

Project Title: Identification of Microcystin Degrading Bacteria in Lake Erie Western Basin and the Grand Lake St. Marys

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The Western Basin of Lake Erie (LEWB) and the Grant Lake St Marys (GLSM) represent two highly eutrophic water systems in Ohio. Despite much Federal and State research and restoration efforts that have been made to regulate and monitor the nutrient loading, periodic nuisance cyanobacterial (blue-green algae, such as *Microcystis*) harmful blooms (cyanoHABs) occur every summer in recent years in these two lakes and with increased affected area, frequency and intensity. One important harmful effect of *Microcystis* blooms is the production and release of microcystin, a hepatotoxin that can be accumulated in invertebrates and cause liver damage of vertebrates, including finfish and human. Heterotrophic bacteria have been suggested as the major agents to degrade microcystin in natural environments. However, the composition and activity of the functional assemblages as well as their regulation factors are not well studied.

HYPOTHESES AND OBJECTIVES

Our proposal aims to study the microbial degradation of microcystin and taxa that carry out this function in the Grand Lake St. Marys and Lake Erie Western Basin.

Hypothesis 1. Cyanotoxin, such as microcystin, can be rapidly degraded by a diverse group of heterotrophic bacteria in lakes that are experiencing frequent harmful cyanobacterial blooms (cyanoHABs).

Objective 1: To estimate and compare microcystin degradation potentials between photo-oxidation and microbial processes in GLSM and LEWB surface water.

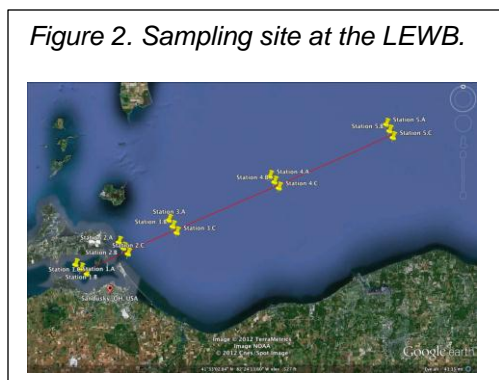
Hypothesis 2. Composition and dynamics of microcystin-degrading bacteria in LEWB and GLSM are different.

Objective 2: To determine the seasonal community structure of microcystin-degrading bacteria in the surface water of LEWB and GLSM using molecular approaches.

Objective 3: To identify environmental factors affecting the distribution and activity of microcystin-degrading bacteria.

METHODOLOGY

Sampling collection and processing. Water samples were taken from the Grand Lake St. Marys (Figure 1) and the Sandusky Bay of Lake Erie (Figure 2) in June and July 2012. Surface



(0.5 m below air-water interface) and bottom water (0.5 m above bottom) were collected (1L) at each sample site. Immediately after sampling, sequential filtration was performed on site to pass whole water samples through 3.0 μm -pore-size and 0.2 μm -pore-size membrane filters. Original water (3 ml) and filtrates (3 ml) that passed through the 3.0 μm -pore-size filter, were preserved using 1% PFA for bacterial cell counting. Filtrates (3 L) that passed both 3 μm -pore-size and 0.2 μm -pore-size membrane filters were immediately cooled on ice and stored at 4 °C before measurements for a number of nutrients. All membrane filters were immediately cooled on ice before stored at -80 °C in the lab for molecular analysis. *In situ* parameters of water temperature, dissolved oxygen concentration (DO), conductivity and pH. Chlorophyll- α concentration were measured using an YSI hydrolab. The photic zone depth was estimated by using a Secchi disk. All samples were taken in triplicates.

Figure 1. Sampling sites and photos taken during GLSM sampling in June 2012. The dark green color of the water indicates an intense *Microcystis* bloom.



