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Evidence against fluvial seeding of recurrent toxic blooms of *Microcystis* spp. in Lake Erie's western basin

Olga A. Kutovaya [a,1], Robert Michael L. McKay [a,2], Benjamin F.N. Beall [a,2], Steven W. Wilhelm [b,3], Douglas D. Kane [c,4], Justin D. Chaffin [d,5], Thomas B. Bridgeman [d,5], George S. Bullerjahn [a,2,*]

[a] Department of Biological Sciences, Bowling Green State University, Life Sciences Building, Bowling Green, OH 43403, USA
[b] Department of Microbiology, The University of Tennessee, Knoxville, TN 37996-0845, USA
[c] Natural Science and Mathematics Division, Defiance College, 701 North Clinton Street, Defiance, OH 43512, USA
[d] Department of Environmental Sciences, University of Toledo, Lake Erie Center, 6200 Bayshore Road, Oregon, OH 34618, USA

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ABSTRACT

For almost two decades, the western basin of Lake Erie has been plagued with recurring toxic algal blooms dominated by the colonial cyanobacterium, *Microcystis* spp. Since the Maumee River is a major source of nutrients and sediment inputs into the lake, and *Microcystis* spp. has been identified as a member of the upstream river algal assemblage, the possibility exists that the river *Microcystis* species serve as a seed population for the toxic blooms occurring in the lake. Genetic profiling of toxic cyanobacteria using the microcystin synthesis gene, *mcyA*, clearly indicates that the toxic cyanobacteria of the river are distinct from the toxic *Microcystis* spp. of Lake Erie. Indeed, *mcyA* sequences are almost exclusively from toxic *Planktothrix* spp., similar to what has been documented previously for Sandusky Bay. UniFrac statistical analysis of cyanobacterial community composition by comparison of 16S–23S ITS sequences also show that the Maumee River and Lake Erie communities are distinct. Overall, these data show that despite the importance of nutrient inputs and sediments from the river, the toxic cyanobacterial blooms of Lake Erie do not originate from toxic species endemic to the Maumee River and instead must originate elsewhere, most likely from the lake sediments.

1. Introduction

Western Lake Erie (Laurentian Great Lakes) has been increasingly affected by toxic *Microcystis* spp. blooms in recent decades (Brittain et al., 2000; Murphy et al., 2003; Rinta-Kanto et al., 2005; Wang et al., 2009). Recognizing the Maumee River as the major source of nutrient inputs (especially phosphorus) to the western basin of Lake Erie (Baker and Richards, 2002), the influence of the river in promoting these blooms cannot be ignored. Indeed, the role of the Maumee River in providing nutrients to support *Microcystis* spp. growth has been widely accepted as an important factor (Wang et al., 2009; Millie et al., 2009; Rinta-Kanto et al., 2009b; Bridgeman et al., in press; Chaffin et al., 2011), along with temperature and turbidity due to sediment plumes (Brannan, 2009; Wang et al., 2009; J. Chaffin and T. Bridgeman, unpublished data). Less understood, however, are the sources of *Microcystis* spp. that comprise the major microcystin producers in the lake. Rinta-Kanto et al. (2005), based on qPCR analyses of *Microcystis* species, suggested that the river could possibly serve as a seed population for toxic blooms. Further, Conroy et al. (2008) proposed the Algal Loading Hypothesis (ALH), where nutrient-replete algae are loaded from riverine systems with high sediment concentrations into the more favorable light conditions of lacustrine systems, such as Lake Erie, and thus can grow rapidly and reach bloom conditions. In support of the ALH, surveys along the Maumee River have identified *Microcystis* spp. upstream in the early spring (Bridgeman et al., in press). In contrast, analysis of Lake Erie sediments demonstrated the presence of viable toxic *Microcystis* spp. that were genetically similar to bloom forming populations (Rinta-Kanto et al., 2009a), consistent with studies from other systems showing that *Microcystis* can overwinter vegetatively in the sediment (Preston et al., 1980; Takamura et al., 1984; Verspagen et al., 2004). Thus, the major reservoir of the toxic blooms in Lake Erie's western basin remains unresolved. However, it has been

