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Genetic Variation of the Bloom-Forming Cyanobacterium *Microcystis aeruginosa* within and among Lakes: Implications for Harmful Algal Blooms

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To measure genetic variation within and among populations of the bloom-forming cyanobacterium *Microcystis aeruginosa*, we surveyed a suite of lakes in the southern peninsula of Michigan that vary in productivity (total phosphorus concentrations of ~10 to 100 μg liter^{-1}). Survival of *M. aeruginosa* isolates from lakes was relatively low (i.e., mean of 7% and maximum of 30%) and positively related to lake total phosphorus concentration ($P = 0.014$, $r^2 = 0.407$, $n = 14$). In another study (D. F. Raikow, O. Sarnelle, A. E. Wilson, and S. K. Hamilton, Limnol. Oceanogr. 49:482–487, 2004), survival rates of *M. aeruginosa* isolates collected from an oligotrophic lake (total phosphorus of ~10 μg liter^{-1} and dissolved inorganic nitrogen:total phosphorus ratio of 12.75) differed among five different medium types (*G* test, *P* of <0.001), with higher survival ($P = 0.003$) in low-nutrient media (28 to 37% survival) than in high-nutrient media. Even with the relatively low isolate survivorship that could select against detecting the full range of genetic variation, populations of *M. aeruginosa* were genetically diverse within and among lakes (by analysis of molecular variance, $\Phi_{sc} = 0.412$ [Φ_{sc} is an F-statistic derivative which evaluates the correlation of haplotypic diversity within populations relative to the haplotypic diversity among all sampled populations], $P = 0.001$), with most clones being distantly related to clones collected from lakes directly attached to Lake Michigan (a Laurentian Great Lake) and culture collection strains collected from Canada, Scotland, and South Africa. Ninety-one percent of the 53 genetically unique *M. aeruginosa* clones contained the microcystin toxin gene (*mcyA*). Genotypes with the toxin gene were found in all lakes, while four lakes harbored both genotypes possessing and genotypes lacking the toxin gene.

The effects of grazers or nutrients on harmful phytoplankton blooms (HABs) or HAB toxins show high temporal and spatial variability (10, 42, 43, 47, 51). One source of this variation could be genetic dissimilarity among HAB populations. For example, toxic and nontoxic genotypes within a HAB species might dominate in different habitats and at different times, which could lead to variation in, for example, the ability of consumers to control HABs. However, few studies have measured the genetic composition of HAB populations across time (28, 29) or space (2, 25), limiting our ability to assess the degree to which environmental variation may select for genotypes with different ecological traits.

In freshwater systems, HABs are largely caused by cyanobacteria of the genera *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, and *Oscillatoria*. Among these taxa, *Microcystis aeruginosa* is one of the most ecologically damaging species due to its prevalence in bodies of water that vary in nutrient loading and its degree of toxicity to aquatic and terrestrial organisms (7, 11). Further, we note recent reports showing that the ongoing invasion of freshwaters in North America by the filter-feeding zebra mussel, *Dreissena polymorpha*, is causing an increase in *M. aeruginosa* in low-nutrient lakes (50, 53, 60). Due to the present and potentially increasing importance of *M. aeruginosa*, we focused our efforts on the genetic diversity of this species.

Cyanobacteria may use a suite of strategies, including morphology and intracellular toxins, to reduce herbivory by filter feeders (12, 31, 45). Of these traits, toxic secondary metabolites are the most frequently studied, and recent analytical techniques have identified genes responsible for the production of microcystins, a group of toxins produced by *M. aeruginosa* (e.g., *mcyA*, *mcyB*) (57). Microcystins are cyclic peptides that have been shown to be potent hepatotoxins for rodents and humans (9) and are considered by many to be grazing deterrent compounds (30). If these compounds are grazing deterrents, it might be expected that intense selective herbivory by freshwater grazers, like cladocerans and invasive mussels, would favor genotypes containing microcystin genes. Selection for these toxic genotypes could thus create cascades with major implications for human health (8).

Traditionally, assessment of diversity within *M. aeruginosa* has focused on morphological variation, such as colony shape and cell size (61). However, the concordance between morphological variation and genetic variation is often not clear, especially for microbe populations (25). Recent advances in molecular techniques (e.g., *nifH*, *cpcBA*-IGS, nucleotide se-

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TABLE 1. Limnological characteristics of the study lakes

