DOE Bioenergy Technologies Office (BETO)
2023 Project Peer Review

Production of triacetic acid lactone

ABF DFO with Pyrone Systems

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Conversion Technologies

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Project Overview

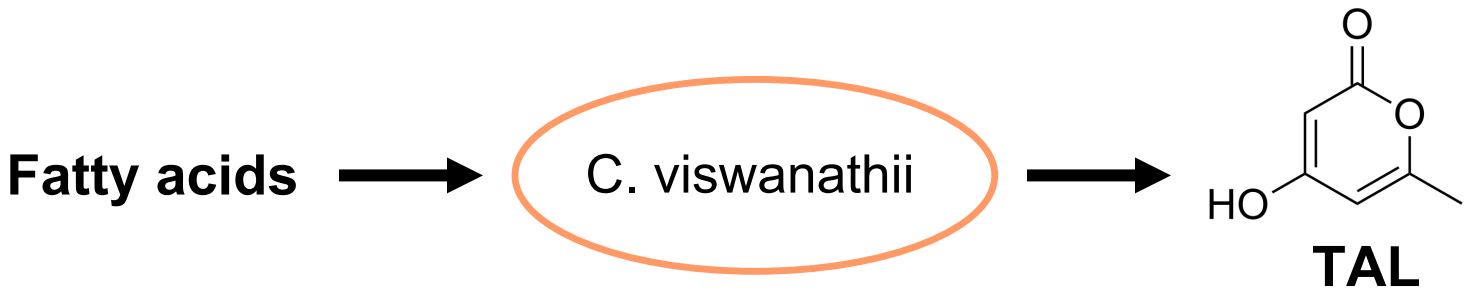
Project Goal: demonstrate high-level production of the bioprivileged molecule triacetic acid lactone (TAL) from fatty acids.

- TAL is a versatile platform chemical to produce compounds with high market values such as caprolactone (polymer precursor), sorbic acid (food preservative), and pogostone (antimicrobial and insecticide).
- Pyrone Systems Inc. is developing bio-based methodologies to create non-toxic biopesticides from TAL.
- Synthesis of TAL occurs via a type-III polyketide synthase enzyme and uses the fatty acid derived molecules acetyl-CoA and malonyl-CoA as substrates.
- The use of fatty acids derived from vegetable oil production processes, water treatment plants, etc., as substrates for microbial fermentation could enable the valorization of waste streams.

Project Overview

- Our strategy involves engineering a yeast with naturally strong fatty acid degradation capabilities, *Candida viswanathii,* to introduce the genes required for TAL biosynthesis and increase the intracellular pool of the direct metabolic precursors.

- This project will support BETO's goal of decarbonizing the industrial sector through research, development, and demonstration to produce cost-effective and sustainable chemicals from biomass and waste resources.



Project duration: 06/01/2022 – 05/31/2024

Approach

Strain engineering (Pyrone, SNL)

- Preserve the acetyl-CoA pool derived from β-oxidation in the peroxisome.
- Compartmentalize TAL production in the peroxisome.

