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Potent toxins in Arctic environments – Presence of saxitoxins and an unusual microcystin variant in Arctic freshwater ecosystems

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ABSTRACT

Cyanobacteria are the predominant phototrophs in freshwater ecosystems of the polar regions where they commonly form extensive benthic mats. Despite their major biological role in these ecosystems, little attention has been paid to their physiology and biochemistry. An important feature of cyanobacteria from the temperate and tropical regions is the production of a large variety of toxic secondary metabolites. In Antarctica, and more recently in the Arctic, the cyanobacterial toxins microcystin and nodularin (Antarctic only) have been detected in freshwater microbial mats. To date other cyanobacterial toxins have not been reported from these locations. Five Arctic cyanobacterial communities were screened for saxitoxin, another common cyanobacterial toxin, and microcystins using immunological, spectroscopic and molecular methods. Saxitoxin was detected for the first time in cyanobacteria from the Arctic. In addition, an unusual microcystin variant was identified using liquid chromatography–mass spectrometry. Gene expression analyses confirmed the analytical findings, whereby parts of the *sxt* and *mcy* operon involved in saxitoxin and microcystin synthesis, were detected and sequenced in one and five of the Arctic cyanobacterial samples, respectively. The detection of these compounds in the cryosphere improves the understanding of the biogeography and distribution of toxic cyanobacteria globally. The sequences of *sxt* and *mcy* genes provided from this habitat for the first time may help to clarify the evolutionary origin of toxin production in cyanobacteria.

1. Introduction

Several phototrophic organisms survive the harsh climate of the high Arctic regions, including some higher plants, mosses, lichens, various algal groups and cyanobacteria. Cyanobacteria are globally distributed, but in high Arctic freshwater ecosystems they represent the dominant primary producers [1,2]. Special features such as resistance to ultraviolet (UV) radiation, freeze–thaw cycle adaptation and nitrogen fixation allow their survival in these extreme environments [2]. During the polar summer, when both light and temperatures above the freezing point prevail, cyanobacterial communities thrive. They develop highly diverse benthic or floating mats in freshwater streams, ponds and on soils continuously fed with melt water that can be several centimeters thick and extend over large areas [2,3]. These extensive mats form the basis of a small but diverse and dynamic ecosystem accommodating a variety of organisms such as nematodes, rotifers, tardigrades [4], mosses and moss-infecting oomycetes such as the recently-described *Pythium polare* [5].

Saxitoxins (STXs) (Fig. 1A) are carbamate alkaloids, a group of fast-acting neurotoxins, inhibiting neuronal signal propagation of most higher organisms [6]. They are typically produced by marine dinoflagellates [7]. However, planktonic and benthic cyanobacteria from temperate and tropical regions, e.g. *Aphanizomenon* spp., *Anabaena circinalis*, *Cylindrospermopsis raciborskii*, *Planktothrix* spp., and *Lyngbya wollei* [8], are also known to produce STXs. Microcystins (MCs) (Fig. 1B) on the other hand, represent a group of ⩾80 structural heptapeptide variants with varying hepato-, renal-, and

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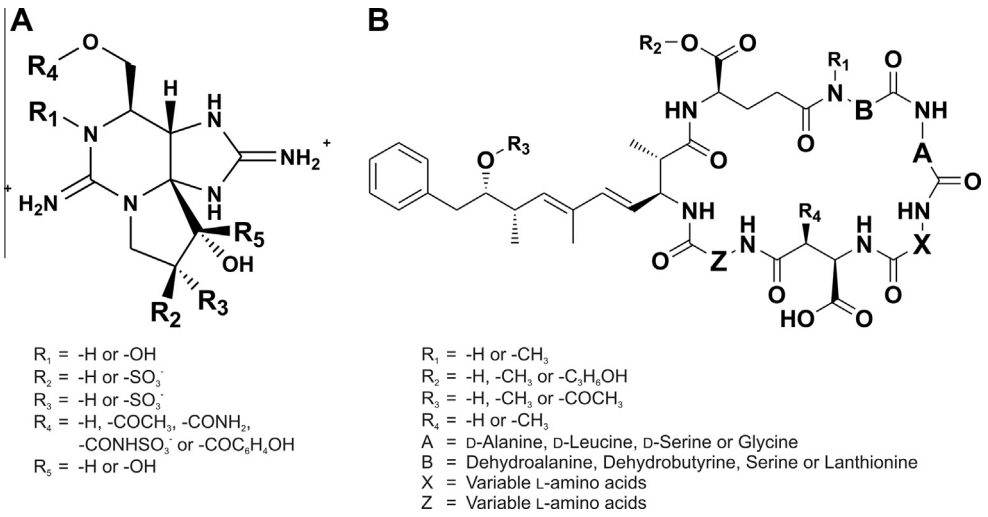