Lake	County (Michigan)	Total phosphorus (μg liter^{-1})	Chlorophyll (μg liter^{-1})[a]	Surface area (ha)[b]	Maximum depth (m)	Mean depth (m)
Bear	Muskegon	66.3	38.6	168[c]	3.7[c]	2.1[b]
Clark	Jackson	17.8	2.4	227	16.8[c]	3.6
Diamond	Cass	23.7	5.3	420	19.5[c]	5.1
Gilkey	Barry	17.1	4.2	32	9.4	5.0
Gravel	Van Buren	22.4	3.8	123	9.5[c]	5.6
Gull	Kalamazoo	19.7	2.0	794	33.5[c]	12.4
Hudson	Lenawee	48.8	55.2	181	9.1[c]	3.1
Magician	Cass	24.6	4.0	202[c]	17.4[c]	2.1[b]
Pine	Barry	27.2	5.5	275	10.4[c]	3.2
Portage	Kalamazoo	28.3	3.6	73[c]	10.4[c]	4.5[e]
Round	Van Buren	35.3	2.9	76	8.2[c]	2.3
Spring	Ottawa	101.8	25.0	424[c]	14.3[c]	6.2[e]
Swan	Allegan	87.0	80.2	81[c]	8.5[c]	3.7[e]
Warner	Barry	12.8	3.8	128	14.0[d]	6.8[d]

[a] Data from 1998–1999 lake survey (47).
[b] Unpublished data from 2000 lake survey.
[c] From STORET database (http://www.epa.gov/storet).
[d] Alan Tessier [Michigan State University], personal communication.
[e] Calculated from following formula (volume/surface area at zero depth) (63).

quences, and highly iterated palindromic [HIP]-PCR) now allow for extensive examinations of genetic differences among harmful phytoplankton genera, species, and strains within a species (26, 35, 36, 41). However, many of these studies have focused on strains of cyanobacteria from culture collections (34, 35, 40; see also references 32 and 37), leaving few reports of the genetic diversity in natural HAB populations (25, 28). Of the latter studies, only a handful provide details about isolate survivorship after sample collection and initial culturing (21, 46, 49; see also references 18 and 52). Many field-collected genotypes may do poorly in culture because abiotic conditions in the lab may not closely match environmental conditions in nature. Limited isolate survivorship may severely constrain assessments of genetic diversity, because only a few isolates may be identified and these may be closely related. It is reasonable to hypothesize that the number of culturable genotypes from an ecosystem might correlate with how well culture conditions match environmental conditions in that particular ecosystem. However, we are unaware of any past studies describing the effect that such conditions have on algal isolate survivorship. If, for example, isolate survivorship in the laboratory is a function of the match between nutrient concentrations in nature versus in the culture medium, the choice of culture medium should presumably be adjusted for differences in nutrient levels among habitats from which isolates are collected. This would reduce biases introduced by the process of genotype isolation and so improve cross-system comparisons of genetic diversity; it could also, however, confound comparative laboratory studies of ecological traits if all strains cannot be maintained on the same culture medium.

Genetic diversity traditionally has been defined as the percentage of distinct genotypes collected from a sampled population. Although past studies describing genetic variation of phytoplankton have shown both little variability (i.e., 0 to 10% [1, 5, 29]) and much variability (i.e., 50 to 100% [2, 19, 33]), recent studies of cyanobacteria suggest considerable genetic diversity both among sites (3, 4, 22, 25, 27, 37) and within lakes (2, 25, 29, 46, 62). In contrast, three recent studies suggest low genetic diversity for certain cyanobacteria (1, 21, 24). Such conflicting results could be a function of natural history, recent anthropomorphic habitat alterations, different sampling and genetic analysis techniques, or culture conditions that bias results in favor of only a few culturable genotypes. We suggest that more-thorough investigations of the genetic makeup of HAB populations among, and especially within, bodies of water are necessary to better predict and understand the genesis of HAB events. To our knowledge, this study presents the most extensive data set describing the genetic composition of *M. aeruginosa* isolates within and among freshwater habitats.

In this paper, we address the following questions. (i) Is there potential isolate bias with respect to the match between nutrient concentrations in standard algal growth media and environmental nutrient concentrations? (ii) Are *M. aeruginosa* populations comprised of one or many genotypes? (iii) If significant within-population genetic variation exists for *M. aeruginosa*, do sympatric genotypes vary in the presence of the microcystin gene (*mcyA*)?

MATERIALS AND METHODS

Study lakes. Fourteen lakes in the lower peninsula of Michigan were sampled for *M. aeruginosa* (Table 1). One lake (Gull Lake) was sampled three times from June to August 2000, and all 14 lakes were sampled in August 2002. The lakes were distributed across a broad range of productivity ($\sim$10 to 100 μg liter^{-1} total phosphorus). Two of the lakes, Bear Lake and Spring Lake, are directly connected to Lake Michigan. Up to three lakes were sampled per day, and at each lake integrated whole water samples of the mixed layer were collected with a tube sampler for chlorophyll *a* analysis. After collecting particles on Gelman attaching and effacing filters, chlorophyll *a* was extracted in 90% ethanol (38) and measured via fluorometry with acid correction. Nutrient data and morphometric estimates were obtained from previous studies (47, 63; L. Knoll, C. Scheele, and S. Hamilton, personal communication).

Collection, isolation, and culturing of *M. aeruginosa*. *M. aeruginosa* colonies were collected with a zooplankton net (30-cm diameter and 100-μm mesh) towed horizontally near the surface. The contents of all tows from each lake were poured into a plastic container and stored in a cooler with lake water until returning to the lab. In 2000, colonies were isolated on the same day as the collection. In 2002, multiple lakes were sampled on a single day, so colony isolation was completed the following day. For overnight storage, samples were poured into individual 1-liter glass containers and provided with gentle aeration.

