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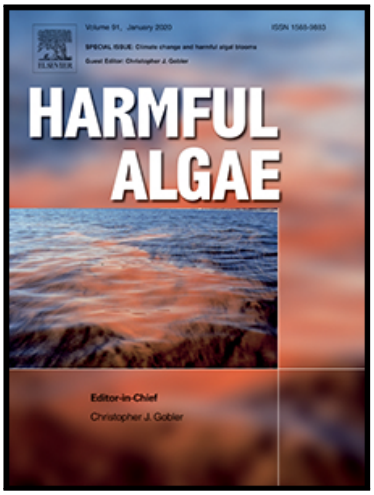

The Western Lake Erie Culture Collection: A promising resource for evaluating the physiological and genetic diversity of Microcystis and its associated microbiome

Colleen E. Yancey , E. Anders Kiledal , Subba Rao Chaganti , Vincent J. Denef , Reagan M. Errera , Jacob T. Evans , Lauren N. Hart , Dragan Isailovic , William S. James , Jenan K. Kharbush , Jeffrey A. Kimbrel , Wei Li , Xavier Mayali , Helena Nitschky , Catherine A. Polik , McKenzie A Powers , Sanduni H. Prematilaka , Nicole A. Rappuhn , Laura A. Reitz , Sara R. Rivera , Claire C. Zwiers , Gregory J. Dick

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Title: The Western Lake Erie Culture Collection: A promising resource for evaluating the physiological and genetic diversity of *Microcystis* and its associated microbiome

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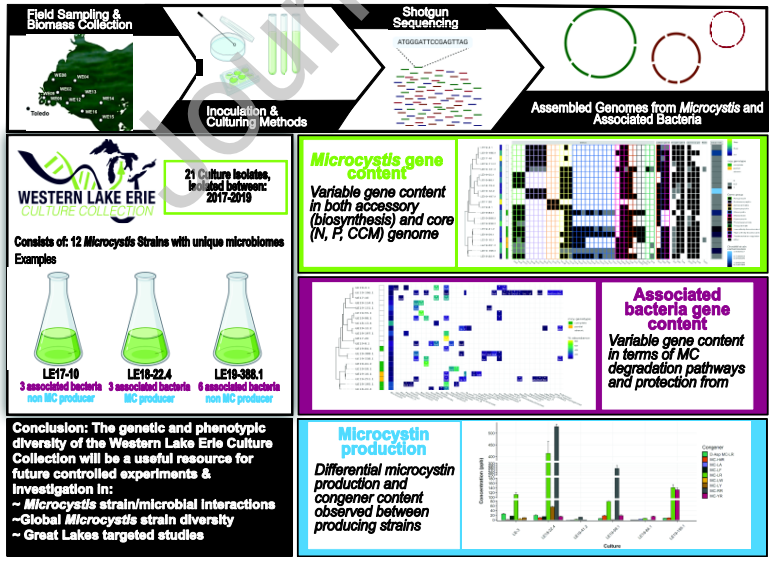
Highlights

- Twenty-one xenic *Microcystis* cultures were isolated from western Lake Erie and capture the diversity of *Microcystis* strains observed in natural populations as well as their associated bacteria.
- *Microcystis* strains within the culture collection show variability in core and accessory gene content, capture much of the strain diversity observed in the 2014 cyanoHAB, and genetically similar strains produce varying concentrations and congeners of microcystins.
- This collection is a valuable resource for studying strain diversity and interactions between *Microcystis* and associated bacteria.
- Our collection increases the availability of environmentally relevant strains from temperate North America, which is historically underrepresented in culture collections.

Graphical Abstract



Abstract

Cyanobacterial harmful algal blooms (cyanoHABs) dominated by *Microcystis* spp. have significant public health and economic implications in freshwater bodies around the world. These blooms are capable of producing a variety of cyanotoxins, including microcystins, that affect fishing and tourism industries, human and environmental health, and access to drinking water. In this study, we isolated and sequenced the genomes of 21 primarily unialgal *Microcystis* cultures collected from western Lake Erie between 2017-2019. While some cultures isolated in different years have a high degree of genetic similarity (Average Nucleotide Identity >99%), genomic data show that these cultures also represent much of the breadth of known *Microcystis* diversity in natural populations. Only 5 isolates contained all the genes required for microcystin biosynthesis while 2 isolates contained a previously described partial *mcy* operon. Microcystin production within cultures was also assessed using Enzyme-Linked Immunosorbent Assay (ELISA) and supported genomic results with high concentrations (up to 900 μg L^{-1}) in cultures with complete *mcy* operons and no or low toxin detected otherwise. These xenic cultures also contained a substantial diversity of bacteria associated with *Microcystis*, which has become increasingly recognized as an essential component of cyanoHAB community dynamics. These results highlight the genomic diversity among *Microcystis* strains and associated bacteria in Lake Erie, and their potential impacts on bloom development, toxin production, and toxin degradation. This culture collection significantly increases the availability of environmentally relevant *Microcystis* strains from temperate North America.

Keywords: cyanoHABs, *Microcystis*, western Lake Erie, metagenomics, phycosphere, microcystin

Abbreviations

CCM: Carbon Concentrating Mechanisms

ELISA: Enzyme-Linked Immunosorbent Assay

MAG: Metagenome Assembled Genome

MC: Microcystin

ROS: Reactive Oxygen Species

WLE: Western Lake Erie

WLECC Western Lake Erie Culture Collection

1. Introduction

The Laurentian Great Lakes are increasingly threatened by cyanobacterial harmful algal blooms (cyanoHABs) capable of producing cyanotoxins that are harmful to human and environmental health (Binding et al., 2020; McKindles et al., 2020; Miller et al., 2017). Of particular concern is western Lake Erie (WLE), which experiences intense cyanoHABs dominated by the microcystin (MC)-producing cyanobacteria *Microcystis* spp. (Berry et al., 2017; Bridgeman et al., 2013; Rinta-Kanto et al., 2005; Steffen et al., 2017; Watson et al., 2016). These annual blooms begin midsummer and can persist through late fall, threatening drinking water supplies to both Canada and the United States. Blooms can also cause hypoxia, fish kills, and other ecosystem disruptions (Berry et al., 2017; Harke et al., 2016; Huisman et al., 2018; Steffen et al., 2017; Watson et al., 2016). CyanoHABs have substantial economic ramifications as well: a single bloom in WLE during August 2014 led to a $65 million loss in the United States (Bingham et al., 2015), and blooms in WLE will cost Canada an estimated $5.3 billion over the next 30 years if left unmanaged (Smith et al., 2019). The threat to potable water access is of greatest concern and became a reality during the 2014 cyanoHAB, resulting in a “do not drink”

advisory in the city of Toledo, Ohio, USA due to high levels of MC in treated drinking water supplies (Steffen et al., 2017).

Microcystis-dominated cyanoHABs are expected to increase in intensity and frequency both globally and within WLE as a result of intensifying climate change (Griffith and Gobler, 2020; Ho et al., 2019; Huisman et al., 2018; Watson et al., 2016). Toxin content per cell within blooms is also expected to increase due to climate change associated increases in atmospheric pCO_2 (Sandrini et al., 2015) and the continuation of unchecked nitrogen (N) loading (Hellweger et al., 2022; Wagner et al., 2019). As climate change continues to alter freshwater systems, it is critical to understand the effects of these shifts to better inform future management strategies.

The diversity of *Microcystis* strains and their interactions with associated bacteria in the phycosphere are potentially important controls on toxin content and concentration (Dick et al., 2021). Natural phytoplankton assemblages are known to contain multiple strains of *Microcystis*, some capable of producing MC while others are not (Rinta-Kanto et al., 2005, 2009; Yancey et al., 2022a). Further, the *mcy* operon, which encodes the synthesis of MCs (Tillett et al., 2000), exhibits genetic substructure between strains which may contribute to differential congener production (Mikalsen et al., 2003; Tooming-Klunderud et al., 2008; Yancey et al., 2022a). For example, the drivers of the emergence of MC-LA in Lake Erie (Birbeck et al., 2019), a congener typically seen on the West Coast of the United States, remains elusive and may be the result of changing strain composition and/or environmental gradients. The microbiome of bacteria physically associated with *Microcystis* colonies impart additional diversity, are *Microcystis* strain-specific (Smith et al., 2021), and likely engage in mutualistic interactions with *Microcystis* (Smith et al., 2022a, 2022b). While such interactions influence toxin production by other algae (Bates et al., 1995), the environmental effects on toxin production by *Microcystis* are largely

unknown. Thus, it is imperative that we understand the intricate dynamics of cyanoHABs including *Microcystis* strain diversity and interactions within the cyanoHAB phycosphere to understand how toxin production and bloom progression may be impacted by anthropogenic forcings.

