DOE Bioenergy Technologies Office (BETO)
2021 Project Peer Review

Development of *Bacillus* as an Industrial Host for Microbial Production of Biopolymers

Feb-19-2021
Agile BioFoundry

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Project Overview

- Development of *Bacillus* as an industrial host for the microbial production of biopolymers of industrial interest from lignocellulosic hydrolysates

- Project Goals
  - Enable ZymoChem's carbon conservation (C^2) technology in non-model organisms to maximize carbon efficiencies (yields) to biopolymers via minimizing loss of CO_2 from lignocellulosic hydrolysates
  - Apply ABF's state of the art metabolic engineering tools/strategies to engineer strains with improved Titer, and Rate, metrics by >4x over base case performance
  - Partner with ABF to utilize fermentation and process development & scale up expertise to scale up production process to pilot scale

Project Overview

- Project Importance
  - Establish non-model organisms as microbial hosts for making biopolymers of industrial interest
  - Develop a scalable production process for making biopolymers using non-model organisms
  - Commercialize bio-based polymers as sustainable and cost-competitive replacements of current fossil-based polymers
- Project Risks
  - Non-model hosts are difficult to engineer due to a variety of reasons
  - Scaling fermentation and downstream processing at metrics that are equal to those achieved at lab scale
- Project Tasks
  - Task 1: Establish C^2 Technology in *Bacillus*
  - Task 2: Strain Engineering
  - Task 3: Genetic Engineering Tool Development
  - Task 4: Fermentation and DSP Scale Up

1 – Management

ZYMOCHEM

1) Project lead

2) Lead Task 1

3) Co-lead Task 2

4) Support all tasks as needed

5) Prepare DOE reports

BERKELEY LAB

1) Lead – omics application on all Tasks

2) Co-lead Task 2

OAK RIDGE National Laboratory

1) Lead Task 3

2) Support Task 1

BERKELEY LAB

1) Lead Task 4

1 – Management

Team Management

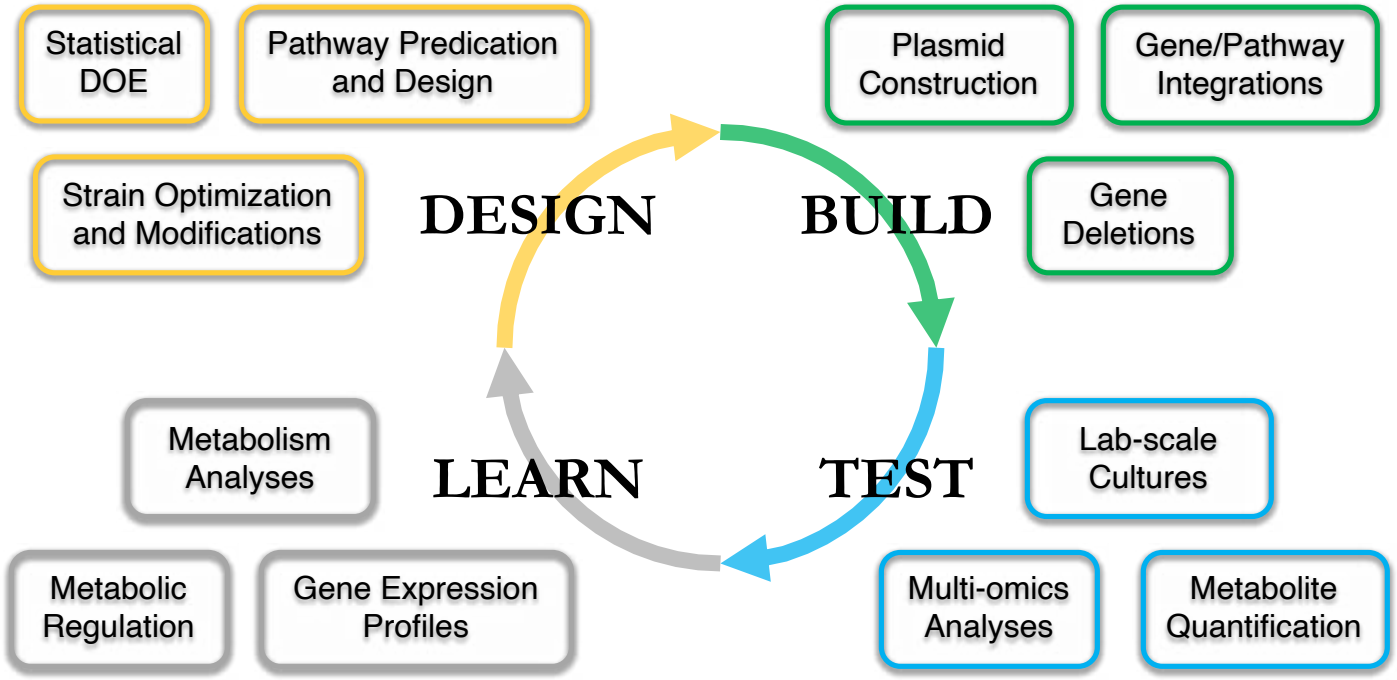
- Monthly all hands meeting – To discuss progress, review milestones and chart next steps for all team members. Technical team members meet more frequently and on as needed basis.
- Clear ownership of project tasks/strategies/milestones.
- Setting expectations on monthly basis for all teams. Each lab PI is responsible for managing technical risk and project risk for their tasks with input from larger team.
- Quarterly updates to program manager

