Microcystin Concentrations and Genetic Diversity of *Microcystis* in the Lower Great Lakes

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ABSTRACT: The resurgence of *Microcystis* blooms in the lower Great Lakes region is of great concern to public and ecosystem health due to the potential for these colonial cyanobacteria to produce hepatotoxic microcystins. A survey of *Microcystis* cell densities and microcystin concentrations during August 2004 showed particularly high concentrations of both cells and toxin in the nearshore regions of Saginaw Bay (Lake Huron) and western Lake Erie, often exceeding the World Health Organization's recommended drinking water limit of 1 μg L^{-1}. The dominant congener of microcystin in both basins was microcystin-LR (MC-LR), whereas the second most abundant congeners, accounting for up to 20–25% of the total microcystin concentrations, were MC-LA in Saginaw Bay and MC-RR in western Lake Erie. Multiplex PCR assays of *Microcystis* colonies isolated from these two regions showed that a much greater percentage of the *Microcystis* colonies from Saginaw Bay carried the *mcyB* gene necessary for microcystin production, in comparison with those from western Lake Erie. The *mcyB* genotypes sequenced separated into two distinct phylogenetic clusters, with *Microcystis* originating from Lake Erie predominantly in one branch and from Saginaw Bay present in both branches. These results indicate that the genetic composition of the bloom could impact the concentrations and congeners of microcystin produced and that the cell count methods currently being used to gauge public health threats posed by *Microcystis* blooms may not sufficiently assess actual bloom toxicity. Environ Toxicol 23: 507–516, 2008.

Keywords: cyanobacteria; Great Lakes; Lake Erie; mcyB; microcystin; *Microcystis*; Saginaw Bay

INTRODUCTION

Bloom-forming cyanobacteria are found in lakes and reservoirs worldwide and can produce a range of toxins, including the hepatotoxic microcystin and cylindrospermopsin as well as neurotoxic anatoxins (Carmichael, 1994). The most common cyanotoxins found in the Great Lakes are microcystins, cyclic heptapeptide compounds that are detrimental to human, animal and ecosystem health (Brittain et al., 2000). The primary source of microcystins is the colony-forming cyanobacteria *Microcystis*, which typically dominates the cyanobacterial harmful algal bloom (HAB) community in the Great Lakes (Carmichael, 1994, 1997; Brittain et al., 2000; Vanderploeg et al., 2001). Preliminary

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studies have documented the presence of microcystins in the Great Lakes, at times exceeding the recommended limit of 1 μg L^{-1} established by the World Health Organization for drinking water supplies (Brittain et al., 2000; Vanderploeg et al., 2001). Due to the dependence on these waters as a drinking water and recreational resource, an increase in widespread *Microcystis* blooms in recent years has caused considerable concern. The ability to accurately measure the distribution and concentration of microcystin in the Great Lakes, including the various microcystin congeners, is therefore essential to protect human and ecosystem health in this region. The purpose of this study was to systematically map intracellular and extracellular microcystin concentrations in eutrophic portions of the Great Lakes (western Lake Erie and Saginaw Bay, Lake Huron) during a summer period (August 2004) when *Microcystis* sp. was abundant.

Additionally, this study investigated the relationship between microcystin concentrations and both *Microcystis* colony abundance and the frequency of toxic genotypes in natural *Microcystis* populations. Toxic strains can be identified by the presence of *mcy* genes, a bidirectionally transcribed complex of 10 open reading frames that encode the polyketide synthases and peptide synthetases involved in microcystin biosynthesis (Dittman et al., 1997; Nishizawa et al., 1999; Tillett et al., 2000; Kaebernick et al., 2002). Studies indicate that microcystins are constitutively produced (Kaebernick et al., 2000; Lyck and Christofferson, 2003), but microcystin cell quotas can vary with growth rate (Orr and Jones, 1998) and between strains (Blackburn et al., 1996; Carmichael, 1997). Although changes in environmental factors that regulate *Microcystis* growth, such as nutrients and light, can result in a 2–10-fold increase in microcystin cell quota (Sivonen and Jones, 1999), these effects can be relatively minor in comparison with the 10–1000 fold increase in bloom toxicity associated with shifts in community composition toward the predominance of toxic strains (Zurawell et al., 2005). The genetic diversity and frequency of toxic genotypes in the Great Lakes is just beginning to be characterized (Wilson et al., 2005; Rinta-Kanto and Wilhelm, 2006). The development of genetic indicators to determine the proportion of potentially toxic *Microcystis* colonies, in combination with mapping the distribution of *Microcystis* and microcystin concentration, is important in assessing the potential risks of algal blooms to public health in the Great Lakes.

