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Agile BioFoundry

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

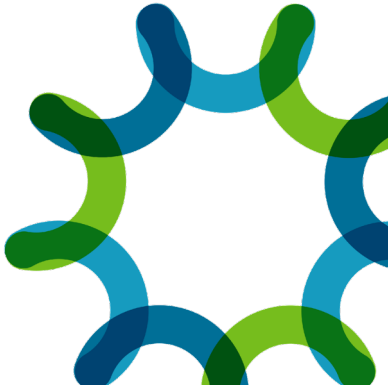
BIOENERGY TECHNOLOGIES OFFICE

DOE Bioenergy Technologies Office (BETO)
2023 Project Peer Review

Agile BioFoundry – DFO with Superbrewed Foods

April 5, 2023 at 9:27 am MST
Conversion Technologies

Adam Guss
Oak Ridge National Laboratory



Project overview

- **Technology:** Fermentation to most traditional products has a maximum carbon yield of 66%. The Superbrewed Foods technology uses an organism that co-ferments sugars and H_2, enabling a theoretical maximum carbon yield of 100%

- **Challenge:** Strain instability and cell-to-cell variation of an engineered isopropanol-producing strain leads to diminished performance within a bioreactor

- **Primary Goal :** Understand the cause and identify potential solutions to strain instability over time, leveraging ABF expertise in anaerobic microbiology and biosensor development

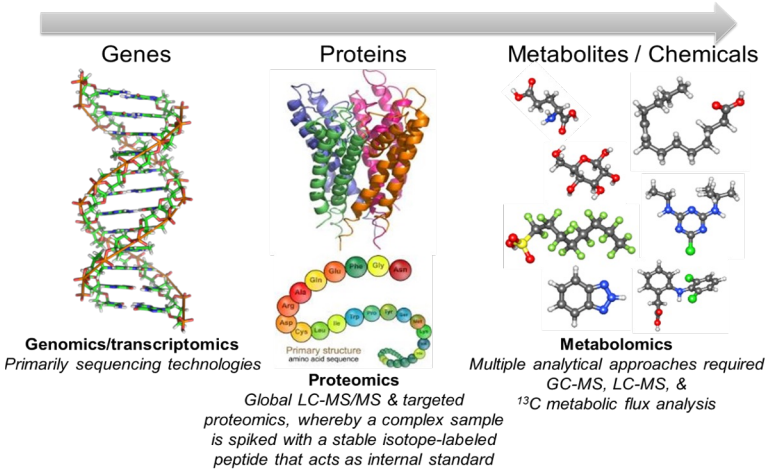
Approach

- **Technical Approach:**
  - Genome resequencing, proteomics, and metabolomics to understand cause of instability
  - Biosensor circuit to allow selection for improved strains
  - Understand and engineer strains for improved and consistent production
- **Challenges:** Work in anaerobic organisms is more difficult and time-consuming than aerobes; bulk measurements may not reflect the reality of individual cells
- **Risks:** ‘omics may not reveal the cause of instability and variation

  Mitigation: The biosensor approach may allow selection of improved strains with the need to first understand it
- **Communication and Collaboration:** This project leverages past and ongoing tool development from the Host Onboarding team within the ABF. Monthly calls are used to coordinate work

Approach – ‘omics

- **Technical Approach:**
  - Sample replicate bioreactors at multiple time points, with different production phenotypes
  - Sequence bulk culture and individual members of the population to look for mutations and copy number variants
  - Perform proteomics and metabolomics to explore differences in enzyme levels and metabolic flux
  - Use results to inform rational engineering targets



Genomics/transcriptomics
Primarily sequencing technologies

Proteomics
Global LC-MS/MS & targeted proteomics, whereby a complex sample is spiked with a stable isotope-labeled peptide that acts as internal standard

Metabolomics
Multiple analytical approaches required GC-MS, LC-MS, & ^{13}C metabolic flux analysis