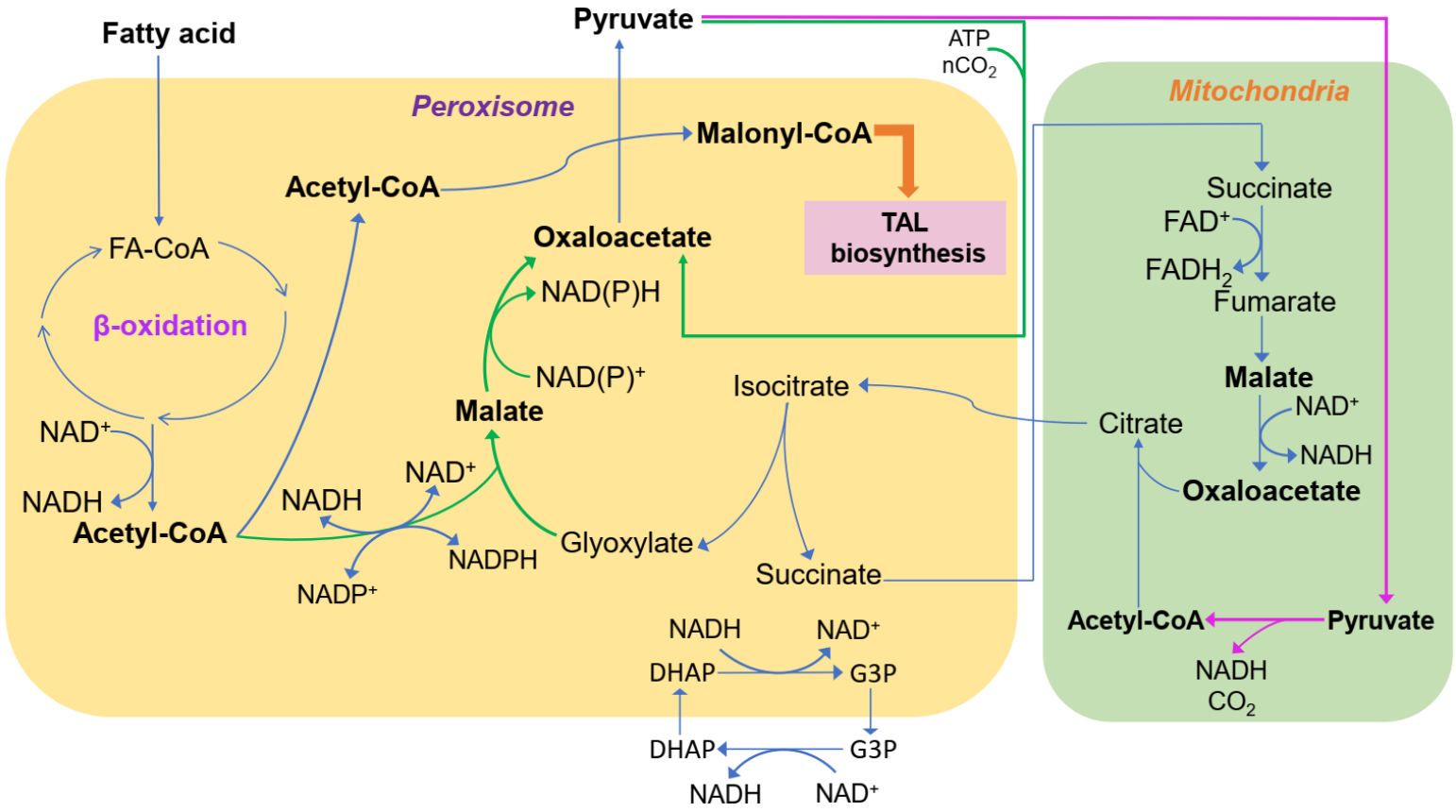
Adaptive laboratory evolution (SNL)

- Obtain TAL producing strains with higher growth on fatty acids.
- Explore the effect of using supplementary carbon sources in ALE experiments.

Characterization of evolved strains (SNL, PNNL)

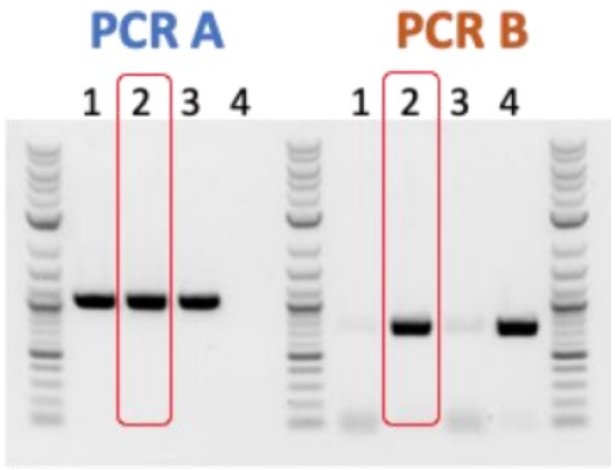
- Optimize cultivation conditions to improve growth and TAL titers.
- Perform genome sequencing and multi-omics approaches to understand changes in evolved strains and find new targets for improvement.