* Corresponding author. Tel.: +1 419 372 8527; fax: +1 419 372 2024.
E-mail addresses: kolga@bgsu.edu (O.A. Kutovaya), rmmckay@bgsu.edu (R.M.L. McKay), bbeall@bgsu.edu (Benjamin F.N. Beall), Wilhelm@utk.edu (S.W. Wilhelm), dkane@defiance.edu (D.D. Kane), Justin.Chaffin@utoledo.edu (J.D. Chaffin), Thomas.Bridgeman@utoledo.edu (T.B. Bridgeman), bullerj@bgsu.edu (G.S. Bullerjahn).
[1] Current address: Environment Canada, 867 Lakeshore Road, P.O. 5050, Burlington, Ontario L7R 4A6, Canada. Tel.: +1 905 836 4746.
[2] Tel.: +1 419 372 8527; fax: +1 419 372 2024.
[3] Tel.: +1 865 974 0665.
[4] Tel.: +1 419 783 2593.
[5] Tel.: +1 419 530 8373; fax: +1 419 530 8399.

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Table 1
Information on sampling sites. MR – Maumee River, MB – Maumee Bay, LE – Lake Erie, 882 – Maumee Bay, 558 – Maumee River mouth, 973 – western basin of Lake Erie. All samples were drawn from surface water (<1 m depth).

Site	Date	Volume (L)	Lat/long
MB/882	12 July 2005	0.3	41 44 02 N 83 23 08 W
MB/558	12 July 2005	0.2	41 41 56 N 83 27 39 W
LE/973	12 July 2005	0.3	41 47 30 N 83 20 20 W
MR/Napoleon	7 May 2009	0.25	41 22 55 N 84 8 19.8 W
	12 May 2009	0.13	–
MB/MB20	23 June 2009	0.8	41 41 58 N 83 28 12 W
	2 July 2009	0.35	–
LE/7 M	23 June 2009	1.5	41 43 97 N 83 17 78 W
	2 July 2009	2	–
	13 July 2009	2	–
MR/Independence Dam	20 March 2010	0.18	41 17 40.5 N 84 17 29.2 W
MR/Farnsworth	20 March 2010		
	20 April 2010	0.12	41 28 36.2 N 83 44 55.4 W
MR/Bend	20 April 2010	0.4	41 16 32.1 N 84 30 53 W
MR/Independence Dam	20 April 2010	0.3	–
MR/Rt66 Bridge	20 April 2010	0.375	41 17 18.8 N 84 21 39.2 W

documented that cyanobacterial 16S and toxin gene PCR amplicons obtained from Sandusky Bay samples differ from those taken from other nearby sites in the western basin of Lake Erie, leading to the conclusion that the toxic cyanobacteria of Sandusky Bay (see Fig. 1) are primarily represented by *Planktothrix* spp. (Ouellette, 2006; Rinta-Kanto and Wilhelm, 2006). Therefore, Sandusky Bay samples were employed to compare with the samples from the Maumee River and Lake Erie's western basin.

The aim of this study was to investigate the relationship between the spatial distribution of toxic cyanobacteria in the Maumee River and western basin of Lake Erie using genetic tools. Given that that microcystin-producing cyanobacteria of Sandusky Bay had been found to be different from microcystin-producing cyanobacteria in the lake, we hypothesized that similarly, microcystin producers in the Maumee River would be different from those in the Lake, indicating that the Maumee River is not a major source of the toxic *Microcystis* spp. that dominates blooms in Lake Erie. The *mcyA* gene, encoding a subunit of the microcystin synthetase complex, was employed as a proxy for identification of toxic cyanobacteria in both lake and river samples. In addition, the cyanobacterial 16S–23S ITS, the internal transcribed spacer of the ribosomal RNA operon, was used to provide an overall picture of the cyanobacterial population in the Maumee River and western basin of Lake Erie. Phylogenetic analysis followed by statistical tests assessed the relationships between the Maumee River and Lake Erie cyanobacterial populations.

