Taxonomical and functional diversity of microbial communities in two hot springs of the Baikal rift zone

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Abstract. The Baikal Rift Zone hosts many hot springs with a wide range of temperature and physical-chemical conditions, which may harbour different niches for the distribution of microbial communities. We investigated microbial community composition and their functional activity in two alkaline hot springs with a temperature range of 34.4 to 73.6°C. Comparative analysis of the composition of the dominant taxa showed significant differences depending on the collection sites. In the community of high-temperature zones with a water temperature of 55-64°C, a high proportion of thermophilic bacteria Acetothermia (up to 57.9%), Deinococcus-Thermus (up to 50%), and Aquificae (up to 10.8%). Proteobacteria (29-77%) and Firmicutes (15-26%) dominate in the sulphide-free Garga spring (73-75°C). The functional analysis of the microbial community showed that the primary producers are cyanobacteria, anoxygenic phototrophs, and chemolithotrophic bacteria. At the terminal stages of the mineralization of organic matter, sulphate-reducing bacteria are the main destructors in the microbial communities in hot springs. The cyano-bacterial and sulfidogenic microbial communities play an important role in the formation of geochemical barriers and mineral formation.

1. Introduction
Terrestrial springs are extreme natural systems that are characterized by high temperatures and the inflow of reduced subsurface substances. The biological community of springs consists mainly of prokaryotes (eubacteria and archaebacteria). Various functional groups in microbial communities are linked by close trophic relationships, which allow them to effectively participate in the transformation of organic and inorganic substances. Fluids are convenient model systems for studying the ecology and diversity of extremophilic prokaryotes. Modern trends in the study of microbial communities are based on the widespread use of molecular biology methods. The microbial community in terrestrial hydrothermal systems has been extensively studied worldwide, including Yellowstone (USA), Tibetan Plateau (China), Iceland, Italy, and Russia [1-5].

The Baikal rift zone (BRZ) contains a large number of mineral springs with different physical, chemical, and gas properties. Our earlier studies of the microbial mats of the BRZ hydrothermal vents revealed cultivated and uncultivated species of bacteria of various physiological groups [4, 5].

The purpose of this study was to explore microbial diversity, the functional activity, and the geochemical role of microbial communities in various biotopes of two hot springs of the Baikal rift zone.
2. Material and methods

For the study, samples of water, bottom sediments, and microbial mats were taken from the hot springs of the Baikal rift zone: Alla (54°41'735'' N, 110°44'710'' E), Garga (54°19'203'' N, 110°59'646'' E) in the summer of 2014. Seven collection sites were selected along the temperature gradient of one major effluent channel each for both Alla Spring (Al-0, Al-1, Al-2, Al-3, and Al-5) and Garga Spring (Ga-1 and Ga-2) (table 1).

Temperature, pH, redox potential (Eh), and TDS (total dissolved solids) were measured at the sampling sites.

Microbial and functional diversity was studied using molecular genetic and isotopic methods. Total DNA was isolated using commercial DNA-sorb kits (AmpliSens, Russia) and a Bacterial Genomic DNA kit (Axygen, USA). Metagenomic analysis of V3-V4 variable regions of 16S rRNA was carried out on a MiSeq sequencer (Illumina, USA) at the Genomics Collaborative Use Centre (Novosibirsk). The sequences obtained were compared with the sequences from the GenBank database using the BLAST program (http://www.ncbi.nlm.nih.gov/blast).

The rates of photosynthesis (PS), dark assimilation of carbon dioxide (DA), and hydrogenotrophic methanogenesis (MG) were determined in isolated samples with radioactive NaH$^{14}$CO$_3$; sulphate reduction (SR) was determined using the labelled Na$_2^{35}$SO$_4$ [6].

The rates of microbial processes were calculated according to the formula:

$$A = r \times [C] \times 24 \div (R \times t), \text{ gC dm}^{-3} \cdot \text{day}^{-1}$$

(1)

where: $r$ – radioactivity of metabolite, count$\cdot$min$^{-1}$; $[C]$ – natural substrate concentration, g$\cdot$dm$^{-3}$; 24 – day, hours; $R$ – radioactivity of injected substrate, count$\cdot$min$^{-1}$; $t$ – time of incubation, hours.

3. Results and Discussion

3.1. Physical-chemical characteristics of the sampling site in the hot springs

The water of the springs had a temperature of 34.4-73.6°C, pH ranged within 8.3-9.7.

The ionic composition of the springs was characterized as sulphate-hydrocarbonate-sodium and sulphate-sodium.