TABLE 2. Summary of results for *M. aeruginosa* isolate survival and genetic analysis[a]

Lake	Sample date (mo/day/yr)	No. of isolates collected	No. (%) of surviving isolates	No. of clones genetically analyzed	No. (%) of distinct genotypes
Bear	08/15/02	80	8 (10)	7	7 (100)
Clark	08/17/02	80	9 (11)	9	9 (100)
Diamond	08/13/02	80	1 (1)	0	0
Gilkey	08/05/02	40	1 (3)	1	1
Gravel	08/09/02	80	0 (0)	0	0
Gull	06/19/00	NR	NA	1	1
	06/29/00	NR	NA	8	5 (63)
	08/23/00	NR	NA	1	1
	08/07/02	80	1 (1)	1	1
Hudson	08/17/02	40	4 (10)	3	2 (67)
Magician	08/11/02	80	2 (3)	2	1 (50)
Pine	08/05/02	40	4 (10)	4	2 (50)
Portage	08/07/02	80	2 (3)	2	2 (100)
Round	08/11/02	80	9 (11)	9	9 (100)
Spring	08/15/02	80	7 (9)	6	6 (100)
Swan	08/09/02	40	12 (30)	12	5 (42)
Warner	08/05/02	40	1 (3)	1	1

[a] Some isolates perished prior to genetic analysis. NA, not applicable; NR, not recorded.

Floating colonies with features characteristic of the *M. aeruginosa* morphotype (61) were pipetted through several wells of either distilled water (isolates in 2000) or BG-11 algal medium (isolates in 2002; medium recipe found in reference 58) on a well plate and finally placed into test tubes filled with 15 ml of BG-11 medium.

The examination of culture bias was confined to the study done in 2002, where between 40 and 80 colonies were isolated per lake (Table 2). All test tubes were placed into an incubator maintained at 23 to 24°C with a 16:8 h light:dark cycle, which closely matched the environmental conditions of the study lakes at the time of collection. Culturable isolates were transferred to 125-ml flasks filled with sterile media approximately every 4 to 8 weeks. Isolates that survived the culture process were recorded at each transfer. Although strains were capable of perishing at any time, we conducted our final isolate survival check on 5 February 2003 (Table 2 and Fig. 1), which allowed any slow-growing isolates ample time (~6 months) to thrive. Although most strains were unialgal, a few strains were maintained with associated rod-shaped bacteria (i.e., Bear W, Bear AY, Bear AG, Clark DV, and Spring CW) or green algae (i.e., Bear AI, Portage EJ, Round S, and Round V); thus, they were clearly not axenic. Minor contamination of this type was not a problem in this study because cyanobacteria-specific genetic-analysis methods were used (HIP-PCR methods described below). Four strains of *M. aeruginosa* purchased from two culture collections (University of Texas Culture Collection, strains 2385, 2664, and 2667; Pasteur Culture Collection, strain 7820) were also genetically analyzed to provide a frame of reference for the field-collected isolates.

Test of nutrient media on isolate survival. To examine the effect of algal nutrient medium on *M. aeruginosa* culturability, we isolated 32 separate colonies from an oligotrophic lake (Gull Lake, Kalamazoo County, Michigan) into each of five medium types (pH of ~7.5): (i) autoclaved BG-11 (2,000 μM N as NH_4^+ and 180 μM P), (ii) filter-sterilized BG-11 (2,000 μM N as NH_4^+ and 180 μM P), (iii) autoclaved BG-11 with reduced N (500 μM N as NH_4^+ and 180 μM P), (iv) autoclaved WC-S (55) with NH_4 as the nitrogen source (500 μM N and 19 μM P), and (v) autoclaved WC-S with NO_3 as the nitrogen source (500 μM N and 19 μM P). These medium types were chosen in part because we suspected that high NH_4^+ concentrations might be inhibitory to some genotypes. *M. aeruginosa* was collected on 27 September 2002, and colonies were isolated over the following 2 days by using the techniques described previously. We isolated half of the colonies (16 colonies each for five medium types) on the day of collection and half the following day. Survivorship was determined by visually inspecting tubes for growth on 8 October 2002 (days 10 to 11) and again on 6 November 2002 (days 40 to 41).

Genetic analyses. Prior to sample collection for genetic analyses, cultures were transferred to acid-washed flasks and allowed to grow until a sufficient population was established. Cultures were then lyophilized in individual 2-ml centrifuge tubes and stored dry until analyzed.

