Freshwater microbial taxa as indicators of anthropogenic impact on the freshwater lakes of Buryatia

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Abstract. Three large freshwater lakes located in the Baikal region (Gusinoye, Shchuchye, and Kotokel) with high touristic significance were studied. Increased anthropogenic pressure is one of the most important causes of water quality deterioration (in particular, high solute and nutrient loads, high productivity). The structural features of microbial communities associated with an increased anthropogenic load have been revealed in different environmental gradients. It was shown that the genera Rhodobacter, Polynucleobacter, and Luteolibacter preferred environments with a higher trophic level and are indicators of possible anthropogenic impact.

1. Introduction

Bacteria are a major component of freshwater ecosystems that play a key role in essential nutrient cycles, such as primary production in aquatic food chains and decomposition of organic compounds [1, 2]. The microbial community is interconnected with water quality parameters and other conditions; it responds rapidly to disturbances in the environment [2]. The influence of nutrients on structural changes of microbial community has been well studied in recreational and urban freshwater ecosystems, where changes of bacterial compositions were observed along the nutrient loading gradients [1, 3].

Lakes Gusinoye, Shchuchye, and Kotokel are large freshwater lakes located in the Baikal region, Republic of Buryatia (Russia). These lakes are used as a drinking water supply, for household needs, fishing, and recreational purposes. The lakes are often influenced by non-point pollution sources from surrounding watersheds, croplands, livestock pastures, forestland, residential areas, and as a result of recreational activities during the summer. Lake Gusinoye was and still is the most important source of drinking water for the Gusinoozersk city, and it has also been impacted by the Gusinozersk thermal power station (Gusinozersk GRES). Other sources of pollution are the treatment facilities of Gusinozersk, Gusinoye Ozero village, ash dumps of the GRES, locomotive depot, and coal industry enterprises. Numerous ecological studies have been carried out in connection with the touristic significance and trophic level changes of the lakes [4-6].

The goal of this study was to determine how the composition of microbial community changes under anthropogenic influence. To this end, we investigated the microbial communities of three freshwater lakes, and examined the entire microbial community by next-generation sequencing. We also assessed relationships between the structure of microbial communities, water quality, and environmental conditions, as well as identified the taxa in the microbial community most susceptible to anthropogenic impact.
2. Materials and methods
Lakes Gusinoye (164 km²; maximum depth 26 m), Shchuchye (4.24 km²; maximum depth 23 m), and Kotokel (68.9 km²; maximum depth 14 m) are large freshwater lakes located in Baikal region, Republic of Buryatia (Russia). A total of 30 water samples were collected from lakes in August 2018. Water temperature, salinity (TDS), pH, concentrations of nutrients, and dissolved oxygen were measured by portable instruments. Cation and anion content, chlorophyll $a$, and total organic matter concentrations were measured in a laboratory according to standard protocols.

Microbial diversity was studied by high-throughput sequencing using the Illumina MiSeq platform in three technical replicates. The research was done using the equipment of the Core Centrum “Genomic Technologies, Proteomics, and Cell Biology” in All-Russian Research Institute for Agricultural Microbiology. Bioinformatics analysis was performed using QIIME ver. 1.9.1 and SILVA software [7]. To reveal potential relationships between the environmental and sequencing datasets, canonical correspondence analyses (CCA) were conducted using the Excel data analysis add-on XLSTAT (Addinsoft) [8].

3. Results and discussion
The environmental parameters of the water samples are presented in table 1. In addition to these data, it should be noted that dissolved oxygen had normal variations (from 11.2 to 15.4 mg/l) without significant differences among the three lakes. The concentration of organic matter varied from 5.5 to 15.3 mg/l. The highest total organic matter concentration and high nitrogen concentration were found in the sample from Lake Kotokel with cyanobacterial bloom (Kotokel 1).

| Sample | Location | Depth, m | pH | $t$, °C | TDS, mg/l | NO$_3^-$, mg/l | SO$_4^{2-}$, mg/l | P$_2$O$_5$, mg/l |
|--------|----------|----------|-----|--------|----------|---------------|----------------|---------------|
| Shch1  | Western part of Lake Shchuchye | 0.5 | 8.42 | 25.0 | 274.92 | 0.55 | 9.88 | 0.10 |
| Shch2  | Centre of Lake Shchuchye | 5.0 | 8.16 | 22.7 | 281.35 | 0.46 | 9.52 | 0.08 |
| Shch3  | Southern part of Lake Shchuchye, 150 m from the beach | 2.5 | 7.71 | 22.6 | 279.83 | 0.78 | 9.88 | 0.08 |
| Gus1   | Northern part of Lake Gusinoye, warm water discharge | 0.5 | 8.45 | 26.0 | 380.99 | 0.23 | 63.30 | 0.07 |
| Gus2   | Northern part of Lake Gusinoye, near the city sewage outlet | 2.3 | 8.74 | 24.1 | 380.42 | 0.35 | 62.5 | 0.07 |
| Gus3   | Centre of Lake Gusinoye | 22.0 | 8.5 | 21.2 | 367.33 | 0.52 | 57.6 | 0.07 |
| Gus4   | Eastern part of Lake Gusinoye, beach | 1.5 | 8.61 | 21.4 | 364.24 | 0.35 | 45.65 | 0.14 |
| Kotoke1| Western part of Lake Kotokel, beach | 0.5 | 6.25 | 20.6 | 145.33 | 7.02 | 3.6 | 0.14 |
| Kotoke2| South-Western part of Lake Kotokel | 1.5 | 7.26 | 19.2 | 121.54 | 0.61 | 4.6 | 0.08 |
| Kotoke3| Centre of Lake Kotokel | 8.0 | 8.23 | 18.7 | 119.62 | 1.16 | 2.96 | 0.09 |