MATERIALS AND METHODS

Study Area and Sampling

The study areas within the Great Lakes were Saginaw Bay (in the western part of Lake Huron) and the western basin of Lake Erie, from Toledo to the Bass Islands (Fig. 1). Stations along a set of transects in each of these areas were sampled in August 2004 from the R/V Laurentian. Three parallel transects running southwest to northeast were sampled in Saginaw Bay, with the "B" transect in the shipping channel, the "A" transect 6–7 km to the west and the "C" transect 6–7 km to the east [Fig. 2(A)]. In western Lake Erie, the "D" transect followed the western coastline of the lake, past the mouth of the Maumee River, the "E" transect continued along the southern coastline and included stations in the Bass Islands and the "F" transect included both stations in the Bass Islands and two stations in the middle of western Lake Erie [Fig. 2(A)]. Surface water from each of these stations was collected once in August 2004 and analyzed for chlorophyll *a*, *Microcystis* cell counts, intracellular and extracellular microcystin concentrations and genetic characterization. At each station, the Secchi depth was measured, temperature data were collected from a shipboard CTD (SeaBird, Bellevue, WA) and pH was measured onboard using an Orion 230A portable pH meter.

Fig. 1. Map of study area showing the location of Saginaw Bay and western Lake Erie within the Great Lakes.

Chlorophyll

Whole water samples for chlorophyll *a* analysis were filtered onto 25 mm GF/F filters, blotted dry and stored at -80°C until processing. Cells were lysed in 90% acetone with a Teflon tissue grinder, extracted in the dark overnight at -20°C and centrifuged before measuring concentrations by fluorometer (Turner Designs, Sunnyvale, CA) (Parsons et al., 1989).

Cell Counts

Algal samples were preserved with 1% Lugols solution. Once in the laboratory, these samples were settled and counted on an inverted microscope at 500×. *Microcystis* colonies with cells of 3–5 μm in diameter (as defined in Komarek and Anagnostidis, 1999) were counted and the volume of each colony, minus the peripheral mucilage, was measured. Because many *Microcystis* species are formed in densely packed colonies, individual numbers of cells cannot

