

Diversity of Microcystin-Producing Cyanobacteria in Spatially Isolated Regions of Lake Erie

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The diversity of microcystin-producing cyanobacteria in the western basin of Lake Erie was studied using sequence analysis of *mcyA* gene fragments. Distinct populations of potentially toxic *Microcystis* and *Planktothrix* were found in spatially isolated locations. This study highlights previously undocumented diversity of potentially toxic cyanobacteria.

The Laurentian Great Lakes contain ~18% of Earth's potable water supply, making them a vital global resource. Lake Erie is the most productive of these lakes, and it maintains a position of regional socioeconomic importance (3, 9). Although the annual appearance of *Microcystis* in the western basin of Lake Erie since 1995 is well documented (2, 11, 14, 15), the presence of other microcystin producers has garnered less attention. *Microcystis* has been thought to be the primary toxin producer in Lake Erie, but observations from 2003 and 2004 suggested that the microcystin-producing community may not be composed entirely of *Microcystis*, as only 0.03 to 4.7% of these cells contained toxin biosynthetic machinery (11). Thus, our goal was to examine the diversity of microcystin producers in locations where high microcystin-LR concentrations were observed in 2003 and 2004. The identities and phylogenetic relationships within the microcystin-producing cyanobacterial community were assessed by the analysis of *mcyA* genes carried by microcystin-producing *Anabaena*, *Planktothrix*, and *Microcystis*. The *mcyA* gene encodes a microcystin synthetase found in these microcystin producers (5).

Water was collected at the 1-m depth from the western basin of Lake Erie in August 2003 and August 2004 (Fig. 1). Chlorophyll *a* and toxin (microcystin-LR) equivalents have been previously described (11). Cells for DNA extraction were collected from known volumes onto 47-mm-diameter, 0.22- μ m-nominal-pore-size polycarbonate filters (Millipore) and DNA extracted as described previously (11). The primer pair *mcyA*-Cd 1R and *mcyA*-Cd 1F (5) was used to amplify 291- to 297-bp *mcyA* gene fragments. Reactions were carried out in EasyStart tubes (Molecular BioProducts) in a final volume of 50 μ l containing 0.4 μ M of each primer, 300 ng μ l⁻¹ bovine serum albumin (Sigma A-7030) (7), 2.5 U *Taq* polymerase (Promega), 0.1% Triton X-100 (Molecular BioProducts), 2 mM MgCl₂, 0.2 mM deoxynucleoside triphosphate mix, 1 \times PCR buffer, and 5 μ l of undiluted or 10-fold-diluted DNA extract. The PCR was as follows: 95°C for 10 min; 40 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 30 s; and 72°C for 5 min. Amplicons were separated by agarose gel electrophoresis (12), and DNA from bands of the correct size was extracted

using a QIAquick gel extraction kit. Clone libraries were generated using a TOPO-TA cloning kit (Invitrogen). Thirty-eight clones from each sample were selected and the inserts sequenced at the Clemson University Genomics Institute by using the M13 forward primer (Invitrogen).

To provide information on a known isolate from Lake Erie, *Microcystis aeruginosa* LE-3 was grown in modified BG11 medium (6) at +25°C under continuous illumination (ca. 80 μ mol of photons m⁻² s⁻¹). The cells were grown in a batch culture for 3 weeks and harvested by centrifugation. DNA was extracted using the previously described xanthogenate method (10). *mcyA* gene fragments were PCR amplified and cloned as described above. Sequencing was completed at the University of Tennessee Molecular Biology Resource Facility.

Sequences from natural samples and cultures were manually examined and then analyzed at both the nucleotide and amino acid levels. Individual DNA sequences from natural samples were queried against GenBank by BLAST and BLASTX searches (1). Phylogenetic relationships among unique *McyA* sequences were inferred through a neighbor-joining analysis using Poisson correction distance. Dendrograms were also created by using the unweighted-pair group method using average linkages and minimum-evolution approaches, which yielded comparable phylogenies (not shown). The sequences were edited, aligned, and analyzed using Bio Edit (4), Clustal W (13), and Mega 3.0 (8).

