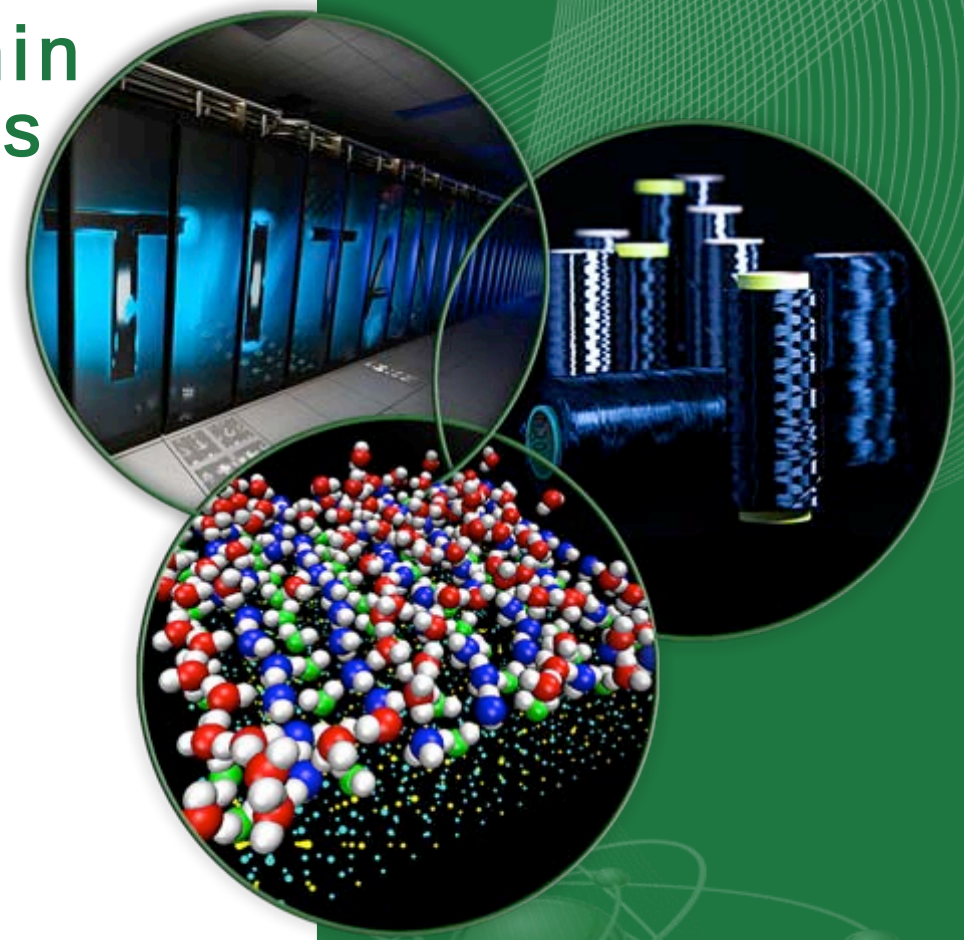
Synthetic Metabolic Pathways for Bioconversion of Lignin Derivatives to Biofuels

WBS: 2.3.2.104



March 25, 2015

Technology Area Review: Biochemical Conversion

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Organization: Oak Ridge National Laboratory

