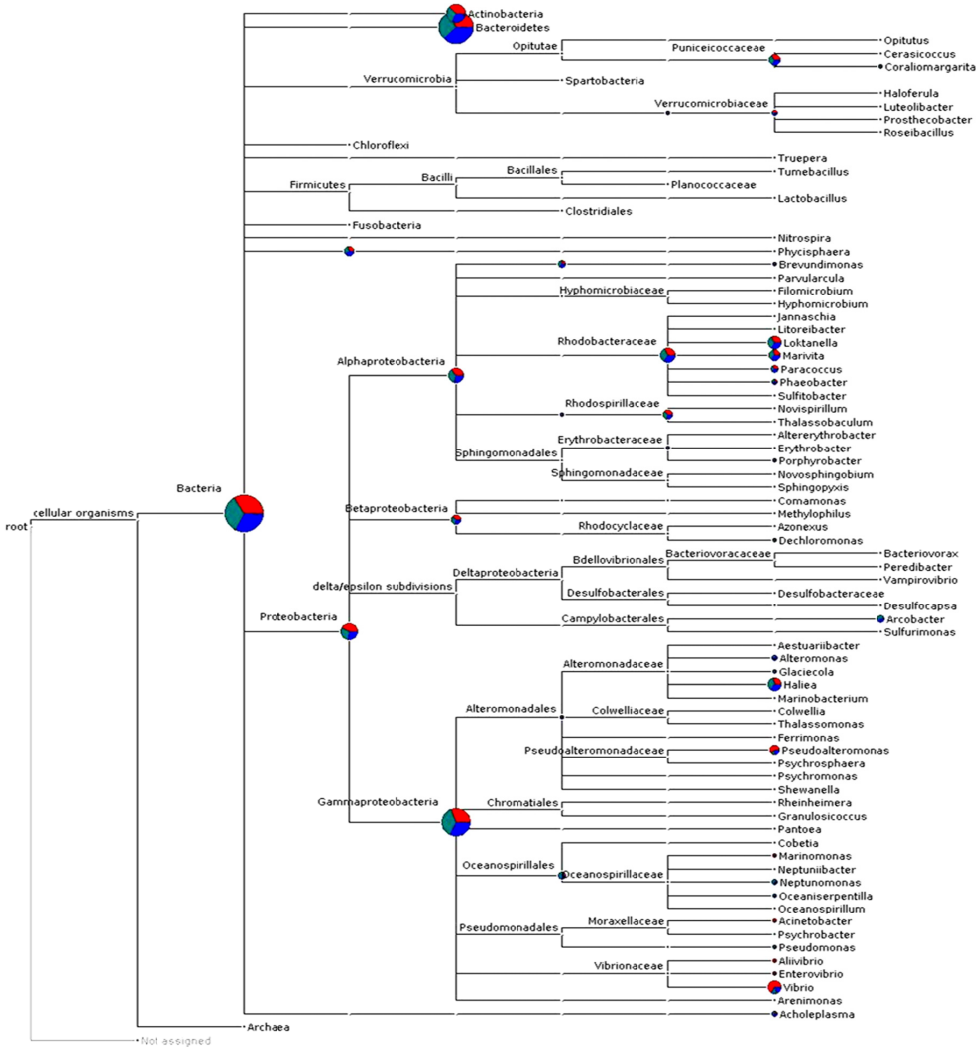

Fig. 6. Phylogenetic tree presented as a cladogram demonstrates the genus relatedness

In the result of the metagenomic 16S rRNA gene analysis, there were detected bacterial populations that inhabit surface waters of the Zmiiniy island. There

were obtained about 3200 OTUs among studied marine samples. There were detected 10 main phyla among *Bacteria* domain: *Proteobactera, Bacteroidetes, Cyanobacteria, Actinobacteria, Verrucomicrobiota, Planctomycetes, Tenericutes, SR1, Fusobacteria* and *Firmicutes*. Among them there were dominant classes: *Gammaproteobacteria, Alphaproteobacteria, Cyanobacteria, Flavobacteria, Actinobacteria, Sphingobacteria, Betaproteobacteria, Opitutae, Planctomycetacia, Epsilonproteobacteria, Verrucomocrobiae, Mollicutes, Deltaproteobacteria, Fusobacteria* and others.