2. Materials and methods

2.1. Sampling and DNA sequencing

Sampling was conducted in the western basin of Lake Erie (2005, 2009), Maumee Bay (2005, 2009), and Maumee River (2005, 2009, 2010) (Table 1, Fig. 1). River samples were collected from the nearshore at each site in water of <1 m depth. The samples were processed by filtration onto 0.2 μm pore-size Sterivex filters (Millipore), followed by DNA extraction as described in Rinta-Kanto and Wilhelm (2006). All PCR amplifications with *Microcystis* spp. *mcyA* (Hisbergues et al., 2003) and ITS (Janse et al., 2003) primers were performed as described earlier (Rinta-Kanto et al., 2005; Rinta-Kanto and Wilhelm, 2006). Clone libraries were generated in the TOPO-TA vector as described by the supplier (Invitrogen). Sequencing of amplicons was performed at the University of Chicago Cancer Research Center using plasmid

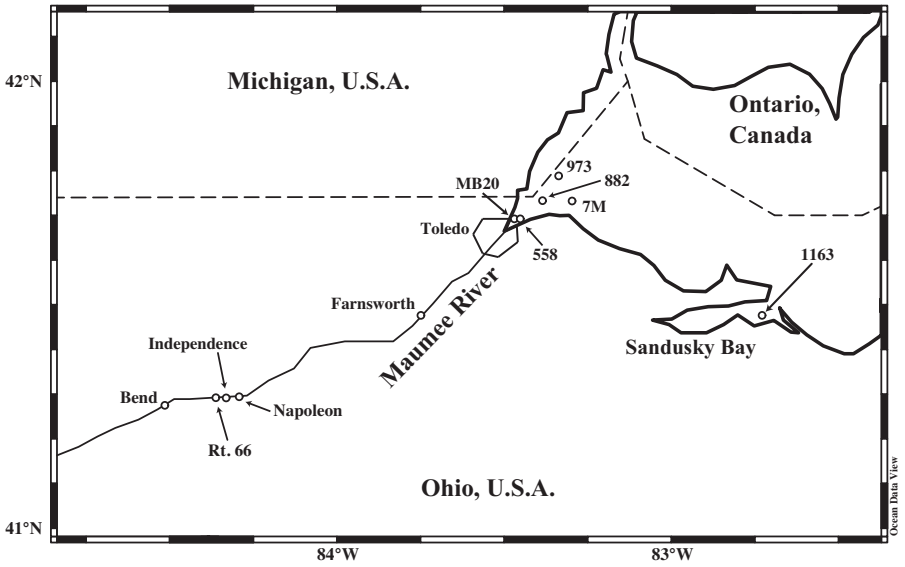

Fig. 1. Sampling locations visited in this study. The Maumee River sites are The Bend, Rt. 66 Bridge, Napoleon, Independence Dam, and Farnsworth Metropark. Maumee Bay sites are MB20 and 7 M. Numbered stations 558, 882 and 973 refer to sites sampled in July, 2005.

