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Genetic variation of the noxious cyanobacterium, *Microcystis aeruginosa*, within and among lakes: implications for harmful algal blooms.

Abstract

nful algal blooms endanger aquatic and marine systems across the globe, ever a thorough understanding of the genetic composition of harmful algal ies both among and, especially, within water bodies is lacking. To better predict e HAB events, we suggest that more studies should determine the genetic sity of natural populations of harmful algal bloom species in space and time and hese data in existing models.

s study, we show that isolate survivorship of the noxious cyanobacterium, *ocystis aeruginosa,* from lakes that range in productivity (total phosphorus entrations ≈ 10 – 100 ug L^{-1}) is typically low (i.e., mean = 7% and maximum = 30%) s positively related to total phosphorus concentration (P = 0.012, R^2 = 0.424, N = Additionally, individual colonies of *M. aeruginosa* collected from an oligotrophic (total phosphorus ≈ 20 ug L^{-1} and DIN:TP ratio = 12.75), Gull Lake (Kalamazoo nty, Michigan), exhibited substantially higher survival (WC media types vs BG a types; P < 0.000) in less-rich algal media with a higher N:P ratio (28 – 37 % val) than lower N:P ratio media (0 – 4% survival).

with relatively low isolate survivorship, most populations of *M. aeruginosa* were vn to be genetically diverse with most clones being distantly related to clones cted from lakes directly attached to the Laurentian Great Lake, Lake Michigan, culture collection strains collected from Canada, Scotland, and South Africa. tionally, analysis of molecular variance (AMOVA) indicated that within-lake tic variation (70%) was significantly greater than the among-lake variation (30%) ne *M. aeruginosa* populations (Φpt = 0.283; P < 0.001.) Subsequently, 93% of the *eruginosa* clones contained the microcystin toxin gene (*mcyA*), however five es from four lakes lacked the toxin gene Thus, four of the *M. aeruginosa* lations were comprised of mixed assemblages containing clones that both lacked ontained the toxin gene. If microcystins are considered to be grazing deterrents, opulation genetic data suggest that natural selection toward grazing resistant *M. ginosa* strains is possible in these habitats given the high genetic variability rved in these water bodies. Additionally, such genetic variation at the local and onal scale could aid in explaining conflicting results regarding important ogical phenomena, such as, dramatic seasonal variation in toxin content of lakes he response of *M. aeruginosa* to the invasive bivalve, *Dreissena polymorpha*.

Questions

is project, we addressed the following questions.

o phytoplankter isolate survival rates relate to lake total phosphorus oncentration?

re populations of *M. aeruginosa* comprised of one or many genotypes within and mong lakes?

within-population genetic variation exists for *M. aeruginosa*, do sympatric enotypes vary in the presence of the microcystin producing gene (*mcyA*)?

oes the presence of the zebra mussel (*Dreissena polymorpha*) affect any of the bove relationships?

Methods

During the summers of 2000 and 2002, fourteen lakes that varied in phosphorus content were sampled for *M. aeruginosa* with a standard 100um mesh zooplankton net. Samples were returned to the lab where individual *M. aeruginosa* colonies were isolated, rinsed, and inoculated into sterile test tubes filled with algal medium. Isolates were monitored for several months to determine isolate survivorship. Survivorship was shown to be positively correlated with lake phosphorus concentration (Fig. 1). Thus, additional isolates from an oligotrophic lake were inoculated into five different media that varied in nitrogen and phosphorus content to determine if these clones would fare better in less rich media (Fig. 2). Once clones were established in the lab, samples from each clone were lyophilized and analyzed via HIP-PCR (highly iterated palindromic polymerase chain reaction) to differentiate among distinct genotypes. To create the phylogenetic tree (Fig. 3), the banding patterns produced by the respective HIPCA and HIPCT PCRs were scored for the presence/absence of bands in 20 discrete positions for all samples and these scores were analyzed via BANDAID (Tim Salmon – UNSW). The clones were also analyzed for the presence/absence of the microcystin toxin gene (*mcyA*) using specific *mcyA* primers.