The genus *Microcystis* is well known for its complex genomes, rich with mobile elements (Harke et al., 2016), low synteny among strains (Humbert et al., 2013), and a large pangenome (Cao et al., 2022; Meyer et al., 2017). *Microcystis* shows substantial strain-level diversity (Harke et al., 2016; Humbert et al., 2013), with extensive heterogeneity of genetic and phenotypic characteristics (Dick et al., 2021). High rates of horizontal gene transfer among *Microcystis* strains (Cao et al., 2022; Pérez-Carrascal et al., 2019) complicate subspecies delineation and may contribute to their environmental success and adaptation to variable environments (Cao et al., 2022; Dick et al., 2021). Their large accessory genomes and potentially global pangenome (Meyer et al., 2017) harbor numerous genes of known and unknown function, many of which may encode toxins (Pérez-Carrascal et al., 2019) or other traits that provide *Microcystis* a competitive advantage in natural communities. While functional groups and key traits within this genus have yet to be fully defined, it is critical to understand how intraspecies *Microcystis* diversity affects the toxicity, persistence, and dominance of *Microcystis* in blooms around the world (Dick et al., 2021).

Several methods are commonly used to investigate cyanoHAB dynamics including long term field monitoring and sampling, and controlled culture experiments in mesocosm and laboratory settings. Field samples provide direct insight into entire cyanoHAB communities, but the complexity of whole communities can complicate the assignment of particular functions to specific taxa. Axenic cultures enable insights into the genetic and physiological traits of specific

Microcystis strains (Dick et al., 2021; Harke et al., 2016; Humbert et al., 2013; Pérez-Carrascal et al., 2019), but may misrepresent how *Microcystis* functions within a natural community, interacts with its environment, and responds to environmental change, as associated bacteria are increasingly recognized to be essential for cyanoHAB dynamics (Hoke et al., 2021; Seymour et al., 2017; Smith et al., 2021). Xenic *Microcystis* cultures are valuable as an intermediary between the full complexity of whole communities and the unrealistic simplicity of axenic cultures and can be valuable for studying interactions between *Microcystis* and its associated bacteria. However, there is currently only one publicly available strain of *Microcystis* from WLE: strain LE-3. This strain was isolated over 20 years ago, produces MCs, and is maintained in culture with associated bacteria, (Brittain et al., 2000), but it has lost its ability to form colonies, which are the dominant phenotype in the field and define the physicochemical habitat of the phycosphere that is packed with heterotrophic bacteria (Smith et al., 2021). Further, LE-3 represents just a single strain, whereas WLE blooms contain multiple diverse strains that play key roles in bloom dynamics (Rinta-Kanto et al., 2005; Yancey et al., 2022a, 2022b).

To address the lack of WLE *Microcystis* isolates in culture collections, we isolated 21 new strains of *Microcystis* from different years, locations, and seasons, and conducted initial characterization and genome sequencing. We find that these xenic cultures capture the known genetic diversity of *Microcystis* and contain several bacterial genera which associate with *Microcystis* in nature. These cultures will serve as a useful resource to better understand the genetic and phenotypic diversity of *Microcystis* and their interactions with associated bacteria.

2. Materials and Methods

2.1 Collection and Isolation

Xenic cultures that contained *Microcystis* were collected for isolation and cultivation during weekly cyanoHAB cruises from various NOAA Great Lakes Environmental Research Laboratory (GLERL) sampling stations within western Lake Erie (Cooperative Institute for Great Lakes Research, 2019) during September 2017, June and August of 2018, and July and August of 2019 at stations WE2, WE4, WE6, WE8, and WE12 (Cooperative Institute for Great Lakes Research, 2019; Den Uyl et al., 2022). Further information regarding collection date, location, and isolation technique is outlined in Table 1. Isolation work was performed on either whole water samples or a >100 µm fraction collected using a net tow. Serial dilutions (1x, 10x, 100x, and 1000x) of water samples were plated (see below for media recipes) and incubated at ~20°C (room temperature) and 50 µmol photons $meter^{-2}$ $second^{-1}$ on a 12:12 hour light:dark cycle. Colonies identified as *Microcystis* by microscopy were then serially streaked onto agarose plates from the initial lawn. Cultures that only contained *Microcystis* colonies and associated bacteria based on visual inspection under the microscope, continued to be transferred on solid plates and were used to inoculate 0.5 mL liquid cultures. All cultures were cryopreserved in a 5% DMSO solution and stored at -150°C in liquid nitrogen.

Cultures are maintained on solid plates and in 10 mL liquid media at room temperature and 40 µmol photons $meter^{-2}$ $second^{-1}$ on a 12:12 hour light:dark cycle. Cultures have successfully been maintained at temperatures from 20 to 26°C and light ranges of 40 to 400 µmol photons $meter^{-2}$ $second^{-1}$. Acclimation changes of no more than 1°C or 20% of the previous set light intensity per day with weekly transfers into fresh media were required to ensure growth and healthy cultures. Various culture media were used during isolation and maintenance of the culture collection including BG-11 2N (Humbert et al., 2013), which is BG-11 (Allen, 1968) modified to contain 2 mM $NaNO_3$ and 10 mM $NaHCO_3$, BG11-2N with 0.22 µm filtered Lake

Erie water instead of MilliQ water (LE BG11-2N), unmodified BG-11, and half strength Wright"s cryptophyte (WC) medium (Guillard and Lorenzen, 1972) modified according to (Stemberger, 1981) and supplemented with 0.6 µM H_2O_3Se. The medium in which each culture was isolated is described in Table 1.

2.2 DNA Extraction and Sequencing

Cultures grown in BG11-2N with a 12:12 hour diurnal cycle were collected around the 7th day after passage, homogenized, and centrifuged for 5 minutes at 15,000 g twice to collect biomass from 4 mL of dense culture. The DNeasy Blood and Tissue Kit with the QIAshredder adapter (Qiagen, Hilden, Germany) was used to extract DNA according to the protocol found at https://www.protocols.io/view/dna-extraction-from-filters-using-qiagen-dneasy-an-j8nlk61wv5r7/v1. Quant-iT™ PicoGreen™ DNA Assay Kit (Invitrogen, Waltham, MA, USA) was used to quantify DNA concentrations. Sequencing was completed at the Advanced Genomic Core at the University of Michigan using an Illumina NovaSeq (S4) (Illumina, San Diego, CA, USA) platform for 300 cycles. The sequencing run was completed with maximum insert possible without compromising read quality.

2.3 Metagenomic Analysis

Raw reads were processed with bbduk from the JGI supported software package bbtools v.38.84 (https://sourceforge.net/projects/bbmap/, (Bushnell, 2014)) to remove adapters, quality trim reads, and remove contamination using the Univec reference collection (https://www.ncbi.nlm.nih.gov/tools/vecscreen/univec/). Duplicate reads were removed using clumpify and dedupe (bbtools; see above). Each sample was assembled separately using Megahit

v1.2.9 (Li et al., 2015) with the meta-sensitive parameter. Contiguous sequences, or contigs, greater than 1 kb in length, were used to create an Anvi'o database for each sample. *Microcystis* bins and bins of the associated bacteria were generated with Concoct v.1.1.0 (Alneberg et al., 2013) then manually refined with Anvi'o v.7 (Eren et al., 2021, 2015). Bin taxonomy was determined using single copy genes in Anvi'o and additionally evaluated with GTDBtk and the GTDB release 202 database (Chaumeil et al., 2019; Parks et al., 2018). A pangenome analysis of 159 *Microcystis* reference genomes and the 21 genomes obtained here determined 26 single copy genes that were present in each of the 180 genomes and the two outgroups (*Anabaena variabilis* ATCC 29413 and *Synechocystis* sp. PCC6803). These single copy genes were concatenated and aligned with MUSCLE v3.8.1551 (Edgar, 2004), and used to produce a tree with fasttree v2.1.11 (Price et al., 2009) using the gamma parameter. Additionally, pyani v.2.12 (Pritchard et al., 2015) was used to calculate genomic pairwise average nucleotide identity (gANI) between each *Microcystis* MAG.

For each sample, the relative abundance of organisms was determined by summarizing reads mapped to bins using CoverM v.0.6.1 and the Minimap2 aligner v.2.24 (Li, 2018; Woodcroft, 2020.). To screen for organisms that did not bin well, taxonomic profiles were also produced for each sample using Kraken 2 version 2.1.2 and Bracken version 2.6.1 with a GTDB release 202 database produced with Struo2 (Lu et al., 2017; Wood et al., 2019; Wood and Salzberg, 2014; Youngblut and Ley, 2021). To reduce spurious hits, only taxa with ≥ 3 minimum-hit-groups and ≥ 150 distinct minimizers were retained; the --report-minimizer-data option was used to obtain these values similar to the unique k-mer counts produced by KrakenUniq (Breitwieser et al., 2018).

