Model-driven analysis of mutant fitness experiments improves genome-scale metabolic models of *Zymomonas mobilis* ZM4

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Project Goals: Our goal is to establish an updated, accurate genome-scale metabolic reconstruction of the bacteria *Zymomonas mobilis* by leveraging high-throughput experimental datasets to fill in knowledge gaps in the organisms's metabolism. This reconstruction will serve both as a knowledge base of our understanding of *Z. mobilis* metabolism and be utilized to enable the design of strains for primary biofuel synthesis as part of the Great Lakes Bioenergy Research Center's larger goals.

Zymomonas mobilis is an industrially relevant, Gram-negative, ethanologen known for high glycolytic fluxes through the Entner Doudoroff pathway, high ethanol production, and exceptionally low biomass yields. Here we present *i*ZM4_478, a genome-scale stoichiometric model of *Z. mobilis* ZM4 metabolism and apply it to analyze a published dataset from pooled mutant fitness experiments. *i*ZM4_478 contains 752 metabolic and transport reactions (of which 625 have gene-protein-reaction associations), 478 genes, and 616 unique metabolites, making it one of the most complete models of *Z. mobilis* ZM4 to date. Model predicted essential genes were compared to fitness data from the pooled mutant experiments. Several discrepancies between the model and dataset were found to be caused by polar effects, mismapped barcodes, or heterozygous mutants, highlighting potential challenges inherent to analyzing these high-throughput datasets. Functionally related modules of reactions and genes in the model were identified via flux coupling analysis. The fitness scores across all 492 experiments in the reported dataset were analyzed in the context of these modules to identify candidate genes for a reaction in histidine biosynthesis lacking an annotated gene and highlight metabolic modules where the fitness scores of mutants in the dataset are poorly correlated. Additional genes for reactions involved in biotin, ubiquinone, and pyridoxine biosynthesis in *Z. mobilis* were identified and confirmed using mutant complementation experiments. These newly identified genes improve our understanding of *Z. mobilis* metabolism, and the updated model provides a platform for future network driven studies of this organism and serves as a starting point for the development of kinetic models of *Z. mobilis* metabolism in the coming years of the Great Lakes Bioenergy Research Center

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