Taxonomic composition and proteolytic potential in the microbial mat of the Uro hot spring (the Baikal rift zone)

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Abstract. We studied the taxonomic diversity of microbial mat in the Uro hot spring (the Baikal rift zone, BRZ) with temperatures of 62°C (Ur-3) and 56°C (Ur-4). According to the results of high-throughput sequencing, the composition of the community is represented by various phylogenetic groups Cyanobacteria, Proteobacteria, Deinococcus-Thermus, Chloroflexi, Firmicutes, and Bacteroidetes involved in different stages of organic matter production and degradation in microbial mats. According to metagenomic analysis, there is a wide variety of natural peptidases, enhancing the potential of their specialised functions of energy and substance conversion in microbial mats.

1. Introduction

In recent decades, many studies have focused on bacteria from extreme habitats. The study of microbial communities from areas of modern volcanic activity and relict biotopes, and formation of terrestrial biota at the molecular level reveals new phylogenetic lineages of prokaryotes and greatly enriches modern fundamental microbiology [1-5].

The trophic system of a microbial community is complex of functionally different, interacting microorganisms, which have specialized enzymes for energy and matter conversion. Proteolysis, despite the uniformity and seeming simplicity of the reactions, is one of the most multi-stage processes in the functioning of biological systems, involving a large number of various proteolytic enzymes and many factors that regulate their activity [6]. The organic matter of any living system includes proteins; therefore, the proteolytic pathway is important for in situ analysis of the trophic system of the microbial community.

At present, the composition of the thermophilic microbial community and its potential metabolic interactions in the microbial community of the BRZ’ systems with extreme conditions are poorly studied, although they are fundamentally important for understanding the structural and functional relationships in ecosystems.

The purpose of this work is to determine the structure of the thermophilic microbial community in the Uro hot spring and to analyse the diversity of peptidases.
2. Objects and methods
The Uro hot spring is located within BRZ (54°19’203” N and 110°59’646” E, altitude 674 m a.s.l.). Thermal water discharge is observed over an area of 200 m². The water is of hydrocarbonate-sulphate-sodium type, low-mineralized (276 mg/dm³), with pH 8.4-9.3 and a temperature of 51.2-70.7°C.

The study of taxonomic diversity and the search for peptidases in the microbial community were performed at “Biospark”, LLC, Moscow.

The search for peptidases in the microbial community was conducted in several stages:
- Isolation of total DNA from the microbial community in 3 stages: cell destruction, washing, and precipitation;
- Creation of a library using a commercial NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, US) in strict accordance with the manufacturer’s recommendations (https://www.neb.com/products/e7370-nebnext-ultra-dna-library-prep-kit-for-illumina);
- Sequencing of the library using the Illumina MiSeq platform;
- Bioinformatic analysis of the data obtained to characterize the diversity of peptidases in the microbial community of Uro.

The assembly of contigs was carried out using the SPAdes v3.9.1 software package (http://bioinf.spbau.ru/spades). A list of all identified types of peptidases was compiled based on the resulting annotation [7].

3. Results and discussion

3.1. The structure of the thermophilic microbial community
Microbial mats were sampled at temperatures of 62°C (Ur-3) and 56°C (Ur-4). The taxonomic composition of the major groups of microorganisms in the Uro hot spring was first described using the amplicon pyrosequencing approach; it was found to be represented by various phylogenetic groups. The presence of a significant proportion of bacteria involved in different stages of formation and destruction of organic matter in microbial mats has been established. Analysis of the samples showed that the composition of microbial mats is similar and is represented by typical species of bacterial mats of low-mineralized fluids. At the sample Ur-3, representatives of the phyla Cyanobacteria (32%), Proteobacteria (25%), Deinococcus-Thermus (11%), Chloroflexi (9%), and Bacteroidetes (7%) were dominating.

Phylum Cyanobacteria unites gram-negative prokaryotes that carry out oxygenic photosynthesis. Cyanobacteria were presented in our study mat by orders Chroococcales, Oscillatoriales, and Stigonematales. 24% of the obtained sequences were attributed to Proteobacteria and represented in the studied community by α-, β-, γ- and δ-classes. Phylum Deinococcus-Thermus (11%) was represented by bacteria of the order Thermales, genus Meiothermus. These bacteria are usually responsible for aerobic degradation of organic matter in the microbial community inhabiting a high-temperature zone of hot springs [8].

