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Two New Strains of *Microcystis* Cyanobacteria from Lake Baikal, Russia: Ecology and Toxigenic Potential

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Abstract

Microcystis, a potentially toxigenic cyanobacterium known to form extensive blooms in eutrophic lakes globally, was investigated in the cold oligotrophic Lake Baikal. We report the isolation of two *Microcystis* strains, *Microcystis aeruginosa* and *M. novacekii*, and document the presence of the latter species in Lake Baikal for the first time. In *M. aeruginosa* strain BN23, we detected the microcystin synthetase gene *mcy*E. Liquid chromatography-mass spectrometry revealed the presence of two microcystin variants in BN23, with microcystin-LR, a highly potent toxin, being the dominant form. The concentration of MC-LR reached 540 µg/g dry weight. In contrast, *M. novacekii* strain BT23 lacked both microcystin synthesis genes and detectable toxins. The habitat waters were characterized as oligotrophic with minor elements of mesotrophy, exhibiting low phytoplankton biomass dominated by the chrysophyte *Dinobryon cylindricum* (76–77% of biomass), with cyanobacteria contributing 8–10%. The contribution of *Microcystis* spp. to the total phytoplankton biomass could not be quantified as they were exclusively found in net samples. The water temperature at both sampling stations was ~19 °C, which is considerably lower than optimal for *Microcystis* spp. and potentially conducive to enhanced microcystin production in toxigenic genotypes.

Keywords: microcystin; phytoplankton; *Microcystis novacekii*; *Microcystis aeruginosa*

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1. Introduction

Cyanobacteria of the genus *Microcystis* are among the most widespread and well-known causative agents of toxic blooms on all continents except Antarctica [1]. The particular interest in these organisms stems from their production of microcystins (MCs), potent hepatotoxic and carcinogenic toxins hazardous to both aquatic organisms and human health [2]. In the Baikal region, toxic *Microcystis* blooms and an outbreak of Haff disease involving the poisoning of humans and piscivorous birds have been previously reported in the shallow, eutrophic Lake Kotokel, located 2 km from Lake Baikal and connected to it by river systems [3].

Lake Baikal, the largest freshwater lake in the world, is located in the temperate zone and classified as an oligotrophic water body. Its great depth (1631 m) and a short warm season contribute to maintaining cold water temperatures [4]. During summer, the average water temperature in the littoral zone of Lake Baikal ranges from 16 to 18 °C, with a recorded maximum of 21.4 °C. The duration of a stable temperature regime averages only

four days due to wind-driven mixing and frequent upwelling of cold, deep water (4 °C) [5]. In shallow bays, the water can warm up to 22–24 °C [6].

Lake Baikal harbors representatives of all major genera of cyanobacteria known to cause harmful algal blooms, including *Aphanizomenon*, *Planktothrix*, *Microcystis*, and *Dolichospermum* [4,7]. However, only the latter genus proliferates extensively in the lake, typically forming blooms dominated by *D. lemmermannii*, particularly in the littoral zone and bays where nutrient inputs from the shoreline promote their growth [8,9].

E. Zapomělová et al. [10] determined the optimal growth temperature for *D. lemmermannii* to be between 14 and 19 °C, a factor likely contributing to the species' success in cold-water Lake Baikal. Species of the genus *Microcystis* are more required for water warming. Experiments have shown that the optimal photosynthetic activity of *M. aeruginosa* and *M. wesenbergii* occurs at 30 °C, while *M. viridis* displays optimal activity at 25 °C [11]. Laboratory studies of *M. aeruginosa* have indicated that increasing the temperature from 20 to 32 °C promotes their growth rates [12,13]. Cold treatment (18–20 °C) not only decreased the growth rates but also significantly increased the toxicity of temperate strains of *M. aeruginosa* in mouse bioassays [12]. Recent findings have confirmed the protective role of microcystins in the oxidative stress response during cool acclimation. A temperature decrease from 26 to 19 °C in toxigenic *M. aeruginosa* PCC 7806 caused a twofold increase in MCs production, whereas a non-toxigenic mutant responded to the oxidative stress by increasing electron sinks and non-photochemical quenching [14]. We hypothesize that suboptimal water temperatures in Lake Baikal can simultaneously suppress *Microcystis* reproduction and stimulate MC synthesis in toxigenic genotypes.

Monitoring of toxin-producing cyanobacteria in Lake Baikal has been ongoing since 2010. Microcystin synthesis genes (*mcy*E) belonging to the genus *Microcystis* have been detected in Barguzin Bay and near the Turka settlement. Based on ELISA data, the concentration of MCs in water from Turka Bay was 0.17 ± 0.01 µg/L, a level below the WHO threshold for drinking water and comparable to values observed in other cold lakes globally [15]. The WHO has established a tolerable daily intake of microcystin-LR of 0.04 µg/kg body weight, and its maximum permissible concentration in drinking water is 1 µg/L [16].

This study aimed to isolate *Microcystis* spp. from Lake Baikal, assess the toxicogenic potential of these strains, and investigate the hydrochemical environmental factors prevalent in *Microcystis* habitats.

2. Materials and Methods

Water samples were collected on 22–24 July 2023 near the settlements of Nizhneangarsk and Turka with a Ruttner water sampler and an Apstein net with a 16 µm mesh size (Figure 1). Phytoplankton samples were fixed with Lugol's solution and concentrated by sedimentation to a final volume of 0.1 L. A 0.1-mL aliquot of the settled sample was pipetted in a Nageotte chamber, and algae were counted in triplicate under a light microscope at magnifications of 400× and 1000× (Axio Imager, Carl Zeiss, Jena, Germany). Cell biovolume was calculated from microphotographs obtained using an AxioCam MRc5 camera and the VideoTest-Size v5.0 software package (https://www.videotest.ru, accessed on 19 April 2023).

