



Heterotrophic Bacteria Dominate Catalase Expression during *Microcystis* Blooms

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ABSTRACT In the oligotrophic oceans, key autotrophs depend on “helper” bacteria to reduce oxidative stress from hydrogen peroxide (H_2O_2) in the extracellular environment. H_2O_2 is also a ubiquitous stressor in freshwaters, but the effects of H_2O_2 on autotrophs and their interactions with bacteria are less well understood in freshwaters. Naturally occurring H_2O_2 in freshwater systems is proposed to impact the proportion of microcystin-producing (toxic) and non-microcystin-producing (nontoxic) *Microcystis* in blooms, which influences toxin concentrations and human health impacts. However, how different strains of *Microcystis* respond to naturally occurring H_2O_2 concentrations and the microbes responsible for H_2O_2 decomposition in freshwater cyanobacterial blooms are unknown. To address these knowledge gaps, we used metagenomics and metatranscriptomics to track the presence and expression of genes for H_2O_2 decomposition by microbes during a cyanobacterial bloom in western Lake Erie in the summer of 2014. *katG* encodes the key enzyme for decomposing extracellular H_2O_2 but was absent in most *Microcystis* cells. *katG* transcript relative abundance was dominated by heterotrophic bacteria. In axenic *Microcystis* cultures, an H_2O_2 scavenger (pyruvate) significantly improved growth rates of one toxic strain while other toxic and nontoxic strains were unaffected. These results indicate that heterotrophic bacteria play a key role in H_2O_2 decomposition in *Microcystis* blooms and suggest that their activity may affect the fitness of some *Microcystis* strains and thus the strain composition of *Microcystis* blooms but not along a toxic versus nontoxic dichotomy.

IMPORTANCE Cyanobacterial harmful algal blooms (CHABs) threaten freshwater ecosystems globally through the production of toxins. Toxin production by cyanobacterial species and strains during CHABs varies widely over time and space, but the ecological drivers of the succession of toxin-producing species remain unclear. Hydrogen peroxide (H_2O_2) is ubiquitous in natural waters, inhibits microbial growth, and may determine the relative proportions of *Microcystis* strains during blooms. However, the mechanisms and organismal interactions involved in H_2O_2 decomposition are unexplored in CHABs. This study shows that some strains of bloom-forming freshwater cyanobacteria benefit from detoxification of H_2O_2 by associated heterotrophic bacteria, which may impact bloom development.

KEYWORDS CHABs, *Microcystis*, catalase, hydrogen peroxide, metagenomics, metatranscriptomics, peroxidase

Interactions between microorganisms are pervasive in aquatic ecosystems, with potentially far-reaching implications for system productivity, the cycling of nutrients, and the toxin production of harmful algal blooms (1). The importance of interactions between microorganisms is illustrated by the detoxification of hydrogen peroxide (H_2O_2). H_2O_2 is a ubiquitous oxidative stressor on microorganisms in natural waters

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(2–4) that can shape microbial community composition and function (5–7). In oligotrophic marine environments, dominant primary producers such as *Prochlorococcus*, heterotrophs such as *Pelagibacter* (SAR11), and nitrifiers such as ammonia-oxidizing *Thaumarchaeota* lack enzymatic defense against H_2O_2 and cannot grow in the presence of H_2O_2 concentrations typical of natural waters (concentrations between 200 nM and $\sim 1 \mu\text{M}$) unless associated with organisms that produce catalases to detoxify the H_2O_2 (7–12). Because H_2O_2 rapidly diffuses across membranes (13), decomposition of H_2O_2 by catalase and peroxidase enzymes produced by these heterotrophic “helpers” reduces environmental H_2O_2 concentrations to tolerable levels and provides community-wide protection to the sensitive “beneficiaries” (8, 10–12, 14). As long as some “helper” bacteria scavenge H_2O_2 and maintain low environmental H_2O_2 levels, adaptive loss of catalase genes in other microbes may proceed and allow for the conservation of resources in the nutrient-scarce open ocean (6, 14).

In eutrophic systems, H_2O_2 has been implicated as a potential factor influencing the toxin concentrations of cyanobacterial harmful algal blooms (CHABs) (15), which are a growing worldwide threat to freshwater ecosystems and associated ecosystem services, including drinking water supplies (16–18). Globally, freshwater CHABs are often dominated by *Microcystis* spp. that can produce microcystins, which are a class of potent liver toxins (19). Microcystins have been shown to increase the resistance of *Microcystis* to H_2O_2 by binding and protecting proteins from oxidative damage (20), leading to the hypothesis that transiently high concentrations of H_2O_2 in the water column may favor microcystin-producing (“toxic”) strains of *Microcystis* over non-microcystin-producing (“nontoxic”) strains (15). Through photochemical reduction of O_2 by chromophoric dissolved organic matter and aerobic microbial metabolisms, H_2O_2 often reaches concentrations in freshwaters (21–23) that can have negative effects on cyanobacterial growth (9, 10, 20, 24). The highest concentrations of H_2O_2 in Lake Erie CHABs precede bloom phases with high microcystin concentrations (25, 26), supporting the idea that high H_2O_2 concentrations favor the proliferation of toxic *Microcystis* strains. Some laboratory experiments in cell culture also support this hypothesis by showing that H_2O_2 disproportionately inhibits the growth of nontoxic *Microcystis* strains (27). However, a more recent study suggested the opposite, that microcystin interferes with H_2O_2 decomposition in *Microcystis* and H_2O_2 disproportionately inhibits growth of toxic *Microcystis* (28).

These contrasting conclusions on the impact of H_2O_2 on the physiology and abundance of *Microcystis* strains may be due to differences in H_2O_2 exposure of the cells between lab experiments or in the lab and field. For example, laboratory studies of *Microcystis* cell cultures generally have not measured natural H_2O_2 production in the growth medium (20, 27) from autoclaving (29) or irradiation (30) of medium components. Without such H_2O_2 quantification, the total H_2O_2 exposure is unknown, and thus the effect of H_2O_2 on the growth of *Microcystis* strains remains uncertain. In addition, most studies to date have measured the response of *Microcystis* to single, large doses of H_2O_2 that exceed natural concentrations by 3 to 6 orders of magnitude but are typical for doses of H_2O_2 used to control CHABs (28, 31–37). Thus, the responses of different *Microcystis* strains to naturally occurring H_2O_2 concentrations are unknown.

Despite the hypothesized role of H_2O_2 in influencing *Microcystis* population dynamics, the microbes responsible for H_2O_2 decomposition in eutrophic systems or during CHABs have not been studied. The distribution of catalase and peroxidase genes in *Microcystis* mirrors that of H_2O_2 -sensitive *Prochlorococcus* and ammonia-oxidizing archaea in that *Microcystis* lacks catalases and heme peroxidases but has multiple peroxiredoxin genes, suggesting that it may benefit from decomposition of natural H_2O_2 by other community members (35, 38). Such interactions could potentially improve the fitness of *Microcystis* strains that are more sensitive to H_2O_2 , thus influencing the relative proportions of *Microcystis* strains in the environment and, by extension, microcystin concentrations during blooms. Indeed, co-culture with other microorganisms can prevent high doses of H_2O_2 that are used for lake treatments

from inhibiting *Microcystis* growth (35, 39). However, there are currently no data showing the impacts of community-wide H_2O_2 detoxification on *Microcystis* growth at naturally occurring H_2O_2 concentrations.

Here, we address these questions: (i) What is the impact of naturally occurring H_2O_2 on the growth of various toxic and nontoxic *Microcystis* strains? (ii) Which genes encoding proteins that detoxify H_2O_2 are expressed in *Microcystis* bloom communities? (iii) Which community members express H_2O_2 detoxification genes? We analyzed the relative abundance of genes and transcripts encoding enzymes that are responsible for H_2O_2 decay in aquatic ecosystems (catalases and peroxidases) (40, 41) during the 2014 western Lake Erie *Microcystis* bloom, which led to a drinking water ban for the city of Toledo, OH (42). Our results show that while catalase transcripts are dominated by heterotrophic bacteria, only some cultured *Microcystis* strains benefit from an H_2O_2 scavenger, and the benefit was unrelated to the potential of the strain to synthesize microcystins. The results of this study suggest that the impact of catalase activity from heterotrophic bacteria on *Microcystis* growth varies between strains and thus may impact *in situ* *Microcystis* strain and species composition but not along a toxic versus nontoxic dichotomy.

RESULTS

Dynamics in pigments, microcystin concentrations, and H_2O_2 in western Lake Erie. The temporal trends in particulate microcystins and phycocyanin concentrations show that a large toxic *Microcystis* bloom in August was followed by a large nontoxic *Microcystis* bloom in the nearshore stations, while the offshore site was impacted only by the toxic *Microcystis* bloom in August (Fig. 1) (as previously reported in the work of Cory et al. [26] and Berry et al. [43]). Changes in concentrations of pigments and microcystin concentrations at three stations (two nearshore and one offshore [see Fig. S1 in the supplemental material]) during the 2014 western Lake Erie CHAB show that the cyanobacterial bloom more heavily impacted nearshore stations (WE2 and WE12) than the offshore station (WE4). Phycocyanin (a pigment found in cyanobacteria) and chlorophyll *a* concentrations were highly correlated at all stations, confirming that the blooms were dominated by cyanobacteria (43).

Concentrations of particulate microcystins correlated with phycocyanin concentrations in July through early August, with the highest microcystin concentrations occurring at the nearshore stations in early August (Fig. 1). Microcystin concentrations decreased substantially starting in mid-August and remained low, despite high cyanobacterial biomass and *Microcystis* relative abundance (43).

H_2O_2 ranged from 20 ± 36 to 590 ± 4 nM (standard error [SE] for technical replicates) in the surface waters of Lake Erie over the course of the bloom (25, 26). The highest concentrations of H_2O_2 were observed at each nearshore station in late July, 1 to 2 weeks prior to peaks in the concentrations of both microcystins and pigments. At nearshore station WE2, a smaller second peak in H_2O_2 (260 ± 51 nM) coincided with the smaller, second peak in microcystin concentrations in September. Peaks in H_2O_2 concentrations at offshore station WE4 were lower than those nearshore, reaching a maximum of 210 ± 94 nM (Fig. 1).

