Metabolic Modeling and Engineering of Enhanced Anaerobic Microbial Ethylene Synthesis

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Project Goals: To develop robust and optimized anaerobic ethylene pathways in photosynthetic and lignocellulosic bacteria for high-yield conversion of renewable CO2 and lignocellulose into bioethylene. This will be accomplished by:

1: First, bioinformatically mining and experimentally screening methylthio-alkane reductase homologs from cultivated and uncultivated organisms for functional enzymes that enhance ethylene yields.

2: Next, constructing and employing predictive systems-level models of ethylene production. Using a physics-based *R. rubrum* model, we predict enzymes that participate in competing or supporting pathways and are thus targets for knockdown or selection studies to increase ethylene yields. This will direct engineering strategies of top-performing genes (Aim 3) to maximize ethylene yield and minimize trade-off costs.

3: Finally, we metabolically engineer bacteria for enhanced, sustained ethylene production from CO2 and lignocellulose. We assemble the best-performing genes under control of optimized active transcription elements on a modular DNA fragment in a combinatorial manner with guidance from predictive models (Aim 2).

Abstract Text: Ethylene is the highest production industrial platform chemical on earth and constitutes a $300 billion global industry. Because of its versatility, ethylene is used to manufacture most plastics including polyethylene films, polyester fabrics, polystyrene packaging, and PVC piping. Plants and select fungi and bacteria naturally produce ethylene in a regulated manner as a signaling hormone. Known aerobic pathways employ one of two oxygen-dependent enzymes that specifically catalyze the oxidation of substrate to ethylene. However, both systems have faced scale-up challenges due to oxygen-ethylene combustion hazards and formation of cytotoxic products (hydrogen cyanide and guanidine). We uncovered a novel and previously uncharacterized methylthio-alkane reductase in *Rhodospirillum rubrum* that produces ethylene in the absence of oxygen (Fig. 1, rxn 4) [1]. The goal of this project is to optimize this anaerobic ethylene production pathway, as outlined above. This report describes predictions from a physics-based model for gene/enzyme engineering and the experimental outcomes.

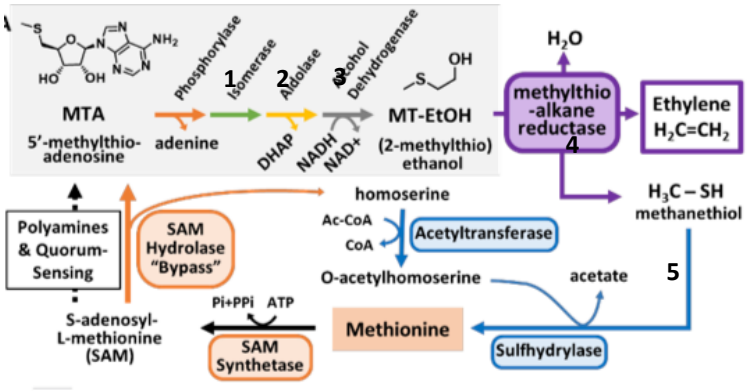

Fig. 1 Anaerobic Ethylene Cycle for microbial synthesis of ethylene. Reactions probed by thermo-kinetic modeling for their effect on pathway flux and ethylene yields are numbered. DHAP, dihydroxyacetone phosphate; CoA, coenzyme-A.

Biology and Physics: The physics-based model is based on recent developments in statistical

thermodynamics of non-equilibrium processes that rigorously describe time-dependent thermodynamic processes, in which classical thermodynamics and kinetics are united in the formulation of entropy production rates. Rate parameters needed for solving the differential equations describing metabolism are obtained by considering that natural selection is a thermodynamic process in which the fittest organisms seek to maximize their entropy production rates, which includes the process of replication, relative to their neighbors. Knowledge of these maximal rates allows for back-calculation of rate parameters. In addition, machine learning/mathematical optimization is used to predict regulation of pathways needed to keep metabolite concentrations in the feasible range and to optimize the activity of the metabolic pathways to be consistent with physiology [2]. Metabolic control analysis, a form of control theory specific for metabolism and systems of coupled reactions, is used to predict which enzymes and reactions influence the production of ethylene the most, either positively or negatively.

Metabolic Engineering: The proposed targets for metabolic engineering to enhance ethylene production were five reactions directly on the ethylene biosynthetic pathway (Fig. 1, rxn. 1-5) and two reactions that act to drain the ethylene pathway of precursors. The proposed on-pathway targets were the (1) isomerase, (2) aldolase, (3) alcohol dehydrogenase, (4) methyl-thioalkane reductase that directly produces ethylene, and (5) the sulfhydrylase that recycles products of the methyl-thioalkane reductase reaction. The proposed off-pathway targets are two enzymes that drain the aldolase substrate, 5-methylthio-ribulose-1-phosphate, from the ethylene biosynthetic pathway: (6) a RubisCO-like isomerase that converts 5-methylthio-ribulose-1-phosphate to 1-methylthio-ribulose-5-phosphate, and (7) a reductase that converts the latter product to deoxyxylulose-5-phosphate for isoprenoid synthesis.

The model suggested that down regulating the off-pathway RubisCO-like isomerase reaction (reaction 6, above) would change flux to ethylene by 2-10 fold, which has experimentally verified (9-fold increase ethylene yield). The model also predicted that the on-pathway isomerase (Fig. 1 rxn 1) would have the least impact on ethylene production, which has so far been the case in on-going experimental studies (max 2-fold increase in ethylene yields). The on-pathway aldolase and alcohol dehydrogenase activities (Fig. 1; rnx. 2, 3) are predicted to have intermediate impacts on ethylene production, and engineering of the aldolase reaction through overexpression or use of more catalytically active homologs has increased ethylene yields up to 4.8-fold, consistent with predictions. Modeling indicated the ethylene and sulfhydrylase reactions (Fig. 1; rxn. 4, 5) would have the largest impact on ethylene production. Indeed, overexpression of the native methylthio-alkane reductase increased ethylene yields directly from substrate, 2-methylthioethanol, by ~7-fold, and the sulfhydrylase reaction is being investigated experimentally now.

References:

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