

## Engineering of bacterial methyl ketone synthesis for biofuels

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### Project Goals:

**The Joint BioEnergy Institute (JBEI) aims to produce a chemically diverse suite of biofuels from lignocellulosic biomass. Many of the target fuels at JBEI rely on well-characterized metabolic pathways (such as the straight-chain fatty acid biosynthetic pathway) to provide precursors for synthesis of biofuel molecules. The goal of this project was to produce methyl ketones from fatty acids for use as blending agents or substitutes for diesel fuel.**

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 are of importance to the flavor and fragrance industry and also have favorable cetane numbers (as we report here). We describe specific improvements that resulted in a 700-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). FadM was previously associated with oleic acid degradation, not methyl ketone synthesis, but outperformed a recently identified methyl ketone synthase (ShMKS2, a thioesterase from wild tomato) in beta-ketoacyl-CoA-overproducing strains tested. Whole-genome transcriptional (microarray) studies led to the discovery that FadM is a valuable catalyst for enhancing methyl ketone production. The use of a two-phase system with decane enhanced methyl ketone production by 4 to 7-fold in addition to increases from genetic modifications.

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