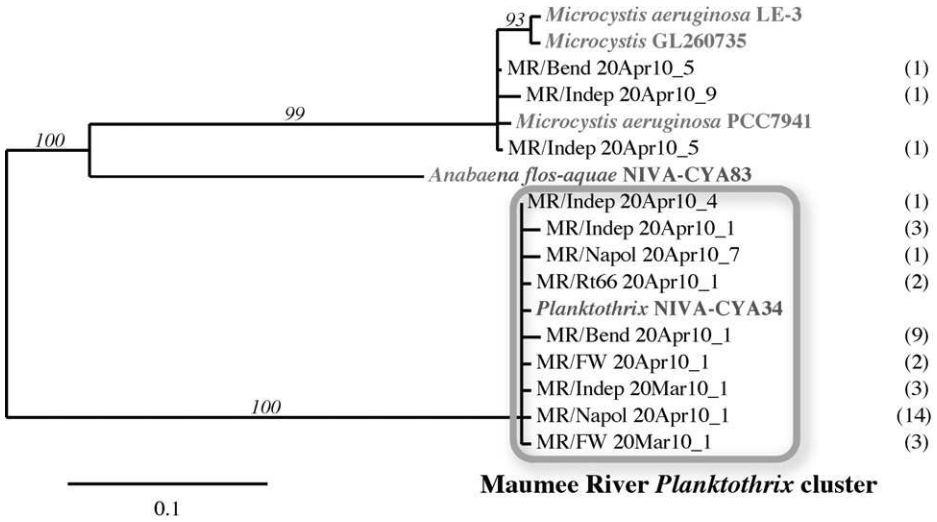

Fig. 2. Phylogenetic tree showing relationship between McyA sequences from the Maumee River and potentially toxic genotypes of cyanobacteria. Samples collected in March and April 2010. Bend, Indep – Independence Dam, Napol – Napoleon, Rt66 – Rt66 Bridge, FW – Farnsworth are sites in the Maumee River. The numbers in parentheses indicate the number of identical sequences represented these sequences.

specific oligonucleotide primers. Obtained *mcyA* sequences were translated, and resulting McyA and 16S–23S ITS sequences aligned using ClustalX-2.0.12 software (Thompson et al., 1997). Phylogenetic analysis was performed using Mega 5.0 software (Kumar et al., 2007) and Phylogeny.fr (Dereeper et al., 2008). PhyML-Approximate Likelihood-Ratio Test (PhyML-aLRT) was applied to compute the phylogenetic trees. UniFrac software was used for comparing microbial community diversity as described in Lozupone and Knight (2005). DNA sequences were deposited into GenBank under accession numbers JN108765–JN108870.

3. Results and discussion

3.1. McyA phylogenic analyses

Phylogenetic analysis of McyA protein sequences from the Maumee River in 2009 and 2010 revealed the majority of the sequences to group with *Planktothrix* spp. (Fig. 2). Indeed, among 41 McyA clones, 38 clustered with *Planktothrix* spp. whereas only three Maumee River clones grouped with *Microcystis* spp. These results indicate that toxic cyanobacteria in the Maumee River were mostly represented by *Planktothrix* spp.

A broader survey of McyA sequences from the Maumee River, Sandusky Bay, and Maumee Bay of Lake Erie demonstrated two distinct clusters (Fig. 3). The Sandusky Bay sequences clustered with the Maumee River sequences and together represent a *Planktothrix* spp. cluster, whereas Lake Erie Maumee Bay sequences grouped separately and appear as a *Microcystis* spp. cluster. Therefore, the presence of two distinct clusters from the Maumee River-Sandusky Bay and Maumee Bay-western Lake Erie, suggests that they exist independently from each other, so that the populations upstream in the Maumee River likely do not serve as a source for *Microcystis* spp. to the western basin of Lake Erie.

We also analyzed a separate set of archived samples obtained in 2005 during a transect toward Toledo, OH from Maumee Bay in order to determine where in the river or bay the population shift from *Microcystis* spp. to *Planktothrix* spp. may occur. Phylogenetic analysis of McyA sequences taken the same day in July 2005 revealed that the sequences from Maumee Bay (sta. 882) and the western basin of Lake Erie (sta. 973) were from *Microcystis* spp. sequences. However, at station 558, nearest the mouth, *Planktothrix* spp. was also detectable as a minor fraction of the total McyA sequences (Fig. 4). This result suggests a shift between *Microcystis* spp. and *Planktothrix* spp. in the downstream reaches of the river near, or at Toledo.

3.2. ITS phylogeny

Since non-toxigenic bloom-forming strains are commonly present in the endemic microbial population, and often bloom in Lake Erie in concert with toxic genotypes (Rinta-Kanto et al., 2009b), analysis of McyA sequences does not provide an overall picture of the cyanobacterial population. To provide a global survey of the major cyanobacterial taxa, the 16S–23S ITS was employed to better understand relationships between the community composition in the Maumee River and Lake Erie, especially with respect to toxic versus nontoxic genotypes. In fact, most cyanobacterial samples taken from the Maumee River (Farnsworth Metropark, Waterville, OH and upstream) in 2009 were shown to be non-toxigenic (T. Bridgeman and G. Winston, unpublished data).