DNA extraction. DNA extraction was performed using a modification of the bacterial lysis method of Tillet and Neilan (56). The lyophilized *Microcystis* sample was resuspended in 500 μl XS buffer containing 0.01% (wt/vol) potassium ethyl xanthogenate (Fluka Chemika), 800 mM ammonium acetate, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, and 1% (wt/vol) sodium dodecyl sulfate. The suspension was incubated at 70°C for 30 min with occasional vortexing and cooled on ice for 30 min. The tube was centrifuged at 12,000 × *g* at 4°C for 10 min and then the supernatant, containing genomic DNA, was carefully removed into a new microcentrifuge tube. The DNA was precipitated by the addition of an equal volume of 2-propanol and incubated at room temperature for 10 min. The DNA was collected by centrifugation at 12,000 × *g* for 20 min at 4°C. The resulting pellet was washed with 70% (vol/vol) ethanol, dried using a vacuum desiccator, and then resuspended in 200 μl TE buffer (10 mM Tris-HCl [pH 8.0], 0.1 mM EDTA).

Detection and amplification of microcystin gene (*mcyA*). The *mcyA*-specific primer pair QMETF and QMETR was applied to each DNA sample in the following reaction. PCR was carried out in 0.2-ml tubes with a final volume of 20 μl and contained 1× F1 *Taq* buffer, 0.2 U F1 *Taq* (Fischer Biotech), 200 μM of each deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 5 pmol of each oligonucleotide primer, and approximately 50 ng genomic DNA. The amplification profile consisted of an initial denaturation at 95°C for 2 min followed by 30 cycles of 94°C for 10 s, 45°C for 20 s, and 72°C for 30 s. This was followed by a final elongation step of 72°C for 2 min and final incubation at 20°C.

Amplification of cyanobacterial-specific HIP profile. Samples of DNA were genetically discriminated via HIP-PCR. The HIP-PCR method has been dem-

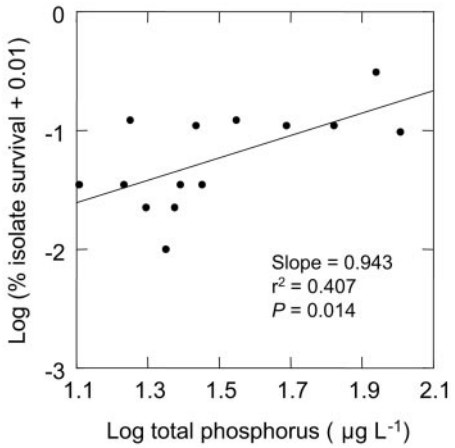

FIG. 1. Relationship between lake trophic status (measured as total phosphorus concentration [μg liter^{-1}]) and log (% isolate survival + 0.01) (% = no. of survivors/no. of total isolates collected).

onstrated experimentally to be specific for cyanobacteria (HIP1) (37), except where sequences analogous to the HIP1 octamer were identified for certain halophilic archaebacteria (HIP2) and a thermophile (HIP3) (48). These organisms were unlikely contaminants of our samples since our study sites are temperate, freshwater lakes. For each sample, a PCR was performed with each of the HIP-targeted primers HIP-CA (GCG ATC GCG CA) and HIP-CT (GCG ATC GCG CT). Each reaction was carried out in 0.2-ml tubes with a final volume of 20 μl and contained 1× F1 *Taq* buffer, 0.4 U F1 *Taq* (Fischer Biotech), 200 μM of each deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 10 pmol of the HIP-CA or HIP-CT primer, and 1 μl of DNA extract. Thermal cycling consisted of a preincubation of 2 min at 94°C, followed by 30 cycles of 10 s at 94°C, 20 s at 40°C, and 2 min at 72°C, with the ramp speed between the last two steps set at 0.1°C min^{-1}. A final incubation at 72°C for 2 min ended the amplification process.

Phylogenetic reconstruction from HIP profiles. The banding patterns produced by the respective HIP-CA and HIP-CT PCRs were scored for the presence or absence of bands in 20 discrete positions for all samples. For each sample, the HIP-CA and HIP-CT scores were concatenated in that order and arranged in a tabular form consistent with that prescribed in PHYLIP (16). These formatted data were submitted to the program BANDAID (50), which calculated pairwise genetic distances in a format compatible with that prescribed above. These data were submitted to the NEIGHBOR program of PHYLIP, which constructed the phylogenetic tree.

Statistical analyses. Linear regression tested for a significant relationship between lake total phosphorus concentration (log total phosphorus) and isolate survivorship [log (% isolate survival + 0.01)] or chlorophyll concentration (log chlorophyll). A row-by-column test of independence used the *G* test (54) to determine survivorship differences among all medium types. Analysis of variance was used to compare isolate survival in the two major different medium types (WC media [$n = 2$] and BG-11 media [$n = 3$]). Analysis of molecular variance (AMOVA [14]) was used to calculate the genetic variation of *M. aeruginosa* within and among populations and to provide pairwise comparisons of genetic structure between different populations of *M. aeruginosa*. All statistical analyses were performed with Systat 9.01 (SPSS) or GenAlEx 5.0 (44). The rejection criterion was set at α of <0.05.