Assembled *Microcystis* genomes were screened for various genes including those involved in biosynthesis of secondary metabolites, nitrogen and phosphorus metabolism, carbon concentrating mechanisms (CCM), vitamin synthesis and requirement, and hydrogen peroxide decomposition by catalase. Assembled genomes of associated bacteria were additionally searched for microcystin degrading genes, vitamin B1 and methionine synthesis, and catalase genes. The list of genes queried with NCBI accession numbers can be found in Table S1. BLAST v.2.9.0 (Madden, 2013) was used to align the gene database to the *Microcystis* and associated bacteria isolates. For each genome, a presence/absence matrix was generated and used for further analysis and visualization; genes with >95% identity and >50% alignment length were considered present. Because genes can be missing from MAGs due to technical artifacts like assembly and binning inefficiencies and low read coverage observed across isolates, QC"ed reads were additionally aligned to the *Microcystis* genes used for BLAST analysis to confirm gene presence or absence. Reads were indexed and mapped with BWA-MEM2 version 2.2.1 (Li and Durbin, 2009; Vasimuddin et al., 2019); alignments were filtered to only include reads with > 80% coverage and > 95% identity. Genes were considered present if at least 50% of the gene was covered by mapped reads and the mean coverage depth was > 1. Based on findings previously described (Yancey et al., 2022a), read coverage patterns were used to determine presence of the complete (all *mcy* genes present), absent (no *mcy* genes present), or partial (truncated *mcyA*, complete *mcyB* and *mcyC*) *mcy* genotypes for the *Microcystis* isolates. Variants in the *mcyB* gene, described in a previous study (Mikalsen et al., 2003), were assessed as well. Additional metabolic functional annotation was done to assess the completeness of pathways using a collection of KEGG ortholog terms (searched with kofamscan version 1.3.0) or custom Hidden Markov models (HMMs, searched with HMMer version 3.3.2). We focused on

metabolism for the cycling of metabolites, growth hormones and vitamins between *Microcystis* and associated bacteria.

Proteins in the MC degradation pathway were identified using the hmmsearch function in HMMER software package (version 3.3.2) based on representative custom HMM profiles including *mlrA-D* genes (Eddy, 2011). A threshold of E-value < 0.001 was used to identify positive hits. To generate the custom profiles, MlrA-D protein sequences available in NCBI were aligned using Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo/) with default parameters to create seed proteins. HMM profiles were then generated from the alignments using the hmmbuild function in HMMER.

Sourmash gather version 4.5.0 was used to compare k-mer sketches (k=31) of the MAGs from the *Microcystis* cultures to metagenomes collected from June-October at three sites during the 2014 bloom in Western Lake Erie (NCBI BioProject: PRJNA464361) (Yancey et al., 2022a). The GTDB release 202 database (released April 27th, 2021) and a database of the 159 *Microcystis* reference genomes were also included to assess the overall relative abundance of Microcystis more accurately and other bacteria and archaea in the samples, and the uniqueness and representation of our MAGs in the field samples. As described in (Irber et al., 2022), the number of unique k-mers attributable to a particular taxon was calculated as (unique_intersect_bp / scaled) * average_abund, and the relative abundance of a particular group was calculated as its unique k-mer count / the total number of unique k-mers in a sample.

2.4 Microcystin Analysis

To determine the overall concentration of MC in cultures, Enzyme-Linked Immunosorbent Assays (ELISA) were used. Cultures were grown in BG-112N on a 12:12 hour light/dark cycle.

After the 7th day of growth, we extracted cultures putatively in the exponential phase based on visible, dense, biomass. 3-10 mL of liquid culture was filtered through 0.2 um Polycarbonate (PC) membrane filters (Merck Millipore Ltd, Tullgreen, Carrigtwohill, Co Cork, Ireland), until clogged. Samples were stored at -80°C prior to extraction following the EPA Method 546 for detection of total microcystins (US EPA, 2019) for particulate microcystin. For comparison, two measurements were completed with independently grown cultures. The first and second measurements were completed in early and late February 2021 respectively. For each measurement, at least 4 technical replicates were collected for each isolate. In addition to ELISA assays, MC congener composition was determined using liquid chromatography-mass spectrometry (LC-MS) via methods previously described (Baliu-Rodriguez et al., 2022; Palagama et al., 2020). Congener identification was confirmed via standards for isoforms MC-LR, RR, YR, LA, LW, LF, HilR, LY and D-Asp MC-LR.

2.5 Quantitative real time PCR (qRT-PCR) assays

To assess for the potential that some isolates may contain multiple *Microcystis* strains with multiple *mcy* genotypes, we performed qRT-PCR assays on a subset of isolates. Primers used included those that would amplify *mcyE*, *mcyA*, and a 16S rRNA universal marker for *Microcystis* sp. and other cyanobacteria. The *mcyA* primer selected amplifies a conserved region that is present in both the complete and partial *mcy* genotype in order to distinguish between these two operon types. A PrimeTime™ qPCR probe-based assay was used for *mcyA*, with forward primer sequence 5‟- TCTATTCTCAGTATTCAGGTTGTCGC-3, reverse prime sequence 5‟-GTAGCTAGTTCAGCAAGAGTAGG-3‟ and probe sequence is SUN-TCGTCAAGCCGGGTTGAAGATTACC-Iowa Black FQ. For 16S rRNA and *mcyE* gene copy

number estimates were obtained by performing total cyanobacteria and Toxin gene assays (Phytoxigene, Inc.; catalog no: 205-0050 and 205-0051) respectively. qRT-PCR The qRT-PCR assays were performed on the QuantStudio™ 6 Flex (Thermo Fisher Scientific) with the following PCR cycling conditions 2 min 50 °C, 5 min 95 °C; 40x (15 sec 95 °C (denaturation), 45 sec 60 °C (annealing/extension). For all gene targets reaction volume were kept at 20 µL, for *mcyA* reaction mixture includes 5 µL of Prime time 1-step 4XBroad range master mix, 2 µL of primers and probe mixture (primers to probe ratio was 1:2), 2 µL of DNA and 11 µL of MilliQ water. For *mcyE* and 16S rRNA 16 µL of Phytoxigene mixture and 2 µL of DNA and 2 µL of MilliQ water was used. No template controls were used as negative controls for all the target genes. Standards for *mcyE* and total cyanobacteria were obtained from Phytoxigene ™ CyanoNAS standard and 80-800,000 gene copies per reaction were used to generate the standard curves. Whereas for *mcyA* gene, complete target gene fragment was synthesized as gBlocks gene fragment (Integrated DNA Technologies) and used for generating standard curve similar to *mcyE*. Gene copies for mcyA and mcyE were normalized to 16S rRNA gene copies using the below formula.

$$mcyE\ or\ mcyA\ gene\ copies\ per\ million\ = (average\ mcyE\ or\ mcyA\ gene\ copies)/average\ 16s\ rRNA\ gene\ copies) * 10^6$$

3. Results

3.1 Culture Collection Overview

Isolation of *Microcystis* strains from five locations (Fig S1) in WLE during 2017-2019 yielded twenty-one unique and xenic *Microcystis* cultures (Table 1) from samples that spanned a variety of environmental conditions (Table S2). The cultures also contain other bacteria, many of

which are physically attached to *Microcystis* colonies in the field (Smith et al., 2021). The majority of cultures were isolated from nearshore stations, where cyanobacterial biomass tends to peak in abundance during WLE cyanoHABs (Berry et al., 2017; Bridgeman et al., 2013; Steffen et al., 2017; Watson et al., 2016). Most isolates were collected during July, although some were collected during August, often the time of peak cyanobacterial abundance, and during June (pre-bloom) and September (post peak) (Table 1).

Currently, all twenty-one cultures are being maintained at the University of Michigan. These cultures have maintained their ability to form colonies, in contrast to LE-3, which grows unicellularly or in duplets in culture. Variations of the BG11-2N media were the most successful for isolation and acclimation to lab conditions (13/21 cultures) (Table 1). We found that BG-11-2N prepared with milliQ water was sufficient for maintenance due to limitation of collection and storage of Lake Erie water. Unmodified BG-11 and half strength WC media yielded variable success in isolation and maintenance of the WLE culture collection (data not shown, Table 1).

3.2 Genome Content and Diversity of WLE Microcystis Isolates

Shotgun metagenome sequencing produced near-complete genomes of *Microcystis* (Table S3) and other bacteria in the cultures. Because other bacteria were present, the shotgun sequence datasets were analyzed as metagenomes. In several cases, the coverage of *Microcystis* genomes was low, around (6-8x), which is below the minimum coverage required for high-quality genome assembly (Dick, 2018), resulting in fragmented assemblies with low N50 (the length of the shortest contig where at least 50% of the assembly is contained in equal or greater length contigs). For example, the *Microcystis* MAG in LE19-196.1 had a reported N50 of 4062 and 1365 contiguous sequences (Table S3). Several of the cultures displayed substantial strain

heterogeneity, particularly LE19-12.2, indicating the likely presence of multiple *Microcystis* strains (Table S3). The fraction of sequences mapping to non-*Microcystis* MAGs varied from 0.5% to 86.9% and the number of MAGs retrieved from non-*Microcystis* bacteria varied from one in LE19-84.1 (*Novosphingobium* sp.) to 15 in LE19-196.1.

WLECC *Microcystis* isolates are scattered across the phylogenetic tree of all sequenced *Microcystis* genomes (Fig. 1). In some cases, WLECC isolates cluster tightly with *Microcystis* strains isolated from other locations around the world. For example, LE19-196.1, LE17-10, LE19-4.1, and LE19-114.1 form a tight clade with strains NIES 87, L211 07 and Sj, which were isolated from Japan, the United States, and Japan, respectively. WLECC *Microcystis* isolates are largely found within groups Mae1, Mae_5, Mfl_ae1, Mflae_ae3, Mae4, Mae3_2, and Mae3_3 (Fig. 1). Groups within this tree were previously described (Dick et al., 2021) and are largely congruent with previously described groups (Pérez-Carrascal et al., 2019).