Phylogenetic analysis of ITS sequences from the Maumee River and Lake Erie did not show distinct clusters (Fig. 5). This was expected, because these data reflect the total composition of the cyanobacteria present, not merely the subset of microcystin producers. Indeed, sites in both Maumee Bay and the Maumee River yielded ITS sequences that clustered with *Planktothrix* spp. and *Microcystis* spp. The results of ITS phylogeny indicated that potentially toxic and non-toxic representatives of both *Planktothrix* spp. and *Microcystis* spp. were present in both the river and bay samples, whereas the McyA data show that toxic *Microcystis* spp. genotypes dominate the toxic cyanobacteria in the Maumee Bay of Lake Erie. Overall, these data suggest that whereas toxic *Microcystis* spp. genotypes blooming in the Bay most likely originate within the Bay itself, nontoxic genotypes could be seeded from the Maumee River population, in accordance with the Algal Loading Hypothesis. Analyzing the ITS data more closely to determine if there was a significant similarity or difference between the river and bay communities, we performed statistical analysis of the community phylogenies.

3.3. UniFrac analysis of ITS sequences

There are a number of statistical programs used to analyze large 16S datasets (Hur and Chun, 2004; Schloss et al., 2004; Eckburg

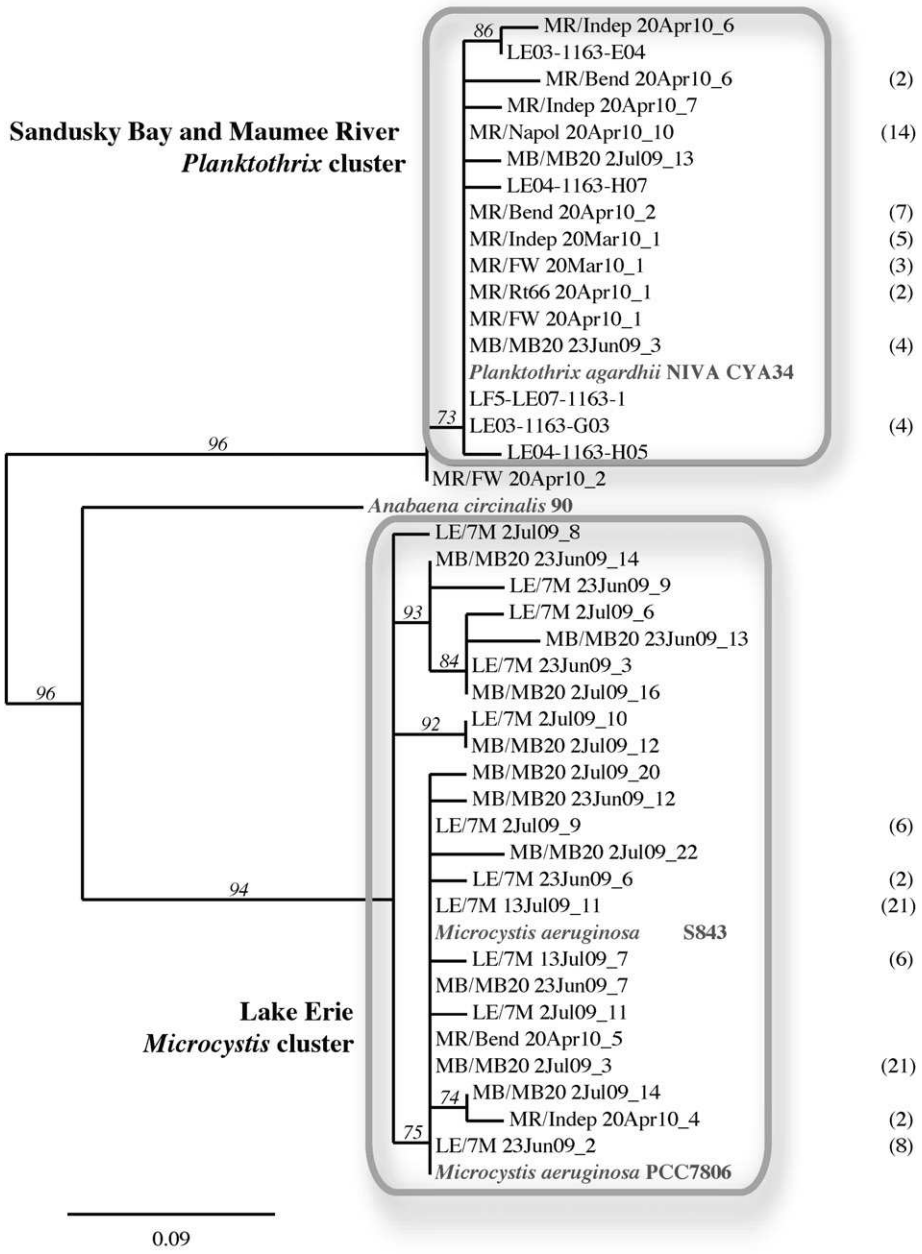

Fig. 3. Phylogenetic tree showing relationship between McyA amplicon sequences from the Maumee River (MR), Sandusky Bay station 1163 and Maumee Bay (MB) of Lake Erie. The coding system for the clones are as follows. Bend, Indep – Independence Dam, Napol – Napoleon, Rt66 – Rt66 Bridge, FW – Farnsworth are sites in the Maumee River. The Maumee River sequences are from April 2010. Lake Erie Maumee Bay samples were collected on 23 June, 1 July, and 13 July 2009. Sandusky Bay sequences (labeled LEO-1163) were obtained from GenBank (Rinta-Kanto 2006). The numbers in parentheses indicate the number of identical sequences represented these sequences.