Microbial diversity was associated with water quality parameters and environmental conditions. At the phylum level, three analysed freshwater lakes had similar microbial community structures; several typical freshwater bacterial groups predominated, although there were also marked differences in their relative abundance (figure 1). The six most common bacterial taxa found in each lake are (from first to sixth): Bacteroidetes (range: 13.7–54.9%), Gammaproteobacteria (range: 17.7–59.8%), Actinobacteria (range: 6.7–27.9%), Alphaproteobacteria (range: 2.3–16.3%), Cyanobacteria (range: 0.6–30.3%) and Verrucomicrobia (range: 2.4–6.5%).
The relative abundance of Planctomycetes-related OTUs was higher in Lake Gusinoye (2.5±1.1%), and was lesser in lakes Shchuchye (1.0±0.3%) and Kotokel (0.8±0.5%). Cyanobacteria had higher relative abundance (30%) in the water sample from Lake Kotokel with high eutrophication and cyanobacterial bloom. Alphaproteobacteria were slightly more abundant in Lake Gusinoye (average for all sampling dates ca. 11.1% of the total abundance) than in lakes Shchuchye (ca. 5.9%) and Kotokel (ca. 4.1%). Their greatest diversity (up to 53 OTUs) was mainly represented by uncultivated bacteria. Considerable variations were observed in the microbial diversity of Gammaproteobacteria, Alphaproteobacteria, Bacteroidetes, Actinobacteria, and Cyanobacteria, where the bacterial community structure was greatly influenced by hydrological conditions and sample locations.

A more detailed bacterial community structure was demonstrated at the genus level. Briefly, dominant members (accounting for > 5% of the total sequences) were: Actinobacteria hgcl clade (18.2%), Flavobacterium (16.2%), Sediminibacterium (7.3%) and Polymucleobacter (6.5%) in Shchuchye Lake; Acinetobacter (14.3%), Flavobacterium (13.1%), Actinobacteria hgcl clade (10.1%) and Sediminibacterium (6.2%) in Kotokel Lake; and Flavobacterium (12.8%), Pseudomonas (12.2%), hgcl clade (10.6%) and Azolla (5.8%) in Lake Gusinoye.

In the sample Gus2 located near the mouth of the Zagustai River microbial community was characterized by an increased anthropogenic influence due to the runoff of household waste, the share of bacteria from the genus Rhodoferax increased up to 9.4%.

After analysing the microbial diversity in general, the distribution patterns of some cosmopolitan freshwater taxa were determined. These freshwater taxa were represented by the Actinobacteria hgcl clade and genus Aquilina, the Bacteroidetes genera: Algoriphagus, Arcicella, Flavobacterium, Fluvicola, and Sediminibacterium, the Proteobacteria lineage SAR11, and genera: Hydrogenophaga, Polynucleobacter, Rhodobacter, Rhodoferax, Sphingopyxis, and the Verrucomicrobia genus Luteolibacter. The distribution of these common freshwater taxa can reflect possible changes in microbial communities in different environmental gradients, a change in dominant taxa, andpatterns
of their distribution associated with an increase in anthropogenic load. Canonical correspondence analysis (CCA) was used to examine potential relationships between microbial community composition and the environmental parameters measured in the study (figure 2). Despite the almost ubiquitous distribution of the 17 studied freshwater genera, we observed significant differences between communities in habitats with high anthropogenic impact (beaches, sewage outlets, etc.) and samples without significant changes.

Figure 2. Canonical corresponding analysis (CCA) showing the relationships between bacterial communities and environmental parameters in three lakes.

Among the parameters, total dissolved solids (TDS) and sulphates were the major component of the first axis in the ordination plot, explaining 57% of the total variance, whereas water temperature, pH, nitrates, and phosphates were major components of the second axis, explaining 16% of the total variance. CCA showed strong correlations of *Fluvicola*, *Pseudomonas*, and *SAR 11* with TDS, while *Rhodobacter* was associated with sulphates concentrations. In addition, the two bacterial taxa, i.e. *Actinobacteria hgcI* clade and *Planktophila*, showed similar directions with temperature in the CCA plot. Previously, it was shown that the genera *Rhodobacter*, *Polynucleobacter*, and *Limnohabitans* preferred environments with a higher trophic level and were indicators of a possible anthropogenic impact [3]. In our study, correspondence analysis (CCA) revealed that the distribution of these freshwater taxa was determined by the influence of factors such as the content of organic matter, nitrates, and phosphates. With an increase in these parameters, the number of sequences representing these genera increased sharply.
4. Conclusion
Microbial diversity along with environmental parameters can be considered as a tool for assessing the status of freshwater lakes. The structural features of microbial communities associated with an increased anthropogenic load have been revealed. We identified some freshwater taxa that can serve as indicators of the quality of freshwater bodies.

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