Within WLECC isolates, the lowest genome-wide average nucleotide identity (gANI) value between two strains was between LE19-84.1 and LE19-8.1 (95.608%) and the highest gANI was between LE17-10 and LE19-4.1 (99.958%). Hierarchical clustering based on pairwise gANI scores revealed that the WLECC comprises two major groups. Within each group, there are several subgroups that have greater than 99% gANI, suggesting closely related or identical strains. For example, in group 1, isolates LE17-10, LE19-4.1, and LE19-196.1 share over 99.9% gANI (Fig. 2). These isolates were collected from various locations throughout the lake, during different months of the year, and in the case of LE17-10, years apart from each other (Table 1), indicating that closely related *Microcystis* strains persist through time and space in WLE. Generally, phylogenomic analyses agree with hierarchical clustering based on pairwise gANI

scores (Fig. 2,3). Phylogenomic analyses indicate 7 distinct clades of *Microcystis* isolated from WLE (Fig. 1,3), further highlighting the extent of the genetic diversity within this system.

We next analyzed the WLECC *Microcystis* MAGs for certain genes of functional interest related to toxin production and/or bloom progression, by comparing the presence or absence of genes between phylogenetically related strains (Fig. 3). Most strains (14/21 strains) lacked *mcy* genes, and therefore microcystin producing potential. Strains that contained *mcy* genes (both partial and complete genotypes) formed a nearly monophyletic clade except for LE19-84.1, which contains a complete *mcy* operon, and is more closely related to strains without *mcy* genes (Fig. 3). The remaining 6 strains form another monophyletic group, with four containing the complete *mcy* operon and 2 that have a partial genotype, in which a partial *mcyA*, complete *mcyB*, and *mcyC* are present (Fig. 3). Of the strains that had the complete *mcy* operon, 2 strains, LE19-84.1 and LE19-195.1, contained a *mcyB* gene with a B1 domain, while 3 strains with the complete *mcy* operon have a B1(C1) *mcyB* genotype in which the C1 domain from *mcyC* replaces the B1 domain, and impacts the type of congener produced (Mikalsen et al., 2003). The B1 adenylation domain incorporates the X-variable amino acid into the microcystin molecule, while the C1 domain and incorporates the Y-variable amino acid (Tillet et al., 2001). The B1 and C1 domains are similar but distinct, with about 91% identity (Mikalsen et al, 2003). LE19-10.1 and LE19-251.1, which contained the partial genotype, both contained an *mcyB* gene with a C1 replacement (Fig. 3B).

Broader gene content also varied across *Microcystis* isolates, with gene presence and absence generally being consistent within clades (Fig. 3A, S3). There was greatest phylogenetic conservation in the presence of genes for decomposition of reactive oxygen species (ROS), nitrogen (N) and phosphorous (P) metabolism, and carbon concentrating mechanisms (CCM).

None of the *Microcystis* strains contained *katG*, an enzyme used for the degradation of hydrogen peroxide (Bernroitner et al., 2009); *katG* was previously shown to be absent from most, but not all cells, in natural WLE *Microcystis* populations (Smith et al., 2022a). However, *t2prx*, a type 2 peroxiredoxin (Schuurmans et al., 2018), was present in every strain analyzed (Fig. S3). Generally, each *Microcystis* strain contained the majority of N and P genes essential for primary metabolism and growth. All *Microcystis* MAGs had genes encoding uptake of nitrate and urea, but seven isolates (LE19-131.1, LE17-20, LE19-8.1, LE19-84.1 LE19-338.1, LE19-388.1, and LE18-22.4) lacked the ammonium transporter gene, *amt* (Fig. 3A). Phosphorus uptake and metabolism genes were largely conserved across *Microcystis,* with a few strains missing *phnD,* which is involved in phosphate transport. However, these strains contained other genes for phosphate transport (Fig 3A, S3).

We also analyzed genes encoding assimilatory pathways in the *Microcystis* genomes (Fig 4A). Many assimilatory pathways, including biosynthesis of certain amino acids (arginine, aspartate, isoleucine, leucine, valine), the Embden-Meyerhof-Parnas pathway, pentose phosphate pathway, and assimilatory ammonia incorporation and nitrate reduction, were complete across most or all strains (Fig 4A, data not shown for Embden-Meyerhof-Parnas pathway found in all strains). Completeness of the methionine biosynthesis pathway was variable; 11 of the 21 strains contained all genes required for methionine biosynthesis by direct sulfurylation via hydrogen sulfide and the *metY* gene product We also evaluated completion of other methionine biosynthesis pathways described by BioCyc annotation of *Microcystis aeruginosa* PCC 9809 and KEGG (Cysteine and methionine metabolism - Reference pathway [map00270]), indicating complete methionine biosynthesis is possible in 11/21 strains (Fig 4A, 4B). Genes for cobalamin (vitamin B12) biosynthesis were consistently present among all strains. Pathways for the

biosynthesis of thiamin (vitamin B1) and biotin (vitamin B7) were partially complete (Fig. 4A), suggesting these strains lack the ability to synthesize these vitamins without metabolic exchange with associated bacteria for precursor molecules.

CCM gene presence and absence was also investigated as variable CCM genotypes can impact fitness in changing bloom conditions (Giordano et al., 2005; Raven et al., 2020; Sandrini et al., 2014). The majority of CCM genes were conserved with some variability in bicarbonate uptake genes *bicA*, *sbtA,* and *sbtB*, which have been suggested to favor variable inorganic carbon conditions (Sandrini et al, 2014, Visser et al., 2016). Ten strains were high bicarbonate affinity specialists and contained only *sbtA* and *sbtB*. These strains were isolated over a wide temporal period from pre-bloom until bloom decline. Only 3 strains, LE19-338.1, LE19-388.1, and LE19-84.1, contained solely *bicA* and are considered high bicarbonate flux specialists (Sandrini et al., 2013); these were isolated during the peak of the bloom (Fig. 3A).

Genes responsible for the synthesis of secondary metabolites were also investigated, as they can be highly variable across *Microcystis* strains and are responsible for the production of secondary metabolites with cytotoxic properties (Dick et al., 2021; Dittmann et al., 2015; Pérez-Carrascal et al., 2019). All strains analyzed contained genes from at least one common *Microcystis* biosynthetic gene cluster (BGC). Aeruginosin and cyanopeptolin genes, which encode metabolites with toxic properties (Ishida et al., 2009, 1999; Martin et al., 1993; Rounge et al., 2007) were commonly detected in WLECC *Microcystis* isolates, observed in 12 and 14 of the 21 genomes respectively. Genes for production of microviridin B were also common – all but 2 isolates (LE17-20, LE19-8.1) had *mdnB* and *mdnD*, while 5/21 had *mdnA*. Piricyclamide encoding genes *pirA* and *pirG,* which have dynamic gene rearrangement and order (Leikoski et al 2012, Welker and Dohren 2008), were more varied among WLE strains in both gene content

and sequence similarity (Fig. 3A). Variation of these genes suggests they may encode for compounds related to piricyclamide rather than the piricyclamide cluster itself.

MAGs from our *Microcystis* cultures were compared to metagenomes generated from samples of the 2014 Lake Erie bloom (Smith et al., 2021; Yancey et al., 2022a, 2022b) and were more representative of field strains than other available *Microcystis* genomes, accounting for much of the *Microcystis* strain diversity observed (Fig. 5). The predominant strains differed by sampling date, site, and size fraction. Strains similar to LE19-41.2, which contains the *mcy* operon, were particularly abundant in early August samples taken near the time a do-not-drink advisory was issued for residents of Toledo, Ohio, USA due to elevated microcystin levels in the municipal water supply. Similar strains were also abundant throughout the rest of that year at site WE12, adjacent to the Toledo water intake crib. LE19-10.1, which contains a partial *mcy* operon, was represented later in the season at all sites, while strains similar to LE19-197.1 and LE19-12.2 were abundant late in the season at sites WE12 and WE2, respectively. In paired samples collected at the same time and site, the strain composition differed between samples collected with an initial >100 μm mesh selection for colonial *Microcystis* and whole water samples collected on 0.22 μm filters without pre-selection. Strains similar to LE19-12.2, which lacks the *mcy* operon, were enriched in whole water samples compared to >100 μm samples. The differences observed between the two size fractions suggest that the >100 μm fraction is not representative of whole water communities and that this size fraction may be biased toward strains that form larger colonies.

3.3 Abundance and Composition of Microcystins

The 5 strains that contained a complete *mcy* operon produced detectable levels of particulate MCs via ELISA measurement, with concentrations varying across strains and to a lesser extent between two independent growth experiments on the same strain (Fig. 6). The 2 strains that contained partial *mcy* operons did not produce MCs above the limit of detection (0.1 μg/L) (Fig. 6A). The 14 strains lacking *mcy* genes, and therefore MC producing potential, did not contain quantifiable amounts of MC, except for LE19-55.1 and LE19-98.1. LE19-196.1, LE17-10, and LE18-13.4 also showed low levels of MC, just above or below detection limits across replicates. Read mapping revealed the presence of just a few sequence reads that mapped to the *mcy* operon from LE19-98.1 and LE17-10 (Table S4), suggesting low level (< 1%) "contamination" of MC producing strains within these cultures.