RESULTS

Thirteen of the fourteen lakes sampled had culturable isolates. Isolate survival rates for *M. aeruginosa* collected from the study lakes in August 2002 varied from 0 to 30% (mean ± standard error, 7.4 ± 2.1%) across all lakes and were positively related to lake total phosphorus concentrations ($P = 0.014$, $r^2 = 0.407$, $n = 14$) (Table 2 and Fig. 1).

We found a significant effect of growth medium on the culturability of *M. aeruginosa* isolates from Gull Lake (*G* test statistic of 38.7, df = 4, $P < 0.001$) with significantly higher survivorship in WC media than in BG-11 media ($P = 0.003$). The highest isolate survival rates were observed in the relatively low-phosphorus WC medium treatments (37.5% survival WC-S with NH_4; 28.1% survival WC with NO_3), with little survival (0 to 3.7%) in the BG-11 medium types that contained higher phosphorus concentrations.

Seventy-nine percent of the 67 cyanobacterial isolates genetically analyzed with HIP-PCR were shown to be genetically distinct. Percent distinct genotypes [(no. of distinct genotypes/no. of isolates analyzed) × 100] from lakes with two or more analyzed isolates ranged from 42% to 100% (mean ± standard error, 78 ± 7.8%). Nine of the 10 lakes where two or more isolates were analyzed via HIP-PCR showed at least two distinct genotypes. In addition, in two lakes, Clark Lake and Round Lake, nine of nine isolates were genetically distinct. Interestingly, one genotype (ClarkB02/PineCD02/PineCF02/PineCG02) was observed in two lakes, Clark Lake and Pine Lake (Table 2), which were separated by 130 kilometers. We did not detect this genotype in any lakes situated near Pine Lake.

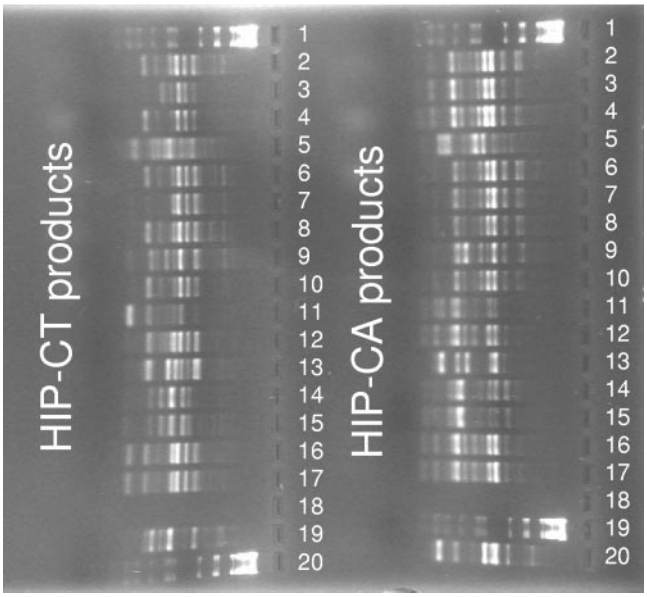

FIG. 2. Example of PCR gel of HIP products. HIP-CT and HIP-CA samples for *M. aeruginosa* isolates from Clark Lake (lanes 2 to 10), Gilkey Lake (lane 11), Gull Lake (lane 12), Hudson Lake (lanes 13 to 15), Magician Lake (lanes 16 to 17), PCC 7806 (lane 19, HIP-CT; lane 20, HIP-CA), and standards (lanes 1 and 20, HIP-CT; lanes 1 and 19, HIP-CA). Note differences among Clark Lake and Hudson Lake strains.

Populations of *M. aeruginosa* exhibited significant genetic variation (Table 2 and Fig. 2 and 3) among lakes (by AMOVA, $\Phi_{sc} = 0.412$ [Φ_{sc} is an F-statistic derivative which evaluates the correlation of haplotypic diversity within populations relative to the haplotypic diversity among all sampled populations], $P = 0.001$ [Table 3]), despite the low culturability of isolated colonies (Table 2). Interestingly, only 41% of the estimated genetic variance could be explained by among-lake variation, while 59% of the genetic variation was explained by within-population variation. AMOVA also revealed many statistically significant pairwise comparisons between *M. aeruginosa* populations in different lakes, with each population being genetically distinct from at least three other populations (Table 4). The extreme examples were the Gull Lake, Round Lake, and Swan Lake populations, which were genetically distinct from all other cyanobacterial populations assessed ($P \leq 0.05$). The Portage Lake and Hudson Lake populations exhibited the least genetic dissimilarity from other populations (only three significant pairwise comparisons between either of these lakes and the other nine lakes).

The phylogenetic analysis of the PCR products also suggested much genetic variation within and among lakes (Fig. 3). Isolates from six (Bear, Clark, Hudson, Portage, Spring, and Swan) of the lakes were dispersed on the phylogenetic tree, while cultures from Gull Lake, Pine Lake, and Round Lake tended to be clustered with sympatric clones. Only one lake, Gull Lake, was sampled multiple times, and the same genotype was never collected on more than one date.

