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Detection and confirmation of saxitoxin analogues in freshwater benthic *Lyngbya wollei* algae collected in the St. Lawrence River (Canada) by liquid chromatography–tandem mass spectrometry

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ABSTRACT

The presence of cyanotoxins in benthic *Lyngbya wollei* algae samples collected in a fluvial lake along the St. Lawrence River, Canada, was investigated using a multi-toxins method. Hydrophilic interaction liquid chromatography (HILIC) and reverse phased liquid chromatography (RPLC) were coupled to triple quadrupole mass spectrometry (LC-QqQMS) for quantification and to quadrupole-time of flight mass spectrometry (LC-QqTOFMS) for screening and confirmation. The presence of two saxitoxin analogues, LWTX-1 and LWTX-6, was confirmed in benthic *Lyngbya wollei* algae samples. Concentration of LWTX-1 was between 209 ± 5 and $279 \pm 9\ \mu g\ g^{-1}$. No other targeted cyanotoxin (such as anatoxin-a, nodularin, microcystin-LR, microcystins-RR and saxitoxin) was found in the samples. The presence of LWTX-6 was observed by using a screening approach based on an in-house database of cyanotoxins, an algorithm of identification and high resolution mass spectrometry measurements on the precursor and product ions. This work demonstrates the need for more research on the fate of benthic cyanotoxins in aquatic ecosystems such the St. Lawrence River.

1. Introduction

Cyanotoxins are among the oldest environmental organic contaminants known as they were first reported in an Australian lake in 1878 [1]. They are metabolic by-products released by cyanobacteria, also called blue-green algae. These species are ubiquitous inhabitants of surface water, but when several physical, chemical and biological mechanisms combine and interact with the ecosystem, they can form dense blooms [2,3]. Harmful algal blooms (HABs) develop when these increased populations release cyanotoxins into the aquatic environment during cell growth or cell lysis. Consequently, surrounding water becomes toxic to animals and humans.

In the last few decades, reports on HABs around the world have increased [2]. Moreover, recent studies suggest that global warming could contribute to a further increase in the formation of HABs [4]. Therefore a growing interest in HABs has arisen in the last few years since these blooms deteriorate water quality thus affecting agricultural and recreational activities and also potentially contaminating drinking water supplies [5,6]. Among the cyanotoxins, two classes of substances have attracted more attention because of their toxicity: the hepatotoxins and the neurotoxins. The hepatotoxins are comprised of three main groups: microcystins, nodularins and cylindrospermopsins. The neurotoxins are comprised of anatoxins and saxitoxins (also called paralytic shellfish poisons or PSP).

For instance in Canada, cyanobacterial research in freshwaters has focused primarily on microcystins and anatoxin-a [7]. However, recent studies on the distribution of benthic macroalgae in fluvial lakes of the St. Lawrence River observed the presence of *Lyngbya wollei,* a filamentous benthic cyanobacterium, at the bottom of the river ecosystem [8]. These cyanobacteria are responsible for the production of several analogues of saxitoxin, such as decarbamoylsaxitoxin and decarbamoylgonyautoxin-2 and -3, as well as six other compounds known as *L. wollei* toxins (LWTXs) [9]. According to mouse bioassays, LWTXs do not appear to be as toxic as other saxitoxins [9]. However given their structural similarity to saxitoxin, abiotic or biotic transformation of LWTXs to other more toxic analogues cannot be ruled out without additional bioassays. This type of transformation has been observed for N-sulfocarbamoyl saxitoxins (C1 and C2) which can be converted to the more toxic decarbamoylgonyautoxins [10].