To further assess if measured levels of MCs via ELISA in strains with the *mcy* absent genotype were due to low-level abundance of *Microcystis* strains with the complete *mcy* operon, RT-qPCR was performed on a subset of isolates. These results revealed quantifiable levels of both *mcyA* and *mcyE* in LE17-20 and LE19-55.1, which had the 2 highest reported levels of MCs in strains heretofore designated *mcy* absent, and detectable levels of *mcyA* gene copies in LE19-196.1 and *mcyE* gene copies in LE17-10 (Fig. 6, S2). Together, these results suggest *Microcystis* strains with the complete *mcy* operon are present in low abundance in LE17-20, LE19-55.1, LE19-196.1, and LE17-10, which are dominated by a *Microcystis* strain with the absent genotype. MCs were analyzed about 3 months after DNA extractions took place, and due to continuous culturing during that time frame, the contaminating strains may have increased in abundance, explaining the substantial abundance of MCs measured by ELISA.

We further analyzed the 5 WLECC isolates with complete *mcy* operons by LC-MS to characterize the microcystin congeners produced. *Microcystis* isolate LE-3 (Brittain et al., 2000)

was included in the analysis for comparison. All 9 congeners screened for were detected in at least one strain with MC-LR being the most common and abundant followed by MC-RR and -YR (Fig. 6B). MC-LR, -RR, -LA, and -YR are the most abundant congeners observed in WLE cyanoHABs in recent years (Chaffin et al., 2021; Palagama et al., 2020). At the time of analysis, LE18-22.4 was producing the greatest amount of MC per congener (over 400 ppb for LR) as well as the greatest diversity with 7 detectable congeners. LE19-84.1 and LE19-41.2 produced 2-4 congeners at detectable levels and were much lower in their concentration compared to other strains (<40 ppb, Fig 6B). In addition to MC-LR, -RR, -LA, and -YR, MC-DAsp-LR, HilR, and -LF were detected in strain LE18-22.4 (Fig 6B). Production of these congeners in culture may be possible due to the abundance of diverse substrates in nutrient replete media and the flexibility of the substrate binding complex in MC biosynthesis enzymes (Tooming-Klunderud et al., 2008).

3.4 Associated Bacteria

Non-*Microcystis* bacteria within the WLE cultures belonged to 34 genera. No two cultures contained identical bacterial communities (Fig. 7) even though some *Microcystis* strains are nearly genetically identical (Fig. 2). The most common associated heterotrophic bacteria, based on presence across the cultures, were known MC degrader *Novosphingobium* sp. (10/21 cultures; Dziga et al., 2013), *Rhabdaerophilium* sp. (5/21 cultures), and *Silanimonas* sp. (4/22 cultures) as determined by mapping reads to MAGs obtained from each sample (Fig. 7). However, taxonomic annotation of sample reads with the k-mer profiling tools Kraken and Bracken (Lu et al., 2017; Wood and Salzberg, 2014) found that *Erythrobacter* (17/21), *Novosphingobium* (12/21), and *Flavobacterium* (12/21) were most abundant across cultures (Fig. S4). These approaches yield slightly different results as the first relies solely on mapping to

completely assembled MAGs specifically from each isolate, while the second may offer a more complete view of culture composition since taxonomy is determined at the read-level and captures taxa that do not assemble or bin well.

In many cases, the associated bacteria were more abundant than the *Microcystis* strains present within each culture (Fig. 7, Table S1), though biases in DNA extraction could influence this result. Associated bacterial genomes were also queried for catalase genes, which degrade hydrogen peroxide, thus potentially ameliorating ROS stress on *Microcystis* (Morris et al., 2008; Smith et al., 2022a). Of the 22 cultures, 17 cultures contained organisms with catalase genes (Fig. 7).

We next examined the genomic potential of associated bacteria to degrade MCs. Nineteen of the *Microcystis* cultures were shown to contain *mlr* genes, which represent a known mechanism to degrade MCs (Bourne et al., 2001), but no single MAG contained a complete *mlr* operon (*mlrA-D*). All four *mlr* genes were detected across multiple taxa in only two cultures, LE19-251.1 and LE19-10.1, which both have the partial *mcy* genotype (Fig. 7). MlrB and MlrC degrade the linearized microcystin product (Dziga et al., 2013) and MlrC is capable of linearizing MCs similar to MlrA (Dziga et al., 2012; Shimizu et al., 2012), suggesting that these bacteria may use intermediate breakdown products as substrates. Of the 7 *Microcystis* strains containing complete or partial sets of *mcy* genes, *mlr* genes were detected in all cultures. MlrD, a putative microcystin transporter or membrane anchoring protein (Bourne et al., 2001), was present in 18 cultures, including all that contained *mcy* genes (Fig 7). The associated bacteria that contained *mlr* genes were distinct across cultures, suggesting multiple taxa have the ability to degrade MC. *MlrA* was detected in three cultures, two with a partial *mcy* operon–LE19-251.1 (*Phreatobacter*), LE19-10.1 (*ELB16-189*), and LE19-12.2 (*Rubrivivax*). *MlrC*, which encodes an

enzyme that can also linearize cyclic MCs (Dziga et al., 2012; Shimizu et al., 2012), was detected in 8 cultures, including both *Microcystis* strains with the partial *mcy* operon and one *Microcystis* strain (LE18-22.4) with the complete *mcy* operon (Fig. 7). The most common associated bacteria with identified *mlr* genes were *Novosphingobium* (11 cultures with *mlrB* and *mlrD)*, *Flavobacterium* (8 cultures with *mlrB* and *mlrD*), *Chryseotalea* (4 cultures with *mlrB* and one with *mlrB and mlrD*), *Gemmobacter*_B (3 cultures with *mlrB* and *mlrC* and one with *mlrB*), and *Silanimonas* (3 cultures with *mlrB* and *mlrD* and one with *mlrB-D*).

4. Discussion

Successfully cultivating axenic *Microcystis* isolates from environmental samples can be labor intensive and often fails to capture the full diversity of organisms observed in natural communities (Schloss and Handelsman, 2005). Due to extensive genetic diversity observed between *Microcystis* strains (Dick et al., 2021; Harke et al., 2016; Humbert et al., 2013), the choice of *Microcystis* culture for experimentation is critical. Here we report the isolation, characterization, and genome sequencing of 21 strains of *Microcystis* from WLE, comprising the WLECC. Although isolation and cultivation methods were largely the same from year to year, we isolated a diverse collection of *Microcystis* strains with variable accessory genomes and associated bacterial communities that capture a large range of *Microcystis* strain diversity (Dick et al., 2021) and colony-associated bacteria (Smith et al. 2021) observed in the WLE. Whereas the 159 publicly available genomes of cultured *Microcystis* are dominated by genotypes with the complete set of *mcy* genes (Dick et al. 2021), with only one partial genotype (Yancey et al., 2022a), the majority of WLECC strains lack *mcy* genes altogether and two strains contain the partial genotype. Further, the WLECC includes a diverse array of *Microcystis* traits (secondary metabolism, CCM, nutrient metabolism) that may be critical in strain succession and community

function in natural environments (Dick et al., 2021). Thus, this collection is a valuable resource for studies of *Microcystis* strain diversity and microbial dynamics in WLE and for the greater cyanoHAB research community as it provides an intermediate between complex environmental samples and pure axenic culture.

Although WLECC cultures contain simplified bacterial consortia relative to complex environmental samples, we encountered challenges in metagenomic data analysis, resulting in draft genome sequences of varying quality (Table S3). However, the majority of *Microcystis* MAGs generated for this study are high quality, with longer contiguous sequence (Table S3) compared to MAGs from environmental samples (Yancey et al., 2022b). Lower-quality draft genomes provide valuable insight into gene content and microbial community diversity, but deeper sequencing and/or long-read technologies would improve assembly quality and enable definitive conclusions about gene absence, gene order, and overall genome architecture. By mapping reads to key genes of interest, we were able to overcome certain binning and assembly challenges, highlighting the importance of targeted approaches like read mapping and high-quality reference genomes. Additionally, genome sequencing revealed that some isolates putatively determined to be "unialgal" via 16S rRNA gene sequencing actually contained genetic diversity at a species and subspecies level. This was evident from a low abundance of *mcy* operon reads (Table S2) in genomes reported to have the absent genotype (Fig. 3A), detectable levels of MCs in those isolates (Fig. 6A), detectable strain heterogeneity via CheckM (Fig. 3, Table S3) and the presence of multiple *mcy* genotypes in a singular culture as evidenced with qPCR (Fig. S2). Further, some of the isolates like LE19-196.1 and LE17-10 have a combination of rare strains with *mcy* genotypes. These results highlight that multiple *Microcystis* strains may be present in cultures derived from single colonies of *Microcystis* picked from the field, and the

potential for the relative abundance of those strains to change over time in culture, underscoring the importance for regular monitoring and purifying of cultures when needed.