Comparing the phylogenetic positions of the *M. aeruginosa* cultures from the study lakes with those of the four culture collection strains revealed interesting relationships. All culture

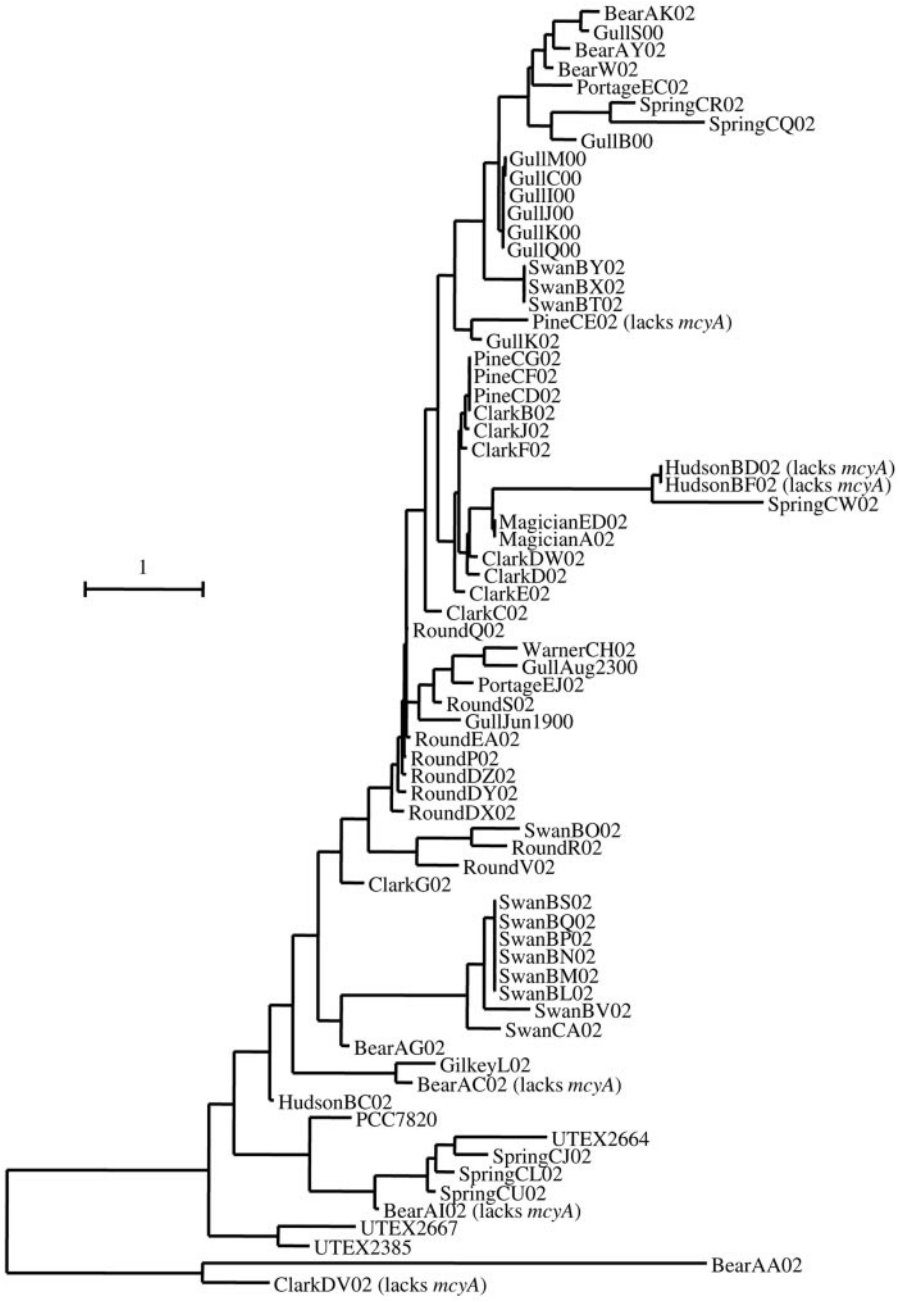

FIG. 3. Phylogenetic tree for *M. aeruginosa* isolates created by calculating the relative "distances" of PCR products in a pairwise fashion between the banding patterns of each HIP-PCR (BANDAID program created by Tim Salmon and Brett Neilan at the University of New South Wales [50]). Toxin gene absence denoted by "lacks *mcyA*" in parentheses following strain name. All other strains contain the toxin gene. Scale = 1 relative distance unit.

collection strains grouped near the origin of the tree and were intermixed with several strains from Bear Lake and Spring Lake, along with one strain from Hudson Lake and one from Gilkey Lake. As might be expected, UTEX 2385 and UTEX 2667 (both from Little Rideau Lake, Ontario, Canada) were neighbors on the tree. Additionally, UTEX 2664 (South Africa) appears to be closely related to PCC 7820 (Scotland) and several isolates from Bear Lake and Spring Lake. Two isolates, BearAA02 and ClarkDV02, were out-groups; however, their colony morphologies suggested that they were *M. aeruginosa*.

