Title: Metabolomic and Proteomic Analysis of *Zymomonas mobilis* During Nitrogen Fixation Reveals Metabolic Remodeling of Biofuel Producing Pathways

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Project Goals: Characterize native metabolic regulation of biofuel producing pathways (such as ethanol production and isoprenoid synthesis) in order to inform genetic engineering of *Z. mobilis* for improved biofuel production.

Abstract text: *Zymommnas mobilis* is a promising biofuel producer capable of rapid glucose consumption and ethanol production. Recently, it was demonstrated that *Z. mobilis* can fix N_2 as a sole nitrogen source (1). Under N_2 fixing conditions, *Z. mobilis* exhibited a higher specific rate of ethanol production than when NH_4^+ was supplied in the media (1, 2). In order to better understand the metabolic remodeling that occurs during N_2 fixation, we performed metabolomics, proteomics, and thermodynamic analysis of *Z. mobilis* under conditions of N_2 fixation compared to replete NH_4^+ availability. We also performed metabolomic and proteomic analysis during the dynamic shift to N_2 fixing conditions (NH_4^+ downshift) and during the shift to NH_4^+ replete conditions (NH_4^+ upshift). We found that intracellular concentrations of intermediates of the Entner-Doudoroff (ED) glycolytic pathway were depleted during N_2 fixation. Protein levels of zinc-dependent alcohol dehydrogenase (encoded by adhA, ZMO1236) increased by 10-fold during the shift to N_2 fixation, helping to explain the previously observed increase in specific ethanol production. Positional stable isotope labeling revealed that labeled forms indicative of reverse flux were more abundant under NH_4^+ replete conditions for all five labeled schemes tested, implying increased thermodynamic favorability of the ED pathway during N_2 fixation. We also observed severe depletion of intermediates of the methylerythritol 4-phosphate (MEP) pathway during N_2 fixation, which was accompanied by decreased protein abundance of deoxyxylulose 5-phosphate synthase (DXS), the first enzyme of the MEP pathway. Unexpectedly, we found that intracellular arginine levels were over 3-fold higher during N_2 fixation and decreased by over 3-fold within 10 minutes of NH_4^+ addition. Based on an overall depletion in intermediates of arginine biosynthesis during N_2 fixation and dynamic changes in protein abundance of a group IV pyridoxal-dependent decarboxylase, encoded in an operon with a deoxyhypusine synthase-like gene, we hypothesize that polyamine synthesis from arginine plays an important role in *Z. mobilis* physiology during changes in NH_4^+ availability. This study has expanded our fundamental understanding of nitrogen metabolism in *Z. mobilis*, identified DXS protein abundance as a native control-point for MEP pathway activity, and demonstrated that metabolic remodeling during N_2 fixation results in increased thermodynamic favorability of

the ED pathway *in vivo*. These results will help to inform future efforts for metabolic engineering in *Z. mobilis* to increase biofuel production.

References/Publications

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2. Palamae S, Choorit W, Chatsungnoen T, Chisti Y. 2020. Simultaneous nitrogen fixation and ethanol production by Zymomonas mobilis. J Biotechnol.

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