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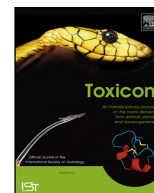

Screening of cyanobacterial cultures originating from different environments for cyanotoxicity and cyanotoxins

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ABSTRACT

Eighty cultures from the Novi Sad Cyanobacterial Culture Collection (NSCCC) were screened for toxicity with *Artemia salina* bioassay and for common cyanobacterial toxins, microcystins/nodularin (MCs/NOD) and saxitoxin (STX), with ELISA assays. The results show that 22.5% (11) of the investigated cyanobacterial cultures in exponential phase exhibited toxicity in the *A. salina* bioassay and 38.7% (31) produced MCs/NOD and/or STX. However, the findings in the two methods applied were contradictory. Therefore, *A. salina* bioassay was repeated on 28 cultures in stationary growth phase, which were positive in ELISA assays but not in the initial *A. salina* bioassay. Seven more cultures exhibited cell-bound toxicity, and only one extracellular toxicity. The observed difference in the toxicity indicates that cyanobacterial growth phase could affect the screening results.

The findings also varied depending on the environment from which the cultures originated. In the initial screening *via* bioassay, 11.8% (6 cultures out of 51) from terrestrial and 17.2% (5 out of 29) from aquatic environment showed cell-bound toxicity. Furthermore, based on the ELISA assay, 31.4% (16) of the cultures from terrestrial ecosystems were positive for the presence of the investigated cyanotoxins, and 51.7% (15) from aquatic ecosystems. Based on all results, more frequent toxin production was observed in cultures originating from aquatic environments. Furthermore, the group of terrestrial cultures that originated from biological loess crusts were basically non-toxic.

The discrepancies in the results by two different methods indicates that the use of several complementary methods would help to improve the assessment of cyanobacterial toxicity and cyanotoxin analyses.

1. Introduction

Cyanobacteria are photosynthetic prokaryotes which inhabit a wide range of aquatic and terrestrial environments throughout the world. They have existed for approximately 2.8–3.5 billion years, and are still to this day one of the most important photosynthetic organism groups on the planet (Schopf and Walter, 1982; Olson, 2006; Whitton, 2012). Even though over 2600 cyanobacterial species have been described so far, it is believed that many more species still remain unknown (Nabout et al., 2013). Biodiversity of the known and collected species or strains can be preserved in cyanobacterial culture collections which represent important repositories and "live gene banks" that can be used for studies of cyanobacterial components or metabolites, as well as ecology, toxicology, and possible biotechnological and medicinal use of these microorganisms.

Cyanobacteria can produce numerous bioactive secondary metabolites including cyanobacterial toxins (cyanotoxins). Strong evidence of the deleterious effects of cyanotoxins on other organisms including humans is continuously emerging (Falconer, 1998; Kuiper-Goodman et al., 1999; Carmichael et al., 2001; Stewart et al., 2008; Saqrane et al., 2009; Peng et al., 2010; Žegura et al., 2011; Drobac et al., 2016, 2017; Svirčev et al., 2013, 2014; 2015, 2017a). Based on their target organs, cyanotoxins can be divided into several groups such as hepatotoxins (e.g. microcystin - MC and nodularin - NOD) and neurotoxins (e.g. saxitoxin - STX). MCs are probably the most widespread and the most studied cyanotoxins, with rich structural variety, encompassing over 240 variants (Spoof and Catherine, 2017), and new analogues are still being discovered.

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As cyanotoxins are regarded as an emerging threat, numerous methods for their detection, identification and quantification have been developed (Kaushik and Balasubramanian, 2013; Meriluoto et al., 2017). However, current routine methods (such as liquid chromatography-mass spectrometry - LC-MS, and enzyme-linked immunosorbent assay - ELISA, bioassays) are not capable of detecting all types and variants of cyanotoxins. While instrumental analysis methods are quite accurate, they are also expensive, laborious and can only detect certain toxins, depending on available standards. On the other hand, test kits and bioassays are sometimes cheaper and provide quick results, but the kits detect groups of toxins and suffer from some degree of unwanted cross-reactivity, while bioassays are not specific/sensitive enough.

Current research emphasis is primarily placed on cyanobacteria in aquatic ecosystems, and little is known regarding cyanobacteria and their toxicity in terrestrial ecosystems. The aim of this paper was to a) investigate the occurrence of toxicity and different cyanotoxins in cyanobacterial cultures from the Novi Sad (Serbia) Cyanobacterial Culture Collection (NSCCC); b) compare the results obtained from cultures originating from terrestrial and aquatic environments; and c) assess the reliability of *Artemia salina* bioassay and ELISA for the detection of toxic secondary metabolites of cyanobacteria.

2. Material and methods

During this investigation, 80 cyanobacterial cultures NSCCC were assessed with an *A. salina* bioassay, and screened for selected cyanotoxins (MCs/NOD and STX) by ELISA. Cultures were isolated from various aquatic (29) and terrestrial environments (51) from Serbia and cultured at the Department of Biology and Ecology in Novi Sad. Cyanobacteria from the cyanobacterial culture collection NSCCC were cultivated in 250 mL Erlenmeyer flasks with BG-11 medium (Rippka et al., 1979) under illumination by white fluorescent light (50 μmol photons $m^{-2} s^{-1}$) with a 12 h photoperiod at 22–24 ± 1 °C. Most investigated cultures belonged to the genera *Nostoc, Anabaena*, *Phormidium, Leptolyngbya, Jaaginema, Chroococcus* and *Planktolyngbya.*

The *A. salina* bioassay was conducted on two separate occasions: firstly, as an initial toxicity screening during the culture's exponential phase (on the 28th day of cultivation), and secondly, for the toxicity screening during the stationary phase. For the first screening, 20 mL of each cyanobacterial culture were filtered through filters. Filters containing the biomass (cell-bound toxin) were then air-dried overnight at 37 °C. The dried filters were extracted with 75% (v/v) methanol for 24 h, sonicated, and the extracts were centrifuged. The supernatants were collected and, after an overnight evaporation in a microtiter plate at 37 °C, used for bioassay. Toxicity of cultures was assessed using *A. salina* larvae according to Kiviranta et al. (1991), and was expressed as the difference (%) between mortalities in the tested and control samples.

