Comparison of Ionic and Molecular Chemicals to Control Harmful Algal Blooms

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Introduction

In freshwater environments, cyanobacterial harmful algal blooms (cHABs) occur when blue-green algae (cyanobacteria) grow in eutrophic conditions and begin to produce toxins. cHABs cause extensive economic and ecological damage when they occur, costing the United States over $1 billion in the last 10 years. Eutrophication of freshwater environments across America coupled with global warming and increased atmospheric carbon dioxide is increasing the occurrence and intensity of cHABs (**Figure 1**). These environmental conditions that are promoting cHAB growth are complex challenges on their own, and while addressing them would indirectly solve exponential cHAB growth, they won't be immediately fixed soon, and methods to control cHAB growth directly must be explored. Currently there are few methods to control or prevent the growth of cHABs, there are no methods that address toxin production by cyanobacteria.

Figure 1. cHAB event. Captured at SIUC Campus Lake in June 2021

Microcystis aeruginosa is one of the major contributors to cHABs in fresh and brackish waters. This microorganism, and others like it, are of specific concern because they produce toxins. *M. aeruginosa* produces a modified polypeptide toxin called microcystin. The physiological importance of this compound to cyanobacteria is unknown, though the toxin poses a major threat to eukaryotic life. Beaches, lakes, and ponds have been closed because livestock, pets, and humans have died from drinking toxic cHAB contaminated water.

The goal of this study is to examine different chemical techniques of controlling microcystin production from *M. aeruginosa*. This study compares the effects of the ionic permeability compound $CaCl_2$ and a designer antisense oligonucleotide (ASO) (**Figure 2**) which was developed by Dr. Brooks and Dr. Hamilton-Brehm from Southern Illinois University of Carbondale.

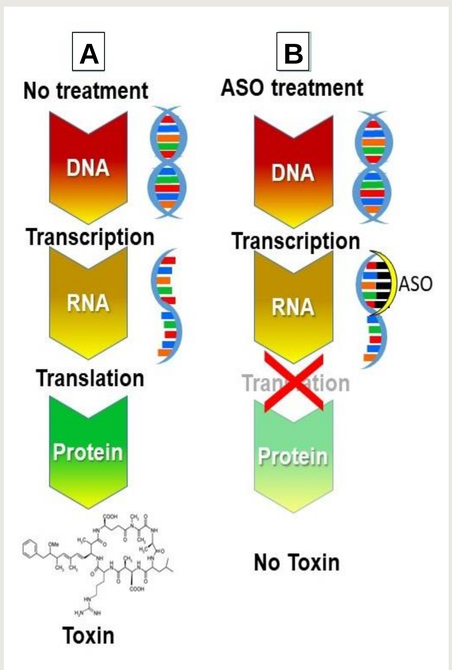

Figure 3. Concept diagram of how ASO's affect central dogma. A) typical pathway DNA -> RNA -> Protein, B) introduction of an ASO complimentary to RNA disrupts the process for making protein product.

In eukaryotic systems, ASO technologies can successfully block messenger RNA transcripts from producing their product (**Figure 2**). They have never been applied to cyanobacteria before, as is done here to stop the production of microcystin in *M. aeruginosa*. Cultures of *M. aeruginosa* were grown in triplicate and monitored by direct cell enumeration and absorbance, and the toxin production quantitatively analyzed by an ELISA microtiter plate assay. Cultures were treated upon reaching 10^7 cell/mL density with distilled water, ASO, ASO + $CaCl_2$, and $CaCl_2$ treatments. Growth of cells were calculated in correlation to measure production of microcystin over the course of 72 hours.

Results

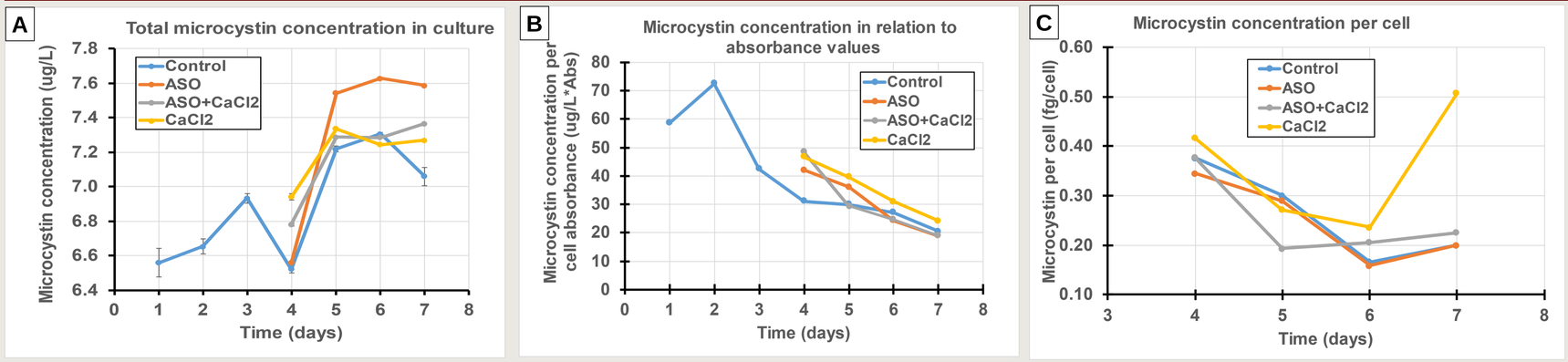

Figure 3. ELISA assay results. A) Microcystin concentration per liter. B) Microcystin concentration related to absorbance. C) Microcystin concentration per cell.

