The Global Prevalence and Biogeochemical Impact of Ancient Phosphorus-Oxidizing Bacteria

Sophia D. Ewens (sdewens@berkeley.edu)* [1, 2], Tyler P. Barnum[1], Kyle Metcalfe[1], Mikayla A. Borton[3], Hans K. Carlson[3], Kelly C. Wrighton[4], **John D. Coates[1, 2]**

[1]Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA

[2]Energy & Biosciences Institute, University of California, Berkeley, CA, USA

[3]Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO, USA

[4]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Lab, Berkeley, CA, USA

Project Goals: This project aims to investigate the role of microbial dissimilatory phosphite oxidation (DPO) in the global phosphorus and carbon biogeochemical cycles. We are examining the prevalence of DPO and phosphite (HPO_3^{2-}) in a broad range of geochemical environments, and to examine fundamental physiological and biochemical aspects of DPO. To achieve this goal we test three specific hypotheses:

1. DPO is an environmentally prevalent metabolism that co-occurs with global phosphite reserves.
2. DPO metabolism is universally conferred by the conserved *ptx-ptd* operon.
3. DPO is universally associated with CO_2 fixation

Phosphite is the most energetically favorable chemotrophic electron donor known, with a half-cell redox potential ($E^{o\prime}$) of −650 mV for the PO_4^{3-}/PO_3^{3-} couple. Dissimilatory phosphite oxidizing microorganisms (DPOM) can harness the free energy from phosphite oxidation for cellular growth and concomitantly use that energy to fix CO_2. At the time of this work, the prevalence of this unique metabolism remained largely uncharacterized since its discovery in 2000, as only two DPOM species had been identified in three discrete locations. A false notion of rarity consequently limited our understanding of the diversity, environmental distribution, and biogeochemical impact of DPOM. However, phosphite has been detected in several environments at concentrations that suggest a contemporary P redox cycle that might sustain a greater diversity of DPOM than is currently recognized.

To survey the prevalence and diversity of DPOM, selective enrichments were inoculated with wastewater sludge from six different wastewater facilities around the San Francisco Bay area. Ion chromatography was used to monitor metabolic activity and identify active DPO enrichments, and metagenomic sequencing of active enrichments allowed for the identification of 21 DPOM. These DPOMs span six classes of bacteria, including the *Negativicutes*, *Desulfotomaculia*, *Synergistia*, *Syntrophia*, *Desulfobacteria*, and *Desulfomonilia_A*. Evolutionary analyses of all binned DPOM genomes suggest that modern DPOM are relics of an ancient ancestor whose capacity to perform DPO originated ~3.2 Gya. We compared the DPO marker genes from these enriched genomes with over 17,000 publicly available metagenomes and found that DPO metabolism exists globally in diverse anoxic environments, including wastewaters, sediments, and subsurface aquifers. We subsequently tested for this metagenomic diversity in an expanded sampling regime that included the Sacramento River, San Francisco Delta watershed, San Francisco Bay, and coastal Pacific Ocean. We found that DPO activity was stimulated in 30% of environmental samples and that the 16S rRNA gene taxonomy mirrored the diversity that was identified in the original wastewater metagenomes. Relatives of *Phosphitivorax anaerolimi* Phox-21 dominated most sampled environments, but we hypothesize that divergent environmental conditions are likely to select for phylogenetically diverse DPOM.

We performed metabolic analyses of metagenome-assembled genomes and found that most DPOM are specialists that use phosphite as their sole electron donor and CO_2 as their preferred electron acceptor. This metabolic niche would poise DPOM to be primary producers while providing nutritional phosphate to their local microbial community, signifying that DPO can sustain geographically isolated microbial communities using exclusively phosphite and CO_2 to generate energy and fixed carbon. However, we find that this pivotal role may be mutually dependent on the activity of the local microbial community, as physiological data suggests that DPOM require symbiotic nutrient exchange to support growth. We explore the potential microbial interactions that could support DPOM activity and find that DPOM enrichments generate diverse corrinoids. One of those corrinoids is novel, and its characterization may lead to crucial insights about DPO activity.

Funding Statement: This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0020156