Improving methyl ketone production in Escherichia coli by heterologous expression of NADH-dependent FabG

We previously engineered Escherichia coli to overproduce medium- to long-chain saturated and monounsaturated methyl ketones, which could potentially be applied as diesel fuel blending agents or in the flavor and fragrance industry. Recent efforts at strain optimization have focused on cofactor balance, as fatty acid-derived pathways face the systematic metabolic challenge of net NADPH consumption (in large part, resulting from the key fatty acid biosynthetic enzyme FabG [β-ketoacyl-ACP reductase]) and net NADH production. In this study, we attempted to mitigate cofactor imbalance by heterologously expressing NADH-dependent, rather than NADPH-dependent, versions of FabG identified in previous studies. Of the four NADH-dependent versions of FabG tested in our previously best-reported methyl ketone-producing strain (EGS1895), the version from Acholeplasma laidlawii (Al_FabG) showed the greatest increase in methyl ketone yield in shake flasks (35-75% higher than for an RFP negative-control strain, depending on sugar loading). An improved strain (EGS2920) attained methyl ketone liters during fed-batch fermentation of 5.4±0.5g/L, which were, on average, ca. 40% greater than those for the base strain (EGS1895) under fermentation conditions optimized in this study. Shotgun proteomic data for strains EGS2920 and EGS1895 during fed-batch fermentation were consistent with the goal of alleviating NADPH limitation through expression of Al_FabG. For example, relative to strain EGS1895, strain EGS2920 significantly upregulated glucose-6-phosphate isomerase (directing flux into glycolysis rather than the NADPH-producing pentose phosphate pathway) and downregulated MaeB (a NADP+-dependent malate dehydrogenase). Overall, the results suggest that heterologous expression of NADH-dependent FabG in E. coli may improve sustained production of fatty acid-derived renewable fuels and chemicals.
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