A Comparison of Cyanobacterial Abundance and Potential Toxicity in Two California Coastal Lakes

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Abstract

Cyanobacteria produce complex cyanotoxins, such as the hepatotoxin microcystin. This project assesses the presence and potential toxicity of cyanobacteria in environmental water samples from Pinto Lake (Watsonville, CA) and Loch Lomond (Santa Cruz, CA). Methods used in this study to determine toxin gene presence and intracellular toxin concentrations include the Polymerase Chain Reaction (PCR), Enzyme Linked Immunosorbent Assay (ELISA), and a modified Utermohl phytoplankton microscopy technique. Preliminary data show higher overall cyanobacteria abundance, presence of the mcyB gene, and elevated intracellular toxin levels in Pinto Lake in relation to Loch Lomond. These data confirm the presence of toxic cyanobacteria in eutrophic Central California Coastal lakes along the Monterey coast.

Introduction

Cyanobacteria are present in most aquatic ecosystems and are essential for many biogeochemical processes including atmospheric oxygen production and the recycling of carbon, nitrogen, sulfur, and phosphorus (Falkowski 2008). In some cases, harmful algal blooms comprised of cyanobacteria release an array of potent hepatotoxins, neurotoxins, cytotoxins and endotoxins which have been linked to liver failure, cancer, and death in humans and animals (Bruja et. al. 2001).

In the Monterey Bay region a variety of cyanobacteria taxa including *Microcystis*, *Aphanizomenon*, *Anabaena*, and *Woronichinia* (Figures 1A-E), have been identified as potential producers of the cyanotoxin microcystin (Figure 2), a cyclic hepatotoxin. Microcystin is synthesized via a non-ribosomal mechanism which brings together products from a complex gene cluster that includes 10 genes called *mcyA-mcyJ* (Figure 3).

Figure 1A: *Anabaena* sp.

Figure 1B: *Aphanizomenon* sp.

Figure 1C: *Microcystis* sp.

Figure 1D: *Aphanizomenon/Anabaena*

Figure 1E: *Woronichinia* sp.

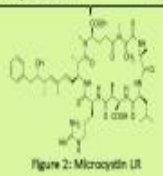

Figure 2: Microcystin LR

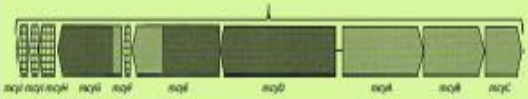

Figure 3: Diagram of the bidirectional mcy gene cluster. Image copyright © 2008, American Society for Microbiology

We used the Polymerase Chain Reaction (PCR) to determine presence or absence of cyanobacteria (16S-rRNA) and the mcyB gene. In addition, Enzyme Linked Immunoabsorbance Assays (ELISA) were used to gauge intracellular toxin levels, and microscopy identified the various cyanobacterial taxa in the samples to determine the presence of cyanobacterial species in Loch Lomond and Pinto Lakes, located in Central California. While much CHAB research has been focused in the Great Lakes Region, the connection between cyanobacterial toxin levels, seasonal variability, and cell density has not been well studied in the Monterey Bay Area. (Figure 4).

This study was designed to assess the abundance of potentially toxic cyanobacteria blooms over a 12 month period in two distinct freshwater habitats using an ecology and molecular biology interdisciplinary approach. This information will assist water management agencies by identifying potentially toxic blooms using molecular and ecological techniques in the Monterey Bay Region.

Methods

Sample Collection and Filtration

- 1L surface samples were collected weekly from Pinto Lake and Loch Lomond Reservoir
- For cyanotoxin analyses 100-300 mL were filtered onto cellulose filters from each sample and stored at -20°C in glass vials.
- For DNA analysis 40 mL of sample was filtered onto 0.2 µM polycarbonate filter and stored at -80°C.
- For cyanobacteria identification and enumeration 50 mL aliquots of unfiltered sample were preserved using a 2% Lugol's iodine solution and stored at 4°C.

Figure 4A: Pinto Lake, Watsonville (CA)
Notice: Agriculturally dominated

Figure 4B: Loch Lomond Reservoir, Santa Cruz (CA)
Notice: Protected Forested

Microscopy

- Cyanobacteria identification based on morphological traits was completed using an Olympus IX71 inverted microscope and a modified Utermohl phytoplankton counting method.
- Preserved samples were centrifuged at 7K RPM for 5 minutes, and 1 mL of pelleted concentrate was viewed in a Sedgewick-Rafter counting cell at 100x magnification.
- To estimate cell count the natural unit counting protocol devised by Watzin et al.(2006) was used.
- Average cell lengths and areas were used to calculate the abundance of each cyanobacterial genus throughout the sampling period.

