Environmental and Biological Constraints on Dissimilatory Phosphite Oxidizing Microorganisms

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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 will test three specific hypotheses:

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

The work here advances hypothesis 1: Phosphorus (P) is an essential nutrient, but the majority of P is trapped in mineral deposits as oxidized phosphate (P^{5+}). Alternative P redox states are often ignored in P cycle models, despite the fact that reduced species have been identified in diverse environments[1]. Phosphite (P^{3+}) is a highly soluble, reduced P compound accounting for up to 30% of total dissolved P in diverse environments[2]. In 2000, Schink et al. isolated the first microorganism capable of dissimilatory phosphite oxidation (DPO) in which phosphite is used as a chemolithotrophic electron donor[3]. This organism, *Desulfotignum phosphitoxidans* FiPS-3, is an autotrophic acetogen for which DPO activity was attributed to the *ptx-ptd* gene cluster[4]. This gene cluster has since been identified in many metagenome-assembled genomes (MAGs) from wastewater enrichments[5], spanning six phylogenetic classes[6]. A search of global metagenomic databases revealed the presence of the *ptx-ptd* cluster in a numerous uncultured microoganisms from diverse environments[6]. Here, we propose new geochemical and biological constraints on DPO microorganisms (DPOM) in the environment, through a synthesis of insights from 1) geochemical modeling, 2) enrichment cultures of DPOM from wastewater, and 3) enrichment cultures of DPOM from estuarine sediment and groundwater. Assuming DPO was coupled to CO_2 reduction to formate,[5] geochemical modeling constrained energy yields for a range of PO_4^{3-}/PO_3^{3-}, which we compared with measurements from a range of environmental settings to identify environments where DPO was likely to provide energy for microbial metabolism. While PO_3^{3-} concentrations and electron accepting capacity provided geochemical constraints for environmental DPOM activity, further evidence from enrichment cultures of DPOM suggests that the community context of DPOM may also biologically constrain activity. Ewens *et al.*[6] found that most DPOM are related to syntrophs, which depend on methanogens to mediate

thermodynamically unfavorable metabolic reactions.[7] While PO_3^{3-} oxidation is too thermodynamically favorable to require syntrophic exchange, a symbiotic nutrient exchange may explain DPOM resistance to isolation.[5] By introducing a variety of inhibitors to a highly enriched PO_3^{3-} oxidizing culture (HEPO), we found that DPO activity is immediately inhibited by 2-bromoethanesulfonate (BES), a specific inhibitor of methanogens. Since methanogens are prolific corrinoid producers, we hypothesized that methanogens may supply DPOM with essential corrinoids in exchange for PO_4^- and reduced carbon products, constraining DPOM to those environments that host methanogens. Genomic analyses revealed that DPOM are incapable of corrinoid synthesis while supporting a role for corrinoids in DPO metabolism. Extractions coupled to HPLC-MS identified four corrinoids in the HEPO culture with a purported role for DPO activity. Enrichment cultures also provided geochemical constraints to DPOM where abundant PO_4^{3-} minerals such as struvite [$MgNH_4PO_4 \cdot 6H_2O$] neogenesis are observed.[8] Precipitation of highly-insoluble minerals should pose a challenge to DPOM by cell encrustation, constraining DPOM to conditions where mineral precipitation on cell surfaces is precluded unless a mechanism exists for enhancing PO_4^{3-} solubility. To further constrain environmental DPO activity, we established 240 DPOM enrichments from sediment samples and a longitudinal transect of the Sacramento River and the Los Angeles Basin, respectively. Results revealed prevalent PO_3^- oxidation in these environments and revealed a correlation between DPO activity and the *in-situ* potential for DPO in a variety of anaerobic environments. Ongoing work will aim to characterize DPO MAGs from enrichments that span the full diversity of environments sampled. By pairing geochemical analyses with metagenomics, we will map the metabolic and phylogenetic diversity of DPOM to their environmental context.

References

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