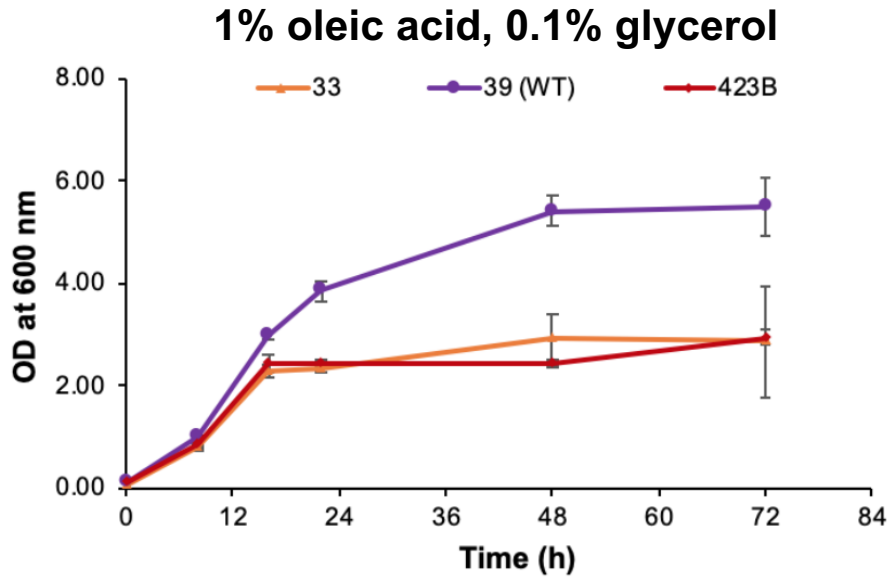
Approach

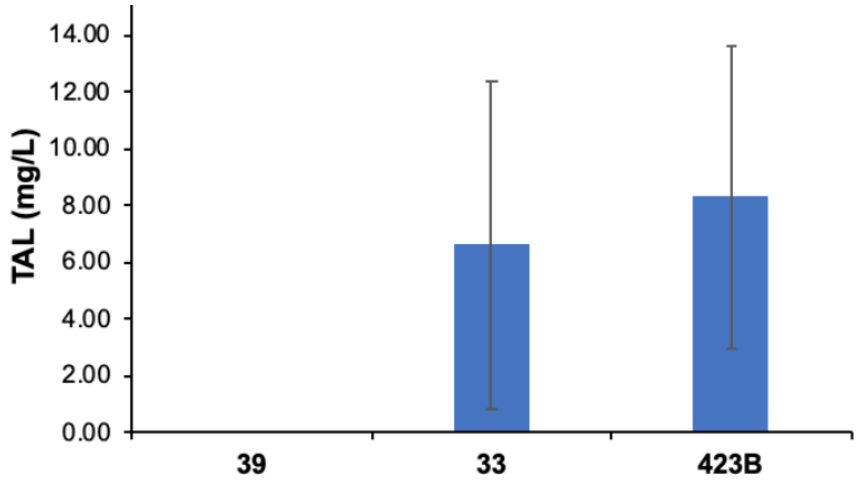


Compartmentalization of β-oxidation and TAL biosynthesis in the peroxisome is our strategy to increase yields

Progress and Outcomes







We generated *C. viswanathii* strains that produce TAL and lost the ability to grow in oleic acid as single carbon source

Progress and Outcomes

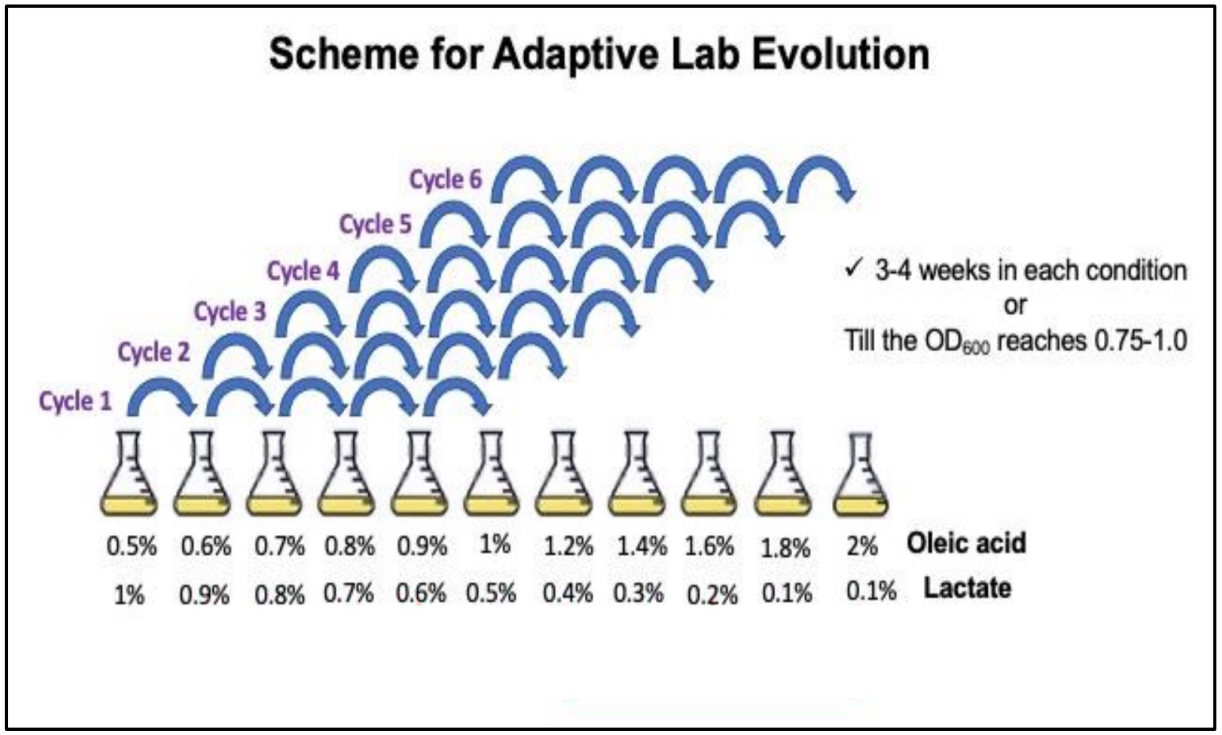
Effect of media components on growth and TAL production in the engineered strain