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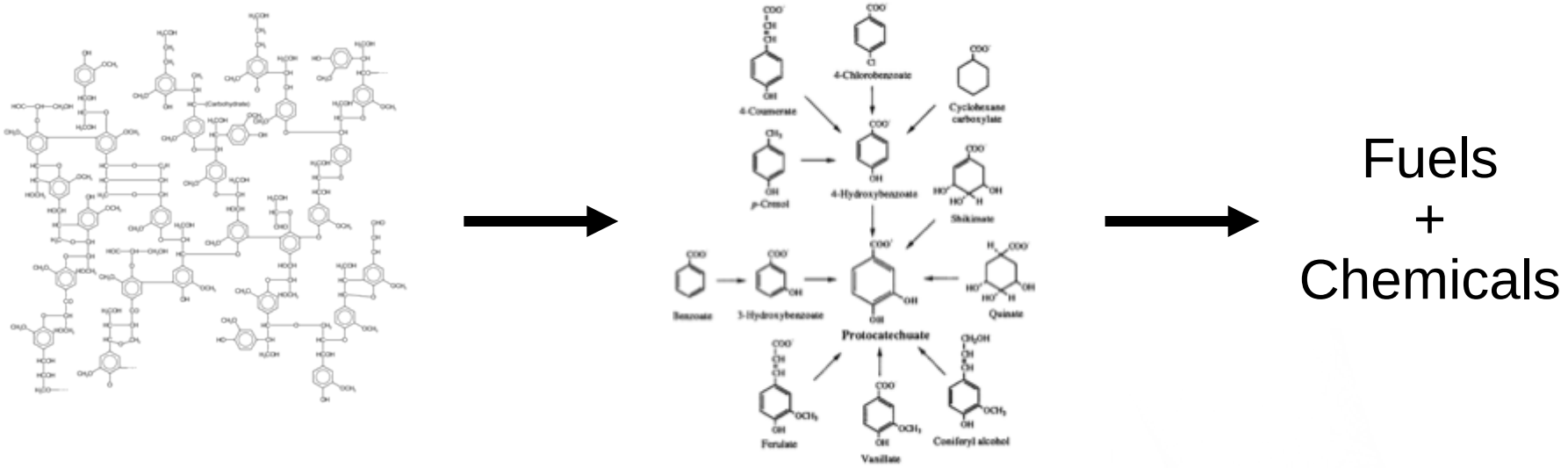
Goal Statement

- Goal: Develop microbial biocatalysts to convert lignin-rich streams into value-added products



- Relevance: Adding value to the lignin fraction of plant biomass will improve the economics of biorefineries to enable a bioeconomy

Quad Chart Overview

Timeline

- Project start date: March 2014
- Project end date: September 2017
- Percent complete: 28%

Barriers

- Barriers addressed
  - Bt-I. Catalyst Efficiency
  - Bt-F. Hydrolytic Enzyme Production

Budget

	Total Costs FY 10 – FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	$0	$0	$205k	$1.2 MM

Partners

- Collaboration and shared milestones with Dr. Gregg Beckham at National Renewable Energy Laboratory BETO-funded project “Lignin Utilization” WBS 2.3.4.100

Project Overview

History:

- Started as ORNL internal investment (LDRD project) to engineer *E. coli* to catabolize aromatic compounds and convert them into value-added products
- BETO Seed project starting in FY14

Context:

- Lignin accounts for ~25% of plant biomass but is underutilized during biofuel production
- Primary current use is for process heat and electricity

Project Objectives:

- Identify best microbial platform for deconstruction of lignin and catabolism of aromatic compounds from biomass
- Develop the genetic tools needed for bioengineering novel microbes
- Develop a biological platform for production of fuels and chemicals from lignin-rich streams

Technical Approach

Task 1: Identify the best lignin-depolymerizing microbes and develop into bioengineering platform

Approach:

- Screen putative lignin-degrading organisms for the ability to reduce the molecular weight of lignin in alkaline pretreated liquor (APL)
- If better candidate(s) than *Pseudomonas putida* are found, develop genetic tools to allow rational engineering
- Genetically modify selected organism(s) to make value-added products directly from polymeric lignin

Challenges:

- May not find sufficiently ligninolytic organism
- Development of genetic tools is often time-consuming

Task 2: Engineer *Pseudomonas putida* to produce value-added chemicals such as polyhydroxyalkanoates (PHAs) from APL

Approach:

- Delete and/or insert genes to redirect carbon and electron flux to product (PHAs, others)
- Measure product formation during growth on APL
- Combine beneficial genetic modifications

Challenges:

- Control of metabolic flux to achieve high yield, titer, and rate → productivity

Management Approach

Critical success factors

- Integration with TEA (Mary Biddy, NREL) to identify top product targets
- Maintaining or improving production rate and titer as yield increases