et al., 2005; Lozupone and Knight, 2005; Bik et al., 2006). The UniFrac web interface has been previously utilized to successfully study dynamics of microbial populations in diverse environments such as hot springs, the mammalian intestinal tract and marine phytoplankton blooms (Lozupone et al., 2007; Jones et al., 2010), and we chose this method to differentiate the cyanobacterial populations herein. UniFrac was applied to the ITS sequences from the Maumee River, Maumee Bay, and the western basin of Lake Erie to examine the similarities in the entire cyanobacterial community between the different locations. For this analysis, 16S–23S ITS sequence data were analyzed from the Maumee River (Napoleon, OH) and Maumee Bay station MB20 and Lake Erie station 7 M. UniFrac tests for differences between locations based on the frequency of sampled sequences.

The Environment Counts analysis shows detailed information on how many Operational Taxonomic Units (OTUs) from each environment were identified in the study. OTUs were set at 97% sequence identity (Drancourt and Raoult, 2005). To evaluate how the three environments (Napoleon, 7M and MB20) related to one another, we used the *Environment Distance Matrix* analysis. Lower values represent communities that were more similar. The analysis demonstrated that sequences from Maumee Bay (MB20) and western Lake Erie (7M) were more similar to each other ($UniFrac_{MB20,7M}$ value = 0.5717) than to the Napoleon site ($UniFrac_{MB20,Napoleon}$ = 0.7238; $UniFrac_{7M,Napoleon}$ = 0.7597).

The robustness of the result of the significant differences between the River, Bay, and Lake Erie was confirmed using the Jackknife Environment Clusters analysis. The UniFrac Environment