Glycerol	Oleic acid	Ammonium sulfate	Yeast extract	Lactate	Growth OD_{600}	TAL conc. (mg/L)
1%	1%	0.5%			4.22±0.08	3.30±0.35
2%	1%	0.5%			5.29±0.64	5.20±0.12
5%	1%	0.5%			5.16±0.05	1.42±0.24
2%	1%	0.1%			6.12±0.24	4.16±0.57
2%	**1%**	**0.2%**			**5.41±0.50**	**10.36±0.51**
1%	0.25%	0.2%	0.05%		5.08±0.25	< 1.00
1%	0.25%	0.2%	0.1%		4.63±0.42	< 1.00
	0.25%	0.2%		1%	4.24±0.05	< 1.00
	0.25%	0.2%		2%	5.21±0.15	< 1.00
	0.25%	0.2%		5%	2.93±0.11	< 1.00

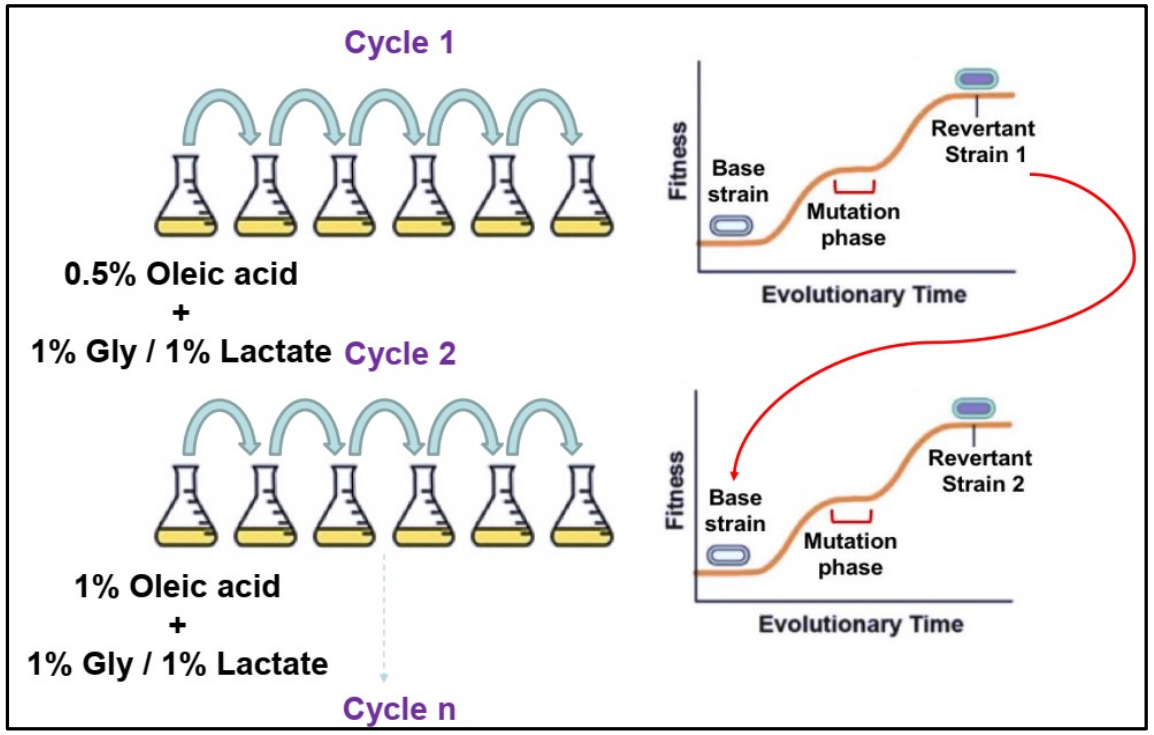
Both glycerol and lactate promote growth of the engineered strain, but TAL concentrations are variable