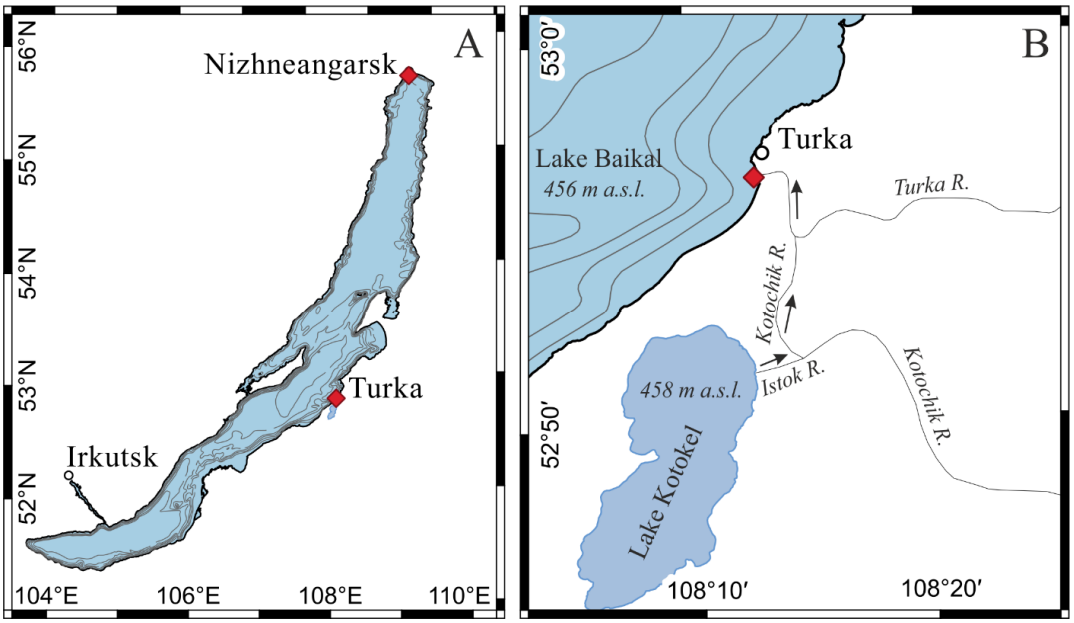

Figure 1. Sampling locations in Lake Baikal, 2023 (**A**). Detailed view of the 'Turka settlement' sampling station, highlighting the rivers connecting Lake Baikal and Lake Kotokel (**B**).

Net phytoplankton samples were fixed with 2% formalin for microscopic observations and frozen at −20 °C for subsequent chemical analysis. The relative abundance of particular phytoplankton species in net samples was determined under the microscope using the Starmach's scale: 1 represents individual occurrence, 2 represents 7 to 16 units on a standard viewing surface, 3 represents 17 to 30 visible units, 4 represents 31 to 50 visible units, and 5 indicates absolute dominance (more than 50 units in every field of view) [17].

Cultures were isolated from single colonies selected from freshly collected net samples under a stereomicroscope. Following three to five washes in sterile water, single colonies were transferred to tubes containing 25 mL MA medium via a small Pasteur pipette (1 mL capacity) [12]. Cultures were maintained at 28 °C under "cool-white" fluorescent illumination providing a light intensity of 50 µmol photons m^{-2} s^{-1} with a light:dark cycle of 12 h:12 h. Strains were identified using a microscope according to the guide [18].

DNA isolation, PCR amplification, and phylogenetic tree construction were carried out according to techniques described in [3]. HepF and hepR primers were applied to detect the *mcy*E gene [19]. *mcy*E gene sequences were obtained using a Nanofor-05 genetic analyzer (Syntol, Moscow, Russia) and BrilliantDye Terminator reagents (NimaGen, Nijmegen, The Netherlands). Whole-genome sequencing DNA libraries were prepared using the NadPrep EZ DNA library preparation kit (Nanodigmbio, Nanjing, China) and sequenced (150 bp paired-end reads) on an MGI DNBSEQ-G50 platform (MGI International Sales Co., Hong Kong, China). Barrnap v0.9 was used to detect 16S rDNA genes (https://github.com/tseemann/barrnap/, accessed on 1 April 2025). Open Reading Frame (ORF) prediction was performed using Prodigal v2.6.3 [20]. The predicted ORFs were then queried against the NCBI non-redundant protein database using DIAMOND v2.1.8 [21]. The sequences obtained have been deposited in GenBank under the accession numbers PV268088-PV268089 for 16S rDNA and PP971142 for *mcy*E [22].

Microcystin variants were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF/TOF) mass spectrometry. Extraction was performed following a previously described method [3]. For MALDI-TOF/TOF analysis (Bruker UltrafleXtreme, Hamburg, Germany), the AnchorChip Standard Target was consequently coated with 0.5 µL of the test sample and 0.5 µL of 2,5-dihydroxybenzoic acid (20 mg/mL) in 30:70

(*v*/*v*) acetonitrile: 0.1% trifluoroacetic acid in water. Detection was performed in positive ion mode, scanning a mass range of 700–3500 Da. MS^2 spectra were registered in positive LIFT mode (100–1200 Da).

Strain productivity was assessed using HPLC-UV. A Milichrom A-02 micro-column liquid chromatograph (EcoNova, Novosibirsk, Russia), 2 × 75 mm column packed with ProntoSil 120-5 C18 AQ (Bischoff Chromatography GMBH, Hamburg, Germany), was used, maintained at 60 °C with a flow rate of 150 µL/min. A gradient elution was employed, using 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The solvent program was as follows: 10% B at time zero, followed by a gradient from 10% to 65% B over 2500 µL. The injected sample volume was 2 µL, and detection was performed with a multi-wavelength UV detector at 230, 238, 250, 260, and 280 nm. Quantification was achieved by measuring the chromatographic signals of MC-LR against a calibration curve generated from a Microcystin LR Standard (Abraxas International Inc., Toronto, ON, Canada).

