## BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

# Identification of Toxic Cyanobacteria in Lake Baikal

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**Abstract**—Cyanobacteria of the genera *Anabaena* and *Microcystis*, containing genes for the synthesis of microcystins (hepatotoxic cyanotoxins) were found for the first time in the coastal zone of Lake Baikal near the village of Turka, where a tourism and recreational complex were constructed. According to the enzyme immunoassay, microcystin concentration in water was $0.17 \pm 0.01$ µg/L. Using 16S rRNA gene pyrosequencing, we found 3936 sequences in the eubacterial community of central basin of Lake Baikal. The summer bacterioplankton in both littoral and pelagic areas of the lake was dominated by the phylum Cyanobacteria, whereas a higher diversity of cyanobacteria was recorded in the plankton of the littoral zone. Moreover, the potentially toxic *Anabaena* and *Microcystis* were detected in this area.

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Cyanobacteria, which are among the most ancient organisms on the Earth (approximately 3.8 billion years old), are widely spread in all ecosystems, including the extreme ones, due to their ability to combine two most important processes in the biosphere: oxygenic photosynthesis and molecular nitrogen fixation. These microorganisms synthesize a wide range of secondary metabolites, some of which are valuable biomedical compounds and some are toxins that are quite hazardous in cases of mass development of cyanobacteria. It was found that approximately 60% of cyanobacterial blooms are toxic [1].

The most common toxins in fresh waters are microcystins (MCs)—cyclic heptapeptides synthesized by cyanobacteria of the genera *Microcystis*, *Anabaena*, *Planktothrix*, *Anabaenopsis*, *Nostoc*, *Gloeotrichia*, and Hapalosiphon [1, 2]. Microcystins are produced by microcystin synthetase, multienzyme complexes encoded by a gene cluster of a modular structure (mcyA-J). Microcystins damage hepatocytes by inhibiting serine/threonine-specific phosphatases, which leads to hyperphosphorylation of cytoskeletal proteins in hepatocytes, loss of cell–cell contacts, and extensive hemorrhages in the liver [1, 2]. Currently, approximately 100 MC isoforms were identified, the most toxic of which is MC-LR [3]. According to WHO recommendations, MC-LR concentration in potable water should not exceed 1 µg/L for a single intake and 2–4 µg/L in bathing water [4].

Lake Baikal is the largest lake in the world, the natural reservoir of pure fresh water. It contains more than 80% of fresh waters of Russia and approximately 20% of world reserves. For the first time in Russia, we started molecular-biological studies of toxic cyanobacteria in Lake Baikal and water bodies of the Baikal region. In 2005, using genetic markers, we identified cyanobacteria containing the genes for MC synthesis in reservoirs of the Angara River [5, 6]. In Lake Baikal, we found no toxicogenic species at that period of research.

However, the presence of bays and shallow water areas in Lake Baikal that are well warmed in summer in combination with the steadily increasing anthropogenic pressure caused by intensive development of tourism and recreational activities and, as a consequence, the mass development of cyanobacteria, led to a continuous monitoring of plankton in the lake (2005–2010).

The aim of this work was to detect cyanotoxins in Lake Baikal by enzyme immunoassay (ELISA) and to identify the potentially toxic cyanobacteria using the genetic marker targeting the aminotransferase (AMT) domain encoded by the *mcyE* gene. This enzyme is a component of all known microcystin synthetases playing a key role in the MC biosynthesis [6]. To identify the dominant genotypes of cyanobacteria, we performed metagenomic analysis of the microbial community for the 16S rRNA gene fragment using the 454-pyrosequencing technology.

Plankton samples were collected in August 2010 in the central basin of Lake Baikal: in the pelagic area in the middle of the Ukhan–Tonkii section (depth 1562 m) and in the coastal area near the Turka village located at the confluence of the Turka River with the lake (depth 7 m). The estuarine area is characterized by a higher concentration of nutrients and phytoplankton biomass compared to the pelagic area [7]. In the vicinity of

