

Report for 2005IN174B: Rapid Detection of Toxic and Taste and Odor Causing Cyanobacteria in Indiana Surface Water

Publications

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 - Britton, C. H., R. E. Pruitt, and C. A. Lembi. 2005. A rapid, quantitative detection assay of cyanobacteria. Abstract booklet produced by the Aquatic Plant Management Society, July 10-13, 2005, San Antonio, TX, page 35.

Report Follows

Title: Rapid Detection of Toxic- and Taste and Odor-causing Cyanobacteria in Indiana Surface Waters

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Problem and Research Objectives: The invasion of *Cylindrospermopsis raciborskii*, a toxin-producing cyanobacterium, into Indiana and other Midwestern waters has caused great concern among state agencies and drinking water providers. At the present time, water treatment involves the use of copper-containing compounds to temporarily reduce the *C. raciborskii* populations. A statement from the Indiana Natural Resources Commission (2003) indicates the concern of the state of Indiana regarding copper: **“In 1989 and for several years since 2000, all major surface sources of drinking water for the city of Indianapolis have been chemically treated to reduce populations of blue-green algae, at a high cost to the utility and its customers, as well as incurring the risks to the ecosystem associated with the use of herbicides.”**

The ability to rapidly detect small numbers of *C. raciborskii* and *P. limnetica* cells before they reach peak populations could greatly reduce the amount of copper used in-water and the amounts of activated carbon and chlorine used at the drinking water treatment plant. It could also greatly enhance ecological studies of these organisms, including their responses to nutrient reduction strategies. Early and rapid detection at the current time is not possible because 1) the filaments are extremely small (the width of a *C. raciborskii* filament is only 2-3 μm wide), making detection with light microscopy extremely difficult, even for experts, 2) other phytoplanktonic species can easily be confused with the two, and 3) sending water samples for identification to a lab such as Phycotech is expensive (>\$150/sample) and time-consuming and beyond the financial ability of managers of surface waters not used for drinking.

The purpose of this project is to develop a quantitative detection method for *Pseudanabaena limnetica* and *Cylindrospermopsis raciborskii* using 5'-exonuclease PCR, which measures the release of a fluorescent dye attached to an oligonucleotide probe in response to amplification of a specific target sequence. This method provides a quantitative measure of the target DNA found in the sample as well as a greater degree of specificity than methods that quantify the total amount of DNA amplification by using a dye that binds non-specifically to all DNA amplified. A properly developed and calibrated assay can be used to accurately calculate the concentration of the cyanobacterial cells present in collected water samples. The project has two primary objectives:

Objective 1 is to verify or determine the DNA sequences for a number of PCR products amplified from *Cylindrospermopsis raciborskii* and to utilize this information to design species-specific 5'-exonuclease PCR assays.

Objective 2 is to validate these new assays using highly purified DNA from cyanobacterial cultures, crude DNA from cyanobacterial cultures, and crude DNA prepared from cyanobacterial cultures mixed with environmental water samples.

Methodology and Principal Findings:

Objective 1: Regions of the cyanobacterial genome that were specific to *C. raciborskii* were found using gene databanks and computer alignment programs. We decided to use a conserved region within the *nifH* gene (associated with nitrogen fixation). We then tested four different DNA extraction methods (with a number of variations) to determine the most reliable method to obtain Real Time PCR-quality DNA from lake water samples. We finally settled on the MoBio UltraClean Soil DNA Isolation Kit. We found that this extraction kit was reliable and sensitive enough to detect cell concentrations within a range of 5000 to 100,000 cells/mL

Using Primer3 software, we focused on a region within the *nifH* gene and tested primers for that fragment for their specificity to *C. raciborskii* over other cyanobacteria (*Anabaena*, *Microcystis*, *Pseudanabaena*) using Sybr Green analysis. The *C. raciborskii* sample amplified after the fewest cycles, showing that the *C. raciborskii* primers were most specific for *C. raciborskii* DNA. In order to more stringently follow the guidelines for ABI's quantitative PCR protocol, we used PrimerExpress software to find a more suitable primer/probe set. Alignment of the primer/probe to known DNA sequences, as well as conventional PCR analysis, allowed us to do this.

Objective 2: Now that we have found a method to extract quality cyanobacterial DNA directly from the lake water and have developed the species-specific primers and probe, we have begun to test the sensitivity and the accuracy of our assay. However, a problem of contamination has arisen during PCR. After attempting to resolve the contamination by switching water sources, buffers, ordering new primers, etc. we have still been unsuccessful in locating the cause of bands in the negative controls. Most recently, the PCR product has been successfully cloned into *E. coli*. From this, sequencing is being conducted to identify whether or not our negative control product is indeed *C. raciborskii* contamination or if it happens to be a random product of similar size. Once the contamination source has been identified, we will be able to resume our calculations of sensitivity and efficiency for the assay.

Significance of the Project: The funding provided by the IWRRC allowed us to begin the process of isolating, identifying, and quantifying the populations of *Cylindrospermopsis raciborskii* in lake water using DNA. Initially, the hardest part was finding a DNA isolation technique that would work in our study lake (Lake Lemon in Monroe Co.), because water constituents such as tannic and humic acids appeared to be inhibiting the assay. This is why we eventually settled on a soil DNA isolation procedure, which effectively removes these types of contaminants. Currently, we are working to solve a second problem that involves a PCR contaminant.

Once the procedures have been developed for *C. raciborskii*, we will then extend them to *Pseudanabaena limnetica*, a cyanobacterium that has caused major taste and odor causing problems in drinking water reservoirs. The early detection of this organism could be of great benefit to water treatment facilities throughout the U.S.

An additional benefit of the IWRRC funding was that it allowed us to sample, on a monthly basis, the *C. raciborskii* populations in Lake Lemon, IN. We now have depth distribution data and water quality parameters at two locations in the lake each month since June 2005. This will be the first time a population of this potentially toxic alga has been monitored, even over the winter months, in a temperate lake in the U.S. In addition, we have begun isolating strains of *C. raciborskii* from Lake Lemon and other Indiana lakes, which, with DNA and toxin characterization, will allow us to eventually study the geographic distribution and potential for toxin production of individual strains in the state.

Student: Clay Britton (graduate student; Ph.D. candidate) was supported on this project and conducted all of this research.

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