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Cyanobacterial Blooms in Highland Lake, ME

William Lambeth
University of Southern Maine

Rachel Henson
University of Southern Maine

Michael Morrison
University of Southern Maine

CYANOBACTERIAL BLOOMS IN HIGHLAND LAKE, ME

William Lambeth[1], Rachel Henson[1], Michael Morrison[1], Faculty Mentor: Lisa R Moore[1]
University of Southern Maine

BACKGROUND

Cyanobacterial blooms, like algal blooms, are a much studied phenomena; and many triggers that cause these blooms are well understood. Usually, excess nutrients (such as soluble nitrogen or phosphate) are prerequisite for a bloom to occur. Since 2013, annual summer cyanobacterial blooms have been observed at Highland Lake (HL), located in Cumberland County, Maine. Data going back more than 15 years, collected by the Highland Lake Association, shows this is a new phenomenon in this body of water. Other investigators have shown HL water chemistry to have high aluminum to phosphorous and aluminum to iron ratios. These metals bind phosphate making it unavailable to most microbes, decreasing the probability a bloom will occur. This raises the questions of what particular cyanobacteria are present, and the source of phosphate.

HYPOTHESIS

We hypothesize that the cyanobacteria involved in the blooms may be capable of solubilizing aluminum phosphate, using it as a phosphate source.

METHODS

To carry out our investigation, we collected a fresh water sample during the winter, and filtered out organisms >30 µm. Using this filtrate, we conducted the following:

- Brightfield microscopy & gram staining.
- Epiflurescence microscopy using 4',6-Diamidino-2-Phenylindole (DAPI stain) for DNA.
- Flow cytometry using SYBR Green I stain for DNA.
- Cell Culture - First to grow any autotrophs present in winter sample, then to isolate specific colonies to grow on media using sterilized lake water with ammonia added as a nitrogen source and 1.5% BD Difco agar (conditions were: no added phosphate, added sodium phosphate, added aluminum phosphate; aerobic & anaerobic; solid and liquid media). Background phosphate level in lake water was measured at ~10 ppm +/- 3ppm.
- PRC & Gel Electrophoresis (18S & 16S), on filtered environmental samples, and isolates.
- Transformation of competent E. coli with plasmid containing 16S PCR products of environmental samples, ampicillin resistance gene, and a β-galactosidase reporter gene.
- 16S rRNA sequencing and comparative analysis.

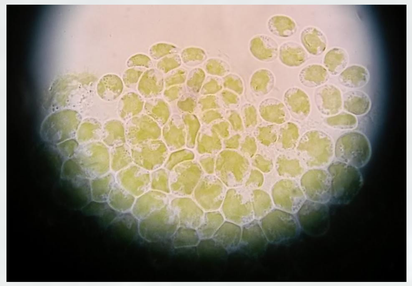

Fig. 1 Brightfield microscopy of isolate cultured on agar.

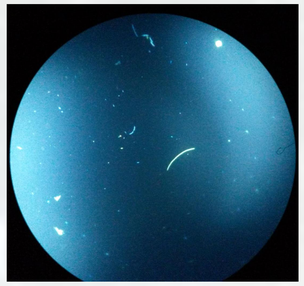

Fig. 2 Fluorescence microscopy under UV with DAPI stain.

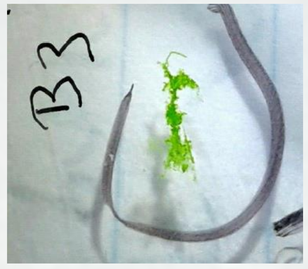

Fig. 3 Isolate grown on experimental condition with added sodium phosphate.

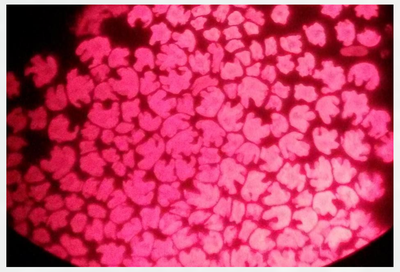

Fig. 4 Isolate stained with crystal violet viewed under fluorescence microscopy.

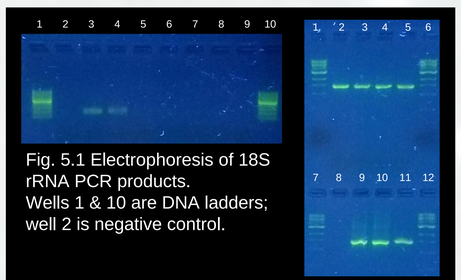

Fig. 5.1 Electrophoresis of 18S rRNA PCR products.
Wells 1 & 10 are DNA ladders; well 2 is negative control.

Fig. 5.2 Electrophoresis of 16S rRNA PCR products.
Wells 1, 6, 7, 12 are DNA ladders; well 8 is neg. ctrl.