Potential challenges

- It is unclear if better lignin-depolymerizing strains exist

Management approach

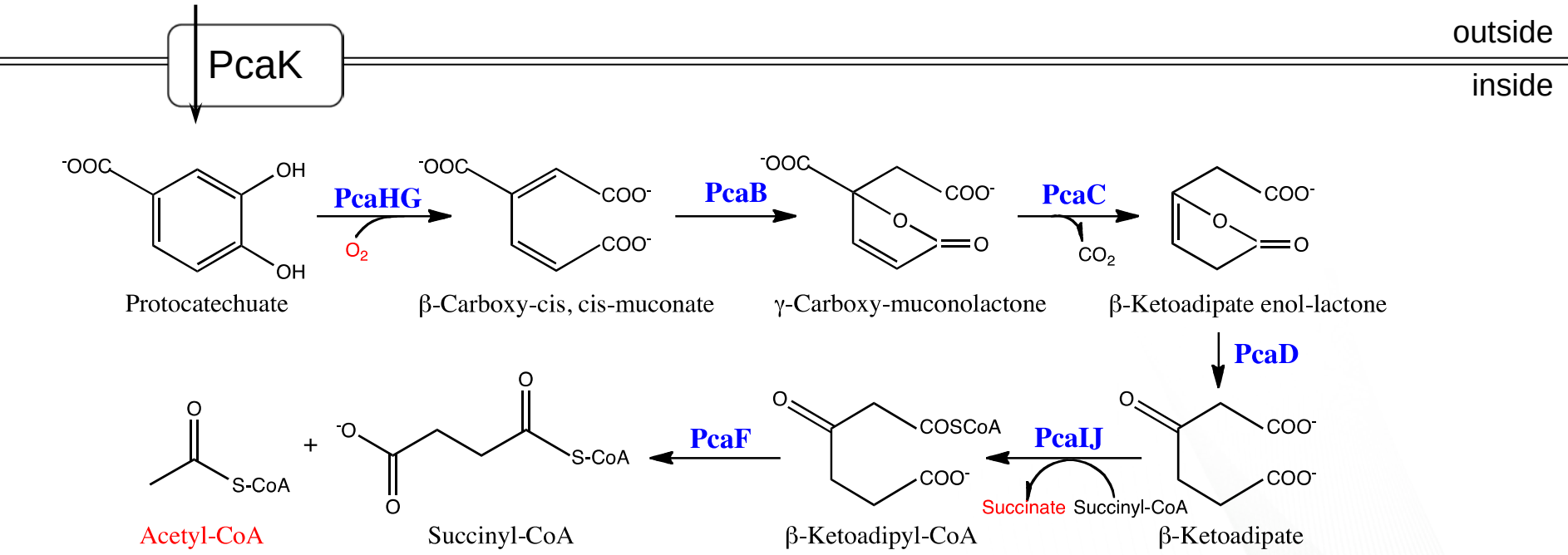
- Regular calls with BETO TPM – first Joyce Yang, now Bryna Guriel
- Regular interactions with Gregg Beckham (NREL)
  - MTA and NDA in place
  - Site visits, phone calls, data sharing, strain exchanges

Technical Accomplishments – Overview

- Initial work focused on engineering *E. coli* to catabolize aromatic compounds
- Further engineering the *E. coli* strain to make value added products, laying the groundwork for work in other organisms
- Possible inhibition of modified *E. coli* by APL and complex lignin-degradation pathways led to a shift to ligninolytic microorganisms that can thrive in APL
- *Pseudomonas putida* progress
  - Identified top targets for modification
  - Have begun metabolic engineering

Technical Accomplishments

- Initially targeted *E. coli* for proof of principle. Introduced nine gene 3,4 ortho protocatechuate (PCA) degradation pathway

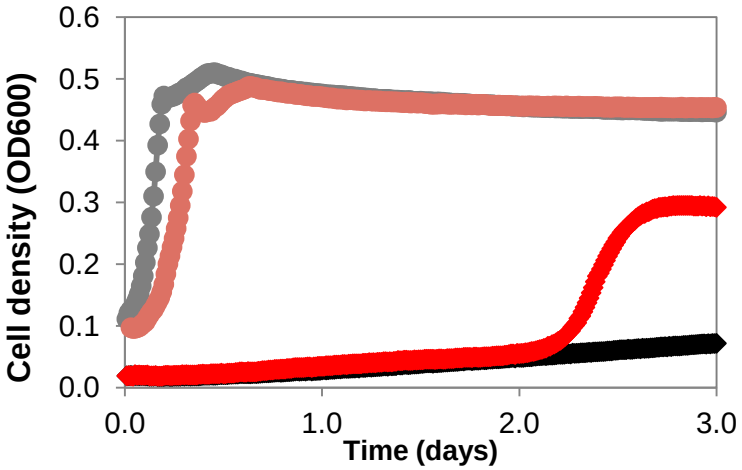


$$PCA + O_2 + CoA + H_2O \rightarrow CO_2 + succinate + acetyl\text{-}CoA + H^+$$

