Blue-Green Biofuels: Engineering Cyanobacteria for Fuel Production

SAND2012-5360P

Sandia National Laboratories

Anne M Ruffing, Christine A Trahan, Howland DT Jones
8622 – Bioenergy and Defense Technologies

LDRD LABORATORY DIRECTED RESEARCH & DEVELOPMENT

Truman Fellow

Problem

Our current dependence on fossil fuels is a threat to national security. Microalgal fuels are a promising technology for the advancement of energy independence:

CBSnews.com

"*We're making new investments in the development of gasoline, diesel, and jet fuel that's actually made from a plant-like substance, algae – you've got a bunch of algae out here. If we can figure out how to make energy out of that, we'll be doing alright. Believe it or not, we could replace up to 17 percent of the oil we import for transportation with this fuel that we can grow right here in America.*"
~ President Barack Obama at the University of Miami, Feb. 23, 2012

- Renewable – uses sunlight for energy
- CO_2 mitigation – uses photosynthesis for carbon fixation
- Simulates the US economy – job creation

… but current microalgal fuel technology is not cost-competitive with petroleum-based fuels.

Approach

Genetically engineer cyanobacteria for fuel production:

- Easier to genetically manipulate compared to eukaryotic algae
  - ➢ Can engineer cyanobacteria to produce a variety of target fuels (FFA, FAEE, alkanes/alkenes, fatty alcohols, ethanol, butanol, isoprene, etc.)
  - ➢ Can optimize the metabolism to maximize fuel production
- Excretes fuel precursors (FFA)
  - ➢ Continuous production system (high productivities)
  - ➢ Lower nutrient requirements (N & P)

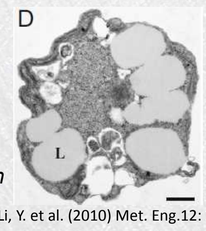

Figure 1 (right): Accumulation of oil (L) in eukaryotic algae
Li, Y. et al. (2010) Met. Eng.12: 387--91.

Figure 2 (below): Simplified schematic of cyanobacterial metabolism, including pathways for FFA production. Genetic modifications are colored.

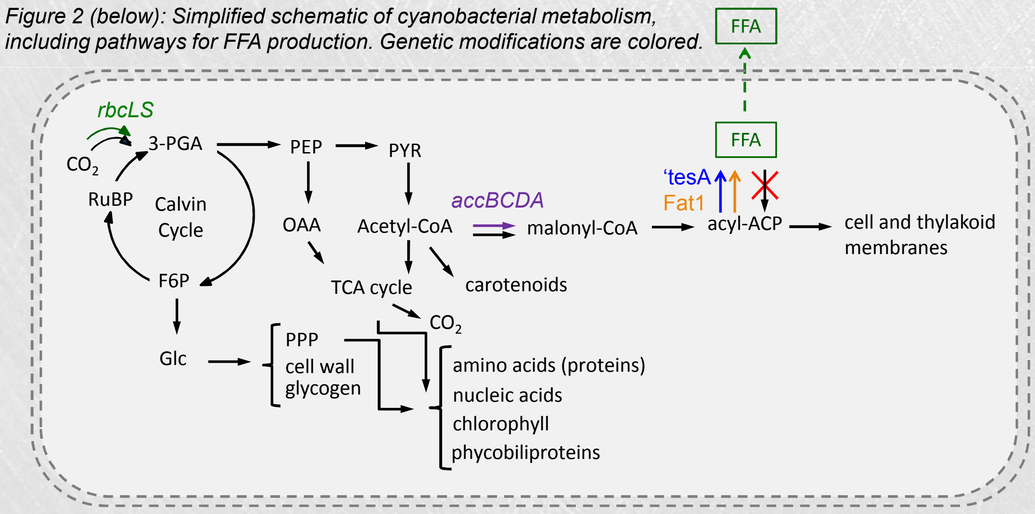

Table 1: Strains constructed for FFA production.

Genetic modifications	*Synechococcus elongatus* 7942	*Synechococcus* sp. 7002
Δaas	SE01	S01
Δaas, 'tesA	SE02	S02
Δaas, Fat1	SE03	S03
Δaas, Fat1, rbcLS	SE04	S04
Δaas, Fat1, rbcLS, accBCDA	SE05	
Δaas, Fat1, P_{psbAI} rbcLS	SE06	
Δaas, Fat1, P_{psbAI} rbcLS, P_{rbc} accBC P_{cpc} accDA	SE07	

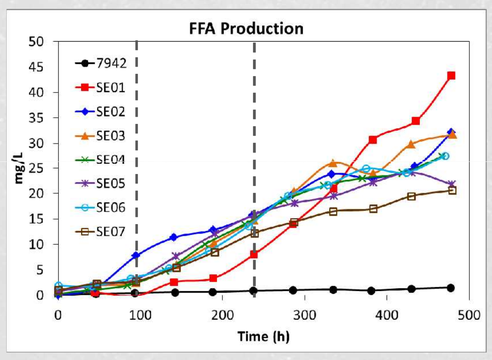

Figure 3: Production and excretion of FFA in 7942 strains.

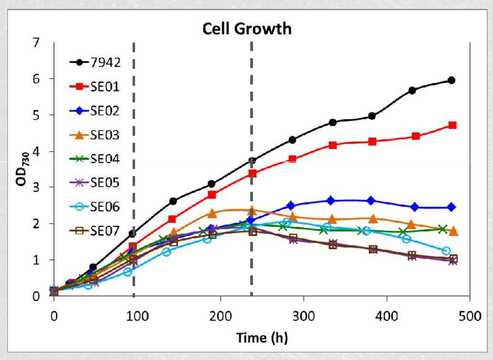

Figure 4: Cell growth during FFA production in 7942 strains.