Catalase and peroxidase gene and transcript relative abundance. The relative abundances of the following genes were quantified in western Lake Erie metagenomes and metatranscriptomes: monofunctional heme catalase (*katE*), catalase-peroxidase (*katG*), manganese catalase (*katM*), cytochrome *c* peroxidase (*ccpA*), ascorbate peroxidase (APX), and alkyl hydroperoxide reductase subunit C (*ahpC*) (see Materials and Methods). These genes were targeted because there is biochemical evidence supporting that they scavenge H_2O_2 as a defense against H_2O_2 toxicity (44) or use H_2O_2 as a terminal electron acceptor, as in the case of *ccpA* (45). However, the cytochrome *c* peroxidases share many structural similarities with the methylamine-utilization protein *mauG*, and many of the genes identified as *ccpA* matched equally well with *mauG* genes in NCBI and paperBLAST (46). Therefore, the role of the genes identified as *ccpA* in H_2O_2 decomposition is uncertain, and we report their abundances separately in Fig. S2 as *ccpA-mauG*. We targeted APX because it can be abundant in marine metatranscriptomes (47). Of the proteins encoded by these genes, the

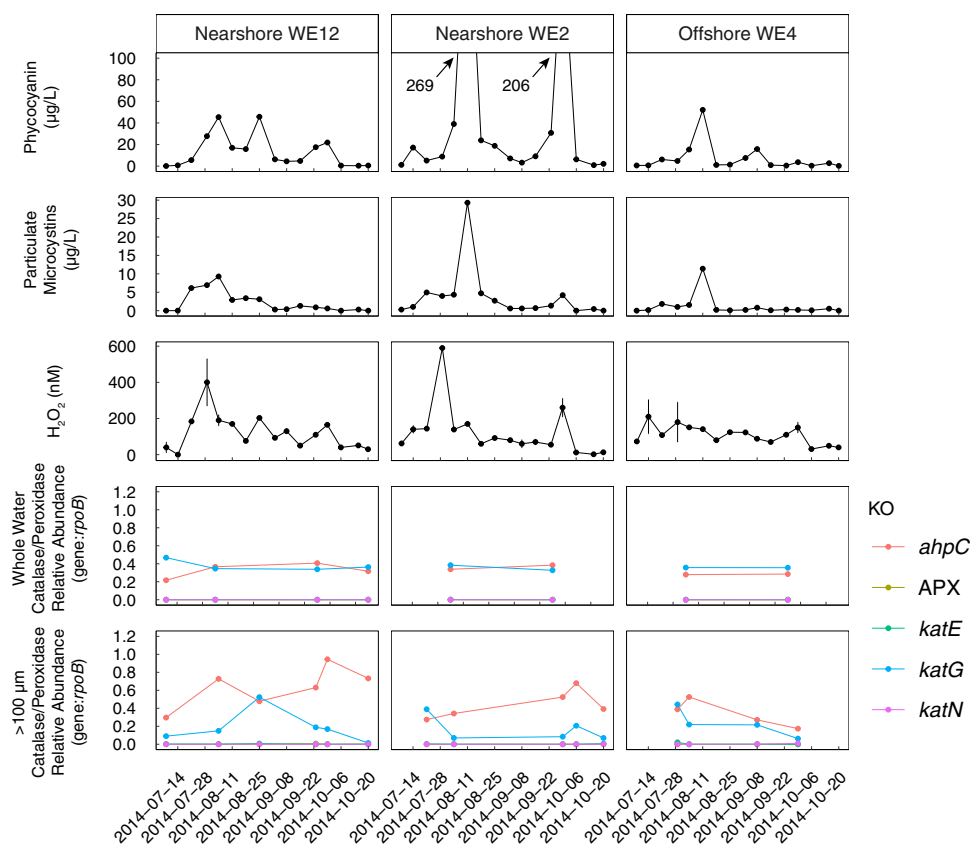


FIG 1 Temporal and spatial trends in the relative abundance of catalase and peroxidase genes in the 2014 western Lake Erie cyanobacterial bloom metagenomes are not correlated with concentrations of phycocyanin, particular microcystins, or H_2O_2 . Genes with KEGG orthology (KO) annotations for catalase-peroxidase (*katG*) and alkyl hydroperoxide reductase subunit C (*ahpC*) had the highest relative abundances. The relative abundance of catalase and peroxidase genes shown is the total of all genes with the specified KEGG orthology ID in metagenomes from the $>100\text{-}\mu\text{m}$ particle size fraction at each station. Read counts are normalized to the length of the matching gene in the database and to the number of reads that mapped to bacterial *rpoB*, encoding the beta subunit of RNA polymerases. Error bars on H_2O_2 concentrations show the standard error of technical replicate measurements ($n = 3$).

periplasmic KatG has the primary function to decompose extracellular H_2O_2 while the primary role of the cytoplasmic AhpC is to decompose intracellularly produced H_2O_2 and organic peroxides (13, 48–51). However, in some bacterial taxa, both KatG and AhpC can compensate for the loss of the other under certain physiological conditions (49–51).

Previous studies have suggested that plankton biomass captured in the $>100\text{-}\mu\text{m}$ particle size fraction is responsible for most of the microcystin production and contains 93 to 99% of the total *Microcystis* biomass during *Microcystis* blooms (52, 53). This particle size fraction is enriched in *Microcystis* and excludes microbes not physically attached to large *Microcystis* colonies and other particles $>100\text{ }\mu\text{m}$ in diameter. In contrast, whole water samples (filtered onto a $0.22\text{-}\mu\text{m}$ filter) contain all microbes present in the water column and thus represent the “total community.”

Genes encoding the catalase-peroxidase KatG and the peroxiredoxin AhpC had the highest abundance relative to the beta-subunit of bacterial RNA polymerase (*rpoB*) in western Lake Erie metagenomes (Fig. 1). *katG* was equally abundant in both the *Microcystis*-enriched and total community size fractions, while *ahpC* had a higher relative abundance in the *Microcystis*-enriched fraction than in the total community in most cases (Fig. 1). Neither *katG* nor *ahpC* gene relative abundances were significantly correlated with microcystins, pigment, or H_2O_2 concentrations in the lake ($P > 0.05$, F-test).

To focus on *Microcystis* and physically-associated bacteria, metatranscriptomes were obtained from the *Microcystis*-enriched fraction. *ahpC* transcripts had the highest

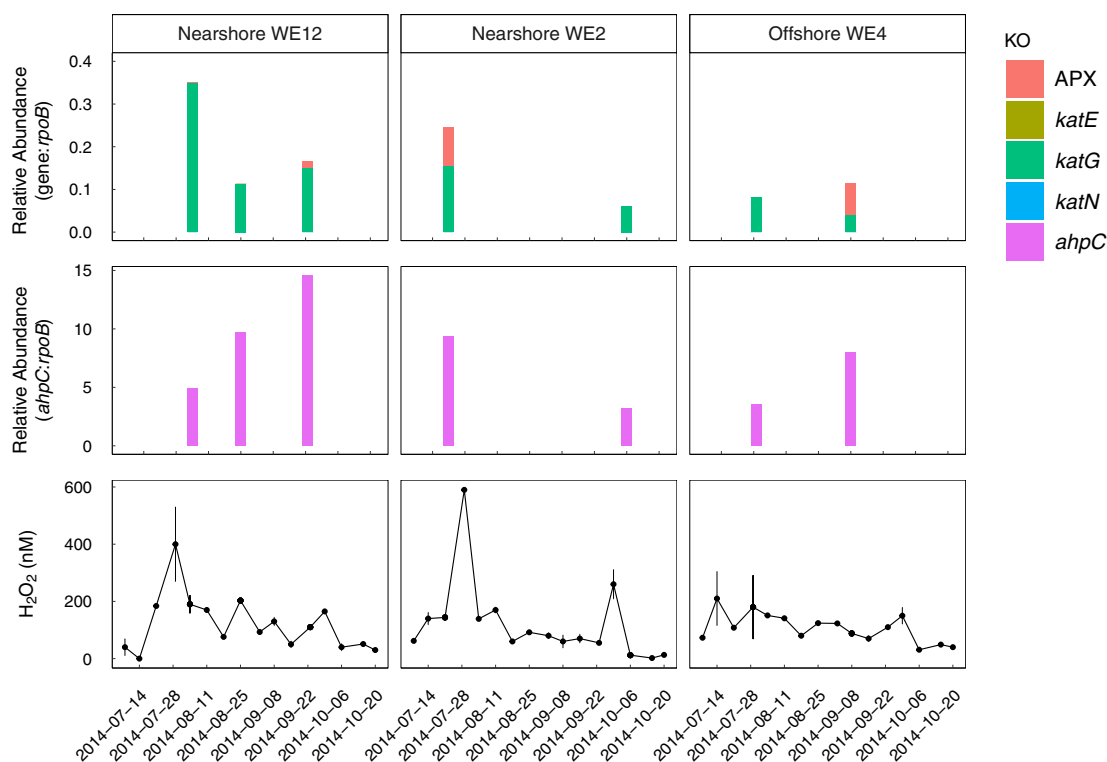


FIG 2 Temporal and spatial trends in the relative abundance of total *katG* and *ahpC* transcripts in particle- and phytoplankton-attached microbial communities show qualitative patterns with H_2O_2 concentration. The relative abundance of catalase and peroxidase genes shown is the total of all genes with the specified KEGG orthology (KO) ID in metatranscriptomes from the $>100\text{-}\mu\text{m}$ particle size fraction at each station. Read counts are normalized to the length of the matching gene in the database and to the number of reads that mapped to bacterial *rpoB*, encoding the beta subunit of RNA polymerases. Relative abundance of *ahpC* is shown separately in the middle panel because it was 2 orders of magnitude higher than the combined total of the other genes. Error bars on H_2O_2 concentrations show the standard error of technical replicate measurements ($n = 3$).

abundance relative to *rpoB*, followed by *katG* (Fig. 2). Transcripts encoding KatE were infrequently present and at low relative abundance, perhaps due to the low relative abundance of *katE* genes. Transcripts encoding CcpA-MauG were present at low relative abundance with no apparent patterns with any bloom parameters, except for one sample collected on 23 September at WE12, where the abundance of *ccpA-mauG* relative to bacterial *rpoB* was comparable to *katG* relative abundance (Fig. S2). No transcripts for KatN-encoding genes were found in the metatranscriptomes (Fig. 2). APX transcripts were rare (<0.01 relative to bacterial *rpoB*) or absent in most samples. No significant correlations were observed between the relative abundance of *ahpC* or *katG* transcripts and H_2O_2 , phycocyanin, or chlorophyll *a* concentrations throughout the field season ($P > 0.05$, F-test).

