Phylogenetic diversity of microbiota of sediments, water and coastal surface technogenic formations of soda sludge storage (Berezniki, Perm region, Russia)

Anna Shilova[1], Aleksandr Maksimov[1,2], Yuliya Maksimova[1,2]

[1]*Institute of Ecology and Genetics of Microorganisms UB RAS, Perm Federal Research Center UB RAS, Goleva, 13, 614081, Perm, Russia, Anechka_Shilova@mail.ru*

[2]*Perm State National Research University, Bukireva, 15, 614990, Perm, Russia*

Abstract

The phylogenetic diversity of the microbial community was evaluated using metagenomic sequencing. The objects of the study were water, sediments and coastal surface technogenic formations of the soda sludge storage facility of the soda plant "Soda", Berezniki (Perm region, Russia). The bacterial communities of the all samples were dominated by representatives of two classes: *Bacilli and Gammaproteobacteria*. At the same time, differences in the species diversity of microbiocenoses were noted. The water samples with a pH of 12.6 were characterized of lowest biodiversity, where the basis of the microbial community was only 3 classes: *Bacilli* (57,51%), *Gammaproteobacteria* (29,79%) and Clostridia (4,55%). The most diverse microbial community (8 classes) was noted in the soda sediments; the pH of these samples was 11.

Keywords: alkalophiles, sludge storage, microbiocenosis, metagenomic analysis

Introduction

Soda Lakes as extreme habitats are the object of study for a long time, and interest in their research does not decrease. Such sources are unique aquatic ecosystems characterized by high salt concentrations and pH values (Kompantseva et al. 2007). Despite these dual extreme conditions, most lakes are highly productive ecosystems with a fully functional microbial community (Sorokin et al. 2011). Considerable interest for understanding the functioning of soda lakes as a separate type of ecosystem is the study of microbiocenosis (Grant 2006).

Microbiological studies that were carried out in soda-salt lakes showed that they have an active alkaliphilic microbial community, which is not found in other ecosystems (Shargaeva et al. 2014; Egorova et al. 2011). Unlike natural alkaline lakes, formed from tens of thousands of millions of years ago, sludge storages with an extremely alkaline and saline environment have existed only decades. The microbiocenosis of alkaline biotopes of anthropogenic origin is interesting in plan of studying the mechanisms of secondary adaptation to alkalization and high salt concentration.

The purpose of this study is to learn the phylogenetic diversity of the sludge storage facility of the Berezniki soda plant (Perm region).

Objects and methods

Samples for physical, chemical and microbiological studies were taken from the territory of the existing sludge storage "Soda" plant in the Berezniki city (Perm region, Russia). The sludge storage is located on the northwestern outskirts of Berezniki city on the left bank of the Kama river. The area of the current map is about 155 hectares. According to rough estimates, the volume of sludge in the storage currently exceeds 10 million m3 (Blinov et al. 2003). Sampling of water, sediment and coastal surface formations was carried out in September 2017. Samples before DNA extraction were transported and stored for a short time at 4°C. Preparations of the chromosomal DNA of bacteria were obtained by the phenol method modified for the isolation of DNA from actinomycetes (Hunter 1985). For screening bacterial microbiota, a metagenomic analysis of the samples was carried out on the 16S rRNA

genes on the MiSeq (Illumina) platform. Sequencing of the V3-V4 region of the 16S rRNA gene was performed following 16S Metagenomic Sequencing Library Preparation protocols.
(https://support.illumina. com / content / dam / illumina-support / documents / documentation / chemistry_documentation / 16s / 16s-metagenomic-library-prep-guide-15044223-b.pdf).

