Phosphate Utilization by Great Lakes (Cyanobacteria)

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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 freshwater (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 is phosphonates, characterized by the presence of a C-P covalent bond, distinct from the C-O-P monooester bond found in organic phosphates. Where all algae can acquire organic phosphorus, some bacteria and some algae (cyanobacteria) can utilize phosphonates (e.g., Myshala et al., 2009). This is of potential importance, because one phosphate 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. Etdronic acid is a phosphonate used by power plants to prevent Ca^{2+} precipitation 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 being caused by high loading 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) 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, 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 µM KH₂PO₄, 100 µM glyphosate and 50 µM etidronic acid. Etdronic acid was used at 50 µM 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 in vivo chlorophyll fluorescence in a Turner TD-700 fluorometer. Growth was monitored as an averaged increase in fluorescence over time.

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.](image)

Growth experiments:Given that many micro-organisms capable of growth on phosphonates can only utilize a few phosphonate compounds, Etdronic acid (etidronic acid) and glyphosate are the phosphonates most likely to simulate algal blooms. Etdronic acid at 50 µM does not support growth of Synechococcus ARC-21, whereas growth of Synechococcus sp. strain LS0503 is significantly reduced. 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 water (Figure 2).

The experiment with LS0503 grew did not yield clear results, because the experiment was halted prior to the rapid growth of the cultures (Figure 2). Nonetheless, the last time point shows that etidronic acid does not yield a growth advantage over the phosphorus free control cultures. This study suggests that etidronic acid does not promote blooms of Synechococcus.

![Figure 2: 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.](image)

Conclusions

• Cyanobacteria in Lake Erie have the capacity to utilize phosphonates.
• Etdronic acid, another important synthetic phosphonate, cannot be utilized by cyanobacteria.
• Glyphosate is more likely to stimulate algal blooms.