Technical Accomplishments – FY14

- Plasmid-based expression allowed for *E. coli* utilization of PCA as sole carbon and energy source
- Growth on glucose was slower than control strain
- Stably integrated the pathway genes into the chromosome
- Chromosome-based expression allowed for faster utilization of PCA and normal growth on glucose

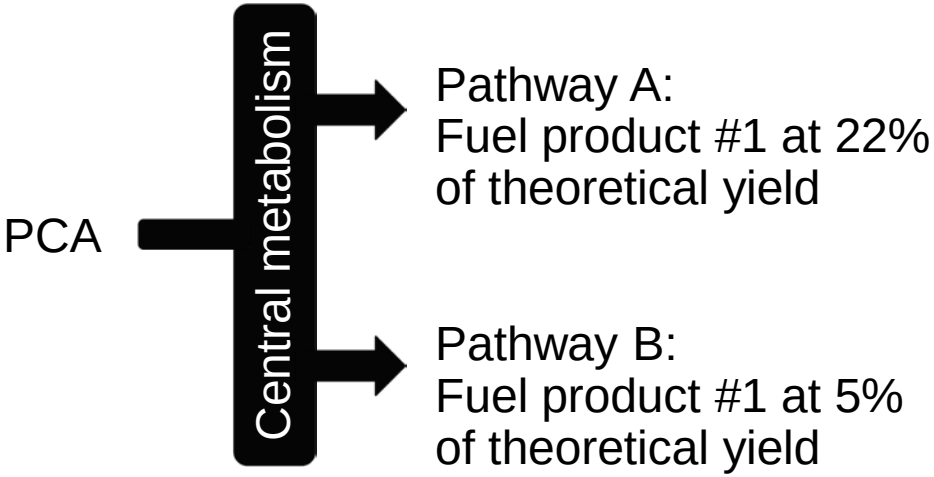
Plasmid-based expression

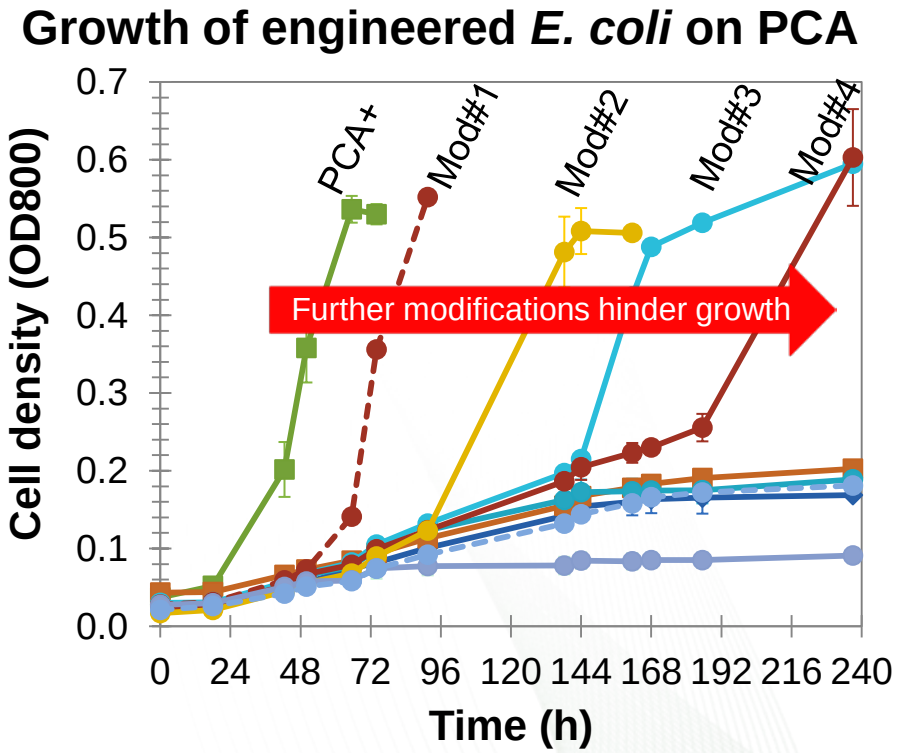


Chromosome-based expression

Technical Accomplishments – FY14

- Further modification of the PCA-degrading *E. coli* strain allowed for flux to be directed toward the product of interest, but at the expense of growth rate and robustness