Sequencing was performed at the the Illumina MiSeq Systems (Illumina, USA) according to the manufacturer's instruction with 2x250 bp paired-end runs. We used software trimming v0.3 for read trimming and FastQC v.0.10.128 for quality control. Raw sequence data were processed and analyzed using QIIME (Quantitative Insights Into Microbial Ecology, Version 1.9.1) software (Caporaso et al. 2010) and Ribosomal Database Project (RDP) http://rdp.cme.msu.edu/. To obtain the necessary PCR fragments, 2 rounds of PCR were used. The nucleotide sequences of the main primers for the 16S rRNA region V3/V4 were used: Forward Primer TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and Reverse Primer = 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC. Primers were synthesized in Evrogen LLC (Russia). PCR was performed with the Tersus PCR kit (Evrogen, Russia). The first round of PCR was performed in 25 µl of the reaction mixture containing 10 µl of 10x buffer; 2,5 µl dNTP; 1 unit of Tersus polymerase activity; 1 µl of chromosomal DNA, 20 pmol of primers, and H_2O up to 25 µl. The reaction was carried out in the amplifier "T-100", the company "Bio-Rad". Amplification mode: 95°C - 5'([95°C - 30"; 57°C - 30"; 72°C - 30"] 25 cycles) 72°C - 5'; 10°C. After amplification, the presence of PCR products was checked in a 1,5% agarose gel. Then a second round of PCR was performed, in which MIDs were added to the obtained PCR fragments. The second round of PCR was performed in 50 µl of the reaction mixture containing 10 µl of 10x buffer; 2,5 µl dNTP; 1 unit of Tersus -polymerase activity; 5 µl of PCR mix after the first round of amplification, 20 pmol each of primers, and H2O up to 50 µl. The reaction was carried out in the amplifier "T-100", the company "Bio-Rad". Amplification mode: 95°C - 2'([95°C - 30"; 57°C - 30"; 72° - 30"] 10 cycles) 72°C - 5'; 10°C. The approximate length of the PCR product (after annealing the adapter and the terminated MID) was 530 bp. After the second round of amplification, the presence of PCR products was also checked in a 1,5% agarose gel. After imaging the fragments, each amplicon was double-cleaned with AMPure XP particles. Then, the concentration of each amplicon was evaluated using the Quant-it Picogreen dsDNA Assay Kit, and the amplicons were mixed in an equimolar amount to a final concentration of each amplicon in the pool of 5 ng.

The ionic composition of the medium was investigated using the Kappel-105 capillary electrophoresis system, and the elemental composition was studied using a Shimadzu AA-6300 atomic absorption spectrometer, in accordance with the manufacturer's instructions.

Results

Metagenomic analysis of samples taken from the territory of the sludge storage (water, coastal surface technogenic formations and sediment) was carried out. Representatives of 8 classes were identified in the studied samples, among which 2 classes of *Bacilli* (up to 57,51%) and *Gammaproteobacteria* (up to 31,51%) occupied a dominant position.

In the soda sediment, at pH 11, the most diverse microbial community was noted, the basis of wich consist representatives of 8 classes: *Gammaproteobacteria* (20.34% of the total), *Bacilli* (18,71%), *Alphaproteobacteria* (16,39%), *Clostridia* (7,49%), *Betaproteobacteria* (6,23%), *Actinobacteria* (4,75%), *Deltaproteobacteria* (3,73%), *Sphingobacteria* (3,53%) (fig.1). The dominant cation was a calcium cation, the dominant anions were carbonates, bicarbonates and chlorides, which was caused by the production process of soda obtaining.

In samples of coastal surface formations, the microbial community turned out to be less diverse. Representatives of 4 classes were found here: *Bacilli* (45,60%), *Gammaproteobacteria* (31,51%), *Betaproteobacteria* (6,89%), *Clostridia* (5,29%), despite on the fact that the conditions were less extreme (pH 8) (fig.2). These formations, obviously, arise when soda

is washed on natural soils. The content of calcium cation in these samples is reduced by 4 times, carbonates are not detected, and the content of hydrocarbonates is comparable to that in the sediment. The concentration of chloride ions is reduced by 7,5 times.

In water samples, the basis of the microbial community was only 3 classes: *Bacilli* (57,51%), *Gammaproteobacteria* (29,79%) and *Clostridia* (4,55%) (fig.3). The decrease in biodiversity can be due both to an extreme increase in pH (up to 12,6) and to the fact that in the adhered state on the sediment particles, microorganisms are more resistant to adverse factors.

