# U.S. Department of Energy (DOE)
# Bioenergy Technologies Office (BETO)
# 2017 Project Peer Review

## Direct Photosynthetic Production of Biodiesel by Growth-Decoupled Cyanobacteria (Bioenergy Technology Incubator 2 Project)

March 9, 2017
Advanced Algal Systems

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Arizona State University

# Goals

- Directly produce excreted, ‘drop in’ ready biofuel (ethyl laurate) by cyanobacteria using $CO_2$, water, and light as the main inputs
- Uncouple growth of the culture (biomass production) from production of the biofuel to increase the ethyl laurate yield
- Further increase ethyl laurate production by (1) boosting metabolic flux through the fatty acid biosynthesis pathway and (2) reducing the production of exopolysaccharides

**Outcome:**

- Successful production and excretion of ethyl laurate
- Good yields (current laurate production is at about 20 mg/L/day)

**Relevance:**

- Direct production of excreted biofuel from $CO_2$, water and light
- Scalable with limited processing needs

# Quad Chart Overview

## Timeline

- Start: October 1, 2016
- End: September 30, 2018
- ~18% Complete

## Budget

| | Total Costs FY 12 –FY 14 | FY 15 Costs | FY 16 Costs | Total Planned Funding (FY 17-Project End Date) |
|---|---|---|---|---|
| DOE Funded | 0 | 0 | 0 | 1,818,000 |
| Project Cost Share (Comp.)* | 0 | 0 | 0 | 252,288 |

## Barriers

- Generating ethyl laurate from co-produced ethanol and laurate
- Scale-up
- Being cost-effective and contribute to sustainable, reliable, and affordable algae-based advanced biofuels

## On the Plus Side

- Potentially disruptive: Direct biofuel production at high yields

## Partner

- Algenol: Providing ethanologenic plasmids

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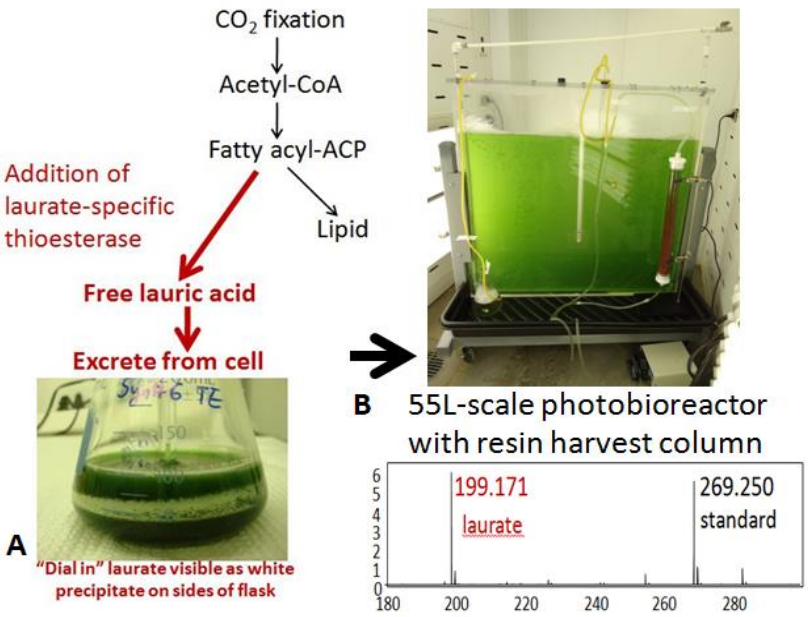
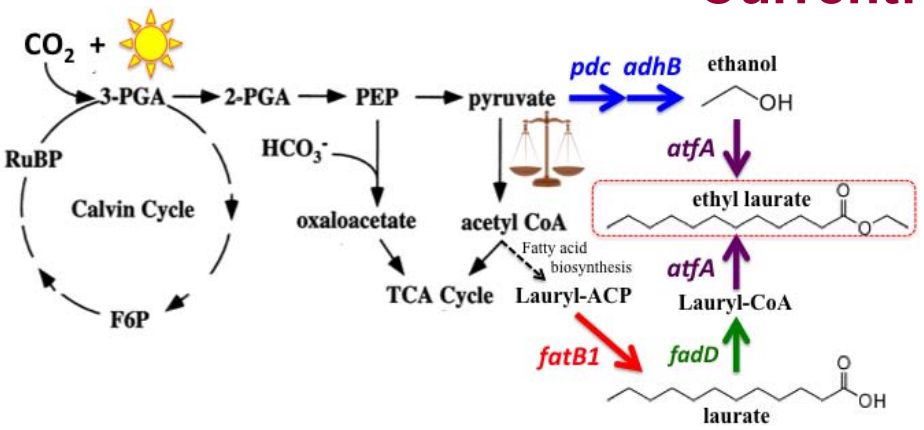
# 1 - Project Overview

