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Short communication

# First detection of benthic cyanobacteria in Lake Baikal producing paralytic shellfish toxins

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ABSTRACT

Cyanobacteria were screened from the surface of diseased sponges, stone and bedrock in Lake Baikal for the presence of saxitoxin using enzyme-linked immunosorbent assay. In sequel, eight paralytic shellfish toxin (PST) variants were identified using a MALDI mass spectrometry. Microscopic examination found that *Tolypothrix distorta* dominated in the biofouling samples. PCR and sequencing detected *sxt*A gene involved in saxitoxin biosynthesis, thereby providing evidence of the PST producing potential of Baikal cyanobacterial communities inhabiting different substrates.



Excessive proliferation of toxin-producing cyanobacteria is a serious environmental problem. Planktonic cyanobacterial bloom and cyanotoxin poisoning events are well documented. In recent decades, anatoxin-a, nodularin, microcystin and saxitoxin have been identified in benthic cyanobacterial mats worldwide (Quiblier et al., 2013).

Among the toxins produced by cyanobacteria, saxitoxin (trialkyl tetrahydropurine, STX) is the most toxic, with a 50% lethal dose of 10 μg/kg body weight (administered intraperitoneally) in mice (Chorus and Bartram, 1999). STX and more than 50 of its analogues are collectively referred to as "paralytic shellfish toxins" (PSTs) (Wiese et al., 2010). The production of PSTs by some benthic representatives of the genera *Anabaena*, *Nostoc*, *Oscillatoria* and species *Lyngbya wollei*, *Phormidium favosum*, *P. autumnale*, and *Scytonema crispum* has been reported (Quiblier et al., 2013). PSTs ingested by herbivorous shellfish are transferred to birds and mammals via food webs causing neurotoxic effects like paralysis or respiratory failure. PST toxicity is mediated mainly through inhibition of nerve conduction by blocking neuronal voltage-gated sodium channels in excitable cells.

Toxin-producing cyanobacteria were found in the pathogenic microbial consortium leading to rapid tissue necrosis in black band disease of corals (BBD) (Myers et al., 2007). Filamentous cyanobacteria were detected in bleaching sponge tissues in fatal sponge orange band (SOB) disease and Aplysina red band syndrome (ARBS) (Olson et al., 2006; Angermeier et al., 2011). However, no information on the presence of cyanotoxins was available.

Since 2011, large-scale changes referred to the "ecological crisis" have been observed in the littoral zone of Lake Baikal. The most alarming events concerned sudden mass mortality of endemic sponges accompanied by abundant development of filamentous cyanobacteria on sick sponges (Fig. 1A-E). The percentage of affected branched sponges *Lubomirskia baicalensis* ranged from 30% to 100% in some areas of the lake (Timoshkin et al., 2014). The causes of rapid ecological changes observed in Lake Baikal, the UNESCO World Heritage Site and the largest world's fresh water reserve (Kozhova and Izmesteva, 1998), are still unknown (Timoshkin et al., 2014, 2015).

The aims of this work were to detect the production of STX (PSTs) by Baikal benthic cyanobacteria inhabiting different substrates by ELISA, identify PST variants by mass-spectrometry and search cyanobacterial genes involved in STX synthesis by molecular methods.

