Agile BioFoundry

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

DOE Bioenergy Technologies Office (BETO)
2023 Project Peer Review

ABF DFO with Technology Holding, Inc.

April 5, 2023
Technology Area Session: Agile BioFoundry

National Laboratory PI: Gregg T. Beckham, NREL
Technology Holding PI: Mukund Karanjikar

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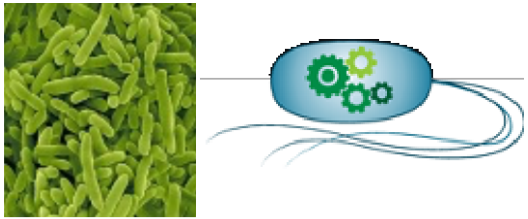
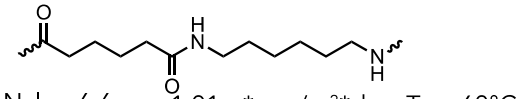
Project overview

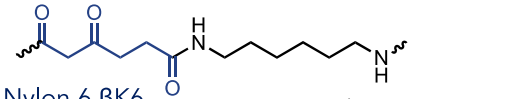
- **Goal:** Develop *P. putida* strains to produce β-ketoadipate from hydrolysate at industrially-relevant levels and scale-up with Technology Holding and partners
- **Motivation:** β-Ketoadipate enables performance-advantaged bio-based nylons & polyesters, which are materials of interest to Technology Holding
- Project started in October 2022



Pseudomonas putida KT2440

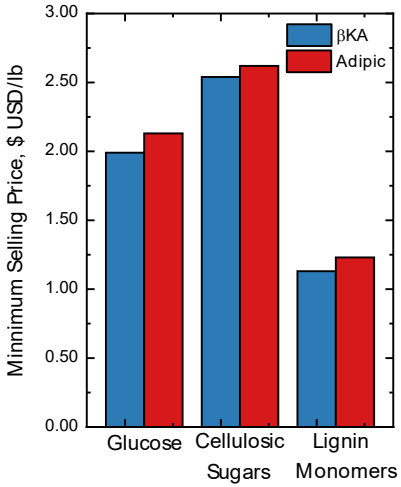


Nylon 6,6 1.01 g*mm/m²*day, T_g = 60°C



Nylon 6,βK6 0.8 g*mm/m²*day, T_g = 130°C

Rorrer *et al. Cell Reports Phys. Sci.* 2022



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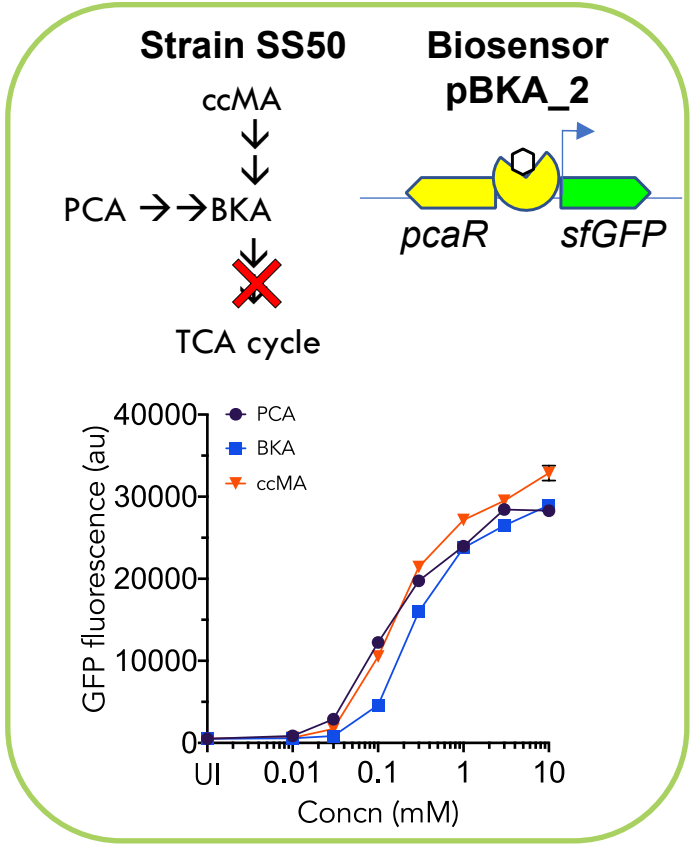
Approach for project