## History:

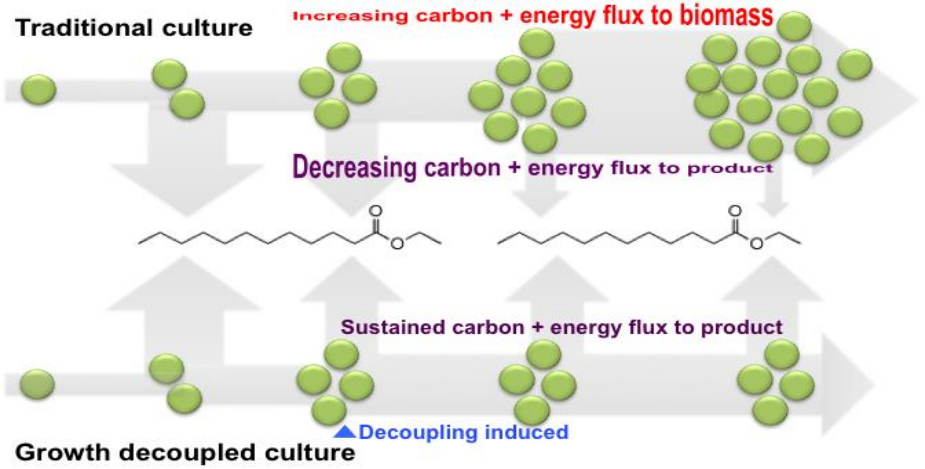



- Previous ARPA-E project: Generate a laurate-producing strain

## Current:




- Introduce ethanol production
- Introduce ethyl laurate production




- Use CRISPR-dCas9 or sRNA to induce growth arrest



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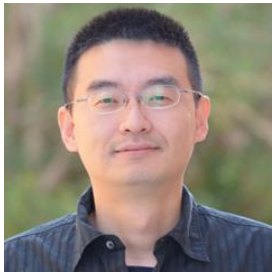
# 2 – Approach (Management)

**Team:**

- Wim Vermaas (PI; ASU, School of Life Sciences)
  - Fatty acid production improvement; ethyl laurate; exopolysaccharide deletion
- David Nielsen (Co-PI; ASU, School of Engineering of Materials, Transport and Energy)
  - Ethyl laurate; growth arrest; metabolic engineering
- Xuan Wang (Co-PI; ASU, School of Life Sciences)
  - Molecular biology; overexpression of fatty acid biosynthesis pathway genes; ethyl laurate balance
- Rob Stirling (ASU School of Sustainable Engineering and the Built Environment)
  - Ethyl laurate TEA once we have production data
- Three postdocs, four graduate students, one laboratory staff member, and a 50%-time project coordinator
- Biweekly meetings of the team



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# 2 – Approach (Technical)

