Regulation of a novel phosphatase common in marine bacteria

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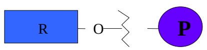
Relevance and Background

Phosphorus (P) is vital for life's processes, such as catabolism, energy transfer, and genetic inheritance. Bacteria are the most abundant group of organisms on Earth, and they shape the biochemical cycles in the oceans. Bacteria prefer P in the form of inorganic phosphate (Pi). When Pi supply is low, they turn to dissolved organic phosphate (DOP), 75% of which is phosphoesters. Microbes produce phosphatases that cleave the ester bond, releasing P from molecules such as glucose-6-phosphate.



Activation of this survival mechanism can be an indication of bacterial P deficiency. Assessment of the *in situ* P status of marine bacteria can help us understand the P cycle and its affect on bacterial ecology and diversity. The gene *phoX* may be an ideal genetic or molecular marker of P status. PhoX is a recently discovered, well-conserved, and widespread phosphatase among marine bacteria[1,2]. In P-limited *Silicibacter pomeroyi*, it represents as much as 90% of the alkaline phosphate activity[2]. However, for it be a useful marker, **PhoX should be primarily regulated by P.**

Methods

•The model organism *Silicibacter pomeroyi* was grown in a minimal basal media under **10 different nutrient conditions** varying in **iron, P, glucose,** and **glucose-6-phosphate (G6P)**

•**Optical density (OD)** measurements of each treatment were taken, as well as a **fluorescence assay** for phosphatase activity.

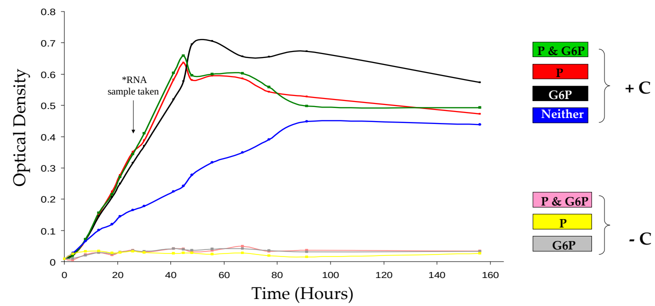
•Samples were taken during exponential growth for **RNA extraction, cDNA synthesis, and PCR analysis of *phoX* expression.**

Objective 1: Carbon

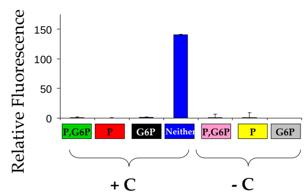
It has been suggested that microbes can produce phosphatases to obtain carbon under carbon limitation[3].

We hypothesize that bacteria do not use *phoX* to overcome carbon limitation.

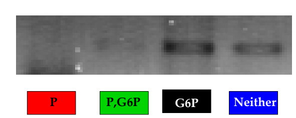
•Growth



•Phosphatase Activity



•*PhoX* Expression



•**Carbon deficiency was not significantly overcome with utilization of G6P.**

•**G6P substrate competition may have interfered with the phosphatase assay**, because the (G6P) treatment showed *phoX* genetic expression, but no activity until it was washed with a G6P-free media.

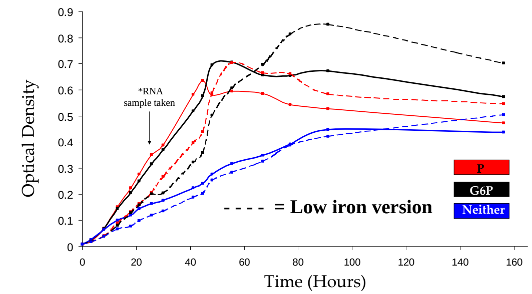
•**Expression by (– C) treatments was assumed to be negligible**, as they did not grow enough for RNA extraction. Treatments (P) & (P,G6P) appear to have no *phoX* expression in this replicate, although previous results have sometimes differed.

Objective 2: Iron

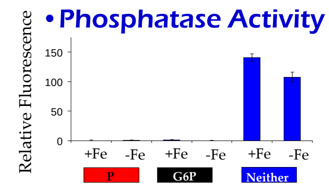
Iron is known to effect the transport of phosphatases (TAT system) to the outside of the cell, where DOP resides[4].

We hypothesize that a minimum iron concentration is necessary for growth and PhoX activity, but not *phoX* genetic expression.

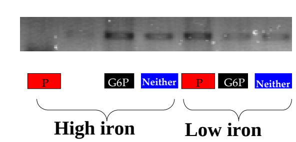
•Growth



•Phosphatase Activity



•*PhoX* Expression



•**Iron deficiency hindered growth**. The (G6P, low Fe) treatment grew to a higher density than expected for unknown reasons, but only after the end of exponential growth.

•**Iron deficiency slowed activity when no form of P was present.** With just P, all activity was negligible, as expected. When just G6P was present, the problem of substrate competition previously mentioned makes the results unclear.

•***PhoX* expression appears to be similar for +Fe and –Fe versions of all P conditions, *although it is not definitive*.** The band on (P, low Fe) may be DNA contamination, which was suspected on an RNA check from a previous gel.

Acknowledgements

We would like to thank M.A. Moran (U. Georgia) for cultures of *S. pomeroyi* and the media recipe, as well as Kim Thamatrakoln for her advice and guidance.

Literature Cited

[1]Majumdar, A., Ghatak, A., & Ghosh, R.K. Identification of the gene for the monomeric alkaline phosphatase of *Vibrio cholerae* serogroup O1 strain. *Gene* **344,** 251-258 (2005).

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Future Directions

Both of our hypotheses were tentatively confirmed, although future studies could strengthen our results and further explain PhoX regulation:

•**In a study with more Fe limitation**, there may be a greater difference between + and –Fe activity. With a **quantitative** PCR analysis of such a study, significant differences may be observed in genetic expression, as well.
•Further study should include **washing of any replicates which have G6P**, and perhaps all treatments, with a control to gauge the effect of washing.
•A similar experiment should be run using **limiting nitrogen concentrations**.