DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

ABF Industry Engagement Lab Call - Visolis: Production of High-Value Chemicals from Renewable Feedstocks

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ABF

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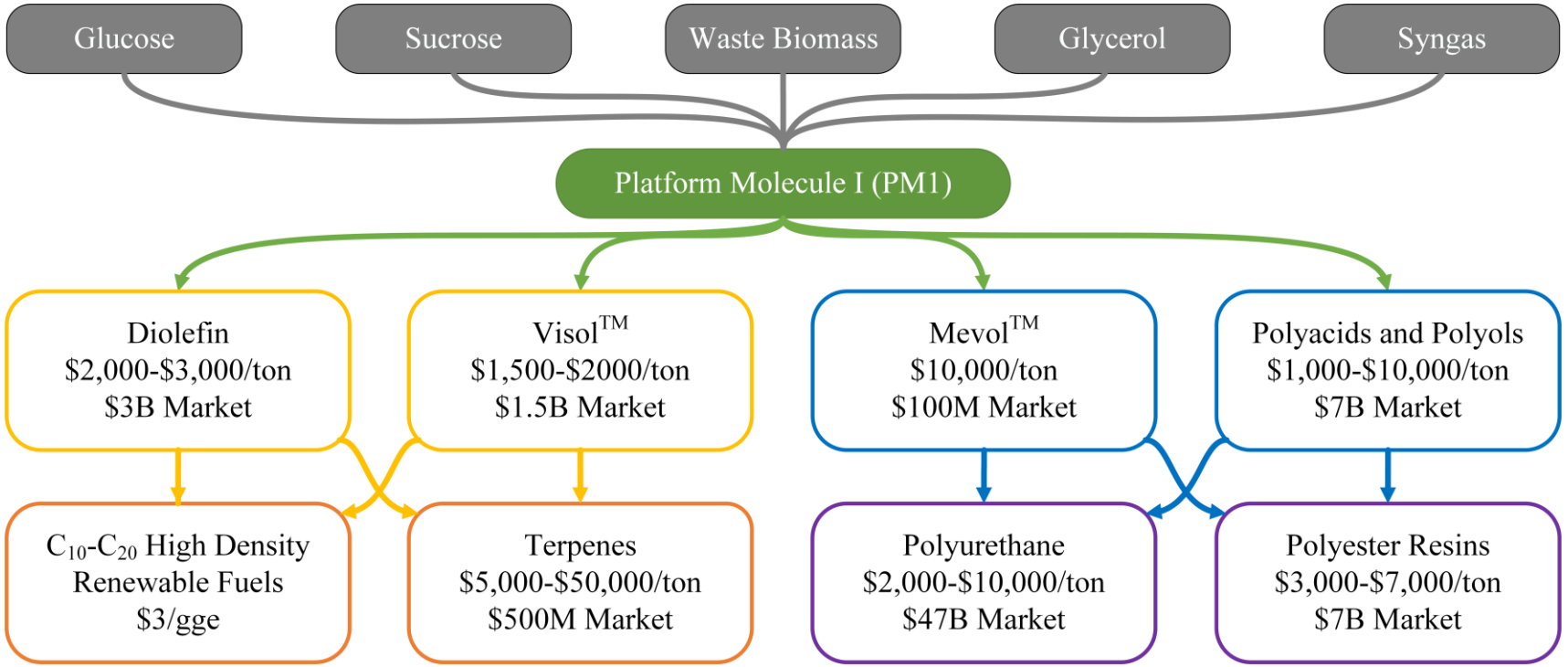
Presentation Outline