## Tasks:

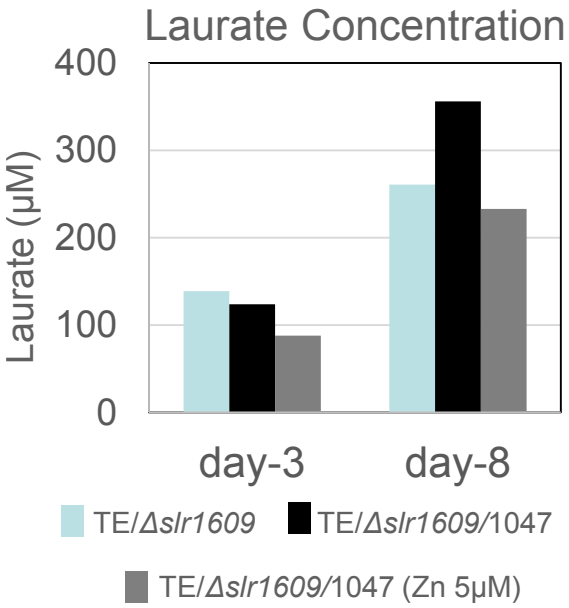
1. Increase laurate production (more laurate means more ethyl laurate)
   - Metabolic engineering (fatty acid biosynthesis regulation, overexpression)
2. Engineer ethyl laurate biosynthesis (Go/No-Go in October)
   - Introduce ethanol production; convert laurate to lauryl-CoA; convert lauryl-CoA and ethanol to ethyl laurate
3. Decouple growth and ethyl laurate production; grow at 55-L scale
   - Readily inducible (e.g., $Ni^{2+}$-inducible) growth arrest (CRISPR-dCas9)
4. Reduce exopolysaccharide production
   - Delete up to three genes involved in exopolysaccharide production
5. Techno-economic analysis on ethyl laurate production in cyanobacteria

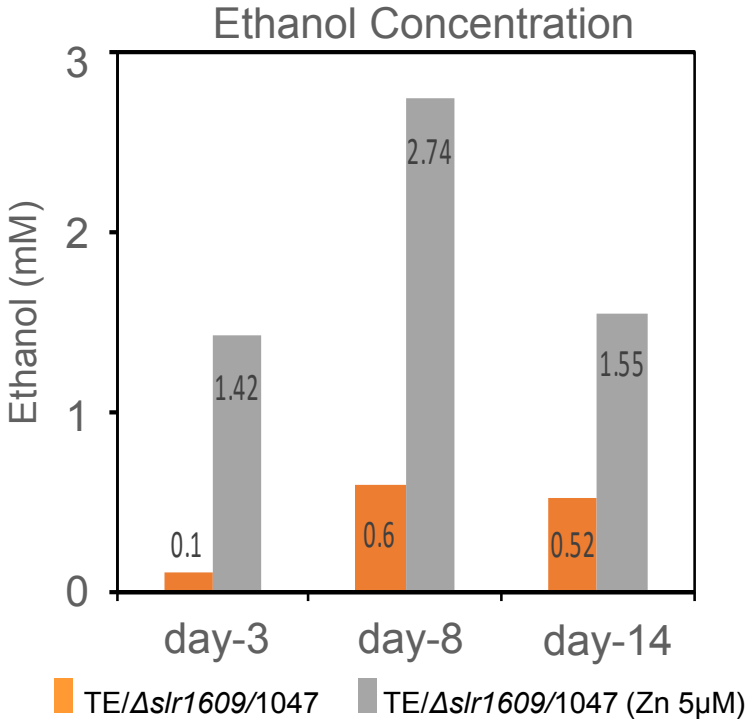
## Potential Challenges:

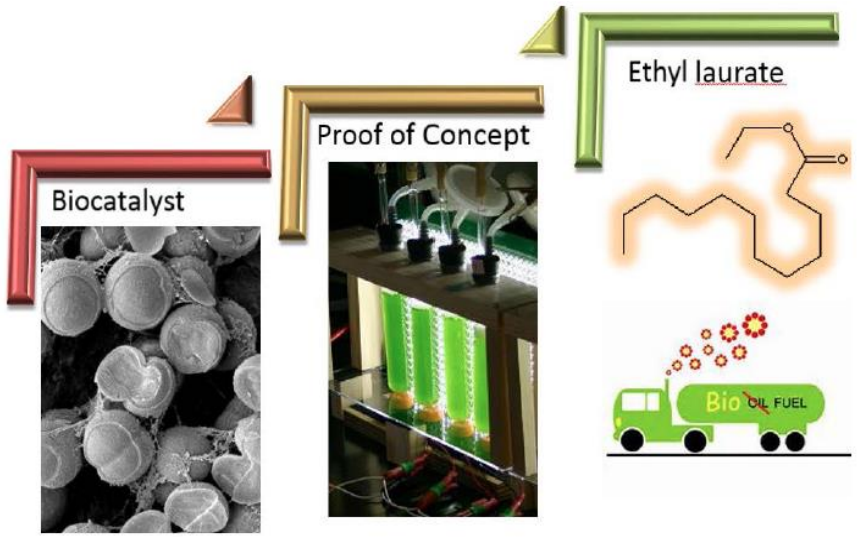
1. Productivity: How much can be made and how well can ethanol and laurate production stoichiometries be balanced in varying conditions?
2. Stability: How well is ethyl laurate excreted from cells and how stable is it in the medium?