DNA extraction and PCR

- DNA from filtered field samples was extracted using the Qiagen DNeasy plant kit (Germantown, MD).
- The PCR was run at standard conditions identified in the primary literature.
- The PCR products were run on a 2.0% agarose gel with a 100 bp ladder at 70V for 90 minutes.

Table 1: Primers used in this study

Primer	Assay	Expected Length
Cyan395F	16S-rRNA	450 bp
Cyan781Ra	16S-rRNA	
Cyan781Rb	16S-rRNA	
mcyB2959F	mcyB	320 bp
mcyB3278R	mcyB	

Intracellular Toxin Assay / ELISA

- Filtered samples were extracted using 50% methanol, manually broken up, and sonicated.
- The extract was diluted to less than 5% methanol in DI water
- 20ul of diluted extract was used for ELISA using the Envirologix Enzyme Linked Immunosorbent Assay (ELISA) QuantiPlate Kit for microcystins and nodularins (Assay quantification range: 0.16 to 2.5 ppb; detects microcystins LR, LA, RR, and YR).

Results

Preliminary results of intracellular toxin levels highlight the large difference in toxin concentrations between Pinto Lake and Loch Lomond. Both Loch Lomond and Pinto Lake experienced large blooms of similar taxa throughout the sampling period (Figures 5A &B).

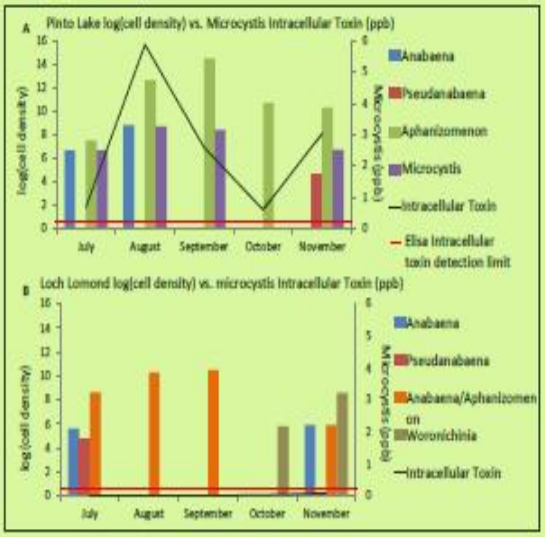

Figure 5A & B: Abundance of cyanobacteria taxa (cells/mL) with a natural log correction, Secondary axis is intracellular toxin levels (ppb) per month. *note: 4(B) toxin levels never above detection limit.

The cyanobacteria specific 16S-rRNA and *mcyB* genes were present in both Pinto Lake and Loch Lomond (Figure 6A & B).

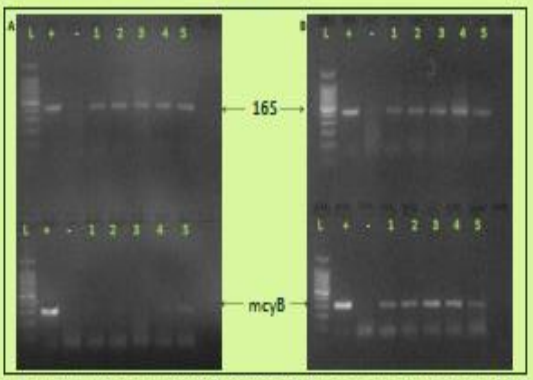

Figure 5A & B: Upper lanes are 16S-rRNA PCR products and bottom lanes are mcyB product. Numbers indicate months with 1: July, 2: August, 3: September, 4: October, 5: November. Loch Lomond Figure 5A shows 16S-rRNA present in all samples and a faint mcyB band was present only in the November sample. Pinto Lake Figure 5B shows 16S-rRNA and mcyB bands in all months.

Discussion

Pinto Lake, a eutrophic shallow lake set in a rich agriculture-dominated watershed, demonstrated a higher overall abundance of cyanobacterial cells, higher abundance of cyanobacteria-containing toxin genes and experienced toxin levels that exceed WHO recommendations for 1 ppb in drinking water. In contrast, Loch Lomond, a mesotrophic reservoir set in an undeveloped, forested watershed, demonstrated a lower overall cyanobacterial abundance, intermittent presence of the cyanobacteria toxin gene mcyB, and toxin levels that were below 1 ppb. These results emphasize that very different freshwater habitats can produce similar cyanobacterial harmful algal blooms. In addition, these data can be used to inform water management agencies about potential seasonal patterns in bloom formation and the presence of cyanobacterial toxins in water bodies used by local communities.

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