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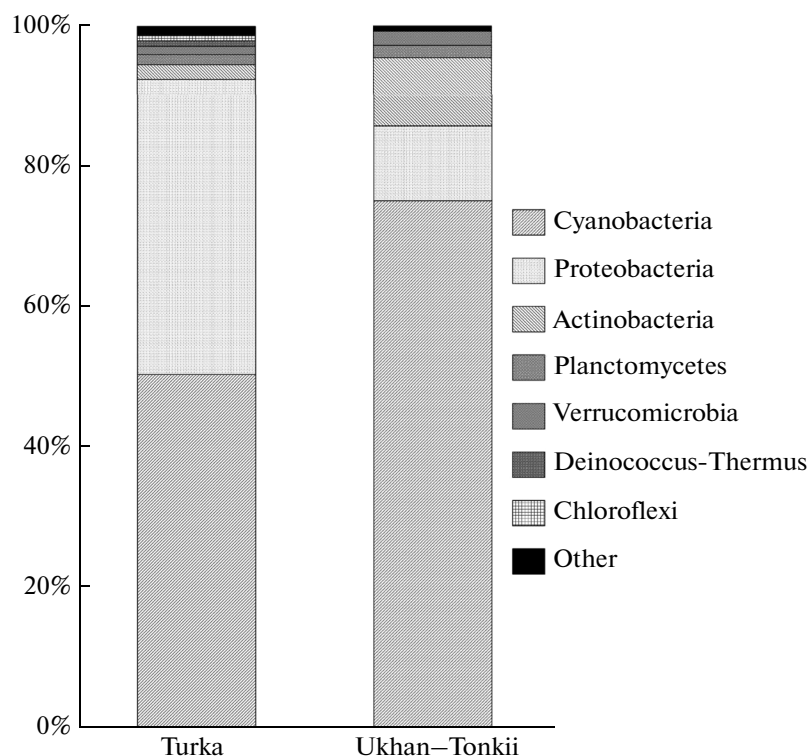
**Fig. 1.** Metagenomic analysis of bacterial communities of plankton from the coastal areas of the Turka Village and Ukhan–Tonkii section according to the results of pyrosequencing of the 16S rRNA gene fragment.

the Turka village, tourist and recreational complex "Baikal Harbour" has been constructed since 2010, which will also become the first modern port on the eastern coast of Lake Baikal.

Plankton samples were collected with bathometers from surface to a depth 25 m every 5 m. Simultaneously, the temperature and water clarity by Secchi disk were measured. For the molecular-genetic studies, samples (volume 1 L) were filtered through polycarbonate filters with a pore diameter of 0.2 μm (Millipore, United States). DNA was isolated by phenol-chloroform extraction. The encoded by the *mcyE* gene was identified using hepF and hepR primers as described in [6, 8]. The sequences were deposited in GenBank (accessions JF837306–JF837311). The 16S rRNA gene fragment was amplified using eubacterial primers 9F and 541R [9]. Pyrosequencing was peformed by the ChunLab Inc. company (Republic of Korea) on a Roche/454 Genome Sequencer FLX Titanium instrument (Roche Diagnostics Corporation, Switzerland). The nucleotide sequences were analyzed using the algorithm proposed earlier [6, 10].

The presence of MCs in water was determined by ELISA using the Microcystins-ADDA ELISA kit (Abraxis LLC, United States). The results were analyzed using the RIDA® SOFT Win software.

In August 2010, the water temperature in the pelagic area was 9.3°C on the surface and 5.9°C at a depth of 25 m; the transparency of water was 6 m. In the coastal zone near the Turka village, the water temperature reached 14°C on the surface and 12.8°C at the bottom; the transparency of water was 3.2 m.

In samples of the littoral zone, pyrosequencing revealed 1906 nucleotide sequences of the 16S rRNA gene fragment belonging to the domain Bacteria. The majority of sequences were referred to the phyla Cyanobacteria, Proteobacteria, Actinobacteria, Planctomycetes, Verrucomicrobia, Deinococcus-Thermus, Chloroflexi, Bacteroidetes, Acidobacteria, Firmicutes, and Armatimonadetes (Fig. 1). Eight sequences belonged to phantom phyla. In total, 191 new phylotypes were revealed; the Chao1 species richness was 370 (cluster spacing 0.03). The basis of the community was formed by the phyla Cyanobacteria and Proteobacteria, which accounted, respectively, for 50% and 42% of the total number of nucleotide sequences. The cyanobacteria were dominated by the picoplankton genera *Synechococcus* (729 nucleotide sequences), *Cyanobium* (119 nucleotide sequences), and *Cyanobacterium* (26 nucleotide sequences). Of the large-cell species, the coastal community includes *Anabaena flos-aquae*, *A. lemmermannii*, *A. solitaria*, *Gloeotrichia echinulata*, *Microcystis* sp., *M. aeruginosa*, *Chamaesiphon* sp., *Phormidium* sp., and *Tolypothrix* sp.