Progress and Outcomes

Approach 1 (lactate or glycerol)



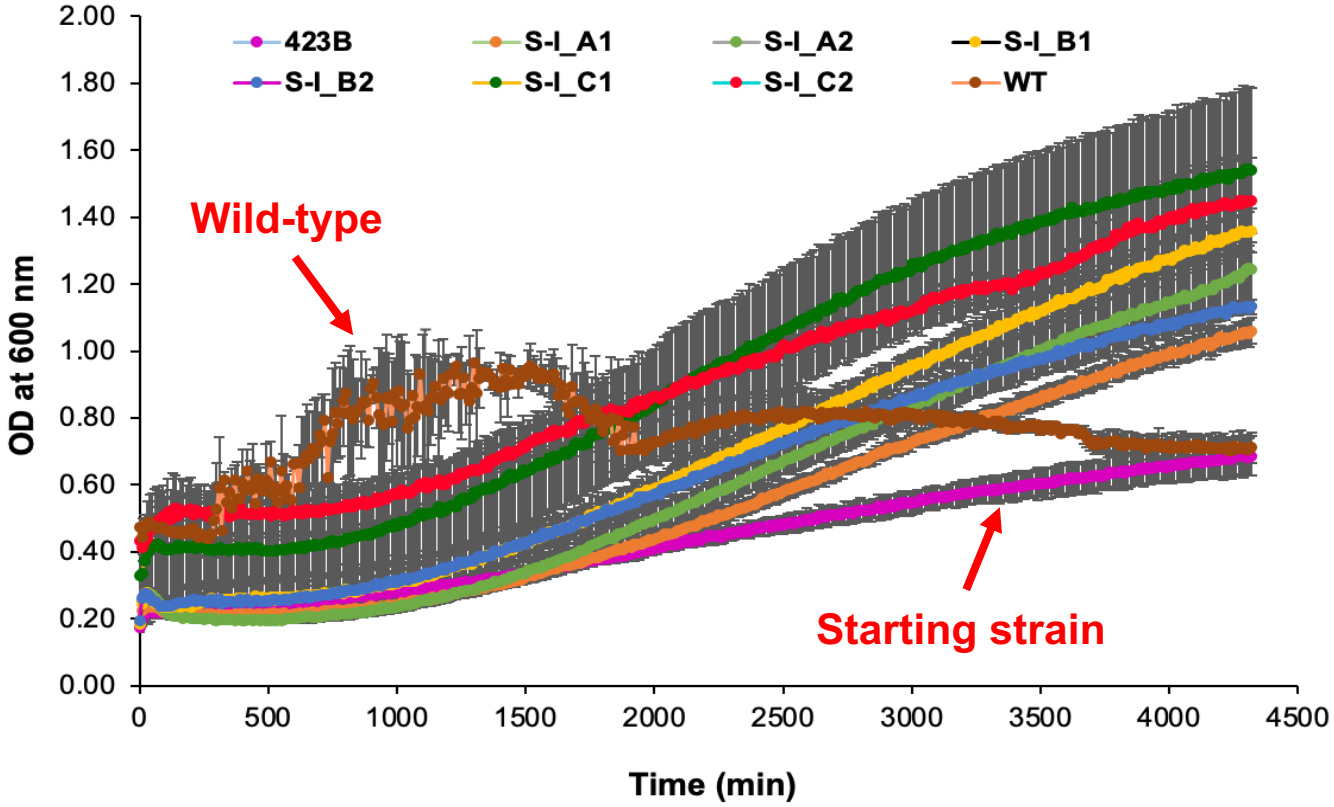
Approach 2 (lactate or glycerol)