VISOLIS
CARBON NEGATIVE MATERIALS

Platform molecule production from diverse feedstocks



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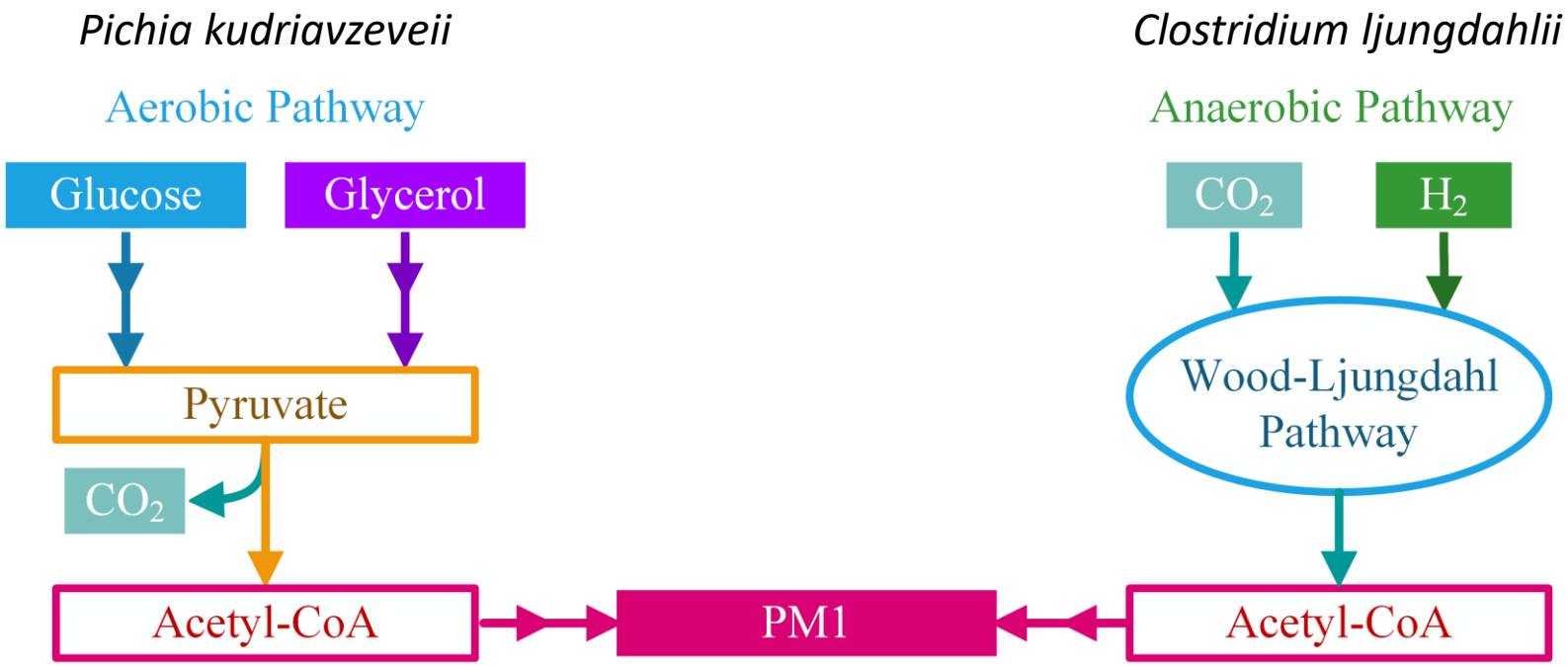
Non-conventional hosts with potential for performance advantage



A New Paradigm: a hybrid biomass-conversion technology integrating thermochemical gasification with syngas fermentation ($CO/H_2/CO_2$) to improve biorefinery economics

- Lower capital and operating cost with low pH fermentation
- Better biomass utilization
- Higher carbon efficiency (compared to carbon loss from glycolysis)