Taxonomy of metagenome-assembled genomes that have and express *katG*.

The taxonomy of metagenome-assembled genomes (MAGs; see supplemental files 1 and 2 for MAG metadata) that contained catalase and peroxidase genes were identified to ascertain which organisms were responsible for H_2O_2 decomposition in the 2014 western Lake Erie cyanobacterial bloom (Table S1). In all metagenomes of the *Microcystis*-enriched fraction, reads from diverse groups of bacteria contributed substantially to the total *katG* read abundance (Fig. 3). Of the genes that could be assigned a taxonomy in the *Microcystis*-enriched fraction, *katG* from *Microcystis*, *Phenylobacterium* (Alphaproteobacteria), *Burkholderiaceae* bin 53603 E6 (Betaproteobacteria), *Rhodobacteraceae* bin 49625 E6 (Alphaproteobacteria), and *Flammeovirgaceae* bins 42896 C11 and 42896 E20 (*Bacteroidetes*) were most abundant in the *Microcystis*-enriched fraction (Fig. 3). *Microcystis katG* comprised 0 to 4.71% (mean $1.0\% \pm 1.4\%$, standard deviation) of the total *katG* reads in the *Microcystis*-enriched metagenomes (Fig. 3). *katG* was

Taxonomic Assignment

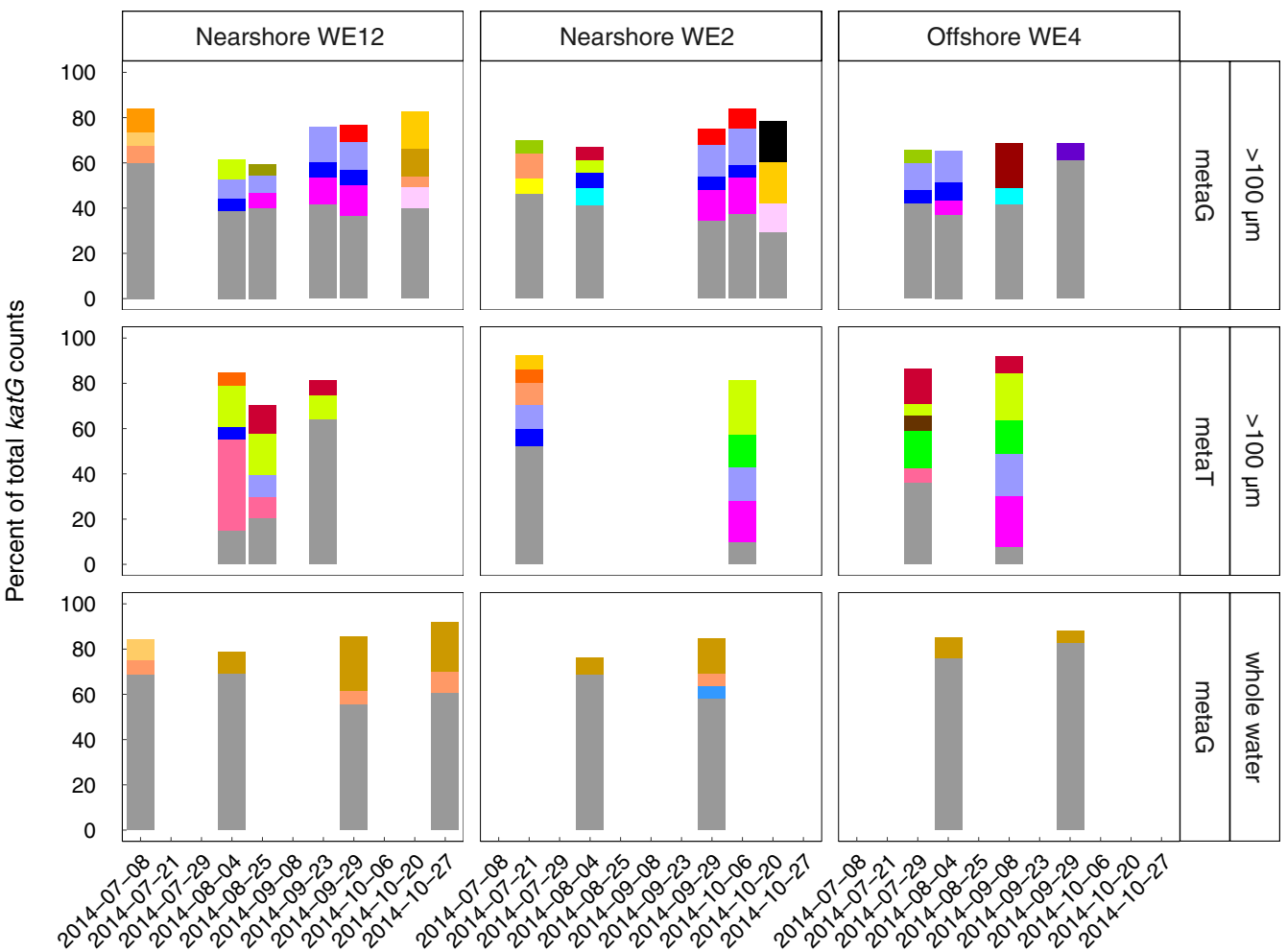
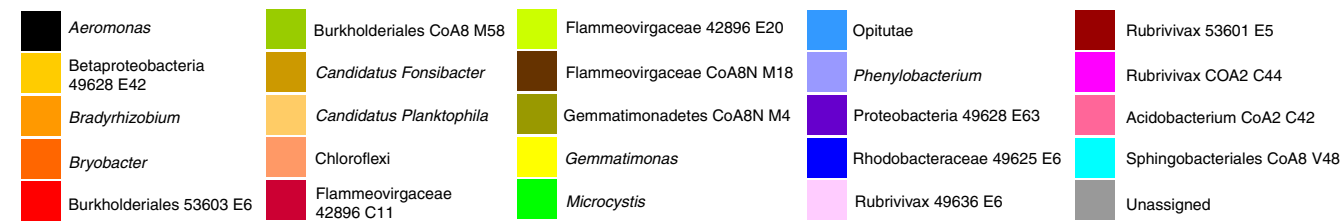


FIG 3 Genes and transcripts encoding *KatG* in the 2014 western Lake Erie cyanobacterial bloom were from a diverse community of microbes, and *katG* from *Microcystis* did not dominate total *katG* reads. *katG* reads were summed by taxonomic assignment. For each sample, only taxa that had percent abundances higher than 5% of the total *katG* reads in the given sample are shown; the remaining white space is made up of taxa with *katG* that had a percent abundance below 5%. Dates without bars indicate that no data are present for the given combination of date and station. metaG, metagenome samples; metaT, metatranscriptome samples.

also detected in *Cyanobium-Synechococcus* group MAGs, an abundant cyanobacterial group in the bloom (43), but *Cyanobium-Synechococcus* group *katG* was only occasionally present at low relative abundance (Fig. 3).

The dominant organisms with *katG* in the whole community were distinct from those in the *Microcystis*-enriched fraction (Fig. 3). In the whole community, the sum of *katG* genes that could not be assigned a taxonomy was most abundant. Of the genes that could be assigned taxonomy, *katG* from "*Candidatus Fonsibacter*" (*Alphaproteobacteria*) and unclassified *Chloroflexi* had the highest abundance consistently throughout the bloom (Fig. 3), but they were largely absent in the *Microcystis*-enriched fraction. Similarly, the most abundant

taxa containing *katG* in the *Microcystis*-enriched fraction were rare in the total community fraction, with *Microcystis* accounting for only 0 to 0.06% of the total *katG* in the total community metagenomes (mean $0.017\% \pm 0.022\%$ [Fig. 3]).

Noncyanobacteria dominated *katG* transcripts in all samples (Fig. 3). *Microcystis katG* transcripts comprised only 0 to 17% (mean for nonzero samples was $10\% \pm 8\%$ [standard deviation]) of the total *katG* reads despite being abundant in the metagenome and accounted for only 0.8% of *katG* reads during peak *katG* relative transcript abundance. *katG* from an uncultured *Flammeovirgaceae* member (bin 42896 E20, *Bacteroidetes*) was a significant portion of total *katG* read counts during peak *katG* relative abundance (18%, 4 August at WE12 [Fig. S3]) and was a major proportion of total *katG* transcripts for all dates, with the exception of 21 July at nearshore station WE2 (Fig. 3).

Highly abundant *katG* transcripts were often from organisms present at low relative abundance in the metagenomic reads (Fig. S3). The most striking example of this result was observed on 4 August at WE12, when total *katG* transcript relative abundance was the highest; *katG* from *Acidobacteria* bin CoA2 C42 and *Bryobacter* (*Acidobacteria*) comprised $\sim 40\%$ and $\sim 6\%$ of the total *katG* RNA reads, respectively (Fig. S3). *Bryobacter* and *Acidobacteria* bin CoA2 C42 comprised a lower proportion of the total *katG* transcripts in other samples, but the relative abundance of transcripts from both organisms was consistently enriched relative to gene abundance when present.

The ratio of *Microcystis katG* to *Microcystis* housekeeping genes (estimated by dividing *Microcystis katG* reads by *Microcystis rpoB* reads) ranged from 0 to 2.6% (mean $0.5\% \pm 0.7\%$, standard deviation), suggesting that most *Microcystis* cells in the bloom lacked *katG*. *Microcystis katG/rpoB* ratios tended to be higher in the early phases of the bloom (Fig. 4A). The abundance of *Microcystis katG* transcripts relative to *Microcystis rpoB* transcripts did not show any patterns with H_2O_2 concentration (Fig. 4B).

Taxonomy of metagenome-assembled genomes that have and express *ahpC*.

