The Effects of Different Insoluble Phosphate Media on Phosphate Solubilizing Bacteria from Mendocino Terrace Soil

Danielle Naiman[1], Alex Aaring[2], Shi Wang[2], Bryson Cwick[2], Romy Chakraborty[2]

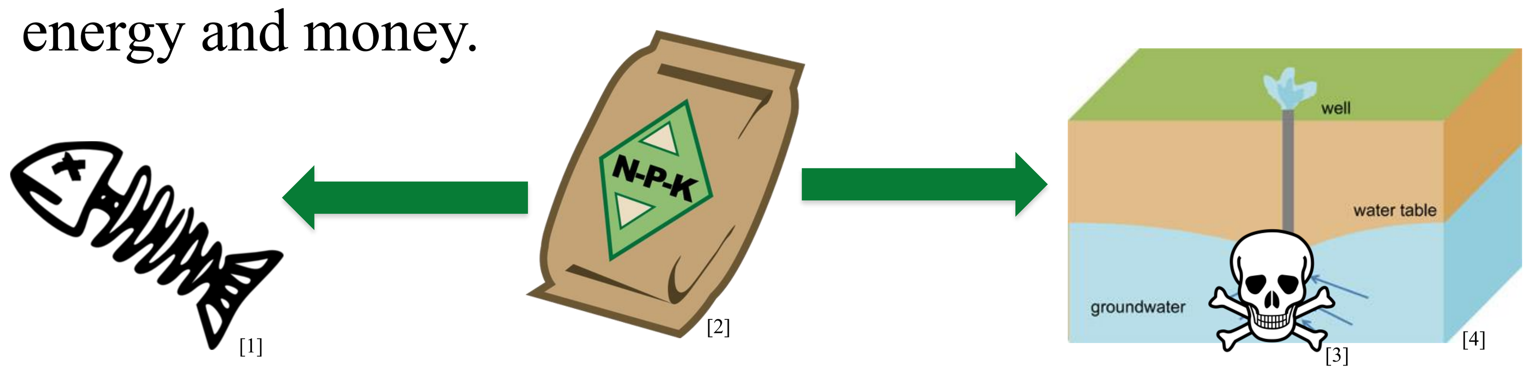
[1]Santa Monica College, [2]Lawrence Berkeley National Laboratory

2015 Transfer-to-Excellence Research Experiences for Undergraduates Program (TTE REU Program)

Abstract: The goal of this project is to test isolated Phosphate Solubilizing Bacteria (PSB) from Mendocino Ecological Staircase soil samples and screen them for their ability to solubilize different insoluble phosphates in growth media: $FePO_4$, $AlPO_4$, and phytic acid. Almost one hundred strains were grown on an LB broth initially for robust growth, and then transferred to $FePO_4$, $AlPO_4$, and phytic acid containing basal media until the bacteria reached the stationary phase. A colorimetric assay using malachite green was used to detect soluble phosphate in the filtrate for each culture. Isolate E3, initially taken from the phytic acid plate, was found to be the quickest grower and produced the most solubilized phosphate on phytic acid. E3 was identified as *Burkholderia sediminicola* using 16s rDNA sequencing. *B. sediminicola* was then inoculated and grown in triplicate in $AlPO_4$ and phytic acid containing basal medium as the sole source of phosphorus. Optical density measurements at 600 nm and the colorimetric assay were used at regular time points to determine growth and solubilized phosphate. It can be concluded *B. sediminicola* grows best in phytic acid containing media when compared to $AlPO_4$ containing media and is efficient at solubilizing phosphate from phytic acid. Further research can be implemented into characterizing this particular strain and the mechanism, for phosphate solubilization.