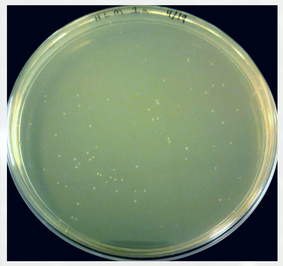

Fig. 6 Transformed E. coli used for metagenomics of environmental samples.

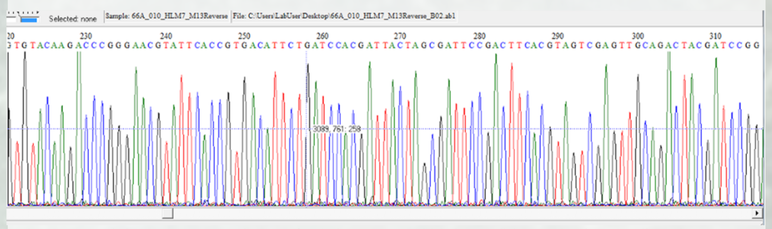

Fig. 7 Examining sequence data to look for areas of high confidence in the predicted nucleotide sequence. (Software BioEdit)

CONCLUSIONS / FUTURE WORK

- Additional 18S PCR & electrophoresis should be done to verify that isolates are not eukaryotes.
- Culturing isolates on defined medium should be conducted to validate hypothesized use of Aluminum Phosphate.
- It is possible that one of the isolates is the same species implicated in the HL blooms; but the small size of our metagenomic sample (n=10) for the summer water sample means this should not be assumed.
- More in depth analysis of existing environmental sequence data is required for identification of microbes.
- Cyanobacteria from samples taken during bloom conditions should be cultured and isolated for additional 16S analysis.

RESULTS

Optical Microscopy

- Both cocci and rods were present
- Gram negative cocci being most predominant.
- Cells cultured on solid agar were large (~10µm) rounded discus shape (Fig. 1.)

EpiFluorescence Microscopy

- Lake water filtered through 30 µm filter and treated with DAPI DNA stain revealed various cell shapes and sizes (Fig. 2).
- A cultured isolate sample stained with crystal violet showed unusual shaped cells (Fig. 4)

Flow Cytometry

- Flow cytometric analysis on a cryopreserved lake water sample from winter was inconclusive for the presence of cyanobacteria, or any particular organisms due to large amounts of detritus (data not shown).

Cell Culture

- Isolation of cells from a winter water sample was very successful
- Growth was slow at first, taking about 1.5 weeks for growth to become without the aid of a microscope (Fig. 3).
- Growth was observed in all conditions tested.
- Clearing of aluminum phosphate was not obvious.
- Growth in the liquid media showed that isolates grew along the bottom of tubes, with the exception of one isolate which grew along the top edge of the media surface. No liquid cultures showed any planktonic characteristics, rather, they displayed an almost plaque-like growth.

16S and 18S rRNA PCR and Gel Electrophoresis

- PCR was conducted on five cultured lines, a lake sample taken during a 2016 summer bloom, and the winter lake sample our group collected.
- Figure 5.1 shows the 18S PCR products.
  - The five cultured isolates in wells 5-9 did not reveal any 18S PCR product.
  - The summer and winter environmental samples in well 3 and 4, respectively, did have 18S PCR products.
- Figure 5.2 shows 16S PCR products.
  - The five cultured isolates in wells 2-5 & 11 had 16S rRNA PCR products.
  - The summer and winter environmental samples in well 9 and 10, respectively, showed 16S rRNA PCR products.

E. coli Transformation

- *E. coli* transformed with plasmids containing 16S rRNA sequences from winter and summer environmental samples were cultured on two amp/IPTG/X-gal plates per sample (Fig. 6).
- All plates showed both blue colonies suggesting X-gal cleavage (lacking proper 16S insertion), and white colonies, suggesting successful insertion of 16S sequences into those plasmids.
- Enough successfully transformed colonies grew to allow selection of 10 colonies each representing winter and summer environmental samples (20 in total).

Initial 16S rRNA Gene Sequence Analysis

- Sequence data for three of the five isolated lines produced usable data between 250-300 nucleotides long. Initial BLAST analysis comparison of the three isolates showed 100% query cover with 97% identical match, suggesting they are likely the same species.
- Sequence data for the 20 sequence from the two environmental samples showed usable, high-confidence nucleotide sequences ranging from 470-650 bp (Fig. 7). Comparison within winter and summer environmental sequences respectively, show several 500+ bp sequences which have matches > 95%, strongly suggesting they came from the same species.
- A comparison between an isolate sequence and winter samples, shows matches using 230 bp from an isolate returns matches with 100% coverage, ~91% identical for two different organisms from the winter environmental samples.
- The same comparison run between the isolate sequence and summer samples shows a 100% coverage, 90% match for two organisms from the summer sample.

ACKNOWLEDGEMENTS

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