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Project Structure

Improving genetic tools for *C. ljungdahlii*

Metabolic Engineering of *C. ljungdahlii*

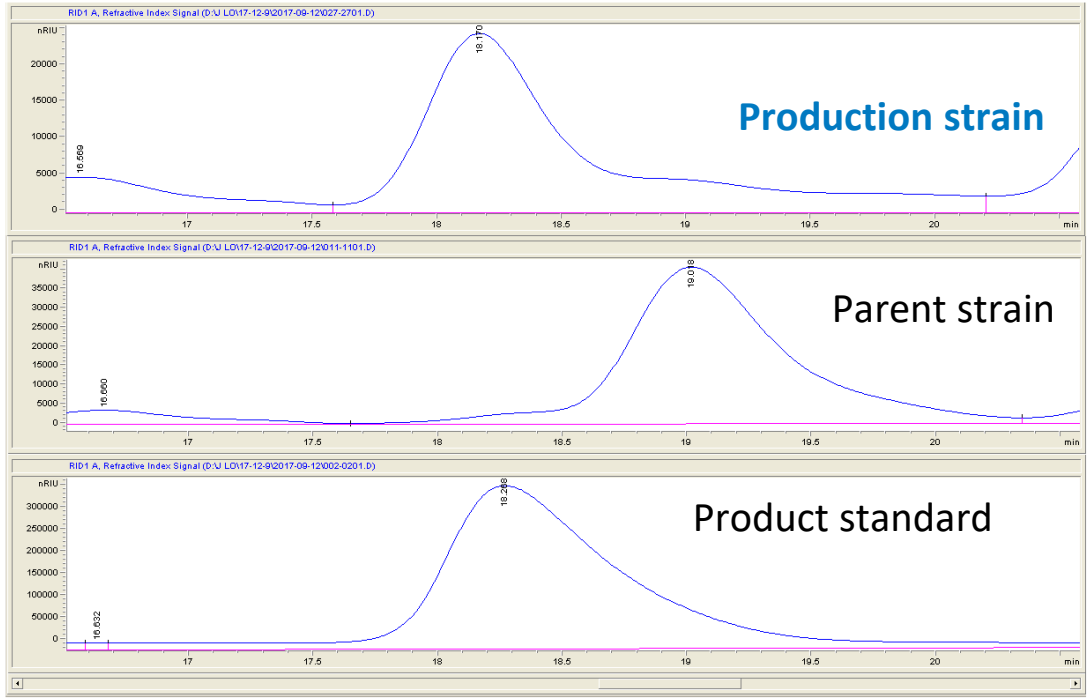
Metabolic Engineering of *P. kudriavzeveii*

Bioreactor Experiments/TEA

Engineering PM1 production in *C. ljungdahlii*

- **Higher Carbon Efficiency:** CO_2-fixing autotrophic microbes such as *Clostridium ljungdahlii* metabolize CO_2, H_2, and CO yielding acetyl-CoA.
- Acetyl-CoA upgrade leads to PM1, a precursor with myriads of applications.
- Initially transformed production related constructs m_1 and m_2 into *C. ljungdahlii* using a replicating plasmid:
- Used *thl* and *araE* promoters to drive expression of genes
- Detected ~80 mg/L PM1 in WT background strain

C. ljungdahlii



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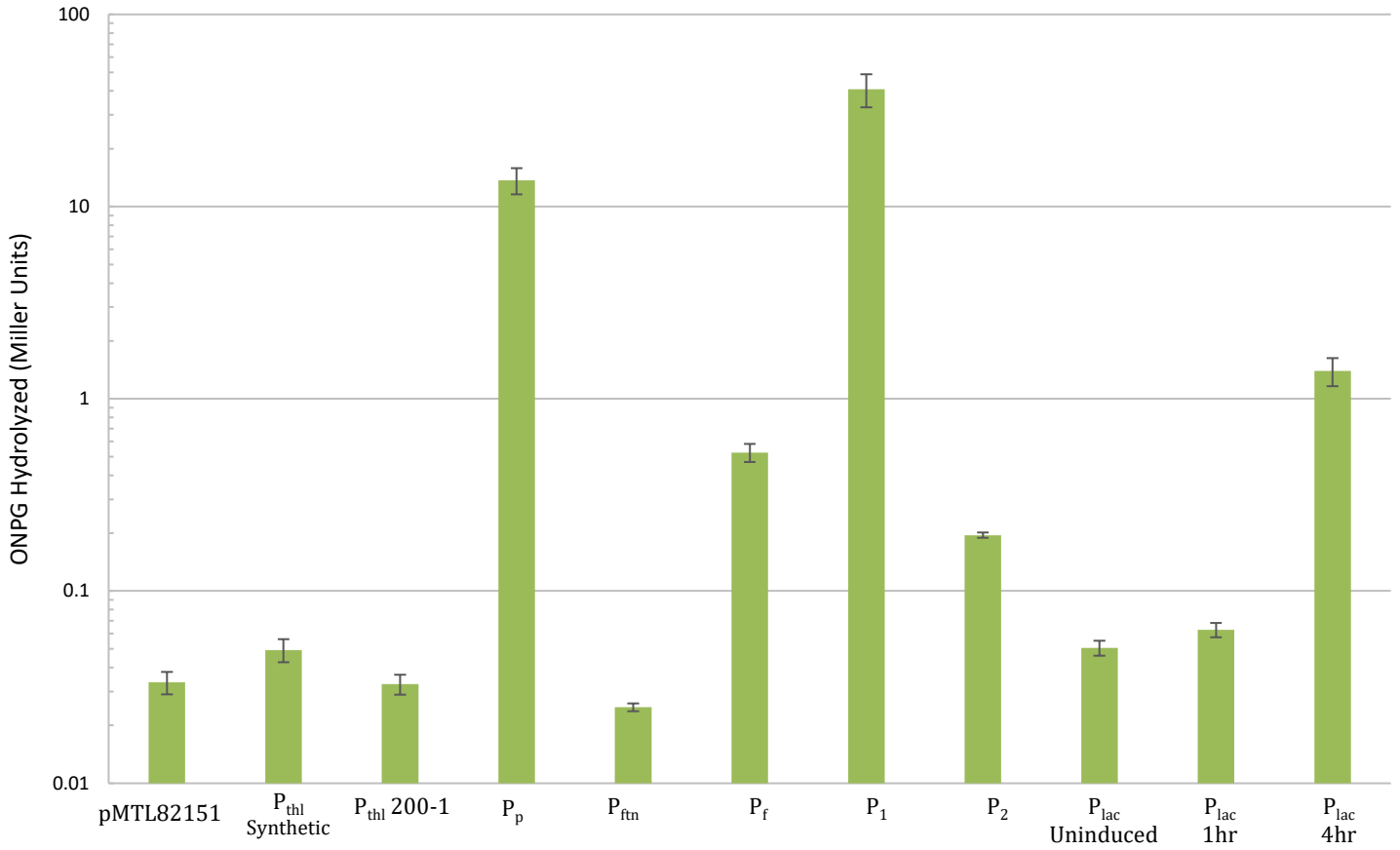
Engineering PM1 production in *C. ljungdahlii*

