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SHORT COMMUNICATIONS

Identification of Cyanobacterial Producers of Shellfish Paralytic Toxins in Lake Baikal and Reservoirs of the Angara River

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Massive development of cyanobacteria often results in the release of toxins into water. Among these toxins the neurotropic saxitoxin (SXT) and its analogues, termed paralytic shellfish toxins (PST), are the most dangerous [1]. Dinoflagellates and cyanobacteria are the major PST producers in marine and freshwater ecosystems, respectively. Shellfish accumulate PST via the food chain and may cause severe poisoning with the symptoms of central nervous system affection if consumed by humans. Out of 2000 cases of PST poisoning, 15% had a fatal outcome [2]. Humans are poisoned by PST mainly via drinking water or during bathing. Saxitoxin (trialkyl tetrahydropurine, $C_{10}H_{17}N_7O_7$) is a highly toxic compound with LD_{50} of 10 μg/kg. Over 57 SXT analogues have been identified [4]. PST block the sodium channel pores of nervous and muscular cells and prevent the generation of the action potential, thus causing muscular paralysis, including that of respiratory muscles [2, 4]. In the course of 2–12 h complete paralysis and death from respiratory failure can occur, or the patients recover in several days with complete disappearance of the symptoms. In spite of high PST toxicity, WHO-approved maximum allowable concentrations exist only for shellfish meat, but not for water. Regional standards exist in the countries where PST-producing blooms are common. In Australia and Brazil, the maximum allowable concentration for SXT in drinking water is 3 μg/L [1]. PST synthesis in dinoflagellates and cyanobacteria is carried out by a modular multienzyme complex containing approximately 26 enzymes, of which polyketide synthase plays the major role [2].

The goal of the present work was to search for PST-producing cyanobacteria in Lake Baikal and reservoirs of the Angara River using a marker for the polyketide synthase gene *stx*A and to detect saxitoxin and its analogues by enzyme-linked immunosorbent assay (ELISA) and mass spectrometry.

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Samples from Lake Baikal were collected in August 2010 in the coastal zone of the Barguzin and Kurkut Bays (Maloe More Strait). Samples were taken from the Irkutsk reservoir (near Patrony village), Bratsk reservoir (near Novo-Dolonovo village), and Ust-Ilimsk reservoir (near Zheleznodorozhnik settlement) in July 2010. Plankton was sampled with a Ruttner sampler from 1-m depth and with an Apstein plankton net by filtration of the upper 1.5-m water layer. The bottle samples were used for qualitative and quantitative assessment of cyanobacteria as described previously [5]. Total DNA was extracted from net samples fixed with 70% ethanol using the DNA-sorb kit (InterLabServis, Russia). The *stx*A gene fragment (555 bp) was amplified according to [6]. DNA of saxitoxin-producing *Aphanizomenon gracile*, which was used as a positive control, was kindly provided by A. Ballot (Stechlin, Germany). PCR fragments were analyzed as described in [5]. The sequences were deposited to GenBank under accession nos. JF739253–JF739268 and JF739275–JF739282. For ELISA, unfixed samples were frozen. The concentrations of STX and its analogues were determined using the Abraxis Saxitoxin ELISA kit (Abraxis LLC, United States). PST screening in phytoplankton extracts was carried out on a tandem time-of-flight mass spectrometer with matrix laser desorption/ionization (MALDI-TOF) (Ultraflex Bruker Daltoniks, Germany).

Microscopy of plankton samples revealed members of two cyanobacterial genera capable of PST production: *Aphanizomenon* and *Anabaena* (table). Cyanobacteria containing the *stx*A genes were detected in Lake Baikal and the Ust-Ilimsk reservoir (table). The obtained *stx*A gene sequences exhibited 97–99% similarity to those of the *Aphanizomenon* and *Anabaena* species isolated in Europe and Australia during PST water bloom. PST were revealed at the same stations by ELISA; their concentrations did not exceed the standards for drinking water (table). MALDI-TOF/TOF analysis revealed four PST types in Lake Baikal: saxitoxin and neosaxitoxin (neoSXT) contain-

PCR analysis of cyanobacteria and measured ELISA-concentrations of saxitoxin and analogues in Lake Baikal and reservoirs of the Angara River

Water body	Potentially toxic cyanobacteria, 10^6 cells/mL		*stx*A gene	PST, μg/L	PST variants found
	Anabaena spp.	*Aphanizomenon* spp.			
Barguzin Bay (Lake Baikal)	0.38	Single cells	+	0.14	SXT neoSXT
Kurkut Bay (Lake Baikal)	2.16	Single cells	+	0.59	SXT dcGTX2,3 dcGTX1,4
Irkutsk Reservoir	0.02	0.05	–	–	ND*
Bratsk Reservoir	3.04	2.71	–	–	ND*
Ust-Ilimsk Reservoir	2.06	71.44	+	1.37	ND*

* ND stands for no data.

ing carbamoyl groups, as well as decarbamoyl derivatives of gonyautoxin dcGTX2,3 and dcGTX1,4 (table).

Our data demonstrate the presence of two cyanobacterial genera capable of synthesis of STX and its analogues in Lake Baikal and the Ust-Ilimsk reservoir: *Aphanizomenon* and *Anabaena*. Since phylogenetic relations between these genera according to the *stx*A gene have not been determined, PST producers were not identified at the species level. Since *Anabaena lemmermannii*, a known SXT producer [7], predominated in Baikal samples, this species is probably the main saxitoxin producer in Lake Baikal. *Aphanizomenon* sp., which was predominant in the Ust-Ilimsk reservoir, was probably responsible for synthesis of shellfish paralytic toxins in this ecosystem.

Thus, cyanobacteria producing shellfish paralytic toxins were revealed in Lake Baikal and reservoir of the Angara River by a combination of various methods. Presently, development of tourism increases the anthropogenic load on the lake, resulting in increased abundance of cyanobacteria. Methods for detection of cyanobacteria capable of producing paralytic toxins were approved, and potential threat of poisoning by saxitoxin and its analogues was confirmed.

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