The Effects of Different Insoluble Phosphate Media on Phosphate Solubilizing Bacteria from Mendocino Terrace Soil
Danielle Naiman¹, Alex Aaring², Shi Wang³, Bryson Cwick², Romy Chakraborty²
¹Santa Monica College, ²Lawrence 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: FePO₄, AlPO₄, and phytic acid. Almost one hundred strains were grown on an LB broth initially for robust growth, and then transferred to FePO₄, AlPO₄, 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 AlPO₄ 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 AlPO₄ 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.⁰ 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, FePO₄, and AlPO₄?
• 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.

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 AlPO₄ 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 AlPO₄ containing media, it possibly secretes organic acids, evidenced by low pH.

Future Directions
• When tested, another strain, labeled as C2, also perhaps could solubilize AlPO₄.
• 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

Contact Information
Danielle Naiman: (818) 605-0917
danielle.naiman@gmail.com

Support Information
This work was funded by National Science Foundation Award ECCS-1309514 
& ECCS-1157069 & ECCS-1461157