- Characterized promoters that span ~1000-fold range of expressions
- Confirmed trends with new anaerobic fluorescent marker (Y-FAST)
- Identified a promoter that is stronger than ones currently used



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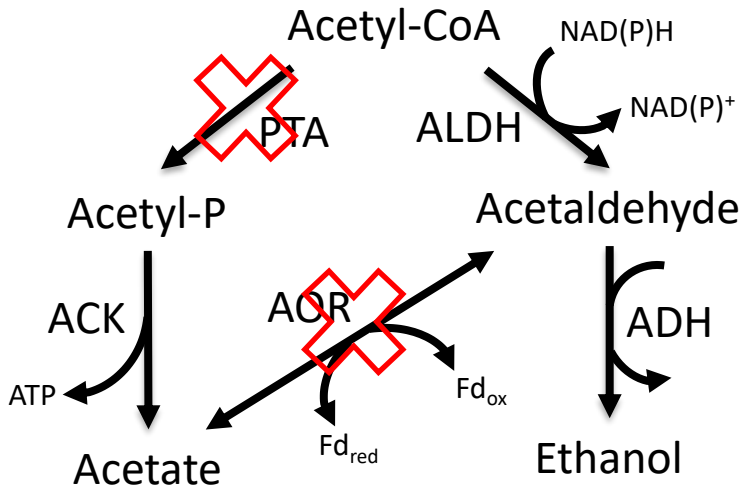
Engineering PM1 production in *C. ljungdahlii*

Using newly characterized promoters, we generated new construct with stronger promoters to drive production genes:

- We put this construct into a Δ*pta* Δ*aor2* strain that has reduced acetate
- Increased PM1 production to ~700 mg/L, up from 80 mg/L
- Provides a proof-of-concept that PM1 production via a synthetic pathway is linked to native cellular metabolic network in chassis strain.



