Engineering of Enhanced Microbial Anaerobic Ethylene Synthesis Through Predictive Modeling and Metagenomic Functional Gene Discovery

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Project Goals: Ethylene is the most widely employed organic precursor compound in industry. The potential to impact ethylene formation via the recently discovered microbial "anaerobic ethylene cycle" is promising. This drives the following specific aims:

1. Construct a thermo-kinetic predictive model of microbial metabolism including the the "anaerobic ethylene cycle" to identify hypothesis-driven targets for engineering optimal regulation and metabolic activity.
2. Perform metagenomic analysis of cultured and uncultured organisms, DNA synthesis of candidate genes, and activity screening to identify functional analogs of ethylene cycle genes.

Abstract Text: Ethylene is the highest production industrial platform chemical, required for the manufacturing of plastics, detergents, and solvents. Supplementing current fossil fuel-based ethylene with ethylene produced biologically from renewable sources is desirable to reduce carbon emission. This is feasible using the anerobic ethylene cycle discovered and characterized by our group (Fig. 1A), which is found in photosynthetic and lignocellulosic bacteria [1,2]. Engineering of high yields in industrially tractable bacteria such as *Rhodospirillum rubrum* and *Clostridium cellulolyticum* requires optimization of ethylene cycle pathway kinetics, synthesis of requisite cofactors by supporting pathways, and coordinated gene expression.

Predictive modeling of ethylene production and identifiable bottlenecks: Microbial engineering of ethylene production and subsequent methionine regeneration is highly complex. Synthetic biology engineering endeavors need to address trade-offs between the production of intended targets and the energetic and regulatory requirements of the cell. To specifically address these needs, we have developed hybrid thermodynamic-kinetic model of microbes, including *R. rubrum*, that allows us to characterize energy requirements of native and engineered pathways as well as the regulatory requirements. This model involves approximately 200 metabolic reactions including central carbon metabolism, generic protein, RNA and DNA synthesis reactions, and the ethylene cycle. Currently, key testable constraints identified by the model that limit ethylene production include:

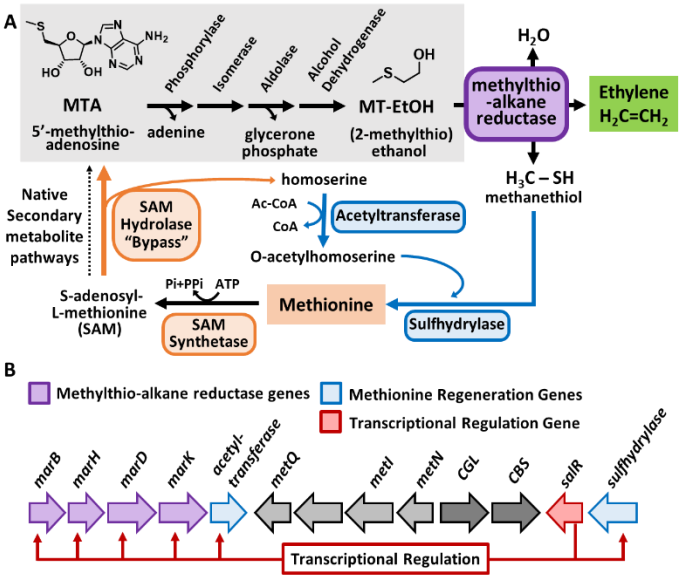

Fig. 1: A) Anaerobic ethylene cycle and B) regulatory network under SalR transcription control.

i. *Ethylene precursory compound synthesis rate:* The ethylene precursor, MTA, metabolized by the DHAP-ethylene shunt (Fig. 1A, gray) is produced natively by multiple pathways. Modeling predicts that increased MTA synthesis rate will increase ethylene production. Initial introduction into *R. rubrum* of the viral enzyme SAM hydrolase (Fig. 1A, bypass) increases ethylene >50-fold.

ii. *Regulation of methionine synthesis:* The model predicts that increased methionine synthesis will increase ethylene production. The methylthio-alkane reductase and methionine regeneration enzymes are under tight transcriptional control by SalR (Fig. 1B) [2]. Replacement of the methylthio-alkane reductase promoter with active promoters increased ethylene yields 10-fold.

Functional diversity of metagenomic ethylene cycle enzymes: Previous studies indicated that aldolase and isomerase orthologous genes could substantially enhance ethylene levels relative to endogenous genes [2]. To scale up the search for ethylene-enhancing orthologs we applied targeted functional metagenomics. Mining of JGI IMG/M genome and metagenome sequence databases for candidate orthologs to the isomerase and aldolase genes (Fig. 2) yielded candidates covering a wide variety of environments including wetlands, forest soils, rhizosphere, and bioreactors. A subset of these genes have been synthesized by the JGI DNA Synthesis Science program and screened via our high throughput *E. coli* lysate activity assay (Fig. 2). Genes for aldolase orthologues with measurable activity in *E. coli* were introduced into the *R. rubrum* aldolase deletion stain. Ethylene was measured and several aldolase orthologues were identified that alone increase ethylene yields 1.3-fold compared to the native enzyme (Fig. 2). Similar approaches will be taken with the isomerase, and combinations of optimal enzymes that increase ethylene yields will be employed.

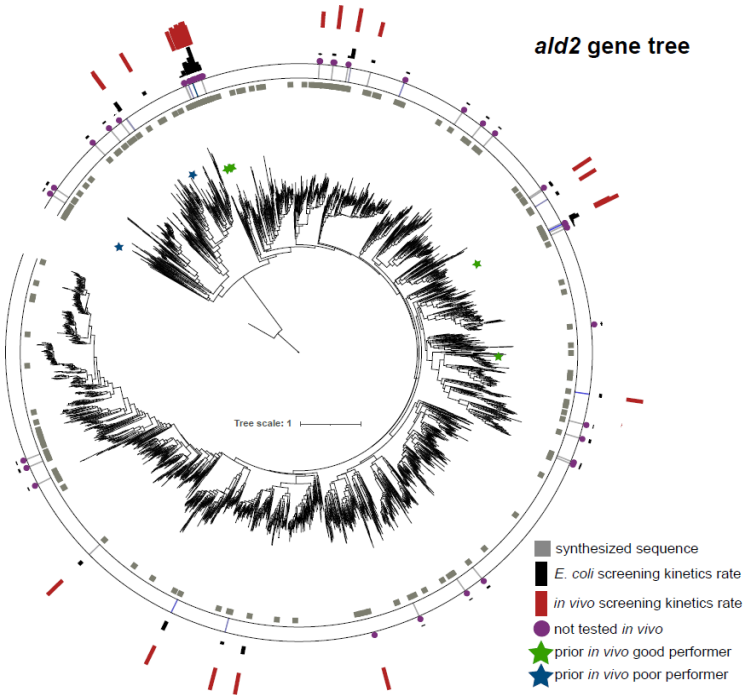

Fig. 2: Functional screen of metagenomic ethylene cycle aldolases

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