The majority of studied bacterial representatives belonged to orders *Flavobacteriales, Alteromonadales, Rhodobacterales, Actinomycetales, Acidimicrobiales, Sphingobacteriales, Pseudomonadales, Rhodospirillales, Planctomycetales, Vibrionales, Puniceicoccales* etc.

In the result of the taxonomic analysis, the majority of bacterial families and genera were identified. The most represented genera among studied samples were: *Marivita, Loktanella, Paracoccus, Pseudoalteromonas, Vibrio, Coraliomargarita, Acholeplasma, Pseudomonas, Phaeobacter, Neptunomonas, Allivibrio, Oceaniserpentilla, Oceanospirillum, Robiginitalea, Acinetobacter, Haliea*.

The results of SILVAngs and QIIME analyses coincide and supplement with each other. Thus, with the help of SILVAngs there were identified sequences belonging to two classes of *Archaea* domain: *Thaumarchaeota* and *Euryarcheota*. In the mentioned above metagenomics study of the Sargasso Sea these two archea classes were also observed in small amounts.

The obtained data showed that the majority of the identified bacterial groups are typical representatives of marine environments. Many of them are poorly investigated and have not been cultivated yet. The obtained results agree with literature data on similar marine investigations [8, 9, 11, 20, 22] and provide far more data on composition and abundances of the microbiota comparing to classical microbiology methods. The provided metagenome of the 16S rRNA gene allowed to evaluate rich taxonomic diversity of prokaryotes in the surface water of the Zmiiniy island.

О.Є. Боброва[1], Й.Б. Крістофферсен[2], В.О. Іваниця[1]

[1] Одеський національний університет імені І.І. Мечникова, вул. Дворянська, 2, Одеса, 65082, Україна, тел.:+38 (0482) 68 79 64, e-mail: o.bobrova@onu.edu.ua
[2]Інститут Морської Біології, Біотехнології та Аквакультури, Грецький Центр Морських Досліджень, Гурнес 71500, 71003 Іракліон, Греція

МЕТАГЕНОМНИЙ 16S РРНК АНАЛІЗ МІКРОБНОГО РІЗНОМАНІТТЯ ЧОРНОГО МОРЯ В РАЙОНІ ОСТРОВА ЗМІЇНИЙ

Реферат

Метою дослідження *було визначення морського мікробної біорізноманітності прибережної морської води острова Зміїний за допомогою метагеномного аналізу.* ***Методи.*** *Для ампліфікації v4 області 16S рРНК гена використовували*

праймери з двома індексами. Секвенування здійснювали на платформі Illumina MiSeq. Нуклеотидні послідовності аналізували за допомогою програм SILVAngs та QIIME. ***Результати.*** *В результаті метагеномного 16S рРНК аналізу в прибережних водах острова Зміїний виявлено близько 3200 OTE. Виявлені основні відділи домену Bacteria: Proteobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria, Verrucomicrobiota, Planctomycetes, Tenericutes, Fusobacteria, Firmicutes і кандидатні відділи некультивованих представників прокаріот: SR1, BD1-5, BHI80-139, OP3, OD1, WS3, NPL-UPA2, SHA-109, SM2F11 та TM6. В результаті таксономічного аналізу ідентифіковано багато бактеріальних родин і родів. Найбільш представленими родами серед досліджуваних зразків були Marivita, Loktanella, Paracoccus, Pseudoalteromonas, Vibrio, Coraliomargarita, Acholeplasma, Pseudomonas, Phaeobacter, Neptunomonas, Allivibrio, Oceaniserpentilla, Oceanospirillum, Robiginitalea, Acinetobacter, Haliea.* ***Висновки.*** *Метагеномний 16S рРНК аналіз дозволив оцінити величезну таксономічну різноманітність прокаріот поверхневих вод Чорного моря в районі острова Зміїний. Багато з них слабо вивчені і не піддаються культивуванню.*

Ключові слова: Чорне море, острів Зміїний, морська вода, 16S рРНК аналіз, таксономічна різноманітність, бактерії.