In contrast to *katG*, *ahpC* from *Microcystis* was the most abundant of the *ahpC* genes in the *Microcystis*-enriched fraction, and *Microcystis* accounted for a larger proportion of the total *ahpC* reads (2 to 82%, mean $45\% \pm 28\%$, standard deviation; Fig. 5) than for total *katG* reads. In contrast to the *Microcystis*-enriched fraction, no single organism dominated *ahpC* reads in the total community (Fig. 5). In the *Microcystis*-enriched fraction, *Microcystis* dominated the *ahpC* transcript pool in all but 2 metatranscriptomes collected on 23 September 2014 at nearshore station WE12 and 21 July 2014 at nearshore station WE2 (Fig. 5).

Catalase and peroxidase genes in published cyanobacterial genomes. To confirm the presence or absence of catalases in dominant bloom-forming cyanobacteria, we surveyed publicly available genomes of cyanobacteria that are regular members of cyanobacterial blooms in Lake Erie, including the dominant potential microcystin producers, *Microcystis* and *Planktothrix* (43, 54–56) (Fig. S4; also see Materials and Methods). We also compared the distribution of catalases and peroxidases in these genomes to that of *Prochlorococcus*, a dominant cyanobacterium in the open ocean that has been shown to be dependent on other community members for H_2O_2 detoxification (9).

In total, 343 public genomes were analyzed, and 99% of the genomes had an *ahpC* gene. Nearly all 150 published *Microcystis* genomes lack genes encoding catalases, except for four genomes that have a *katG* gene. Similarly, catalase genes were absent in all published *Prochlorococcus* genomes, consistent with previous genomic studies and the low tolerance of H_2O_2 by this genus (10, 57). In contrast, 57% of *Pseudanabaena* genomes possess catalases, while 43% of *Anabaena-Dolichospermum* genomes contain manganese catalases. All the *Anabaena-Dolichospermum* genomes lack heme catalases. A majority of *Synechococcus* genomes have either or both *katE* and *katG*, consistent with previous observations of the relatively high H_2O_2 tolerance of this genus as well as previous intergenomic comparisons (9, 57).

Axenic culturing experiments. To assess how bacterial cross-protection from natural H_2O_2 concentrations impacts the growth of toxic and nontoxic *Microcystis* strains, we grew axenic *Microcystis* cultures for 12 to 13 days in the presence and absence of sodium pyruvate, a rapid scavenger of H_2O_2 that is used to remove H_2O_2 in cell cultures

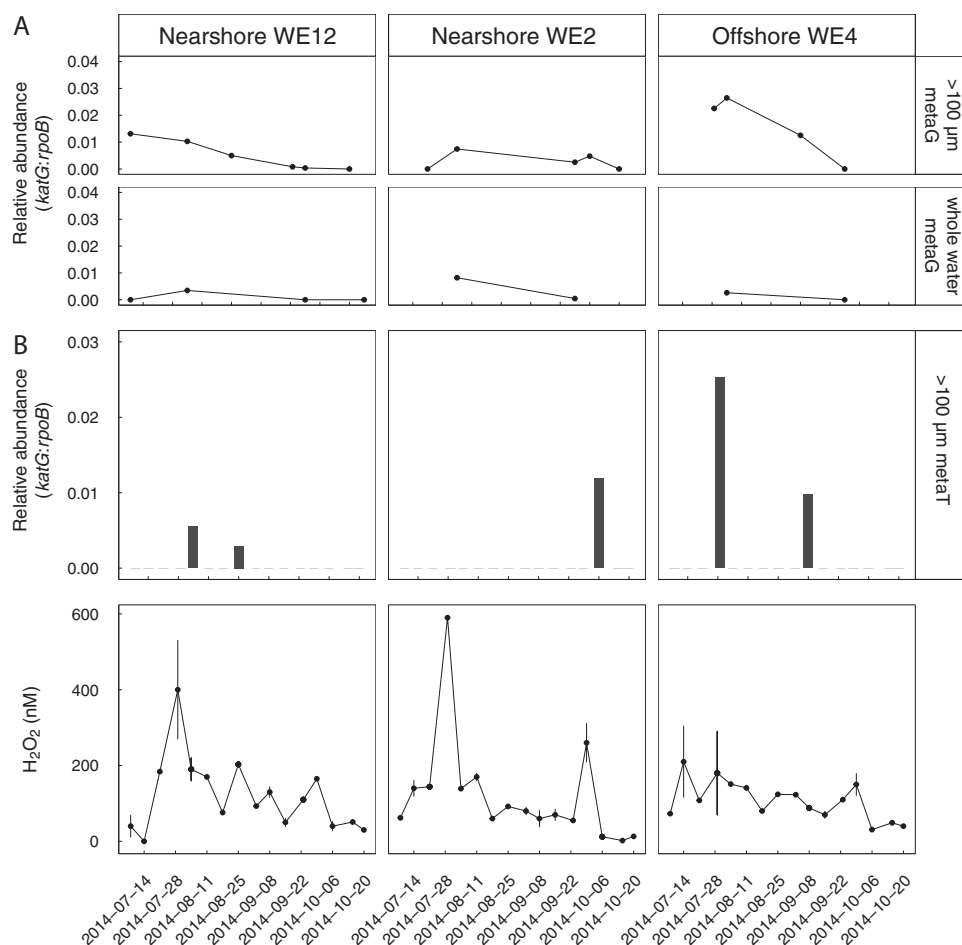


FIG 4 Spatial and temporal variation in *Microcystis* *katG* abundance relative to *Microcystis* *rpoB* in metagenomes and metatranscriptomes from the 2014 western Lake Erie cyanobacterial bloom. (A) Low and decreasing *katG*/*rpoB* ratios with time suggest that a minority of *Microcystis* genomes in the 2014 bloom have *katG* and fewer *Microcystis* genomes have *katG* in the late summer and fall. (B) There are no quantitative or qualitative relationships between the relative abundance of *Microcystis* *katG* transcripts and H_2O_2 concentrations.

(8, 24, 29, 58, 59). In unamended *Microcystis* cultures, H_2O_2 concentrations at the start of the experiment ranged from 910 to 1,330 nM (mean $1,000 \pm 148$ nM, 95% confidence interval; Fig. 6A). In four strains, H_2O_2 concentrations declined to 170 ± 40 nM. The other three strains apparently degraded H_2O_2 less efficiently, and H_2O_2 concentrations in these cultures declined to 370 ± 70 nM. In the pyruvate-treated cultures, H_2O_2 concentrations could not be quantified because pyruvate degraded the H_2O_2 standard added to the samples during standard addition by 10 to 25% during the flow injection analysis (Fig. S5; see also Materials and Methods). However, the rapid decay of the standard suggests that H_2O_2 concentrations were maintained at very low levels in the pyruvate treatments.

Although there were slight increases in the cell densities of four strains when cultured with sodium pyruvate, final cell densities at the end of the experiment were not significantly different in most strains when cultured with pyruvate ($P > 0.05$, Welch's *t* test; Fig. 6A). One strain (strain NIES-843) had significantly lower mean final cell densities when cultured with sodium pyruvate ($P = 0.01$, Welch's *t* test; Fig. 6A). Only one strain (PCC 7806) had a significant improvement in maximum specific growth rate (μ_{max}) when cultured with sodium pyruvate, while the remaining strains showed no differences in maximum specific growth rate ($P = 0.05$, Welch's *t* test; Fig. 6B). Neither *Microcystis* strain toxigenicity (determined by the presence or absence of the *mcy* gene cluster in the genome) nor the presence of catalase and peroxidase genes in the