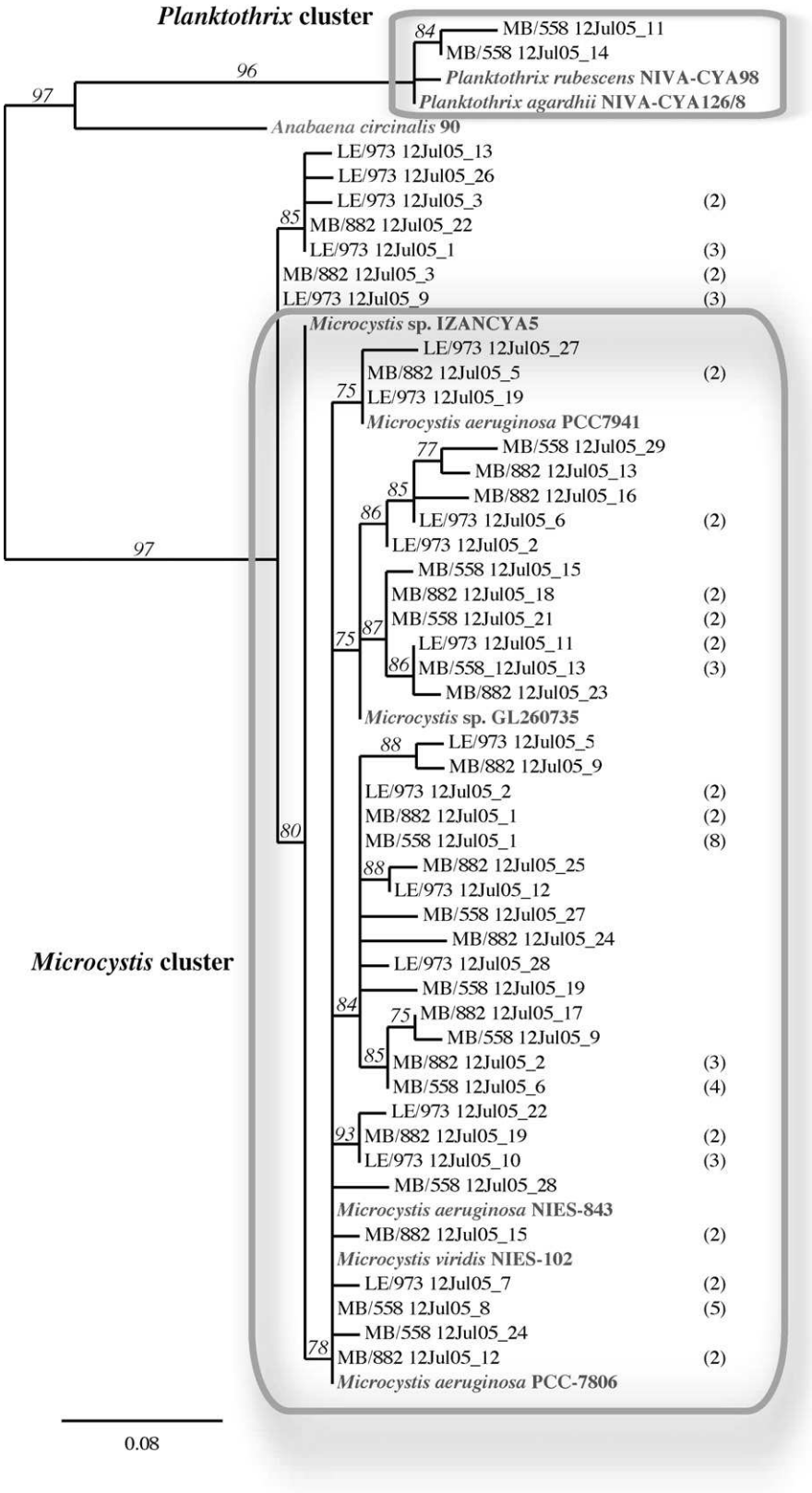

Fig. 4. Phylogenetic tree showing relationship between McyA amplicon sequences from Maumee Bay (MB 558, MB 882) and western Basin of Lake Erie (MB 973) collected on 12 July 2005.

Clusters analysis estimates the robustness of the results with respect to sampling effort and evenness. The number of sequences was set to 30, which was the number of sequences from the environment with the fewest OTUs (MB 7M), and number of permutations to 100. These reduced sets of sequences were analyzed in an identical manner to the complete sequence set in UniFrac to establish the robustness of the UniFrac distances between the sampling sites. The Maumee Bay and Lake Erie