А.Е. Боброва[1], Й.Б. Кристоффersен[2], В.А. Иваныця[1]

[1] Одесский национальный университет имени И.И. Мечникова, ул. Дворянская, 2, Одеса, 65082, Украина, тел. (0482) 68 79 64, e-mail: o.bobrova@onu.edu.ua
[2]Институт Морской Биологии, Биотехнологии и Аквакультуры, Греческий Центр Морских Исследований, Гурнес 71500, 71003 Ираклион, Греция

МЕТАГЕНОМНИЙ 16S рРНК АНАЛИЗ МИКРОБНОГО РАЗНООБРАЗИЯ ЧОРНОГО МОРЯ В РАЙОНЕ ОСТРОВА ЗМЕИНЫЙ

Реферат

Целью исследования *было определение морского микробного биоразнообразия прибрежной морской воды острова Змеиный с помощью метагеномного анализа.* ***Методы.*** *Для амплификации v4 области 16S рРНК гена использовали праймеры с двумя индексами. Секвенирование производили на платформе Illumina MiSeq. Нуклеотидные последовательности анализировали при помощи программ SILVAngs і QIIME.* ***Результаты****. В результате метагеномного 16S рРНК анализа в прибрежных водах острова Змеиный обнаружено около 3200 OTE. Выявлены основные отделы домена Bacteria: Proteobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria, Verrucomicrobiota, Planctomycetes, Tenericutes, Fusobacteria, Firmicutes и кандидатные отделы некультивируемых представителей прокариот: SR1, BD1-5, BHI80-139, OP3, OD1, WS3, NPL-UPA2, SHA-109, SM2F11 и TM6. В результате таксономического анализа идентифицировано множество бактериальных семейств и родов. Наиболее представленными родами среди исследуемых образцов были Marivita, Loktanella, Paracoccus, Pseudoalteromonas, Vibrio, Coraliomargarita, Acholeplasma, Pseudomonas, Phaeobacter, Neptunomonas, Allivibrio, Oceaniserpentilla, Oceanospirillum, Robiginitalea, Acinetobacter, Haliea.* ***Выводы.*** *Метагеномный 16S рРНК анализ позволил оценить богатое таксоно-*

мическое разнообразия прокариот поверхностных вод Черного моря в районе острова Змеиный. Многие из них слабо изучены и не поддаются культивированию.

Ключевые слова: Черное море, остров Змеиный, морская вода, 16S рРНК анализ, таксономическое разнообразие, бактерии.

LITERATURE

1. *Васильєва Т.В., Іваниця В.О., Васильєва Н.Ю., Бобрешова Н.С., Юргелайтіс Н.Г.* Біологічні властивості тіонових бактерій північно-західної частини Чорного моря // Вісник ОНУ. – 2005 . – Т. 10, В. 3. – С. 123–135.

2. *Іваниця В.О.* Стан та мінливість мікробних ценозів морських екосистем: Автореф. дис. докт. біол. наук. К., 1996. – 47 с.

3. *Крисс А.Е., Мишустина И.Е., Мицкевич И.Н., Земцова Э.В.* Микробное население Мирового океана. – М.: АН СССР, 1964. – 300 с.

4. *Лісютін Г.В., Бухтіяров А.Є.,Білоіваненко С.О., Пономарьова Л.П., Гудзенко Т.В., Іваниця В.О.* Нафтове забруднення і гетеротрофна мікробіота акваторії острова зміїний Мікробіологія і біотехнологія // Мікробіологія і біотехнологія. – 2009. – № 1. – С. 88–94.

5. *Цыбань А.В.* Бактерионейстон и бактериоплактон в прибрежной зоне Черного моря – К.: Наукова думка, 1970. – 272 с.

6. *Acinas S.G., Klepac-Ceraj V., Hunt D.E., Pharino C., Ceraj I., Distel D.L., Polz M.F.* Fine-scale phylogenetic architecture of a complex bacterial community // Nature. – 2004. – V. 430. – P. 551–554.

7. *Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N.* QIIME allows analysis of high-throughput community sequencing data // Nature Methods. – 2010. – V. 7. – P. 335–336.

8. *Caporaso J.G., Lauber C.L., Walters W.A., Berg-Lyons D., Huntley J., Fierer N., Owens S.M., Betley J., Fraser L., Bauer M.* Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms // The ISME Journal. – 2012. – V. 6. – P. 1621–1624.

9. *Cottrell M.T., Mannino A., Kirchman D.L.* Aerobic Anoxygenic Phototrophic Baceria in the Mid-Atlantic Bight and the North Pacific Gyre // Applied Environmental Microbiology. – 2006. – V. 72(1). – P. 557–564.

