Development of metabolomic methods for investigating metabolic regulation of the MEP pathway in *Zymomonas mobilis* and *Escherichia coli*

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Project Goals: The mission of the Great Lakes Bioenergy Research Center is to perform research that generates technology to convert cellulosic biomass to advanced biofuels. In alignment with this goal, we have developed a method to examine the metabolic regulation of the MEP pathway using metabolite quantification with LC-MS. Understanding the metabolic response of the MEP pathway to environmental and genetic changes will help to guide targeted metabolic engineering for high-yield production of advanced biofuels such as isoprene and isoprene-derived alcohols.

The methyl-erythritol-phosphate (MEP) pathway responsible for producing isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) in most bacteria (Fig 1). These metabolites play an integral role in cellular metabolism as they are the building-blocks for biosynthesis of isoprenoids, a diverse class of compounds encompassing quinones, carotenoids, hopanoids, and various secondary compounds. Additionally, IPP and DMAPP are direct precursors to isoprene, a high-value chemical which can be used for the production of synthetic polymers, or converted to high-grade fuels such as methylbutenol. In recent years, efforts have been made to metabolically engineer bacteria for over-production of isoprene and isoprene-derived alcohols via the MEP pathway. However, directed engineering has been limited by an incomplete understanding of *in vivo* regulation of the MEP pathway. Our work aims to understand the metabolic regulation of the MEP pathway in both *E. coli* and the emerging biofuel-producer *Zymomonas mobilis*. We utilize a metabolomic approach to monitor metabolic changes in the MEP pathway, and connected pathways of central metabolism, in response to environmental and genetic perturbations.

In order to interrogate the metabolic regulation of the MEP pathway, we have developed a method using LC-MS to quantify all eight intermediates of the MEP pathway: 1-deoxy-D-xylulose 5-phosphate (DXP), 2-C-methyl-D-erythritol 4-phosphate (MEP), cytidine diphosphate-methlyerythritol (CDP-ME), cytidine diphosphate-methylerythritol 2-phosphate (CDP-MEP), methylerythritol 2,4-cyclodiphosphate (MEcDP), hydroxyl-methrylbutanol diphosphate (HMBDP), IPP and DMAPP as well as downstream products such as geranyl pyrophosphate (GPP) and farnesyl pyrophosphate (FPP) (Fig 1). This method also allows for quantification of a broad range of central metabolites including intermediates of glycolysis, the pentose phosphate pathway, and the TCA cycle as well as amino acids, nucleotides, and electron carriers such as NAD(P)H. With this method, it is possible to monitor depletion and accumulation of intermediates of the MEP pathway in response to changes in nutrient conditions or abiotic stressors, and to identify interactions between the MEP pathway and the rest of central metabolism. To date, we have examined the effects of growth stage, nitrogen depletion, aerobic

growth, and exposure to ethanol on the MEP pathway in the context of central metabolism. We have observed a dramatic effect of exposure to oxygen on the abundance of intermediates in the MEP pathway in *Z. mobilis*, indicative of a bottleneck at the final two reductive steps (Fig 1).

In addition to environmental perturbations, we are interested in examining the metabolic consequences of genetic changes to the MEP pathway. With our LC-MS method and engineered strains of *E. coli* and *Z. mobilis* generated by the Landick Lab, we are able to quantify the effects of over-expressing or silencing genes of the MEP pathway on intermediate metabolites of the pathway, as well as central carbon metabolism. To date, we have performed metabolomic analysis on *E. coli* and *Z. mobilis* strains containing plasmids that overexpress the first five genes of the MEP pathway, ending with the production of MEcDP, and have observed a significant increase in the abundance of MEcDP upon induction of the plasmids. This overexpression is accompanied by changes in central metabolism that may reveal key carbon, energy, and redox inputs that limit the activity of the MEP pathway.

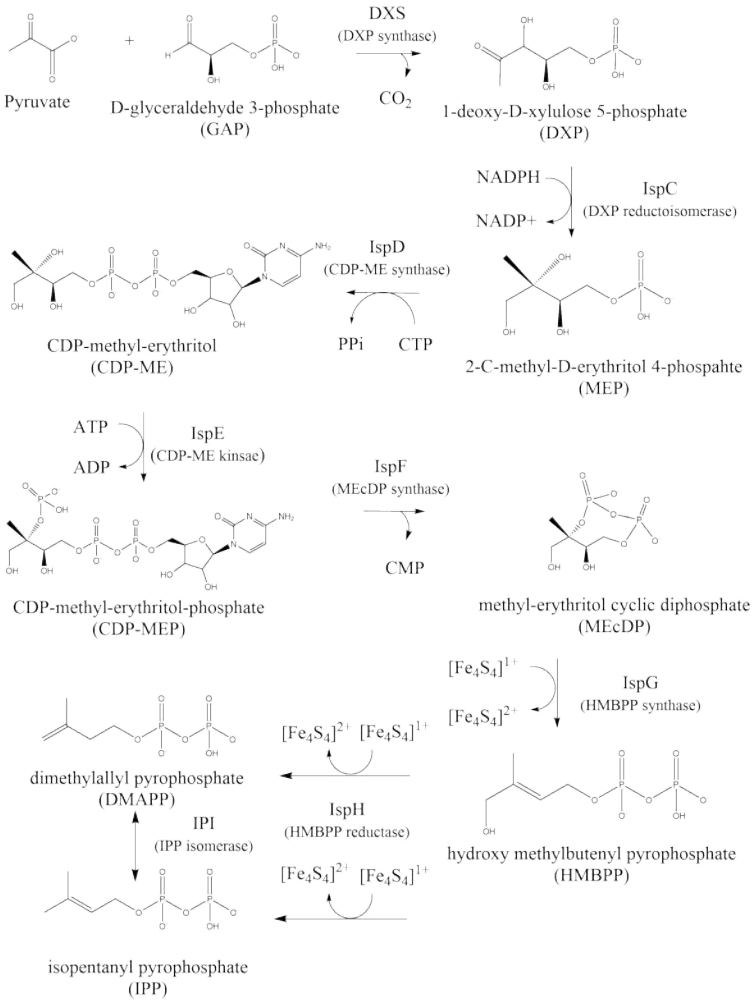

Figure 1. The seven enzymatic steps of the MEP pathway.

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