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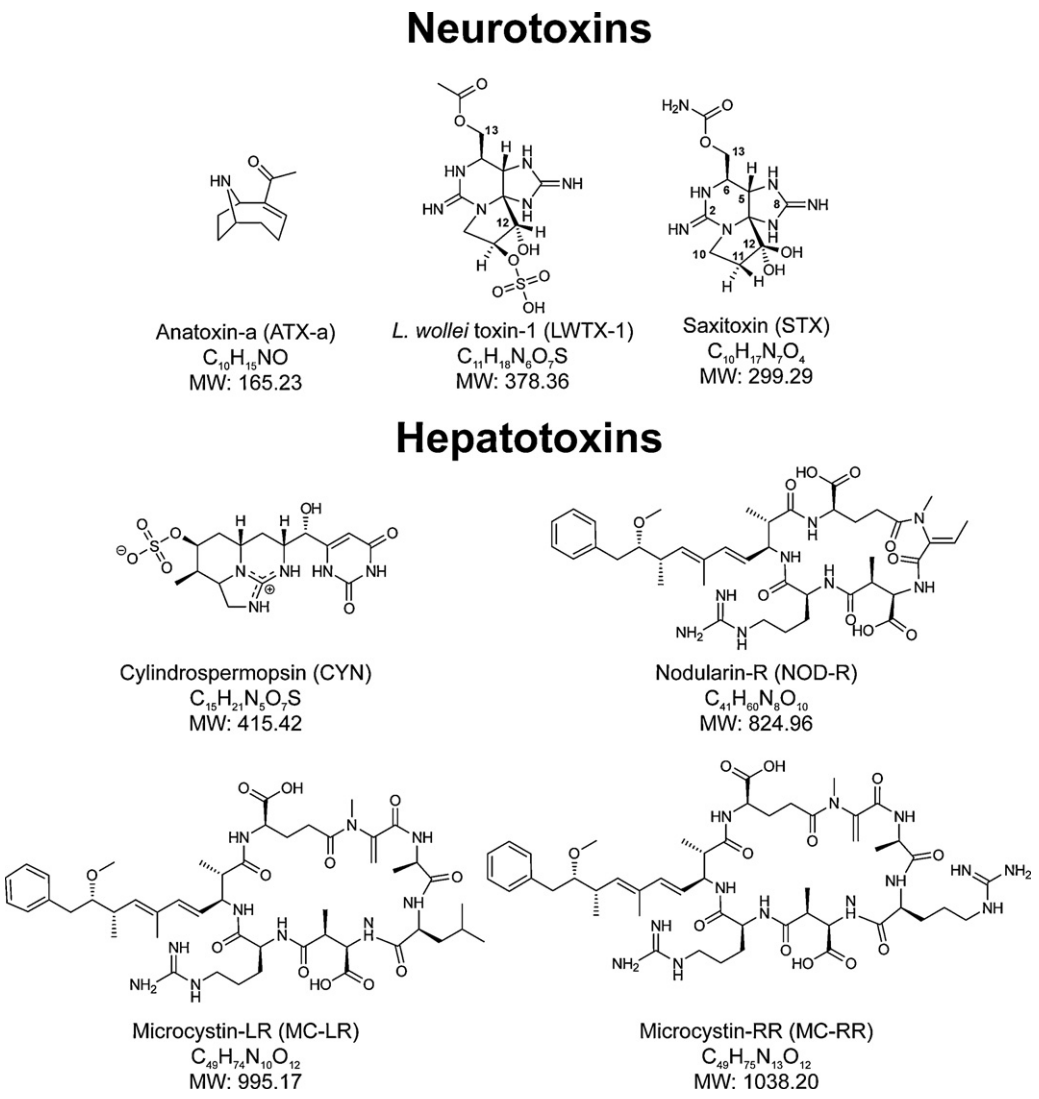

Fig. 1. Chemical structures of studied cyanotoxins along with their respective molecular formula and molecular weight.

Cyanotoxins present several challenges that have hindered the development of analytical methods. The scarcity of certified calibration standards makes quantitative analysis difficult since scientists must also extract and purify target cyanotoxins from HABs samples. Also, the chemical diversity of these substances further complicates the development of multi-toxin methods of analysis, which is now required for complex samples containing a variety of toxins.

Since HABs can release mixtures of cyanotoxins rather than just single toxins [11], therefore methods of analysis capable of identifying different analogues concurrently are necessary. Since cyanotoxins have been studied for decades, numerous methods have been developed for their detection [12,13] but most lack the specificity required to unambiguously identify and quantify cyanotoxins in complex matrices such as algae. In the last few years, specific and quantitative multi-toxin methods of analysis based on liquid chromatography combined with tandem mass spectrometry (LC–MS/MS), have been published [14–17]. However, these methods often require a number of time-consuming sample preparation steps, thus further complicating their analysis.

The goal of our research was threefold: (a) to develop and validate a common and simple multi-toxins method for LC–MS/MS analysis of six representative hepatotoxins and neurotoxins (Fig. 1) in different types of algae (e.g., *L. wollei* field samples and *Anabaena flos-aquae* and *Microcystis aeruginosa* culture samples); (b) to investigate and confirm the presence of these cyanotoxins in *L. wollei* field samples; and (c) to screen for the presence of other cyanotoxins using high resolution-mass spectrometry and a database of cyanotoxins.

2. Material and methods

2.1. Reagents and materials

Standard (≥95% purity grade) anatoxin-a (ATX-a), nodularin (NOD), microcystin-RR (MC-RR), and microcystin-LR (MC-LR) were purchased from Enzo Life Sciences Inc. (Plymouth Meeting, PA, USA). Ampoules of certified standard solutions of cylindrospermopsin (CYN, 30 ± 2 μM in purified water), saxitoxin dihydrochloride (STX, 65 ± 3 μM in 2 mM hydrochloric acid), and an in-house reference materials containing *L. wollei* toxin-1 (LWTX-1, 39.9 μM in 0.01 M acetic acid) and 7-desmethylmicrocystin-LR (7dmMC-LR, 10.0 μM in 50% methanol/water) were obtained from the Certified Reference Materials Program (NRC, Halifax, NS, Canada). The internal standards *p*-aminobenzoic acid-d_4 (PABA-d_4) and clarithromycin-d_3 (CLA-d_3) were provided from Toronto