Analyses with the two ELISA assays followed, where about 2 mL of each cyanobacterial cultures in the stationary phase were freeze-thawed and sonicated to ensure cellular decomposition and release of intracellular content. The extract was then centrifuged (NF 800 R, Nüve, Turkey) at 2348 × *g* for 15 min and the supernatant was used in two assays. The Microcystins-ADDA ELISA and Saxitoxin ELISA (Abraxis LLC, USA) are immunoassays for the quantitative and sensitive congener-independent detection of MCs/NOD and STX, respectively. The ELISA plates were read using a microplate reader (Asys Expert Plus UV, Biochrom, UK).

In 28 cultures which were positive in the ELISA assays but not in the initial *A. salina* bioassay, a second bioassay for cell-bound and extracellular toxicity was performed again in the stationary growth phase. For the second bioassay, 20 mL of cyanobacterial cultures were filtered and the preparation of the cell-bound fraction was the same as in the first bioassay. However, the filtrate (extracellular part) was also collected from cultures in stationary phase and used as such for the bioassay.

Table 1
Results from *Artemia salina* bioassay and ELISA assay.

No.	Code	Genus	*Artemia salina* bioassay	ELISA assay	
			CB exponential phase (48 h)	MC/NOD	STX
1	T1	*Anabaena* sp.	–	–	–
2	T2	*Anabaena* sp.	–	–	–
3	T3	*Anabaena* sp.	–	–	–
4	T4	*Anabaena* sp.	–	–	–
5	T5	*Anabaena* sp.	–	–	–
6	T6	*Anabaena* sp.	–	–	–
7	T7	*Anabaena* sp.	–	–	–
8	T8	*Anabaena* sp.	+	–	–
9	T9	*Anabaena* sp.	+	–	–
10	T10	*Anabaena* sp.	–	+	+
11	T11	*Anabaena* sp.	–	+	–
12	T12	*Anabaena* sp.	–	+	–
13	T13	*Anabaena* sp.	–	+	–
14	T14	*Anabaena* sp.	–	+	–
15	T15	*Calothrix* sp.	–	–	–
16	T16	*Chroococcus* sp.	–	–	–
17	T17	*Chroococcus* sp.	–	–	–
18	T18	*Chroococcus* sp.	–	–	–
19	T19	*Chroococcus* sp.	–	–	–
20	T20	*Chroococcus* sp.	–	–	–
21	T21	*Chroococcus* sp.	–	–	+
22	T22	*Leptolyngbya* sp.	–	–	–
23	T23	*Leptolyngbya* sp.	–	–	–
24	T24	*Leptolyngbya* sp.	–	–	–
25	T25	*Leptolyngbya* sp.	–	–	–
26	T26	*Leptolyngbya* sp.	–	+	–
27	T27	*Nostoc* sp.	–	–	–
28	T28	*Nostoc* sp.	–	–	–
29	T29	*Nostoc* sp.	–	–	–
30	T30	*Nostoc* sp.	–	–	–
31	T31	*Nostoc* sp.	–	–	–
32	T32	*Nostoc* sp.	–	–	–
33	T33	*Nostoc* sp.	–	–	–
34	T34	*Nostoc* sp.	–	–	–
35	T35	*Nostoc* sp.	–	–	–
36	T36	*Nostoc* sp.	–	–	–
37	T37	*Nostoc* sp.	–	–	–
38	T38	*Nostoc* sp.	–	–	–
39	T39	*Nostoc* sp.	–	–	–
40	T40	*Nostoc* sp.	–	–	–
41	T41	*Nostoc* sp.	+	–	–
42	T42	*Nostoc* sp.	–	+	+
43	T43	*Nostoc* sp.	+	–	+
44	T44	*Nostoc* sp.	–	+	–
45	T45	*Nostoc* sp.	–	+	–
46	T46	*Nostoc* sp.	–	+	–
47	T47	*Nostoc* sp.	–	+	–
48	T48	*Nostoc* sp.	–	+	–
49	T49	*Nostoc* sp.	–	+	–
50	T50	*Phormidium* sp.	+	+	+
51	T51	*Synechocystis* sp.	+	–	–
52	A1	*Anabaena* sp.	–	–	–
53	A2	*Aphanizomenon* sp.	–	–	–
54	A3	*Geitlerinema* sp.	–	+	–
55	A4	*Gloeocapsa* sp.	–	–	–
56	A5	*Gloeocapsa* sp.	–	–	–
57	A6	*Gloeocapsa* sp.	–	+	–
58	A7	*Jaaginema* sp.	–	–	–
59	A8	*Jaaginema* sp.	–	–	–
60	A9	*Leptolyngbya* sp.	–	+	–
61	A10	*Leptolyngbya* sp.	–	+	–
62	A11	*Nostoc* sp.	–	–	–
63	A12	*Nostoc* sp.	+	–	–
64	A13	*Nostoc* sp.	–	+	+
65	A14	*Nostoc* sp.	–	–	+
66	A15	*Nostoc* sp.	–	+	–
67	A16	*Nostoc* sp.	–	+	–
68	A17	*Oscillatoria* sp.	–	+	–
69	A18	*Phormidium* sp.	–	–	–
70	A19	*Phormidium* sp.	+	–	–

(*continued on next page*)

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