In the pelagic samples, 2030 nucleotide sequences of the 16S rRNA gene fragment belonging to the domain Bacteria were found, which were representative mainly of the phyla Cyanobacteria, Proteobacte-

ria, Actinobacteria, Verrucomicrobia, and Plancto-mycetes. The minor phyla were represented by Bacteroidetes, Thermobaculum_p, Armatimona-detes, Acidobacteria, TM7, and GN02. Cyanobacteria accounted for more than 75% of the community; Proteobacteria and Actinobacteria, 11 and 10%, respectively. The indices of bacterial species diversity at the pelagic and littoral areas were similar: in the pelagic samples, 198 phylotypes were revealed; the Chao1 coefficient was 405. The dominant phylotype in the deep-water station was *Synechococcus* sp. (1346 nucleotide sequences, 66.3%); the proportion of species of *Cyanobium* and *Cyanobacterium* was 8.8% (178 nucleotide sequences). Representatives of nano-plankton were not found in the pelagic area of the lake. It should be noted that, in July–August, massive development of picocyanobacteria is annually observed in Lake Baikal; their contribution to the total amount of bacteria is 60% [7]. However, in June the microbiome is determined by the phyla Bacteroidetes, Actinobacteria, and Proteobacteria [10].

Thus, the analysis of the microbial diversity showed that the contribution of nontoxic picocyanobacteria to the coastal community was more than 90%, the proportion of potentially toxic *Microcystis* and *Anabaena* accounted for 3%, and 100% of genotypes in the pelagic area were represented by picocyanobacteria. Our data obtained for the littoral area were similar to those for lakes Erie and St. Marys, where, according to metagenomic analysis data, up to 70% of nucleotide sequences during toxic blooms belonged to picocy-anobacteria, whereas *Microcystis* spp., the main producer of MCs, accounted for less than 3% [11].

In PCR analysis, using total phytoplankton DNA as a template and primers to the AMT-domain gene, a positive response was detected in the samples collected in the littoral area, whereas the response of the samples collected from the deep-water station was negative. Fifty clones obtained from the positive samples were representative of six different genotypes, half of which had a 99% similarity with the nucleotide sequences of the *Microcystis* genus, and the remaining were similar to *Anabaena*. On the phylogenetic tree, the nucleotide sequences of the AMT domain form stable clusters corresponding to individual genera of MC-producing cyanobacteria [6, 8]. In the *Microcystis* cluster, Baikal genotypes BaT10-3 and BaT10-9 grouped together with *M. wesenbergii* NIES-107 from Lake Kawaguchi, and BaT10-12 grouped with *Microcystis* sp. B5-4 from the Baltic Sea (Fig. 2). In the cluster *Anabaena*, the nucleotide sequence BaT10-1 was related to the isolate from Lake Kotokel and strains *Anabaena* from Scandinavian lakes and the Baltic Sea, whereas BaT10-13 and 10-14 formed single lines.

Thus, the genes of MC synthesis belonging to species *Microcystis* and *Anabaena* were identified in Lake Baikal for the first time during the observation period from 2005 to 2010.

The concentration of MCs in the vicinity of the Turka village was $0.17 \pm 0.01$ μg/L, which is well below the threshold set for drinking water. In the pelagic zone of the lake, MCs were not found. The concentration of MCs in Lake Baikal is very low even compared to other cold-water lakes, in which the toxic species *Anabaena* and *Microcystis* were also found. For example, the maximum concentration of MCs in Finnish lakes was 5200 μg/L [1, 12]. It is known that the ambient temperature is one of the factors determining the abundance of cyanobacteria and the production of toxins by cells [1, 2]. The highest MC concentration was detected at 18−25°C, whereas at low temperatures (10°C) the synthesis of toxins is decelerated 2−3 times [1]. The water temperature on the surface in the coastal area of Lake Baikal was close to the lower limit, at which both the abundance of cyanobacteria and the synthesis of MCs significantly decrease. Intensive vegetation of cyanobacteria in the coastal areas might also be prevented by the mixing of water masses due to the runoff of the Turka River, the fourth tributary of Lake Baikal in terms of water content [13].

The Turka River through two small tributaries connects lakes Baikal and Kotokel, a shallow eutrophic reservoir located 2 km from Lake Baikal. In this lake, toxic blooms of species *Anabaena* and *Microcystis* have been annually observed since 2008. The number of cyanobacteria during blooms reached 7.13 million cells/L, their biomass was 3.87 g/m$^3$, and the concentration of MCs was 53 μg per gram dry weight [8]. In summer 2008, a mass-scale death of animals and 16 cases of human poisoning after eating fish caught in the lake were registered at Lake Kotokel. The presence of a direct water connection between lakes Baikal and Kotokel makes possible the drift of toxic genotypes with the river run-off into Lake Baikal. As was noted above, the toxic genotypes found in the littoral area near the Turka village were closely related to those of Lake Kotokel. It is known that one of the factors contributing to the distribution of toxigenic cyanobacteria is their introduction into large lakes from the neighboring small lakes and puddles during storms as well as due to external factors such as the movement of fishing boats, equipment, etc. [14].

The development of toxic blooms is associated with the eutrophication and global warming [1, 2]. Long-term studies have shown that climate in Lake Baikal also changes. During the observation period from 1896, the annual air temperature has increased at an average rate of 1.2°C/100 years. Over the past 60 years, the temperature of surface and upper layers of water in the warm months of the year gradually increases [15]. The development of tourism business at Lake Baikal leads to an increase in the anthropogenic pressure, which inevitably causes the eutrophication of shallow bays and mass-scale development of cyanobacteria.

