Phosphonates - an unrecognized source of P for endemic microorganisms?

Irina Ilikchyan

Mike McKay

Grace Miner

George Bullerjahn

(Bowling Green State University)

Claudia Benitez-Nelson (Univ. South Carolina)

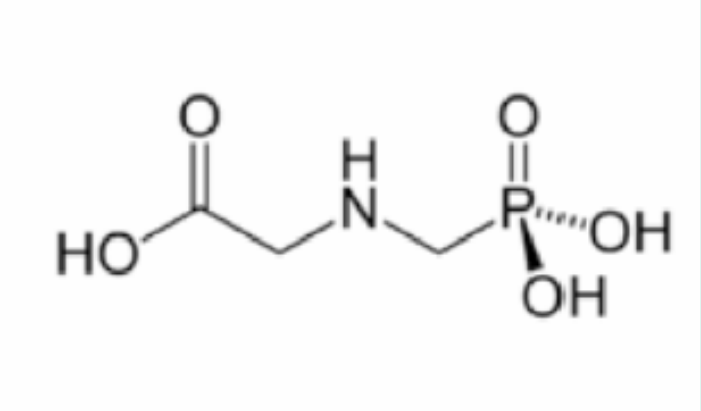
Ohio Sea Grant R/ER-080

Phosphonates

- Organic P containing a C-P bond
- Thought to be more recalcitrant than phosphate monoesters
- Sources
  - Natural: antibiotics, phosphonolipids
  - Anthropogenic: glyphosate (Roundup®)
- Prokaryotes primarily utilize phosphonates
- 20-25% of HMW DOP pool in the ocean
- Do glyphosate-derived phosphonates contribute to the DOP pool in Lake Erie?

