DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

Engineering fatty acid synthesis in *Rhodosporidium toruloides* to produce mid-chain fatty acids and fatty alcohols

ABF DFO with C16 Bio

04-05-2023
Conversion Technologies

DI LIU
Sandia National Laboratories

This presentation does not contain any proprietary, confidential, or otherwise restricted information

Project Overview

Palm oil

- 38% global vegetable oil market
- Widely used across consumer products

- Environmental damage
- Increased greenhouse emissions

Project Goal: Engineering *R. toruloides* to produce mid-chain fatty acids and fatty alcohols as an alternative to palm kernel oil

BETO relevance:

- CO_2 emission reduction
- Sustainable biomanufacturing

c16 BIOSCIENCES

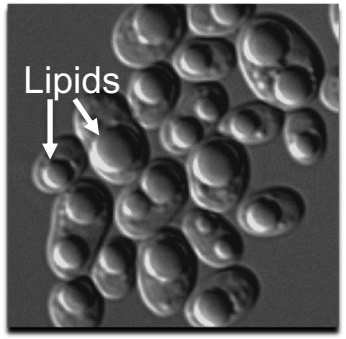
Sandia National Laboratories

Pacific Northwest NATIONAL LABORATORY

Project duration: 01/04/2021 – 01/04/2024

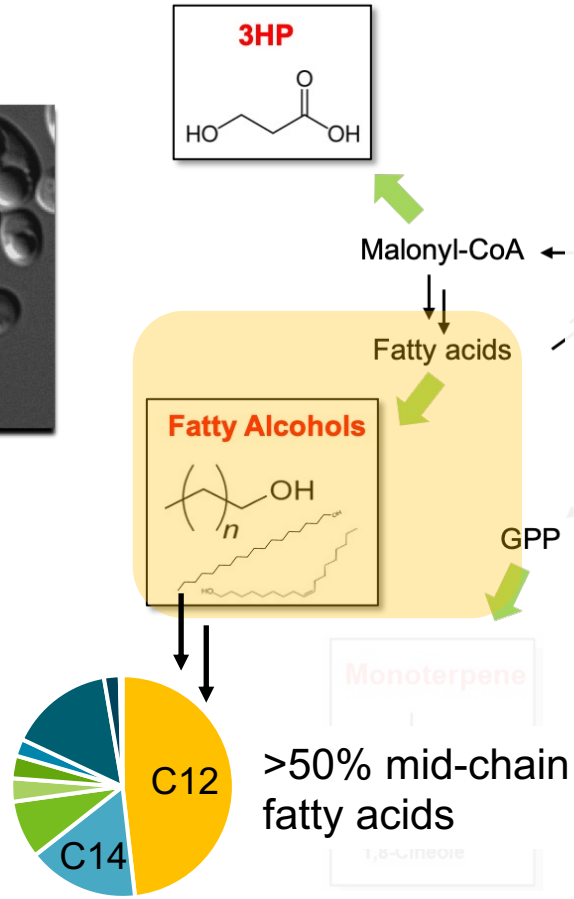
Project Approach

The project leverages previous research and capabilities at ABF:



Rhodosporidium toruloides

- Utilizes lignocellulose
- Fast growing
- Oleaginous, carotenogenic
- Metabolically versatile
- Genetically tractable



>50% mid-chain fatty acids

Palm kernel oil & its derived products

- Previously engineered fatty alcohol producing *R. toruloides* strains
- Previously synthesized plasmids
- Knowledge developed on the fatty acid pathway
- Established genetic tools and protocols
- A *R. toruloides* functional genomics library
- Multiomic capabilities