Methodology and Investigation

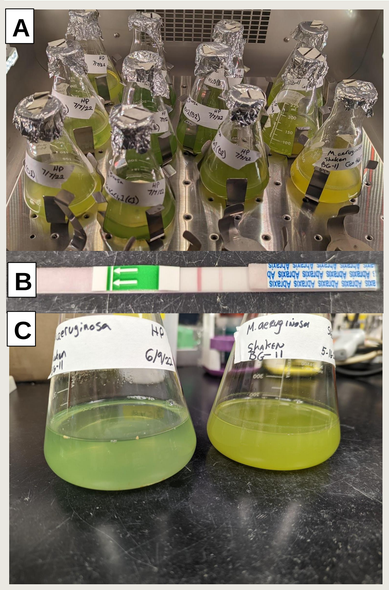

Figure 4. *M. aeruginosa* cultures and microcystin test strip. A) typical cyanobacterial growth under 12-hour light/dark cycle. B) Microcystin confirmation by test strip. C) healthy culture have a bright green color, while 'old' or sickly cultures begin to lose their brightness.

Analytical determination of microcystin production. *M. aeruginosa* cultures were cultured in standard BG-11 medium until 4, 6, and 13 days and assayed for presence of microcystin (**Figure 4A**). Eurofin antibody-based test strips were used to qualify presence of microcystin (**Figure 4B**). Further study revealed that the toxin is associated with the cells and not the supernatant at time points 4 and 6 days (data not shown). Cultures of *M. aeruginosa* need to be maintained and fresh media transfers to prevent culture collapse (**Figure 4C**).

Growth of *M. aeruginosa* cultures

Using aseptic technique, 15 serum bottles of *M. aeruginosa* were grown to a density of 10^7 cells/mL over the course of 4 days (**Figure 4A**). In triplicate flasks were sampled for cell enumeration, absorbance (685nm), and ELISA. Sampling occurred on days 1, 2, and 3. On 4th day, three treatments were administered to each flask. The treatments included distilled water (control), 1X ASO, 1X ASO + 20mM $CaCl_2$, and 20mM $CaCl_2$. Samples continued for three days after resulting in a post treatment of 24, 48, and 72 hours.

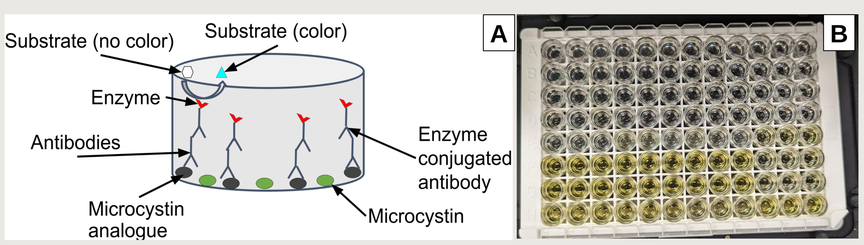

Figure 5. Microcystin ELISA diagram and plate. A) Explains how the ELISA test works to quantify microcystin levels within a sample. B) Example of ELISA plate with samples.

Microcystin quantification through ELISA assay

Total microcystin levels of each sample collected were quantified using a Eurofin Abraxis microcystin-ADDA ELISA microtiter plate (**Figure 5**). ELISA assays function as a congener-independent competitive method for microcystin detection based on recognition of the congener by specific antibodies.

Standard solutions of microcystin were assayed in triplicate to generate a standard curve by which the total concentration of microcystin from all experimental sample concentrations were calculated from. (**Figure 3A**). Toxin concentrations were then evaluated against cell absorbance at 685nm and direct enumeration by cell counting chamber (**Figure 3B and 3C**).

The challenge becomes how to attribute the concentration of microcystin to the number of cells. Cell density by absorbance may not be precise enough and portrays a false impression that toxin is decreasing. When the toxin concentration is adjusted by direct cell counts by cell counting chamber the results appears to be more accurate to culture performance. Unexpectedly, the control follows a decreasing trend of toxin production over 48-hour time period. After 72 hours all cultures seem to exhibit a trend to higher toxin concentrations. Interestingly, at the 24-hour time point the ASO + $CaCl_2$ combination appears to have the lowest reported toxin.

Conclusion and Future

- Administered exclusively, $CaCl_2$ is not a suitable agent for ionic permeability in *M. aeruginosa.*
- Combined administration of $CaCl_2$ with ASO resulted in lower microcystin production than other treatments.
- $CaCl_2$ appears to increase microcystin production in *M. aeruginosa*.
- Other methods of delivering ASO treatment should be explored in future studies.
- Absorbance values are not reliable for cell enumeration due to lack of specificity to *M. aeruginosa*.
- Other methods for enumeration should be explored for future studies.
- Future studies should be conducted to observe the timeline of microcystin production, to determine the time frame in which microcystin begins associating in extracellular media instead of within the cell.

Acknowledgements

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