The values of redox potential (Eh) varied from $-297$ to $+55$ mV. Total dissolved solids (TDS) of thermal spring water did not exceed 800 ppm. The sulphide content in water of the hot Alla Spring was equal to 1.25-13.5 mg$\cdot$l$^{-1}$ (table 1).

| Table 1. Physical-chemical parameters of springs water. |
|-------------------------------------------|
| Hot spring | Sites | $t$, °C | $pH$ | $Eh$, mV | TDS, ppm | $S^2$, mg$\cdot$l$^{-1}$ | Type of water |
| Alla       | Al-0  | 68    | 9.8  | $-297$ | 280     | 13.5                | $SO_4^- - HCO_3^- - Na$ |
|           | Al-1  | 64.4  | 9.7  | $-297$ | 270     | 3.35                | $SO_4^- - HCO_3^- - Na$ |
|           | Al-2  | 38.9  | 9.8  | $-250$ | 238     | 5.5                 | $SO_4^- - HCO_3^- - Na$ |
|           | Al-3  | 58.1  | 9.6  | $-267$ | 184     | 1.25                | $SO_4^- - HCO_3^- - Na$ |
|           | Al-5  | 34.4  | 9.26 | $-250$ | 144     | 0                   | $SO_4^- - Na$ |
| Garga      | Ga-1  | 73.6  | 8.3  | $+30$  | 700     | 0                   | $SO_4^- - Na$ |
|           | Ga-2  | 55.2  | 8.75 | $+55$  | 744     | 0                   | $SO_4^- - Na$ |
3.2. Microbial communities

The Alla Spring (t 34.4 – 68°C / pH 9.26-9.93 / S² 1.25-13.25 mg·l⁻¹) pours out in the bed of the river of the same name and has thermal sulphate-hydrocarbonate-sodium water, the temperature reaches 75°C.

The microbial community of water at high temperatures (sample Al-1w, 64.4°C, S² 13.5 mg·l⁻¹) was dominated by representatives of the phylum Deinococcus-Thermus (55.5%). Phototrophic bacteria Chloroflexi (10%), and thermophilic bacteria Aquificae (7.7%) were co-dominants. Gammaproteobacteria (5.9%), Cyanobacteria (5.4%), Firmicutes (5%), Acetothermia (4.6%), and Nitrospirae (3.5%) are represented in approximately equal. Archaea accounted for 0.25% of the total community. At lower temperatures, the microbial community of water Al-2w (38.9°C / S² 3.35 mg·l⁻¹) and Al-3w (58.1°C / S² 5.5 mg·l⁻¹) was dominated by Cyanobacteria, whose proportion reached 55.7 and 70.7% respectively. The share of Gammaproteobacteria increased and the share of thermophilic bacteria Deinococcus-Thermus decreased. Archaea have 0.2% of the total community been identified.

The diversity of the microbial community was studied in five sediment samples, where the temperature varied from 45 to 68°C, pH 9.7-9.9. The microbial community was dominated by representatives of the phyla Acetothermia, Firmicutes, Proteobacteria, and Chloroflexi, the ratio of which varied depending on the sampling site. Archaea were found in all sediment samples (up to 4.7%). The microbial communities of sediments in samples from the high-temperature zones Al-0s (68°C) and Al-1s (64.4°C) had a similar structure: representatives of Acetothermia (31.6 and 18.3%, respectively), Firmicutes (28.6 and 38.5%, respectively) and Gammaproteobacteria (16.5 and 24.5%, respectively), Bacteroidetes (4.2 and 7.4%, respectively). The structure of the community of samples of sediments Al-2s (45°C) differed from the structure of samples from the high-temperature zone in that the codominant representatives of Acetothermia are Deltaproteobacteria (14.9%), as well as phototrophic Chloroflexi (6.2%).

The Al-3s sediment community (64.2°C) differed from the community of other accessions by the dominance of Acetothermia sequences (58%). The phyla Atribacteria (11.2%), Aminicenantes (9.5%), Chloroflexi (6.3%), Nitrospirae (4.6%) were next in prevalence in this sample.

The Garga Spring (t 55.2-73.6°C / pH 8.3-8.8) has thermal sulphate-sodium water. The water temperature reaches 73.6°C. Water flowing down the creek forms travertine up to 50 m long and up to 25 m wide. The composition of the travertines is almost entirely carbonate-calcium, with high SiO₂ content.