The microcystin toxin gene (*mcyA*) was detected in most isolates (92.5%) and unique genotypes (90.6%) and in all 12 lakes that had *M. aeruginosa* genotypes (Table 2 and Fig. 3). Only five genotypes lacked the toxin gene. Four lakes harbored both toxic and nontoxic genotypes (Bear, Clark, Hudson, and Pine).

TABLE 3. AMOVA outputs for population genetic comparisons of *M. aeruginosa* clones from the 10 study lakes for which multiple clones were analyzed[a]

Source	df	SS	MS	Estimated variance	% variance explained	Φ_{sc} value	*P* value
Among populations	9	71.07	7.90	1.02	41	0.412	0.001
Within populations	55	80.37	1.46	1.46	59		

[a] SS, sum of squares; MS, mean square error; Φ_{sc} value, F-statistic derivative which evaluates the correlation of haplotypic diversity within populations relative to the haplotypic diversity among all sampled populations (14).

DISCUSSION

We found considerable genetic variation within and among populations of the bloom-forming phytoplankter, *M. aeruginosa*, in southern Michigan lakes. Fifty-three of the 67 isolates analyzed via HIP-PCR were shown to be genetically distinct. In the four lakes with larger sample sizes of isolates (six to nine isolates analyzed), virtually all isolates were genetically distinct. We found only one instance of the same genotype being present in two separate lakes (ClarkB02/PineCD02/PineCF02/PineCG02). Although genetic variability of cyanobacteria (13, 37), including *M. aeruginosa* (2, 25, 62), has been documented previously, this data set represents the most comprehensive genetic analysis of *M. aeruginosa* strains collected from within and among lakes in North America (see reference 25 for a survey of *Microcystis* morphotypes throughout Europe). Furthermore, we note that if different colony morphologies (e.g., *M. botrys*, *M. flos-aquae*, *M. ichthyoblabe*, *M. viridis*, and *M. wesenbergii*) represent different strains within *M. aeruginosa*, then we may have underestimated the genetic diversity of this bloom-forming phytoplankter, because we limited our collections to only those morphologies that are typical of *M. aeruginosa* (i.e., our conclusion of "high" diversity is therefore conservative).

Low isolate survival in culture may lead to underestimation of genetic variability, yet few studies have quantified isolate survival. Given the relatively low isolate survival in our study, we caution that genetic variability of *M. aeruginosa* in Michigan lakes may be substantially greater than we report. Newer techniques enable determination of genetic diversity based on freshly collected individual colonies (15, 25, 28) and so eliminate problems related to culture bias. However, genetic analysis of individual colonies precludes culturing (since the colonies are destroyed in the process) to assess isolate survivorship as a function of medium type or isolation procedures.

We found that the average survivorship rate observed for isolates collected from 14 lakes (7.4%) was higher than the rate determined by some studies (46, 49) but much lower than that determined by others (18, 52). Although our survivorship estimates were low, survivorship of *M. aeruginosa* isolates cultured in a nutrient-rich medium was positively related to ambient nutrient concentrations found in our study lakes. In other words, isolates from oligotrophic lakes showed lower survivorship when cultured in nutrient-rich algal medium than did isolates collected from more-eutrophic lakes (Fig. 1 and Table 2). Our observation that *M. aeruginosa* isolates from an oligotrophic lake survived better in less-rich WC media than in more-rich BG-11 media provides further support for this pattern. Such results have important implications for scientists studying the population genetic structure of phytoplankters in freshwater, and possibly marine, habitats that vary in nutrient concentration. Furthermore, scientists interested in collecting isolates from the field to be used in later experiments may benefit from choosing an algal medium based on the nutrient regime of the bodies of water sampled.

Our lake survey revealed substantial genetic diversity within *M. aeruginosa* both within and among lakes. Bittencourt-Oliveira et al. (2) provided similar results for *M. aeruginosa* strains from four Brazilian reservoirs. In that study, nine distinct genotypes were collected from four sites, with (at most) six genotypes collected from one site and one specific genotype collected from two sites. Additionally, one unique genotype of *M. aeruginosa* was collected from the same site over time and along a depth gradient at the same sampling time (2). Most recently, Janse et al. (25) surveyed the genetic diversity of 107 *Microcystis* colonies (seven morphospecies) from 15 European lakes and characterized 59 distinct genetic classes, demonstrating significant genetic variation within and across habitats. For example, all but one *Microcystis* population (93%) was comprised of at least two distinct classes, and 24% (14 of 59 classes) of the *Microcystis* classes were found from at least two different lakes, with one group being found in lakes located in the Czech Republic, Germany, Italy, and Scotland. Addi-

TABLE 4. Pairwise population Φ_{sc} values and *P* values for comparisons of *M. aeruginosa* clones from the 10 study lakes for which multiple clones were analyzed[a]