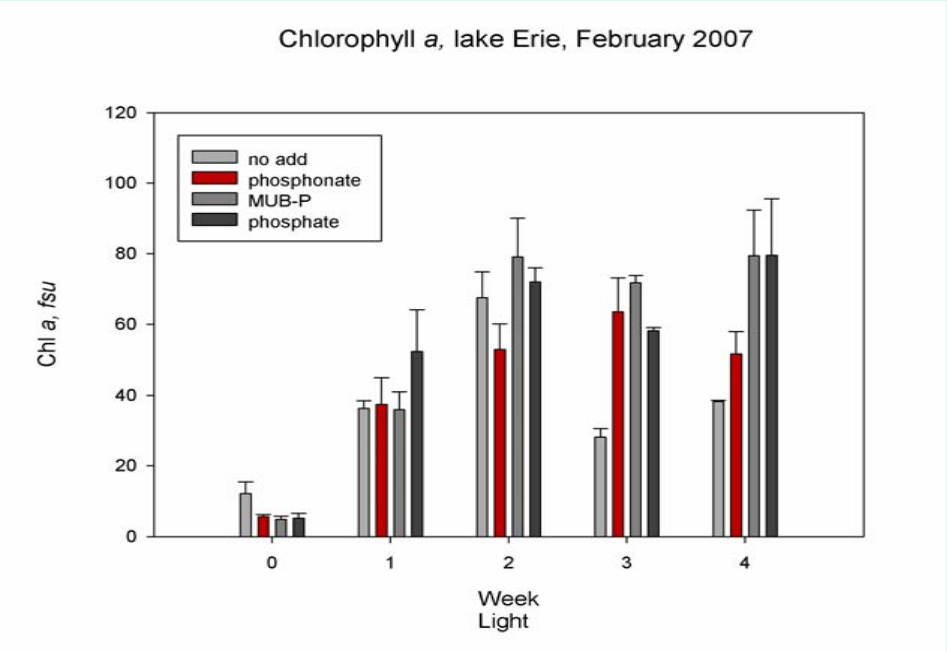
Glyphosate is a phosphonate

- N-(phosphonomethyl) glycine
- Potential P (and N?) source for microbes



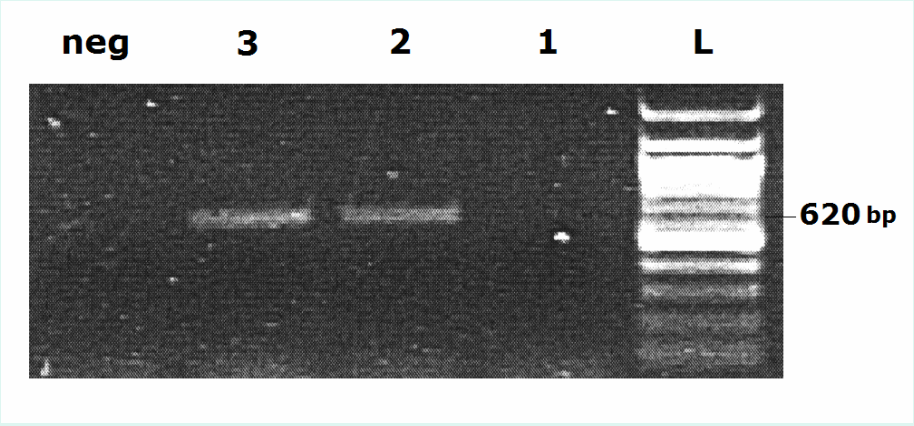
Lake Erie and phosphonates

- Glyphosate in the watershed - does it have an impact?
  - Up to 1000 metric tons per year applied to fields within the watershed; Roundup Ready crops planted starting in 1996
  - Phosphonate loadings have been confirmed in streams adjacent to glyphosate-treated fields (Scribner USGS 2003)
- Lake Erie endemic microbes can utilize phosphonates as a P source



Lake Erie and phosphonates

- Lake Erie cyanobacteria and bacteria have the genetic potential to utilize phosphonates
- *phn* genes are present in cultured cyanobacteria and bacteria and Erie environmental DNA
- Could glyphosate loadings stimulate the microbial community?
  - Addition of glyphosate to water samples has been shown to stimulate picocyanobacteria over eukaryotic algae (Perez, 2007)