The dominant groups of bacteria in the microbial community of water at the outlet at 73.6°C (Ga-1w) were representatives of Gammaproteobacteria with 77.9% of the sequences. The proportion of Firmicutes bacteria was also high at 15.6%. In the microbial community of sediments at 75°C (Ga-1s), Gammaproteobacteria (29.3%) and Firmicutes (26.7%) were equally represented, with Bacteroidetes (15.7%) being the next numerous representatives. The thermophilic representatives Deinococcus-Thermus and Aquificae accounted for 7.7 and 6.6% respectively.

The dominant bacterial groups in the microbial community of the spring creek water (Ga-2w, 55.2°C) were representatives of the phyla Deinococcus-Thermus (50%), Proteobacteria (20%), and Aquificae (10.8%).
Table 2. Characteristics of microbial communities of BRZ hot springs  
(w – water, m – microbial mat, s – sediments).

| Hot spring site | site | $t$, °C | $pH$ | TDS, ppm | The main phyla, % |
|-----------------|------|---------|------|-----------|-----------------|
| Alla Al-1w      |      | 64.4    | 9.7  | 0.25      | Deinococcus-Thermus 50 |
|                 |      |         |      |           | Chloroflexi 9.9   |
|                 |      |         |      |           | Aquificae 7.7    |
|                 |      |         |      |           | Gammaproteobacteria 5.9 |
|                 |      |         |      |           | Cyanobacteria/Chloroplast 5.4 |
| Al-2w           | 38.9 | 9.9     | 0.30 |           | Cyanobacteria/Chloroplast 55.7 |
|                 |      |         |      |           | Gammaproteobacteria 18.3 |
|                 |      |         |      |           | Chloroflexi 5.3    |
| Al-3w           | 58.1 | 9.6     | 0.34 |           | Cyanobacteria/Chloroplast 70.7 |
|                 |      |         |      |           | Gammaproteobacteria 8 |
|                 |      |         |      |           | Chloroflexi 4.5    |
| Al-1m           | 41   | 9.6     | 0.25 |           | Atribacteria 28.4 |
|                 |      |         |      |           | Nitrospirae 27     |
|                 |      |         |      |           | Chloroflexi 16.2   |
|                 |      |         |      |           | Deinococcus-Thermus 4.9 |
| Al-3m           | 58.1 | 9.93    | 0.34 |           | Chloroflexi 56     |
|                 |      |         |      |           | Nitrospirae 9.7    |
|                 |      |         |      |           | Deltaproteobacteria 2.1 |
|                 |      |         |      |           | Firmicutes 2.1     |
| Al-5m           | 34.4 | 9.26    | 0.35 |           | Deltaproteobacteria 27 |
|                 |      |         |      |           | Gammaproteobacteria 26.4 |
|                 |      |         |      |           | Alphaproteobacteria 16.3 |
|                 |      |         |      |           | Acidobacteria 17.5 |
| Al-0s           | 68   | 9.8     | 0.28 |           | Acetothermia 31.6 |
|                 |      |         |      |           | Firmicutes 28.6    |
| Al-2s           | 45   | 9.7     | 0.30 |           | Gammaproteobacteria 16 |
|                 |      |         |      |           | Acetothermia 32.2 |
|                 |      |         |      |           | Deltaproteobacteria 14.9 |
|                 |      |         |      |           | Chloroflexi 6.2   |
|                 |      |         |      |           | Acetothermia 57.9 |
|                 |      |         |      |           | Chloroflexi 6.3   |
|                 |      |         |      |           | Nitrospirae 4.6 |
| Garga Ga-1w     |      | 73.6    | 8.3  | 0.88      | Gammaproteobacteria 77.9 |
|                 |      |         |      |           | Firmicutes 15.6    |
|                 |      |         |      |           | Deinococcus-Thermus 2.3 |
|                 |      |         |      |           | Gammaproteobacteria 49.5 |
|                 |      |         |      |           | Aquificae 10.8 |
| Ga-2w           | 55.2 | 8.75    | 0.98 |           | Deinococcus-Thermus 1.7 |
|                 |      |         |      |           | Gammaproteobacteria 49.5 |
|                 |      |         |      |           | Aquificae 10.8 |
| Ga-2m           | 55   | 8.5     | 0.98 |           | Proteobacteria 46 |
|                 |      |         |      |           | Cyanobacteria 31.7 |
|                 |      |         |      |           | Deinococcus-Thermus 10.6 |
|                 |      |         |      |           | Gammaproteobacteria 29.3 |
|                 |      |         |      |           | Firmicutes 26.7    |
|                 |      |         |      |           | Bacteroidetes 15.7 |
|                 |      |         |      |           | Deinococcus-Thermus 7.7 |
|                 |      |         |      |           | Aquificae 6.6     |
| Ga-1s           | 75   | 8.8     | 0.88 |           | Bacteroidetes 49 |
|                 |      |         |      |           | Alphaproteobacteria 33.4 |
The majority of *Deinococcus-Thermus* was represented by bacteria of the genus *Thermus* (94.6%), the genera *Meiothermus* and *Truepera* constituted 5.2% and 0.1%, respectively, of the microbial community. The dominant genera of the phylum *Proteobacteria* in water were *Enhydrobacter* (12.2%, class *Gammaproteobacteria*) and *Acinetobacter* (2.6%, class *Gammaproteobacteria*). The phylum *Aquificae* was represented by the genus *Hydrogenobacter*. Bacteria of this genus are obligate chemolithoautotrophs that oxidize hydrogen and are characteristic of thermal ecosystems [1].

