Overexpression and Metabolic Regulation of *Z. mobilis* MEP Pathway Enzymes

Mehmet Tatli[1,2*] (mtatli@wisc.edu) and **Daniel Amador-Noguez**[1,2]

[1]Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin, USA;
[2]DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, Wisconsin, USA

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Project Goals: Understanding metabolic regulation of *Z. mobilis* MEP pathway and engineering ZM4 strains to overproduce targeted isoprenoids.

Zymomonas mobilis, a facultative anaerobe, can convert 96% of the glucose consumed to ethanol at high yields. This highly catabolic metabolism can also be redirected towards generation of isoprenoid-derived biofuels via the 2-C-Methyl-D-erythritol 4-phosphate (MEP) pathway. Here, we have individually over-expressed the MEP pathway enzymes (DXS, DXR, IspDF, and IspE) of *Z. mobilis* in the ZM4 strain to better understand the metabolic regulations in the first part of the MEP pathway, and investigate the effect of the enzymes on directing carbon flux into the MEP pathway and down to the cyclic intermediate, MEcDP. Initial results showed that DXS2 over-expression increases flux through MEP pathway, leading to a 70 fold increase in intracellular MEcDP levels, and also increases levels of the two end products of this pathway, IDP/DMADP. These results have indicated that DXS2 is a rate limiting enzyme in the MEP pathway of *Z. mobilis*. Moreover, coupling DXS2 with isoprene synthase, IspS, allows for production of isoprene in ZM4. This over-expression strategy also revealed interesting metabolic changes in the MEP pathway, which might bring new insights into understanding metabolic regulation of the MEP pathway.

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