It is established that the dominant species are *Staphylococcus* sciuri and *Acinetobacter baumannii*, the DNA of which in different samples ranges from 14.38 to 47.21% and from 7.91 to 24.60% of the secreted metagenome, respectively. It should be noted that the number of representatives of *Staphylococcus* in all samples was higher than *Acinetobacter*.

Representatives of the genus *Staphilococcus* are gram-positive facultative anaerobes, chemo-organotrophs with an oxidative and enzymatic type of metabolism.

Staphylococcus sciuri is able to ferment sugar to cellobiose, which is a distinctive feature of this species. It also possesses pronounced saccharolytic, caseolytic and gelatinase activities. These properties and the absence of a pathogenicity factor characterize them as the most ancient, capable of existing in an abiotic environment (Deryabin 2000).

The genus *Acinetobacter* includes strictly aerobic non-fermenting catalase-positive oxidase-negative gram-negative immobile prototrophic bacteria with G + C content in DNA from 39 to 47% (Wang et al. 2014). Acenetobacteria are characterized by a universal metabolic activity, which ensures their ecological plasticity. Substances used as source of nutrition of acinetobacteria are very diverse: simple carbohydrates, oil, human tissues (Bergogne-Berezin et al. 2008).

Also in the literature note the high lipolytic activity of acinetobacteria. They have a set of lipases, at which the optimum action of most lipases lay in an alkaline environment. High activity and a wide range of substrates for acinetobacterial lipases justified their use as industrial detergents (Aehle 2007).

Clostridia are very important organisms for modern biotechnology. Extracellular enzymes produced by these bacteria are able