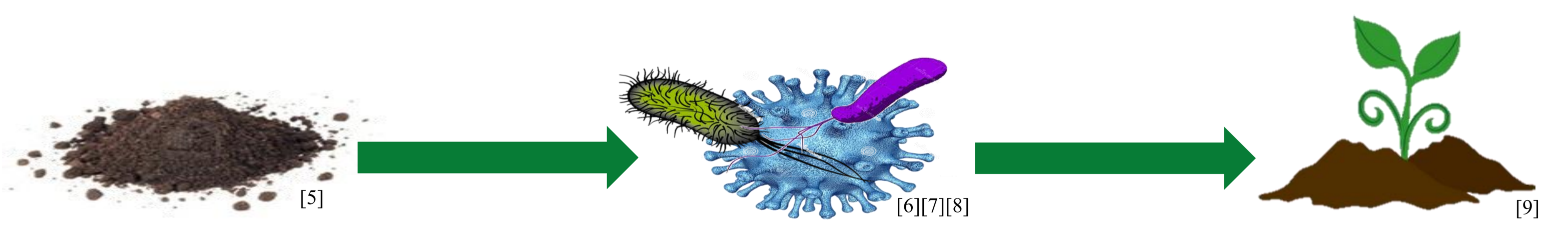
Background

Plants need phosphorus for growth; however, less than 0.1% is in accessible forms for plant uptake. Man-made fertilizers are used to combat this issue but frequently lead to pollution in bodies of water from run-offs, and fertilizer uses extensive energy and money.



A Solution

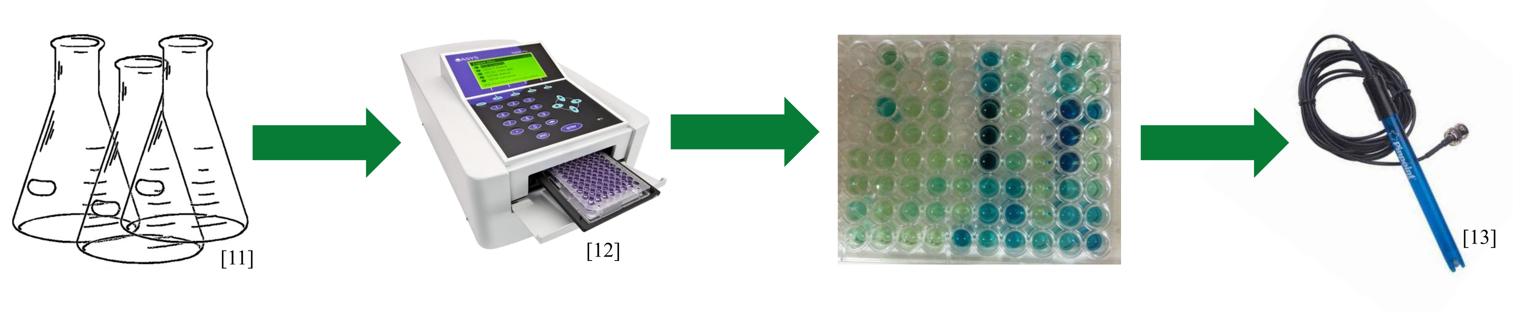
- A environmentally sustainable solution lies with Phosphate Solubilizing Bacteria (PSB)
- PSB can naturally break down insoluble phosphates in soil into soluble forms using a variety of mechanisms



The Process

PSB can break down and solubilize phosphates by using enzymes or secreting organic acids.[10] It has been shown that these specific strains break down organic phosphates during stationary phase. Regular time points were measured for optical density to determine stationary phase, along with measurements of phosphate and a final pH measurements.

