ISSN 0026-2617, Microbiology, 2014, Vol. 83, No. 5, pp. 709–711. © Pleiades Publishing, Ltd., 2014.
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SHORT COMMUNICATIONS

Molecular Genetics Identification of Microcystin-Producing Cyanobacteria Taxa in Lake Nero (Russia)

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Received March 6, 2014

DOI: 10.1134/S0026261714050245

Microcystins are natural hepatotoxins produced by different cyanobacteria, most frequently by species from the genera *Microcystis, Anabaena*, and *Planktothrix* [1]. Since they are harmful for human health according to the recommendations of the World Health Organization, the presence of cyanotoxins in drinking and recreational waters is controlled in many countries of the world [2]. In the Russian Federation there are no standards of safe microcystin levels in drinking or natural waters. To develop the national system of cyanotoxin monitoring, the most common microcystin-producing cyanobacteria in Russian reservoirs are to be identified. Cyanobacterial blooms are not necessarily accompanied by the production of toxins in water, because non-toxigenic strains, morphologically indistinguishable from the toxigenic ones, can dominate bacterial population. The detection and sequencing of the *mcy* gene cluster, responsible for biosynthesis of microcystins, has led to the "molecular era" in cyanotoxin research [3]. At present, with the use of the molecular approach, it becomes possible to detect the presence of toxigenic cyanobacteria in water long before the microcystin level exceeds the safe values. Application of the molecular techniques in the monitoring program is appropriate if the microscopic analysis has revealed the presence of potentially toxic cyanobacterial genera in the reservoir. However, the data about the main cyanotoxin producers in Russian waters are rare and have been obtained with the use of laborious molecular techniques (cloning and DNA sequencing, phylogenetic analysis) not feasible for the purposes of routine environmental monitoring [4].

The aim of the study was the molecular identification of microcystin-producing cyanobacteria taxa directly in environmental water samples from Lake Nero, the largest lake in the Upper Volga region.

Water samples from Lake Nero were collected in the period of July–September 2009–2011. Cyanobacteria species were identified using appropriate determinants [5–7]. Cyanobacteria biomass was determined by counting the cells in a Nageotte chamber. DNA was isolated from filter concentrated samples using the Metagenomic DNA Isolation Kit for Water (Epicentre). Amplification of the specific site of the *mcyA* gene responsible for microcystin synthesis was performed by the polymerase chain reaction (PCR) using *mcyA*-Cd primers according to the conditions described in [1]. All PCR products resulting from the *mcyA*-Cd primers were analyzed using the Restriction Fragment Length Polymorphism (RFLP) analysis, established by Hisbergues and co-authors to identify the microcystin-producing genera/species [1]. After being purified using the GeneJET PCR Purification Kit (Thermo Scientific), PCR products from environmental samples with mixed cyanobacterial composition were digested with *Hind*III and/or *Eco*RV (Fermentas). Restriction profiles were compared with those of the reference cyanobacterial strains, as well as with the theoretical restriction fragment lengths of the *mcyA*-Cd fragments described in [1]. Two strains were used as reference strains: *Microcystis aeruginosa* PCC 7806 and *Planktothrix agardhii* NIVA-CYA 126 (provided by Elke Dittmann, University of Potsdam). Since the reference strain of the genus *Anabaena* was not available, potential microcystin-producing species of the genus were determined using the theoretical values of the *mycA*-Cd gene restriction fragment length characteristic of *Anabaena* as reported in [1]. Additionally, to confirm the restriction analysis results, phytoplankton DNA from the same samples was amplified using genus-specific primers mcyE-F2/AnamcyE-2R, which allowed for the detection of the *Anabaena* microcystin synthetase gene *mcyE* [8]. Digested DNA were fractionated by electrophoresis in 3% agarose gel and analyzed under UV light after staining with ethidium bromide. The fragment size was determined using the O'GeneRuler Low Range DNA Ladder (Thermo Scientific).

When DNA templates were tested using the *mcyA*-Cd primers, a 297-bp fragment of the microcystin synthesis gene was detected in all samples, thus having confirmed the presence of hepatotoxic cyanobacteria in Lake Nero. Microscopic analysis revealed simultaneous presence of potentially toxigenic genera *Micro-*

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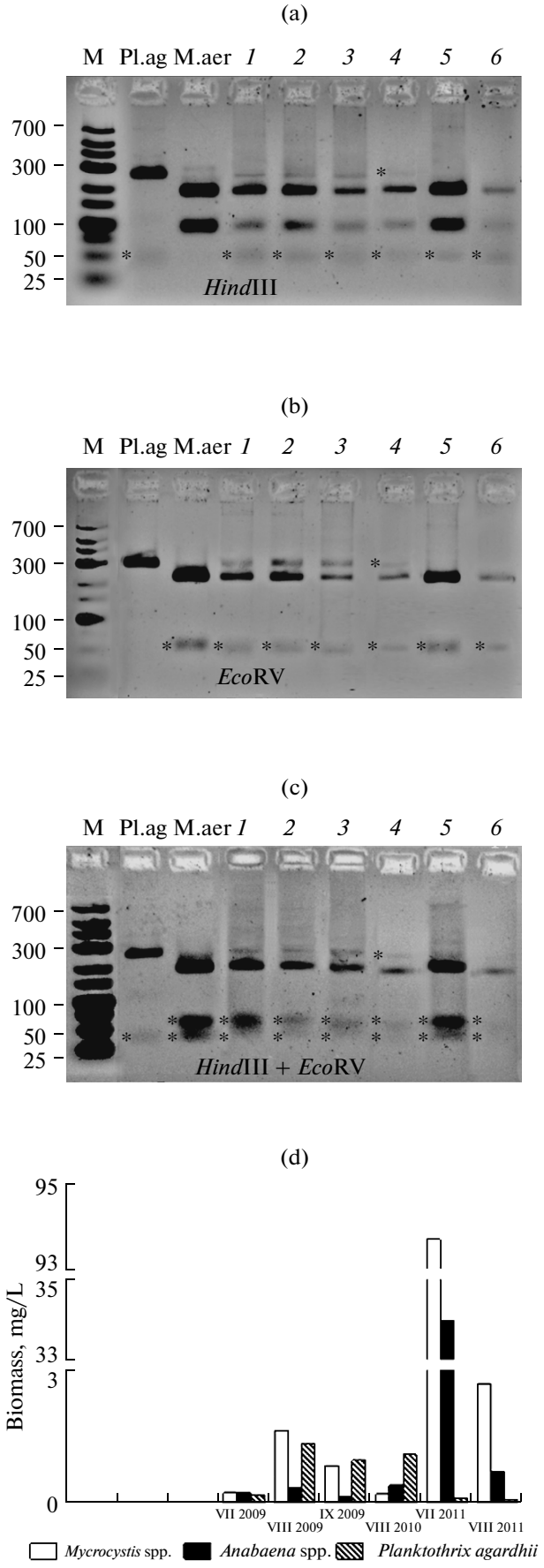