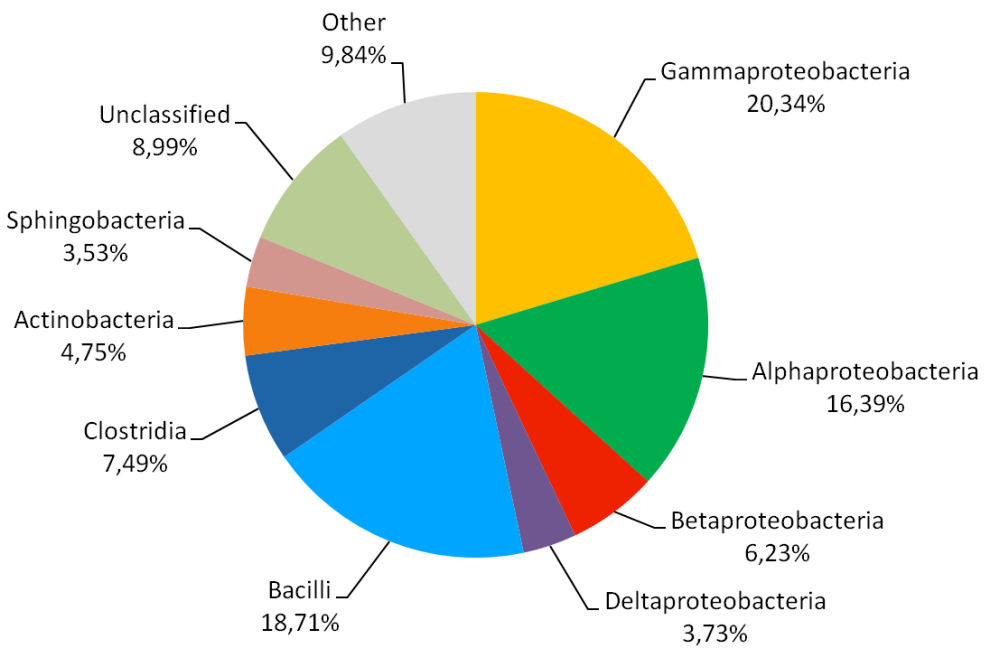

Figure 1 *Diversity of microbial community in the soda sediment.*

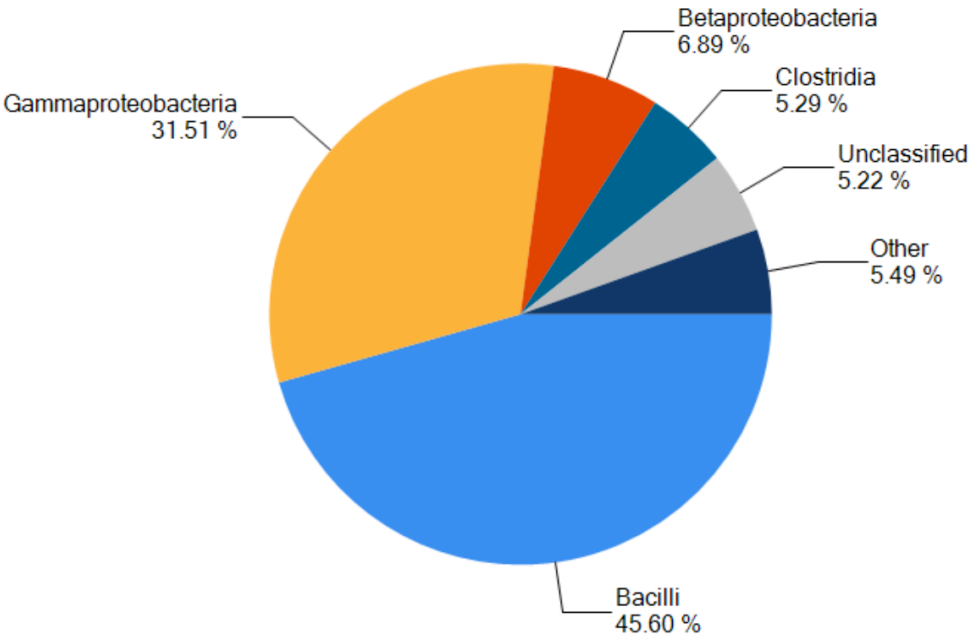

Figure 2 *Diversity of microbial community in samples of coastal surface formations of soda sludge storage.*

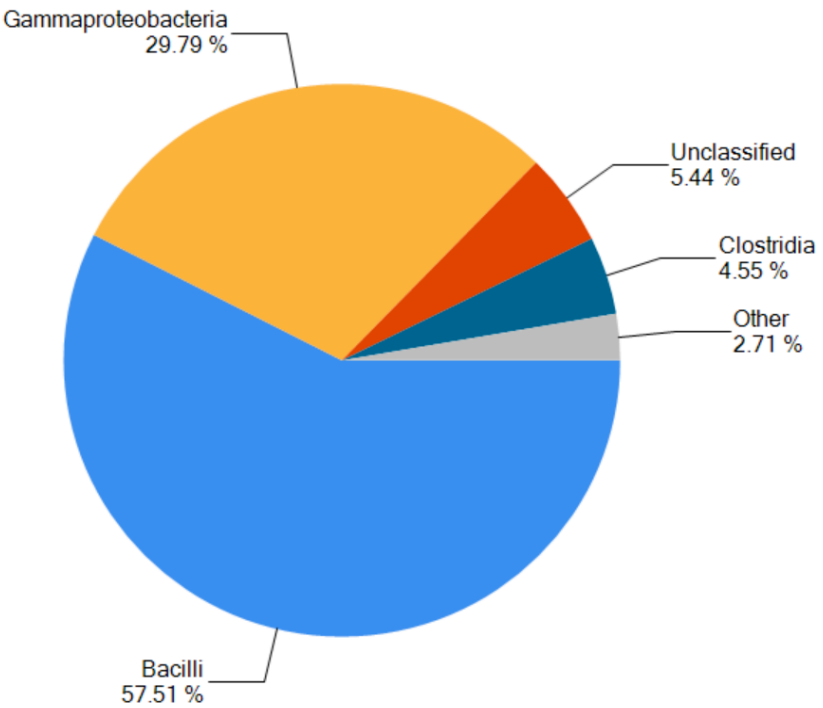

Figure 2 *Diversity of microbial community in samples of coastal surface formations of soda sludge storage.*

to degrade or hydrolyze biopolymers such as starch or cellulose (Schwarz et al. 2004).

Representatives of the genus *Sphingomonas*, chemogeterotrophic, strictly aerobic alphaproteobacteria, known for their ability to decompose hydrophobic polycyclic aromatic hydrocarbons and express hydrolytic enzymes (Zhou et al. 2016), also constitute a substantial proportion (3.77%) of soda sediment among the dominant microorganisms.