Key learnings & Successes:

- Multiple strategies implemented to address go/no go milestones. Early success achieved.
- COVID and project kick off delays – dynamic changes in project roles due to overlapping skills + leverage 3rd party services vs doing it inhouse
- Redistribution of effort on a specific task, which ZymoChem was originally responsible – but LBL and ORNL had substantial expertise in due to prior efforts on other projects

2 – Approach

We will utilize the expertise at the Agile BioFoundry to develop and implement various facets of the ***DBTL*** cycle to achieve the project **Objectives**.



2 – Approach

To enable facile application of DBTL approach to our non model host our technical approach involves four tasks

- Task 1 – Establish C^2 Technology in *Bacillus*
  - To be accomplished in coordination with Tasks 2 and 3
  - Enables carbon lossless conversion from feedstock to product improving theoretical maximum yields by > 20%
- Task 2 – Strain engineering
  - Multi-omics & metabolic flux analysis to understand what's going on inside the cell and help identify strain engineering targets
- Task 3 – Genetic engineering tool development
  - Robust systems to enable engineering within our non-model *Bacillus* host including recombinant biosynthetic pathway expression, gene deletions, and chromosomal gene integrations
  - Critical capability that enables all tasks
- Task 4 – Fermentation and DSP scale up
  - Demonstrate scalability of the entire production process at metrics similar to those achieved at lab scale and make product samples for testing

2 – Approach – Go/No-Go Milestones

- By 30 June 2021, demonstrate >2x improvement in both titer and rate metrics over benchmarking values.

- By 30 June 2022, demonstrate >4x improvement in both titer and rate metrics over benchmarking values.

