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SHORT COMMUNICATIONS

Detection of Microcystin-Producing Cyanobacteria in the Upper Volga Reservoirs

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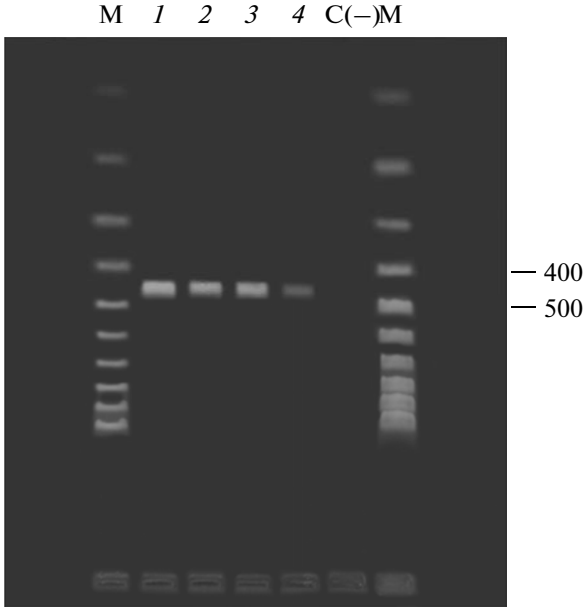
Microcystins are hepatotoxic cyclic peptides; their synthesis does not involve ribosomes and is carried out by the multienzyme complex including peptide synthetases (NRPS), polyketide synthases (PKS), and a number of other enzymes [1, 2]. The capacity for microcystin production has been found in plankton cyanobacteria from the genera *Microcystis, Anabaena, Planktothrix, Anabaenopsis, Oscillatoria, Phormidium*, and *Nostoc* [2]. The gene cluster encoding the enzymes responsible for microcystin synthesis (*mcy*) has a modular structure [1]. At present, over 90 structural variants of microcystins are known, among which microcystins LR, RR, and YR are more prevalent and microcystin LR is the most toxic [2]. The World Health Organization (WHO) therefore established the maximum permissible concentration (MPC) for microcystin LR in drinking water (1 μg/L) [2].

In Russia, systematic investigations of cyanotoxins using modern techniques were initiated several years ago [2–4]. Morphologically indistinguishable toxic and nontoxic strains of the same species may be present in the water during mass development of cyanobacteria. The molecular biological approach may be a relatively efficient instrument for detection of toxigenic cyanobacteria. However, so far such investigations have been carried out in Russia only for the Baikal region [3]. The aim of this work was to reveal microcystin-producing cyanobacteria in the Upper Volga reservoirs.

Water from Lake Nero, the Volga River in the vicinity of Yaroslavl, and in the estuary of the Solonitsa River, a right tributary of Volga, was sampled in July and September 2009–2011 (table). The cyanobacterial biomass was determined by counting the cells in a Nageotte chamber. The microcystin LR concentrations were determined by immunoenzyme assay (IEA) using the ELISA kit (Abraxis LLS) according to the manufacturer's instructions. In 2009, the phytoplankton sample from the Solonitsa River was found to contain intracellular microcystin LR; later, microcystin LR dissolved in water was determined in the remaining samples. DNA was isolated from the samples using the standard Diatom DNA Prep 200 reagent kit (Izogen Ltd.). Amplification of the specific site of the *mcyE* gene responsible for microcystin synthesis was performed by the polymerase chain reaction (PCR) using HEP primers according to the conditions described in [1]. The total DNA of the cyanobacteria from the Solonitsa River, which were earlier found to contain microcystin LR, was used as the positive control. The DNA of cyanobacteria not producing microcystin (the non-axenic culture of *Gloeocapsa decorticans* provided by the Department of Botany and Microbiology, Yaroslavl State University) was used in the negative control



Electrophoregram of the DNA fragments of the Upper Volga basin phytoplankton amplified with HEP primers. Lanes: Solonitsa River (positive control) (*1*); Lake Nero, July 9, 2011 (*2*); Volga River (*3*); Lake Nero, July 14, 2010 (*4*). C (–) is the negative control (*Gloeocapsa decorticans*); M is the molecular mass marker (bp).

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Results of PCR analysis, concentrations of microcystin LR (MC-LR), and the quantitative indices of abundance of cyanobacteria in the Upper Volga reservoirs

Sampling date	Water body	Biomass in a sample, mg/L				Presence of the *mcyE* gene	MC LR, μg/L
		Cyanobacteria	*Microcystis*	*Anabaena*	*Planktothrix*		
Sept. 3, 2009	Solonitsa River	7704.0	6954.0	307.0	–	+	>5.0
July 14, 2010	Lake Nero	18.0	0.8	2.6	2.9	+	>5.0
July 9, 2011	Lake Nero	136.9	93.7	34.0	–	+	>5.0
July 25, 2011	Volga River	1481.8	158.8	0.9	–	+	0.5

reaction. The PCR products were fractionated electrophoretically in 2% agarose gel and analyzed under UV illumination after staining with ethidium bromide. The size of the DNA amplified fragments was determined using external standards of molecular masses.

This is the first search for toxigenic cyanobacteria containing the microcystin synthetase gene *mcyE* been carried out for the Volga basin. Cyanobacteria from the genera *Microcystis, Anabaena, Planktothrix, Gloeotrichia, Aphanizomenon*, and *Limnothrix* dominated in the phytoplankton samples. The species of the genera *Microcystis* (*M. aeruginosa, M. wesenbergii, M. viridis, M. flos-aquae, M. novacekii*), *Anabaena* (*A. spiroides, A. affinis, A. flos-aquae*), and *Planktothrix agardhii* were potentially toxigenic cyanobacteria (table). The PCR product of expected length (about 470 bp) was revealed in both the positive control and in all the remaining samples, which indicated the presence of the gene responsible for microcystin synthesis by cyanobacteria in the reservoirs investigated (figure). The results of molecular genetic analysis were verified by direct determination of microcystin LR in the water of Lake Nero and the Volga River using IEA (table). The concentration of this hepatotoxin in most samples exceeded both the upper threshold of sensitivity of the method and the MPC established by the WHO by at least more than fivefold (table).

Thus, it was the first time the presence of the population of cyanobacteria synthesizing the hepatotoxin microcystin was determined for the Upper Volga reservoirs using the molecular genetic and immunoenzyme assays.

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