- Leverage learnings from muconic acid to produce β-ketoadipic acid from hydrolysate
- Biosensors to detect β-ketoadipic acid production in genome-scale libraries
- Biosensors with RB-TnSeq and overexpression libraries
- ALE with growth-restored β-ketoadipic acid strains
- Metabolomics to identify and eliminate production of off-target compounds
- Bioprocess development, TEA, and LCA to increase β-ketoadipic acid TRY and scale



Shin *et al. ACS Syn Biol.* 2022

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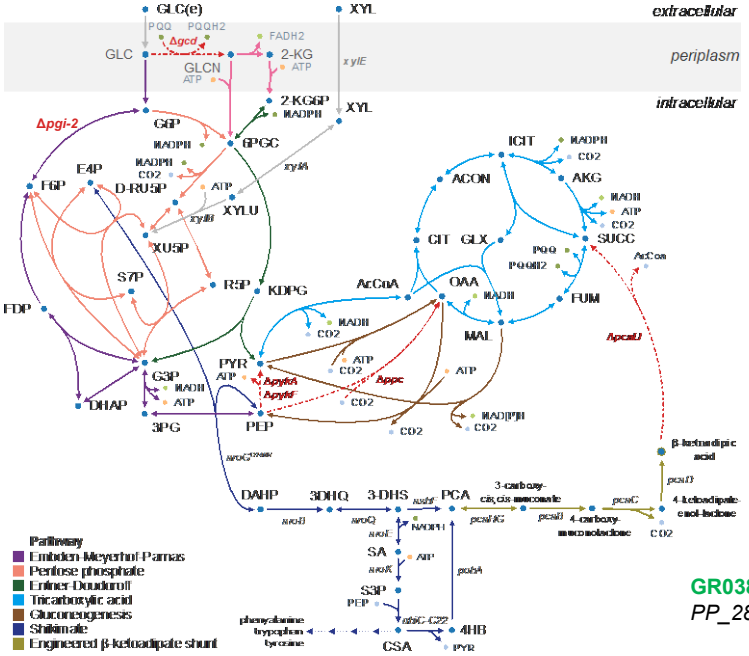
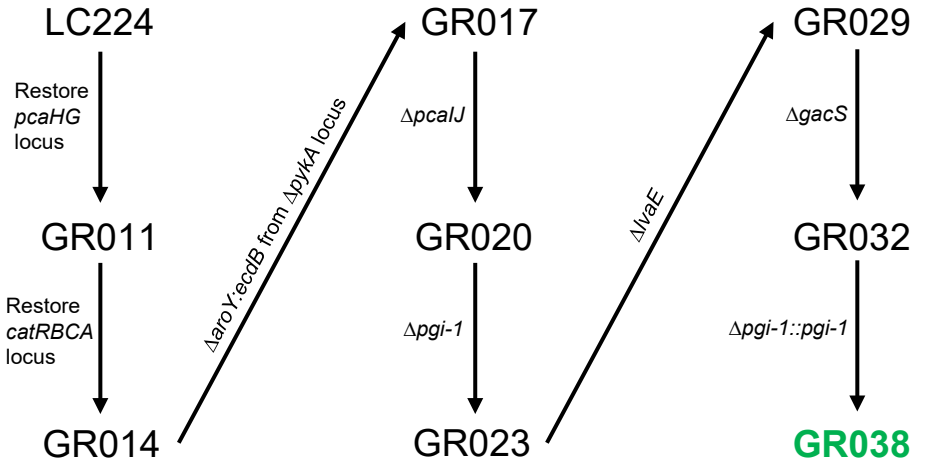
Progress and outcomes: Strain engineering