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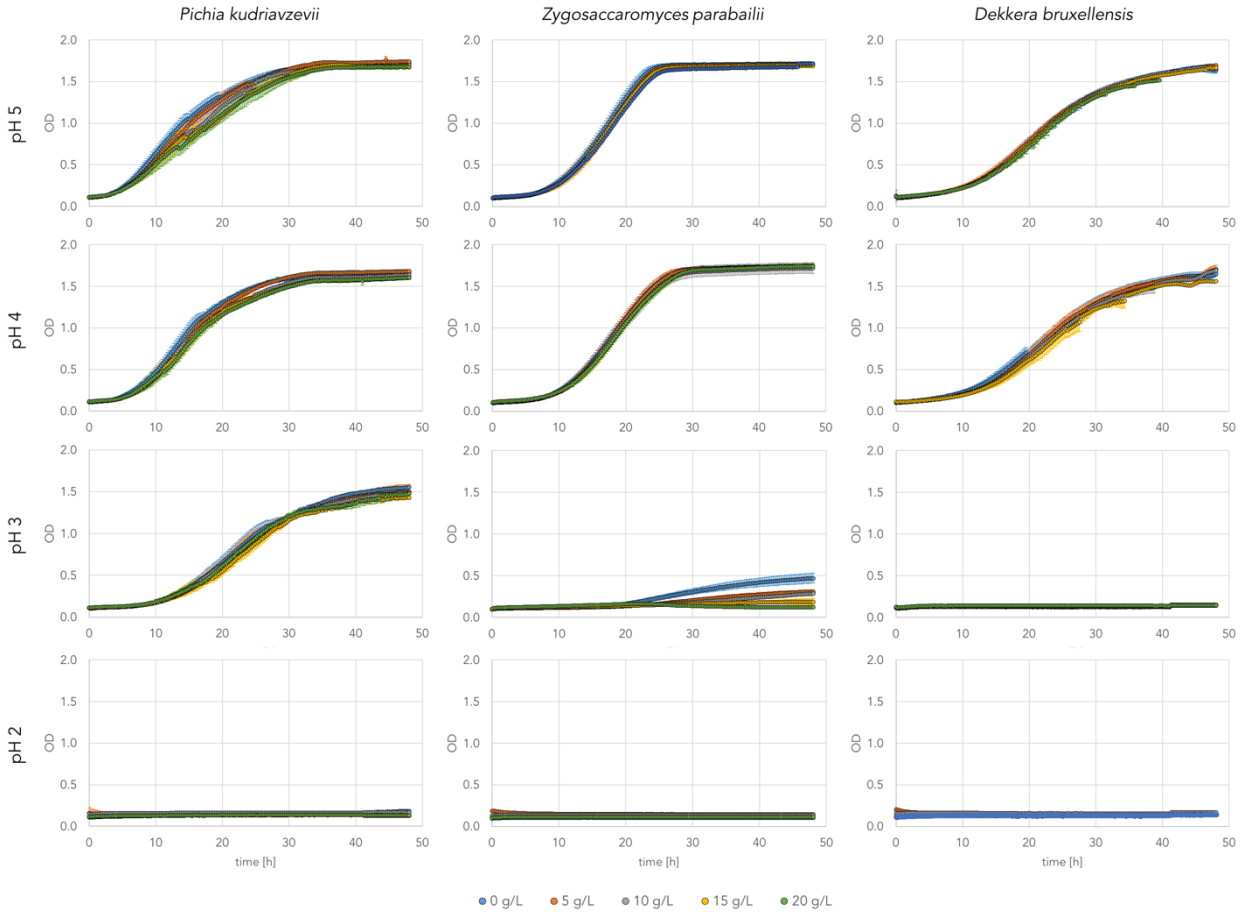
Engineering PM1 production in a low pH yeast

- Cultivations of *P. kudriavzevii*, *Z. parabailii*, and *D. bruxellensis* were performed in the presence of 0, 5, 10, 15 g/L PM1 at initial pH of 5.0, 4.0, 3.0 and 2.0.
- While *Z. parabailii* and *D. bruxellensis* exhibited no growth at pH 3, *P. kudriavzevii* growth profiles were largely unaffected, confirming that this yeast is a suitable chassis for PM1 production under low pH conditions.