- Optical density measurements at 600 nm were taken twice daily until stationary phase was observed from isolate
- BioAssay Malachite Green Phosphate Assay was used to detect µM concentrations of phosphate after bacteria sample was filtered through a 0.2 µm filter
- pH was measured at the end to help determine possible process behind phosphate solubilization

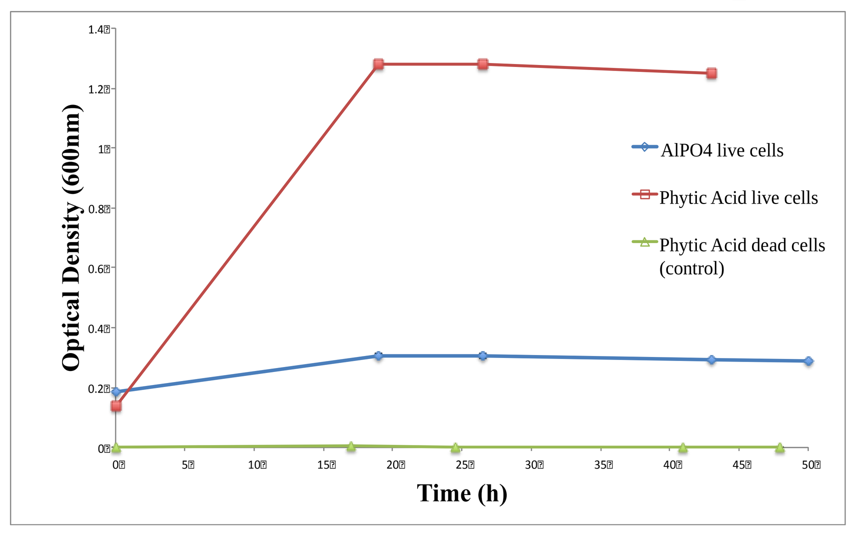


My Question

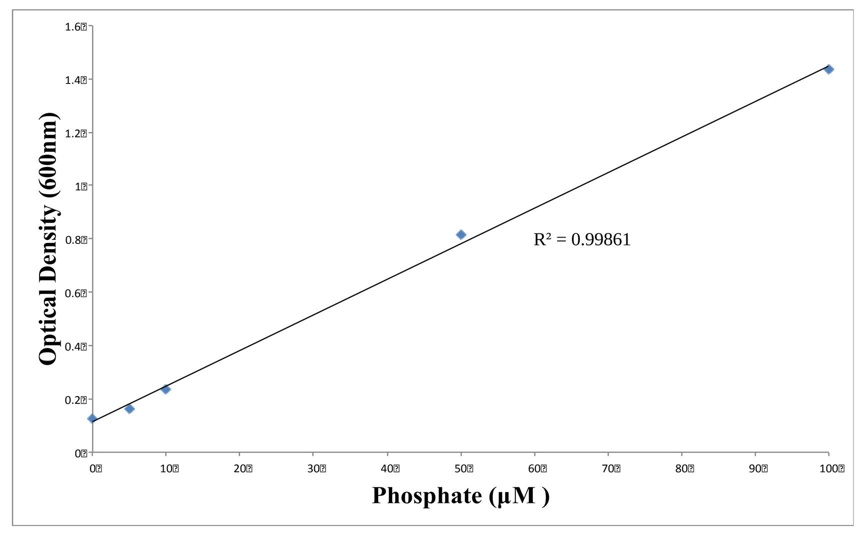
- Which bacteria isolated from Mendocino terrace soil can solubilize phytic acid, $FePO_4$, and $AlPO_4$?
- Do these strains prefer certain insoluble phosphorus containing compounds to others and how does their growth compare?

