Toxicon 130 (2017) 47–55

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Dolichospermum and *Aphanizomenon* as neurotoxins producers in some Russian freshwaters

Ekaterina Chernova [a, *], Sergey Sidelev [b], Iana Russkikh [a], Ekaterina Voyakina [a], Olga Babanazarova [b], Roman Romanov [c], Anton Kotovshchikov [d], Hanna Mazur-Marzec [e]

[a] Saint-Petersburg Scientific Research Centre for Ecological Safety, Russian Academy of Sciences, Korpusnaya str., 18, Saint-Petersburg, 197110, Russia
[b] P.G.Demidov Yaroslavl State University, Sovetskaya str.,10, Yaroslavl, 150000, Russia
[c] Central Siberian Botanical Garden, Siberian Branch of Russian Academy of Sciences, Zolotodolinskaya str., 101, Novosibirsk, 630090, Russia
[d] Institute for Water and Environmental Problems, Siberian Branch of Russian Academy of Sciences, Molodeznaya str., 1, Barnaul, 656038, Russia
[e] Department of Marine Biotechnology, Institute of Oceanography, University of Gdańsk, Al. Marszałka Piłsudskiego 46, 81-378 Gdynia, Poland

ARTICLE INFO

Article history:
Received 24 November 2016
Received in revised form
13 February 2017
Accepted 16 February 2017
Available online 21 February 2017

Keywords:
Cyanotoxins
LC-MS
PCR
Neurotoxin producers
Aphanizomenon
Dolichospermum

ABSTRACT

Last decades, cyanobacterial blooms have been commonly reported in Russia. Among the boom-forming species, potential toxin producers have been identified. The aim of this paper was to study the presence of neurotoxic compounds - saxitoxins and anatoxin-a - in water bodies from different regions of Russia. We also made attempts to identify the neurotoxin-producing genera. The good convergence of the results obtained by light microscopy, PCR and LC-MS/MS analyses indicated the presence of active neurotoxin producing species in all investigated water bodies. Saxitoxin was detected in phytoplankton from 4 water bodies in Central European Russia and West Siberia, including lake and reservoirs used as a source for potable water. The water bodies differed with the respect of saxitoxin producers which belonged to *Aphanizomenon* and/or *Dolichospermum* genera. For the first time, we obtained quantitative data on the intracellular saxitoxin concentration in Russian freshwaters using LC-MS/MS. Anatoxin-a was detected only in lakes of Northwestern Russia. In the eutrophic shallow Lower Suzdal Lake, *Aphanizomenon* was the stated anatoxin-a-producing genus. In the large shallow artificial hypertrophic Sestroretskij Razliv Lake, it was very likely that both dominant species - *Aphanizomenon flos-aquae* and *Dolichospermum planctonicum* - were anatoxin-a producers.

1. Introduction

Last decades, cyanobacterial blooms have been commonly reported in Russia. Limnologic studies documented the domination of the genera *Aphanizomenon, Dolichospermum, Planktothrix, Microcystis* in most of examined Russian water bodies (Babanazarova et al., 2011; Belykh et al., 2013, 2015; Chernova et al., 2016; Gaevsky et al., 2011; Korneva et al., 2014; Matishov and Kovaleva, 2010; Russkikh et al., 2012; Sidelev et al., 2015; Trifonova and Pavlova, 2008). It has been stated that some species from these genera could produce toxic secondary metabolites (Pearson et al., 2010). However, the investigations of toxin-producing cyanobacteria and cyanotoxins in Russian freshwaters using modern methods are scarce. Studies of toxigenic cyanobacterial species using molecular genetic analysis and determination of cyanotoxin concentration have been conducted since the early 2000s and have shown the presence of microcystins in all investigated blooming water bodies (Babanazarova et al., 2011; Belykh et al., 2013; Chernova et al., 2016; Gaevsky et al., 2011; Korneva et al., 2014; Matishov and Kovaleva, 2010; Russkikh et al., 2012; Sidelev et al., 2015). The number of records on the occurrence of neurotoxins, such as anatoxin-a and saxitoxins, are significantly lower as compared to microcystins. This can be due to lower frequency of neurotoxin-producing cyanobacteria, or less common studies on this group of cyanotoxins.