Physicochemical parameters of the water were determined using standard chemical analysis methods generally accepted in freshwater hydrochemistry [23,24]. The hydrogen index (pH), specific electrical conductivity (EC), total phosphorus, and total nitrogen concentration were determined in unfiltered samples, while biogenic elements were determined in samples filtered through acetate cellulose membrane filters with a pore size of 0.45 µm (Vladisart, Vladimir, Russia). The concentrations of dissolved oxygen and biogenic elements, as well as pH and EC, were determined using titrimetric, spectrophotometric (PE-5400VI spectrophotometer, Ekroskhim, St. Petersburg, Russia), potentiometric (Expert-pH pH meter, Econix-Expert, Moscow, Russia), and conductometric (Expert-002 conductometer) methods, respectively.

For the determination of chlorophyll *a*, water samples (0.5 L) were filtered through 0.45 µm polycarbonate membranes (Sartorius, Göttingen, Germany), and the pigment was extracted with methanol for 12 h at 8 °C. After centrifugation of the supernatant, chlorophyll *a* concentration was measured at 665 nm using a Cintra-2020 spectrophotometer (GBC Scientific Equipment, Braeside, Australia).

3. Results

Strain BN23, isolated from the Nizhneangarsk station, formed lobate colonies with distinct holes. Strain BT23, isolated from the Turka station, exhibited weakly aggregated, compact colonies with densely packed cells in the center of the colony and sparsely distributed solitary cells within the enveloping mucilage. The average cell size for both strains was 5 µm, and gas vacuoles were abundant throughout the cells (Figure 2A,B and Figure A1). Based on morphological characteristics, the strains were identified as *Microcystis aeruginosa* BN23 and *M. novacekii* BT23. Phylogenetic relationships among *Microcystis* species on the 16S rRNA gene are unresolved, and in the phylogenetic tree, the sequences of the isolated strains clustered within the common clade of the genus *Microcystis* (Figure A2).

PCR amplification using primers targeting the aminotransferase domain of the microcystin synthetase gene (*mcy*E) yielded positive results for both natural samples and the *M. aeruginosa* BN23 strain. The *M. novacekii* BT23 strain lacked the *mcy*E gene. The sequence of strain BN23 exhibited 98.5–98% identity to *mcy*E sequences from *M. aeruginosa* S1–S4 strains isolated from the Hilla River in Iraq, NIES-843 from Lake Kasumigaura in Japan, and FCY-26 from Lake Paldang in Korea. The highest similarity (98.7%) was observed with the uncultured clone KF219506 obtained from Lake Baikal in 2012 [15,22]. In the phylogenetic tree, these sequences clustered within the common clade of the genus *Microcystis* (Figure 2C). The concentration of MC-LR in *M. aeruginosa* BN23 was 540 µg/g dry weight, with MC-YR detected as a minor variant (Figure 2D).