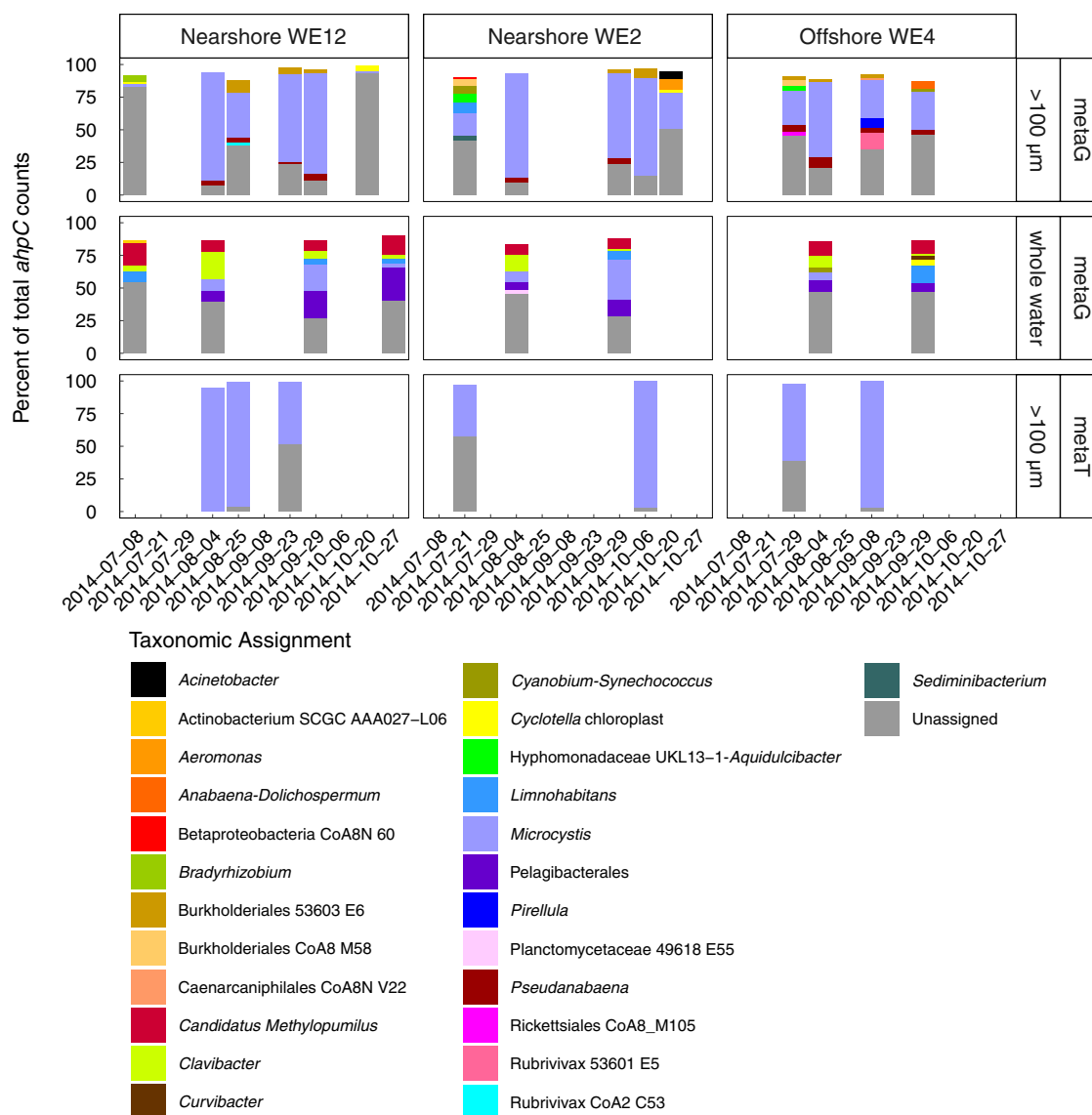


FIG 5 *Microcystis ahpC* was abundant in most particle- and phytoplankton-associated metagenomic samples and dominated particle- and phytoplankton-associated *ahpC* transcripts in the 2014 western Lake Erie cyanobacterial bloom, but *Microcystis ahpC* was less abundant in whole water metagenome samples. *ahpC* reads were summed by taxonomic assignment. For each sample, only taxa that had percent abundances higher than 2% of the total *ahpC* reads in the given sample are shown; the remaining white space is made up of taxa with *ahpC* that had a percent abundance below 2%. Dates without bars indicate that no data are present for the given combination of date and station. metaG, metagenome samples; metaT, metatranscriptome samples.

genome predicted whether a strain benefited from addition of sodium pyruvate to the growth medium (Fig. 6B).

In sterile BG-11 2N medium, H_2O_2 concentrations showed complex dynamics, decreasing to 580 ± 50 nM within 2 days and subsequently increasing to $1,700 \pm 100$ nM (Fig. 6C). Therefore, declines in H_2O_2 below ~ 580 nM were attributed to the presence of *Microcystis* cells. The H_2O_2 concentrations observed in sterile BG-11 2N medium required both citrate and transition metals present in the medium (Fig. S6).

DISCUSSION

The presence of microcystin biosynthesis genes does not predict the response of a *Microcystis* strain to an exogenous H_2O_2 scavenger. Variations in H_2O_2 concentrations have been hypothesized to impact the relative proportions of toxic and non-toxic *Microcystis* strains in the environment (15). However, there is conflicting evidence on whether the growth of toxic *Microcystis* is either more (20, 27) or less (28) favored

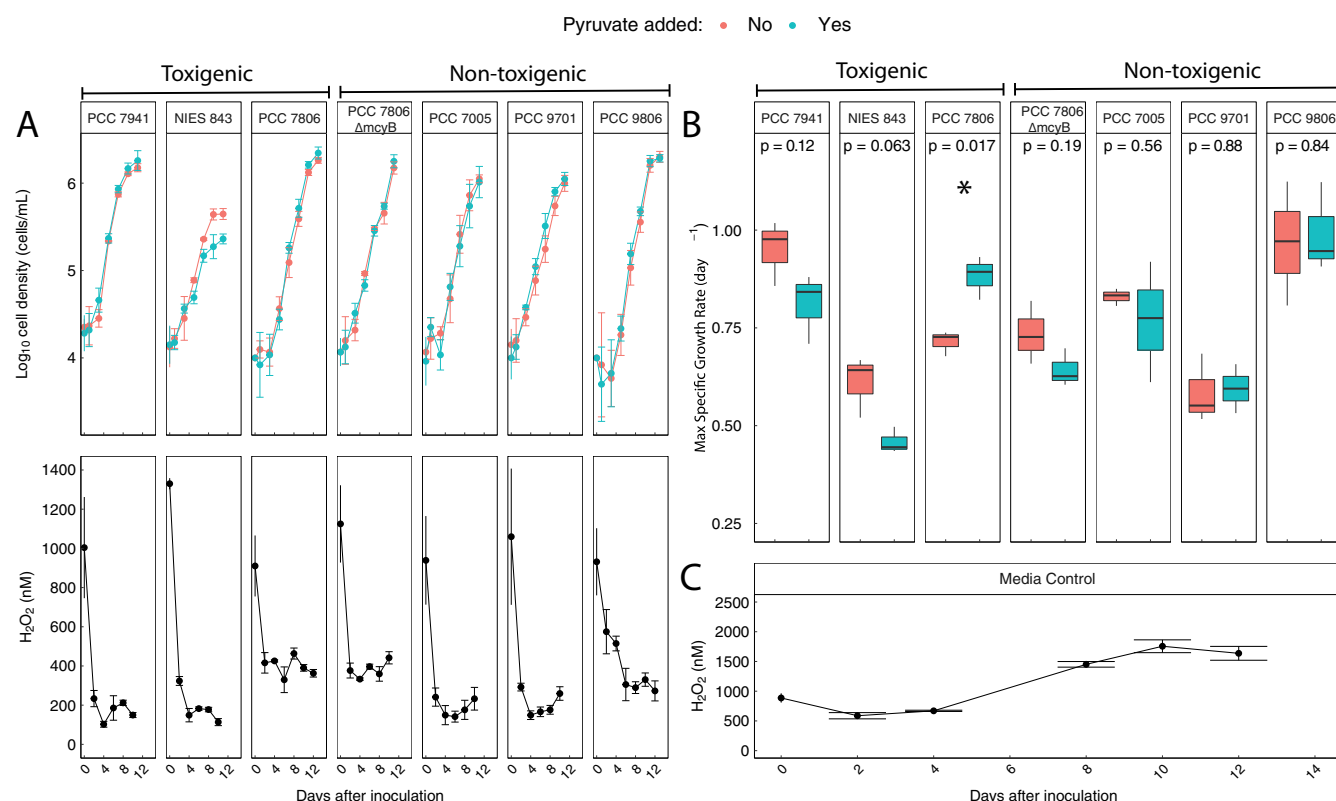


FIG 6 (A) Small differences in cell density between pyruvate and control treatments and the decomposition of initially high, but environmentally relevant, H₂O₂ concentrations suggest that *Microcystis* does not require help degrading H₂O₂ under the experimental conditions despite lacking *katG*. Error bars depict 95% confidence intervals of biological triplicates. (B) Some *Microcystis* strains had significant ($P < 0.05$) improvements in maximum growth rates when cultured with pyruvate, suggesting a benefit from receiving help with H₂O₂ decomposition. Strain toxigenicity is not an indicator of whether a strain benefits from help decomposing H₂O₂. Significance was determined with Welch's two-sided *t* test. (C) In sterile growth medium, H₂O₂ concentrations increase over time rather than decrease. Error bars depict 95% confidence intervals of three technical replicates.

by H₂O₂ than that of nontoxic *Microcystis*. The *Microcystis* strains tested here (3 naturally toxic, 3 naturally nontoxic, and 1 nontoxic mutant) showed different impacts of the H₂O₂ scavenger pyruvate on growth. The maximum growth rate of most strains was not significantly impacted by pyruvate (Fig. 6B), which suggests no fitness benefit from the removal of H₂O₂ from the growth medium. However, strain PCC 7806 (toxic) showed a significantly higher maximum growth rate when cultured with pyruvate (Fig. 6B; $P < 0.05$), suggesting that H₂O₂ in the growth medium reduced growth rates of this strain despite its ability to decompose H₂O₂ (Fig. 6A). While the H₂O₂ scavenger had little impact on the carrying capacity of the culture (Fig. 6A), the reduced growth rate may be important in determining the outcome of competition with other *Microcystis* strains and phytoplankton taxa in natural communities.

It is possible that the breakdown products of the reaction between pyruvate and H₂O₂ (acetate and carbon dioxide) account for the differences in the maximum growth rates measured for strain PCC 7806. However, previous studies that used sodium pyruvate as an H₂O₂ scavenger found no evidence that uptake of the reaction products significantly impacts growth or the amount of carbon fixation in other autotrophic organisms (8). Furthermore, the finding that H₂O₂ reduces PCC 7806 maximum growth rate is consistent with other laboratory culture experiments with this strain that showed growth inhibition of PCC 7806 at higher H₂O₂ concentrations than those used in this study (20, 27, 28, 60).

In contrast to the wild-type strain, the mutant strain of PCC 7806, incapable of microcystin biosynthesis (61), did not display any significantly different growth rates with pyruvate treatment (Fig. 6B), indicating that the environmentally relevant concentrations of H₂O₂ in the medium had no detectable effect on growth of the mutant. The observation that H₂O₂ exposure impacted wild-type growth rates but not microcystin

knockout mutant growth rates conflicts with the hypothesized protection from H_2O_2 provided by microcystins and with previous results showing higher growth impairments of H_2O_2 additions to the mutant strain than of those to the wild type (20, 27) but is consistent with other experiments that used single, highly concentrated H_2O_2 additions (28). However, the two strains degraded environmentally relevant H_2O_2 concentrations similarly (Fig. 6A), which does not support the idea that production of microcystins directly impairs the ability of *Microcystis* to degrade H_2O_2 at environmentally relevant concentrations (28).

When grown with the H_2O_2 scavenger, wild-type PCC 7806 had significantly higher maximum growth rates than the mutant strain (Fig. 6B; $P < 0.05$, Welch's two-sided t test). The lower growth rate of the mutant is unrelated to extracellular H_2O_2 exposure but may be related to its altered metabolome (62, 63), proteome content (64), assembly and subcellular localization of RubisCO (63), or expression of peroxiredoxins and other genes involved in the oxidative stress response (28) compared to the wild type. Such metabolic differences between the wild-type and mutant PCC 7806 may also explain how the two strains differ in whether they benefit from an exogenous H_2O_2 scavenger despite having similar growth rates when grown without the H_2O_2 scavenger.