Fig. 1. Chemical structures of saxitoxin (STX) and microcystin (MC). General structure of the STXs (A); adapted from Humpage et al. [8] and the general structure of the MCs (B); adapted from Puddick [67].

neurotoxicity [9,10]. They appear to act primarily via specific inhibition of serine/threonine phosphatases [8,10], thereby interfering with one of the most important regulatory mechanisms of the cell. MCs are produced by a large variety of planktonic and benthic cyanobacterial genera including *Microcystis*, *Nostoc*, *Planktothrix*, *Anabaena*, *Synechococcus* and *Snowella* [8,11].

The molecular basis for the production of both toxins are large and variable gene clusters, encoding enzymes involved in secondary metabolite production such as polyketide synthetases and/or non-ribosomal peptide synthetases [12–14]. The distribution of these gene clusters among different strains of cyanobacteria does not necessarily correlate with the actual production of the toxins [15]. Therefore the presence of these genes in a given ecosystem is only an indication for the presence of the toxins and is therefore considered in this study as providing evidence for the 'potential to produce toxins'. These biosynthetic steps are energetically expensive for cyanobacteria [16], and this has prompted considerable speculation on their ecological function. To date the physiological function and ecological regulation of both STXs and MCs are poorly understood [17–19]. The current hypotheses aiming to explain this relatively enormous investment of energy in the synthesis of these toxins, include protection against grazing pressure, UV-radiation, and reactive oxygen species, as well as their function as signaling molecules in a quorum sensing-like manner [16,19–22]. The development of toxins as protection against grazers appears to be the least plausible hypothesis as the corresponding gene clusters appear to have been present in ancestral cyanobacteria species that have existed prior to the mesoproterozoic period [23–25], i.e. millions of years prior to the emergence of potential eukaryotic grazers of cyanobacteria. However it cannot be excluded that new functions have developed in the course of evolution.

In view of the ancient origin and the high conservation of the toxin gene clusters it is not surprising that cyanobacteria inhabiting remote and pristine areas, e.g. the rudimentary environments of the polar regions, could produce toxins. The presence of MCs in cyanobacterial mats has been reported for several locations in Antarctica [26–28], whereas this has only recently been demonstrated for Arctic cyanobacteria from northern Baffin Island [29]. Kleinteich et al. [29] demonstrated that culturing of cyanobacterial mats in the laboratory at increased temperatures caused a marked rise in the concentration of MCs in concert with shifts in the diversity of the cyanobacterial mat community composition. Whether the latter is a response to temperature stress, changing diversity of community structures or indeed a marker of a growth advantage of toxin producing cyanobacteria still needs to be ascertained. Saxitoxins, on the other hand, have never been reported in polar environments and cyanobacterial toxins in general remain understudied in this habitat.

In this study five cyanobacterial communities from the Arctic were screened for the presence of STX using enzyme-linked immunosorbent assay (ELISA) and further confirmation was undertaken using high performance liquid chromatography with fluorometric detection (HPLC-FLD). Furthermore an unusual MC variant was identified using liquid chromatography–mass spectrometry (LC–MS). Samples were also screened for selected genes involved in the synthesis of MC and STX, thereby providing evidence of the toxin-producing potential of Arctic cyanobacterial communities.

2. Materials and methods

2.1. Study sites and sampling

Five samples of cyanobacterial communities were collected during an expedition to northern Baffin Island in the vicinity of Cape Hatt (72°30′ N and 79°47′ W) in August–September 2009 from microbial communities on wet soil, small streams and ponds (see Supplemental Fig. 1 for GPS coordinates). Samples for DNA extraction and toxin analysis were sealed in sterile tubes and those for RNA analysis immersed in RNAlater (Qiagen, Hilden, DEU). DNA and RNA samples were frozen (−20 °C) within 24 h after collection and stored for approximately 6 months until further analysis.

2.2. Screening for saxitoxin and microcystin

2.2.1. Saxitoxin extraction

Frozen cyanobacterial material was lyophilized and their dry weight recorded. Samples for STX analysis were extracted as described by Smith et al. [30]. Briefly, 50 mg of lyophilized material was homogenized in 5 mL H_2O using a mortar and pestle and dried under nitrogen flow. The dried material was dissolved methanol (4 mL) acidified with acetic acid (0.1%), vortexed (15 min), and placed in an ultrasonic water bath (15 min, ice cold). The suspension was centrifuged (30 min, 4000*g*) and the supernatant transferred into a separate tube for HPLC-FLD analysis.

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