Chloroflexi (34%) dominated in the community of the sample Ur-4 and presented order Chloroflexales (29%). The metabolic flexibility of various representatives of the phyla Chloroflexi allows us to determine them as a mat-forming component in many investigated hot springs and contributes to their competitiveness for nutrients and/or physical space [9]. Cyanobacteria (17%) were represented by the genus Chlorogloeopsis only. Another dominant component of the community was Proteobacteria (14%) among which the classes α-Proteobacteria and γ-Proteobacteria prevailed. 11% of the sequences belong to Deinococcus-Thermus, order Thermales. A large number of bacteria of this group are responsible for the first stage in the destruction of organic matter in natural habitats. Phyla Chlorobi and Firmicutes each accounted for 6% of the community. Armatimonadetes (4%) had no any cultivated representatives among the closest homologs. The phylogenetic diversity and a large number of metabolically divergent groups indicate a balanced and complex structure of the community, where each group occupies a separate ecological niche.
3.2. Metagenomic analysis of peptidases in Uro microbial mats

In natural habitats, hydrolytic bacteria occupy the niche of primary destructors due to their ability to grow heterotrophically on biopolymers of various nature.

As a result of the metagenomic analysis, the diversity of peptidases in natural samples of the studied microbial mats has been revealed. Table 1 presents the data of bioinformatic analysis for the studied samples.

Based on the analysis of the collected metagenomic sequences, we systematized and characterized the identified peptidase genes in the microbial mats of the Uro hot spring (figure 1).

![Figure 1. Diversity of peptidases in microbial mats Ur-3 and Ur-4 on basis of identified peptidase genes.](image)

Comparison of metagenomic sequences of representative data showed a predominance of enzymes of the serine peptidase class. Serine peptidases are known to be generally active at neutral and alkaline medium, with pH optimum at 7-11 [10].

The metagenomic analysis also revealed the wide distribution of peptidases of the metallopeptidase class. The optimum pH values for metallopeptidases are within the range of 7 to 9. Metallopeptidases are also known to have thermostability properties [11].
Table 1. Contig assembly statistics for each sample.

| Sample | Ur-3          | Ur-4          |
|--------|---------------|---------------|
|        | Number of contigs | %     | Cumulative length of contigs | %   |
|        | 488,049       | 477,523,726  | 107,747                   | 93,329,928 |
|        | Median of lengths | 326 | Average length | 978 |
|        | Maximum length | 2,110,788    | 632,882                   |
|        | N50           | 3,712        | 4,215                     |
|        | N90           | 294          | 272                       |
|        | Number of annotations | 376,788       | 78,185 |
|        | Coding        | 370,394      | 76,782                     | 98.30 | 98.21 |
|        | tRNA          | 4,550        | 976                       | 1.21 | 1.25 |
|        | tmRNA         | 70           | 17                        | 0.02 |
|        | Iterations    | 1,774        | 410                       | 0.47 | 0.52 |
|        | Others        | –            | –                         | 0.00 |
|        | With title    | 149,382      | 31,876                     | 39.65 | 40.77 |
|        | With EC enzyme code | 101,934      | 21,491                     | 27.05 | 27.49 |

Single sequences of genes of the class of cysteine peptidases were found in the microbial community Ur-3. Perhaps cysteine peptidases are effective at neutral and weak acid medium and depending on the nature of protein. The microbial communities under the study develop in high-temperature zone and alkaline pH range. Threonine peptidase was expectedly found in the microbial community Ur-4. Threonine peptidases belong to the class of proteolytic enzymes. The prototype of this class of enzymes is peptidases – catalytic subunits of proteasomes [12], which are intracellular complexes that degrade proteins.

Serine, metallo- and cysteine peptidases are the dominant (~90%) intracellular and extracellular proteolytic enzymes of archaea and bacteria, while aspartic and threonine peptidases account for <10% of the total [13].

4. Conclusion

Thus, based on the metagenomic analysis, we obtained data indicating a high diversity of natural peptidases, which indicates a high potential of specialized functions of peptidases in the conversion of energy and matter in microbial mats. In the metagenomic sequences, potential peptidases were identified, which may be of biotechnological interest in various fields of industry, medicine, and bioremediation.

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