Traits other than the production of microcystins, catalases, and peroxidases must contribute to the overall impact of H_2O_2 on the growth of a given *Microcystis* strain. All of the tested strains have *ahpC* (38), lack *katG* (38), and degrade H_2O_2 in the growth medium (Fig. 6A). Thus, the presence or absence of catalases and peroxidases alone does not determine whether a given strain benefits from help decomposing H_2O_2 . While the cell quotas of microcystins vary among *Microcystis* strains and growth conditions (65–67), microcystins are constitutively produced in a growth-rate-dependent manner (66–68). Therefore, our results cannot be explained by a lack of microcystins in strains containing microcystin biosynthetic genes.

Several other mechanisms may explain the differences in the response of the *Microcystis* strains to H_2O_2 . Differences between *Microcystis* strains in exopolysaccharide composition and production (69), which may degrade H_2O_2 (37) and influence H_2O_2 permeability (12), could modulate the effect of H_2O_2 on growth rates. Strain-dependent susceptibility of cellular components to H_2O_2 damage could also play a role. For example, the proteome varies between *Microcystis* strains (70), and H_2O_2 damage is specific to certain protein structures.

Some *Microcystis* strains benefitted from receiving help with H_2O_2 decomposition despite their ability to degrade and tolerate H_2O_2 alone. All the *Microcystis* strains tested here could grow to high cell density despite initial H_2O_2 concentrations in the culture representative of some of the highest concentrations detected in Lake Erie (Fig. 1 and 6A; see also reference 26). H_2O_2 decomposition by *Microcystis* has been detected previously (28) and is presumably from *ahpC*. Others have reported that *ahpC* can compensate for the loss of *katG* in some bacteria at the submicromolar concentrations of H_2O_2 typically observed in natural waters (48–51) and in our experiments (Fig. 6A). These results support that AhpC protein can scavenge extracellular H_2O_2 for some organisms and suggest that *Microcystis* can lower environmental H_2O_2 to some extent even without *katG*. Thus, the absence of *katG* may not indicate dependence on helper bacteria as predicted by previous results from *Prochlorococcus* (9, 10).

The higher maximum growth rates of strain PCC 7806 when cultured with sodium pyruvate occurred despite its ability to degrade H_2O_2 in the growth medium (Fig. 6A). Growth impairment from H_2O_2 despite its decomposition has also been observed in other *Microcystis* strains (34, 39) and may indicate that a portion of H_2O_2 decay comes from reactions that damage cellular structures or that there is a fitness cost to H_2O_2 decomposition in some *Microcystis* strains. Indeed, AhpC protein activity requires NADH and thioredoxin or glutathione as electron donors (44, 51, 71, 72). Thus, H_2O_2 decomposition in *Microcystis* may impart a cost to growth under conditions under which electron donor availability is reduced. For example, the form and concentration of nitrogen in the environment change the intracellular concentrations of NAD(P)H in *Microcystis* (73), which may impact H_2O_2 decomposition by AhpC. Additionally, if the environmental conditions that cause electron donor limitation vary

between *Microcystis* strains, electron donor availability could be another factor that determines differences in the impact of H_2O_2 on the growth of different *Microcystis* strains.

Sources of H_2O_2 in BG-11 2N medium and caveats concerning H_2O_2 exposure.

H_2O_2 production in sterile BG-11 2N medium is likely from visible-light reactions involving trace metal complexes rather than reactions involving UV light and dissolved organic matter as expected in natural waters (see the supplemental material). In lake systems with photochemical H_2O_2 production and rapid biological decay of H_2O_2 , the total H_2O_2 exposure of *Microcystis* spp. may be higher than in these cultivation experiments. For example, if a high H_2O_2 production rate countered by a high rate of decomposition by other bacteria keeps H_2O_2 concentrations within a tolerable range, the *Microcystis* populations may be more dependent on other bacteria for H_2O_2 decomposition than shown in our lab experiments. The total H_2O_2 concentrations during the growth phase of our cultures (Fig. 6A) do not provide information on the absolute rates of H_2O_2 production or decay during the experiment. Thus, we cannot rule out that natural H_2O_2 production may inhibit growth of the *Microcystis* strains tested here more broadly or severely than shown by our lab experiments.

Other biological factors complicate extrapolation of the experiment results to natural waters. First, the impact of natural H_2O_2 concentrations on *Microcystis* growth and physiology likely depends on light intensity and wavelength (31, 60, 74), nutrient availability (75), and temperature (assuming that *Microcystis* responds to the interactive effect between temperature and H_2O_2 in the same manner as *Prochlorococcus* [24]). Second, the *Microcystis* strains included in our experiments may not be representative of abundant strains found in western Lake Erie, in part due to inhibition of strains sensitive to H_2O_2 present in culturing media (29, 30), which exerts a strong bias on the cultivation of bacteria from the environment (76), including some cyanobacterial strains (9, 10).

The relative contribution of attached bacteria to H_2O_2 decomposition in phytoplankton assemblages. Metatranscriptomics revealed that *Microcystis katG* comprised very few total *katG* transcripts (<1% of total transcripts during peak relative abundance [Fig. 3; see also Fig. S3 in the supplemental material]) in the *Microcystis*-enriched fraction, and a small fraction of *Microcystis* cells in Lake Erie had *katG* (Fig. 4). The most highly expressed *katG* genes in the *Microcystis*-enriched fraction were from heterotrophic bacteria, suggesting that they may be responsible for most of the H_2O_2 degradation in phytoplankton assemblages and thus provide protection from H_2O_2 to other community members. However, some actinobacteria have catalases with low specific activity and catalytic efficiency (k_{cat}/K_m) and still require helpers in order to tolerate naturally relevant H_2O_2 concentrations (60, 77). Thus, the detection of *katG* transcripts may not necessarily be indicative of efficient H_2O_2 decomposition in some bacteria. Given that the organisms responsible for the majority of *katG* expression are uncultured, no axenic isolates of *Microcystis* with *katG* are currently available, and *ahpC* can compensate for *katG* at the H_2O_2 concentrations measured in Lake Erie, it is unclear how *katG* expression of these organisms relates to rates of H_2O_2 decomposition in the environment. However, *Microcystis* colonies are encapsulated by exopolysaccharide slimes (37, 69, 78) that can scavenge H_2O_2 (37) and are colonized by heterotrophic bacteria (79–81). Thus, bacterial cells attached to the colony perimeter are likely exposed to more exogenous H_2O_2 than *Microcystis* cells in the colony interior, perhaps making them larger sinks for exogenous H_2O_2 than *Microcystis* cells in the colony interior.

Conclusions. This study provides evidence that questions the current thinking on the effect of H_2O_2 on *Microcystis* physiology and the relative abundance of *Microcystis* strains in natural waters. The results do not support the hypothesized protection from H_2O_2 provided by microcystins (20, 27) nor the idea that microcystins directly impair the ability of *Microcystis* to degrade H_2O_2 in the environment (28). Every *Microcystis* strain tested was able to tolerate H_2O_2 concentrations within the range of some of the highest concentrations observed in Lake Erie blooms (Fig. 1) without *katG*. Such tolerance of H_2O_2 by *Microcystis* is different from H_2O_2 -sensitive *Prochlorococcus* (9, 10) and ammonia-oxidizing archaea (8, 12) that lack catalases and heme peroxidases. Together, the results suggest that (i) the presence of catalase and peroxidase genes in a genome alone is insufficient for determining the susceptibility of an organism to growth

inhibition from H_2O_2 in natural systems and (ii) changes in H_2O_2 concentrations in freshwater lakes due to natural biological and photochemical processes do not favor certain *Microcystis* strains based solely on their ability to produce microcystins.

Although the results suggest that *Microcystis* spp. are not fully dependent on “helper” bacteria to grow in the H_2O_2 concentrations observed in freshwater blooms, *Microcystis* growth rates may be impacted by rates of community-wide H_2O_2 production and decomposition. Natural variation in H_2O_2 concentrations in freshwater lakes (26) may have differential impacts on growth rates of *Microcystis* strains, which have diverse ecophysiological traits (38). Understanding the diversity of these *Microcystis* strains and how their response to H_2O_2 is linked with other traits and with the environment may improve our understanding of the widely observed seasonal transitions from high to low microcystin concentrations that are underpinned by successions in toxic and nontoxic *Microcystis* strains (82–84).

MATERIALS AND METHODS

Field sampling and water chemistry. Weekly sampling was conducted at three locations in the western basin of Lake Erie in conjunction with the NOAA Great Lakes Environmental Research Laboratory long-term harmful algal bloom (HAB) monitoring program. These sites correspond to NOAA stations WE2, WE12, and WE4 (see Fig. S1 in the supplemental material). The average depth at nearshore stations WE2 and WE12 is 4.8 ± 0.1 m and 5.9 ± 0.1 m, respectively, while the average depth at offshore station WE4 is 7.8 ± 0.1 m. Water chemistry measurements (pH, conductivity, and nutrients) are detailed elsewhere (43). At all stations, pH ranged from 7.80 to 9.29 (mean = 8.55 ± 0.06), conductivity ranged from 175.5 to 314.6 $\mu\text{S}/\text{cm}$ (mean = 250.6 ± 5.2 $\mu\text{S}/\text{cm}$), and water temperature ranged from 25.0°C (mid-August) to 12.5°C (mid-October) with a mean of 20.6 ± 0.6 °C from June to October. Total N as nitrite/nitrate and ammonium ranged from 0 to 1.1 mg/L and 0.1 to 36.4 $\mu\text{g}/\text{L}$ while soluble reactive phosphorus and total phosphorus ranged from 0.04 to 21.2 and 9.9 to 132.7 $\mu\text{g}/\text{L}$, respectively. These values are typical of Lake Erie (82, 85) and other eutrophic lakes impacted by CHABs (86, 87). Water quality data were collected by the NOAA Great Lakes Research Lab and are available under NCEI accession no. 0187718.