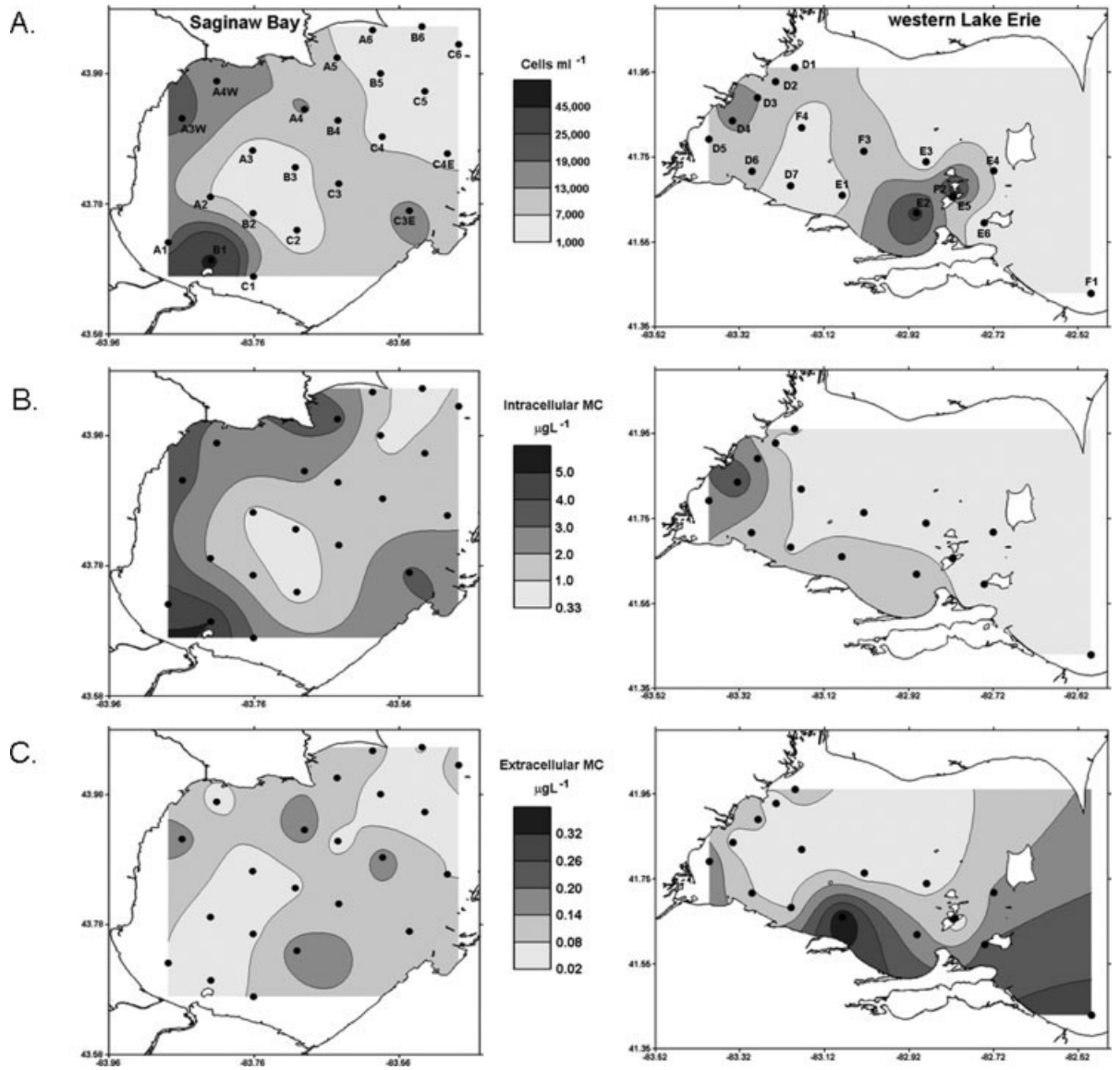

Fig. 2. Data from the August 2004 sampling cruise. Saginaw Bay is in the left column and western Lake Erie is in the right column with the scale bar for each particular dataset in the middle. The data from each station (marked by the black dots in the top set of maps) is plotted in Surfer (version 7.0, Golden Software, Golden, CO), which extrapolates between these known data points to create the contours shown. The data represented in these maps are (A) cell counts (cells per milliliter), (B) total intracellular microcystin concentrations as measured by HPLC (microgram per liter), and (C) extracellular microcystin concentrations as measured by ELISA (microgram per liter).

be counted directly for all colonies. A relationship between colony size and number of cells was derived by breaking apart individual colonies by sonication and enumerating cell numbers in those colonies. Colony volume was used to calculate colony Equivalent spherical diameter (ESD) which was then regressed against cell number to develop the following equation: $\log Y = 2.83\ (\log_{10} X)^{-2.50}$ where X = ESD and Y = cell number. This equation was very similar to that calculated by Reynolds and Jaworski (1978) and was used to calculate cell number (in cells per milliliter) from ESD measurements for each sample.

Microcystin Analysis

Most *Microcystis* colonies are in the >53 μm fraction (Vanderploeg et al., 2001), so samples for intracellular microcystin analyses were measured from cells filtered

onto a 53-μm nitex mesh (Small Parts, Miami Lakes, FL) and stored at -80°C until processing. To extract the intracellular microcystin, samples were lyophilized under vacuum at -40°C for 24 h, then sonicated in 75% MeOH (Fastner et al., 1998) for 3 min (VirSonic, VirTis, Gardiner, NY), extracted overnight at -20°C and sonicated a second time for 2 min just prior to analysis. The lysate was centrifuged to remove filter and cell debris, then the supernatant was filtered through a 0.2 μm syringe filter to remove any smaller particulates remaining.

HPLC analyses of microcystin concentrations employed an Agilent series 1100 system with a quaternary pump through a Gemini C18 column (5 μm, 150×4.6 mm^2, Phenomenex, Torrance, CA). Acetonitrile and water plus 0.05% (v/v) trifluoroacetic acid (TFA) were used as the mobile phases with a flow rate of 1 mL min^{-1}. UV detection was performed with a diode array detector at 238 nm. The gradient used was a variation of the standard method by Lawton et al. (1994): 0–30 min, 15% acetonitrile; 30–40 min, linear gradient of 15–60% acetonitrile (85–40% water plus TFA); 40-45 min, 60% acetonitrile. Toxin peaks were identified by their UV spectra, retention times and by spiking the samples with microcystin standards. The number of congeners that can be analyzed is limited by the availability of microcystin standards. The microcystin congeners that could be obtained for use in this study were MC-LR (Sigma), MC-RR, MC-YR and MC-LA (from Wayne Carmichael, Wright State University, Dayton, Ohio). Samples were periodically spiked with a known amount of a single standard to verify that the sample peaks were being correctly identified. The microcystin concentrations in the samples were quantified based on standard curves and the total concentration was calculated by summing the individual concentration of each of the four congeners.

Samples were prepared for measuring extracellular microcystin concentrations by filtering $\sim$1 L of sample water from each station through a 0.45 μm Supor filter (Polyethersulfone, Pall, East Hills, NY) and concentrating onto a SPE column (HLB Oasis, Waters, Milford, MA) that had been preconditioned with 100% MeOH followed by water. Microcystin was eluted from the column with 100% MeOH, concentrated under heat and vacuum, and diluted to $<$5% final MeOH concentration. Extracellular microcystin concentrations were assayed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit from Abraxis (Abraxis LLC, Hatboro PA), according to manufacturer's instructions. Samples that exceeded the 0.15–5 μg L^{-1} optimal linear range for the ELISA assay were diluted and re-run.