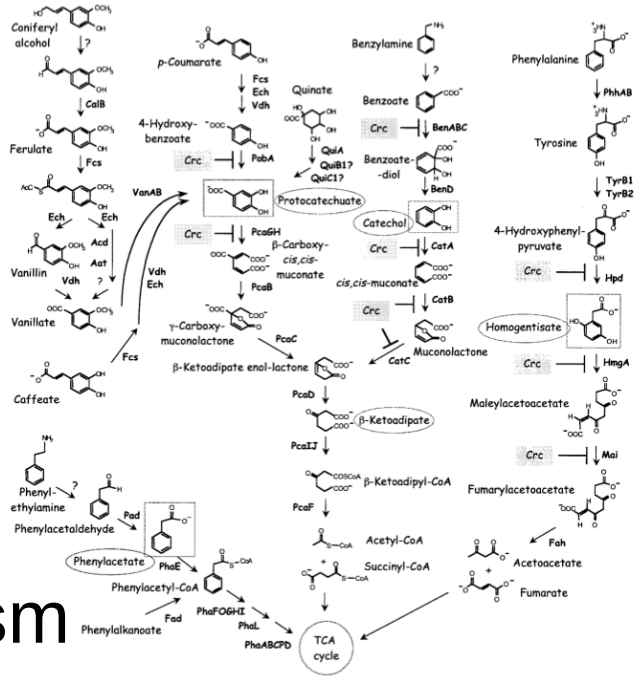




Technical Accomplishments

- *E. coli* is unlikely to be deployed for lignin conversion
  - Complexity of funneling reactions
  - Difficulty in simultaneous expression

    of dozens of pathways
  - Low tolerance to aromatics



- Identified need for a better organism
  - Identifying organisms that depolymerize lignin and catabolize many of the aromatic compounds present in Alkaline Pretreated Liquor (APL)
  - Established collaboration and strain sharing with NREL (Beckham)
  - *P. putida* has been shown to grow on APL and depolymerize lignin (Beckham)