# 3 – Technical Accomplishments/Progress/Results

This is considered to be a new project (started October 1, 2016) but there already are some results to share: A strain producing both laurate and ethanol, a prerequisite for ethyl laurate production. Also, the three-month milestone has been reached.

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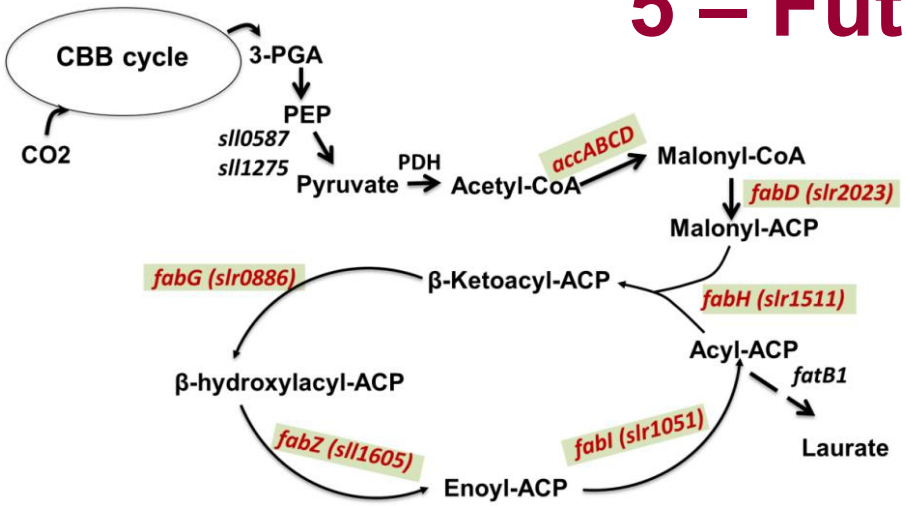
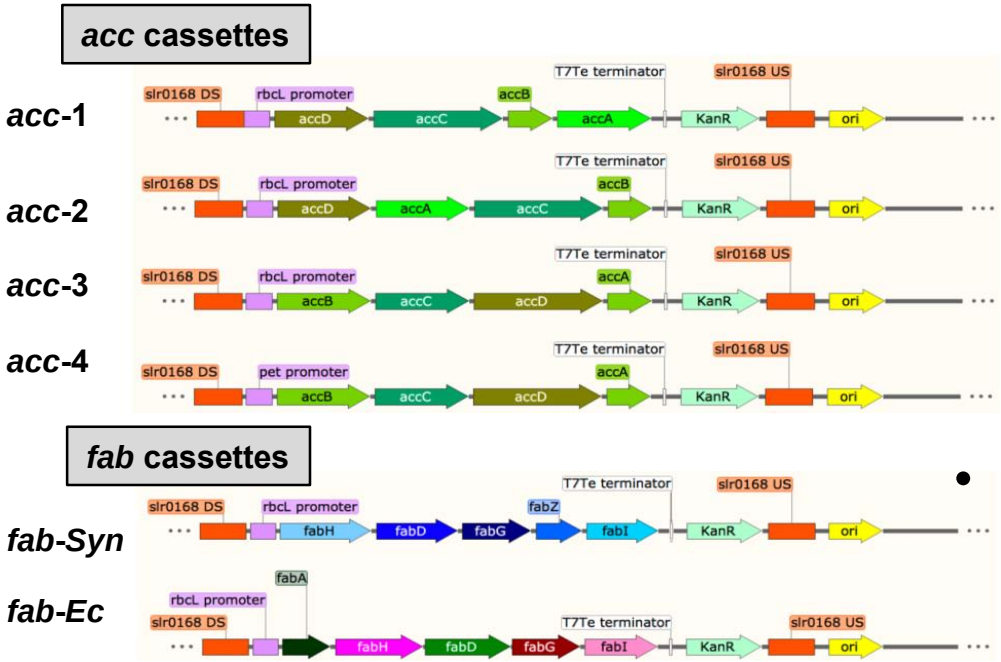
# 4 – Relevance