Genetic Analyses

A multiplex PCR reaction capable of amplifying both the *mcyB* gene and a region of the internal transcribed spacer (ITS) from individual *Microcystis* colonies was developed. The ITS primers were designed to amplify most cyanobacterial genera, including *Microcystis*, and were used to confirm that the extracted genomic DNA was cyanobacterial in origin and not degraded. A *mcyB*-specific PCR product indicated the colony was carrying the genes necessary to synthesize microcystin. To assay a given population, $\sim$20 individual *Microcystis* colonies were isolated using a Pasteur pipette and a stereoscope. Each colony was washed three times in sterile deionized water, and stored in a separate 0.2 mL tube containing sterile water at -20°C until processing. DNA was extracted from these colonies using three freeze-thaw cycles (-20°C to 55°C) and the DNA extract was added to the following multiplex PCR reaction: 1$\times$ reaction buffer, 2.5 mM $MgCl_2$, dNTPs, 2% DMSO, 100 ng each of the primers 16C ITS, 23C ITS, *mcyB* F, *mcyB* R, and Taq polymerase (Invitrogen^{TM}, Carlsbad, CA) in a final volume of 50 μL. The *mcyB* F primer (5′ TTC AAC GGG AAA ACC BAA AG) and *mcyB* R primer (5′ CYT GAT TAT CAA TSC GYC CT) were designed in this study to specifically amplify a $\sim$800-bp product from the *mcy* gene complex. The ITS primers 16C and 23C were developed by Neilan et al. (1997) to amplify a $\sim$530-bp cyanobacterial-specific PCR product. The multiplex PCR was run in a Strategene Robocycler (La Jolla, CA) under the reaction conditions: 94°C for 5 min, 30 cycles of 94°C 1 min, 55°C 1 min, 72°C 1 min, followed by a 7-min extension at 72°C. Colonies for multiplex analysis were collected from stations A2 and A4 (Saginaw Bay) and E2 (western Lake Erie).

In addition to the colony isolations, *mcyB* was amplified and sequenced from environmental samples to investigate the association between *mcyB* genotypes and the relative abundances of different microcystin congeners. From each of 10 stations (A4W, B1, C3E, C5 in Saginaw Bay and D2, D7, E2, F2, F2B, F4 in western Lake Erie), DNA was extracted and purified from 100 to 200 mL of water filtered onto a 0.45 μm Supor filter, as described previously (Piehler et al., 2002). Briefly, filtered material was subjected to heat (90°C) and bead beating (150–200 μm glass beads) to lyse the cells and the DNA was purified using DNAzol (MRC, Cincinnati, OH) with a chloroform extraction and the DNeasy Plant Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The presence of microcystin-producing cyanobacteria was identified by amplifying *mcyB*, using the primers *mcyB* F and *mcyB* R listed above. PCR products were cloned using ultracompetent TOP10 cells (Invitrogen) and 3–5 cloned inserts from each station were sequenced in both the forward and reverse directions. Resulting sequences were aligned and compared to other *mcyB* sequences in the GenBank database using the BioEdit program and checked manually. Phylogenic trees were generated by distance methods and the neighbor-joining algorithm (Saitou and Nei, 1987) with PHYLIP software (University of Wisconsin Genetic

Computer Group, Felsenstein, 1989) and the distribution of *mcyB* sequences was compared to the microcystin congener frequency determined using HPLC in each of the basins.

RESULTS

Environmental Parameters

Water temperatures during the August 2004 sampling period ranged from 18 to 21.6°C in Saginaw Bay and were slightly warmer (21–23.2°C) in western Lake Erie. Stations in Saginaw Bay also had slightly higher pH values of 8.2–8.7 *versus* 8–8.6 in western Lake Erie. There were no visible blooms at the stations sampled and cells densities were not sufficiently high to discolor the water or form surface scums. Secchi depths in Saginaw Bay ranged from 0.5–0.9 m at the nearshore stations, increasing to 1.4–1.8 m in the middle of the bay and up to 5 m at the easternmost stations close to the outer bay. Similarly in western Lake Erie, nearshore stations had Secchi depths of 0.8–1.7 m, increasing to 2.5 m in the middle of the basin.

Cell Counts

In Saginaw Bay, densities of *Microcystis* sp. were highest across the southwestern end of the bay (13,000–18,000 cells mL^{-1}), with the highest cell density for the entire system during this transect series (50,000 cells mL^{-1}) found at the station (B1) closest to the entrance of the Saginaw River to the bay [Fig. 2(A)]. Other high cell densities (14,000–22,000 cells mL^{-1}) were measured at the western edge of the inner Bay, whereas cell densities in the central section of the bay were much lower (1000–9000 cells mL^{-1}). In western Lake Erie, highest cell densities (27,000–34,000 cells mL^{-1}) were found around South Bass Island and southeast of Cataba Island. There were generally higher cell densities along the western and southern edge of the lake in comparison with the central lake stations (5000–12,000 cells mL^{-1}). Cell counts were conservatively reported as *Microcystis* sp. in recognition of the fact that even though the colonies counted met the standard morphological definition for *M. aeruginosa*, colonies of other species may have been inadvertently included.