Saxitoxins (SXTs), known as paralytic shellfish toxins, are among the most potent natural toxins (Aráoz et al., 2010). They include a group of 57 structurally-related carbamate alkaloid neurotoxins (trialkyl tetra hydropurines) (Pearson et al., 2010). The toxins were initially most commonly associated with three genera of marine dinoflagellates: *Alexandrium, Gymnodinium* and *Pyrodinium* (Wiese et al., 2010). Blooms of these dinoflagellates have led to mass kills of

* Corresponding author.
E-mail addresses: s3561389@yandex.ru (E. Chernova), sidelev@mail.ru (S. Sidelev), yanarussk@gmail.com (I. Russkikh), katerina.voyakina@gmail.com (E. Voyakina), baba@bio.uniyar.ac.ru (O. Babanazarova), romanov_r_e@ngs.ru (R. Romanov), kotovschik@iwep.ru (A. Kotovshchikov), biohm@ug.edu.pl (H. Mazur-Marzec).

http://dx.doi.org/10.1016/j.toxicon.2017.02.016
0041-0101/

fish, native animals and livestock (Pearson et al., 2010). The major toxic effect of STXs is due to the blockage of voltage-gated sodium channels of neuronal cell membranes (Pearson et al., 2010). STXs typically accumulate through the food chain and can be present in organisms consumed as seafood (Shumway, 1995). Intraperitoneal LD_{50} is 10 μg kg^{-1} body weight in mice (Halstead and Schantz, 1984), while human death occurred after ingestion of 1 mg of the toxin (Evans, 1969).

In Russia, the investigations of STXs were mainly conducted in marine environment; the earliest documented case of paralytic shellfish poisoning was in September 1945, when six crew members of fishing fleet "Aleut" were poisoned and two of them died after eating mussels collected in Pavla Bay on the western coast of the Bering Sea (Lebedev, 1968). Later the cases of human poisoning by STXs were recorded along the Russian Pacific cost (Kisselev, 1959; Konovalova, 1993; Kurenkov, 1974; Orlova et al., 2007). STXs can be also produced by some fresh water species of cyanobacteria. *Aphanizomenon flos-aquae* as a STXs producer was first reported in Canada in the late 1960s (Jackim and Gentile, 1968). To date, it has been stated that STXs are also synthesized by *Dolichospermum*, *Cylindrospermopsis*, *Lyngbya*, *Scytonema*, *Planktothrix* and *Raphidiopsis* (Aráoz et al., 2010; Pearson, 2010; Wiese et al., 2010). As Russian freshwaters were considered, STXs were recorded in Lake Baikal in 2010 (Belykh et al., 2015) and Rybinsk Reservoir in 2013 (Sidelev et al., 2016).

Neurotoxic anatoxin-a (ANTX-a, 2-acetyl- 9-azabicyclo(4-2-1) non-2-ene) is a low molecular weight alkaloid. It is a potent post-synaptic cholinergic nicotinic agonist, which acts as a depolarizing neuro-muscular blocking agent (Osswald et al., 2007). The LD_{50} of ANTX-a is 200–250 μg kg^{-1} dw (Devlin et al., 1977). Initially it was isolated from toxic *Dolichospermum* (*Anabaena) flos-aquae* (Devlin et al., 1977). The identified ANTX-a producing genera include *Oscillatoria*, *Planktothrix, Cylindrospermum*, *Phormidium*, *Raphidiopsis*, *Aphanizomenon*, *Arthrospira* (Ballot et al., 2010a; Gugger et al., 2005; Lyra et al., 2001, Rantala-Ylinen et al., 2011; Sivonen and Jones, 1999; Viaggiu et al., 2004).

The occurrence of ANTX-a in Russian water bodies was reported in a few works (Chernova et al., 2016; Matishov et al., 2006), and only in one work attempts were made to identify the toxin producer using molecular methods (Sidelev et al., 2015.).

The aim of the present work was to extend the knowledge about the occurrence of cyanobacterial neurotoxic compounds (SXTs and ANTX-a) in water bodies from different geographical regions of Russia, namely Northwestern, Central European parts and West Siberia of Asian part of Russia.