- Breakthrough concept: Direct production of excreted biofuel from $CO_2$, water and light
  - Ethyl laurate is nearly immiscible with $H_2O$ and is stable, thus opening the perspective of open-pond cultures (after GMO approval)
  - Ethyl laurate is an immediate biodiesel equivalent
  - Reduced processing costs
  - No need for biomass extraction
  - Less biomass provides more fixed carbon for ethyl laurate
- Scalable with limited processing needs
  - Helps reaching the 5 bil gal/yr advanced algae biofuel by 2030
  - Helps reaching the 2022 $3/gasoline gal equivalent goal
- Biomass remains unaltered, yields valuable co-products, and may be used as feedstock
- Potential to provide a very positive techno-economic analysis

# 5 – Future Work




## Task 1:

- Increase laurate production:
  - Overexpress acetyl-CoA carboxylase (ACC) and fatty acid biosynthesis genes
  - Overexpress acyl carrier protein (ACP)
  - Reduce palmitoyl-ACP levels, a feedback inhibitor
  - Delete *glnB*, a negative regulator of ACC
- Develop a rapid high-throughput assay to detect laurate

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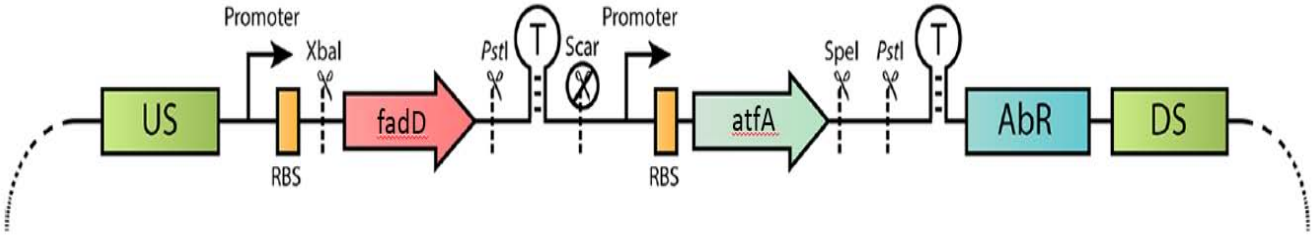
# 5 – Future Work

**Task 2:**

- Engineer ethyl laurate biosynthesis:
  - Introduce genes to enable ethyl laurate formation from laurate and ethanol
  - Demonstrate de novo ethyl laurate production
  - Control the carbon flux distribution from pyruvate, the last common metabolic intermediate in the ethanol and laurate pathways, to optimize ethyl laurate production
- Go/No-Go decision point (October 2017): Demonstrate ethyl laurate production

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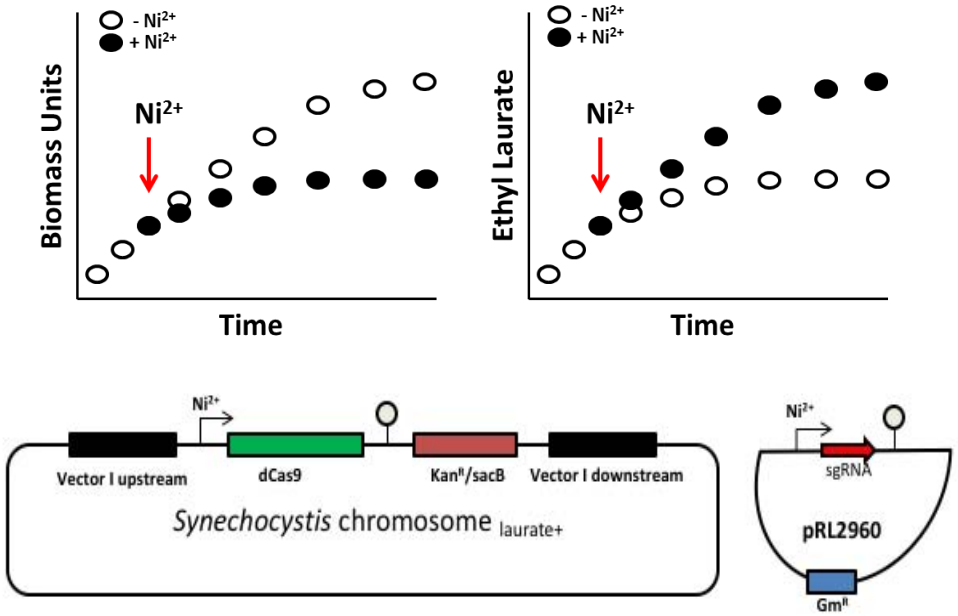
# 5 – Future Work