Project Approach

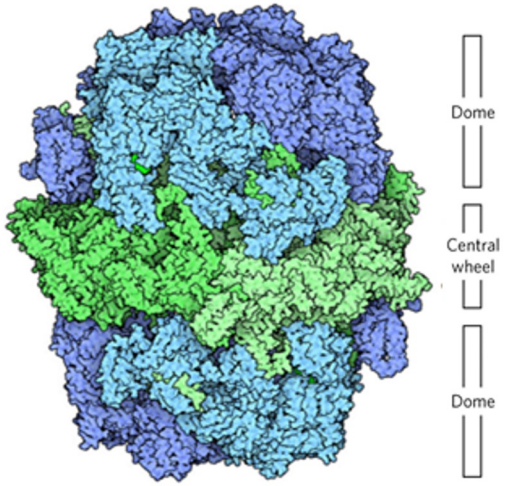
Technical Approach:

- Engineer fatty acid synthase
- Identify and bridge bioconversion gaps
- Address product degradation

TWO TYPES OF FATTY ACID SYNTHASE (FAS)

Type I Fatty Acid Synthase

- Pathway components form an **enzyme complex**
- Found in **yeast**, **fungi**, animals
- Only outputs mostly **C16-C18** fatty acyl CoA (occasionally little C14)

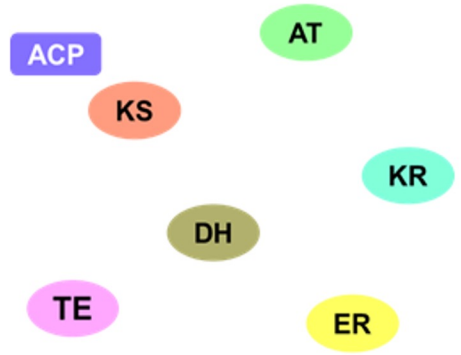


⊕ More efficient catalysis due to metabolic channeling

⊖ More difficult to modify individual domains. Significant changes may alter quaternary structure

Type II Fatty Acid Synthase

- Pathway components are **independent enzymes**
- Found mostly in archaea, bacteria, algae, and plants
- Acyl-ACPs of intermediate chain lengths (<C16) are well exposed



⊖ Pathway is less efficient (diffusion-limited)

⊕ More amenable to metabolic engineering and to modifications of individual enzymes

Project challenges, risks and mitigation

	Challenges / Risks	Mitigation
Engineer fatty acid synthase	• Directly modifying native FAS intractable • Heterologous enzymes unable to access intermediates in FAS	• Pursue Type II FAS systems • Design multiple strategies to modify native FAS • Employ additional routes (fatty acid catabolic pathways)
Identify and bridge bioconversion gaps	• Unknown if biochemical gaps exist within the multi-step conversion	• Implement functional genomics and multiomics to understand metabolism and guide pathway design
Address product degradation	• Many redundancies in proteins encoding product degradation • Multiple cellular compartments involved	

Project Approach

Go / No-Go checkpoints (18 months)

Target the production of ~5% mid-chain fatty acids or mid-chain fatty alcohols (C10-C14) as a proportion of overall lipid or fatty alcohol profile.

Technical metrics:

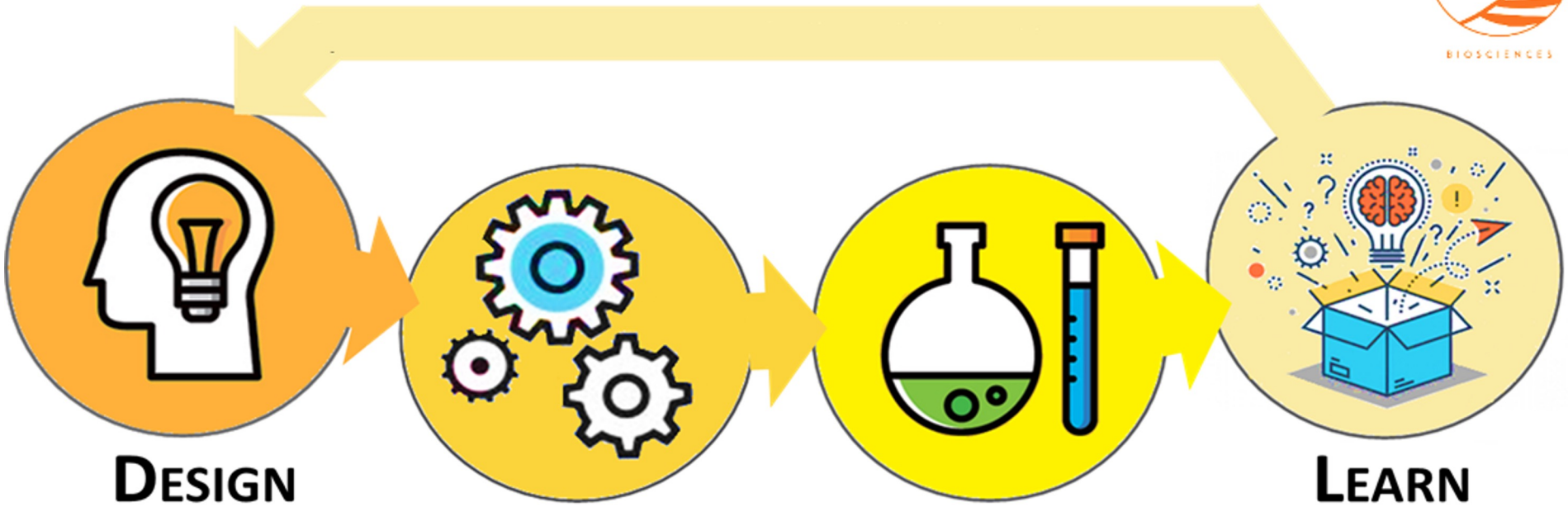
- Total lipid content and fatty alcohol production.
- Percentages of mid-chain fatty acids or mid-chain fatty alcohols.

Why critical:

- Proves the feasibility the basic design principles.
- Enables further optimization to address potential bioconversion gaps.

