### ABSTRACT

Harmful algal blooms (HABs) have been recorded in Lake Erie for decades, caused by years of eutrophication from agricultural runoff and effluent from municipal waste treatment plants. In the 1970s, the condition of the lake became so poor that the International Joint Commission implemented regulations to diminish phosphorus loading, which greatly improved the health of the lake, but HABs eventually returned to Lake Erie. In August 2014, the city of Toledo, Ohio was forced to issue a "do not drink" order that affected nearly 500,000 residents for three days due to the presence of unsafe levels of the HAB toxin microcystin LR (MC-LR) in drinking water. MC-LR and other cyanobacterial toxins pose significant health risks to humans and the increasing incidences of Lake Erie HABs are a major public health concern. Given that MC-LR is composed of seven amino acids (or modified amino acids), we hypothesized that naturally-occurring Lake Erie bacteria could use MC-LR as an energy source. To test this hypothesis, water samples were collected from HABs in western Lake Erie during the summers of 2014-2017, MC-LR was continuously added to each water sample for 3-6 weeks to select for MC-LR degrading bacteria, and MC-LR levels were quantitated during the experiment. A total of 66 individual bacterial clones were isolated from MC-LR degrading water samples and were genotyped to exclude potential human pathogens. Next, bacterial clones were examined either individually, or in groups, for their ability to degrade MC-LR or microcystin congeners MC-RR and MC-LA, and to form biofilms. Selected groups of bacterial clones also were tested for their ability to degrade MC-LR in lab-scale biofilters. Due to previous studies demonstrating that *mlrABC* were responsible for MC-LR degradation, we next examined our MC-LR degraders for *mlrABC* but these genes were not detected, indicating that alternative MC-LR degradation pathways must be present in Lake Erie bacteria. Collectively, these studies isolated and identified bacterial clones that degraded microcystins MC -LR and MC-RR and these bacterial clones formed robust biofilms that degraded MC-LR on lab-scale biofilters. The results of these studies indicate the potential use of bacterial clones to remove and degrade MC-LR from drinking water.

# THESIS COMMITTEE

Jason Huntley, Ph.D. (Mentor) Robert Blumenthal, Ph.D. Jyl Matson, Ph.D. R. Mark Wooten, Ph.D.

R. Travis Taylor, Ph.D., Graduate School Representative



The University of Toledo College of Medicine and Life Sciences

Medical Microbiology and Immunology (MMI) Track

Department of Medical Microbiology & Immunology



# THESIS PRESENTATION by Alison Thees

April 20, 2018

Identification and Characterization of Lake Erie Bacteria that Degrade the Microcystin Toxin MC-LR

> M.S. in Biomedical Sciences

#### **PUBLICATIONS**

<u>Alison Thees</u>, Ealla Atari, Johnna Birbeck, Judy A. Westrick, Jason F. Huntley. Isolation and Characterization of Lake Erie bacteria that Degrade Cyanobacterial Microcystin Toxins. *Applied and Environmental Microbiology* (Under Revision)

#### AWARDS

Master of Science in Biomedical Sciences Basic Science Tracks Outstanding Student

#### **FUTURE PLANS**

Alison plans to work in the field of contract microbiology.

## INVITED ORAL PRESENTATIONS

<u>Brandel A</u> and Huntley JF. Isolation and Characterization of Lake Erie Bacteria that Degrade the Microcystin Toxin MC-LR. Presented at the International Association for Great Lakes Research (IAGLR) 59<sup>th</sup> Annual Conference, University of Guelph, June 2016, Guelph, Ontario, Canada.

<u>Brandel A</u> and Huntley JF. Characterizing the Ability to Degrade the Microcystin Toxin MC-LR in Bacteria Isolated from Lake Erie. Presented at the International Association for Great Lakes Research (IAGLR) 60<sup>th</sup> Annual Conference, May 2017, Cobo Center, Detroit, Michigan.

# OTHER PRESENTED ABSTRACTS

<u>Brandel A</u> and Huntley JF. Isolation and Characterization of Lake Erie Bacteria that Degrade the Microcystin Toxin MC-LR. Presented at the 23<sup>rd</sup> Annual Midwest Microbial Pathogenesis Conference (MMPC), University of Illinois at Urbana-Champaign, September 2016, Champaign, Illinois.

<u>Brandel A</u> and Huntley JF. Isolation and Characterization of Lake Erie Bacteria which Degrade the Microcystin Toxin MC-LR. Presented at the 2017 Graduate Research Forum, University of Toledo Health Sciences Campus, March 2017, Toledo, Ohio.

<u>Brandel A</u> and Huntley JF. Isolation and Characterization of Lake Erie Bacteria that Degrade the Microcystin Toxin MC-LR. Presented at Understanding Harmful Algal Blooms: State of the Science Conference, Sponsored by Ohio Sea Grant, The Ohio State University, and U.S.D.A., Stranahan Theater, September 2017, Toledo, Ohio.