DO SINGLE CELL CYANOBACTERIAL BLOOMS IN CONESUS LAKE POSE A RISK TO PUBLIC HEALTH?

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Abstract:

Previously unreported blooms dominated by single-celled cyanobacteria have taken place in Conesus Lake, NY since 2015. The dominant bloom organisms are range in size from about 1.0-2.0 µm and are thus referred to as picocyanobacteria. During the summer 2020, a bloom of picocyanobacteria persisted from mid-July to mid-August, reaching peak cell densities of 3.5 x 10^5 cells/mL. Field samples were filtered through 1.0 micron filters and grown in Alga-Gro liquid culture media at 25°C. Samples were plated in Alga-Gro agar plates and individual colonies were used to grow additional liquid cultures. DNA obtained from these cultures was sequenced and the results were analyzed by Dr. Logan Peoples using bioinformatics techniques. The dominant autotrophs in culture were a previously unreported strain of *Synechococcus* sp., most closely related to a strain from estuarine waters (15 ppt) in the Chesapeake Bay. The heterotroph *Sediminibacterium* sp.(Phylum Bacteroidetes) was also present in the sample. Culture samples tested under different salinities grew well at 2.5 ppt and freshwater media controls but not in 5 or 10 ppt media, indicating that the species must live exclusively in freshwater. Experiments to grow monocultures of the heterotrophic species are currently taking place.

Introduction:

Harmful Algal Blooms dominated by toxin-producing cyanobacteria (also known as blue-green algae) are a major problem in lakes world-wide. This study is focused on the algal blooms in Conesus Lake, NY (*Figure 1 & Figure 2*). The specific colonial species of toxin-producing cyanobacteria are commonly colonial filamentous forms such as *Microcystis* and *Dolichospermum*. Species in these and other genera are known to produce a large range of neurotoxins and hepatotoxins. These toxins can cause serious side effects to humans such as rashes, upper respiratory issues, vomiting and even chronic effects like liver, kidney, and nervous system damage (Harmful Algal Blooms 2019). During July of 2020 and 2019, new cultures of single cell cyanobacteria were isolated from Conesus Lake bloom.

The objectives of our study are the following: (i) to identify the dominant 2020 photosynthetic and heterotrophic isolates using DNA extracted from mass cultures followed by sequencing in the Microbial Genome Sequencing Center (Pittsburgh, PA) and (ii) to characterize the 2019 and 2020 isolates in terms of their salinity and temperature responses. Experiments to determine temperature preferences and to selectively isolate heterotrophic strains are presently under way.

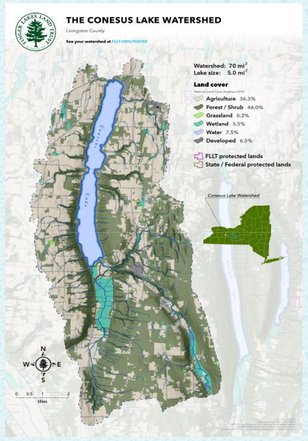

Figure 1. Map of Conesus Lake watershed (https://www.fllt.org/maps-the-finger-lakes-watersheds/)

Figure 2. Algal bloom on Conesus Lake (https://www.dec.ny.gov/docs/water_pdf/conesushabplan.pdf)

Methods:

(i) Isolation and Sequencing of Genome

Cultures from 2020 samples were successfully plated in Agar made with AlgaGro Media (Carolina Biological Supply Co.). and colonies growing on the plate were identified microscopically using a fluorescence filter fitted with a Texas Red filter set. Colonies identified as having autotrophic single cells were transferred individually into test tubes filled with autoclaved growth media. To extract the DNA from the cyanobacteria, we used a DNeasy Power Water Kit (Qiagen) and followed this manufacturer's protocol. Extracted DNA was measured using a Nanodrop Spectrophotometer and a sample with DNA concentration greater than 10 µg per µL was shipped the Microbial Genome Sequencing Center (MiGS) in Pittsburgh, PA for analysis. The results from the MiGS were then processed by Dr. Logan Peoples using bioinformatics techniques.

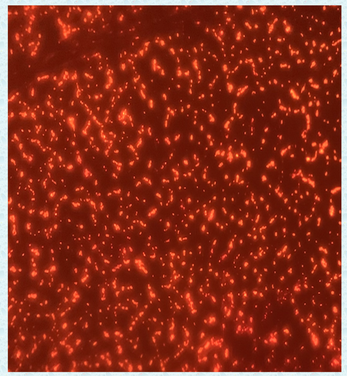

Figure 3. 2019 Conesus Lake *Synechococcus* isolate under fluorescence microscopy (400X).

(ii) Salinity

The growth rate of 2019 isolates under different conditions of salinity were observed at regular intervals during a 10 day experiment. Nine test tubes were filled with media made to specific salinities using NaCL. Three replicate tubes of 0ppt, 2.5ppt, 5.0ppt and 10ppt were used for the experiment. A HOBO sensor and datalogger were used to check the conductivity and salinity was calculated from these values. Each test tube was inoculated from a culture to an initial concentration of 0.16 x 10^6 cells per mL as determined by fluorescence microscopy using a hemocytometer (*Figure 3*).