Intracellular Microcystin Concentrations

Intracellular microcystin concentrations generally followed a pattern of lake wide distribution similar to *Microcystis* sp. cell densities. In Saginaw Bay, intracellular microcystin concentrations, as measured by HPLC, were consistently higher than 1 $\mu g\ L^{-1}$ with concentrations as high as 5 $\mu g\ L^{-1}$ at stations where the Saginaw River enters the bay and along the shallow southern and western edges of the bay [Fig. 2(B)]. In the channel through the middle of Saginaw Bay (transect "B"), microcystin concentrations were at or just below 1 $\mu g\ L^{-1}$. Overall, microcystin concentrations in western Lake Erie were lower than in Saginaw Bay. A few stations along the western and southern shoreline of Lake Erie, near the mouth of the Maumee River, had microcystin concentrations of 1.4–4 $\mu g\ L^{-1}$, but most stations in the central part of the basin and around the Bass Islands showed intracellular microcystin concentrations of 0.5–1 $\mu g\ L^{-1}$ [Fig. 2(B)]. In a wind-accumulated scum on the south shore of South Bass Island, microcystin concentrations of 58 $\mu g\ L^{-1}$ were measured. Although *Microcystis* cell numbers and intracellular microcystin concentrations had the same general patterns of distribution in both western Lake Erie and Saginaw Bay, there was not a strong correlation between the two when compared at individual stations ($r^2 = 0.34$). Intracellular microcystin concentrations also did not correlate highly with chlorophyll *a* concentrations ($r^2 = 0.39$), but had a slighter stronger correlation with the cyanobacterial-specific photopigment zeaxanthin ($r^2 = 0.59$) (data not shown).

Concentrations of individual microcystin congeners (MC-LR, MC-RR, MC-YR, and MC-LA) were measured for each station by HPLC. Of these four congeners, MC-LR comprised the greatest proportion (50–100%) of total microcystin pool (defined as the sum of the four congeners measured), with the second most abundant congener being MC-RR or MC-LA, depending on the location (Fig. 3). Lake Erie stations generally had a higher percentage of MC-RR (with an average of 18.5% and maximum of 36% of the total microcystin) than those stations in Saginaw Bay (with an average of 3.7% and maximum of 10.5%). MC-RR was present in all but one station in Lake Erie. MC-LA made up an average of 14.6% of the total microcystin for Saginaw Bay (maximum 28%) and 13.1% for western Lake Erie (maximum 36%). MC-YR was generally a minor component of the microcystin pool, only found at seven

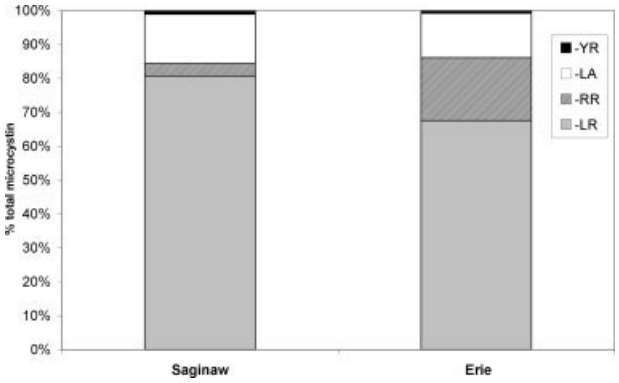

Fig. 3. Average distribution of microcystin congeners in Saginaw Bay and western Lake Erie. The concentrations of each congener, as assessed by HPLC, were averaged for all the stations in each basin and expressed as the percentage of total microcystin.

stations and at no more than 9% of the total microcystin concentration.

Extracellular Microcystin Concentrations

Extracellular microcystin concentrations were measured by ELISA instead of HPLC because sampling constraints made it difficult to concentrate sufficient quantities of toxin for accurate HPLC analysis. ELISA-based measurements of dissolved microcystin concentrations in the water column ranged from 0.01 to 0.18 $\mu g\ L^{-1}$ in Saginaw Bay in August 2004, with concentrations greater than 0.1 $\mu g\ L^{-1}$ only measured at stations in the middle of the outer transects (A4, A5, A6, A3W, C2, C3, C4, C6, C3E) [Fig. 2(C)]. Extracellular microcystin concentrations were higher in western Lake Erie, ranging from 0.02 to 0.38 $\mu g\ L^{-1}$, with the higher concentrations predominantly on the western shore where the Maumee River enters the basin (0.19 $\mu g\ L^{-1}$), along the southern shore (with the highest concentration at station E1 of 0.38 $\mu g\ L^{-1}$) and outside the mouth of Sandusky Bay (0.28 $\mu g\ L^{-1}$). Extracellular microcystin concentrations in the middle of western Lake Erie were 0.02–0.04 $\mu g\ L^{-1}$. The overall distribution of extracellular microcystin did not match the *Microcystis* cell density distribution in either basin. The percentage of total microcystin that is found in the extracellular fraction ranged from 1 to 58%. The dissolved fraction contributed a high percentage to the total only in samples with an overall low total microcystin concentration. In environmental samples with $>1\ \mu g\ L^{-1}$ total microcystin, extracellular microcystin concentrations did not comprise more than 10% of the combined intra and extracellular pool.

