BRC Science Highlight
June 2020

Metabolic Engineering of β-Oxidation to Leverage Thioesterases for Production of 2-Heptanone, 2-Nonanone, and 2-Undecanone

Background/objective

Methyl ketones are industrially and biologically relevant as insecticides, flavors, and fragrances and have the potential to be used as liquid transportation fuels. While methyl ketones have been a metabolic engineering target in bacteria and yeasts, few studies have investigated production of medium-chain length methyl ketones. Here, researchers used bioprospecting and metabolic engineering studies in *E. coli* as a model organism to identify key enzymes and to develop metabolic pathways for producing 2-heptanone, 2-nonanone, and 2-undecanone.

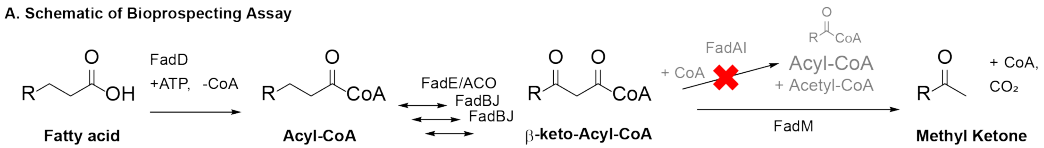
Approach

- Used bioprospecting to identify and test *E. coli* β-ketoacyl-CoA thioesterase (FadM) homologs with high activity toward substrates with fewer than 12 carbons.
- Compared two beta-oxidation pathways for producing beta-ketoacyl-CoA's (oxidation of acyl-CoAs vs. thiolase-mediated elongation).
- Used bioprospecting to identify best beta-oxidation enzyme variants for developing methyl-ketone pathways.
- Established product volatility and tested condenser and *in situ* extraction protocols for capturing methyl ketones from bioreactors.

Results

- Identified FadM homolog from *Providencia sneebia* as having highest activities toward medium-chain substrates.
- Achieved production of up to 4.4 g/L total medium-chain methyl ketones.