In addition, the profile of the neurotoxins detected with chemical and molecular methods was compared with the structure of cyanobacterial community. We tried to elucidate which cyanobacteria could be a source of the toxins. Frequent occurrence of *Aphanizomenon* and *Dolichospermum* in the biomass of STX and ANTX-a containing samples indicates that a representative of these genera could produce the toxins.

2. Material and methods

2.1. Chemicals

The STX and GTX2/3 standards were from Institute of Marine Science National Research Council in Halifax, Canada; () anatoxin-a fumarate was obtained from Tocris Bioscience (Bristol, UK). Acetonitrile and methanol (hypergrade for LC-MS LiChrosolv) were purchased from Merck (Darmstadt, Germany). Water was purified to 18.2 MΩ cm^{-1} in a Millipore Direct-Q water purification system (Bedford, MA, USA). Formic acid and ammonium formate (≥97%) were obtained from Fluka Chemika (Buchs, Switzerland). Membrane filter discs (nylon 66, 47-mm diameter) were obtained from Supelco (Bellefonte, PA).

2.2. Study sites and sampling

The present study was conducted in water bodies located in different parts of Russia (Fig. 1). The description of the studied reservoirs has been presented in Table 1.

Sampling was performed in the period of 2013–2015. From the water bodies with visible blooming, biomass samples were collected with a net (85 μm). Cyanobacterial material from net samples was transported to laboratory in screw-cap tubes (Axigen, California, USA) and immediately frozen at −20 °C until freeze-drying and further processing. Additionally, *Dolichospermum lemmermannii* and *D. circinale* single colonies were gathered from Rumnikovo Lake. They were picked up from diluted samples with a sterile needle under binocular microscope and washed 3 times in sterile water. Then, the colonies were analyzed. The absence of other cyanobacteria was checked under the microscope.

From water bodies with the lack of visible bloom, surface water samples (1 L) were collected with plastic bottles. In laboratory, the samples were filtered using Whatman GF/C filters. The filters with biomass were immediately frozen at −20 °C until extraction. Later the filters with biomass were used for isolation of environmental DNA and cyanotoxins extraction for LC-MS analysis.

For microscopic investigation, the surface water samples in 1 L bottles were fixed with a Lugol-formalin solution. In total, 54 phytoplankton samples were collected.

2.3. Analysis of cyanobacteria

The samples were preserved with Lugol's solution with addition of formalin, ice acetic and chromic acids. Samples were left for sedimentation for 2 weeks. Then sample volume was reduced to 10 ml by decantation. Qualitative and quantitative analyses of cyanobacteria were carried out with the aid of a light microscope (Axioscop 40L, Carl Zeiss, Germany). Cells were counted in an Uchinskaya counting chamber № 2. The gridded area (100 mm^2) of the chamber consists of 40 narrow strips of 2.5mm2. The chamber depth is 0.1 mm, the volume is 10 mm^3 (Guseva, 1956). The biomass of cyanobacteria was calculated using species-specific geometric formulas (Olenina et al., 2006). The biomass was determined using the results of species-specific counts and individual volumes. Species comprising more than 10% of total phytoplankton biomass were considered to be dominant. The species identification was performed according to Komárek and Anagnostidis (1998, 2005), Komárek (2013).

2.4. DNA extraction and PCR amplification

Environmental DNA from phytoplankton samples collected in lakes and reservoirs was isolated using the Diatom™ DNA Prep 200 reagent kit (Isogene Lab ltd, Russia) according to the manufacturer's recommendations. DNAs from the isolated colonies of *Dolichospermum lemmermannii* and *D. circinale* were extracted with the InstaGene Matrix (Bio-Rad, USA) according to the manufacturer's instruction.

The samples were searched for the *stxA* (additionally *stxI*) and *anaC* genes, involved in synthesis of STX and ANTX-a, respectively. Polymerase chain reaction (PCR) was performed using specific primers *anaC-genF/anaC-genR* (366 bp), *sxtaf/sxtar* (600 bp) and *sxtIf/sxtIr* (910 bp) under amplification conditions described in Ballot et al. (2010a), Rantala-Ylinen et al. (2011), Casero et al. (2014). For direct genus-level identification of potential ANTX-a producers in the environmental samples with mixed cyanobacterial