Completed in FY22

Project Structure



DESIGN

Develop strategies and designs

C16

SNL

BUILD

Build DNA constructs and transform designs into *R. toruloides* strains

C16

SNL

TEST

Perform bench-scale fermentation and omic analysis of selected strains

SNL

PNNL

LEARN

Analyze and interpret data. Provide inputs for next round of designs

PNNL

C16

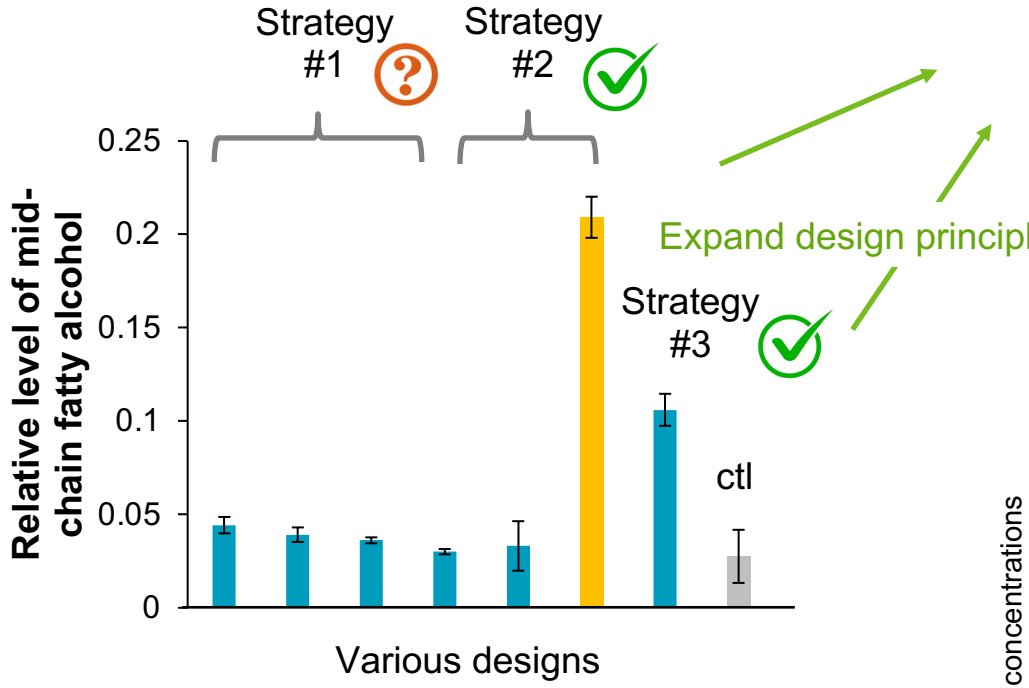
SNL

Progress and Outcomes

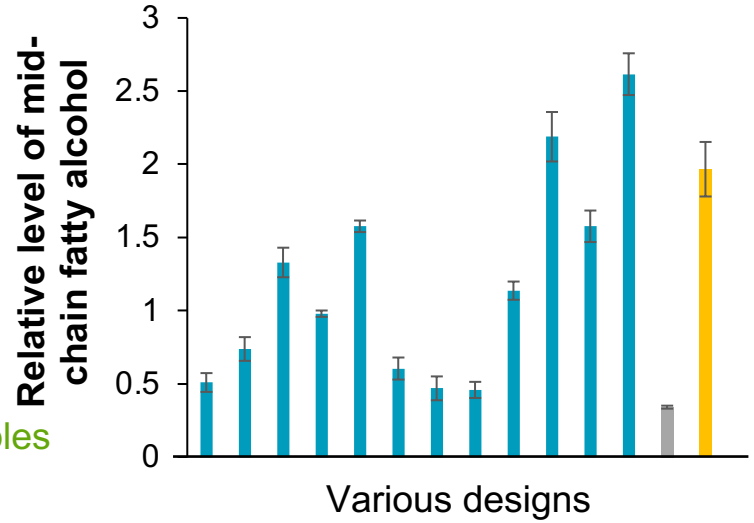
Engineer fatty acid synthase

Identify and bridge bioconversion gaps

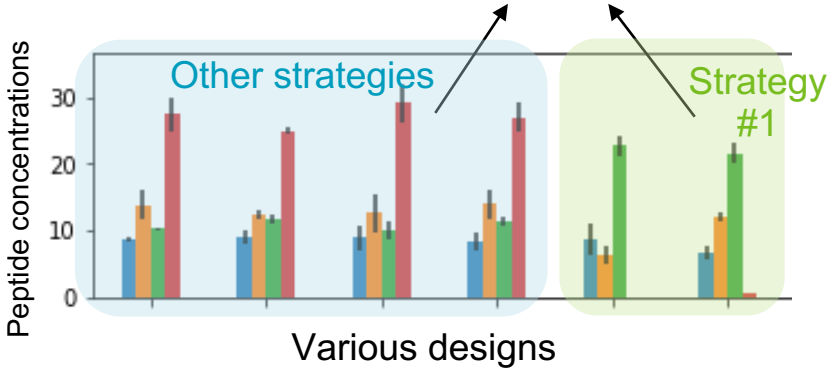
Address product degradation



Expand design principles



Targeted proteomics suggest disruption of protein expression



Milestones achieved

- Design, build and test of first round of engineered FAS strains (FY21 Q3 & Q4, FY22 Q1)
- Multiomic analysis of the engineered FAS strains (FY22 Q2)
- Generate second round of engineered FAS strains (FY23 Q1)