## Task 3:

Decouple *Synechocystis* growth and ethyl laurate production:

- Inhibit cell growth (but not metabolism) within about 6 h when "all" light is absorbed by the culture (9-month milestone)
- Integrate growth decoupling with ethyl laurate production
- Demonstrate growth in larger-scale photobioreactors in year 2

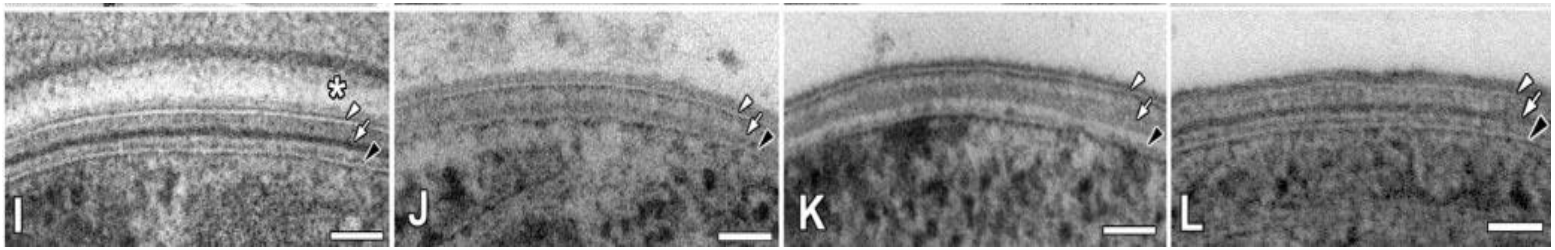


# 5 – Future Work

**Task 4:**

Reduce exopolysaccharide production:

- Delete three genes known to be involved in exopolysaccharide production or transport; together they should reduce exopolysaccharides by about 90%
- Combine this trait with growth-arrest-inducible, ethyl laurate producing strain
- Less exopolysaccharides means:
  - Less sugar sources for opportunistic heterotrophic contaminants
  - More fixed carbon is available for ethyl laurate production



# 5 – Future Work

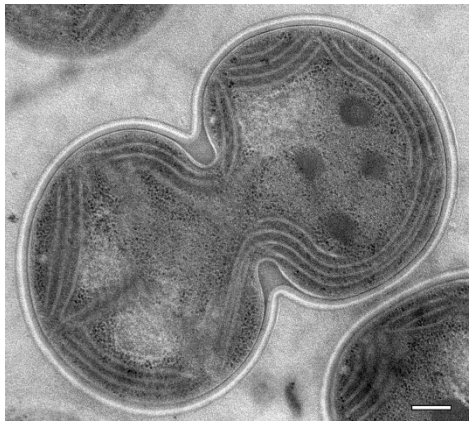
**Task 5:**

Perform a techno-economic analysis on ethyl laurate production

- Will be performed toward the end of the project when there are sufficient data on ethyl laurate productivity potential
- The last project milestone (month 24) is a techno-economic model of ethyl laurate production in cyanobacteria

# Summary

- Cyanobacterial one-stop-shop for producing excreted drop-in biodiesel from $CO_2$, water and light
- Combined production of laurate and ethanol by the same cell already has been demonstrated
- Laurate production will be maximized, and laurate and ethanol stoichiometries will be calibrated to optimize ethyl laurate production
- Combination with inducible growth arrest and reduced exopolysaccharides will maximize ethyl laurate production
- The project has breakthrough potential because of its immediate production of a biodiesel alternative that will separate from the aqueous phase and float, thus minimizing processing



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# Additional Slides

# Responses to Previous Reviewers' Comments

- This is a new project (started in October 2016) that has not been reviewed in a previous BETO meeting
- There have been no Go/No-Go decision points in the project yet

# Publications, Patents, Presentations, Awards, and Commercialization

- This is a new project that started in October 2016, and no publications, patents, presentations, awards and commercialization is available yet