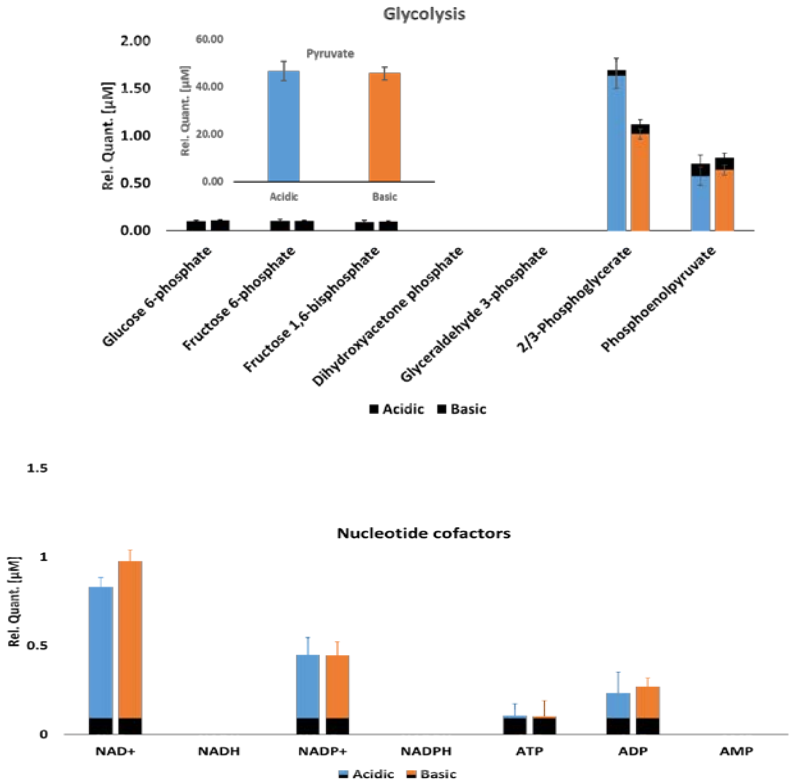
3 – Impact

- Project has already generated more follow-on funding for ZymoChem to further develop and scale this technology
- Genetic tools, resources, and expertise created as part of this project for engineering this *Bacillus* species will help researchers for future engineering endeavors with other closely-related species of this genus, as they are great production hosts for proteins, biopolymers, and small molecules
- Successful development of a C^2 biotechnology that is designed to improve yields by minimizing or eliminating carbon loss will help change the paradigm and motivate future researchers to pursue novel ideas on pathway and microbial designs tailored for carbon conservation to enable more economical production of products

4 – Progress and Outcomes

- Successful development of genetic tools and recombinant protein expression within the host
- Successfully demonstrated >2x improvement in titers and rates of production – **Go/No Go milestone completed 4 months ahead of schedule**
- Successfully ran scale up of fermentation and process development of the strain at pilot level

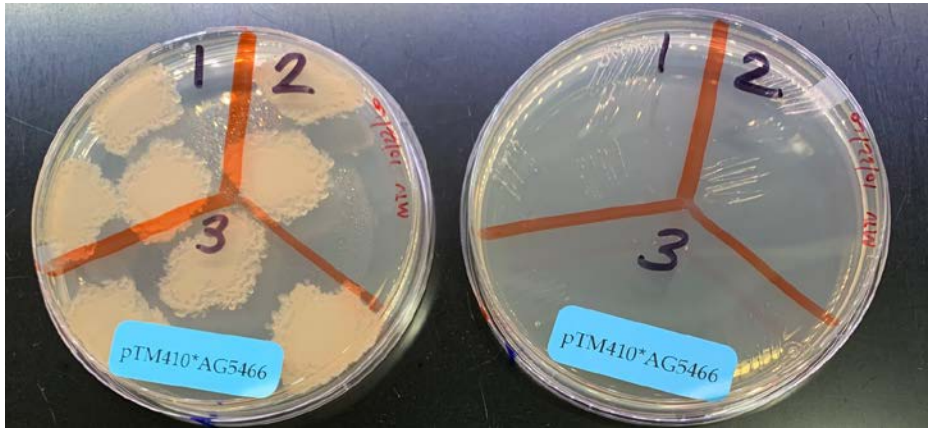
Task 2 Progress: Strain metabolome characterization



- Various methods of metabolite extraction and analysis have been tested. One method has been validated to monitor biosynthetic pathways and cofactors of interest inside the cell.