The genome sequencing results in this study underscore the substantial genetic diversity observed in *Microcystis* at a subspecies level, highlighting our limited understanding of the extent of genotypic and ecophysiological diversity of this genus. The 21 isolates in the WLECC can be categorized into 12 unique genotypes that form distinct phylogenetic clusters with a minimum gANI of 99.8% between any of the cluster members (Fig. 2,3). Of these 12 proposed strain groups, 6 are represented by more than one isolate and 6 are singletons (Table 1, Fig. 2). The WLECC isolates are scattered phylogenetically across the *Microcystis* tree, interspersed with *Microcystis* isolated from around the world (Fig. 1), consistent with global dispersal of *Microcystis* strains (Marmen et al., 2016; Moreira et al., 2014). Together with our isolation of near-identical strains at different stations and years and their similarity to strains present in the 2014 WLE bloom, these results are consistent with the notion of a persistent pool of *Microcystis* strains with wide spatial and temporal distribution. The WLECC strains are genetically diverse, with variability in N and P metabolism, CCMs, and secondary metabolite genes (Fig. 3) suggesting each strain is specifically adapted to microniches resulting from spatiotemporal environmental gradients observed in WLE (Cooperative Institute for Great Lakes Research, 2019.; Dick et al., 2021). Whether such microniches exist, and what the parameters are that determine strain success remains to be determined. Apart from adaptation to different nutrient conditions among some clades of *Microcystis* (Jackrel et al., 2019), no clear delineation of ecological adaptations has been identified.

Our results also reveal our limited understanding of the multi-faceted controls on differential MC congener production and the need for further studies to constrain the role of both

genotype and associated bacteria. *Microcystis* has the ability to produce a wide range of MC congeners with varying degrees of toxicity (Chernoff et al., 2020; Bouaïcha et al., 2019; Díez-Quijada et al., 2019). Our findings are consistent with previous work that demonstrates the ability of *mcyB* B2 genotypes to produce at least MC-LR, while C1 genotypes may produce at least MC-LR and -RR (Fig. 6B) (Mikalsen et al., 2003). Our results also reveal the ability of these strains to produce several other congeners including MC-LA and -YR, which are observed in natural WLE cyanoHABs along with -LR and -RR (Chaffin et al., 2021, Palagama et al., 2020), as well as others not commonly observed (-LF, -HilR) (Fig. 6B). Previous studies have established *mcy* operon architecture as an important component of differential congener production (Mikalsen et al., 2003; Tooming-Klunderud et al., 2008). However, several other factors can influence congener production such as flexibility of the substrate binding complex (Tooming-Klunderud et al., 2008), N form and availability (Puddick et al., 2014), and available intracellular amino acid substrates (Tonk et al., 2008). Since measurements were completed in culture, abiotic variables were initially controlled for, but biotic transformation of substrates from distinct consortia of associated bacteria were not, which may be responsible for observed differences in congener production. For example, LE19-195.1 and LE18-22.4 are genetically similar (>99.8% gANI) yet produce varying ratios of the 9 measured MC congeners (Fig. 6B). While it is possible these differences may be attributed to subtle differences between *mcy* genotypes, these cultures also contain distinct consortia of associated bacteria (Fig. 7), which may impact substrate availability and metabolic exchange (Baran et al., 2015).

Indeed, the gene content of the WLE *Microcystis* strains suggests possible important interactions with other bacteria. For example, incomplete pathways for methionine biosynthesis in 10 of our cultures may indicate methionine auxotrophy, and previous work implicated

heterotrophic bacteria in supplementing *Microcystis* with methionine (Jung et al., 2022). In turn, the conserved capacity for vitamin B12 biosynthesis across all strains is consistent with evidence that vitamin B12 from *Microcystis* may support heterotrophic bacteria (Smith et al., 2022b). The lack of genes for vitamin B1 (thiamin) biosynthesis in WLE *Microcystis* strains parallels findings in HAB-forming dinoflagellate species, which are suggested to be thiamin auxotrophs (Tang et al., 2010). Cyanobacterial blooms have been implicated in interfering with trophic transfer of B1 (Fridolfsson et al., 2018), but we are not aware of previous studies showing that *Microcystis* lacks B1 biosynthesis.

The WLECC provides a unique means to further investigate this and other possible mutualistic interactions between *Microcystis* and their microbiome. The WLECC captures a breadth of diversity of physically associated bacteria, with minimal overlap between cultures, even for *Microcystis* strains that are genetically similar (Fig. 7). This agrees with previous studies that showed WLE *Microcystis* colonies have different associated bacteria between strains and across time, lacking a "core" or conserved bacterial community found across all strains (Smith et al., 2021). While *katG,* which encodes an enzyme used by cyanobacteria to degrade hydrogen peroxide (Bernroitner et al., 2009; Mutsuda et al., 1996), was absent in every *Microcystis* strain (Fig. 3A), a large portion of associated bacteria in these cultures contained catalase genes, suggesting they are likely important in alleviating ROS stress from the cyanoHAB phycosphere. During periods of rapid growth in WLE cyanoHABs, concentrations of hydrogen peroxide can reach high levels of 600-800 nM (Cory et al., 2016; Smith et al., 2022a), which can cause oxidative stress and cell death in microorganisms (Imlay, 2003; Latifi et al., 2009). Our results are consistent with previous work suggesting "helper" heterotrophic bacteria facilitate rapid growth of cyanobacteria in aquatic systems by decomposing hydrogen peroxide

(Morris et al., 2008; Smith et al., 2022a). Although the proportions of associated bacteria within culture isolates may not reflect the proportions of associated bacteria and *Microcystis* observed in the field, these cultures will provide a useful resource for studying microbial interaction and exchange between known members of the phycosphere.

In contrast to the nearly universal presence of catalase genes among associated bacteria, the distribution of *mlr* genes for MC degradation was patchy with no bacterial genome containing a complete suite of *mlr* genes (Fig. 7). Further, there was no obvious relationship between *mcy* genotype and *mlr* genes present (Fig. 3,7). Previous studies have shown actinobacteria (Manage et al., 2009) and a variety of bacteria isolated from WLE (Krishnan et al., 2020) are able to degrade MCs despite lacking *mlr* genes, suggesting undescribed alternative pathways for biodegradation (Dziga et al., 2017). Additionally, most cultures lack *mcy* genes and MC producing potential, but still encode for the synthesis of other secondary metabolites (Fig. 3A). A recent study has demonstrated the ability of *mlr*- bacteria to degrade both MCs and other cyanobacterial peptides (Santos et al., 2021) suggesting that associated bacteria within the WLECC may be capable of degrading other cyanopeptides beside MC through unknown pathways. Future research should address the substrate specificity of *mlr*-encoded enzymes, as well as cryptic pathways beyond *mlr* that can degrade MCs and/or other cyanobacterial peptides.

5. Conclusion

The WLECC will be a valuable resource for several directions of cyanoHAB research. First, the diversity and variable gene content of strains present opportunities to study how various physiological traits are distributed across phylogenetic clusters of *Microcystis* and how these traits are underpinned at the genetic level. Second, cultures of *Microcystis* strains with variable

arrays of biosynthetic gene cluster content enable studies of the toxicology of diverse secondary metabolites from *Microcystis* and efforts to link "orphan" BGCs (Humbert et al., 2013; Yancey et al., 2022b) with biosynthesized metabolites. These cultures may also be used to study potential toxicological synergisms of various combinations of secondary metabolites (Fernandes et al., 2019; Kust et al., 2020; Pawlik-Skowrońska and Bownik, 2022), which often occur in complex mixtures in cyanobacterial blooms (Beversdorf et al., 2018, 2017). Third, this collection can be used to address how different assemblages of associated bacteria can impact aspects of growth, toxin production and degradation, and colony formation for genetically similar/identical strains of *Microcystis*. For example, LE17-10, LE19-196.1, and LE19-4.1 all belong to strain group 2 (Table 1, Fig. 2), but these cultures consist of very different combinations of heterotrophic bacteria, with only *Novosphingobium* sp. being present in all three. Finally, despite decades of frequent and relatively well studied Lake Erie cyanoHABs, only a few Great Lakes *Microcystis* genomes are available and only one strain, LE-3, is routinely used for laboratory studies. The WLECC greatly expands the number of *Microcystis* strains isolated from Lake Erie, and we demonstrate they are more representative of abundant Lake Erie strains than existing cultures and include highly relevant strains like those responsible for the 2014 Toledo drinking water crisis. Thus, we expect the WLECC to serve as a valuable resource for bridging field-based observations with laboratory-controlled experiments to characterize the dynamics of diverse *Microcystis* strains and their microbiomes.

Declaration of Competing Interest

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data and Culture Availability

Raw reads, and metagenome assembled genomes (MAGs) were deposited at the respective NCBI repositories and are available in BioProject PRJNA903891. Raw reads are

available under the SRA accession numbers: SRR22360640-SRR22360660. Any researcher can submit requests for culture isolates from the WLCC by sending an email to GreatLakesCC@umich.edu.

Figure Captions and Tables

Figure 1: Phylogenetic tree based on concatenation of 26 housekeeping genes of all publicly available *Microcystis* genomes as well as metagenomes from the WLECC. Groups shown by color were previously defined and described by Dick et al., 2021. WLE strains are distributed throughout the tree, clustering with groups Mae1, Mae5, Mfl_ae1, Mfl_ae3, Mae4, and Mae3. WLE strains are bolded and denoted with a green circle. *Anabaena variabilis* ATCC 29413 and *Synechocystis* sp. PCC 6803 were used as the outgroup.