In situ hyperspectral light profiles were collected at each sampling site using a Sea-Bird HyperPro II (Sea-Bird Scientific, Philomath, OR) profiler equipped with up- and down-facing HyperOCR radiometers measuring wavelengths 348 to 801 nm (bin size = 3.3 nm), plus an identical fixed-surface radiometer to record sky conditions. The profiler was deployed on the sunny side of the vessel and allowed to free-fall through the water column to avoid the vessel shadow. A duplicate cast was collected as soon as the profiler was returned to the surface to capture identical light conditions. Profiles were collected after the vessel had been drifting without power for several minutes, so the water column through which the profiler traveled had as little artificial disturbance as possible. Light profile data were processed using ProSoft (Sea-Bird Scientific, Philomath, OR). Output spectral light intensities in power units (W/m^2) were converted to photon flux units ($\mu\text{mol photons}/\text{m}^2/\text{s}$) by dividing by the photon energy, which was calculated using the following equation: $E_{\text{ph}} = hc/\lambda$, where h is Planck's constant (6.626×10^{-34} J/s), c is the speed of light (per m), and λ is the wavelength of light in meters. Total photon flux was calculated as the sum of the spectral photon fluxes from a single Sea-Bird cast.

Microbial samples were obtained from a 20-L depth-integrated water sample collected from the surface to 1 m above the lake bottom. To collect the whole community fraction, 150 mL of depth-integrated sample was filtered onto a 47-mm Millipore Express Plus membrane with a 0.22- μm pore size, placed into 2-mL cryovials with 1 mL RNAlater, and kept on ice during cruise transit. To collect the colony-associated fraction, 2 L of depth-integrated sample was filtered through a 100- μm -pore-size mesh, and the retentate was backwashed into a Falcon tube using altered BG-11 medium (Table S2). RNAlater was added in a 2:1 ratio with the backwash. The backwash was filtered onto a 1.6- μm -pore-size glass fiber filter with a syringe. After filtration, all filters were placed into 2-mL cryovials with 1 mL of RNAlater and kept on ice during cruise transit. Upon arrival at the lab, all filters were frozen at -80°C until extraction. H_2O_2 , chlorophyll, and microcystin measurements were conducted as described in reference 26, and phycocyanin measurements were conducted as described in reference 43.

DNA and RNA extraction and sequencing. Filters with collected biomass were thawed, folded with biomass facing inward, and rinsed with sterile phosphate-buffered saline (PBS) to remove RNAlater preservative. Filters were pretreated following the Qiagen DNeasy protocol for Gram-negative bacteria. Filters were incubated in 100 μL Qiagen ATL tissue lysis buffer, 300 μL Qiagen AL lysis buffer, and 30 μL proteinase K for 1 h at 56°C on a rotisserie (Qiagen, Hilden, Germany). Cells were further lysed by vortexing in this lysis buffer for 10 min. Addition of lysozyme for Gram-positive bacteria was omitted because previous studies showed that lysozyme addition had variable impacts on DNA yields, often reducing the DNA yield, with minimal impacts on bacterial community composition (88). However, the omission of lysozyme could have biased the extraction against Gram-positive bacteria.

The resulting lysates were homogenized using a QIAshredder column, and DNA was purified from the filtrate using the Qiagen DNeasy blood and tissue kit according to the manufacturer's standard protocol. The quantity and quality of DNA in each sample were determined using a NanoDrop Lite spectrophotometer (Thermo Scientific). DNA extracts were frozen at -80°C until analysis.

For RNA extraction, the filters were incubated in 600 μL Qiagen RLT+ buffer and 6 μL β -mercaptoethanol for 90 min on a rotisserie. The filters were then vortexed for 10 min and homogenized using a QIAshredder

column. RNA was purified from the homogenized solution using the RNeasy kit according to the manufacturer's standard protocol.

All sequencing was performed at the University of Michigan Sequencing Core. Paired-end DNA sequencing (2×125) was conducted on Illumina HiSeq 2000 with V4 chemistry reagents with "low-input prep" using the Rubicon ThruPlex kit. RNA single-read sequencing (1×50) was performed on Illumina HiSeq 2000 with V4 chemistry reagents. Before sequencing, RNA libraries were prepared with a 50/50 mix of plant and bacterial Ribo-Zero kits to remove rRNA sequences. In total, 23 metagenomes and 7 metatranscriptomes were sequenced, and the 7 metatranscriptomes were paired with 7 of the metagenomes collected at the same date and sampling location. Of these, 15 metagenomes are from the 100- μ m fraction and 8 are from the total community fraction. All metatranscriptomes are from the 100- μ m fraction.

Metagenomic assembly. Metagenomes were obtained from the $>0.22\text{-}\mu\text{m}$ size fraction (targeting the whole microbial community) and from the $>100\text{-}\mu\text{m}$ *Microcystis*-enriched fraction (targeting *Microcystis* colonies, microplankton, and attached bacteria). Metagenomic short reads from each sample were individually processed and assembled *de novo*. Before assembly, metagenomic short reads from each sample were dereplicated at 100% length and identity using a custom-made dereplicate.pl script, and sequencing adaptors were removed with Scythe (<https://github.com/vsbuffalo/scythe>). Sickle was then used to remove low-quality reads (89), and the forward and reverse reads were interleaved using the custom script interleave.pl. The *de novo* assembly for each sample was constructed using IDBA-UD with the following parameters: min kmer = 55, max kmer = 115, kmer step size = 10 (90).

Because the quality of the assembly of a genome from a metagenome can be reduced when its read coverage exceeds $20\times$ (91), we performed a subsampling approach to optimize *Microcystis* coverage, which could be as high as $1,200\times$ in some samples. *Microcystis* coverage in each sample was estimated by mapping the unprocessed reads to the complete *Microcystis aeruginosa* NIES-843 genome using the Burrows-Wheeler Aligner version 0.7.9a (92). Our results confirmed that the longest contigs containing *Microcystis* housekeeping genes were obtained when *Microcystis* coverage was between $20\times$ and $35\times$. Therefore, for each sample in which the *Microcystis* coverage was greater than $35\times$, an additional *de novo* assembly was constructed by obtaining a randomized subset of the reads to normalize the *Microcystis* coverage to $35\times$ using IDBA-UD with the following parameters: min kmer = 52, max kmer = 92, kmer step = 8 (90). In total, 42 assemblies were constructed from the metagenomic reads.

To improve the assembly of lower-abundance bacteria, combined-sample assemblies (coassemblies) were generated with MEGAHIT (93) using kmin 21, kmax 141, and kmer step size of 12. We performed 3 coassemblies in total: one from the two samples with the highest *Acidobacteria* CoA2 C42 abundance (Aug-4 and Aug-25 particle metagenomes from WE12), another containing all the samples in which *Acidobacteria* CoA2 C42 was present (8 samples total), and another with the same 8 samples but in which the read kmer coverage was normalized to $20\times$ prior to assembly with BBnorm in the BBTools package (94). Paired-end short reads were quality and adaptor screened and dereplicated with BBTools prior to coassembly (94). An additional single-sample MEGAHIT assembly was constructed on the particle size fraction sample from 4 August at WE12 following the same pipeline.

Read mapping, contig binning, and MAG quality assessment. Assembly and binning of sequence reads produced metagenome-assembled genomes (MAGs) of the dominant cyanobacteria (*Microcystis*, *Cyanobium-Synechococcus*, *Pseudanabaena*, and *Anabaena-Dolichospermum*) and other bacteria (supplemental files 1 and 2), allowing us to assess the distribution and expression of genes encoding catalases and peroxidases among specific microbial taxa during the bloom. The metagenomic read coverage of contigs in each assembly was obtained by mapping reads to their corresponding assembly using Bowtie2 (95). Differential coverage across samples was determined by iteratively mapping reads from each sample used in the assembly to the coassembled contigs.

Binning of contigs in single-sample IDBA-UD assemblies was iteratively performed by clustering assembled contigs by the Euclidean distance of tetranucleotide frequencies and visualized in Databionics Emergent Self-Organizing Maps (ESOMs) (96). Genome sequences of *Microcystis aeruginosa* NIES-843, *Rhizobiales* bacterium AUSA02, and *Brevundimonas subvibrioides* were included in all ESOMs as reference genomes. Each sample received its own ESOM, but contigs generated from full and subsampled reads were binned together in the same ESOM. For most 100- μ m fraction samples, contigs greater than 5,000 bp in length were split into 5,000-bp pieces, while contigs below 2,500 bp in length were not included in the binning process. For the total community fraction and the 100- μ m sample from 29 September at WE4, a contig size window of 4,000 to 10,000 bp was used because the larger number of contigs above 2,500 bp was computationally unmanageable.

In the coassemblies, contigs were binned using a multialgorithm binning approach. Contigs were binned using differential coverage and tetranucleotide frequencies in CONCOCT (97) and MetaBAT 2 (98) and with tetranucleotide frequencies alone using VizBin (99) and ESOMs. The contig size window for ESOM was 4 to 10 kbp and 2.5 to 10 kbp for the other binners. The resulting redundant bin data sets from each assembly were dereplicated using DASTool (100). The single-sample MEGAHIT assembly was binned using the above coassembly workflow. For this assembly, differential coverage was estimated by mapping reads from 4 August WE12 and 25 August WE12 to the contigs.

The completeness, contamination, and redundancy estimates of each genomic bin were generated using the lineage workflow in CheckM (101). The bins from single-sample IDBA-UD assemblies with contamination metrics greater than or equal to 5%, which is the established benchmark for a draft genome of low contamination (101), were refined by plotting GC content versus the read coverage of all scaffolds within the target bin and removing outlier contigs. Coverage and tetranucleotide frequencies of the coassembly bins were visualized for manual refinement in Anvi'o (102). Refined bins were reanalyzed using the lineage workflow in CheckM (101). All bins with contamination scores greater than 5% after refinement were eliminated from downstream analysis. Contamination scores of the final bins were

considered while ignoring the amount of contamination due to strain heterogeneity. Redundant marker genes were considered to be from closely related strains if their shared amino acid identity (AAI) was 95% or more. In total, 315 MAGs were generated with low contamination (<5%) and various levels of completeness (supplemental file 1). This redundant bin data set was dereplicated using dRep (103) with 97% average nucleotide identity (ANI) and 60% alignment coverage cutoffs and skipping the MASH pre-clustering step. Only MAGs with completeness above 75% were included in the final dereplicated MAG data set, leaving a total of 136 MAGs (supplemental file 2). To improve completeness of one incomplete *Acetobacteraceae* bin (Bin 42896M_V12), sequences within 50 nodes of binned contigs in the assembly De Bruijn graph were included to the bin.