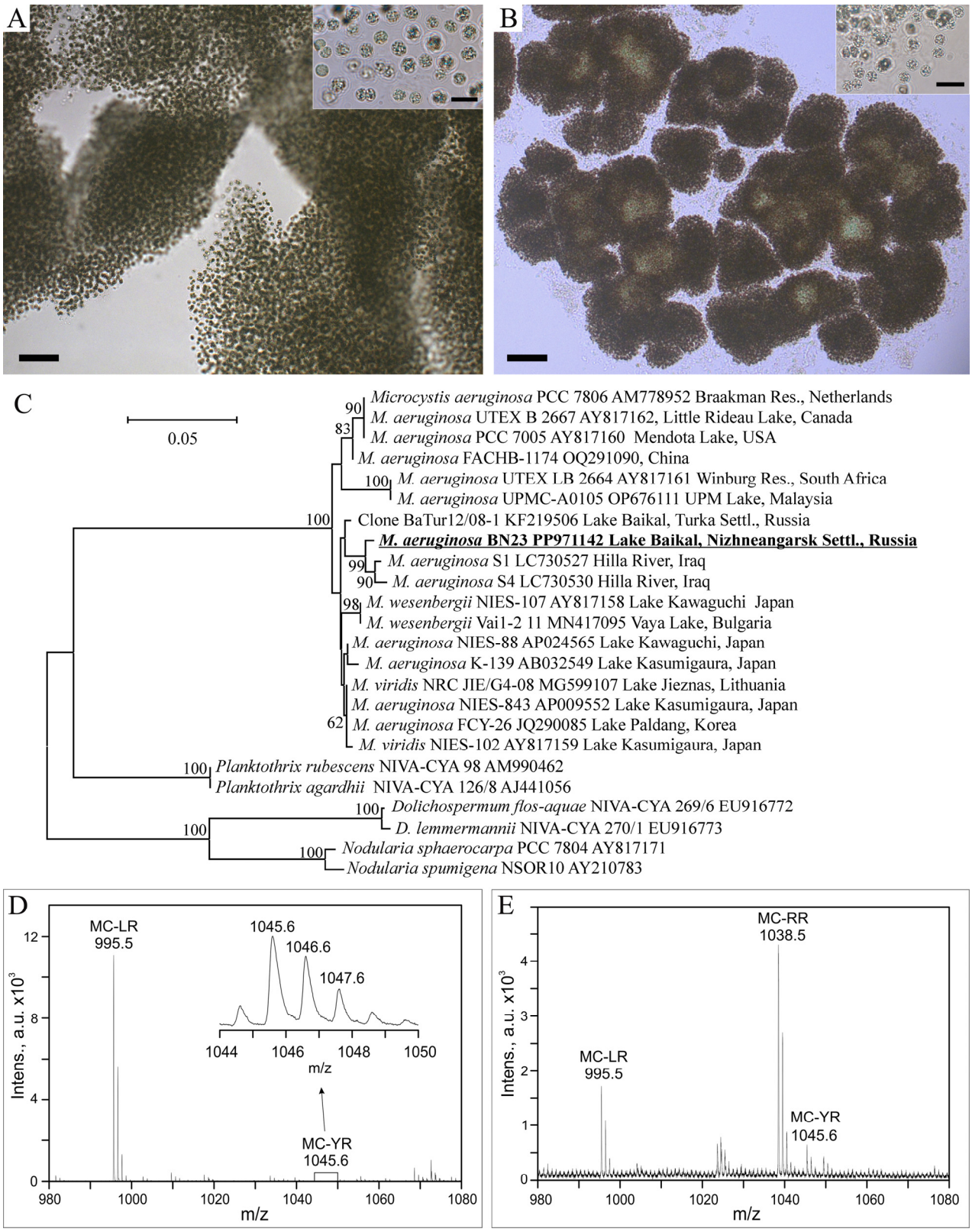

Figure 2. Morphological and genetic features of microcystin-producing and non-toxic *Microcystis* strains from Lake Baikal: light microscopy of colonies and cells (insets) of *M. aeruginosa* BN23 (**A**) and *M. novacekii* BT23 (**B**), scale bar = 50 µm, insets = 10 µm. (**C**) Unrooted neighbor-joining tree of *mcy*E and *nad*F gene sequences (447 bp) from planktonic cyanobacteria. Bootstrap values are based on 1000 replicates; the sequence obtained in this study is shown in bold and underlined. (**D**) Mass spectrum of the biomass extract of *M. aeruginosa* BN23 isolated from the Nizhneangarsk station. (**E**) Mass spectrum of plankton extract from the Turka station.

In the littoral zone of Lake Baikal, where net samples were collected for culture isolation, water quality, as indicated by total nitrogen and phosphorus concentrations, was consistent with an oligotrophic water body (Table 1) [25]. While Secchi disk transparency and chlorophyll *a* concentration indicated mesotrophic conditions. The water temperature in the surface layer did not exceed 19 °C.

Table 1. Mean values of limnological variables and phytoplankton in Lake Baikal in July 2023.

Sampling Stations / Variables	Nizhneangarsk Settlement (55°45.23′ N, 109°34.87′ E)		Turka Settlement (52°56.3′ N, 108°12.7′ E)			
	0 m	5 m	0 m	5 m	10 m	17 m
Water temperature, °C	19.0	17.8	18.7	14.9	10.3	9.0
Secchi depth, m	4.0	-	4.7	-	-	-
Dissolved oxygen, mg/L	9.4	9.7	9.9	10.9	11.2	11.2
EC (25 °C), µS/cm	91.7	96.9	130.6	128.6	129.7	127.4
pH	8.01	8.01	8.02	7.94	7.87	7.67
Total phosphorus, mg/L	0.012	0.009	0.008	0.010	0.011	0.012
Total nitrogen, mg/L	0.18	0.18	0.15	0.16	0.12	0.17
Dissolved inorganic nitrogen, mg/L	0.03	0.02	0.01	0.04	0.01	0.11
PO_4^{3-}, mg/L	0.003	0.003	0.003	0.002	0.005	0.028
NH_4^+, mg/L	0.002	0.001	<lod *	0.001	0.002	0.038
NO_2^-, mg/L	0.001	0.001	<lod	<lod	0.002	0.003
NO_3^-, mg/L	0.12	0.09	0.06	0.06	0.17	0.35
Chlorophyll *a*, µg/L	3.1	2.9	1.5	2.1	2.8	4.5
Phytoplankton abundance, cells/L	171,000	-	321,000	-	-	-
Cyanobacteria abundance, cells/L	71,000	-	211,000	-	-	-
Phytoplankton biomass, mg/m^3	90	-	74	-	-	-
Cyanobacteria biomass, mg/m^3	9	-	5.6	-	-	-
Microcystin variants	<lod	-	MC-LR, -YR, -RR	-	-	-

* lod—limit of detection.

Phytoplankton abundance at the Nizhneangarsk station was 1.71 × 10^5 cells/L (Table 1), with cyanobacteria and green algae contributing equally, each accounting for 41% of the total abundance; Chrysophyta contributed 12%. Total phytoplankton biomass was 90 mg/m^3. The large-celled Chrysophyta alga *Dinobryon cylindricum* accounted for the largest proportion of the biomass (76%), while cyanobacteria and Chlorophyta each contributed only 10%. *Dolichospermum lemmermannii* (4.3 × 10^4 cells/L), *Monoraphidium contortum* (3.3 × 10^4 cells/L), and *Dinobryon cylindricum* (1.9 × 10^4 cells/L) were the dominant phytoplankton species at the Nizhneangarsk station. The contribution of *Microcystis aeruginosa* to phytoplankton abundance was not quantified due to its rarity (score of 1 on the Starmach scale) and presence only in the net samples. Mass spectrometry analysis of the plankton biomass indicated the absence of MCs in the sample.

Phytoplankton abundance at the Turka station was 3.21 × 10^5 cells/L (Table 1), with cyanobacteria contributing the largest proportion (66%), primarily due to the small-celled colonial *Aphanocapsa* spp. Similar to the Nizhneangarsk station, phytoplankton biomass was low (74 mg/m^3). *Dinobryon cylindricum* comprised the largest proportion of biomass (77%), whereas members of the phyla Chlorophyta and Cyanobacteria, despite high species richness, contributed only 12% and 8% of the total biomass, respectively. *M. aeruginosa*, *M. novacekii*, and *D. lemmermannii* were dominant in the net sample from the Turka station (score of 3 on the Starmach scale), while species of *D. flos-aquae*, *D. crassum*, *M. viridis*, *Aphanizomenon flos-aquae*, and *Gloeotrichia echinulata* were present in low numbers. MALDI-

TOF/TOF mass spectrometry analysis indicated the presence of MC-LR, -RR, and -YR in the plankton biomass extract (Figure 2E).