Analysis of the taxonomic composition of the Ga-2m microbial mat community developing in a hot creek along the outlet showed that the community was dominated by representatives of three phyla: *Proteobacteria* (46%), *Cyanobacteria* (31.7%), and *Deinococcus-Thermus* (10.6%). Among *Proteobacteria*, aerobic chemoorganotrophic bacteria of the genus *Sandaracinobacter* (12.2%, class *Alphaproteobacteria*) and thermophilic chemolithoautotrophic bacteria of the genus *Elioraea* (5.7%, class *Alphaproteobacteria*) dominated. Thermophilic organotrophic bacteria of the genus *Meiothermus* dominated in the phylum *Deinococcus-Thermus* (99%).

The phyla *Proteobacteria* (42%) and *Bacteroidetes* (49%) were most abundant in the microbial community of Ga-2s sediments (55.2°C). *Alphaproteobacteria* accounted for 33.4% of the community. They were represented by bacteria of the genus *Caulobacter* (20.2%), which are widely distributed in oligotrophic freshwater lakes and streams, the chemoheterotrophic obligate aerobic bacteria *Sphingomonas sp.* (9.1%). The phylum *Bacteroidetes* was 95% represented by the genus *Sediminibacterium* (family *Chitinophagaceae*).

Our results are consistent with data on the taxonomic diversity of the hot springs of Yellowstone, Tibetan Highlands, and India. *Deinococcus-Thermus*, *Proteobacteria*, or *Firmicutes* dominate in the alkaline hot springs [1, 7]. Some mesophilic bacteria including *Cyanobacteria*, *Chloroflexi*, or *Proteobacteria* are vital members in lower temperatures.

### 3.3. The rate of microbial processes

The rate of oxygenic and anoxygenic photosynthesis in microbial mats of alkaline hot springs of the Baikal rift zone varies widely and was equal to 0.007-1.27 gC m⁻² ⋅ day⁻¹ and 0.01-0.46 gC m⁻² ⋅ day⁻¹, respectively (table 3). The value of dark carbon dioxide fixation was equal to 0.06-1.15 gC m⁻² ⋅ day⁻¹. The maximum rate of dark fixation of CO₂ – 1.69 gC m⁻² ⋅ day⁻¹ was detected in the hydrogen sulphide thermal Alla spring.

| Hot spring | PS* (gC m⁻² ⋅ day⁻¹) | DA** (gC m⁻² ⋅ day⁻¹) | SR* (gS m⁻² ⋅ day⁻¹) | MG* (µgC m⁻² ⋅ day⁻¹) |
|------------|-------------------|-----------------|-----------------|-----------------|
| Oxygentic  | Anoxygentic       |                 |                 |                 |
| Garga      | 0.01 - 1.27       | 0.03 - 0.46     | 0.06 - 1.15     | 0.004 - 2.13    | 1.78 - 1,560.0 |
| Alla       | 0.007 - 0.278     | 0.01 - 0.12     | 0.28 - 1.69     | 0.01 - 4.1      | 0.28 - 1.7     |

*Photosynthesis; **Dark assimilation of CO₂; *Sulphate reduction; *Methanogenesis

The terminal stages of organic matter destruction are the microbial processes of sulphate reduction and methanogenesis competing for electron donors.

Sulphate reduction in the mats of alkaline hydrotherms reaches 4.1 gS m⁻² ⋅ day⁻¹ and creates conditions for the active functioning of the sulfur cycle microorganisms in the community. The rates of methanogenesis are low (up to 1.5 mgC m⁻² ⋅ day⁻¹).