The taxonomy of each dereplicated bin was obtained with CheckM's tree qa function and GhostKoala taxonomy annotations of gene calls (104). Taxonomic classification with these methods remained coarse in many cases, so EMIRGE was used to assemble 16S rRNA genes (105), which were then assigned to bins by matching fragments of the 16S at the ends of contigs via the blastn function of the Basic Local Alignment Search Tool (BLAST, version 2.2.28+) (106). The mean insert size and accompanying standard deviation input used in the EMIRGE 16S assemblies were estimated from the mapping results of the raw metagenomic reads to their corresponding assembled contigs. The SILVA SSU 16S rRNA database version 128 was curated by the emirge_makedb.py script provided in the EMIRGE package and then used as the reference in EMIRGE to map short reads for assembly (107). The final assembled 16S rRNA sequences were compared to the original reference database via BLAST version 2.2.28+, and taxonomy was assigned with the method of Wang et al. (108) in mothur v. 1.43.0 (109). An EMIRGE sequence was considered to belong to a bin only if the percent match to a bin 16S fragment covered greater than 10% of the assembled 16S rRNA gene, had a bit score of at least 50 and an E value of at most 1×10^{-5} , and did not conflict with taxonomy as determined by CheckM and GhostKoala. Final bin taxonomy was assigned using EMIRGE whenever available; otherwise, the CheckM and GhostKoala taxonomy was used.

Gene annotations and abundance. Gene calls and functional annotations of metagenome-assembled contigs were obtained using the IMG annotation pipeline (110). Genes of interest were obtained from the IMG database by conducting a KEGG orthology search of bacterial genomes in the IMG online interface (111). The following KEGG orthology identifiers (IDs) were used to target our specific genes of interest: K03781 (*katE*, *katA*, and *catB*, here listed collectively as *katE*), K07217 (*katN*, *ydbDA*, *cotG*, and *yjqC*, here listed collectively as *katN*), K03782 (*katG*), K00428 (*ccpA*, *yhjA*, and *mauG*, here listed collectively as *ccpA*), K03386 (*ahpC*), K00434 (ascorbate peroxidase [APX]), and K03043 (*rpoB*). Custom BLAST databases were constructed from the gene calls with KEGG orthology annotations of interest if their final IMG annotation matched that of the KEGG orthology ID. The taxonomy of genes was assigned to match the taxonomic assignments of the dRep bin cluster in which they were found. Unbinned genes were assigned a taxonomy if they had a $\geq 95\%$ match to a protein in NCBI nr (as of 17 October 2018) with blastx (106). All other unbinned genes were included as unassigned. Genes in the custom databases were sorted by gene length (longest to shortest) and clustered at 95% identity with vsearch (112) prior to read mapping.

To determine the abundance of catalases and peroxidases in the metagenomes and metatranscriptomes, BBTools-screened short reads were mapped to the assembled gene databases with BLAST version 2.2.31+ (106). The quality-screening pipeline for metatranscriptomic reads did not include a dereplication step. The alignments to the assembled genes were filtered to exclude hits with a percent identity less than 95% and an E value greater than 1×10^{-5} using the postBlast.pl script. To avoid double counting reads, only the best matches were counted for each read using the top5.pl script. Only read counts to genes with at least 70% amino acid identity (AAI) and 50% alignment coverage to other proteins with the same functional annotation in the NCBI nr protein database with blastx version 2.2.31+ (106) were included. Because more reads inherently map to longer genes, the read counts of each gene were normalized by gene length.

To correct for various library and genome sizes between samples, the gene abundances were reported as ratios to the number of reads that mapped to a custom database of the single-copy housekeeping gene *rpoB*, encoding the beta subunit of the bacterial RNA polymerase. BLAST mappings against *rpoB* were filtered and quality checked as described above for the catalase and peroxidase blast searches. We chose to normalize to bacterial *rpoB* rather than total library size to reduce the impact of eukaryotic genomes on the gene and transcript abundance values for the bacterial community. However, we checked the assumption that *rpoB* is constitutively expressed and a good normalization strategy by normalizing metatranscriptomic read counts via total library size, expressed as reads per kilobase of transcript per million mapped reads (RPKM). We found that the patterns of *katG* relative abundance in metatranscriptomes were generally the same, with the exception of the two early samples that showed much lower gene relative abundance overall due to the dominance of eukaryotic genomes (Fig. S7). *ahpC* relative abundance at station WE12 was lower in September when expressed as RPKM (Fig. S7).

Culturing experiments. The growth of seven axenic *Microcystis* cultures was assessed with and without the presence of 1 mM sodium pyruvate, a scavenger of extracellular H_2O_2 (8, 24). Six strains were purchased axenic from the Pasteur Culture Collection of Cyanobacteria (PCC 7806, PCC 7806 $\Delta mcyB$, PCC 7941, PCC 9806, PCC 9701, and PCC 7005). Strain NIES-843 was generously provided by Steve Wilhelm at the University of Tennessee. The cultures were maintained at room temperature under cool white fluorescent lights (30 to 60 $\mu\text{mol photons/m}^2/\text{s}$, 12-h light-dark [L:D] cycle) in BG-11 2N, a variant of standard BG-11 medium (113) with the sodium nitrate concentration reduced to 2 mM. Axenicity was monitored by light microscopy and 4',6-diamidino-2-phenylindole (DAPI) fluorescence microscopy and by inoculating *Microcystis* cultures into LB and tryptic soy broth (TSB) media and monitoring for the development of turbidity after 3 to 4 weeks.

The experimental cultures were grown in 1-L glass Pyrex bottles at 24.4°C (average in July to August of 2014 to 2017) and 330 $\mu\text{mol photons/m}^2/\text{s}$ on a 12-h L:D cycle (average photosynthetically active radiation

[PAR] intensity at 0.5-m depth in Lake Erie during July to August of 2017 as determined from approximately biweekly hyperspectral profiles) for 12 to 14 days in a Caron plant growth chamber (model 7311-50; Caron, Marietta, OH). The growth medium was BG-11 2N. The positions of the bottles were randomized to correct for potential differences in light intensity across incubator shelving. Cell densities and H_2O_2 concentrations were monitored every other day. Cell density was measured via cell counts under a compound microscope using a Reichert Bright-Line hemacytometer (Hausser Scientific, Horsham, PA). Maximum growth rates were determined by taking the first derivative of a spline curve fit to the cell density data of each biological replicate (average $R^2 = 0.994 \pm 0.006$, 95% confidence interval).

H_2O_2 concentration was measured using the chemiluminescent reaction of the conjugate base of hydrogen peroxide with 10-methyl-9-(*p*-formylphenyl)acridinium carboxylate trifluoromethanesulfonate in a FeLume flow-injection analysis (114). Standard additions of a nominal 200 μM H_2O_2 solution were added to each sample to correct for matrix effects (final nominal concentrations of added standard were 0, 500, and 750 nM). The concentration of the H_2O_2 standard solution was measured from its absorbance at 240 nm in a 5-cm quartz cuvette and the molar absorptivity of H_2O_2 ($38.1 \text{ M}^{-1} \text{ s}^{-1}$) (114). A new standard solution was prepared from a 30% H_2O_2 stock solution on each day measurements were performed. Decay of the standard additions during the flow-injection analysis was used to confirm H_2O_2 degradation by sodium pyruvate-treated samples. Signal decay was monitored by taking 4 replicate injections of each sample, and the normalized signal decay was calculated as the difference in the peak integrals of the chemiluminescent signal in the first and final replicate injections divided by the peak integral of the first injection.

Sodium pyruvate was used as an H_2O_2 scavenger in the cell culture experiments because bovine liver catalase was not appropriate for these experiments. The light levels used to grow the *Microcystis* strains resulted in significant reduction of catalase activity within 4 to 5 h (Fig. S8 in the supplemental material) (9, 30).

Scripts and data availability. All custom bioinformatics scripts and pipelines used in this study can be found on the geomicrobiology lab GitHub page at <https://github.com/Geo-omics/scripts>. All read data sets are publicly available in NCBI SRA under BioSample accession numbers: SAMN09102072 to SAMN09102087. All metagenomic assemblies were submitted to IMG and are publicly available Gold Study ID Gs0113966. The dereplicated MAG sequences are deposited in NCBI under BioProject no. PRJNA464361 (see supplemental file 2 for how accession numbers are assigned to specific bins and assemblies). The R package growthrates v. 0.8.1 (<https://github.com/tpetzoldt/growthrates>) was used to calculate growth rates, and Welch's *t* tests were computed with base R v. 4.0.2. Data tables and analysis code are available on the geomicrobiology lab GitHub page at <https://github.com/Geo-omics/Heterotrophic-Bacteria-Dominate-Catalase-Expression-During-Microcystis-Blooms>.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.04 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.1 MB.

SUPPLEMENTAL FILE 3, XLSX file, 0.2 MB.

SUPPLEMENTAL FILE 4, PDF file, 0.6 MB.

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ADDENDUM IN PROOF

While the current paper was in press, a modeling study was published that proposes the different H_2O_2 defense strategies of toxigenic and nontoxigenic *Microcystis* strains, and their differing susceptibility to nitrogen limitation, influences the strain composition of *Microcystis* blooms in Lake Erie (115). While the results of the Hellweger et al. paper are inconsistent with our finding that H_2O_2 at environmentally relevant

concentrations does not impact *Microcystis* strains according to their ability to produce microcystins, we note two important caveats. First, in contrast to Hellweger et al., the current paper did not consider the role of nitrogen availability, as all experiments were conducted under nitrogen replete conditions. Second, while H_2O_2 concentrations in the current study were in the range of natural conditions, the sources and sinks of H_2O_2 in our lab experiments differed compared to those in the field, where the main sources of H_2O_2 are photochemical production and biological production by bacteria other than *Microcystis* (116). In the lab the sources and sinks of H_2O_2 included photochemical production and decay from media constituents (not present in the field) along with biological production and decay from *Microcystis*. Thus, different dynamics of H_2O_2 production and decay in natural settings could impart greater fitness costs to *Microcystis*, and varying nitrogen availability could differentially affect the growth of toxigenic versus nontoxigenic strains as suggested by the model in Hellweger et al. (115). The Smith (116) and Hellweger et al. (115) findings highlight the need for better understanding of the physiological diversity of *Microcystis* strains.

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