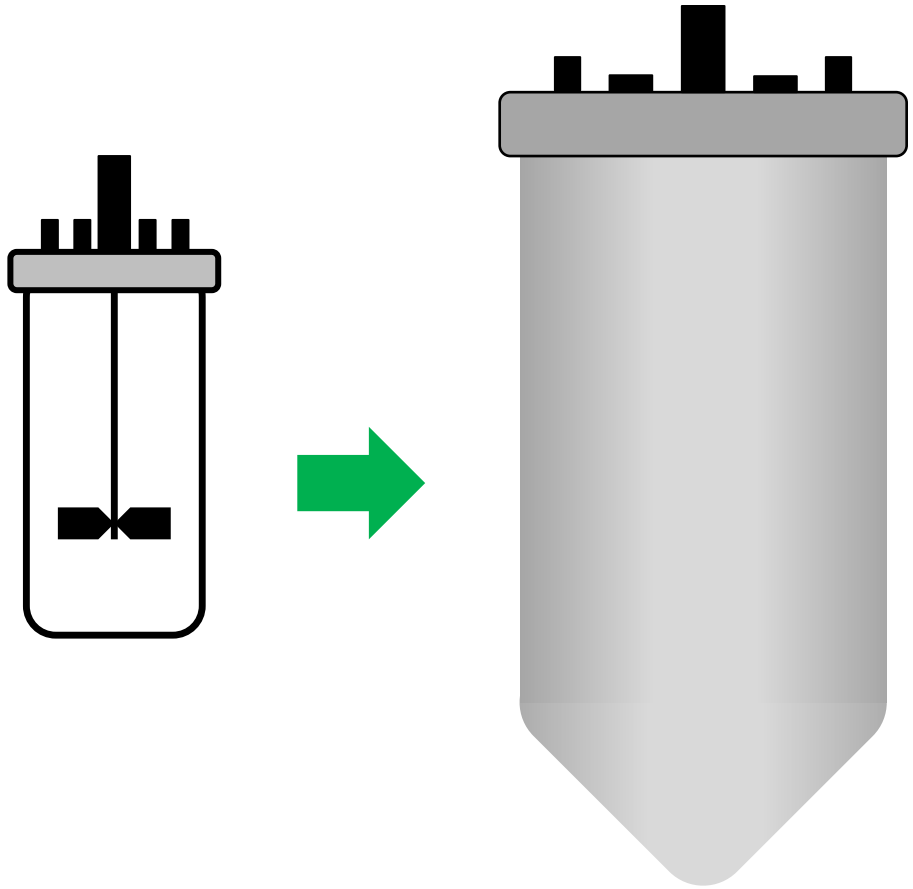
Task 3 Progress: An example of genetic tool development

This system has been used successfully to delete genes and insert genetic elements into the chromosome.



