Short Communication

First Report of Microcystin-LR in the Cyanobacterium *Gloeotrichia echinulata*

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ABSTRACT: *Gloeotrichia echinulata* is a bloom-forming cyanobacterium that is common in eutrophic lakes, and less prevalent but increasing in oligotrophic lakes. We used an enzyme-linked immunosorbent assay analysis to test for the presence of the hepatotoxin microcystin-LR (MC-LR) in *G. echinulata* collected from an oligotrophic lake in central New Hampshire, USA. We found that *G. echinulata* contained MC-LR at mean concentrations of 97.07 $\pm$ 7.78 (1 s.e.) ng MC-LR g^{-1} dry wt colonies. This suggests that recent outbreaks of *G. echinulata* in oligotrophic lakes used as water sources throughout New England (USA) may pose a health concern. The toxicity of *G. echinulata* reported here suggests the need for future monitoring of microcystins in oligotrophic lakes. © 2007 Wiley Periodicals, Inc. Environ Toxicol 22: 337–339, 2007.

Keywords: *Gloeotrichia echinulata*; cyanobacteria; microcystin-LR; ELISA; toxicity; oligotrophic lakes; drinking water

INTRODUCTION

Gloeotrichia echinulata is a nitrogen-fixing cyanobacterium that is well-known for blooming in eutrophic lakes in northern Europe (Pierson et al., 1992; Istvanovics et al., 1993; Pettersson et al., 1993; Jacobsen, 1994; Forsell and Pettersson, 1995; Pettersson, 1998; Hyenstrand et al., 2001; Karlsson, 2003; Karlsson-Elfgren et al., 2003, 2005). Since 2002, *G. echinulata* blooms in oligotrophic lakes have occurred across northern New England (Maine Department of Environmental Protection, New Hampshire Department of Environmental Services). Because of their historically high water quality, many of these lakes are used for drinking water and recreation. Therefore, determining whether *G. echinulata* contains toxins is relevant for human health.

Previous research indicates that *G. echinulata* may contain toxins. Cronberg (1999) and Cronberg et al. (1999) established that recreational human exposure to *G. echinulata* blooms in Lake Ringsjön, Sweden can create skin irritation, and Gromov et al. (1996) found that injections of *G. echinulata* isolated from Lake Ladoga, Russia, were fatal to mice. *G. echinulata* is in the order Nostocales and family Rivulariaceae (Prescott, 1973), and toxicity surveys of phylogentically-related cyanobacteria such as *Aphanizomenon* and *Nostoc* (Dos S Vieira et al., 2005) suggest that

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G. echinulata may contain the hepatotoxin microcystin. However, the toxicity of *G. echinulata* has never been definitively identified or measured. Here, we test whether *G. echinulata* contains the hepatotoxin microcystin-LR (MC-LR).

METHODS

We tested for MC-LR in *G. echinulata* colonies collected from Lake Sunapee, a large, deep, oligotrophic lake in central New Hampshire, USA, at 43°24′N, 72°2′W. The lake has an area of 16.55 km^2, a volume of 1.88×10^8 m^3, and a mean depth of 10 m. *G. echinulata* colonies were first identified in Lake Sunapee in July 2004 (Lake Sunapee Protective Association, 2006, personal communication). The lake is a water source for many local residents.

G. echinulata colonies were collected from the upper 1 m of the water column with an 80 μm plankton net on August 22 and September 14, 2005. On each collection day we isolated two samples of 100 colonies each (four total samples) with an Olympus SZH10 dissecting microscope and estimated each colony's biovolume from its radius. Samples were frozen and transported to the Center for Freshwater Biology Analytical Laboratory at the University of New Hampshire for analysis by enzyme-linked immunosorbent assay (ELISA). Suspensions of *G. echinulata* were subjected to three freeze-thaw cycles followed by sonification to disrupt the cells and release any microcystins. We passed these samples through a 13 mm, 0.2 μm Whatman PTFE syringe filter to remove particulates immediately before analysis (Sasner et al., 2001). ELISA analyses were performed using following the instructions for the Microcystin 96-Well-Plate Kits (EnviroLogix, Portland, ME).

RESULTS

G. echinulata contained low levels of the hepatotoxin MC-LR equivalence. On August 22, 2005, the mean toxicity of *G. echinulata* was 94.91 $\pm$ 4.66 (1 s.e.) ng MC-LR g^{-1} dry wt colonies, and on September 14, 2005, the mean toxicity of *G. echinulata* was 99.24 $\pm$ 18.23 ng MC-LR g^{-1} dry wt colonies. Although expressed as MC-LR, it is not possible to state which of the 47 or more microcystins (Rinehart et al., 1994) were present in *G. echinulata* as the ELISA is sensitive to a broad range of microcystin analogs.

DISCUSSION

This study demonstrates that *G. echinulata* can produce the toxin MC-LR. MC-LR is a slow-acting cyclic heptapeptide hepatotoxin that has wide-ranging effects on vertebrates that drink or are in contact with contaminated water (Bischoff, 2001; Sasner et al., 2001). The concentration of MC-LR in *G. echinulata* is lower than microcystin concentrations measured in other cyanobacteria such as *Microcystis aeruginosa* (concentrations ranging from 0.01 to 1.73 mg g^{-1} freeze-dried cells, reviewed in Vezie et al., 1997) or *Radiocystis fernandoi* (2.47 mg g^{-1} d.w., Dos S Vieira et al., 2005).

In oligotrophic Lake Sunapee, *G. echinulata* blooms do not appear to cause an immediate threat to human health because colony density is low (maximum density in the upper 1 m was 2.96 colonies L^{-1} in 2005, Carey et al., in preparation), contributing only 1.16×10^{-5} μg MC-LR L^{-1}. This is well below the 1 μg MC-LR L^{-1} toxicity threshold for drinking water set by the World Health Organization (Sasner et al., 2001). However, *G. echinulata* could have negative effects on humans or aquatic ecosystems when colony densities are sufficiently large to create high total MC-LR concentrations. For example, in Lake Erken, Sweden, *G. echinulata* abundance can be as high as 5000 colonies L^{-1} (Eiler et al., 2006). If each *G. echinulata* colony has a biovolume of 5.46×10^{-4} mL (the mean 2005 colonial biovolume in Lake Sunapee), then we would expect that MC-LR concentrations in this lake could be as high as 0.027 μg MC-LR L^{-1}, within two orders of magnitude of the WHO drinking water guideline.

MC-LR has the potential to negatively affect aquatic food webs, humans, and livestock that are in contact with cyanobacterial blooms (Carmichael et al., 2001; Dittman and Wiegand, 2006). Traditionally, microcystins are only measured in eutrophic systems where the frequency and intensity of cyanobacterial blooms are high (reviewed in Sasner et al., 2001). *G. echinulata*'s toxicity in samples from Lake Sunapee, albeit low, indicates that microcystin toxicity in oligotrophic systems deserves more study, especially as *G. echinulata* outbreaks occur in oligotrophic lakes throughout New England. As *G. echinulata* has the potential for exponential growth (Carey et al., in preparation), long-term monitoring of *G. echinulata* recruitment and toxicity in Lake Sunapee and other oligotrophic lakes is necessary to evaluate potential microcystin health concerns.

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