Fatty acid-related research at JBEI: methyl ketones and branched fatty acids in *E. coli*

Ee-Been Goh[1]* (EGoh@lbl.gov), Robert Haushalter,[1] Helcio Burd,[1] Taek Soon Lee,[1] Hector Garcia Martin,[1] Jay D. Keasling,[1] and **Harry R. Beller[1]**

[1]Joint BioEnergy Institute (JBEI), Emeryville, CA

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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 goals of the project presented here is to (1) 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, and (2) improve the cold-temperature properties of these fatty-acid derived compounds by incorporating methyl-branching.

Accelerated research-and-development activity in biofuels in recent years has facilitated the development of metabolic pathways that enable biochemical conversion of fatty acids (and intermediates of fatty acid biosynthesis) to a range of industrially relevant compound classes, including aliphatic methyl ketones (Beller et al. 2015). Here, we describe two fatty acid-related projects at JBEI in which we engineered *Escherichia coli* for improved biofuel performance: (1) efforts to improve the titers, rates, and yields (TRY) of diesel-range methyl ketones (a class of fatty acid-derived molecules that have high cetane numbers), and (2) efforts to produce methyl-branched fatty acids, which would yield diesel fuels with better cold-temperature properties than straight-chain fatty acids.

Methyl ketones: We have engineered *E. coli* to overproduce aliphatic methyl ketones (MK) in the C_{11} to C_{15} (diesel) range; this group of MK includes 2-undecanone and 2-tridecanone, which have favorable cetane numbers and are also of importance to the flavor and fragrance industry. Overall, we have made specific improvements that resulted in more than 10,000-fold enhancement in MK titer relative to that of a fatty acid-overproducing *E. coli* strain. The first generation of engineered *E. coli* (Goh et al., 2012) produced ~380 mg/L of MK in rich medium and had modifications that included: (a) overproduction of β-ketoacyl-coenzyme A (CoA) thioesters achieved by modification of the β-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). 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 latest modifications have led to a MK 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 3.4 g/L after ~45 h of fed-batch glucose fermentation (the best values reported to date).

Using another approach for improving MK production, metabolic modeling was used to identify gene deletions that could improve flux through the MK pathway. One of the specified knockouts, Δ*scgE*, which was annotated as a homolog of Rpe (ribulose-5-phosphate epimerase) in the pentose phosphate pathway, improved MK production by >50% relative to the base strain (EGS1710). ^{13}C-glucose experiments have recently been performed on the knockout strain along with the DH1 wild type and base strain (EGS1710) to obtain more comprehensive metabolic flux profiles that will refine the genome-scale model and enable additional improvements in MK production.

Branched fatty acids: We have engineered *E. coli* to produce *iso*- and *anteiso*-branched fatty acids. From a biofuel perspective, the straight-chain fatty acids produced by wild-type *E. coli* are not ideal because they have relatively high melting points. Although *E. coli* generates unsaturated fatty acids, which have far lower melting points than their saturated analogs, the unsaturated fatty acids are more susceptible to oxidation during storage. Other bacteria, such as *Bacillus subtilis*, naturally produce branched fatty acids, rather than unsaturated fatty acids, to modulate membrane fluidity. We have retooled *E. coli* fatty acid biosynthesis with heterologous enzymes to produce > 20% *anteiso*-branched fatty acids, which exhibit lower melting points than straight-chain and *iso*-branched isomers (Haushalter et al., 2014). The ability to synthesize methyl-branched fatty acids in *E. coli* will be important for optimizing fatty acid-derived biofuels, such as methyl ketones, for use in cold climates.

References

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