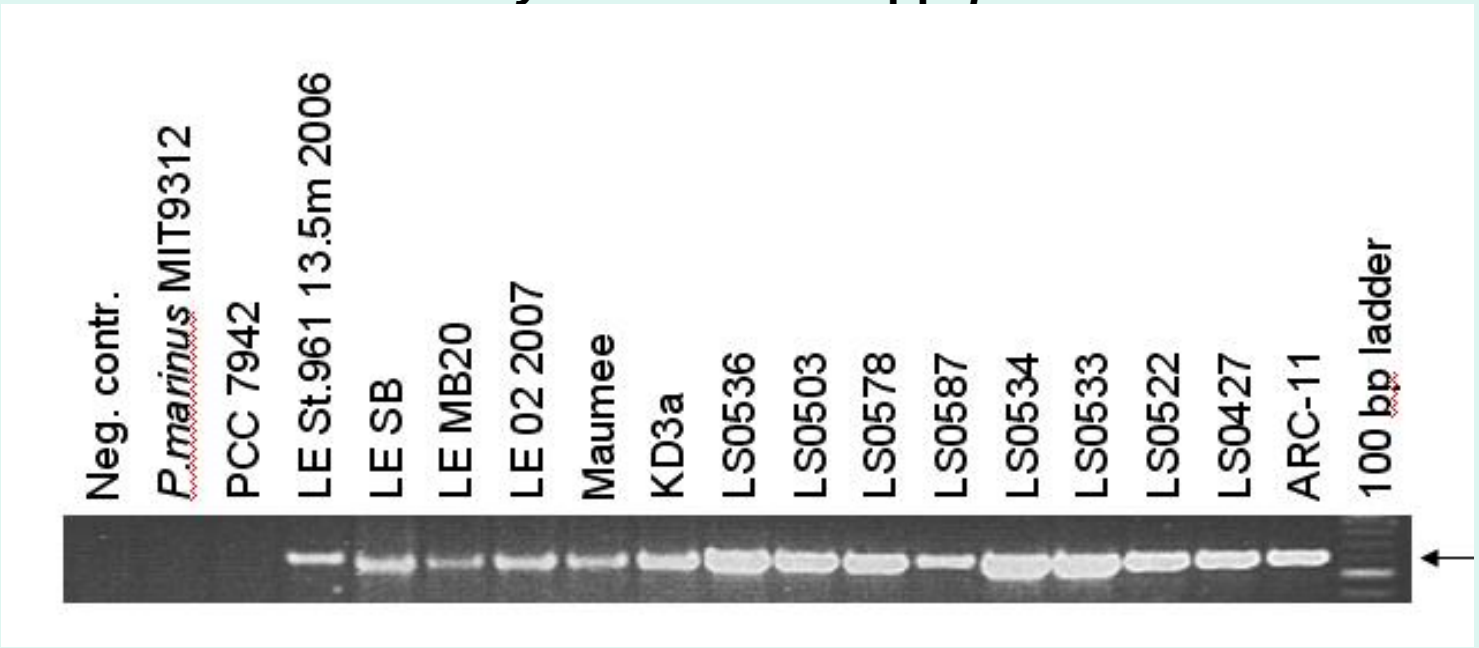


RT-PCR of RNA from *Synechococcus* sp. ARC-11
Lane 1 - PO_4 replete ARC-11 culture
Lane 2 - PO_4-depleted culture
Lane 3 - PO_4-depleted culture + phosphonoacetic acid

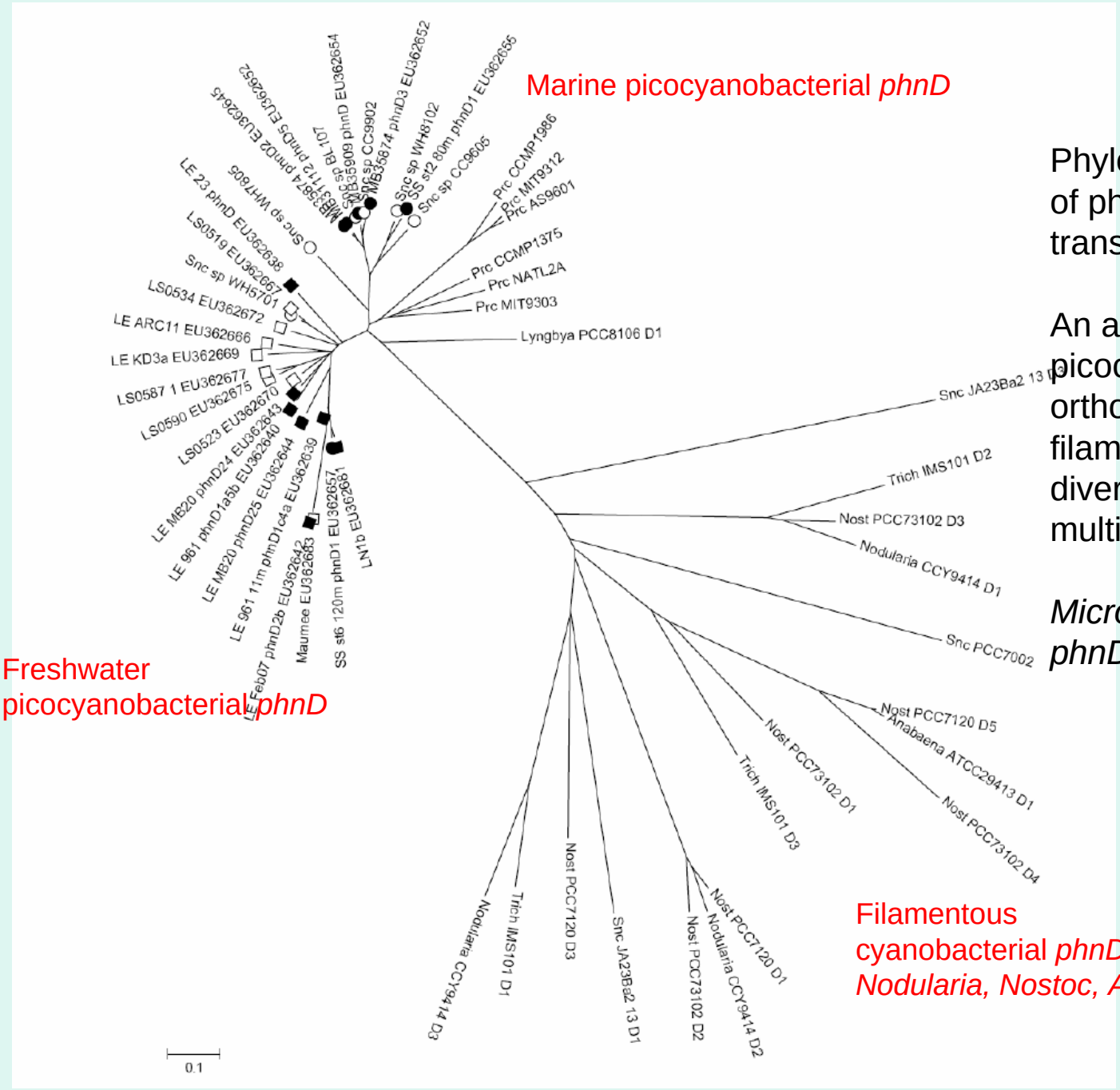
From: Ilikchyan, Zehr, McKay, Dyhrman & Bullerjahn, Appl. Environ. Microbiol., submitted