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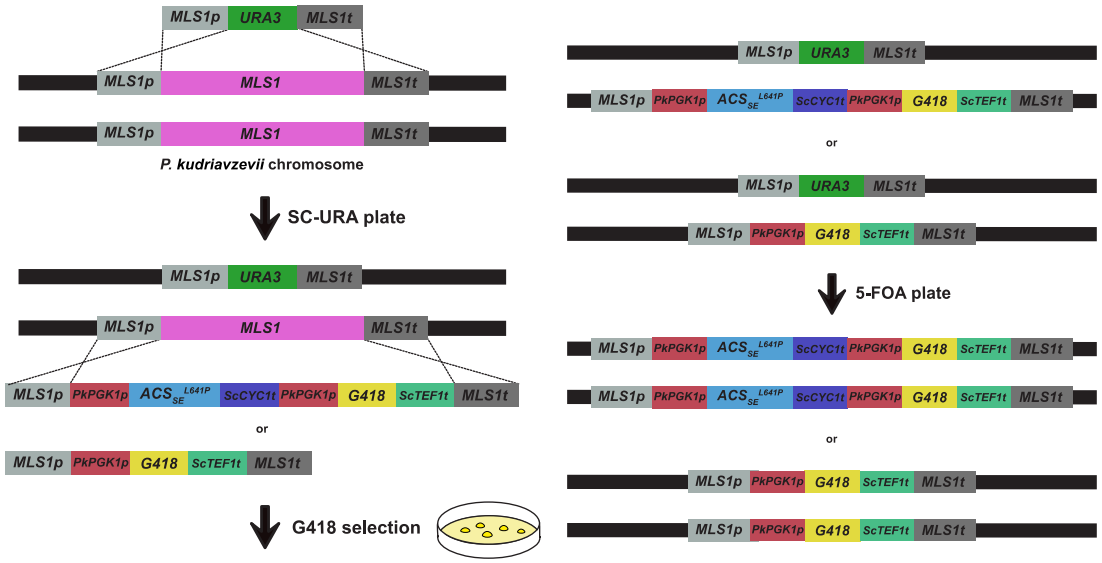
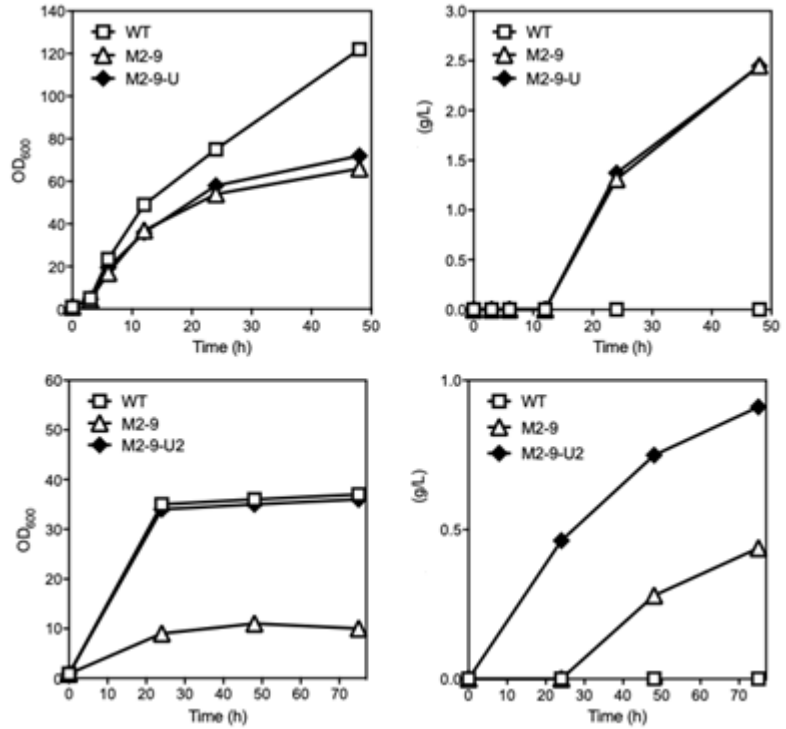
Engineering PM1 production in a low pH yeast





- Using URA3 as a selection/counterselection system, the following genetic edits were introduced into the low pH yeast *Pichia kudriavzeveii*:
- Extra copies of PM1 production genes on both chromosomes to increase production
- Deletion of competing malate pathway gene MLS1 by replacement with URA3 to improve growth at low pH