Rubinstein, Mokwatlo *et al. in preparation*





GR038 *P. putida KT2440 ΔpykA::aroG-D146N:asbF Δpgi-2 Δppc Δgcd ΔampC::xylEA455V,A62V:xylAB:tktA:talB PP_2834:Plac:ubiC-C22:PP_2835 ΔhexR G to A of P PP_2569 ΔpykF::Ptac:aroB ΔpcaIJ ΔlvaE ΔgacS*

- An ABF muconic acid production strain (LC224) was repurposed to produce β-ketoadipic acid from glucose and xylose
- 8 engineering steps to generate the best-performing strain

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Library generation

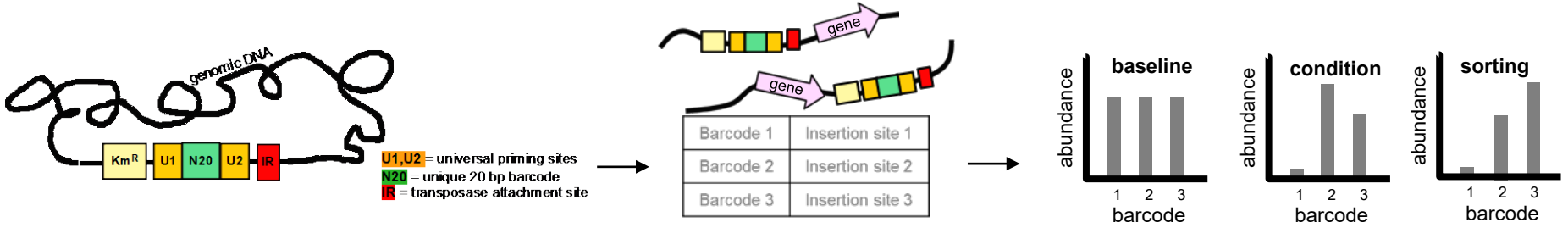
Amendola, Bleem *et al. ongoing*

RB-TnSeq Library

Generate mariner insertions in *P. putida* genome with randomly barcoded vector library

Fragment and map the location of barcodes

Perform fitness and sorting assays



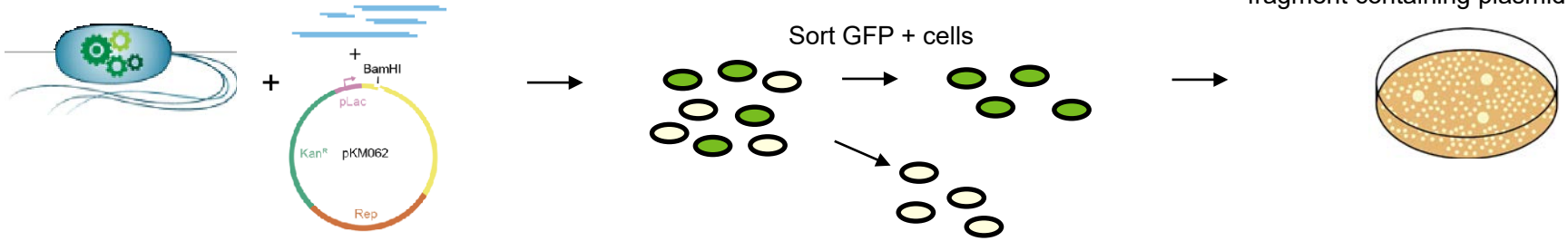
Fragment overexpression Library

Transform *P. putida* with overexpression library

Perform fitness and sorting assays

Sort GFP + cells

Pick single colonies and sequence fragment containing plasmid



Generating libraries will enable the identification of non-intuitive targets for improved production of β-ketoadipate