Results

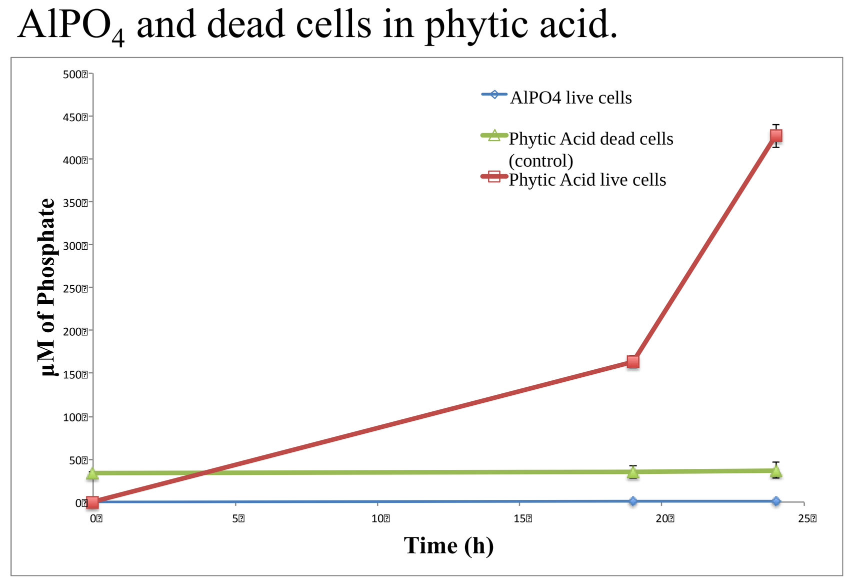
From 96 isolated strains, preliminary results showed promising phosphate solubilizing ability for one strain, later identified as *Burkholderia sediminicola,* from the phytic acid containing media. Further characterization and experiments were performed.



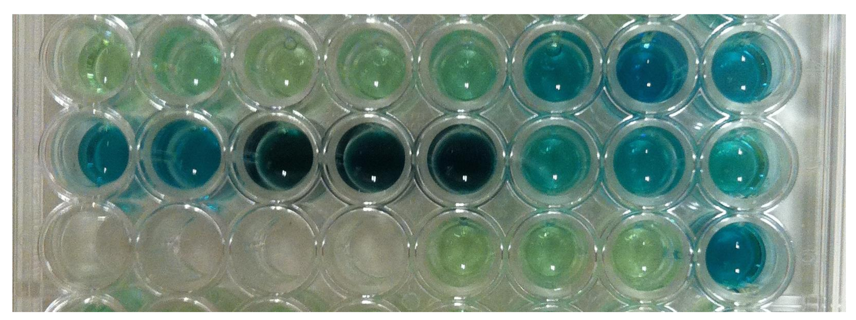
Optical density measurements of *B. sediminicola* were taken at daily time points. The phytic acid with live cells reached stationary phase at approximately18 hours, while the $AlPO_4$ cells started dying after a day. Live cells in phytic acid grow the most when compared to live cells in $AlPO_4$ and dead cells in phytic acid.



The standard curve for the colorimetric phosphate assay kit was taken at every measurement. All standard curves had at least a 0.95 R^2 value, with a different line equation. This is an example of one of the readings taken at 19 hours.

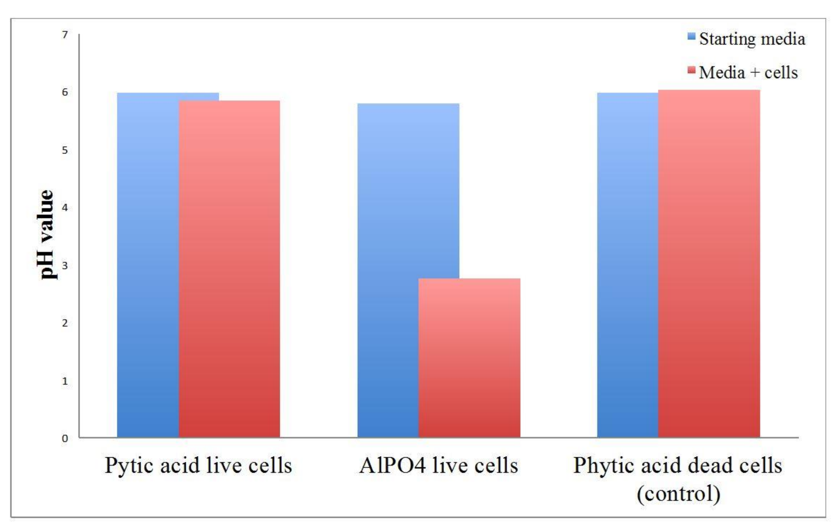


Amount of phosphate solubilized from *B. sediminicola.* At time 24 hours, phytic acid with live cells experiences greater error due to the limitations of the assay kit and the possibility of cells lysing and releasing phosphate. Phytic acid with dead cells has phosphate due to lysed cells that released phosphate into the sample.