Endemic freshwater picocyanobacteria have *phn* genes

A. PCR of freshwater *Synechococcus* spp. *phnD*



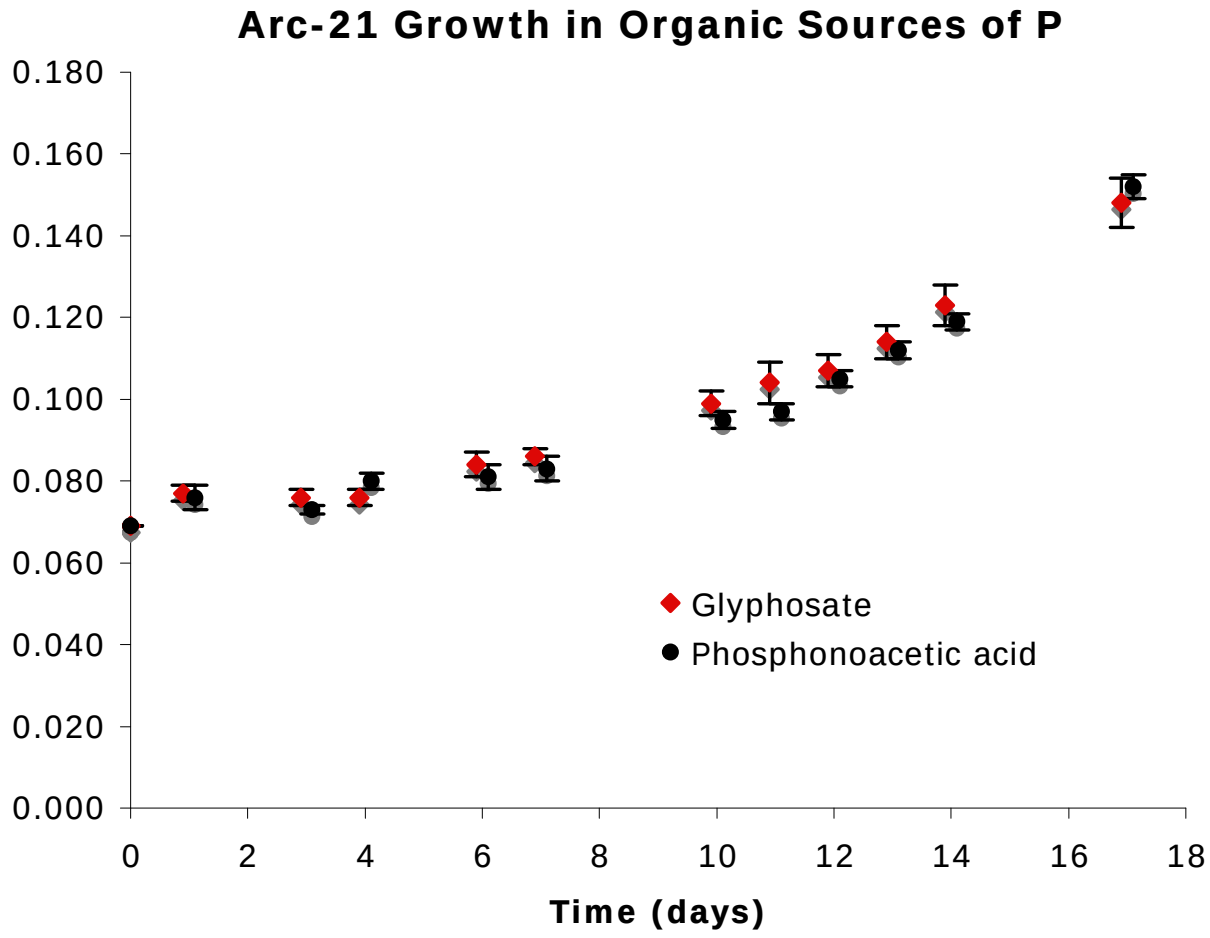
Ilikchyan, Zehr, McKay, Dyhrman & Bullerjahn, Appl. Environ. Microbiol., submitted