Genetic Analysis of mcyB

Multiplex PCR was used to identify the number of colonies positive for *mcyB* for stations A2 and A4 in Saginaw Bay and station E2 in western Lake Erie (Fig. 4). All of the isolated colonies amplified using ITS primers, indicating that the DNA was intact. In Saginaw Bay, 83% of the 18 colonies isolated from station A2 and 95% of the 22 colonies isolated from station A4 were positive for *mcyB*. In contrast, only 25% of the 16 *Microcystis* colonies isolated from station E2 in western Lake Erie were positive for *mcyB*.

A phylogenetic analysis of *mcyB* amino acid sequences isolated from bulk water samples taken from stations in western Lake Erie and Saginaw Bay showed two major clusters of sequences and one small outlying cluster. The two major clusters contained sequences with a high similarity to other *Microcystis mcyB* sequences in the GenBank database. The first cluster of sequences was most similar to those defined as *mcyB1* (*C*) by Mikalsen et al. (2003) and included sequences from Saginaw Bay stations and all but three of the sequences from western Lake Erie (Fig. 5). The

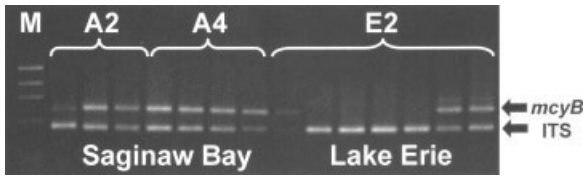

Fig. 4. Representative results from multiplex PCR using PCR primers for *Microcystis* ITS and *mcyB*. The presence of the smaller (~530 bp) ITS PCR product indicates that the colony is cyanobacterial and the DNA is not degraded. The presence or absence of the larger (~800 bp) *mcyB* PCR product indicates whether this colony is capable of microcystin production. The molecular weight marker (M) is a phi X-174RF HaeIII digest.

second major cluster of sequences mostly originated from Saginaw Bay (with only one sequence from western Lake Erie) and was genetically similar to the *mcyB1* (*B*) cluster (Mikalsen et al., 2003). Two additional sequences from Lake Erie (D7-2 and F2B-4) formed a small distinct cluster, and when compared with other *mcyB* sequences in GenBank, were most similar (74% based on amino acid sequences) to *Planktothrix* (Fig. 5).

DISCUSSION

Visible blooms of *Microcystis* were generally not evident during this August 2004 survey and yet intracellular microcystin concentrations during this time were consistently higher than the World Health Organization (WHO) recommended limit of 1 $\mu g\ L^{-1}$ for drinking water in many locations throughout Saginaw Bay (Lake Huron) and western Lake Erie. The risk for human exposure to microcystins increases when *Microcystis* accumulates in high concentrations near piers and shorelines where recreational fishing, swimming and boating activities are focused. Though not prevalent in nonbloom periods, these surface scums can accumulate quickly in favorable conditions. One such scum sampled at Bass Island, Lake Erie had an intracellular microcystin concentration of 58 $\mu g\ L^{-1}$ and, in bloom years, microcystin concentrations of up to 400 $\mu g\ L^{-1}$ have been measured in the Great Lakes (Murphy et al., 2003).

Actively growing *Microcystis* retains most of the synthesized microcystin within the cell [Fig. 2(B,C)], but lysis-driven release of microcystins from senescing cells could account for the relatively high extracellular microcystin concentration in regions where *Microcystis* densities were low in this study [Fig. 2(C)]. Although microcystin remains intracellular, there is a slightly lower risk for drinking water supplies because standard coagulation and sedimentation methods used in water treatment processes will remove many cyanobacterial cells (Hitzfeld et al., 2000). However, dissolved microcystin that has been released by cell lysis during water treatment, or as a result of bloom senescence,

