The Lineages of Dissimilatory Phosphite Oxidation Genes Indicate an Ancient, Vertically Transferred Metabolism

Sophia Ewens[1, 2], Alexa Gomberg* (agomberg@berkeley.edu)[1], Tyler P. Barnum[1], and **John D. Coates[1, 2]**

[1] Department of Plant and Microbial Biology, University of California, Berkeley
[2] Energy & Biosciences Institute, University of California, Berkeley

Project Goals: This project investigates the role of microbial dissimilatory phosphite oxidation (DPO) in the global phosphorus and carbon biogeochemical cycles. As part of this we are investigating the natural occurrence of DPO and phosphite in a broad range of geochemical environments, and examining the fundamental physiological and biochemical aspects of DPO. We are combining systems biology and -omics approaches with physiological and geochemical studies to elucidate the geochemical impact, environmental prevalence and metabolic machinery underlying DPO. To achieve our goals, we are testing 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**

The work described here advances hypothesis 2.

Phosphorus (P) is essential for all life, and predominantly exists on Earth as oxidized phosphate (P^{+5}). The majority of this is trapped in mineral deposits, resulting in biological P limitation for most ecosystems. Global P cycle models consistently overlook alternative P redox states, yet reduced P species have been identified in diverse environments and frequently serve as a biological P source when soluble orthophosphate is limited[1]. In 2000, Schink et al. identified a novel microbial metabolism in which phosphite was used as a chemolithotrophic electron donor in cellular energy metabolism[2]. This metabolism, denoted dissimilatory phosphite oxidation (DPO), was first identified in *Desulfotignum phosphitoxidans* FiPS-3, where DPO activity was attributed to the *ptx-ptd* gene cluster. The *ptx-ptd* gene cluster of FiPS-3 is composed of seven genes (ptxDE-ptdCFGHI), whose functions have been assigned based on proteomics, heterologous gene expression, and homology assignments[3]. However, the biochemical mechanism and evolution of DPO is still poorly understood. In FiPS-3, the *ptx-ptd* gene cluster occurs on a genomic island, and its closest relatives are incapable of DPO, suggesting that this metabolism was acquired via horizontal gene transfer. However, this gene cluster has since been identified in two instances of *Ca. Phosphitivorax anaerolimi*: strain Phox-21, which was identified in an active DPO enrichment metagenome; and strain F81, which was identified in a full scale anaerobic digester metagenome[4,5]. Both strains of *Ca. P. anaerolimi* only harbor six of the seven *ptx-ptd* genes, and neither strain shows classical signatures of horizontal gene transfer. Furthermore, *Ca. P. anaerolimi* is in a distinct taxonomic clade from FiPS-3, with no characterized close relatives. DPO is the most energetically favorable chemotrophic electron donating process known to date, yet *D. phosphitoxidans* and *Ca. P. anaerolimi* are the only

identified representatives, thereby limiting our understanding of the diversity and evolutionary history of DPO. In this work, we have used the ptxD as a marker gene to probe metagenomic databases for the *ptx-ptd* operon. We found that the DPO ptxD is prevalent in global metagenomes, is phylogenetically diverse, and forms a monophyletic clade with the ptxD from known DPO representatives. We evaluated the gene neighborhoods of the contigs within the DPO ptxD clade and found that the *ptx-ptd* gene cluster also showed diversity in synteny and gene inclusion. In addition to the ptxD identified in global metagenomes, Ewens et al. (unpublished) recently enriched for several new DPO representatives whose ptxD fell within the database-generated DPO clade. The host taxonomy of these enrichment representatives spanned gram-positive and gram-negative bacteria, and also corresponded with the evolutionary lineages of the ptxD, suggesting that ptxD is vertically inherited. To test whether this pattern was true for the *ptx-ptd* operon as a whole, we repeated our database search using the ptdC and ptdF as probes, and we found that the phylogenetic relationships of those genes corresponded with those of the ptxD. We also identified divergent ptxD with unique *ptx-ptd* operon structures, indicating a potentially greater diversity of ptxD than was previously recognized. This data collectively suggests that DPO is a vertically transferred metabolism, and that the genomic island found in FiPS-3 is an exception. Given the broad taxonomic diversity of DPO hosts, DPO is likely to be an ancient metabolism that dates back to the last common ancestor of gram-positive and gram-negative organisms, with present-day metabolic diversity attributed to subsequent adaptive radiation. These hypotheses are corroborated by work from Pasek et al., suggesting that early metabolisms may have relied on reduced phosphorus compounds[6]. Today's DPO microorganisms may therefore be the fossils of an ancient metabolism that has otherwise been lost since the oxidation of Earth's phosphite following the great oxygenation event[7].

References

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