Marine picocyanobacterial *phnD*
Freshwater picocyanobacterial *phnD*
Filamentous cyanobacterial *phnD: Nodularia, Nostoc, Anabaena* spp.
Phylogenetic analysis of phosphonate transporter genes
An ancient trait in picocyanobacteria - orthologous genes in filamentous cyanos are divergent and present in multiple copies
Microcystis genome lacks *phnD* as best we know
Snc sp WH8102 SS st2 80m phnD1 EU362655
Snc sp CC9605
Prc CCMP1986
Prc MIT9312
Prc AS9601
Prc CCMP1375
Prc NATL2A
Prc MIT9303
Lyngbya PCC8106 D1
Snc JA23Ba2 13 D3
Trich IMS101 D2
Nost PCC73102 D3
Nodularia CCY9414 D1
Snc PCC7002
Nost PCC7120 D5
Anabaena ATCC29413 D1
Nost PCC73102 D4
Trich IMS101 D3
Nost PCC73102 D1
Nost PCC7120 D1
Nodularia CCY9414 D2
Nost PCC73102 D2
Snc JA23Ba2 13 D1
Nost PCC7120 D3
Trich IMS101 D1
Nodularia CCY9414 D3
LE 23 phnD EU362638
LS0519 EU362667
Snc sp WH5701
LS0534 EU362672
LE ARC11 EU362666
LE KD3a EU362669
LS0587 1 EU362677
LS0590 EU362675
LS0523 EU362670
LE MB20 phnD24 EU362643
LE 961 phnD1a5b EU362640
LE MB20 phnD25 EU362644
LE 961 11m phnD1c4a EU362639
LE Feb07 phnD2b EU362642
Maumee EU362683
SS st6 120m phnD1 EU362657
LN1b EU362681
0.1

Proposed experiments

- Seasonal detection of phosphonates in stream/lake water by ^{31}P NMR
  - Do total phosphonates parallel the springtime application of Roundup®?
- Amendment of lake water with phosphonates
  - Monitor community shifts by phylogenetics (16S rDNA amplicons)
- Monitor cultured cyanobacteria for their ability to use different phosphonates
  - Glyphosate, phosphonoacetic acid, etc.

Growth of *Synechococcus* sp. ARC-21 in phosphonates



Lake Erie *Synechococcus* sp. ARC-21 grown in 100 μM phosphonates

^{31}P NMR, Sta. MB20 July

(Claudia Benitez-Nelson)