Microcosm setup and incubation

Lake water was filtered through 3.0 μm -pore-size membrane filters immediately after sampling, in order to obtain free-living bacterioplankton proportion, and to exclude bacterivores and other large particles. Filtrate was then amended with a mixture of inorganic nitrogen and phosphorus compounds and incubated in the dark at room temperature with occasional agitation for 7 days. This pre-incubation was done to allow the bacterioplankton community to consume labile dissolved organic carbon compounds and to become growth limited by carbon availability.

At the end of the pre-incubation period, microcosms were set up in 20 L acid washed carboys. Two microcosms, designated as MC-1 and MC-2, were constructed of pre-incubated lake water and amended with MC-LR ($\sim 15 \mu\text{g L}^{-1}$, final concentration). Two microcosms, designated as CT-1 and CT-2, served as controls and were constructed of pre-incubated lake water without further amendments. Microcosms were incubated in the dark at room temperature with occasional agitation for a total of 48 hours. At the end of incubation, water was filtered through 0.2 μm pore-size membrane to collect bacterial cells.

DNA extraction and pyrotag sequencing.

DNA was extracted from frozen filters using the PowerMax Soil DNA Isolation Kits (MO BIO Laboratories Inc, Carlsbad, CA) following the manufacturer's instruction. The V6 hyper-variable region of 16S rRNA genes was PCR amplified from extracted DNAs with Illustra PuRe Taq Ready-to-go PCR beads (GE Healthcare, Piscataway, NJ) using primers that were designed specifically for 454 high throughput pyrosequencing. For each sample, triplicate PCR amplifications were performed and the resulting amplicons were pooled and subsequently

examined by gel electrophoresis. PCR amplicons were purified and quantified. Equal quantity of the PCR amplicons from each sample were combined and sequenced using a Roche GS FLX sequencer. Obtained sequences will be annotated using a pipeline established by Mou Lab.

Nutrient analysis.

Concentrations of organic and inorganic nutrients, including dissolved organic carbon (DOC), total dissolved nitrogen (TND), nitrate and nitrite ($\text{NO}_3^- + \text{NO}_2^-$), ammonium (NH_4^+) and soluble reactive phosphorus (SRP) were measured using standard methods for chemical analysis for water and wastewater (APHA, 1999).

Bacterial culturing.

Bacteria in water samples collected from Sandusky Bay and Maumee Bay of Lake Erie and Grand Lake St. Mary's were isolated using the plating method. The obtained isolates were screened for their ability in degrading microcystin using MT2MicroPlatesTM (Manage et al. 2009). Each well of the MT2MicroPlatesTM is carbon limited, pretreated with nutrients and tetrazolium violet dye. Four concentrations (0 $\mu\text{g}/\text{mL}$, 0.1 $\mu\text{g}/\text{mL}$, 1.0 $\mu\text{g}/\text{mL}$, 10.0 $\mu\text{g}/\text{mL}$) of microcystin-LR or microcystin-RR were added to each well depending on the isolate, which acts as the carbon source for the bacteria. Color change of the well indicated that the carbon source (Microcystin-LR or Microcystin-RR) added was being used by the bacteria.

PROJECT PROGRESS AND PRINCIPLE FINDINGS

Most of the lab work has been finished, including the sampling, incubation experiment, obtaining bacterial pure culture, microcystin-degrading bacteria screening, DNA extraction, PCR, bacterial cell counting. We are currently working on performing sequencing analysis of obtained 16S rRNA gene amplicons (expected to finish by early June 2013). Nutrient analyses were delayed due to a foreseen problem with the instrument. The nutrient analysis was expected to finish by July 2013.

Based on incubation experiment, we found that both GLSM and SBLE bacteria have high potential in degrading microcystin. We have also obtained >200 pure culture of bacteria. Of the 50 isolates screened based on the BIOLOG assay, one isolate from Lake Erie was found to degrade Microcystin-LR (Isolate MCLR M26 A; Figure 3). Figure 3 also shows false positives for microcystin degradation, because the negative control wells turned purple even though no carbon source was added (such as MCLR M21 B10). To troubleshoot this issue, re-isolation of bacteria is currently happening. Figure 4 and 5 show average growth curve for MCLR M26 A and MCLR M21 B10 in the BIOLOG wells, respectively. Isolate MCLR M26 A has been confirmed with its ability of MC-degradation and will be sent out for sequencing for taxonomic identification.