Applying selection

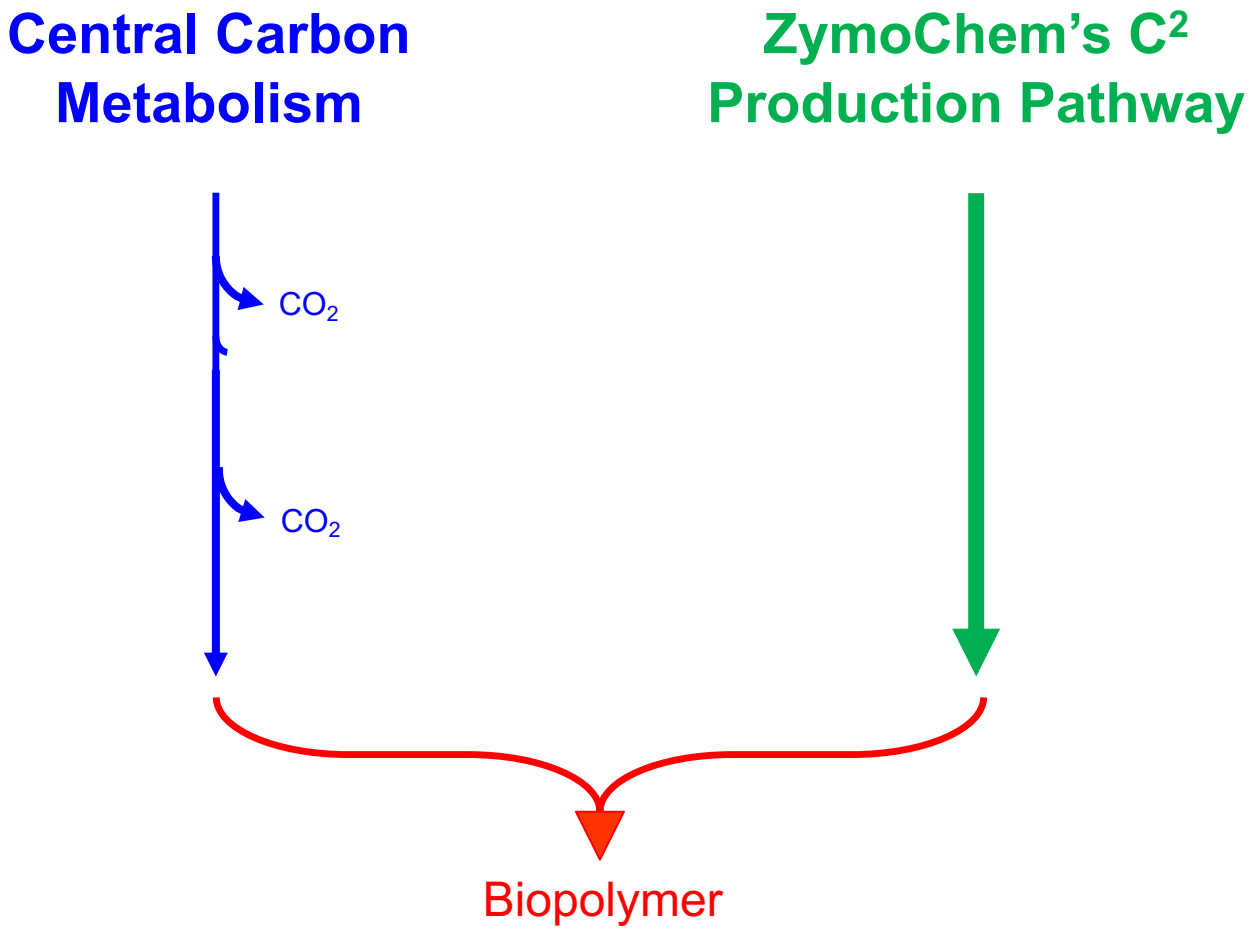
Task 4 Progress: Process scale up



In our first attempt, we successfully scaled our 2 L bench-scale process to a 300 L pilot-scale fermenter.

Summary

- *ZymoChem's Carbon Conserving (C^2) Pathway minimizes carbon loss, thereby lowering overall production costs.*
- *Project is on track even with COVID related delays.*
- *Demonstrated Go/No-Go milestone 4 months ahead of schedule.*
- *Success demonstrated on key enabling technologies in non-model organism, thereby enabling application of DBTL tools to further develop improved strains and processes*
- *Successful process scale up to 300 L*



Quad Chart Overview

Timeline

- Jan 1 2019
- June 30 2022

	FY20 Costed	Total Award
DOE Funding	$317,289	$1,321,381
Project Cost Share	$79,551	$331,615

Project Partners

- Lawrence Berkeley National Lab
- Oak Ridge National Lab

Project Goal

The overall goal of this project is to develop a *Bacillus*-based bioprocess for production of biopolymer from lignocellulosic-derived C5 and/or C6 sugars at 4x the titer and rate over benchmarking values.

End of Project Milestone

- Demonstrate >4x improvement in both titer and rate metrics over benchmarking

Funding Mechanism

DE-FOA-0001916, *BioEnergy Engineering for Products Synthesis (BEEPS)*, and 2019.