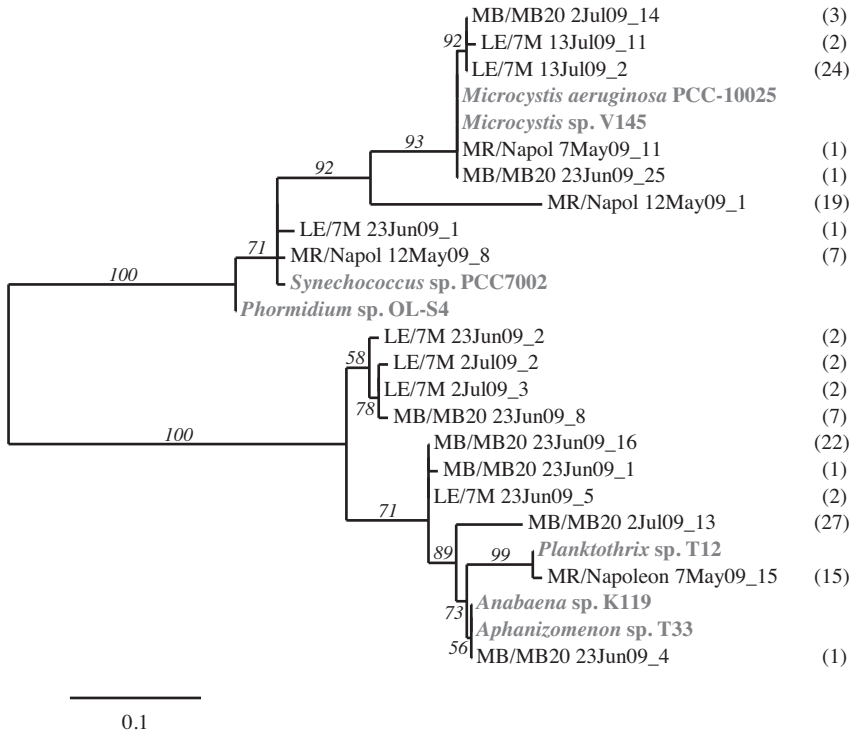

Fig. 5. Phylogenetic tree displaying relationship between ITS amplicon sequences from the Maumee River site Napoleon, Maumee Bay station MB20, and Lake Erie station 7 M. Napoleon samples are from different dates in May 2009. Lake Erie and Maumee Bay samples are from 23 June, 1 July, and 13 July 2009. The numbers in parentheses indicate the number of identical sequences represented these sequences.

sequences (MB20 and 7M) were more similar to each other than to sequences from the River, and these nodes were supported in all 100 permutations.

The UniFrac significance test, set to compare *Each Environment Individually* with 100 random permutations, compared the abundance of specific sequences to determine the similarity of cyanobacteria communities in the river and the two bay environments. Of the three environments, Maumee River sequences were significantly different (MR_Nap $p = 0.01$) from Maumee Bay and Lake Erie sequences (MB_MB20 $p = 0.43$ and MB_7 M $p = 0.51$).

The cyanobacteria ITS sequences recovered from the River and Bay/Lake were statistically different in the relative abundance and the statistically predicted phylogeny of specific sequences. The UniFrac analysis provided robust evidence for significant differences between the cyanobacterial communities of Maumee River and Maumee Bay based on their ribosomal ITS.

4. Conclusions

Phylogenetic analysis of McyA sequences from the Maumee River and western basin of Lake Erie revealed that the Lake Erie Maumee Bay sequences were primarily *Microcystis* spp. sequences, whereas the river sequences form a separate cluster that is homologous to *Planktothrix* spp. The results showing that Sandusky Bay and Maumee sites are populated by *Planktothrix* spp. points to future studies of these locations that might reveal common physical and chemical parameters that could yield toxic *Planktothrix* instead of toxic *Microcystis* blooms.

Overall, we find little evidence for the hypothesis that the Maumee River serves as a source of toxic *Microcystis* spp. to western Lake Erie. The ITS sequences argue for some metapopulation connectivity for cyanobacteria between the River and the Bay, but our results point to toxic *Planktothrix* in the river and toxic *Microcystis* in Lake Erie. Nonetheless, even though Unifrac shows that the river and bay cyanobacterial communities are significantly different, we recognize the possibility that river nontoxic *Microcystis* spp. genotypes could contribute to blooms once they enter Maumee Bay. Whereas the river is a major source of nutrients supporting bloom events, the most likely origin of the toxic Lake Erie *Microcystis* spp. blooms are seed populations within the lake itself. Given that *Microcystis* spp. DNA that is genetically consistent with blooms is detectable in sediments, and that *Microcystis* can be cultured directly from Lake Erie sediments (Rinta-Kanto et al., 2009b), we propose that toxic blooms arise primarily from an endemic lake cyanobacterial community.

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