RFLP analysis of *mcyA*-Cd PCR products digested by *Hind*III (a), *Eco*RV (b), or both enzymes *Hind*III + *Eco*RV (c) and biomass dynamics of toxigenic cyanobacterial genera in Lake Nero (d). Lines: M, size marker (bp); Pl.ag, *Planktothrix agardhii* strain NIVA-CYA 126; M.aer., *Microcystis aeruginosa* strain PCC 7806; 1–6, DNA from environmental samples of Lake Nero (1, VII 2009; 2, VIII 2009; 3, IX 2009; 4, VIII 2010; 5, VII 2011; 6, VIII 2011), * Asterisks indicate image fragments poorly visible on the gel.

cystis and *Anabaena*, as well as *Planktothrix agardhii* Gom. (figure, d), in all samples. Among the representatives of the genus *Microcystis, M. aeruginosa*, and *M. wesenbergii* Kom. prevailed in the samples, and *M. flos-aquae* (Wittr.) Kirchn., *M. novacekii* (Kom.) Comp., *M. viridis* (A. Br.) Lemm., and *M. smithii* Kom. et Anagn. occurred sporadically. The most significant among the *Anabaena* were *A. spiroides* Kleb., *A. sphaerica* f. *conoidea* Elenk., *A. sigmoidea* Nyg., *A. viguieri* Denis et Fremy, *A. flos-aquae* (Lyngb.)Breb., and *A. circinalis* (Kutz.) Hansg. In 2009–2010, all three genera of toxigenic cyanobacteria were present in the phytoplankton in approximately equal proportions (figure, d), but in 2011 *P. agardhii* was observed in the samples only in the form of individual filaments; its biomass was extremely low (about 0.1 mg/L), while species of the genera *Microcystis* (up to 94 mg/L) and *Anabaena* (up to 34 mg/L) reached high densities (figure, d). The RFLP patterns identified *Microcystis* as the main microcystin-producers in Lake Nero, since the restriction profiles obtained for all samples appeared to be similar to those obtained for the strain *M. aeruginosa* PCC 7806 (*Hind*III: 2 DNA bands of about 191 and 100 bp; *Eco*RV: 2 DNA bands of about 232 and 59 bp; *Hind*III + *Eco*RV: 3 DNA bands of about 191, 59, and 41 bp) (Figures a–c). Additionally, the presence of microcystin-producing populations of *P. agardhii* was revealed in samples collected in 2009–2010 (lines 1–4); RFLP profiles showed patterns identical to those obtained for the strain *P. agardhii* NIVA-CYA 126 (*Hind*III: 2 fragments with the size of about 261 and 36 bp; *Eco*RV: single fragment with the size of about 297 bp; *Hind*III + *Eco*RV: 2 fragments with the size of about 261 and 36 bp) (figures, a–c). In 2011 (lines 5–6), it was difficult to detect the presence of toxigenic populations of *P. agardhii* in Lake Nero (figures, a–c), presumably due to the extremely low concentration of *P. agardhii* compared to the dominant genera *Microcystis* and *Anabaena* (figure, d). This is the first report on the detection of hepatotoxic *P. agardhii* in Russia; earlier microcystin-producing strains of this species failed be detected in Russian reservoirs [5]. The *mcyA*-Cd fragments typical for microcystin-producing strains of the genus *Anabaena* [9] have not been identified in Lake Nero. Besides, amplification of the total DNA of phytoplankton from the same samples with mcyE-F2/AnamcyE-2R primers did not yield

any PCR products, which confirmed the results of the RFPL analysis.

Thus, this is first time the coexistence of hepatotoxic populations of *Microcystis* and *P. agardhii* was detected in the phytoplankton of Lake Nero; *Anabaena* strains from Lake Nero were found to be unable to synthesize microcystins. Simple molecular techniques were successfully tested in the identification of the microcystin-producing genera/species of cyanobacteria directly in environmental samples.

ACKNOWLEDGMENTS

The work was supported by the grant of the President of the Russian Federation (project no. MK-1284.2013.5), German–Russian Interdisciplinary Science Center (G-RISC; project no. C-2012a-10), Russian Foundation for Basic Research (project no. 12-04-31280), and the Ministry of Education and Science of the Russian Federation (project no. 4.4532.2011). Special thanks to anonymous reviewer for helpful comments on the manuscript and Prof. Dr. Elke Dittmann for her support of this research.

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Translated by N. Kuznetsova