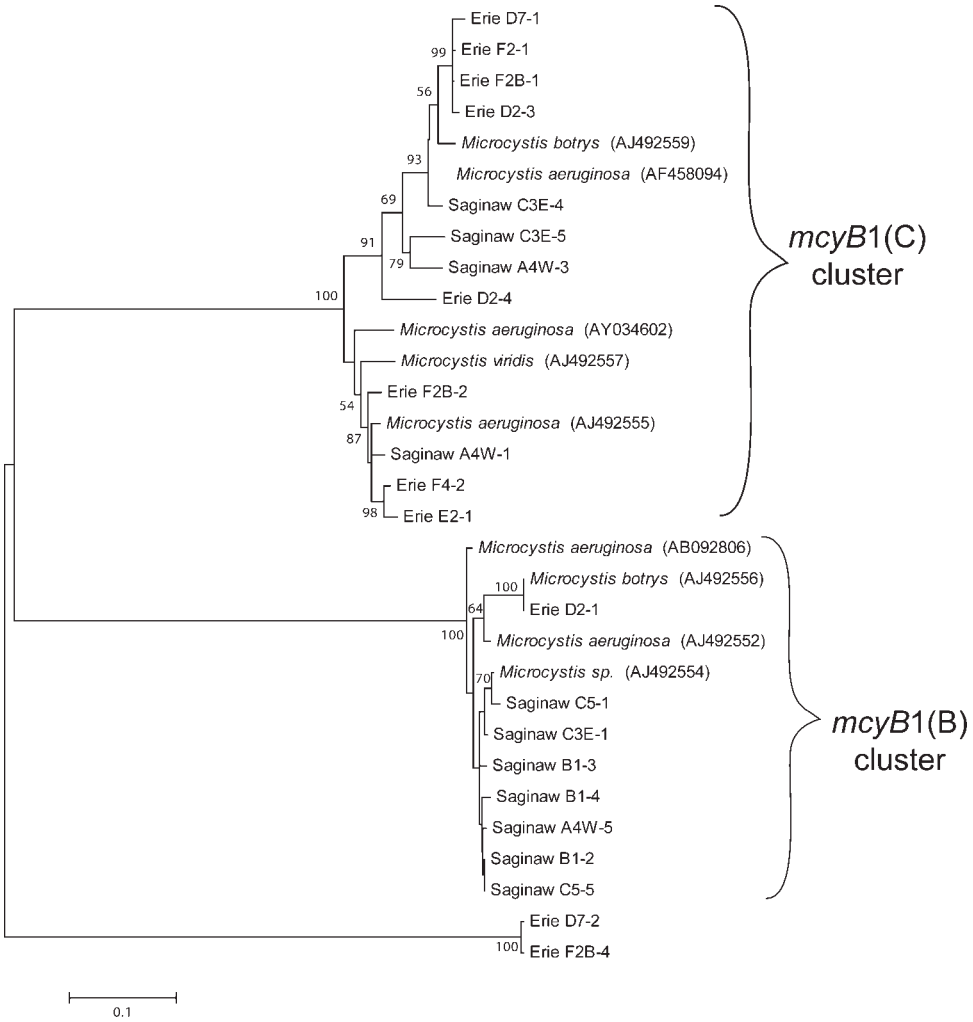

Fig. 5. Phylogenetic tree of *mcyB* sequences isolated from Saginaw Bay and western Lake Erie. The station number is given (i.e., D7) followed by the clone number (i.e., −1). The other *Microcystis* sequences are from GenBank and the accession numbers are given in parentheses. Bootstrap values greater than 50% are indicated at each node. The cluster designations [*mcyB1*(*B*) and *mcyB1*(*C*)] are in reference to Mikalsen et al. (2003).

can be much more difficult to remove from drinking water supplies efficiently (Codd et al., 1989). Chlorination is a standard treatment method employed by many water treatments plants in the Great Lakes region and, unless there are high cell densities or a high organic load, is generally effective at removing dissolved microcystins if used at sufficient concentrations and contact times (Keijola et al., 1988; Hitzfield et al., 2000). Powdered activated carbon is considered the most effective means to remove cyanobacterial toxins from drinking water, but the high expense of this treatment prevents it from being routinely employed in most water treatment facilities (Himberg et al., 1989). Direct ingestion of water during recreational activities or consumption of minimally treated or nontreated water supplies at camps, campgrounds and summer cottages may be another possible route of human exposure to dissolved microcystins. Microcystins are extremely stable compounds and may persist for weeks after being released from the cells. Photochemical degradation by UV in the presence of humics and exposure to microcystin degrading bacteria may speed up their removal from the water (Sivonen and Jones, 1999; Park et al., 2001). Extracellular microcystin concentrations measured in this study were frequently between 0.1 and 0.38 μg L^{-1} in near shore areas without the presence of a visible bloom, so it is reasonable to expect dissolved microcystin concentrations in surface scums to regularly exceed the WHO recommended regulatory limits (1 μg L^{-1}) for drinking water [Fig. 2(C)].

Guidelines for the probability of adverse health effects are often based on *Microcystis* cell counts as a proxy for toxicity. In many locations, recreational use of waters is generally restricted when cyanobacterial cell densities

exceed 100 000 cells mL^{-1} (Falconer et al., 1999). Cell counts alone, however, are often poorly correlated to actual toxicity. In this study, *Microcystis* cell densities only explained 34% of the variation in total microcystin concentration [Fig. 2(A,B)]. A similarly weak relationship between *Microcystis* cell density and microcystin concentration has also been observed in other systems (Kotak et al., 1995; Ozawa et al., 2005). Several factors likely contribute to this poor correlation, including: (1) the inclusion of both toxic and nontoxic strains of *Microcystis* in cell counts, (2) variability in toxin production within a toxic strain related to cell growth, and (3) changing proportions of toxic to nontoxic genotypes in bloom populations. Toxic and nontoxic strains of *Microcystis* are morphologically indistinguishable and the amount of toxin produced can vary even within toxic strains. Additionally, morphological plasticity and similarity between *Microcystis* and other colonial cyanobacterial species makes unequivocal identification of *Microcystis* by microscopy difficult at times, especially for those without detailed taxonomic training. Non-*Microcystis* microcystin-producing cyanobacteria, including *Planktothrix* and *Anabaena* spp., are also commonly found in the Great Lakes and may play an important role in modulating bloom toxicity, particularly in mixed bloom situations. The presence of these species during this study was indicated by several *mcyB* sequences from two western Lake Erie stations (D7 and F2B) that were more closely related to *Planktothrix* than to sequences known from *Microcystis* species (Fig. 5). Another indicator that other microcystin producing cyanobacteria were present in the system was the stronger correlation between microcystin and zeaxanthin concentration, as an indicator of total cyanobacterial biomass, than between microcystin and *Microcystis* cell counts alone ($r^2 = 0.59$ *vs*. 0.34). In the future, it may be possible to better assess the presence of these non-*Microcystis* microcystin-producing species by using a more inclusive *mcyB* PCR primer set or *mcyA* (Hotto et al., 2007).