Lake	Result for lake									
	Bear	Clark	Gull	Hudson	Magician	Pine	Portage	Round	Spring	Swan
Bear	—	0.013	0.002	0.186	0.050	0.029	0.528	0.001	0.329	0.001
Clark	0.237	—	0.001	0.097	0.240	0.188	0.248	0.001	0.011	0.002
Gull	0.278	0.327	—	0.004	0.011	0.003	0.026	0.001	0.005	0.001
Hudson	0.099	0.193	0.467	—	0.199	0.064	0.405	0.010	0.142	0.006
Magician	0.316	0.074	0.611	0.444	—	0.060	0.332	0.015	0.100	0.019
Pine	0.212	0.078	0.490	0.391	0.442	—	0.137	0.002	0.064	0.002
Portage	0.000	0.059	0.424	0.172	0.500	0.000	—	0.037	0.378	0.024
Round	0.528	0.355	0.612	0.616	0.794	0.648	0.557	—	0.002	0.001
Spring	0.011	0.296	0.383	0.192	0.363	0.282	0.000	0.475	—	0.001
Swan	0.392	0.482	0.592	0.563	0.615	0.652	0.550	0.677	0.400	—

[a] *P* values for pairwise comparisons between lakes are shown above diagonal (represented by —), and Φ_{sc} values are shown below diagonal.

tionally, no relationship was found between morphospecies designation and genotypic class. Although our study was more restricted geographically, we also found substantial genetic variation within and among *Microcystis* populations. Thus, populations of *M. aeruginosa* from diverse habitats and within individual lakes are genetically heterogeneous, which could have major ecological implications for the detection, development, mitigation, and ecological impact of HABs.

Recent reports have documented higher genetic similarity for nearby bacterial communities than for more-distant communities (20, 23). Although few studies have determined how genetic variance is partitioned within and among habitats for phytoplankton, AMOVA estimated that 41% of the variation for our *M. aeruginosa* clones was attributed to among-lake differences, while the remaining variation (59%) could be explained by within-lake differences. We found no other studies that used this statistical technique to partition variance among and within populations for cyanobacteria, but our results are consistent with those provided by Shankle et al. (52) for dinoflagellate populations off the coast of Southern California. They found that the genetic variation for *Prorocentrum* sp. populations was almost entirely attributed to within-population differences (93%), while very little of the variation could be explained by among-population differences (10%). Although the surveyed habitats in these two studies are very different biologically, chemically, and physically, both studies show that most of the genetic variation could be attributed to within-habitat differences, with less variation being explained by among-habitat differences.

Four strains of *M. aeruginosa* from three freshwater lakes in North America, Africa, and Europe maintained at two culture collections were genetically analyzed as reference strains (Fig. 3). The culture collection strains were most related to each other and to several, but not all, strains of *M. aeruginosa* from Bear Lake and Spring Lake. The similarity between the culture collection strains and those strains positioned near the origin of the phylogenetic tree from Bear Lake and Spring Lake could be due to the similar nutrient levels in these bodies of water. Bloom-forming cyanobacteria are most prevalent in eutrophic lakes, so it is not surprising that the culture collection strains used in this study were isolated from mesotrophic to hypereutrophic lakes (6, 17).

We also found that populations of *M. aeruginosa* in four lakes contained genotypes with and without one of the genes responsible for microcystin production. Several other studies provided similar results (25, 28, 60, 62). Vezie et al. (60) isolated strains of *M. aeruginosa* from three freshwater sites in France in 1994 and showed that at least one strain from each site produced microcystins and at least one strain did not. Kurmayer and Kutzenberger (28) showed seasonal variation for the occurrence of a microcystin gene (*mcyB*) in a natural population of *M. aeruginosa* in Lake Wannsee from June 1999 to October 2000, with the lowest proportion of colonies containing the toxin gene occurring in the spring. Janse et al. (25) showed that at least 7 of the 15 European lakes surveyed contained sympatric *Microcystis* classes that tested either positive or negative for microcystin or its biosynthetic genes. Finally, Welker et al. (62) used two methods (agar plating and liquid media) to isolate colonies of *M. aeruginosa* from Lake Müggelsee and showed that both techniques produced contrasting results when evaluating strains for toxin production. Agar plating selected for nontoxic strains (96% nontoxic strains) while liquid media selected for toxic strains (5% nontoxic strains). Thus, different isolation and culturing techniques could select for toxic or nontoxic strains of *M. aeruginosa*, and these differences should be considered when developing an isolation/culturing protocol for HAB species.

In conclusion, we show that *M. aeruginosa* populations in the southern peninsula of Michigan are genetically diverse and that isolate survival in a nutrient-rich culture medium is positively related to the total phosphorus concentrations of the source lakes. We encourage future studies aimed at addressing population-level genetic diversity of harmful algal species in both space and time (2, 22, 46). Such information could be useful for predicting and mitigating future HABs and explaining unusual phenomena such as seasonal variation in toxin type and content of lakes (10, 39, 43, 59).

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