4. Discussion

The phytoplankton species list for Lake Baikal includes the cosmopolitan species *M. aeruginosa*, *M. flos-aquae*, *M. wesenbergii*, and *M. ichtioblabe* [7]. This study represents the first documented occurrence of *M. novacekii* in the lake. While considered a tropical species, *M. novacekii* occasionally occurs in the temperate zone during the summer season [18]. In water bodies of Eastern Siberia, *M. novacekii* has been recorded as a component of toxic *Microcystis* blooms in a lake located north of 62 °N in the permafrost zone [26]. This species has been demonstrated to survive prolonged winters both in under-ice water and through freezing into ice, thereby expanding our knowledge of the distribution and ecology of *M. novacekii*.

Microcystins are frequently detected in cultures of *M. aeruginosa*. Compared with other strains with known MC concentration in dry biomass, the MC-LR concentration in the Baikal strain (540 μg/g d.w.) was two-fold higher, although total MC concentrations in some toxic strains can be an order of magnitude higher, reaching 7600 μg/g d.w. [27,28].

To date, approximately 280 microcystin variants have been isolated [29]. However, MC diversity in Lake Baikal plankton is limited. Including the MC-LR, -YR, and -RR variants detected in this study, only seven variants have been reported [9]. The same three MC variants were previously identified in eutrophic Lake Kotokel, which has an outflow to Lake Baikal via the Turka River [3]. Typically, MC-LR, -RR, and -YR are the most abundant variants during *Microcystis* blooms [30]. Therefore, *M. aeruginosa*, which was abundant in the Turka station net sample along with *M. novacekii*, is the likely producer of these toxins. This assumption is further supported by the detection of rarer microcystin variants (MC-LA, -LF, -YM(O), and dmMC-LR) alongside MC-YR during a *Dolichospermum* bloom in Lake Baikal [9].

Molecular studies have revealed the predominance of MC-producing *Dolichospermum* genotypes over *Microcystis* in Lake Baikal [31], and an inverse relationship in the shallow lakes of Eastern Siberia and Finland [3,32,33]. The abundance of MC-producing genes in Baikal was correlated with temperature and nitrogen concentration [31].

The determined phytoplankton biomass (up to 90 mg/m^3) corresponds to average values for July–August in Lake Baikal according to G.I. Popovskaya's records [34]. The low phytoplankton biomass, water transparency, and nutrient concentrations coupled with high chlorophyll *a* values observed in this study are characteristic of Lake Baikal during summer and are attributed to the mass development of autotrophic picoplankton [35]. The picoplankton community is dominated by small organisms (cell size $\leq$ 2 μm), primarily cyanobacteria of the genera *Synechococcus* and *Cyanobium* and the species *Synechocystis limnetica*, with green algae contributing negligibly. In some years, the abundance of autotrophic picoplankton in Lake Baikal reaches 1 million cells/mL [35].

Hydrochemical parameters for nutrients in the pelagic zone of Lake Baikal have remained consistently low over the years of observation [36]. However, recent increases in total nitrogen, total phosphorus, and chlorophyll concentrations have been observed in the coastal zone, and the littoral zone is now recognized as exhibiting signs of mesotrophy [37].

Microcystis species were first described in Lake Baikal in the early 20th century, primarily in shallow bays, rivers, and coastal areas [38]. Despite a gradual increase in the average annual air and water temperature in Lake Baikal (up to 2.24 °C/100 years) [39], temperature conditions are still suboptimal for *Microcystis*, and their habitats have largely remained unchanged. In the study areas near Nizhneangarsk, the shallow Angarskiy Sor bay represents a source of *Microcystis* introduction into the littoral zone [39]. Near Turka, Lake Kotokel, connected to Lake Baikal via the Istok, Kotochik, and Turka rivers, serves as another source [3].

Numerous studies indicate that oligotrophic lakes worldwide are susceptible to cyanobacterial blooms [40]. Cyanobacteria are well adapted to low nutrient concentrations and a wide temperature range. Factors such as summer warming of the surface water layer and increased nutrient inputs from terrestrial sources due to rainfall, polluted groundwater, etc., can trigger cyanobacterial blooms in oligotrophic lakes [8,9,40]. The example of Lake Erie, where *Dolichospermum* blooms have been replaced by *Microcystis* [41], highlights a potential scenario for Lake Baikal in the future. Given our annual observations of toxigenic cyanobacteria in the littoral zone of Lake Baikal, information on toxin-producing species obtained through culturing is crucial for monitoring and predicting harmful blooms.

5. Conclusions

We report the isolation of two *Microcystis* cyanobacterial strains—*M. aeruginosa* and *M. novacekii*—from the littoral zone of Lake Baikal. The *M. aeruginosa* strain BN23 produced a high concentration of microcystin-LR (540 μg/g d.w.), with microcystin-YR present in minor quantities. The *M. novacekii* strain BT23 was non-toxic; however, the detection of microcystin-LR, -RR, and -YR in plankton biomass from the net sample from which it was isolated suggests that *M. aeruginosa* was likely the microcystin producer in this area. Lake waters at the species' habitats are characterized as oligotrophic based on hydrochemical parameters, with low phytoplankton biomass, but were closer to mesotrophic conditions based on chlorophyll *a* concentrations and low transparency. Although current thermal conditions in Lake Baikal are suboptimal for mass development of *Microcystis*, they may stimulate toxin production. Even small quantities of these species are therefore sufficient to pollute the water with highly toxic microcystin-LR, which has been repeatedly recorded at the Turka station.

