Revealing the Prevalence and Diversity of a "Rare" Phosphorus Metabolism through Selective Enrichments and Genome Resolved Metagenomics

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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 1.

Phosphorus (P) is an essential biological nutrient, but the majority of P is biologically unavailable because it is trapped in mineral deposits as oxidized phosphate (P^{+5}). Alternative P redox states are often ignored in global P cycle models, despite the fact that reduced P species have been identified in diverse environments[1]. Phosphite (P^{+3}) is a highly soluble, reduced P compound that can account 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 in cellular energy metabolism[3]. This organism, *Desulfotignum phosphitoxidans* FiPS-3, is an autotrophic acetogen for which DPO activity was attributed to the *ptx-ptd* gene cluster[4]. However, the biochemical mechanism of DPO is still poorly understood. In FiPS-3, the *ptx-ptd* gene cluster occurs on a genomic island, and its closest relatives were incapable of DPO, suggesting that this metabolism was acquired via horizontal gene transfer. This gene cluster has since been identified in two instances of *Ca. Phosphitivorax anaerolimi*: strain Phox-21 was identified in an active DPO enrichment metagenome; and strain F81 was identified in a full scale anaerobic digester metagenome[5,6]. Both strains of *Ca. P. anaerolimi* harbor six of the seven *ptx-ptd* genes, and neither strain shows classical signatures of horizontal gene transfer. Both *Ca. P anaerolimi* strains are missing genes for any known electron accepting pathway, host only partial Wood Ljungdahl pathways for

carbon fixation, and have the genomic capacity to perform syntrophic butyrate oxidation[5,6]. Furthermore, *Ca. P. anaerolimi* is in a distinct taxonomic clade from FiPS-3, with no characterized close relatives[6]. 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 phylogenetic and taxonomic diversity of DPO. In an effort to understand the diversity and prevalence of this metabolism, we established 42 DPO enrichments from six different wastewater facilities throughout the San Francisco Bay area. Over 70% of our enrichments showed DPO activity, with representative enrichments from each of the six sample sites. No phosphite oxidation occurred in heat-killed controls. Genome resolved metagenomics of 11 active enrichments yielded 237 high quality MAGS, 19 of which hosted *ptx-ptd* marker genes (DPO MAGS). The DPO MAGS dominated our selective enrichments, and the taxonomy of DPO MAGS spanned six phylogenetic classes (including both gram positive and negative bacteria) and yielded 8 novel DPO hosts, 6 of which are classified as well-characterized syntrophic bacteria. Given the diversity of DPO hosts in our enrichments, we used the ptxD as a marker gene to identify DPO members in global metagenomic databases. We found that the DPO ptxD occurs worldwide, in diverse environments, and forms a distinct phylogenetic clade. Furthermore, when the ptxD from our enrichment DPO MAGS were placed into this database, we discovered that the phylogenetic relationships of the ptxD followed the same evolutionary pattern as the host taxonomy. Prior to this work, DPO was considered a rare metabolism. Our work now shows that DPO is environmentally prevalent, phylogenetically diverse, and hosted by a broad range of organisms with a propensity for syntrophic metabolism. Future work will involve comprehensive analysis of DPO MAG metabolism, including studies on energy conservation, carbon assimilation, and syntrophic partnerships within each community.

References:

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