10. *Davis J.P., Youssef N.H., Elshahed M.S.* Assessment of the Diversity, Abundance, and Ecological Distribution of Members of Candidate Division SR1 Reveals a High Level of Phylogenetic Diversity but Limited Morphotypic Diversity // Applied and Environmental Microbiology. – 2009. – V. 75, № 12. – P. 4139–4148.

11. *DeLong E.F., Karl D.M.* Genomic perspectives in microbial oceanography // Nature. – 2005. – 437: 336–342.

12. *Gilbert J., Jansson J., Knight R.* The Earth Microbiome project: successes and aspirations // BMC Biology. – 2014. – V. 12:69. – 4 p.

13. *Handelsman J., Liles M., Mann D., Riesenfeld C., Goodman R.M.* Cloning the metagenome: Culture-independent Access to the Diversity and Functions of the Uncultivated Microbial World // Methods in Microbiology. – V. 33. – P. 240–252.

14. *Kantor R.S., Wrighton K.C., Handley K.M., Sharon I., Hug L.A., Castelle C.J., Thomas B.C., Banfield J.F.* Small Genomes and Sparse Metabolisms of Sediment-Associated Bacteria from Four Candidate Phyla //mBio. – 2013. – V. 4, № 5. – P. 1–11.

15. *Kelly K.M., Chistoserdov A.Y.* Phylogenetic analysis of thesuccession of bacterial communities in the Great South Bay (Long Island) // FEMS Microbiol Ecol. – 2001. – 35: 85–95.

16. *Kozich J.J., Westcott S.L., Baxter N.T., Highlander S.K., Schloss P.D.* Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform // Applied and Environmental Microbiology. – 2013. – V. 79, № 17. – P. 5112–5120.

17. *Navas-Molina J., Peralta-Sanchez M., Gonzales A., McMurdie P.J., Vazquez-Baeza Y., Xu Z., Ursell L.K., Lauber C., Zhou H., Song S.J.* Advancing Our Understanding of the Human Microbiome Using QIIME // Methods in Enzymology. – 2013. – V. 531. – P. 371–444.

18. *Pommier T., Canback L., Riemann., Bostrom K.H., Simu K., Lundberg P., Tunlid A., Haqstrom A.* Global patterns of diversity and community structure in marine bacterioplankton // Mol Ecol. – 2007. – 16. – P. 867–880.

19. *Quast C., Pruesse E., Yilmaz P., Gerken J., Schweer T., Yarza P., Peplies J., Glockner F.O.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools // Nucleic Acids Research. – 2013. – V. 41. – P. 591–596.

20. *Sala M.M., Terrado R., Lovejoy C., Unrein F., Pedros-Alio C.* Metabolic diversity of heterotrophic bacterioplankton over winter and spring in the coastal Arctic Ocean // Environ Microbiol. – 2008. – V. 10. – P. 942–949.

21. *Tsyban A.V., Panov G.V., Ivanitsa V.A., Khudchenko G.V.* Taxonomic Composotion of heterotrophic bacteria // In Results of the Third Joint US-USSR Bering and Chukchi Seas Expedition (BERPAC), HUMMER, 1988. – Nagel P.A. (ed.) US Fish and Mildlife Service, Mashington, DC, – 1992. – P. 87–90.

22. *Urios L., Intertaglia L., Lesongeur F., Lebaron P.* Haliea rubra sp. nov., a member of the Gammaproteobacteria from the Mediterranean Sea // International Journal of Systematic and Evolutionary Microbiology. – 2008. – V. 58, № 5. – P. 1233–1237.

23. *Vieira R., Gonzales A.M., Cardoso* ***A.M., Oliveira D.N., Albano R.M., Clementino M.M., Martins O.B., Paranhos R.*** Relationships between bacterial diversity and environmental variables in a tropical marine environment, Rio de Janeiro // Environmental Microbiology. – 2008. – V. 10. – P. 189–199.

24. *Zhang J., Kobert K., Fluori T., Stamatakis A.* PEAR: A fast and accurate Illumina Paired-end read merger // Bioinformatics . – 2013. – V. 30, № 5. – P. 614–620.

Стаття надійшла до редакції 06.05.2015 р.