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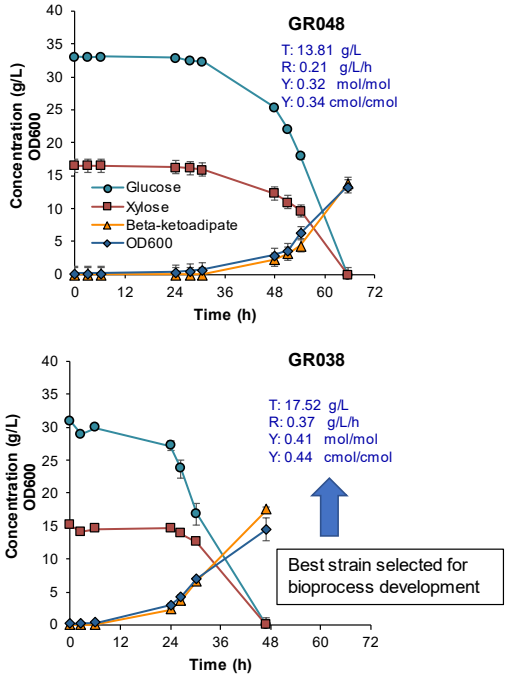
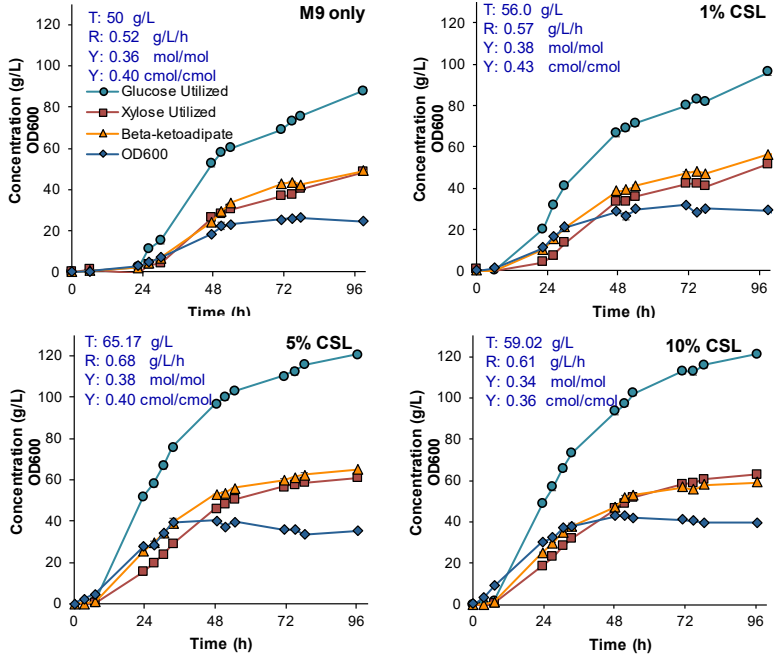
Bioprocess development

Rubinstein, Mokwatlo
et al. in preparation

Strain Evaluation in Bioreactors



Fed batch bioreactor profiles: rich media optimization



Bioprocess optimization improved TRY for the best strain (65.17 g/L at a rate of 0.68 g/L/h and yield of 0.40 C-mol/C-mol)