Author Contributions: Conceptualization, E.S. and I.T.; methodology, E.S., G.F. and N.C.; investigation, E.S., I.T., Y.N., G.F., N.C., M.S., S.P., A.K. (Andrey Krasnopeev), A.G. and A.K. (Anton Kuzmin); writing—original draft preparation, E.S., A.K. (Andrey Krasnopeev) and G.F.; writing—review and editing, E.S. and O.B.; visualization, G.F., E.S., S.P. and A.K. (Anton Kuzmin); funding acquisition, O.B. and N.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

EC	Electrical conductivity
ELISA	Enzyme-linked immunosorbent assay
HPLC-UV	High-performance liquid chromatography-ultraviolet
MALDI-TOF/TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

MC	Microcystin
WHO	World Health Organization

Appendix A

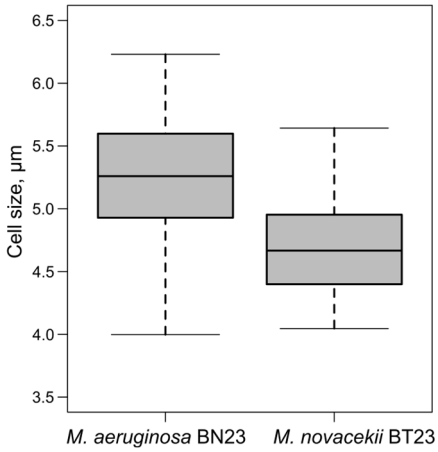

Figure A1. Box–whisker plots depicting the cell diameter of *Microcystis* strains. Data are based on measurements of 60 cells per strain. Boxes represent ± standard deviation; whiskers denote minimal and maximal values; and internal lines indicate mean values.

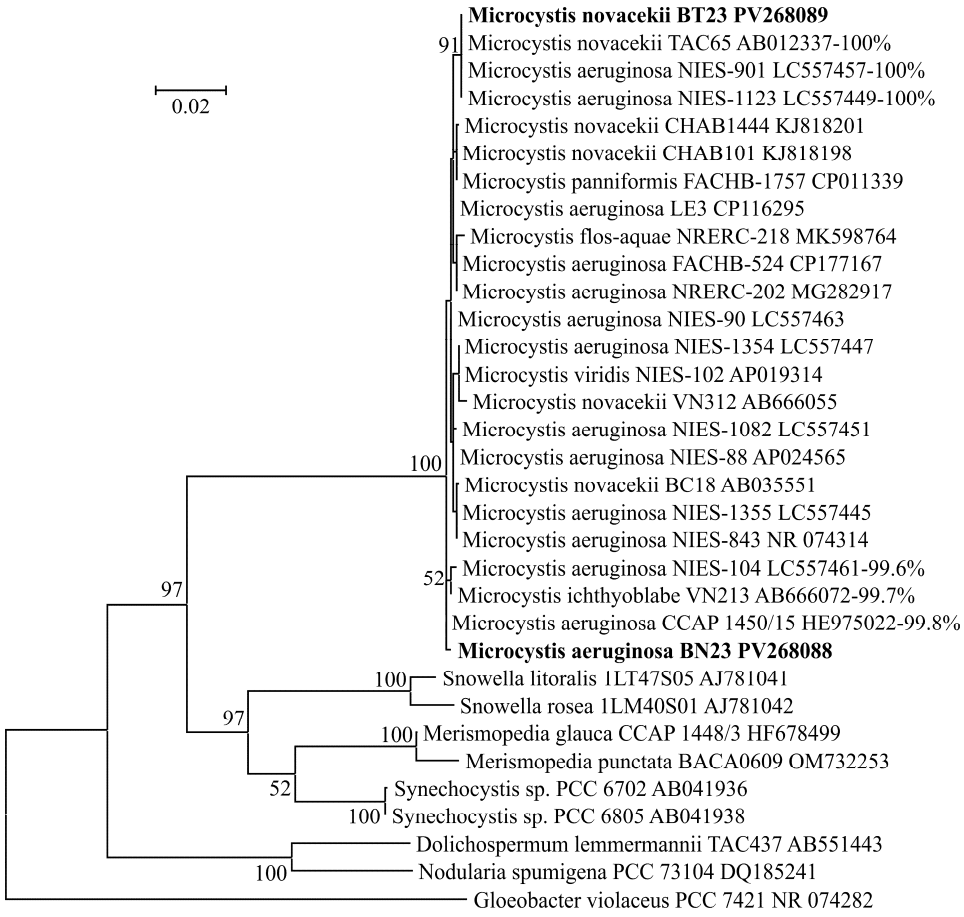

Figure A2. Phylogenetic tree of *Microcystis* strains BT23 and BN23, and related taxa, obtained by maximum likelihood analysis of the 16S rRNA gene (1467 bp). Percentage bootstrap values of 1000 replicates are indicated at each node. For sequences closely related to strains BN23 and BT23 (shown in bold), the percentage of similarity is indicated.