A. Schematic of Bioprospecting Assay



B. Evaluation of FadM Homologs

	C7 MK Titer (mg/L)	C8 FFA Consump. (mg/L/h)	C9 MK Titer (mg/L)	C10 FFA Consump. (mg/L/h)	C11 MK Titer (mg/L)	C12 FFA Consump. (mg/L/h)	C13 MK Titer (mg/L)	C14 FFA Consump. (mg/L/h)	C15 MK Titer (mg/L)	C16 FFA Consump. (mg/L/h)	
Control	0.4 ± 0.1	14.5 ± 2.7	2.3 ± 0.1	2.1 ± 0.4	0.5 ± 0.0	2.3 ± 0.2	7.4 ± 1.2	6.8 ± 1.5	1.2 ± 0.8	3.5 ± 0.2	Control
SeFadM	4.7 ± 0.7	18.1 ± 3.5	10.9 ± 0.6	3.9 ± 0.4	16.0 ± 2.3	9.7 ± 0.6	22.3 ± 2.7	17.5 ± 0.9	36.2 ± 4.7	6.0 ± 1.0	SeFadM
NgFadM	4.9 ± 0.5	5.2 ± 3.4	8.1 ± 0.4	6.0 ± 0.3	22.8 ± 1.5	13.7 ± 0.9	18.5 ± 2.5	15.4 ± 3.8	22.0 ± 10.1	7.6 ± 1.0	NgFadM
MtFadM5	5.3 ± 0.3	5.2 ± 2.9	10.0 ± 0.4	5.3 ± 0.2	27.7 ± 0.1	12.9 ± 1.0	22.9 ± 1.7	17.4 ± 0.5	35.1 ± 1.6	6.2 ± 1.8	MtFadM5
MuFadM	5.6 ± 0.0	10.2 ± 1.3	6.5 ± 0.7	6.5 ± 0.4	17.7 ± 0.0	10.9 ± 0.5	21.9 ± 2.1	14.4 ± 1.2	17.1 ± 1.0	7.0 ± 0.4	MuFadM
PaFadM	5.9 ± 0.1	2.5 ± 2.1	2.3 ± 0.1	2.1 ± 0.4	13.9 ± 2.3	7.0 ± 0.5	18.1 ± 0.1	9.1 ± 2.7	6.0 ± 4.2	5.7 ± 0.1	PaFadM
BaFadM	7.9 ± 0.4	17.2 ± 2.0	6.3 ± 0.4	16.4 ± 0.5	21.9 ± 2.1	13.2 ± 0.2	32.2 ± 7.7	14.5 ± 0.7	31.8 ± 4.2	7.5 ± 0.8	BaFadM
MtFadM1	8.0 ± 0.3	26.9 ± 7.8	14.4 ± 1.1	26.2 ± 0.6	37.9 ± 9.1	15.0 ± 0.2	29.8 ± 3.2	17.0 ± 1.7	28.1 ± 4.7	9.1 ± 1.0	MtFadM1
MtFadM4	10.0 ± 0.2	25.8 ± 2.6	12.0 ± 0.2	25.8 ± 0.7	31.6 ± 1.2	14.7 ± 0.4	29.8 ± 4.2	16.3 ± 2.6	28.4 ± 10.6	7.9 ± 3.5	MtFadM4
MtFadM3	11.4 ± 0.0	26.2 ± 9.3	9.0 ± 0.6	26.9 ± 0.6	13.9 ± 1.4	6.1 ± 0.6	19.8 ± 4.0	4.3 ± 0.5	32.8 ± 2.4	6.2 ± 0.2	MtFadM3
MtFadM2	11.8 ± 0.3	41.9 ± 3.6	18.8 ± 0.8	28.9 ± 0.2	43.5 ± 5.0	14.4 ± 0.9	33.2 ± 1.2	15.2 ± 2.4	35.4 ± 1.5	8.2 ± 0.8	MtFadM2
SoFadM	13.9 ± 0.1	104.9 ± 17.1	25.3 ± 0.8	77.4 ± 1.2	32.0 ± 1.0	15.6 ± 1.4	39.0 ± 6.6	15.4 ± 0.5	40.2 ± 12.0	8.3 ± 2.9	SoFadM
BbFadM	15.5 ± 0.5	89.2 ± 4.8	25.2 ± 1.2	64.2 ± 0.4	20.2 ± 4.4	8.4 ± 0.3	29.1 ± 6.4	13.4 ± 0.3	34.2 ± 1.5	8.3 ± 1.9	BbFadM
EclFadM	24.7 ± 0.5	102.5 ± 7.6	29.1 ± 0.2	71.7 ± 1.0	41.6 ± 5.2	14.8 ± 0.3	39.9 ± 0.3	17.2 ± 0.8	38.7 ± 0.5	11.6 ± 2.7	EclFadM
CtFadM	26.4 ± 0.7	115.6 ± 3.1	20.5 ± 0.4	32.9 ± 1.1	20.6 ± 3.8	12.3 ± 2.1	27.8 ± 7.3	9.6 ± 1.5	38.6 ± 8.4	11.0 ± 2.0	CtFadM
EcFadM	33.3 ± 0.2	105.7 ± 0.8	30.3 ± 1.0	58.9 ± 0.8	40.0 ± 1.4	14.5 ± 1.0	44.6 ± 9.7	17.1 ± 0.0	45.3 ± 11.6	9.5 ± 1.2	EcFadM
PsFadM	50.0 ± 0.2	142.9 ± 7.0	43.3 ± 1.0	137.0 ± 0.2	49.5 ± 1.3	13.6 ± 0.3	39.0 ± 3.4	15.4 ± 3.5	36.9 ± 0.7	7.4 ± 0.8	PsFadM

Bioprospecting experiments use engineered *E. coli* to compare activity of FadM homologs against different chain length substrates.

Significance

This work identified a high-activity FadM variant via bioprospecting and demonstrated a novel thiolase-condensation route for producing the FadM substrate, while simultaneously demonstrating metabolic engineering strategies for selective synthesis of 2-heptanone, 2-nonanone, and 2-undecanone in *E. coli*. In future work, this foundational approach will be transferred to novel yeasts.

Yan, et al., 2020. "Metabolic Engineering of β-Oxidation to Leverage Thioesterases for Production of 2-Heptanone, 2-Nonanone, and 2-Undecanone." ***Metabolic Engineering***. DOI: 10.1016/j.ymben.2020.05.008.

CABBI
CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION
U.S. DEPARTMENT OF ENERGY