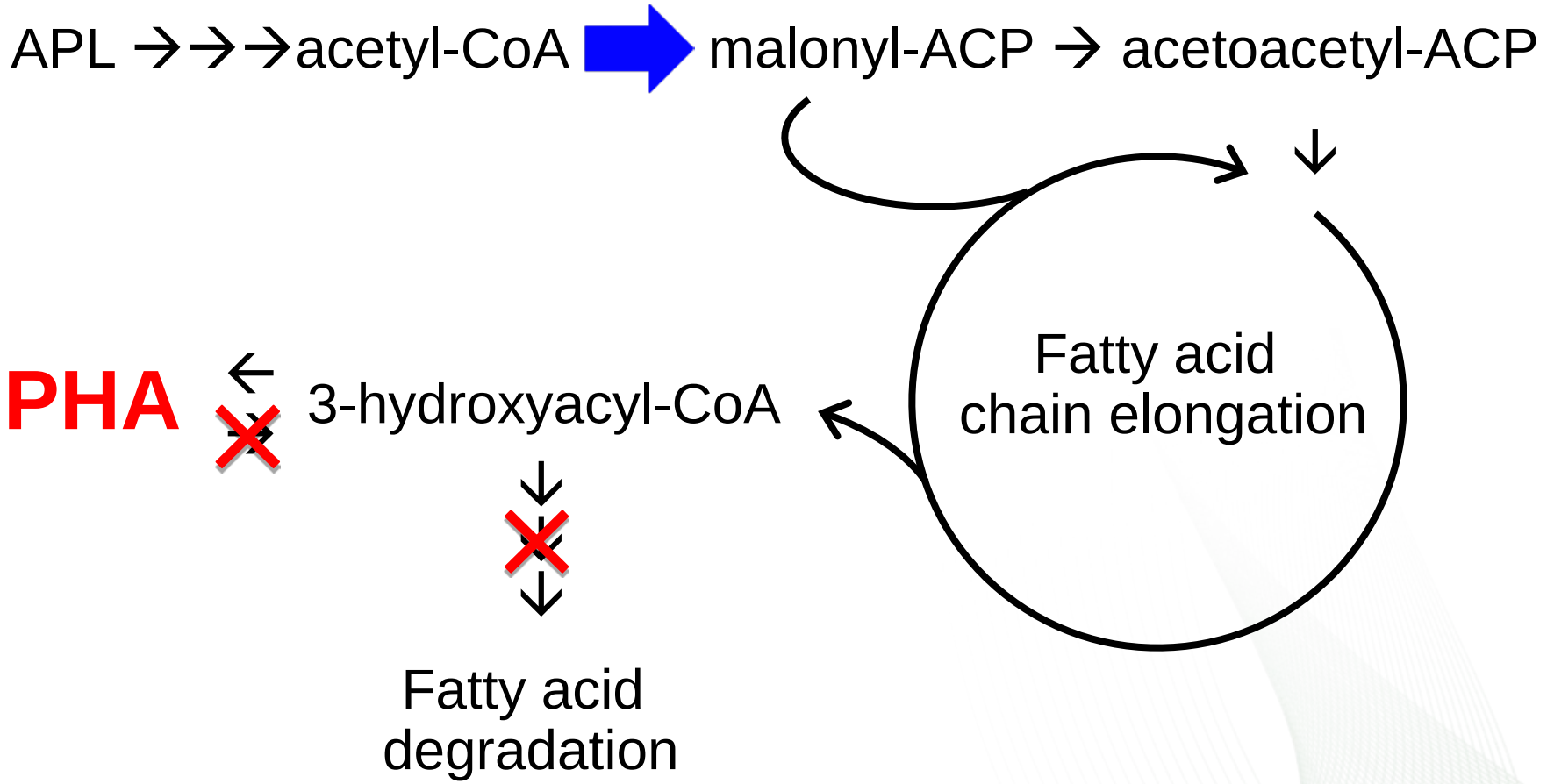
Technical Accomplishments – FY15

- Identification of lignin-deconstructing microbes
  - In collaboration with Gregg Beckham at NREL, we are screening putative lignin-depolymerizing microbes to identify the best for lignin depolymerization and catabolism of aromatic compounds
  - Have sent 16 putative lignin-degrading strains to NREL for characterization

- *Pseudomonas putida* engineering
  - We have identified the highest priority mutations for increasing mcl-PHA production and producing another target molecule from APL
  - We have begun engineering *P. putida* to improve production of these compounds

Technical Accomplishments – FY15

- *Pseudomonas putida* engineering



Relevance

- A mature cellulosic biofuels industry will produce an estimated 300 million tons of lignin-rich material
- Adding value to the lignin fraction of biomass will be critical to meeting cost targets
- Biological lignin conversion will require robust biocatalysts, which are currently lacking
- Tech transfer – Early in the research project.
  - Near term – Intellectual property and publications.
  - Will work with tech transfer and NREL as technology matures to move technology to industrial partners

Future Work

The next 18 months will focus on improving product yield in *P. putida* and identifying the best lignin-degrading strains for further development

Milestones:

- FY15 Q3 (Task 2) Implement at least three metabolic engineering strategies for increasing product yield from APL
- FY15 Q4 (Task 2) Demonstrate at least 20% increase in yield of target product
- FY16 Q2 (Task 1) Go/No-Go Point. Identify a lignin-degrading organism that demonstrates at least 15% greater lignin depolymerization and catabolism
- FY16 Q4 (Task 1) demonstrate genetic modification of the top lignin-degrading organism
  - Will leverage technologies developed within the BioEnergy Science Center

Summary

- Goal: Convert polymeric lignin and aromatic compounds into value-added products using engineered microorganisms
- Approach
  - Identify the best lignin deconstructing and catabolizing microbe
  - Engineer it to convert the lignin into higher value products
- Technical Accomplishments
  - Engineered *E. coli* to consume the aromatic compound PCA and produce a fuel product at 22% of theoretical yield
  - Sent putative lignin-deconstructing strains to NREL
  - Begun engineering *P. putida* to produce value added products from lignin-rich streams
- Relevance
  - Lignin valorization needed to improve economics of biorefinery
  - Biological upgrading of lignin can provide this value
- Future work
  - Increase product yield in *P. putida* from lignin and aromatics in APL
  - Identify best lignin-deconstructing strains; begin metabolic engineering

Acknowledgements

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- Mary Biddy
- Chris Johnson
- Davinia Salvachua

Abbreviations

- APL – Alkaline Pretreated Liquor
- mcl-PHA – Medium chain length Polyhydroxyalkanoate
- MTA – Material Transfer Agreement
- NDA – Non-Disclosure Agreement
- NREL – National Renewable Energy Laboratory
- PCA – Protocatechuate
- TEA – Techno-Economic Analysis
- TPM - Technical Project Manager