The potential to predict bloom toxicity based on the proportion of *Microcystis* strains containing microcystin synthesis genes was supported by the preliminary data collected in this study. Saginaw Bay stations had both a higher proportion of *mcyB* containing colonies as well as higher intracellular microcystin concentrations in comparison to the Lake Erie populations. Though suggestive, more definitive laboratory and mesocosm studies will be needed to fully understand how the frequency of toxic genotypes in the population affects overall bloom toxicity. Our preliminary data indicate that the genetics-based assays used in this study will prove a useful complement to conventional cell count methods for determining the potential toxicity of blooms comprised of morphologically indistinguishable toxic and nontoxic strains.

The toxicity of a *Microcystis* bloom is affected not only by proportion of toxic strains and the promotion of toxin production by environmental factors, but also by which microcystin congeners are being produced. MC-LR is typically the predominant congener produced by *Microcystis* (Carmichael, 1992; Sivonen and Jones, 1999) and consistently comprised the highest percentage of the total microcystin pool in both Saginaw Bay and western Lake Erie during August 2004 (Fig. 3). MC-RR was the second most common congener in Lake Erie, accounting for up to 25% of the total microcystin pool at some stations and MC-LA was the second most common in Saginaw Bay, contributing up to 20% of the total microcystin. Which microcystin congeners are produced is important to overall bloom toxicity because these congeners vary dramatically in degree of toxicity. MC-LR and MC-LA, for example, are 3–4 times more toxic than MC-YR and up to 10 times more toxic than MC-RR, as measured by LD_{50} in mice exposed by intraperitoneal injection (Botes et al., 1982; Krishnamurthy et al., 1986; Namikoshi et al., 1992; Lee et al., 1998; Zurawell et al., 2005). This is especially significant for Saginaw Bay where not only were microcystin concentrations generally higher, but the microcystin congeners present (MC-LR and MC-LA) have more potent toxicities. However, toxicity to mice is not the only measure of the potential impact to human and ecosystem health. There is some evidence that even though it is less toxic, MC-RR may be more bioavailable and preferentially taken up in some biota (Xie et al., 2005).

Recent studies indicate that genotypic variation may influence which of these specific microcystin congeners are produced by a given strain. Mikalsen et al. (2003) identified two major clusters of *mcyB* sequences in *Microcystis* isolates and designated them as *mcyB1*(*B*) and *mcyB1*(*C*). They noted that there was a "strong correlation" between the genetic sequences of the *mcyB* module and the microcystin congener produced, particularly in regards to strains producing MC-LR and MC-RR. Isolates that synthesized MC-RR generally belonged to the C-type subgroup and the B-type subgroup generally produced MC-LR. In this study, the sequences isolated from stations in Lake Erie and Saginaw Bay also fell into these two clusters. Saginaw Bay *mcyB* sequences were evenly distributed between the two phylogenetic subgroups, but sequences originating from western Lake Erie fell predominantly (14 out of 15 clones sequenced) within the *mcyB1*(*C*) cluster. Interestingly, HPLC analysis also showed that MC-RR comprised a higher percentage of the total microcystin in western Lake Erie stations, which is consistent with the predominance of sequences in the *mcyB1*(*C*) cluster and the results of Mikalsen et al. (2003). Therefore, this study suggests that genotypic variation within microcystin synthetase genes may also be important in assessing overall bloom toxicity.

Summary

This survey showed that even in a non bloom year, both *Microcystis* and microcystin were widespread in Saginaw

Bay and western Lake Erie and microcystin concentrations could be significantly above the WHO recommended limit of 1 μg L^{-1}, particularly in nearshore regions and surface scums. The concentration of both *Microcystis* cells and microcystin nearshore is of particular concern because the potential for human and animal contact is much higher for these regions than in mid-lake locations and indicates a need for continued monitoring of microcystin concentrations in these parts of the Great Lakes, as well as other lakes, reservoirs and water supplies used for drinking water and recreation (Brittain et al., 2000; Murphy et al., 2003). Furthermore, genetic data, used for determining the proportion of toxic strains in a population and linking the genotypes of the toxin strains to the specific microcystin congeners produced, may allow the toxin-producing potential of *Microcystis* blooms to be predicted with much greater accuracy than currently possible.

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