Phosphate Utilization by Great Lakes (Cyanobacteria)

Myshala Cummings, Dr. George Bullerjahn, Department of Biological Sciences

Introduction

- The Great Lakes, as in most freshwater systems, are depleted in phosphorus. Phosphorus is an essential nutrient for algae and microbes that is limiting in freshwaters. (Schindler, 1977). When phosphorus in the form of phosphate is limiting, some organisms may adapt to utilize other chemical forms of phosphorus. One such form of organic phosphorus are phosphonates, characterized by the presence of a C-P covalent bond, distinct from the C-O-P monoester bond found in organic phosphates. Whereas all organisms can acquire organic phosphates, only bacteria and some algae (cyanobacteria) can utilize phosphonates (*e.g.* Ilikchyan et al., 2009). This is of potential importance, because one phosphonate in particular, glyphosate, is the herbicide (Roundup®) most widely used in the region and can enter groundwater within the Lake Erie watershed. Knowing this, two questions have been proposed:
- Do cyanobacteria in the Great Lakes routinely utilize phosphonates?
- What phosphonates are being utilized? Can they use some industrial phosphonates such as glyphosate (Roundup®) and etidronic acid?
- Glyphosate is an important herbicide that is typically applied to Lake Erie watershed at an average of 2000 metric tons per year. Etidronic acid is a phosphonate used by power plants to prevent Ca^{2+} buildup in cooling water pipes.
- This work ultimately can lead to the question, if the Great Lakes bacteria and cyanobacteria are utilizing certain phosphonates, are algae blooms are being created by high loadings of these compounds?

Specific Aims

- To conduct this project, two studies were completed. First, we tested whether the cyanobacteria of Lake Erie have the genetic capacity to utilize phosphonates. This was done by PCR-dependent screening of environmental DNA from Lake Erie, examining whether the gene *phnD* is detected. *phnD* encodes a protein necessary for phosphonate uptake.
- Second, two Great Lakes cyanobacteria (*Synechococcus* spp. ARC-21 and LS0503)we have in culture were tested for their ability to use the phosphonates glyphosate and etidronic acid as their sole source of phosphorus.

Methods

- The *phnD* sequences were amplified according to Ilikchyan et al. (2009) using a PTC-100 Programmable Thermal Controller (MJ Research, Inc). Each PCR reaction (25 µL) contained 1 x PCR buffer (Promega), 0.2 mM of each deoxynucleotide (Promega), 0.5 µM of each primer, and 1.0 unit of GoTaq DNA polymerase (Promega), and ca 10 ng of the template DNA. For *Synechococcus* spp. *phnD* amplification, the temperature profile was 95°C for 5 min, 40 cycles of 95°C for 1 min, an initial annealing temperature of 65°C for 1 min decreasing by 0.5°C each cycle until 55°C was reached, 72°C for 1 min, followed by extension at 72°C for 20 min.
- Growth assays of *Synechococcus* spp. strains ARC-21 and LS0503 were performed in sterile phosphorus-free BG-11 media supplemented with the following phosphorus sources: control (no addition); 100 uM K_2HPO_4, 100 uM glyphosate and 50 uM etidronic acid. Etdironic acid was used at 50 uM because each molecule has 2 P atoms, compared to one P in glyphosate. Cultures were prepared in triplicate for each treatment, and measured daily for growth by *in vivo* chlorophyll fluorescence in a Turner TD-700 fluorometer. Growth was monitored as an averaged increase in fluorescence over time.

Literature Cited

Irina N. Ilikchyan,1 R. Michael L. McKay,1
Jonathan P. Zehr,2 Sonya T. Dyhrman3 and
George S. Bullerjahn1,2009 , Detection and expression of the phosphonate transporter gene *phnD* in marine and freshwater Picocyanobacteria

Schindler,1997

Results and Discussion

PCR of environmental DNA from Lake Erie water samples yields a *phnD* PCR amplicon, demonstrating that the cyanobacteria of the lake have the ability to transport and assimilate phosphonates (Figure 1).

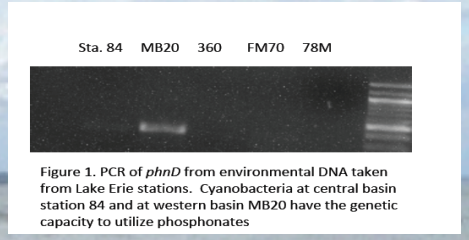

Figure 1. PCR of *phnD* from environmental DNA taken from Lake Erie stations. Cyanobacteria at central basin station 84 and at western basin MB20 have the genetic capacity to utilize phosphonates

Growth experiment. Given that many microbes capable of growth on phosphonates can only utilize a few phosphonate compounds, it may be that glyphosate and etidronic acid cannot be used as phosphorus sources by cyanobacteria.
To test this, we used two different phosphonates as a P source for two *Synechococcus* strains from the Great Lakes.
Growth curves of *Synechococcus* sp. ARC-21 clearly demonstrate that glyphosate can be used this cyanobacterium as a P source because growth rates in glyphosate approach those of the PO4 treated culture (Figure 2). Etidronic acid at 50 uM does not support growth of *Synechococcus* ARC-21. These data suggest that glyphosate runoff could contribute to microbial/algal blooms in freshwaters, whereas etidronic acid would not yield blooms of this organism in power plant cooling lakes.
The experiment with LS0503 shows did not yield clear results, because the experiment was halted prior to the rapid growth of the cultures (Figure 3). Nonetheless, the last time point shows that etidronic acid does not yield a growth advantage over the phosphorus-free control cultures. This further suggests that etidronic acid does not promote blooms of *Synechococcus*.

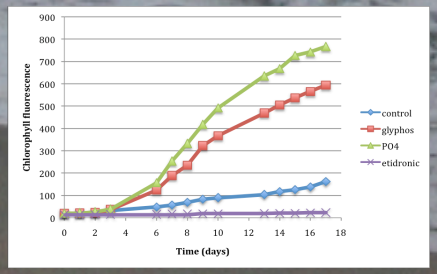

Figure 2 Growth of *Synechococcus* sp. strain ARC-21 (Lake Erie) in various phosphorus sources, including the synthetic phosphonates glyphosate and etidronic acid.

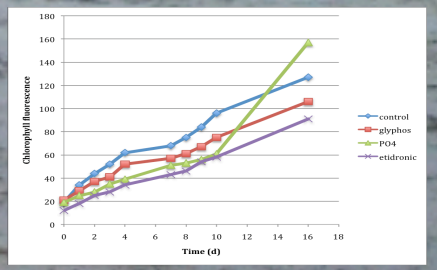

Figure 3. Growth of *Synechococcus* sp. strain LS0503 (Lake Superior) in selected phosphorus sources. The growth experiment was halted prematurely, but etidronic acid yields no growth advantage over the control cultures.

Conclusions

- Cyanobacteria in Lake Erie have the capacity to utiilze phosphonates
- The herbicide Glyphosate is a phosphonate that can be used by cyanobacteria as a source of phosphorus
- Etidronic Acid, another important synthetic phosphonate, cannot be utilized by cyanobacteria
- Glyphosate I smore likely to stimulate algal blooms