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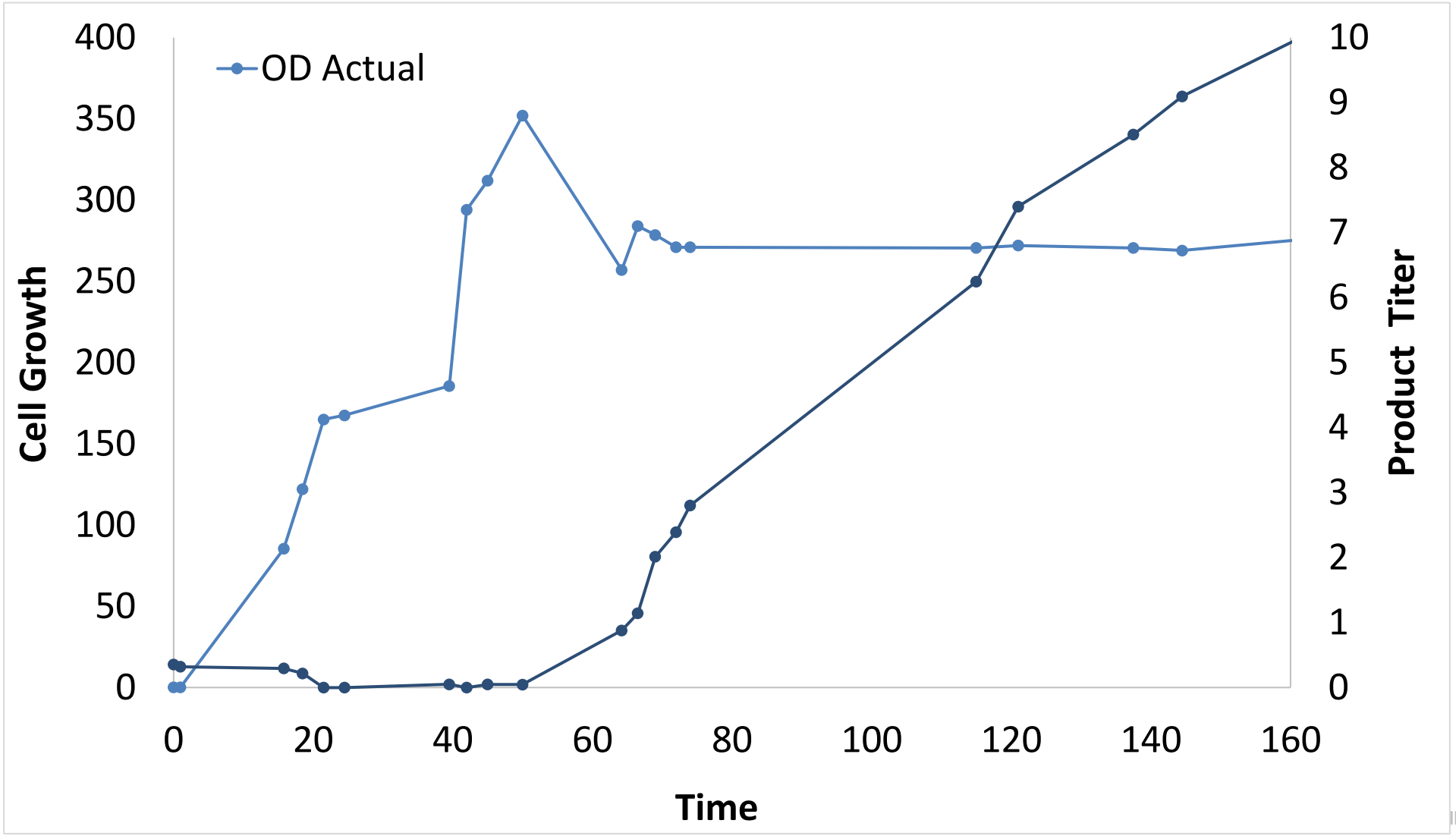
Engineering PM1 production in a low pH yeast



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Progress and Outcomes- Summary and Impact

C. Ljungdahlii chassis

- Improved transformation protocol for *C. ljungdahlii*.
- Initial *C. ljungdahlii* strain with PM1 production validated.
- Construction of promoter libraries for *C. ljungdahlii.*
- *Development of genomic integration protocol for C. ljungdahlii.*

P. kudriavzevii chassis

- Improved pH tolerance of *P. kudriavzevii* for PM1 production.
- DBTL cycle to produce a *P. kudriavzevii* strain with PM1 production.
- Demonstration of PM1 production at 3X project target in bench top bioreactors at low pH

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Quad Chart Overview

Timeline

- March 1, 2018
- Sept 30, 2020

	FY20	Project
DOE Funding	(10/01/2019 – 9/30/2020)	$0 *$500,000 (project)*

Project Partners

- National Renewable Energy Lab
- Oakridge National Lab

Barriers addressed

1. Develop cost-effective biological synthesis technologies.

Project Goal

Demonstrate use of non-traditional hosts for production of platform molecule

End of Project Milestone

Developed tools for engineering or two non-traditional hosts and demonstrated production of key platform molecule

Funding Mechanism

ABF Industry Engagement Lab Call

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U.S. DEPARTMENT OF ENERGY