

## 127. Substantial Improvements in Methyl Ketone Production in E. coli from Rational Metabolic Engineering and Random Mutagenesis

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**Project Goals:** The Joint BioEnergy Institute (JBEI) aims to produce a chemically diverse suite of biofuels from lignocellulosic biomass. Some JBEI fuels use fatty acids as precursors, as these biomolecules are highly reduced, aliphatic compounds that, when modified (e.g., decarboxylated), can have properties comparable to those of petroleum- derived fuel components. The goal of the project presented here is to engineer E. coli to produce diesel-range methyl ketones in the gram-per-liter range with yields of at least 40% of maximum theoretical yield.

We have engineered *Escherichia coli* to overproduce saturated and monounsaturated aliphatic methyl ketones in the C11 to C15 (diesel) range; this group of methyl ketones includes 2- undecanone and 2- tridecanone, which have favorable cetane numbers and are also of importance to the flavor and fragrance industry. We have made specific improvements that resulted in more than 10,000-fold enhancement in methyl ketone titer relative to that of a fatty acid-overproducing E. coli strain, including the following: (a) overproduction of  $\beta$ -ketoacyl-coenzyme A (CoA) thioesters achieved by modification of the  $\beta$ -oxidation pathway (specifically, overexpression of a heterologous acyl-CoA oxidase and native FadB, and chromosomal deletion of fadA) and (b) overexpression of a native thioesterase (FadM). The first generation of engineered E. coli (Goh et al. 2012) produced ~380 mg/L of methyl ketones in rich medium. We have subsequently made additional genetic modifications that included balancing overexpression of fadR and fadD to increase fatty acid flux into the pathway, consolidation of the pathway from two plasmids into one, codon optimization, and knocking out key acetate production pathways (Goh et al. 2014). These modifications have led to a methyl ketone titer of 1.4 g/L with 1% glucose in shake flask experiments, which represents 40% of the maximum theoretical yield, and also attained titers of g/L after ~45 h of fed-batch glucose fermentation (the best values reported to date).

In addition to rational metabolic engineering, we have conducted chemical (MNNG) mutagenesis studies of a high methyl ketone-producing strain and screened using NIMS (Nanostructure-Initiator Mass Spectrometry) technology. Shake-flask incubations of 10 of the most promising mutants resulted in as much as 3- to 4-fold increases in titer relative to the control (un-mutated) strain. We are currently carrying out re-sequencing of the mutants to identify which mutations resulted in the greatest increases in methyl ketone production.

We have also conducted in vitro assays with purified pathway enzymes, which confirmed that a decarboxylase is not required to convert  $\beta$ -keto acids into methyl ketones and that FadM is promiscuous and hydrolyzes not only  $\beta$ -ketoacyl-CoAs but also other CoA-thioester pathway intermediates. These in vitro results have provided insight on how to fine-tune expression of pathway genes for further optimization of methyl ketone production.

### References

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2. Goh, E.-B., E. E. K. Baidoo, H. Burd, T. S. Lee, J. D. Keasling, H. R. Beller. 2014. Substantial improvements in methyl ketone production in E. coli and insights on the pathway from in vitro studies. *Metabolic Engineering* 26:67-76.

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