A Nitrogenase-like Methylthio-alkane Reductase Complex Catalyzes Anaerobic Methane, Ethylene, and Methionine Biosynthesis

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Project Goals: The goal of this project is to identify and characterize the specific enzyme(s) that catalyze anaerobic ethylene synthesis. This is part of a larger project to develop an industrially compatible microbial process to synthesize ethylene in high yields. The specific goals are:

1. Identify the genes and gene products responsible for anaerobic ethylene synthesis.
2. Probe the substrate specificity and metagenomic functional diversity of methylthio-alkane reductases to identify optimal bioproduct generating systems.
3. Characterize the enzymes and the reactions that directly generate anaerobic ethylene.

Abstract Text: Our previous work identified a novel anaerobic microbial pathway (DHAP-Ethylene Shunt) [1] that recycled 5'-methylthioadenosine (MTA) back to methionine with stoichiometric amounts of ethylene produced as a surprising side-product. MTA is a metabolic byproduct of methionine utilization in a multitude of cellular processes. The initial steps of the DHAP-ethylene sequentially converts MTA to dihydroxyacetone phosphate (DHAP) and ethylene precursor (2-methylthio)ethanol (Fig. 1; gray). However, the terminal enzyme(s) responsible for ethylene biogenesis and regeneration of methionine we unknown.

Genes and gene products MarHDK responsible for anaerobic ethylene synthesis: We sought to identify the genes and proteins responsible for ethylene production via proteomics, transcriptomics, and specific gene deletion studies [2]. Ethylene production from (2-methylethio)ethanol is highly regulated by the presence of exogenous sulfate. Therefore, cells were grown under sulfate replete (ethylene suppressing) and sulfate limiting (ethylene inducing) anaerobic growth conditions. Cells were harvested and differential proteome analysis via HPLC-MS/MS was performed to identify proteins that increased in abundance during ethylene inducing conditions. Proteins with the highest increase in abundance during production of ethylene from (2-methylthio)ethanol corresponded to novel nitrogenase-like proteins and previously characterized O-acetyl-L-homoserine sulfhydrylases [2,3] (Fig. 1; rxns 1, 3). This nitrogenase-like complex is named methylthio-alkane reductase, with components MarB and MarHDK based on homology to *bona fide* nitrogenase systems. We further probed the substrate specificity for this novel methylthio-alkane reductase process. Indeed, other small volatile organic sulfur compounds (VOSCs) required the nitrogenase-like gene products to be utilized as a sulfur source by *R. rubrum* for growth and methionine metabolism. Utilization of dimethyl sulfide, the most abundant VOSC in the environment, resulted in stoichiometric production of methane (Fig. 1), and ethylmethyl sulfide led to stoichiometric production of ethane gas [2]. This is the first indication of a nitrogenase-like complex responsible for the reduction of a carbon-sulfur bond, and the first observation of a nitrogenase-like complex involved in sulfur (methionine) metabolism.

Diversity of Organisms with MarHDK: The nitrogenase superfamily is composed of the *bona fide* nitrogenases (groups I-III), which reduce N2 into ammonia, and the Nitrogen Fixation-Like proteins (groups IV-VI), which catalyze a diverse number of reactions of known an unknown function. The methylthio-alkane reductase gene products MarHDK are homologous to nitrogenase proteins NifH (nitrogenase reductase iron protein), and NifDK (catalytic subunits α/β). Methylthio-alkane reductase sequences mined from metagenome databases form a distinct clade (Group IV-C) within the Group IV family of Nitrogen Fixation-Like sequences, and initial tested representatives exhibit activity.

Structure and mechanism of MarHDK: A central part of determining the mechanism of methylthio-alkane reductase is the characterization of its catalytic cofactor and structure of the surrounding protein active site. In collaboration with the JGI FICUS program and the Brookhaven National Laboratory NSLS-II, we are working to solve the structure of the *R. rubrum* methylthio-alkane reductase to understand how ethylene and methane are produced by this system. Initial subunit isolation and purification endeavors reveals that MarH is indeed analogous to NifH and MarDK is analogous to NifDK. This suggests a similar yet distinct structure and mechanism between the two, resulting in their unique respective catalytic functions.

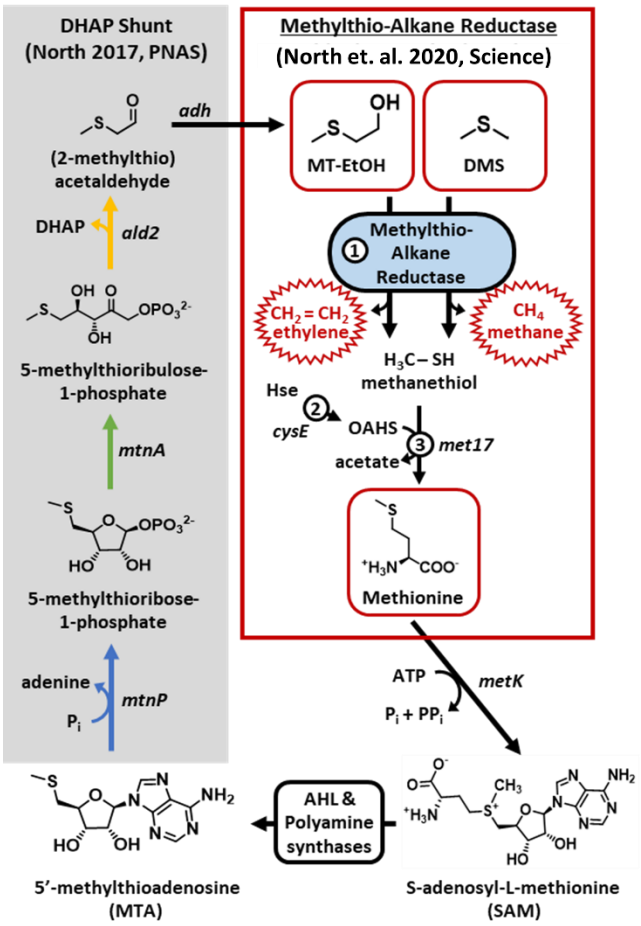

Fig. 1. Full elucidation of the Anaerobic Ethylene Cycle involving a novel nitrogenase-like methylthio-alkane reductases with wide substrate specificity for volatile organic sulfur compounds [2]. (1) Methylthio-alkane reductase (*marHDK*), (2) homoserine acetyltransferase (*cysE)*, (3) acetylhomoserine Sulfhydrylase (*met17*).

References:

1. North JA, Miller AR, Wildenthal JA, Young SJ, Tabita FR. Microbial pathway for anaerobic 5'-methylthioadenosine metabolism coupled to ethylene formation. *Proc. Natl. Acad. Sci. U S A.* **2017**. 114: E10455-E10464
2. North JA, Narrowe AB, Xiong W, Byerly KM, Zhao G, Young SJ, Murali S, Wildenthal JA, Cannon WR, Wrighton KC, Hettich RL, Tabita, FR. A nitrogenase-like enzyme system catalyzes methionine, ethylene, and methane biogenesis. *Science.* **2020.** 369(6507):1094-1098
3. Erb TJ, Evans BS, Cho K, Warlick BP, Sriram J, Wood BM, Imker HJ, Sweedler JV, Tabita FR, Gerlt JA. A RuBisCO-like protein links SAM metabolism with isoprenoid biosynthesis. *Nat Chem Biol.* **2012,** 8:926–932

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