Colorimetric Phosphate Assay. The darker green indicates higher levels of phosphate. From top to bottom and left to right: Phytic acid media and $AlPO_4$ media followed by the phosphate standard; phytic acid with live cells diluted and not diluted followed by phytic acid with dead cells; $AlPO_4$ with live cells and one more diluted phytic acid with live cells.

After the conclusion of the growth experiment, pH was measured to determine whether the strains had changed the pH of the media while growing on these phosphorus sources.



Analysis and Conclusion

- Strain *B. sediminicola* grows best on media containing phytic acid as the sole source of phosphorus with high levels of solubilized phosphate compared to growth on $AlPO_4$ containing media.
- When *B. sediminicola* grows in phytic acid containing media, it may use an enzymatic break down of the phosphate.
- When *B. sediminicola* is in $AlPO_4$ containing media, it possibly secretes organic acids, evidenced by low pH.

Future Directions

- When tested, another strain, labeled as C2, also perhaps could solubilize $AlPO_4$.
- This strain produced noticeable amounts of phosphate when tested with the assay.
- The same experiment should be conducted using this strain to confirm solubilization of phosphate and test the mechanism it uses.
- Both E3 and C2 should also be tested on other sources of phosphates found in soil, and further tested for the mechanism of phosphorus solubilization.

Acknowledgments

Thank you Dr. Romy Chakraborty for mentoring me and providing a wonderful environment to conduct research in. Thank you Shi Wang for providing materials and procedures in my experiment. Thank you Alex Aaring and Bryson Cwick for your wisdom and helpfulness around the lab. My appreciation to Shaila Kotadia for pairing me with my mentor and supporting me through research. Special thanks to Lea Marlor for managing the TTE REU program, and to Jeff Bokor for providing me this opportunity.

References

[1] http://www.clker.com/cliparts/D/P/G/v/I/R/dead-fish-md.png
[2] http://ian.umces.edu/imagelibrary/albums/userpics/101505/normal_ian-symbol-fertilizer-factory.png
[3] http://www.motiondecals.com.au/sc_images/products/436_large_image.jpg
[4] http://www.shef.ac.uk/polopoly_fs/1.305890!/image/groundwater380.jpg
[5] https://media.licdn.com/mpr/mpr/p/2/005/06a/16b/04141a8.jpg
[6] http://images.wisegeek.com/salmonella.jpg
[7] http://previews.123rf.com/images/lightwise/lightwise1110/lightwise111000420/10945951-Virus-and-bacterium-medical-symbol-represented-by-a-single-microscopic-bacteria-intruder-cell-causin-Stock-Photo.jpg
[8] http://thumbs.dreamstime.com/z/e-coli-bacterium-9580396.jpg
[9] http://www.clipartbest.com/cliparts/LTK/kn6/LTKkn6bqc.jpeg
[10]Y. P. Chen et al, "Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities", Dept. of Soil and Env. Science, Nat. Chung Hsing Univ., Taichung, Taiwan., Dec. 19, 2005.
[11] https://classconnection.s3.amazonaws.com/141/flashcards/797684/png/erlenmeyer-flask.png
[12] http://lakshmiclinics.com/admin/upload_images/image575.jpg
[13] http://ecx.images-amazon.com/images/I/41bYH4mHPOL._SX342_.jpg

Contact Information

Danielle Naiman: (818) 665-9017
danielle.naiman@gmail.com

Support Information
This work was funded by National Science Foundation Award ECCS-0939514 & ECCS-1157089 & ECCS-1461157