Overall, the results from BLAST and BLASTX searches confirmed that the cloned sequences originated from the *mcyA* gene of the microcystin synthetase complex. Relative to known sequences, the natural samples clustered into two distinct groups. Phylogenetic analyses confirmed that 100% of the sequences from station 1163 clustered with *Planktothrix aghardii*, whereas 100% of the sequences from stations WLE1, 882, and 974 clustered with *Microcystis* isolates (Fig. 2), although sequences within the *Microcystis* clade were remarkably diverse. All *Microcystis* *McyA* sequences from GenBank as well as most sequences from natural samples were 76 amino acids in length, whereas a subset of *Microcystis*-like sequences (20 sequences from station 974 and 1 sequence from station WLE1) appeared to have two extra amino acids. Sequence alignment indicated that these two additional amino acids occurred at the same positions where similar residues are located in *Planktothrix*, *Anabaena*, and *Nostoc* (Fig. 3). Due to the systematic location

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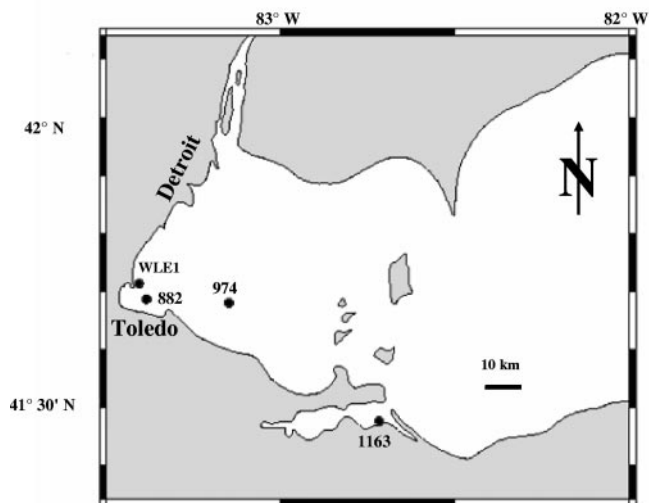


FIG. 1. Map of station locations and corresponding station codes in the western basin of Lake Erie.

of the extra amino acids, we consider it unlikely that these residues in the *Microcystis*-like sequences are PCR artifacts. Despite the extra amino acids, the Lake Erie sequences were most similar to *Microcystis* (93 to 97% identity at the nucleotide level and 93 to 96% identity at the amino acid level), although no exact matches were found.

Microcystis, *Planktothrix*, and *Anabaena* are the most common microcystin-producing cyanobacteria, and their potentially toxic genotypes can be detected and identified by the unique sequences within the *mcyA* amplicons (5). In the context of this study, we have examined this conserved genetic element to determine which organism(s) within a population may be responsible for microcystin production in spatially separated regions of a lake. This analysis has identified a genetic element not associated with any currently known toxic *Microcystis* organism, suggesting the existence of previously unknown sequence diversity within the *mcyA* genes of this genus. The effect of the additional two residues on microcystin production