In the hot spring sediments, the intensity of the sulphate reduction process varied from 0.002 to 0.7 mgS dm⁻³ per day (Table 4). In the sediments, the intensity of methanogenesis does not exceed 29.8 µlCH₄ dm⁻³ ⋅ day⁻¹. The most intense methane is formed in the Alla hot spring. Most of the methane was synthesized from carbon dioxide and hydrogen, and no more than 30% of the resulting methane was formed from acetate. The most intense terminal degradation processes occurred at temperatures below 50°C. In the high-temperature Garga spring (52-74°C), the process intensities were minimal.
Table 4. The rate of sulphate reduction (SR) and methanogenesis (MG) in the hot spring sediments.

| Hot spring | $t$, °C | $pH$ | $SO_4^{2-}$, mg l$^{-1}$ | $S^2-$, mg l$^{-1}$ | SR, mgS dm$^{-3}$ day$^{-1}$ | MG, µl CH$_4$ dm$^{-3}$ day$^{-1}$ |
|------------|--------|------|----------------------|-------------------|-----------------------------|-----------------------------|
| Alla       | 35-71  | 9.9  | 49 - 86.4            | 5 - 16.5          | 0.002 - 0.3                 | 0.35 - 29.8                 |
| Garga      | 67-74  | 8.4  | 110.5 - 270.5        | 0.1 - 1.0         | 0.046                       | 1.55                        |

Quantitative estimation of microbial activity showed that cyanobacteria, anoxygenic phototrophs, and chemolithotrophs are the main primary producers. Analysis of microbial communities mats taxonomic composition of hot springs, the ratio of the main bacteria groups and its functional activity showed a change of microbial community with decreasing temperature. Chemosynthetic thermophilic microbial community, which develops at 65°C are changed by photosynthetic thermophilic communities at 50°C with anoxygenic photosynthesis predominance. Following a decrease in temperature to 40°C leads to increasing oxygenic photosynthesis and dominance of moderately thermophilic/mesophilic communities. Most rate of the organic matter production is observed in the range from 40 to 50°C. Microbial mat producers assimilated atmospheric and volcanogenic CO$_2$, which is more enriched with $^{13}$C. Fractionation of stable isotopes was varied from +8.62 to +25.25‰. In bottom sediments, most CO$_2$ carbons were fixed due to chemosynthesis – up to 83%. The contribution of heterotrophic microorganisms to CO$_2$ assimilation did not exceed 17%. Sediment microbial community producers synthesize organic matter using volcanogenic carbon dioxide enriched with the heavy isotope $^{13}$C [8].

At the terminal stages of organic matter mineralization in BRZ hot springs sulphate reducers are the main organic matter destructors in the microbial communities of hot springs. The sulfidogenic microbial community plays an important geochemical role by participating in the oxidation of organic matter under anaerobic conditions and the synthesis of carbon dioxide and hydrogen sulphide. In hot springs, sulphate reducers are involved in the formation of geochemical barriers on which concentration, component solubility, and pH gradients are created. The formation of geochemical barriers in the microbial mat leads to the deposition of some minerals, such as calcites and pyrites [9].

4. Conclusion

Using high throughput sequencing of the 16S RNA gene of microbial communities of water, microbial mats, and bottom sediments of BRZ hot springs and studying the metabolic activity of the community revealed a wide range of thermophilic and mesophilic microorganisms. They are capable of utilizing a wide variety of carbon sources. The thermophilic bacteria of phyla Acetothermia and Deinococcus-Thermus, as well as a significant proportion of extremely thermophilic representatives of phylum Aquiferae, constituted a significant part of the microbial community of high-temperature zones. In the microbial mats at high temperatures, the anoxygenic Chloroflexi bacteria were the producers, while at lower temperatures the oxygenic Cyanobacteria were the producers. The thermophilic bacteria of phyla Acetothermia and Deinococcus-Thermus, as well as a significant proportion of extremely thermophilic representatives of phylum Aquiferae, were a significant part of the microbial community of high-temperature zones. In the microbial mats at high temperatures, the anoxygenic bacteria Chloroflexi were the producers of organic carbon, while at lower temperatures the oxygenic Cyanobacteria were the producers. The highest rates of organic carbon production are observed in the range from 40 to 50°C. The main terminal process of destruction in BRZ hot springs is sulphate reductions, the intensity of this process reaches high values. The data obtained are important for assessing the biogeochemical potential of the microbial community and the ecological role of prokaryotes in extreme habitats.

The presence in the microbial community of bacteria and archaea with a wide metabolic potential, the ability to use various types of both organic and inorganic compounds, and a diverse range of temperature adaptation makes this microbial consortium important for biotechnological applications.
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