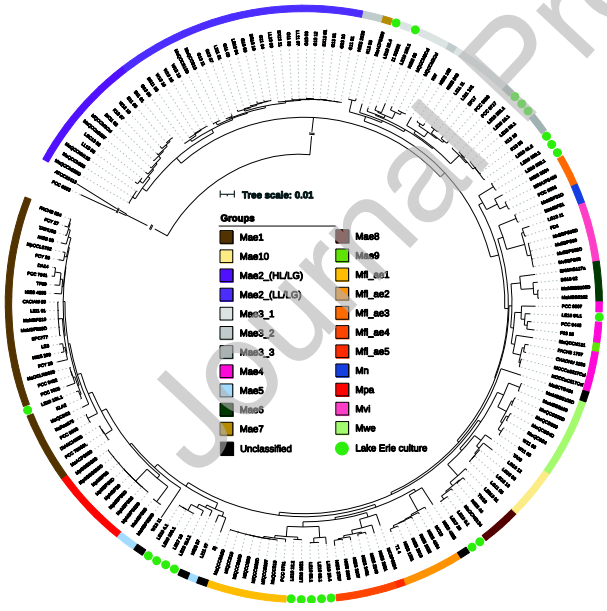

Figure 2: Heatmap of the genome-wide Average Nucleotide Identity (gANI) pairwise comparisons for the WLE *Microcystis* strains. Strain groups 1-12 (see Table 1) are indicated on the bottom.

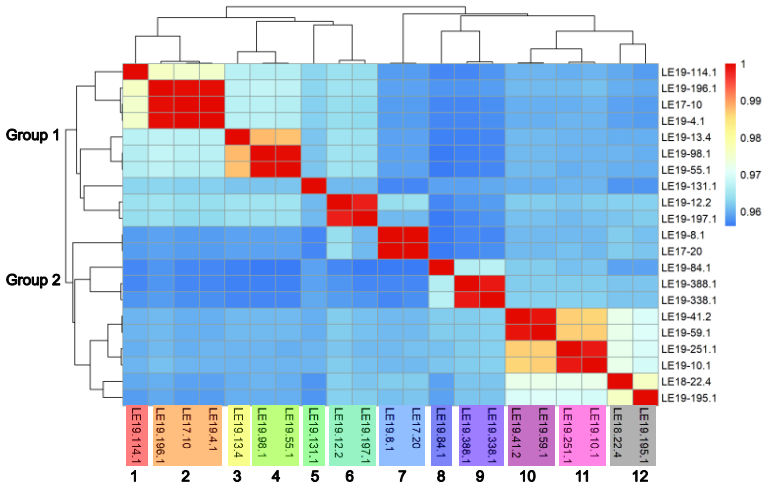

Figure 3: WLE *Microcystis* strains differential gene content (A). The presence/absence of various genes is shown in relationship to strain phylogeny inferred from the concatenated housekeeping gene tree (Fig. 1). Six clade groups are highlighted on the tree. Genes present in MAGs are colored black; to overcome assembly and genomic binning inefficiencies genes not present in MAGs but with >50% coverage by reads are colored gray and absent genes are white for each culture. Only genes with variable presence are shown here, while Figure S3 contains all the searched genes including those present or absent in all cultures. Strain contamination (contamination * strain heterogeneity) determined with CheckM is shown on the right, as strain heterogeneity directly reported by CheckM corresponds to the proportion of contamination predicted to originate from closely related organisms. The partial genotype (yellow) includes truncated *mcyA* and complete *mcyB* and *mcyC*, while the complete genotype (green and purple) includes all *mcy* genes. B) Gene schematics for detected *mcy* operon genotypes. B1) 2 WLE *Microcystis* strains had the complete *mcy* operon with the B2 domain intact in *mcyB* (purple). B2) 4 WLE *Microcystis* strains contained a complete *mcy* operon with a C1 replacement in *mcyB* (green). B3) 2 strains contained a partial *mcy* genotype in which part of *mcyA*, and complete *mcyB* and *mcyC* genes were intact. This genotype also had a C1 replacement.

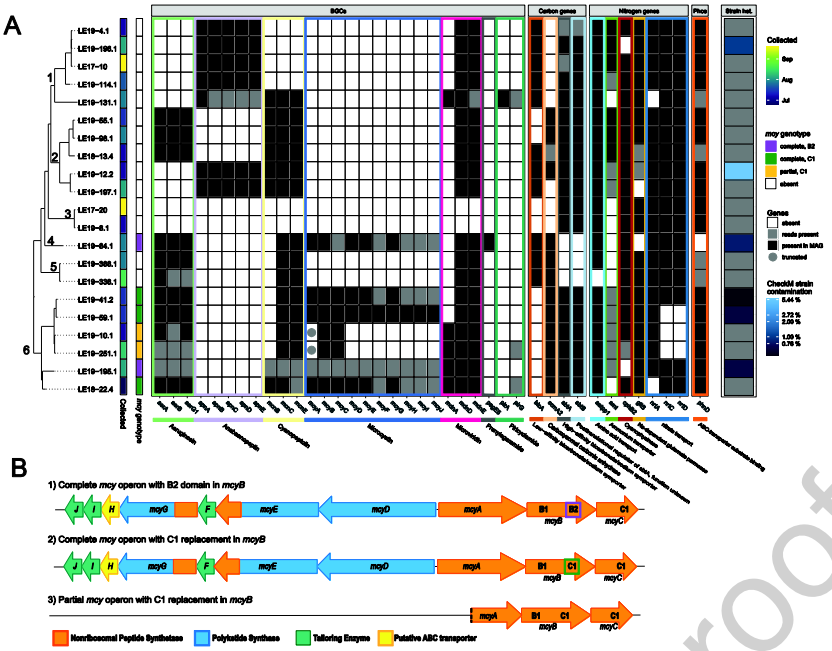

Figure 4: Select metabolic functional pathways for each *Microcystis* isolate. A) Pathway completion percentage is defined as the ratio of genes present for a pathway compared to the total genes required. B) Read mapping to genes was used to further validate completeness of the methionine biosynthesis pathway in each *Microcystis* culture.

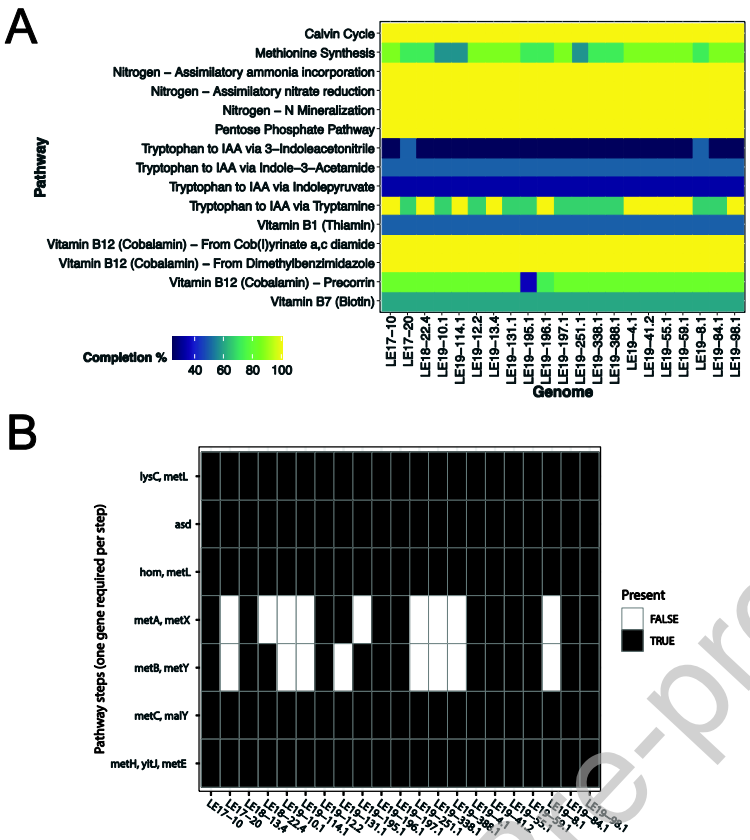

Figure 5: Relative abundance of *Microcystis* cultures in WLE field samples. *Microcystis* MAGs from the Western Lake Erie Culture Collection (WLECC) and the full set of *Microcystis* reference genomes (Other *Microcystis*) were compared via k-mer sketches (k=31, see Methods) to metagenomes obtained from 2014 bloom samples. Genomes in the Genome Taxonomy Database release 202 were also included to determine the non-*Microcystis* proportion of the microbial community (white space). Samples from 2014 were collected from three sites (WE2, WE4, and WE12), and in two size fractions, >0.22 µm (whole water) and >100 µm, which selects for colonial *Microcystis*. The WLECC MAGs are more representative of *Microcystis* in the 2014 bloom than other *Microcystis* genomes and represent a substantial portion of the *Microcystis* community present. The *Microcystis* strain composition varied by date and station and included strains similar to WLECC strains with and without the *mcy* operon.

