High Throughput Screening of Enzymes that Bolster Anaerobic Ethylene Synthesis

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Project Goals: The overall long-term objective is to develop an industrially compatible microbial process to synthesize ethylene in high yields from CO_2 and lignocellulose. To optimize the activity of the recently discovered dihydroxyacetone phosphate (DHAP) ethylene pathway for increased ethylene yields, the following specific goals are:

1. Discover effective and active DHAP ethylene pathway enzymes encoded in cultured and uncultured organisms from anoxic environments. (Wrighton and North)
2. Construct a modular set of optimized genes on a DNA fragment containing specific regulatory elements that will allow high level gene expression in model organisms. (North and Cannon)

Abstract Text: Our previous work identified a novel anaerobic microbial pathway (DHAP-Ethylene Pathway) [1] that converted 5'-methylthioadenosine (MTA) to stoichiometric amounts of ethylene. MTA is a metabolic byproduct of methionine utilization in a multitude of cellular processes. The initial steps of the DHAP-ethylene pathway sequentially convert MTA to dihydroxyacetone phosphate (DHAP) and ethylene precursor (2-methylthio)ethanol (Fig. 1; gray), followed by reduction to the methionine precursor, methanethiol, and ethylene via a novel nitrogenase-like methylthioalkane reductase (Fig. 1; red box) [2]. Kinetic characterization of the initial DHAP-ethylene pathway enzymes from the photosynthetic bacterium, *Rhodospirillum rubrum*, revealed that while the phosphorylase had relatively high specific activity (MtnP = 3.2×10^5 M^{-1} s^{-1}; Fig. 1, blue arrow), the subsequent MTR-1P isomerase and MTRu-1P aldolase had relatively lower specific activity (MtnA = 0.75×10^3 M^{-1} s^{-1}; Ald2 = 1.7×10^3 M^{-1} s^{-1}; Fig. 1, green and yellow arrows, respectively) [3]. This suggested that introduction of more catalytically efficient isomerase and aldolase homologs could increase ethylene yields by increase the DHAP-ethylene pathway flux.

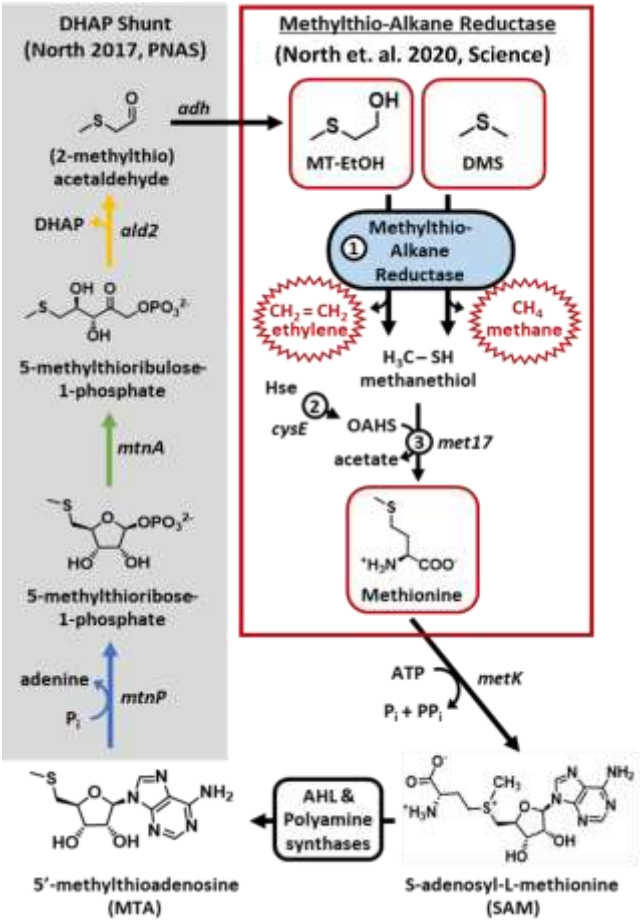

Fig. 1. Anaerobic ethylene cycle (DHAP-ethylene pathway), which is composed of the DHAP shunt (gray box) and methylthio-alkane reductase methionine synthesis pathway (red box). (1) Methylthio-alkane reductase (*marHDK*), (2) homoserine acetyltransferase (*cysE)*, (3) acetylhomoserine Sulfhydrylase (*met17*). MT-EtOH, (2-methylthio)ethanol; DMS, dimethylsulfide.

Indeed, introduction of more catalytically active aldolases from *E. coli* increased ethylene yields by 2-fold in initial trials [1,3].

High-throughput screening of isomerases and aldolases from genome sequences of isolated and environmental bacteria – To identify and isolate enzymes with robust catalytic properties that enhance ethylene production, we adopted a high-throughput cell lysate activity screening approach for enzymes heterologously produced in *E. coli*. Mining of JGI IMG/M genome and metagenome sequence databases for candidate orthologs to the MTR-1P isomerase (*mtnA*) and the MTRu-1P aldolase (*ald2*) genes yielded 1,371,813 and 96,049 candidate genes for *mtnA* and *ald2*, respectively. We selected two hundred syntenous *mtnA / ald2* gene pairs that represented the phylogenetic breath of sequences and considered proximity to active orthologs. Gene product activity in *E. coli* was quantified by colorimetric detection of phenylhydrazine adducts with (2-methylthio)acetaldehyde produced from MTA via coupled enzyme assay in 96-well plate format. ***The isomerase library screen***: recovered 42 soluble and active isomerases out of 250 sequences with *in extracto* specific activity ranging from 3.3-8800 nmol/min/mg total protein. Introduction of a subset of these isomerases into the *R. rubrum* isomerase deletion strain via a plasmid resulted in restored conversion of MTA into ethylene. Select sequences increased ethylene yields by 30% and 60% compared to when the native *R. rubrum* isomerase was expressed from the plasmid or chromosome, respectively. ***The aldolase library screen*****:** recovered ~120 soluble and active aldolases out of 250 sequences with *in extracto* specific activity ranging from 2.0-7996 nmol/min/mg total protein. Introduction of a subset of these aldolases into the *R. rubrum* aldolase deletion strain via a plasmid similarly resulted restored conversion of MTA into ethylene. Select sequences increased ethylene by 35% and 5-fold compared to the *R. rubrum* aldolase expressed from the plasmid or chromosome, respectively.

Conclusions and future directions: Orthologs of DHAP-ethylene pathway isomerases and aldolases with potentially increased catalytic properties were recoverable from bacteria isolates and metagenomes spanning firmicutes, alpha, beta, gamma, and deltaproteobacteria. An increase in DHAP-ethylene pathway isomerase activity, either through overexpression and/or introduction of more active orthologs in *R. ruburm* increased ethylene yields by only 60%, indicating a potential limitation in pathway flux enhancement that can be achieved through modifying the isomerase alone. In contrast, increasing aldolase activity clearly increased pathway flux, resulting in a 5-fold increase in ethylene production. Future studies will quantify the combined effect of efficient isomerases and aldolases.

References:

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