Samples were collected in September of 2015 in the littoral zone

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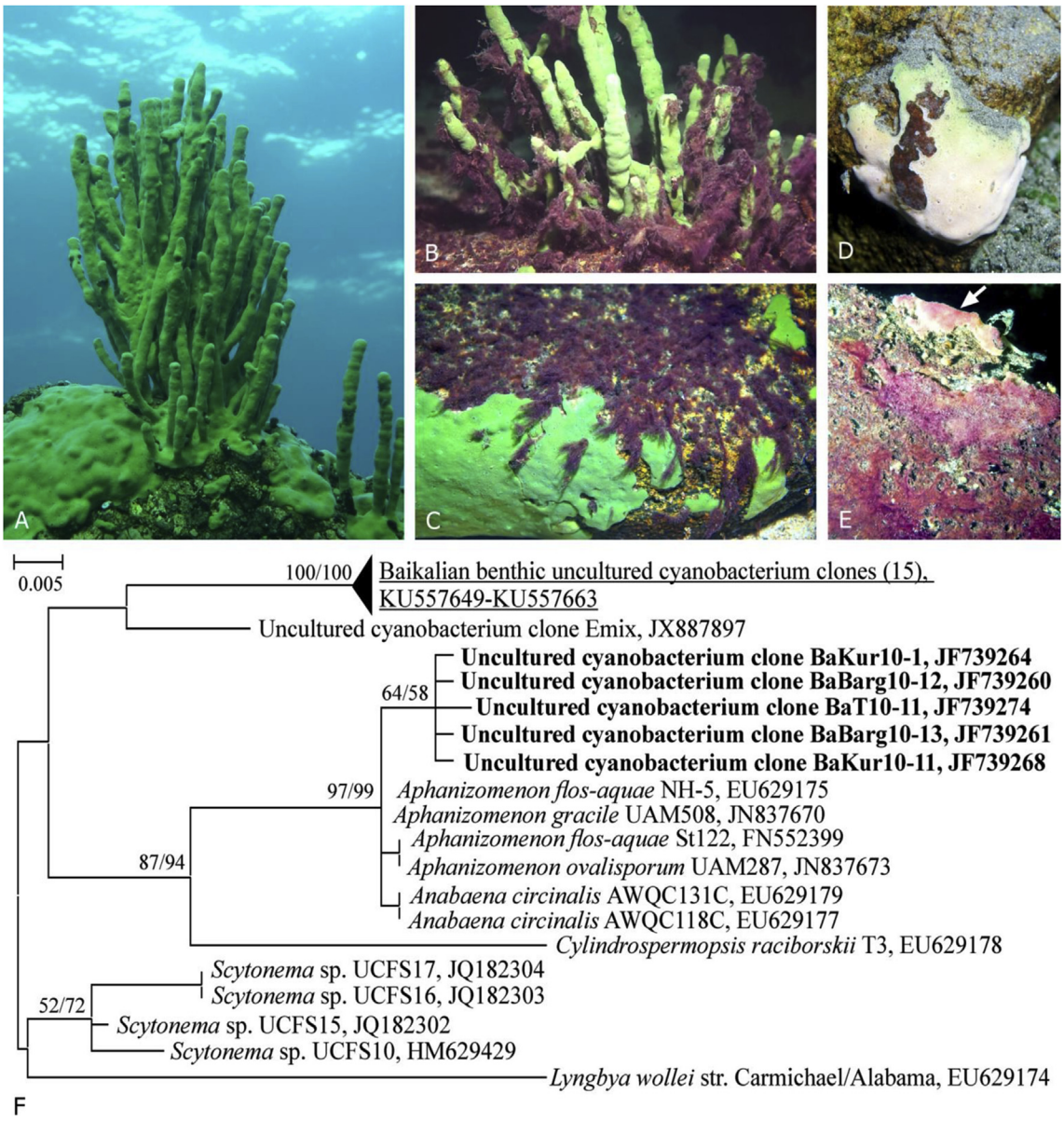


**Fig. 1.** (A) Typical view of the underwater landscape in September of 2010 before "ecological crisis" (6–8 m deep); cyanobacterial fouling on different substrates in September of 2015: (B) on sponge *L. baikalensis*, (C) on stone and intact crustose sponge *Baikalospongia* sp., (D) on diseased *Baikalospongia* sp. and (E) on bedrock and dead sponge (arrow). (F) Maximum likelihood tree of *sxt*A gene sequences for Baikal benthic cyanobacteria. Numbers in branching nodes show bootstrap values in 1000 replications (ML/NJ). Sequences obtained in this study are underlined. Sequences of planktonic cyanobacteria from previous studies are in bold.

of Southern Baikal near the settlements of Listvyanka and Bolshiye Koty. Scuba divers sampled biofouling from sponge surface, stones and bottom detritus and biofouling together with body fragments of sick or decomposed dead sponges at depths of 10–15 m. In total, 12 samples were obtained: 31, 36, 46 – from the sponge *L. baicalensis* (Fig. 1B); 33, 42, 44 – from the crustose sponge *Baikalospongia* sp. (Fig. 1C-E); 32, 34, 35, 45 – from stones (Fig. 1C), 41 – from the bedrock (Fig. 1E), and 43 – from detritus (Table 1). Samples were fixed with 4% formalin for microscopic examination and were frozen in liquid nitrogen for molecular analysis. Species of cyanobacteria were identified using light and epifluorescence microscopy (Sorokovikova et al., 2013). Sponges were identified according to Rezvoj (1936).

Search of saxitoxin synthesis genes was performed in PCR with primers according to Ballot et al. (2010). DNA isolation, PCR, cloning, sequencing, and tree construction were carried out according to techniques described earlier (Belykh et al., 2011). Decoded sequences of 65 cloned amplicons contained 50 identical sequences, which were excluded from the analysis. Unique sequences were deposited in GenBank under accession numbers KU557649-KU557663.

Immunoenzyme assay was employed using an Abraxis Saxitoxin ELISA kit (Abraxis LLC, USA) according to the manufacturer's protocol. The analysis consisted of two stages: sample screening for the STX presence performed on board the research vessel and measurement of STX concentrations in the laboratory. Cyanobacterial material was lyophilised and weighed. Results were processed using the programme RIDA® SOFT Win. Some frozen material was extracted according to Lagos et al. (1999). PST variants were identified using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (UltraflexBrukerDaltoniks, Germany) as described earlier (Belykh et al., 2015a).