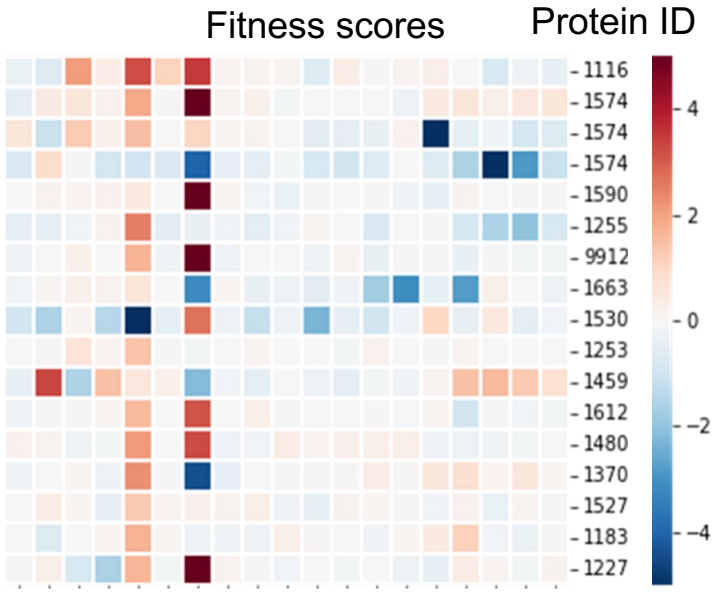
Progress and Outcomes

Substrates → Intermediates → Products

Engineer fatty acid synthase

Gap #1: Enzymes catalyzing the intermediate formation and its cellular compartments are not known in *R. toruloides*

Functional genomics & metabolic model (both developed in ABF) suggest potential genes

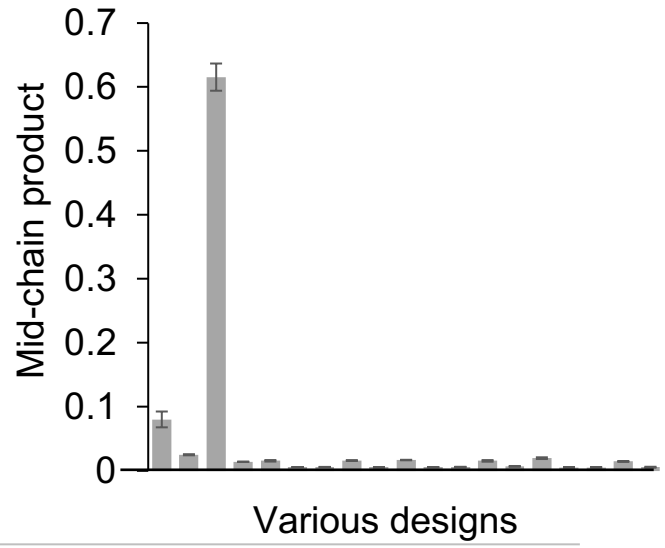


Identify and bridge bioconversion gaps

Gap #2: Enzymes with activities on mid-chain intermediates have not been established in *R. toruloides*

