70. Linking Iron and Nitrogen Cycling in *Anaeromyxobacter dehalogenans*

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Project Goals: The goals of this project are to fill existing knowledge gaps in our understanding of N-flux and associated C-turnover in soils and sediments. Novel information about the diversity, distribution, abundance and expression of genes contributing to N-transformation is required to link desirable (i.e., N-retention) and undesirable (i.e., N_2O emission) activities with measurable microbial parameters. Linking molecular- and organismal-level information with environmental factors that control N- and C-turnover are desirable to interpret field-scale observations, and predict the impact of land management practices on greenhouse gas (N_2O, CO_2) emissions. Such integrated approaches generate novel information at multiple scales of resolution and contribute to system-level understanding of key nutrient cycles in soils. In the present work, we examined the contributions of the common soil bacterium, *Anaeromyxobacter dehalogenans* to N-cycling under different conditions, and examined the *c*-type cytochromes expressed by this organism.

Anaeromyxobacter dehalogenans strains gain energy from the reduction of a variety of electron acceptors including oxidized metals and nitrogen species. *A. dehalogenans* lacks the ability to denitrify (i.e., *nirK* and *nirS* are absent), but reduces nitrate via the dissimilatory nitrate reduction to ammonium (DNRA) pathway, with nitrite as an intermediate. Interestingly, *A. dehalogenans* strains possess a complete and functional "atypical" *nos*Z gene cluster conferring the ability to reduce N_2O to dinitrogen (1). The addition of 1.0 mM nitrite to ferric iron-grown cultures resulted in stoichiometric conversion of nitrite to N_2O via an abiotic mechanism (i.e., chemodenitrification). In contrast, nitrite added to fumarate-grown cultures did not result in N_2O formation.

Abiotic control vessels containing medium without cells and amended with nitrite and ferrous iron (as ferrous chloride) resulted in N_2O production. These observations suggest that the abiotic reaction of ferrous iron with nitrite led to N_2O formation (i.e., chemodenitrification). The N_2O produced in live cultures was reduced by *A. dehalogenans* to dinitrogen. Cell enumeration using quantitative real-time PCR (qPCR) demonstrated growth with abiotically produced N_2O in cultures that had reduced ferric to ferrous iron. Despite the absence of key denitrification genes (i.e., *nirK* and *nirS*), *A. dehalogenans* contributed to the conversion of nitrite (a DNRA intermediate) to dinitrogen via a coupled abiotic-biotic process involving ferrous iron, the end product of ferric iron reduction catalyzed by the same organism. Ferric iron minerals are common to soils and sediments suggesting that coupled abiotic-biotic processes (i.e., chemodenitrification followed by enzymatic N_2O reduction) contribute to N-cycling and affect N_2O flux.

Additional studies attempted to link *c*-type cytochrome expression profiles with *A. dehalogenans* ecophysiology. *c*-type cytochromes are key components for electron transfer to terminal electron

acceptors in *A. dehalogenans* and other metal-reducing populations. Deletion mutant and biochemical studies identified a number of *c*-type cytochromes involved in electron transfer to oxidized metals in *Shewanella* spp. and *Geobacter* spp.; however, only a fraction of the entire *c*-type cytochrome pool has been functionally characterized. The genome of *Anaeromyxobacter dehalogenans* strain 2CP- C encodes 69 *c*-type cytochromes, including two *nrfA* gene copies (Adeh_0910, Adeh_2902) and two *nrfH* gene copies (Adeh_0911, Adeh_2903) involved in DNRA. To identify and subsequently characterize unique *c*-type cytochromes involved in soluble and amorphous ferric iron reduction and N-metabolism, *A. dehalogenans* strain 2CP-C was grown in batch cultures with ferric citrate, ferric oxyhydroxide, and nitrate. Fumarate-grown strain 2CP-C cells were included as a control. Whole cell lysates were subjected to trypsin proteolysis and analyzed using a biphasic LC-MS/MS (Liquid chromatography-tandem mass spectrometry) setup. Distinct *c*-type cytochrome expression patterns were observed in cells grown with the different electron acceptors. Interestingly, several *c*-type cytochromes, including NrfA (Adeh_2902) were expressed in cells grown with ferric iron or nitrate but could not be detected in control cultures grown with fumarate. The second *nrf*A gene copy (Adeh_0910) was only expressed in cells grown with nitrate.

These results indicate that the *A. dehalogenans* strain 2CP-C uses the same *c*-type cytochromes for electron transfer to nitrate and to ferric iron, thus linking iron and N- cycling. Further the data illustrate that the role of a microorganism or a microbial community in N-cycling cannot be predicted based on gene content (i.e., the genome sequence or metagenomic datasets) alone, and confirm that non-denitrifiers possessing atypical *nosZ* genes contribute to N_2O consumption (1). In experimental systems, *A. dehalogenans* effectively contributes to denitrification, even without enzymes converting nitrate to N_2O, by coupling biotic with abiotic reactions. The contributions of non- denitrifying populations such as *Anaeromyxobacter* spp. to complete denitrification (i.e., N_2 formation) and associated C-turnover in soil ecosystems is being explored.

Reference:

1. Robert A. Sanford, Darlene D. Wagner, Qingzhong Wu, Joanne C. Chee-Sanford, Sara H. Thomas, Claribel Cruz-García, Gina Rodríguez, Arturo Massol-Deyá, Kishore K. Krishnani, Kirsti M. Ritalahti, Silke Nissen, Konstantinos T. Konstantinidis, and Frank E. Löffler. **Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils.** *Proc. Natl. Acad. Sci. U. S. A.* **109**: 19709-14.

This research was supported by the US Department of Energy, Office of Biological and Environmental Research, Genomic Science Program, Award DE-SC0006662.