Thus, the dominant microorganisms of the studied microcenoses are heterotrophic microorganisms capable of producing various hydrolytic enzymes and utilizing various organic substrates. The data obtained by us give an representation of the diversity of bacteria in the soda sludge storage, which is an environment with an extreme anthropogenic load. The smallest biodiversity was found in water samples with a pH of 12,6. Despite less extreme conditions of coastal zone (pH 8), phylogenetic diversity was reduced, which may be due to lower humidity compared to sediments (8% versus 52%). In general, the phylogenetic diversity of microorganisms of soda sludge storage is substantially different from natural biotopes, that may be due to a different elemental composition and higher pH values.

The work was performed as part of the state task, the state registration number: 01201353247.

References

Aehle W (2007) Enzymes in Industry. Production and Applications. Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA. 517 p.

Bergogne-Berezin E, Friedman H, Bendinelli M (2008) Acinetobacter. Biology and Pathogenesis. New York: Springer. 236 p.

Blinov SM, Maksimovich NG, Naydanova NF, Shlykov VG, Potapov SS (2003) Mineralogicheskie osnovy utilizatsii otkhodov OAO «Bereznikovskiy sodovyy zavod». Mineralogiya tekhnogeneza 4:51–55.

Caporaso JG, Kuczynski J, Stombaugh J et al. (2010) QIIME allows analysis of high-throughput community sequencing data. Nat. Methods. 7(5):335–336. doi:10.1038/nmeth.f.303

Deryabin DG (2000) Staphylococcus: Ecology and Pathogenicity. Ekaterinburg: UB RAS. 240 p.

Egorova DV, Anan'ina LN, Kozyreva LP, Zakharyuk AG, Plotnikova EG (2011) Physical-chemical properties and microbial diversity of lake Solenoe (Republic of Buryatia). Microbiology 1:55–59. https://elibrary.ru/item.asp?id=17664593

Grant WD (2006) Alkaline environments and biodiversity. Extremophiles – Vol III. Ed.: Gerday EC, Glansdorff N. Oxford, UK: UNESCO, Eolss Publishers. http://www.eolss.net/ebooks/sample%20chapters/c03/e6-73-05-01.pdf

Hunter IS (1985) Gene cloning in Streptomyces. In Cloning. A Practical Approach – Vol 2. Ed.: DM Glover. Oxford, IRL Press. P. 19-44.

Kompantseva EI, Bryantseva IA, Komova AV, Namsaraev BB (2007) The Structure of Phototrophic Communities of Soda Lakes of the Southeastern Transbaikal Region. Microbiology 76(2):243–252, doi:10.1134/S0026261707020130

Schwarz WH, Zverlov VV, Bahl H (2004) Extracellular Glycosyl Hydrolases from Clostridia. Advances in Applied Microbiology. 56: 215-261. doi: 10.1016/S0065-2164(04)56007-0

Shargaeva OV, Abidueva EYu (2014) Microzonal destribution of cellulolytic bacteria in bottom sediments of the soda lake Khilganta (South-East Zabaikalie). Bulletin of the Buryat State University 4:113–115, https://elibrary.ru/item.asp?id=15629318

Sorokin DY, Kuenen JG, Muyzer G. (2011) The microbial sulfur cycle at extremely haloalkaline conditions of soda lakes. Frontiers in Microbiology. Microbial Physiology and Metabolism.. 2: 44, doi:10.3389/fmicb.2011.00044 .

Wang X, Zhang Z, Hao Q, Wu J, Xiao J, Jing H (2014) Complete Genome Sequence of Acinetobacte baumannii ZW85-1. Genome Announc. 2 (1): e01083-13. doi: 10.1128/genomeA.01083-13

Zhou L, Li H, Zhang Y, Han S, and Xu H (2016) Sphingomonas from petroleum-contaminated soils in Shenfu, China and their PAHs degradation abilities. Braz J Microbiol. 47(2): 271–278. doi:10.1016/j.bjm.2016.01.001