26. Metal Metabolism in ENIGMA: The Environmental Role of and Regulation by Molybdenum under Denitrifying Conditions

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Project Goals: The overarching goal of the Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) is to understand environmentally-relevant microbial communities and processes through an integrated field-to-laboratory approach. A critical component of microbial community interactions is assimilation and metabolism of metals. The Metal Metabolism campaign is developing an interdisciplinary platform to elucidate the fundamental mechanisms that drive metal assimilation. Our initial focus is on the role of molybdenum in denitrification. Our functional goal is to understand fully the pathways and gene networks that coordinate Mo homeostasis. Of particular interest are the identities of proteins and regulatory elements that provide competitive advantages or disadvantages to microbes in defined biogeochemical environments.

Molybdenum (Mo) is an essential component of all nitrate reductases, the enzyme that reduces nitrate to nitrite, while copper (Cu) and iron (Fe) are essential components of other enzymes in the pathway for complete denitrification of nitrate to nitrogen gas. We originally hypothesized that biological nitrate reduction in contaminated wells at Oak Ridge National Laboratory (ORNL) is limited by the environmental availability of Mo, Cu and/or Fe. We have demonstrated in the ENIGMA 100-well Global Survey that in many of the contaminated wells containing high nitrate concentrations (>10 mM), the concentrations of Mo are very low (<10 nM) and in a range that severely limits nitrate reduction by the model denitrifer *Pseudomonas stutzeri* RCH2 under laboratory conditions. In contrast, concentrations of Cu and Fe measured in these wells are sufficient for denitrification. The first goal of the new platform will be to study the effects of Mo concentrations on denitrification at the protein and genomic level. Techniques and tools that have been developed and refined to investigate Mo metabolism include a barcoded transposon mutant library of *P. stutzeri* RCH2 and computationally-reconstructed regulons. These will be used together with μscale growth experiments with environmentally-relevant concentrations of Mo (1–100 nM) and nitrate (1–500 mM) to develop draft regulatory networks of cellular processes. Fifteen *Pseudomonas* strains have been isolated from samples obtained through the ORNL 100- well survey and, while closely related by 16S rRNA sequence, these isolates vary greatly in their tolerance and ability to reduce nitrate. Barcoded transposon mutant libraries will be developed for some isolates and dentrification and Mo limitation experiments will be carried out in order to gain genetic insights into Mo uptake, Mo homeostasis and denitrification. It is anticipated that this platform will be extended to field isolates from other genera such as *Castellaniella* and *Rhodanobacter* that appear to play important metal-based roles in well communities. To establish the environmental relevance of RCH2- and the field isolates, a pilot study with environmentally-based laboratory bioreactors will be conducted in collaboration with the Natural and Synthetic Ecology campaign of ENIGMA. The bioreactors will use synthetic groundwater mimicking the geochemistry of

the ORNL wells and will be inoculated with groundwater supplemented with various Mo concentrations. The experimental duration is expected to be 30 days with temporal measurements of metals (40 elements), metabolites, 16S rRNA to determine changes in community structure, and by qPCR of key denitrification genes. End-point samples will be used for the isolation and characterization of new denitrifying strains.

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