The first data on the presence of toxigenic cyanobacteria and their dangerous metabolic products in the coastal zone of Lake Baikal indicate the threat of toxic
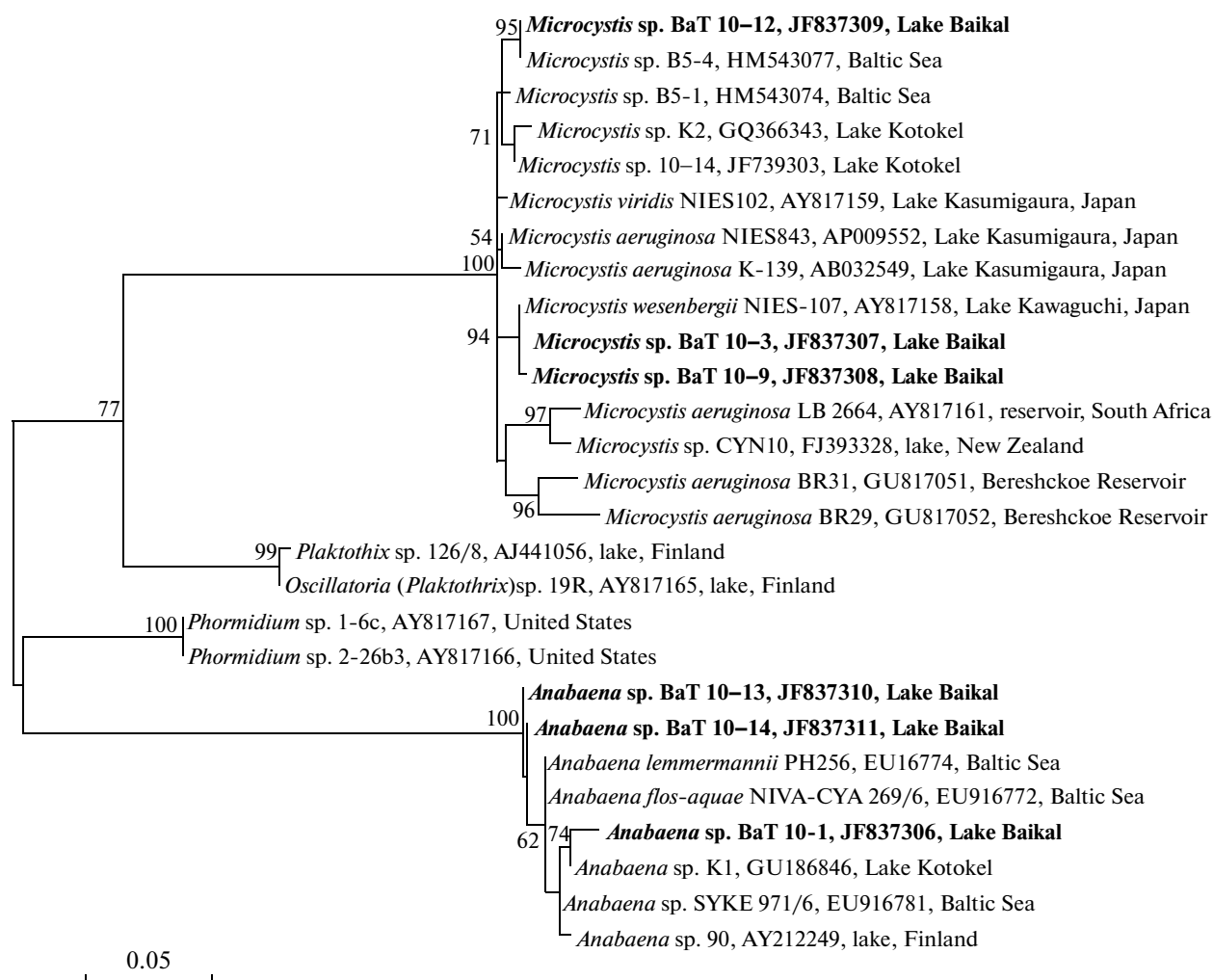
**Fig. 2.** Phylogenetic tree of cyanobacteria based on the results of analysis of sequences of *mcyE* gene fragments, built by the method of maximum likelihood (ML). The numerals at nodes indicate the results of bootstrap analysis of 1000 replicas. The sequences of the *mcyE* gene from Lake Baikal are shown in bold.

blooms and their possible spread to the unique ecosystem of the lake. To timely detect the foci of potentially toxic cyanobacteria, the monitoring of plankton, especially in shallow areas of the lake, should be continued.

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