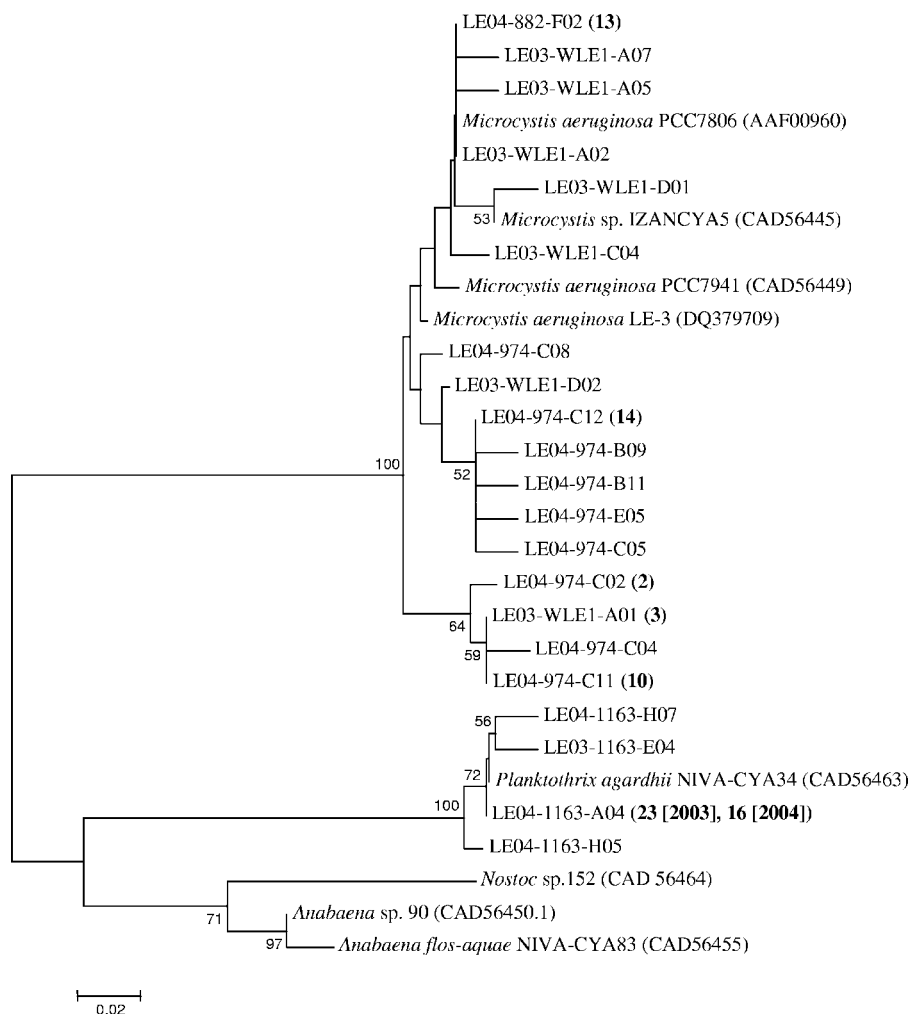


FIG. 2. Neighbor-joining tree displaying the relationship between partial *McyA* sequences (76 to 78 amino acids). The coding system for the clones is as follows: LE03-1163, Lake Erie, year 2003, station 1163; LE03-WLE1, Lake Erie, year 2003, station WLE1; LE04-1163, Lake Erie, year 2004, station 1163; LE04-974, Lake Erie, year 2004, station 974; LE04-882, Lake Erie, year 2004, station 882. The numbers in parentheses after the codes for the clones indicate the number of identical sequences represented by these sequences. Accession numbers are in parentheses after the names for reference sequences obtained from GenBank. Bootstrap values of >50% (for 2,000 iterations) are displayed at the branch nodes. The scale bar represents substitutions per site.

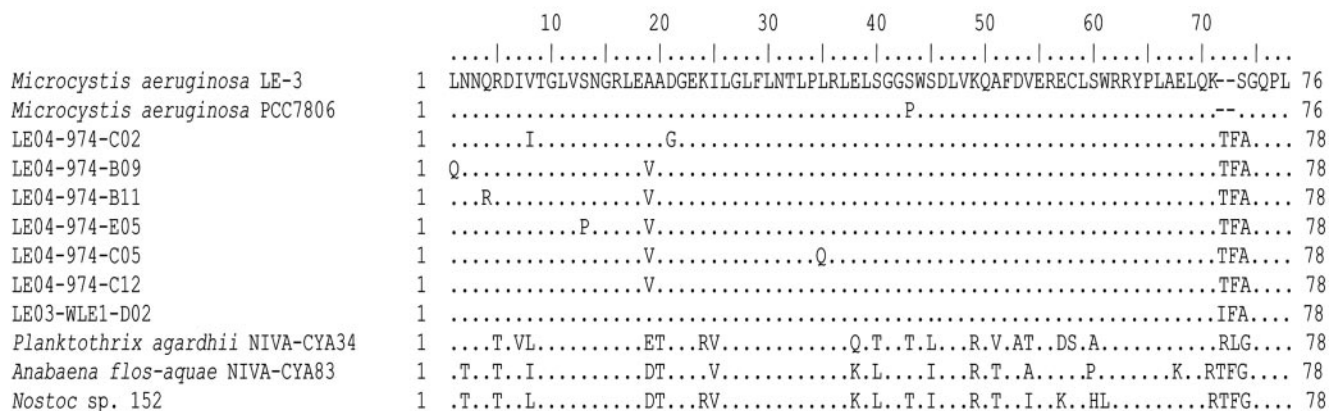


FIG. 3. Multiple alignment of McyA amino acid sequences of cultured isolates of *Microcystis aeruginosa* LE-3 (translated from the *mcyA* gene sequence under accession no. DQ379709), *Microcystis aeruginosa* PCC7806 (accession no. AAF00960), *Planktothrix aghardii* NIVA-CYA34 (CAD56463), *Anabaena flos-aquae* NIVA-CYA83 (CAD56455), and *Nostoc* sp. strain 152 (CAD56464) with novel *Microcystis*-like McyA sequences from natural samples that contain two extra amino acids, unlike *Microcystis* sequences from cultured isolates.

is also unknown. Analysis of McyA sequences from natural samples indicates a spatial separation of these toxigenic populations within Lake Erie. Although the data presented here indicate no mixing between *Planktothrix* and *Microcystis* populations, this may not be entirely the case. It is possible that *Planktothrix mcya* fragments were preferentially amplified from samples collected at station 1163 due to a high abundance of toxic *P. aghardii* relative to toxic *Microcystis*. Regardless, these data reveal a new diversity of microcystin-producing cyanobacteria in Lake Erie, although the conditions supporting and separating these populations are not yet understood. Overall, we couch the observations within the framework of developing a better understanding of the factors that regulate cyanobacterial blooms in Lake Erie and other environments.

Nucleotide sequence accession numbers. The GenBank accession numbers for the sequences reported here are DQ379674 to DQ379709.

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