

Characterization of photoautotrophic microbial communities in the coastal water of Lake Baikal

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ABSTRACT. In this study, we determined the concentration of chlorophyll *a* in biofilms formed on rocky substrates, in the surface water microlayer, water column, and near-bottom water layer of the coastal zone of Lake Baikal. High throughput sequencing of 16S rRNA and *rbcl* gene libraries revealed high genetic and taxonomic diversity of photoautotrophic microbial communities in the studied ecotopes of the lake. The UPGMA analysis indicated that the composition of photoautotrophic communities largely depends on both the geographical location and ecotope. Using electron microscopy, we described the structure and composition of biofilms.

Keywords: cyanobacteria, algae, 16S rRNA, RuBisCO, high throughput sequencing, Lake Baikal

1. Introduction

Photoautotrophic oxygenated microorganisms, mainly cyanobacteria and algae, convert carbon dioxide and water into an organic matter during photosynthesis using visible light as an energy source and, thus, release oxygen. Thanks to the activity of photoautotrophs, the existence of heterotrophic organisms that consume organic substances and aerobic organisms that require oxygen becomes possible. Moreover, cyanobacteria fix molecular oxygen and are highly resistant to the adverse factors caused by morphological and physiological properties. These unique properties of photoautotrophic microorganisms contribute to their ubiquitous distribution: they are the first to occupy various aquatic ecotopes, often forming benthic and plankton blooms as well as biofouling of different substrates.

We aimed to study the composition and structure of photoautotrophic microbial communities in the coastal zone of Lake Baikal using microscopy methods and high throughput sequencing of amplicons of 16S rRNA and RuBisCO (ribulose biphosphate carboxylase/oxygenase) genes.

2. Material and methods

Samples of biofilms from rocky substrates, surface water microlayer, water column, and near-bottom water layer were taken in August 2019 in the coastal zone opposite the settlements of Bolshiye Koty and

Bolshoye Goloustnoye. Chlorophyll *a* concentration was measured by spectrophotometric method (Jeffrey and Humphry, 1975). Genomic DNA was extracted using phenol chloroform solution. DNA amplification was performed using primers targeting V3 and V4 regions of the 16S rRNA gene (Martemyanov et al., 2016). The large subunit of RuBisCO (*rbcl*) gene fragments was amplified with previously published *rbcl* primers (Wawrik et al., 2002). Sequencing of 16S rRNA and *rbcl* gene libraries was conducted on a MiSeq genome sequencer (Illumina). Sequence fragments of 16S rRNA and *rbcl* gene were filtered, trimmed and merged using DADA2 R language package following standard operating procedure (SOP) (Callahan et al., 2016). Taxonomic assignment of 16S RNA gene fragments was performed with SILVA SSU database v. 132 (Quast et al., 2013). Sequence fragments of *rbcl* gene were annotated with using BLAST toolkit search. AlgaeBase was used for systematic and ecology information about the algae (Guiry and Guiry, 2020).

Scanning electron microscopy (SEM) was used for the detailed analysis of the biofilm structures (Sorokovikova et al., 2013).

3. Results and discussion

The results of determining the chlorophyll *a* concentrations as an indicator of the productivity of photoautotrophs revealed the maximum values in the upper and near-bottom water layers (2.78-5.88 µg/l).

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The minimum concentrations were recorded in the water column (1.34-4.3 µg/l). However, they were higher than those in the pelagic zone of the lake at that time. The chlorophyll a concentrations in biofilms averaged 342.7 µg/l near Bolshoye Goloustnoye and 445.7 near Bolshiye Koty. On the contrary, plankton showed the highest chlorophyll a concentration near Bolshoye Goloustnoye.

We obtained 169497 16S rRNA gene sequences with an average length of 418 bp. The phyla Cyanobacteria, Actinobacteria, Proteobacteria, Bacteroidetes, Verrucomicrobia, Firmicutes, Fusobacteria, and Acidobacteria dominated bacterial communities. The proportion of Cyanobacteria in the total number of sequences averaged 17.5%; the number of OTUs was 37.

Picoplankton species of the cluster *Synechococcus/Cyanobium* (14 OTUs) and *Dolichospermum lemmermannii* (3 OTUs) mainly represented cyanobacteria in neuston and plankton. Benthic and periphyton species, *Synechococcus* sp., *Calothrix* sp., *Tychonema* sp., and *Pseudanabaena* sp., were very few in the water. *D. lemmermannii* was the most numerous near Bolshiye Koty. Previously, we showed that this mass species in Baikal phytoplankton is a producer of paralytic mollusc toxins (Belykh et al., 2015). Among the three aquatic microbiocenoses, water column had the greatest biodiversity (16 OTUs).

In fouling of rocky substrates, we identified 20 OTUs of cyanobacteria belonging to the orders Nostocales, Oscillatoriales, Chroococcales, and Synechococcales. The main species were *Tychonema* sp., *Phormidium* sp., *Symplocastrum* sp., and *Pseudanabaena* sp. Near Bolshiye Koty, we detected *Chamaesiphon* sp., *Leptolyngbya* sp., *Synechococcus* sp., and *Calothrix* sp. In biofilms, chroococcal cyanobacteria, which are closely related to the clones that we previously detected in the fouling of sick sponges (Sorokovikova et al., 2020), as well as picoplankton cyanobacteria, were abundant

Based on the analysis of the chloroplast 16S rRNA gene, benthic diatoms and planktonic green algae prevailed in biofilms. In plankton, we detected *Nannochloropsis limnetica* (Eustigmatophyceae), *Nannochloris bacillaris* и *Choricystis* sp. (Chlorophyta), *Chrysochromulina parva* (Haptophyta), *Spirogyra* sp. (Charophyta), *Teleaulax* sp. (Cryptophyta), *Synura* sp. (Ochrophyta, Synurophyceae), Dictyochophyceae, and *Chlorokybus* sp. (Charophyta).