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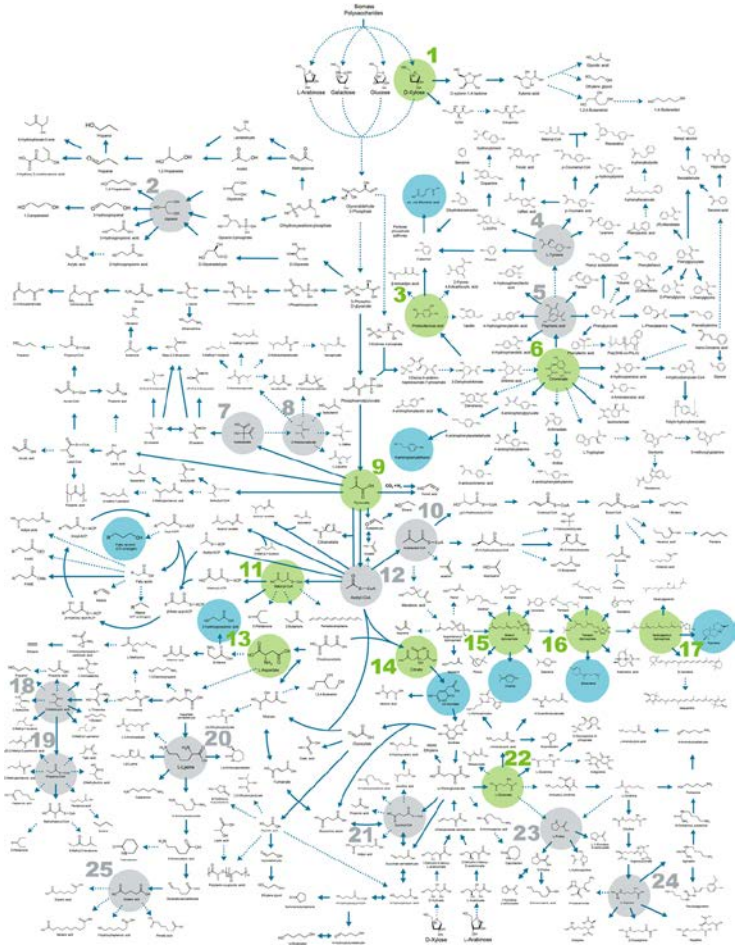
Impact

Scientific

- Peer-reviewed manuscripts and patent applications to manufacture a performance-advantaged C6 diacid, β-ketoadipic acid

Industry

- Working with Technology Holding and partners to scale-up
- Have sent 100 grams of β-ketoadipic acid for testing so far, plan for 1 kg by project end



Quad chart overview

Timeline

- Project Start: 10/1/2022
- Project End: 9/30/2024

	FY22 costed	Total Award (FY23-24)
DOE Funding	$0	$ 1,400,000 LANL - $360k PNNL - $300k NREL – $740k
Cost Share	$0	Technology Holding - $350k

Project Partners

ABF Labs: LANL, PNNL, NREL

Industry Partner: Technology Holding, LLC

Project Goal

Develop P. putida strains to produce b-ketoadipate from hydrolysate at industrially-relevant levels and scale-up with Technology Holding and partners

End of Project Milestone

Conduct 10-L bioreactor cultivations in triplicate to be able to produce > 1 kg of βKA to deliver to Technology Holding for separation, purification, and polymerization into βKA-nylons.

Funding Mechanism

FY21 ABF Directed Funding Opportunity

TRL at Project Start: 3

TRL at Project End: 4

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DOE Technology Manager **Gayle Bentley**

Project Contributors:
LANL: Taraka Dale, **Ramesh Jha**, Tari Kern

NREL: Caroline Amendola, Gregg Beckham, Alissa Bleem, Christopher Johnson, Megan Krysiak, Charles Mokwatlo, Michelle Reed, Davinia Salvachúa Rodriguez, Gabe Rubinstein, and Sean Woodworth

PNNL: Kristin Burnum-Johnson, Yuqian Gao, **Young-Mo Kim**, Nathalie Munoz

Technology Holding: **Mukund Karanjikar**, Robert Price

NREL/PR-2A00-85695

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Additional Slides

Publications

- Gabriel M. Rubinstein, Sekgetho Charles Mokwatlo, Chen Ling, Sean P. Woodworth, Kelsey J. Ramirez, Davinia Salvachúa, Christopher W. Johnson, Gregg T. Beckham, Production of β-ketoadipic acid from glucose and xylose with *Pseudomonas putida* KT2440. Pending submission to *Metabolic Engineering*.