Prevalence of autotrophy and the characterization of carbon reduction in dissimilatory phosphite oxidizing microbes

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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* operon.**
3. **DPO is universally associated with CO_2 fixation**

The work here advances hypothesis 3.

Phosphite (P^{3+}) is a highly soluble, reduced P compound that can account for up to 30% of total dissolved P in diverse environments[1]. 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[2]. This organism, *Desulfotignum phosphitoxidans* FiPS-3, is an autotrophic homoacetogen for which DPO activity was attributed to the *ptx-ptd* gene cluster, and genomic analyses suggested that FiPS-3 fixed CO_2 via the Wood-Ljungdahl pathway[2]. Following the isolation of FiPS-3, *Phosphitivorax anaerolimi* Phox-21 was identified in the metagenome of a wastewater digester enrichment, serving as the second known DPO microbe[3]. However, unlike FiPS-3, Phox-21 contained no known pathways for CO_2 fixation, despite being grown autotrophically in the absence of alternative electron acceptors[3]. Genomic analysis revealed that Phox-21 must fix CO_2 via the reductive glycine pathway[3]. At the time, the reductive glycine pathway had been proposed as a synthetic pathway for CO_2 fixation[4], making Phox-21 the first natural representative identified to harbor this novel CO_2 fixation pathway[3]. Recently, *Desulfovibrio desulfuricans* was biochemically proven to use the reductive glycine pathway to fix CO_2, validating its legitimacy as the seventh known carbon fixation pathway[5]. Since the identification of Phox-21, our group has identified 21 novel DPOM through metagenomics of wastewater enrichments (Ewens, *et al.* PNAS, 2021)[6]. Taxonomic analyses revealed that DPOM span six taxonomic classes, but despite this diversity, physiological and analyses of the metagenome assembled genomes (MAGs) suggests that the typical DPOM is a chemolithoautotroph that specializes in phosphite oxidation coupled to CO_2 reduction[6].

CO_2 as an Electron Acceptor: Enrichment biochemistry revealed that DPOM preferentially grew in microcosms in which CO_2 was the only exogenous electron acceptor, and DPO was not definitively coupled to any electron acceptor other than CO_2 [6]. A physiological survey of one of our highly enriched DPO cultures further showed that CO_2 was necessary and sufficient to support phosphite oxidation and growth[6]. The final product of CO_2 reduction in Phox-21 remains enigmatic, as the genes for pyruvate conversion to acetate (phosphotransacetylase and acetate kinase) are missing from the genome[3]. Lactate is a possibility, as the genomes of Phox-21 and several other DPO MAGs contain D-lactate dehydrogenase, which converts pyruvate to lactate at the expense of NADH. This is an energetically favorable reaction that accounts for all reducing equivalents produced via phosphite oxidation:

$6PO_3^{3-} + 3CO_2 + 3H_2O \rightarrow 6PO_4^{3-} + C_3H_6O_3 \;\; \Delta G^{\circ\prime} = -29$ kJ/mol e^- (-348 kJ/mol lactate)

CO_2 Fixation to Biomass: In addition to serving as the electron acceptor for DPOM, CO_2 is also fixed into biomass as the carbon source. We supplemented our physiological observations with genomic analyses and found that, as observed in Phox-21, comparative genomics of DPO MAGs revealed a notable absence of any canonical CO_2-reduction pathways[6]. While none of the DPO MAGs contained any canonical CO_2 fixation pathways, the majority of DPOM genomes appear capable of CO_2-fixation to pyruvate via the reductive glycine pathway[6]. Even if not a universal carbon fixation pathway in DPOM, our analyses suggest the reductive glycine pathway might be an important autotrophic mechanism across diverse DPO taxa.

Ongoing work is focused on parsing out the detailed mechanisms of the carbon reduction and carbon fixation pathways of DPOM using HPLC and carbon tracing studies. These studies will also be critical to understanding how nutrients are being exchanged in the DPOM communities, as evidenced in parallel work by our group.

References:

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