Results

Figure 1. Isolate survivorship vs. lake productivity for fourteen lakes sampled in 2002.

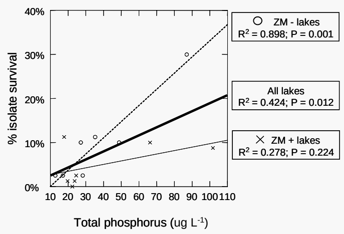
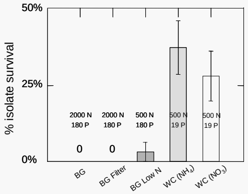


Figure 2. Isolate survivorship vs. algal media types (all clones collected from oligotrophic Gull Lake).



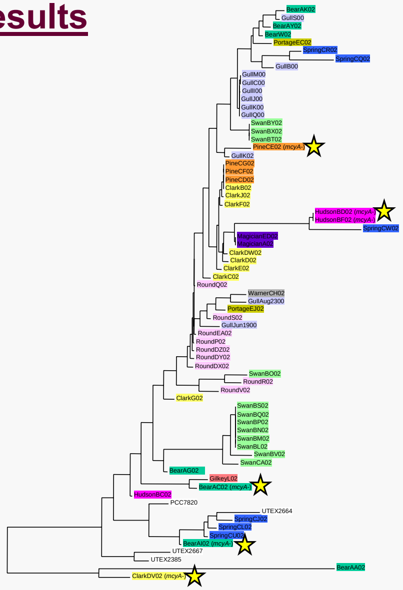


Figure 3. Phylogenetic tree of *M. aeruginosa* clones.
★ = clone that lacks toxin gene (*mcyA*)
Clones color-coded according to lake.

Results

AMOVA data output for population genetic comparisons of *M. aeruginosa* clones from nine of the fourteen study lakes. In the analysis, only unique genotypes from each lake (i.e., no duplicate genotypes were included) and clones from the 2000 and 2002 collections were included. Φpt values below diagonal. P-values based on 999 permutations above diagonal. P-values < 0.05 bolded and in red.

Source	df	SS	MS	Estimated variance	% variance explained	Φ_{pt} value	P-value
Among Pops.	8	46.122	5.765	0.745	30%	0.298	**0.001**
Within Pops.	41	71.998	1.756	1.756	70%		

	Bear	Clark	Gull	Hudson	Pine	Portage	Round	Spring	Swan
Bear	- -	**0.008**	**0.006**	0.475	0.379	0.509	**0.001**	0.348	**0.006**
Clark	0.237	- -	**0.005**	0.231	0.235	0.252	**0.001**	**0.011**	**0.003**
Gull	0.211	0.261	- -	**0.023**	**0.047**	0.068	**0.001**	**0.014**	**0.003**
Hudson	0.000	0.075	0.299	- -	0.348	0.338	**0.022**	0.366	0.102
Pine	0.039	0.000	0.304	0.000	- -	0.661	**0.001**	0.306	**0.031**
Portage	0.000	0.059	0.304	0.000	0.000	- -	**0.036**	0.334	0.105
Round	0.528	0.355	0.564	0.543	0.591	0.557	- -	**0.002**	**0.002**
Spring	0.011	0.296	0.305	0.001	0.114	0.000	0.475	- -	**0.045**
Swan	0.263	0.364	0.461	0.247	0.406	0.363	0.611	0.240	- -

Summary

Survivorship of *M. aeruginosa* isolates from a suite of lakes throughout southern Michigan was positively related to total phosphorus content. Additionally, isolates from an oligotrophic lake exhibited greater survivorship in less rich media (lower phosphorus content) vs. more rich media.

Although overall isolate survivorship was relatively low (7% average), we found huge genetic diversity within and among populations of *M. aeruginosa*. Also, four populations contained sympatric clones that lacked or contained the microcystin toxin gene (*mcyA*).

Thus, lake managers should not assume that harmful algal bloom species are genetically homogenous. Instead managers should understand that algal populations maintain incredible levels of genetic diversity and this information should be considered when deciding on bloom prevention, identification, and mitigation plans.

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