References

1. Preece, E.P.; Hardy, F.J.; Moore, B.C.; Bryan, M. A review of microcystin detections in Estuarine and Marine waters: Environmental implications and human health risk. *Harmful Algae* **2017**, *61*, 31–45. [CrossRef]
2. Svirčev, Z.; Drobac, D.; Tokodi, N.; Mijović, B.; Codd, G.A.; Meriluoto, J. Toxicology of microcystins with reference to cases of human intoxications and epidemiological investigations of exposures to cyanobacteria and cyanotoxins. *Arch. Toxicol.* **2017**, *91*, 621–650. [CrossRef] [PubMed]
3. Belykh, O.I.; Sorokovikova, E.G.; Fedorova, G.A.; Kaluzhnaya, O.V.; Korneva, E.S.; Sakirko, M.V.; Sherbakova, T.A. Presence and genetic diversity of microcystin-producing cyanobacteria (*Anabaena* and *Microcystis*) in Lake Kotokel (Russia, Lake Baikal Region). *Hydrobiologia* **2011**, *671*, 241–252. [CrossRef]
4. Kozhova, O.M.; Izmest'eva, L.R. (Eds.) *Lake Baikal. Evolution and Biodiversity*; Backhuys Publishers: Leiden, The Netherlands, 1998; p. 447.
5. Fedotov, A.P.; Khanaev, I.V. Annual temperature regime of the shallow zone of Lake Baikal inferred from high-resolution data from temperature loggers. *Limnol. Freshw. Biol.* **2023**, *4*, 119–125. [CrossRef]
6. Timoshkin, O.A. (Ed.) *Guide and Key to Pelagic Animals of Baikal*; Nauka: Novosibirsk, Russia, 1995; p. 694.
7. Bondarenko, N.A. List of Planktonic Algae of Lake Baikal. In *Guide and Key to Pelagic Animals of Baikal*; Timoshkin, O.A., Ed.; Nauka: Novosibirsk, Russia, 1995; pp. 621–630.
8. Bondarenko, N.A.; Tomberg, I.V.; Shirokaya, A.A.; Belykh, O.I.; Tikhonova, I.V.; Fedorova, G.A.; Netsvetaeva, O.G.; Eletskaya, E.V.; Timoshkin, O.A. *Dolichospermum lemmermannii* (Nostocales) bloom in world's deepest Lake Baikal (East Siberia): Abundance, toxicity and factors influencing growth. *Limnol. Freshw. Biol.* **2021**, *1*, 1101–1110. [CrossRef]
9. Tikhonova, I.; Kuzmin, A.; Fedorova, G.; Sorokovikova, E.; Krasnopeev, A.; Tsvetkova, A.; Shtykova, Y.; Potapov, S.; Ivacheva, M.; Zabortzeva, T.; et al. Toxic cyanobacteria blooms of Mukhor Bay (Lake Baikal, Russia) during a period of intensive anthropogenic pressure. *Aquat. Ecosyst. Health Manag.* **2023**, *25*, 85–97. [CrossRef]
10. Zapomělová, E.; Řeháková, K.; Jezberová, J.; Komárková, J. Polyphasic characterization of eight planktonic *Anabaena* strains (Cyanobacteria) with reference to the variability of 61 *Anabaena* populations observed in the field. *Hydrobiologia* **2010**, *639*, 99–113. [CrossRef]
11. Yagi, O.; Ohkubo, N.; Tomioka, N.; Okada, M. Effect of irradiance and temperature on photosynthetic activity of the cyanobacterium *Microcystis* spp. *Environ. Technol.* **1994**, *15*, 389–394. [CrossRef]
12. Watanabe, M.F.; Oishi, S. Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. *Appl. Environ. Microbiol.* **1985**, *49*, 1342–1344. [CrossRef]
13. Yang, Z.; Zhang, M.; Yu, Y.; Shi, X. Temperature triggers the annual cycle of *Microcystis*, comparable results from the laboratory and a large shallow lake. *Chemosphere* **2020**, *260*, 127543. [CrossRef]
14. Stark, G.F.; Martin, R.M.; Smith, L.E.; Wei, B.; Hellweger, F.L.; Bullerjahn, G.S.; McKay, R.M.L.; Boyer, G.L.; Wilhelm, S.W. Microcystin aids in cold temperature acclimation: Differences between a toxic *Microcystis* wildtype and non-toxic mutant. *Harmful Algae* **2023**, *129*, 102531. [CrossRef] [PubMed]
15. Belykh, O.I.; Gladkikh, A.S.; Sorokovikova, E.G.; Tikhonova, I.V.; Butina, T.V. Identification of toxic Cyanobacteria in Lake Baikal. *Dokl. Biochem. Biophys.* **2015**, *463*, 220–224. [CrossRef] [PubMed]
16. WHO. *Guidelines for Drinking-Water Quality*, 4th ed.; World Health Organization: Geneva, Switzerland, 2011; p. 631.
17. Vasser, S.P.; Kondrat'eva, N.V.; Masyuk, N.P. (Eds.) *Algae: A Handbook*; Naukova Dumka: Kiev, USSR, 1989; p. 608.
18. Komárek, J.; Anagnostidis, K. *Süβwasserflora von Mitteleuropa, Band 19/1. Cyanoprokaryota. 1. Teil/Part 1: Chroococcales*; Springer: Heidelberg, Russia, 1999; p. 554.
19. Jungblut, A.D.; Neilan, B.A. Molecular identification and evolution of the cyclic peptide hepatotoxins, microcystin and nodularin, synthetase genes in three orders of cyanobacteria. *Arch. Microbiol.* **2006**, *185*, 107–114. [CrossRef] [PubMed]
20. Hyatt, D.; Chen, G.-L.; LoCascio, P.F.; Land, M.L.; Larimer, F.W.; Hauser, L.J. Prodigal: Prokaryotic gene recognition and translation initiation site identification. *BMC Bioinform.* **2010**, *11*, 119. [CrossRef]
21. Buchfink, B.; Xie, C.; Huson, D.H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **2015**, *12*, 59–60. [CrossRef]
22. Sorokovikova, E.G.; Tikhonova, I.V.; Naidanova, Y.A.; Belykh, O.I. Identification of microcystin producing cyanobacteria in the plankton of Lake Baikal and Irkutsk Reservoir. *Limnol. Freshw. Biol.* **2024**, *4*, 1101–1108. [CrossRef]
23. Lozovik, P.A.; Efremenko, N.A. (Eds.) *Analytical, Kinetic and Calculation Methods in Hydrochemical Practice*; Nestor-Istoriya Publ.: St. Petersburg, Russia, 2017; p. 272.
24. Boeva, L.V. (Ed.) *Guide to Chemical Analysis of Land Surface Waters*; NOK Publ.: Rostov-on-Don, Russia, 2009; p. 1150.
25. Likens, G.E. Primary Production of Inland Aquatic Ecosystems. In *Primary Production of the Biosphere*; Springer: Berlin/Heidelberg, Germany, 1975; pp. 185–202. [CrossRef]
26. Gabyshev, V.A.; Sidelev, S.I.; Chernova, E.N.; Vilnet, A.A.; Davydov, D.A.; Barinova, S.; Gabysheva, O.I.; Zhakovskaya, Z.A.; Voronov, I.V. Year-Round Presence of Microcystins and Toxin-Producing Microcystis in the Water Column and Ice Cover of a Eutrophic Lake Located in the Continuous Permafrost Zone (Yakutia, Russia). *Toxins* **2023**, *15*, 467. [CrossRef]