Heterologous gene expression identified genes with mid-chain activities

Address product degradation



Milestones achieved

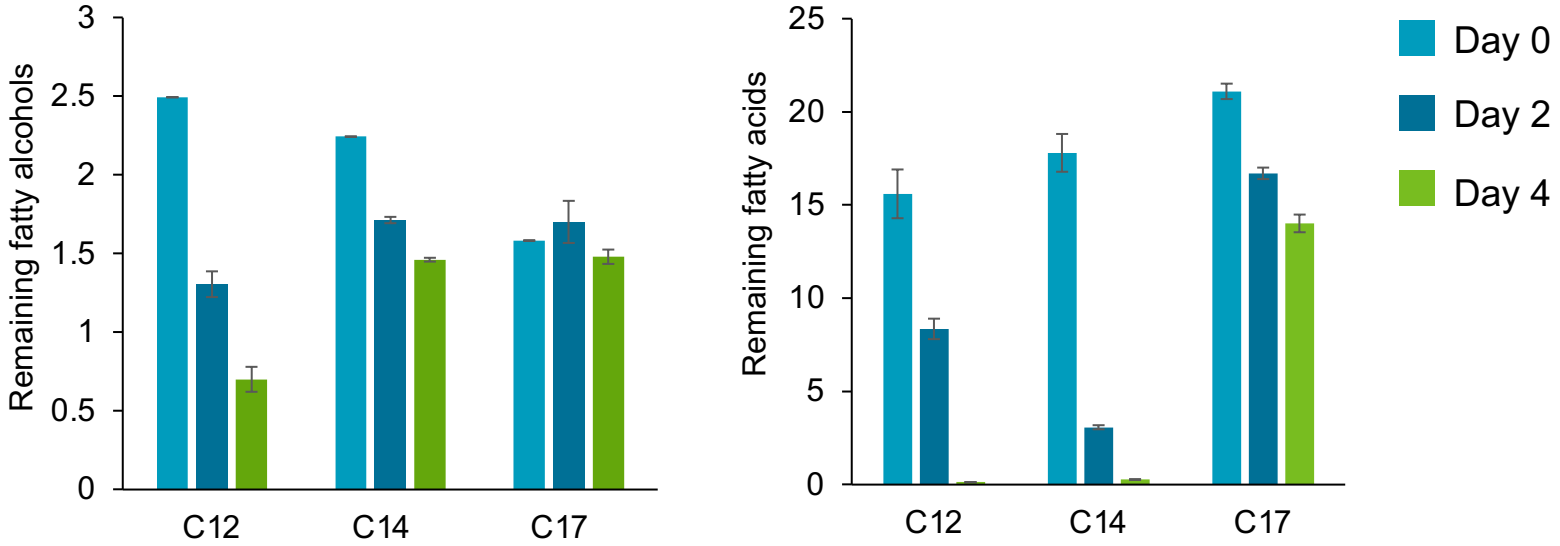
- Assess chain length specificity of enzymes for mid-chain fatty alcohol production (FY22 Q3)

Progress and Outcomes

Engineer fatty acid synthase

Identify and bridge bioconversion gaps

Address product degradation



Feeding study suggests **strong degradation of mid-chain products**

Functional genomics suggest potential gene candidates encoding product degradation (strains built, testing underway)

Milestones achieved

- No milestones for this thrust in Year 1 & 2
- Directly contributing to enhancing the final strain titer, rate and yield

Project Impact

Enable sustainable production of palm kernel oil:

- 8 million metric tons palm kernel oil per year (over $12mm market size)
- Dominant source of mid-chain triglyceride oils
- Mid-chain fatty alcohols make up the majority of the global fatty alcohol market

Reduce CO_2 emission towards industrial decarbonization

Demonstrate transfer of technology from ABF to industry

C16 Bio recently release their first consumer-facing brand platform: Palmless

Palmless.™

Meet Palmless™ Torula oil

First Palmless brand ingredient, torula oil (Beauty sector)

Summary

Leveraging previous work and capabilities in ABF

R. toruloides strains engineered to produce fatty alcohols and accumulate various amount of lipids

R. toruloides barcoded strain library for functional genomic studies

A well-curated *R. toruloides* metabolic model

ABF multiomic capabilities

Genetic tools to engineered *R. toruloides*

Main approaches in this project

Engineer fatty acid synthase

Identify and bridge bioconversion gaps

Address product degradation

Outcome/Impact

Established novel metabolic routes to produce mid-chain fatty acids and fatty alcohols

Achieved Go/No-Go checkpoint (>5 % mid-chain products)

Furthered knowledge on lipid metabolism in *R. toruloides*

Towards sustainable biochemical production

Quad Chart Overview

Timeline

- *Project start date: 01/04/2021*
- *Project end date: 01/04/2024*

	FY22 Costed	Total Award
DOE Funding	*(10/01/2021 – 9/30/2022)* *$383,142* *SNL - $264,142* *PNNL - $119,000*	*$1,449,000*
Project Cost Share *	*$235,000*	*$598,000*

TRL at Project Start: 2

TRL at Project End: 4

Project Goal

Engineering *R. toruloides* to produce mid-chain fatty acids and fatty alcohols as an alternative to palm kernel oil

End of Project Milestone

The production of ~10% medium chain fatty acids and fatty alcohols (C10-C14) as a proportion of overall lipid profile

Funding Mechanism

ABF DFO

Project Partners*

- SNL
- PNNL
- C16 Bio

*Only fill out if applicable.

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