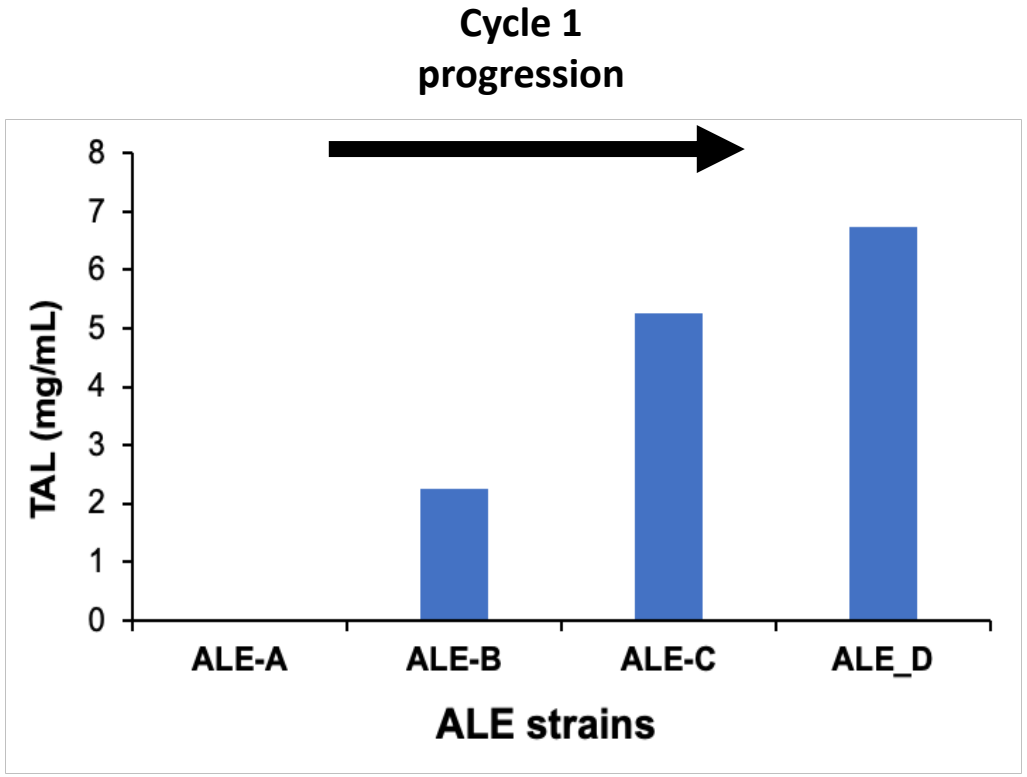


We used two ALE approaches with two different supplementary substrates

Progress and Outcomes

Preliminary characterization of evolved strains (isolated mixed cultures)





Positive trends are visible, sampling is ongoing

Impact

- If successful, this project will be the first to report TAL production from fatty acids using a peroxisomal compartmentalization approach and higher titers of TAL would have a direct impact on the commercialization potential of the process.

- If higher titers (>5 g/L) and growth rates are achieved, *Candida viswanathii* could be further engineered to produce other polyketide molecules or the engineering strategy ported to other hosts.

- This project will contribute to developing commercially viable technologies to enable the sustainable, nationwide production of bioproducts.

Summary

- Pyrone Systems partnered with the ABF to evaluate the potential of *C. viswanathii* to convert fatty acids to triacetic acid lactone.
- The microbial engineering strategy involved peroxisomal compartmentalization of TAL precursors and biosynthetic enzymes.
- Engineered strains were subjected to adaptive laboratory evolution to select for mutants with faster growth in oleic acid.
- Evolved strains will be characterized by multi-omics approaches to inform the next round of genetic engineering and cultivation conditions to increase TAL production.

Quad Chart Overview

Timeline

- *Project start date: 06/01/2022*
- *Project end date: 05/31/2024*

	FY22 Costed	Total Award
DOE Funding	*(10/01/2021 – 9/30/2022)* *Total - $32,917* *SNL - $30,917* *PNNL - $2,000*	*$600,000* *SNL - $400,000* *PNNL - $200,000*
Project Cost Share *	*$150,000*	*$150,000*

TRL at Project Start: 2
TRL at Project End: 3

Project Goal

Pyrone Systems will partner with SNL and PNNL to build and optimize engineered yeast strains that can efficiently produce the bioprivileged compound triacetic acid lactone (TAL) from renewable fatty acids.

End of Project Milestone

Report at least one strain that produces 5-10 g/L of TAL from fatty acids with or without additional supplementation.

Funding Mechanism

ABF DFO

Project Partners

- Pyrone Systems Inc.
- PNNL

Acknowledgements

- Alex Hutagalung (Pyrone Systems)

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Agile BioFoundry

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