27. Long, B.M.; Jones, G.J.; Orr, P.T. Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate. *Appl. Environ. Microbiol.* **2001**, *67*, 278–283. [CrossRef]
28. Oh, H.M.; Lee, S.J.; Jang, M.H.; Yoon, B.D. Microcystin production by *Microcystis aeruginosa* in a phosphorus-limited chemostat. *Appl. Environ. Microbiol.* **2000**, *66*, 176–179. [CrossRef]
29. Bouaïcha, N.; Miles, C.O.; Beach, D.G.; Labidi, Z.; Djabri, A.; Benayache, N.Y.; Nguyen-Quang, T. Structural diversity, characterization and toxicology of microcystins. *Toxins* **2019**, *11*, 714. [CrossRef]
30. Ibelings, B.W.; Kurmayer, R.; Azevedo, S.M.F.O.; Wood, S.A.; Chorus, I.; Welker, M. Understanding the occurrence of cyanobacteria and cyanotoxins. In *Toxic Cyanobacteria in Water: A guide to Their Public Health Consequences, Monitoring and Management*; Chorus, I., Welker, M., Eds.; Taylor & Francis: Abingdon, UK, 2021; pp. 261–262.
31. Li, X.; Tikhonova, I.V.; Potapov, S.A.; Krasnopeev, A.Y.; Zhuchenko, N.A.; Niao, X.; Wang, L.; Sorokovikova, E.G.; Wang, W.; Belykh, O.I. World's largest oligotrophic Lake Baikal: Concerns about cyanobacterial blooms and potential microcystin producers along the littoral zone. *Harmful Algae* **2025**, *144*, 102841. [CrossRef]
32. Barinova, S.; Gabyshev, V.A.; Gabysheva, O.I.; Naidanova, Y.A.; Sorokovikova, E.G. Phytoplankton Diversity, Abundance and Toxin Synthesis Potential in the Lakes of Natural and Urban Landscapes in Permafrost Conditions. *Land* **2025**, *14*, 721. [CrossRef]
33. Vaitomaa, J.; Rantala, A.; Halinen, K.; Rouhiainen, L.; Tallberg, P.; Mokelke, L.; Sivonen, K. Quantitative real-time PCR for determination of microcystin synthetase E copy numbers for *Microcystis* and *Anabaena* in lakes. *Appl. Environ. Microbiol.* **2003**, *69*, 7289–7297. [CrossRef] [PubMed]
34. Popovskaya, G.I. Phytoplankton. In *Problems of Baikal. Proceedings of the Limnological Institute*; Galaziy, G.I., Votintsev, G.Y., Eds.; Nauka: Novosibirsk, USSR, 1978; Volume 16, pp. 158–168.
35. Belykh, O.I.; Sorokovikova, E.G. Autotrophic picoplankton in Lake Baikal: Abundance, dynamics, and distribution. *Aquat. Ecosyst. Health Manag.* **2003**, *6*, 251–261. [CrossRef]
36. Khodzher, T.V.; Domysheva, V.M.; Sorokovikova, L.M.; Sakirko, M.V.; Tomberg, I.V. Current chemical composition of Lake Baikal water. *Inland. Waters* **2017**, *7*, 250–258. [CrossRef]
37. Domysheva, V.; Vorobyeva, S.; Golobokova, L.; Netsvetaeva, O.; Onischuk, N.; Sakirko, M.; Khuriganova, O.; Fedotov, A. Assessment of the Current Trophic Status of the Southern Baikal Littoral Zone. *Water* **2023**, *15*, 1139. [CrossRef]
38. Meyer, K.I. An introduction to flora of algae of Lake Baikal. *Byul. MOIP, Otd. Biol., Nov. Ser.* **1930**, *39*, 179–396.
39. Shimaraev, M.N.; Domysheva, V.M. Trends in Hydrological and Hydrochemical Processes in Lake Baikal under Conditions of Modern Climate Change. In *Climatic Change and Global Warming of Inland Waters*; Goldman, C., Kumagai, M., Robarts, R.D., Eds.; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2013; pp. 43–66. [CrossRef]
40. Reinl, K.L.; Brookes, J.D.; Carey, C.C.; Harris, T.D.; Ibelings, B.W.; Morales-Williams, A.M.; De Senerpont Domis, L.N.; Atkins, K.S.; Isles, P.D.F.; Mesman, J.P.; et al. Cyanobacterial blooms in oligotrophic lakes: Shifting the high-nutrient paradigm. *Freshw. Biol.* **2021**, *66*, 1846–1859. [CrossRef]
41. Chaffin, J.D.; Mishra, S.; Kane, D.D.; Bade, D.L.; Stanislawczyk, K.; Slodysko, K.N.; Jones, K.W.; Parker, E.M.; Fox, E.L. Cyanobacterial blooms in the central basin of Lake Erie: Potentials for cyanotoxins and environmental drivers. *J. Great Lakes Res.* **2019**, *45*, 277–289. [CrossRef]

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