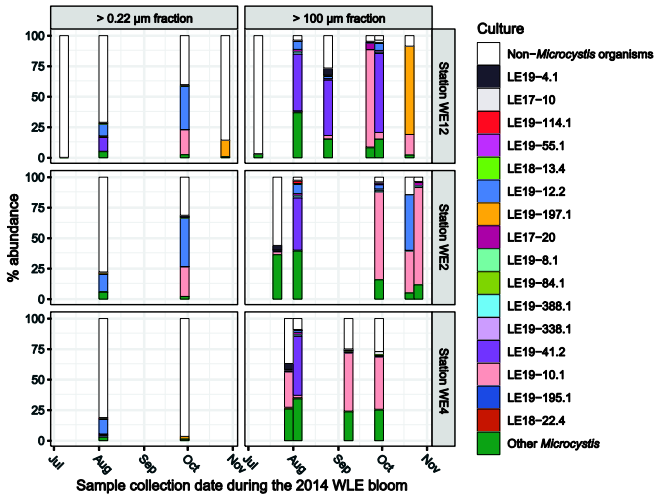

Figure 6: Measured particulate microcystin concentrations in all WLE Culture Collection isolates as measured via ELISA. Strains with the complete *mcy* genotype are shown on the left, the absent are in the middle, and the partial *mcy* genotype containing strains are on the right. Two separate biological replicate measurements were completed for ELISA analysis and are distinguished by gray and black columns. The limit of detection, 0.1 ug/L, is shown on the absent and partial plots with a dotted red line. B) Congener detection and quantification of MCs from LE-3 and the 5 WLE culture isolates with the complete *mcy* operon. The discontinuous Y-axis in panel B serves to better illustrate the broad range of observed microcystin concentrations.

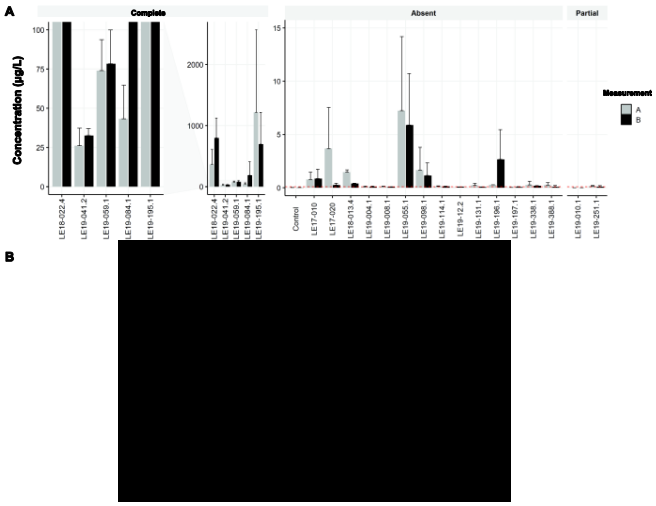

Figure 7: Taxonomy, relative abundance, and key functions of bacteria for which metagenome assembled genomes were retrieved in each *Microcystis* culture. The phylogenomic tree on the left was generated via concatenated single copy housekeeping genes for *Microcystis* as seen in Figure 1 and 3. Some cultures only contain 1 heterotroph (LE17-20 and LE19-84.1) while others contain more than 10 genera (LE19-196.1). Asterisks indicate the presence of catalase genes and a-d represents the presence of respective *mlr* genes, part of a microcystin degradation pathway.

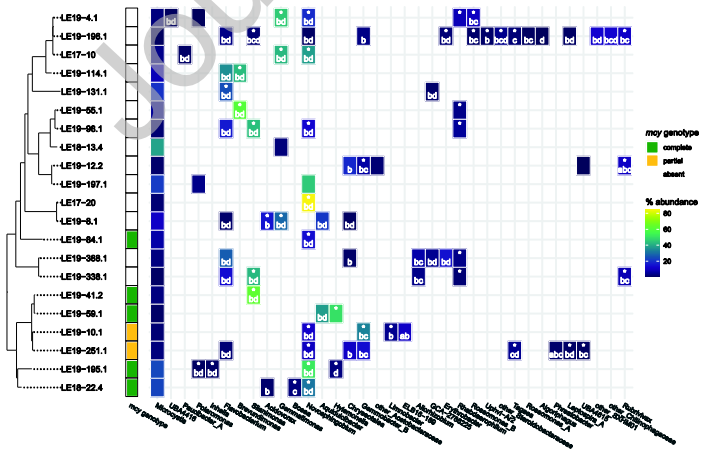

Table 1: Summary of Culture Isolation and Maintenance

Strain group	Culture	Isolation Method	Isolation Media	Collection Date	Isolation Location	*mcy* genotype	Associated bacteria catalase
1	LE19-114.1	Solid 100X Dilution	LE BG11-2N	7/22/2019	WE8	Absent	Yes
2	LE17-10	Solid Dilution	BG11-2N	9/18/2017	WE8	Absent	Yes
2	LE19-196.1	Solid 1000X Dilution	LE BG11-2N	8/5/2019	WE6	Absent	Yes
2	LE19-4.1	Solid 100X Dilution	LE BG11-2N	7/8/2019	WE8	Absent	Yes
3	LE18-13.4	Solid 10X Dilution	WC (1/2X)	6/12/2018	WE2	Absent	No
4	LE19-55.1	Solid 100X Dilution	LE WC (1/2X)	7/15/2019	WE2	Absent	Yes
4	LE19-98.1	Solid 10X Dilution	BG11-2N	7/29/2019	WE4	Absent	Yes
5	LE19-131.1	Solid 1000X Dilution	BG11-2N	7/29/2019	WE8	Absent	Yes
6	LE19-12.2	Solid 1000X Dilution	LE WC (1/2X)	7/8/1919	WE8	Absent	Yes
6	LE19-197.1	Solid 1000X Dilutio	BG11-2N	8/5/2019	WE8	Absent	No

		n					
7	LE17-20	Solid Dilution	BG11-2N	9/18/2017	WE8	Absent	Yes
7	LE19-8.1	Solid 1000X Dilution	WC (1/2X)	7/8/2019	WE8	Absent	Yes
8	LE19-84.1	Solid 100X Dilution	LE WC (1/2X)	7/29/2019	WE4	Complete (B1)	No
9	LE19-338.1	Solid 100X Dilution	WC (1/2X)	8/20/2019	WE6	Absent	Yes
9	LE19-388.1	Solid 100X Dilution	BG11 (no nitrate)	7/29/2019	WE6	Absent	Yes
10	LE19-41.2	Solid 1000X Dilution	LE BG11-2N	7/15/2019	WE2	Complete (C1)	Yes
10	LE19-59.1	Solid 1000X Dilution	WC (1/2X)	7/15/2019	WE12	Complete (C1)	Yes
11	LE19-10.1	Solid 100X Dilution	BG11-2N	7/8/2019	WE8	Partial (C1)	Yes
11	LE19-251.1	Solid 10X Dilution	LE BG11-2N	8/12/2019	WE8	Partial (C1)	Yes
12	LE18-22.4	Solid 10X Dilution	WC (1/2X)	6/25/2018	WE12	Complete (C1)	Yes
12	LE19-195.1	Solid 100X Dilution	LE BG11-2N	8/5/2019	WE6	Complete (B1)	Yes

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Supplemental Tables and Figure Captions

Figure S1: Western Lake Erie cyanoHAB monitoring stations as part of the long-term monitoring efforts by the NOAA Great Lakes Environmental Research Laboratory (GLERL), and Cooperative Institute for Great Lakes Research (CIGLR). WLECC isolates were collected and isolated from WE2, WE4, WE6, WE8, and WE12.

Figure S2: qPCR results using *mcyA* and *mcyE* primers on a subset of WLECC isolates. Isolates selected for analysis were those that had measurable levels of microcystin via ELISA, but were annotated to contain the absent *mcy* genotype. The limit of quantification is depicted by a dashed lined at 80 gene copes/million copies of 16S rRNA.

Figure S3: WLE *Microcystis* strains gene content without removal of fully present and absent genes.

Figure S4: Culture composition by read profiling with Kraken and Bracken

Table S1: List of Genes used to query *Microcystis* and associated bacteria genomes

Table S2: Physicochemical measurements at WLE stations during sample collection for culture isolates

Table S3: Bin statistics for *Microcystis* MAGs: Completion, Contamination, and Strain Heterogeneity are measured using CheckM

Table S4: Read counts for *mcy* genes from *Microcystis* metagenomes. Read counts are normalized by gene length and shown in units reads/kb.

Declaration of Competing Interest

For the manuscript titled *The Western Lake Erie Culture Collection: A promising resource for evaluating the physiological and genetic diversity of* Microcystis *and its associated microbiome* by Colleen E. Yancey, E. Anders Kiledal, Vincent J. Denef, Reagan M. Errera, Jacob T. Evans, Lauren Hart, Dragan Isailovic, William James, Jenan K. Kharbush, Jeffrey A Kimbrel, Wei Li, Xavier Mayali, Helena Nitschky, Catherine Polik, McKenzie A Powers, Sanduni H. Premathilaka, Nicole Rappuhn, Laura A. Reitz, Sara R. Rivera, Claire C. Zwiers, Gregory J. Dick:

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.