(iii) Light Response

Subcultures of 2020 samples were grown in the dark on media-free agar plates and on plates with heat killed *Synechococcus* added to the agar. The *Synechococcus* agar plate was made by filtering 50 mL of a culture onto a 0.45 micron Nucleopore membrane filter, allowing smaller bacteria to travel through while retaining most of the *Synechococcus* (*Figure 4*). The cells were then resuspended in distilled water by shaking followed by vortexing to assure maximum resuspension. This solution of organic material was then added to 1g of Agar and 100 mL of distilled water. The control also received a 1g of agar and distilled water but not *Synechococcus* organic material. Two 2020 subcultures were then plated on the control and experimental media plates using a triple streak and experimental plates were placed in a dark box at 24 C. Control plates (for autotroph survival) were grown in light. Plates were observed and growth recorded after a week.

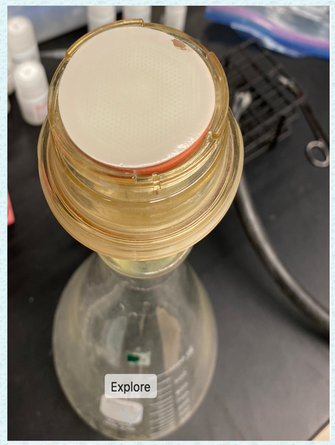

Figure 4. Filter covered in *Synechococcus* cells that were used as organic enrichment for agar plates. Control plates were made with distilled water.

Results:

(i) Isolation and Sequencing of Genome

The DNA samples, analyzed by Dr. Logan Peoples, showed that the dominant autotroph were the single-celled cyanobacteria *Synechococcus* sp. and the heterotroph was *Sediminibacterium* sp.(Phylum Bacteroidetes). Both of the isolates seem to be unique strains/species. There was no evidence of toxin-producing genes in the genome sequence. The species are described in more detail by Peoples and colleagues (2020) and in a full manuscript currently in preparation.

(ii) Salinity

The 0ppt NaCl samples had the highest growth rates consistently attaining a final total of 36.4 x 10^6 cells per mL. The 2.5 ppt NaCl samples had the second highest growth rates and reached a maximum cell number of 28.9 x 10^6 cells per mL. At the higher salinities of 5.0 ppt 10 ppt autotrophic cells decreased rapidly and by 4 days there were no living cells in the culture (*Figure 5*).

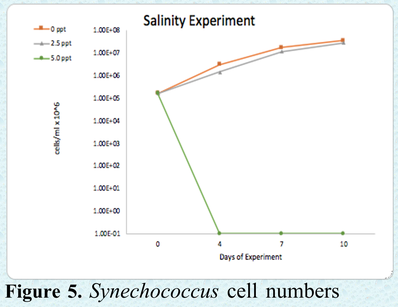

Figure 5. *Synechococcus* cell numbers over time at salinities of 2.5 ppt, and 5 ppt and in the 0 ppt control.

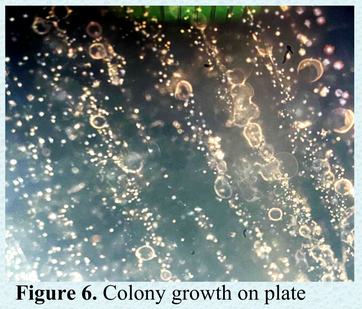

Figure 6. Colony growth on plate media containing autoclaved *Synechococcus* cell material.

(iii) Light Response

The first plated 2020 subcultures were grown under both light and dark conditions. Colony growth was only observed for the plates grown in the light conditions. The second set of experiments 2020 subcultures were only observed in the dark. The plates with agar containing organic material showed growth (*Figure 6*) whereas the plates without organic material in the agar did not show growth.

Discussion

The genomics analyses revealed previously unreported strains of single celled cyanobacteria *Synechococcus* sp. and heterotrophic *Sediminibacterium* sp.(Phylum Bacteroidetes) consistent with that reported from the 2019 isolates (Peoples *et al*., 2020). Preliminary analysis of gene sequences did not reveal toxin producing genes in the *Synechoccocus*, contrary to what has been found in studies of other strains (Yew-Hoong Gin *et al*., 2021). Thus, preliminarily we conclude that blooms of this species do not pose a risk to public health. The results from the salinity experiment are logical in that the the 2019 *Synechococcus* samples grew best under low to no salinity, indicating it is adapted to freshwater conditions contrast to its genetically closest strain from the Chesapeake Bay, which lives at salinities of up to 15 ppt. Culture samples raised in the dark, with organic material in the plate medium, were observed to have colonies of heterotrophs. RNA-sequencing analysis of these heterotrophs will allow us to better understand the trophic interactions that might exist between the two groups and it will create opportunities for further study.

References:

Fucich D, Marsan D, Sosa A, Chen F. 2019. Complete genome sequence of subcluster 5.2 *Synechococcus* sp. strain CB0101, isolated from the Chesapeake Bay. Microbiol Resour Announc 8:e00484-19.

Harmful Algal Blooms. (2019, December 19). Retrieved October 31, 2020, from https://www.epa.gov/nutrientpollution/harmful-algal-blooms

Peoples LM and Colleauges. 2020. Draft genome sequences of four bacterial species as part of an experiential microbiology project at SUNY Geneseo. Microbiology Resource Announcments Vol 9:1-3

Gin KYH, Sim ZY, Goh KC, Kok JWK, Te SH, Tran NH, Li W, He Y. Novel cyanotoxin-producing Synechococcus in tropical lakes. Water Research 192:116828.