Analysis of fragments of the gene coding ribulose biphosphate carboxylase/oxygenase, the main enzyme of photosynthesis (rbcL), revealed the high algal diversity in the studied ecotopes. We obtained 34531 rbcL gene sequences with an average length of 554 bp. The number of OTUs in the samples was 141. Among them, 70 OTUs were diatoms, which were the most diverse in benthic samples (55 OTUs). In biofilms, members of the genera *Ellerbeckia*, *Amphora*, *Cocconeis*, *Nitzschia*, *Cymbella*, *Diploneis*, *Didymosphenia*, *Navicula*, and *Achnanthes* dominated. In plankton and neuston, haptophyte *Ch. parva* and Chrysophyta (*Ochromonas* spp., *Chrysophaerella* sp., *Mallomonas* spp., and *Urogenopsis* sp.) prevailed. Additionally, we also

often detected *Pedinella* spp. (Dictyochophyceae), *N. limnetica*, *Nitzschia draveillensis*, *Nitzschia* sp., *Ulnaria* sp. (previously *Synedra acus*), *Lindavia* spp., (previously *Cyclotella baicalensis* and *C. minuta*), *Fragilaria* sp. (Bacillariophyta), and *Teleaulax* (Cryptophyta).

According to the UPGMA analysis, algae and cyanobacteria from biofilms sampled near Bolshiye Koty and Bolshoye Goloustnoye form a common “benthic” clade, and planktonic and neustonic representatives form a common “aquatic” clade. Benthic communities tend to cluster depending on the geographical location, like planktonic and neustonic cyanobacteria from the Bolshiye Koty area (Fig.). Neustonic and planktonic algae do not manifest this.

The study of biofilms using the scanning electron microscopy indicated that diatoms and cyanobacteria were the structural basis of biofilms. In some cases, biofilms were mainly formed by diatoms of the genus *Cocconeis*, and in other cases – by filamentous cyanobacteria of the orders Nostocales and Oscillatoriales.

4. Conclusions

Cyanobacteria and algae in the coastal zone of Lake Baikal have high productivity from the bottom to the surface, including fouling of rocky substrates. High genetic and taxonomic diversity characterises photoautotrophic communities from various ecotopes of the coastal zone. Geographic location and ecological niche significantly affect the composition of communities. The main feature of the microbial communities from the coastal zone of Lake Baikal in the summer is a predominance of cyanobacteria. In plankton, picocyanobacteria and nitrogen-fixing potentially toxic representatives of Nostocales are numerous. In benthos, species of the order Oscillatoriales dominate. Diatoms, the most diverse and numerous among photoautotrophic eukaryotes, mostly inhabit rocky substrates. Like cyanobacteria, diatoms form complex and structurally organised biofilms. The sufficient supply of nutrients and trace elements from the sub-bottom water and substrates contributes to their successful development in the coastal zone of the lake.

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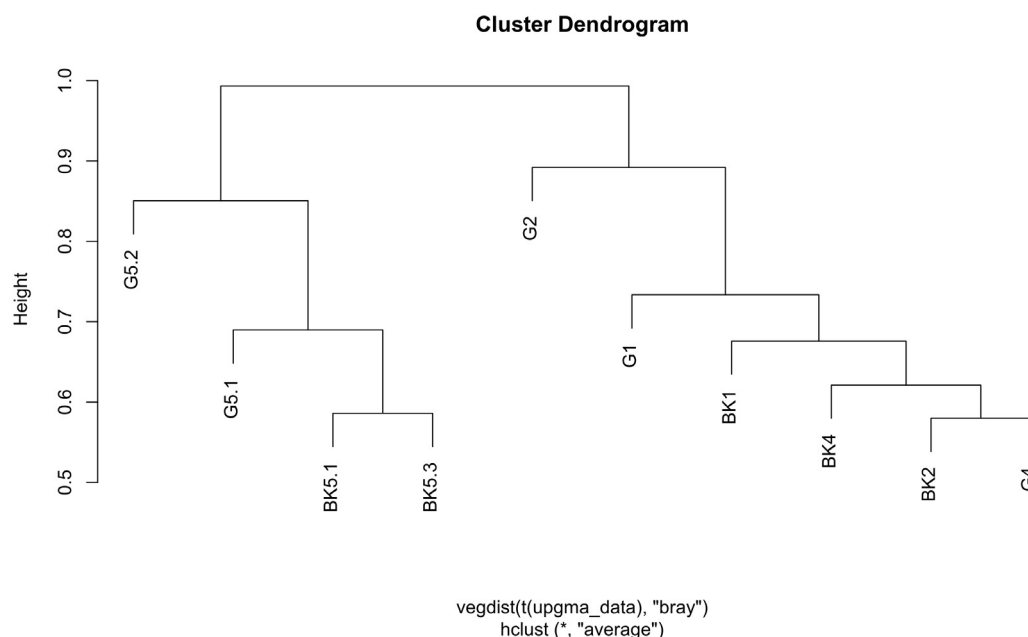


Fig. Dendrogram of the *rbcL* communities from water, surface microlayer and epilithic biofilms constructed using hierarchical cluster analysis (hclust) by the UPGMA method. G – Bolshoye Goloustnoye, BK – Bolshiye Koty; G1, BK1 – surface water microlayer, G2, BK2 – water column, G4, BK4 – near-bottom water, and G5.1, G5.2, BK5.1, BK5.3 – epilithic biofilms.

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