Single-Sample Extraction

➡Metabolites
➡Proteins

➡Lipids

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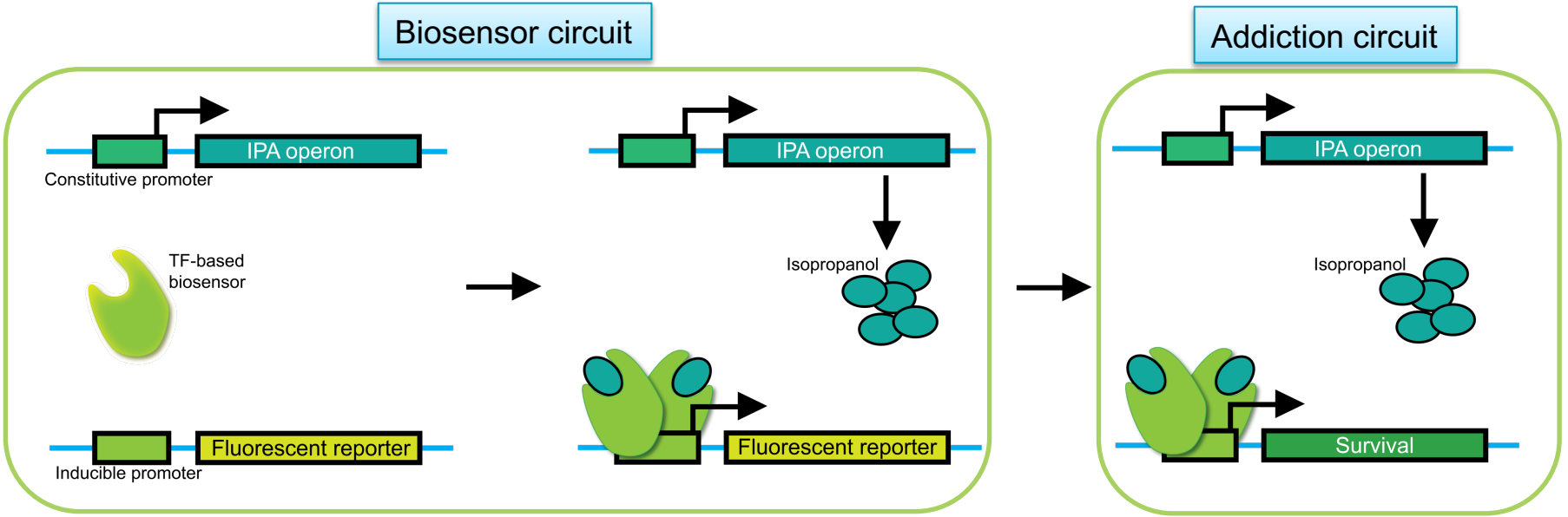
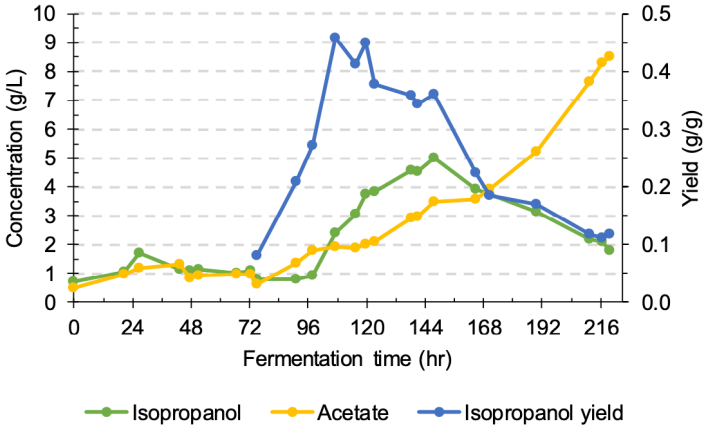
Approach - biosensor

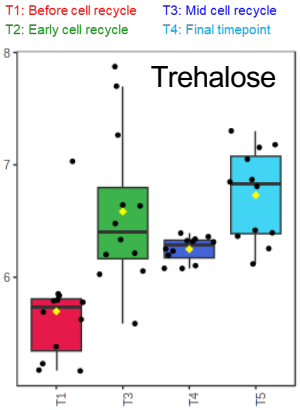
- Design and demonstrate an isopropanol biosensor in *C. ljungdahlii*
- Modify biosensor circuit into “addiction” circuit for cellular fitness or survival
- Use this untargeted approach to identify new genetic targets

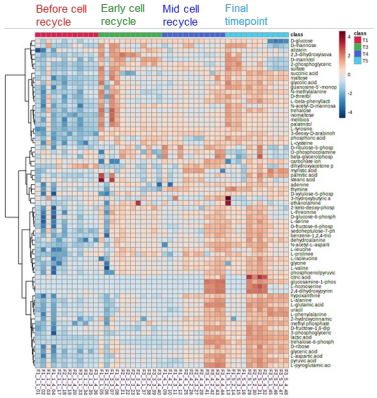


Progress and Outcomes

- **Technical Approach:**
  - We are in Q3 of the project and have run replicate bioreactors and sampled at multiple time points, targeting the different production phenotypes
  - Samples were sent to ORNL for sequencing analysis, which is ongoing
  - Samples were sent to PNNL for proteomics and metabolomics, which is also ongoing
- This will hopefully identify targets for genetic manipulation for improved production







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Impact

- **Positive impacts for Superbrewed Foods (SBF)**
  - Generate an improved, more stable isopropanol strain
  - Gain knowledge from the changes the strain undergoes during the continuous, cell-retention process
- **Positive impacts for ABF**
  - First ABF sensor circuit developed for a *Clostridium* species and a strict anaerobe, helping guide future efforts
  - Develop a set of approaches to assist in future ABF projects probing culture heterogeneity
- **Positive impacts for the American bioeconomy**
  - SBF is targeting isopropanol as a commercialization biochemical
  - MixoFerm technology can be applied to any number of other biochemicals
  - Successful commercialization of isopropanol will lead to other MixoFerm-based processes for other products and further grow the American bioeconomy

Summary

- **Approach**
  - Use multi-omics to better understand the cause of strain instability and loss of production over time

- **Technical progress**
  - We are currently analyzing the genetics, proteins, and metabolites from multiple fermenter runs

- **Impact**
  - Potential to commercialize a process for decarbonization of isopropanol production

Quad Chart Overview

Timeline
- *July 1, 2022*
- *June 30, 2024*

	FY23 Costed	Total Award
DOE Funding		$1,450,000
Project Cost Share *		$370,000

Project Goal
- Understand the cause of and identify potential solutions to strain instability over time, leveraging ABF expertise in anaerobic microbiology, omics, and biosensor development

End of Project Milestone
- Combine biosensor circuit with engineered strain for improved and sustained production of isopropanol

Funding Mechanism
DFO

Project Partners*
- PNNL
- LANL
- ORNL

*Only fill out if applicable.

Acknowledgements:

DOE Technology Manager Gayle Bentley

Project Contributors:

LANL: Ellin-Kristina Triola, Taraka Dale, Ramesh Jha

ORNL: Adam Guss, Melissa Tumen-Velasquez

PNNL: Yuqian Gao, Kristin Burnum-Johnson

Suberbrewed Food, Inc: Emily Crawford, Carrissa Wiedel

Additional Slides

Responses to Previous Reviewers' Comments

- N/A: not previously reviewed at Peer Review

Publications, Patents, Presentations, Awards, and Commercialization

- None to date