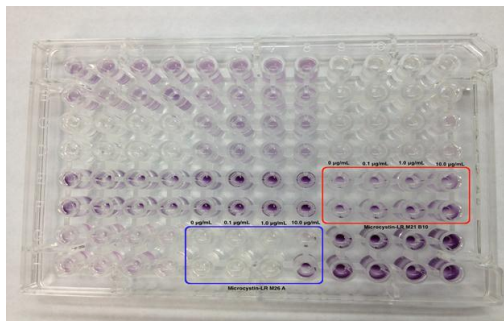


Figure 3: MT2MicroPlate™ results. The red box denotes the microcystin-LR M21 B10 isolate that showed negative results for microcystin degradation. The blue box denotes the microcystin-LR M26 A isolate that showed positive results for microcystin-LR degradation. Each well is carbon limited and is pre-treated with nutrients and tetrazolium violet. Four concentrations (0 µg/mL, 0.1 µg/mL, 1.0 µg/mL, 10.0µg/mL) of microcystin-LR or microcystin-RR were added to each well depending on the isolate, which acts as the carbon source for the bacteria. Color change indicates the usage of microcystin.

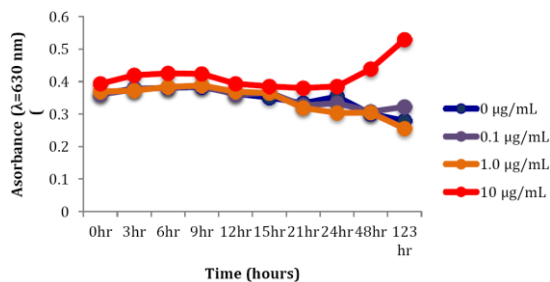


Figure 4: Average growth curve for isolate MCLR M26 A. The concentration 10.0µg/mL shows positive growth. All other concentrations followed the pattern, decreasing over time.

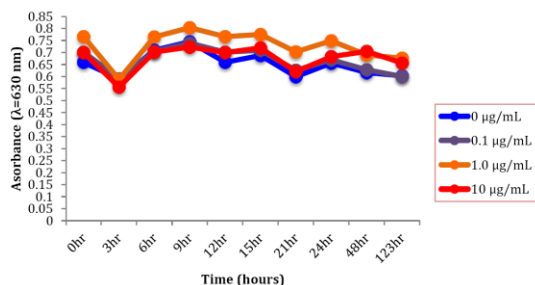


Figure 5: Average growth curve for isolate MCLR M21 B10. This is an example of an isolate screened that had negative results. The concentration 0µg/mL shows a false positive showing color change despite no carbon source was added to that well. All other concentrations followed a similar pattern, decreasing over time.

Publication citations

Mou X, Jacob J, Robbins S, Lu X, Sun S and Ortiz J. 2013. Diversity and distribution of free-living and particle associated bacterioplankton community in Sandusky Bay and Adjacent Waters of Lake Erie Western Basin. *Journal of Great Lakes Research*. Doi: 10.1016/j.jglr.2013.02.014.

Ormiston A and **X Mou**. Temporal and spatial variability of microbial community compositions along a transect from the western basin to the central basin of Lake Erie. International Association for Great Lakes Research Annual Conference 2013. Jun 2013, West Lafayette, IN.

Ormiston A and **Mou X**. Culture-based identification of microcystin-degrading bacteria in the Sandusky Bay and Maumee Bay of Lake Erie. American Geophysical Union 2013 Fall Meeting. Dec. 2012. San Francisco, CA.

STUDENTS SUPPORTED

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