Exposure to oxygen induces a transient bottleneck in the methylerythritol 4-phosphate pathway in *Zymomonas mobilis*: the role of iron-sulfur cluster assembly proteins and flavodoxin reductase in recovering pathway activity

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Project Goals: The goal of this research is to inform metabolic engineering efforts to improve yields of isoprenoid-based bioproducts via the methylerythritol 4-phosphate (MEP) pathway. By examining native responses to a severe metabolic bottleneck in the MEP pathway, we hope to gain a new perspective about the key regulatory enzymes controlling MEP pathway activity. www.glbrc.org/research/conversion

The methylerythritol 4-phosphate (MEP) pathway generates isoprenoid precursors in bacteria. Isoprenoids are a diverse class of molecules, encompassing several desirable bioproducts including pharmaceuticals, synthetic polymers, and high-grade fuels. In recent years, efforts have been made to metabolically engineer microbes for over-production of isoprenoid commodity molecules via the MEP pathway. However, directed engineering has been limited by an incomplete understanding of the regulatory modules controlling pathway activity.

Our work investigates the metabolic regulation of the MEP pathway in the emerging biofuel-producer *Zymomonas mobilis* by monitoring metabolic changes in the MEP pathway and connected pathways in response to oxygen exposure. Using metabolite quantification with UHPLC-MS, we have observed a dramatic and transient metabolic bottleneck in the MEP pathway induced by exposure to oxygen. Metabolite profiling indicates this bottleneck is caused by inactivation of the final two enzymes of the pathway, IspG and IspH, likely due to oxidative damage of their Fe_4S_4 iron-sulfur cluster cofactors. In *E. coli*, and likely in *Z. mobilis*, these Fe_4S_4 clusters are reduced in by flavodoxin, which is in turn reduced by an NADPH-dependent flavodoxin reductase.[1,2].

Proteomics and transcriptomics data suggest the bottleneck in the MEP pathway is relieved by increased expression of the SUF iron-sulfur assembly operon and flavodoxin reductase, recovering IspG and IspH function by maintaining in-tact and reduced iron-sulfur cluster cofactors in both enzymes. Our findings have thereby identified the SUF iron-sulfur cluster assembly operon and flavodoxin reductase as potential targets for overexpression to increase microbial production of isoprenoid commodity molecules.

References:

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