Results

FFA production has negative physiological effects in engineered *Synechococcus elongatus* PCC 7942

FFA-producing strains show:

- Reduced cell growth
- Decrease in photosynthetic yield
- Degradation of chlorophyll-a
- Changes in subcellular pigment location
- Increased saturation of membrane lipids

Publication: Ruffing AM, Jones HDT. 2012. Biotech. Bioeng. Early view .

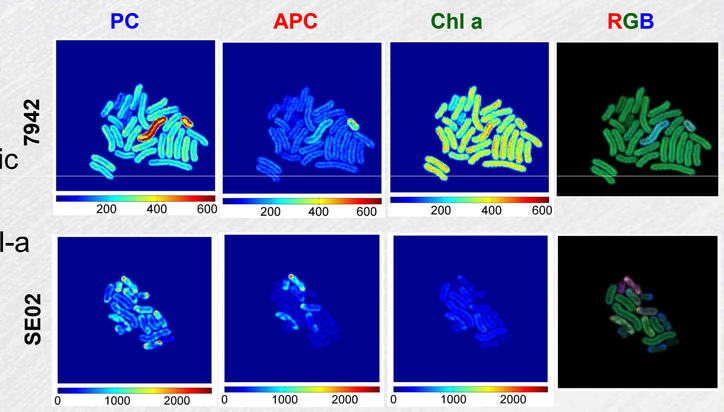

Figure 5: Concentration plots and RGB images of photosynthetic pigments in wild type (7942) and engineered strains (SE01 and SE02). PC: phycocyanin (blue); APC: allophycocyanin (red); Chl a: Chlorophyll-a (green).

Seq-ing Gene Targets to Enhance FFA Production in *S. elongatus* 7942

Applied RNA-Seq to identify genetic response to FFA production

- 3 strains: 7942, SE01, SE02
- 2 time points: 100h, 240h
- 3 biological replicates

Differential gene expression analysis (high vs. low FFA) revealed increased expression of:

- Nitrogen acquisition genes
- Stress response genes
- Potential FFA exporters (transporters/porins)

Table 2: RNA-seq comparisons

Low FFA	High FFA
SE01, 100h	SE02, 100h
7942, 100h	SE02, 100h
7942, 240h	SE01, 240h
7942, 240h	SE02, 240h
SE01, 100h	SE01, 240h

An Alternative Cyanobacterial Host: *Synechococcus* sp. PCC 7002

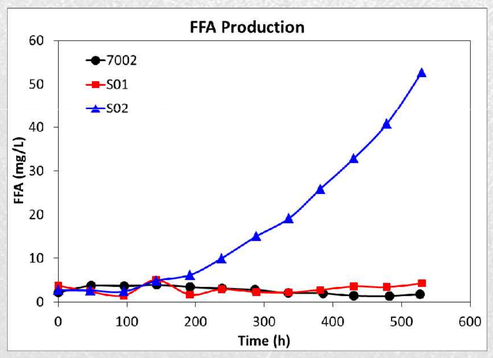

Figure 6: Production and excretion of FFA in 7002 strains.

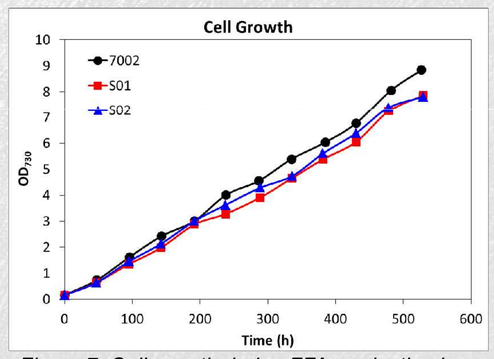

Figure 7: Cell growth during FFA production in 7002 strains.

Screening Biofuel Toxicity for Model Cyanobacteria

Tested cyanobacterial growth inhibition of:

- Short chain alcohols
- Long chain alcohols
- Alkanes/alkenes
- Fatty acids

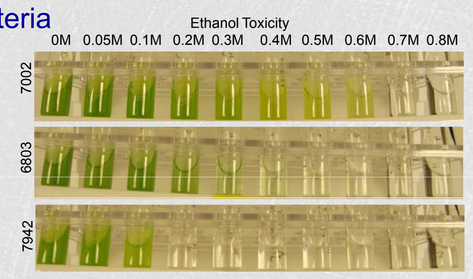

Figure 8 (right): Cyanobacterial growth inhibition with increasing ethanol concentration

Significance

1. Proof-of-concept demonstration of engineering cyanobacteria for FFA production and excretion.
2. Identification of gene targets for improving FFA production.
3. Biofuel toxicity screens will identify optimal hydrocarbon targets for fuel production and the best model cyanobacterial strain for genetic engineering.
4. Long term goal: Strain optimization for economically-competitive fuel production from cyanobacteria.

Acknowledgements

- Howland DT Jones – hyperspectral imaging and analysis
- Christine A Trahan – biofuel toxicity screening
- Anthony Martino (6124), Eric Ackerman (8635) – mentors
- Los Alamos National Laboratories – Sequencing
- Kansas Lipidomics Research Center – lipid analysis (ESI-MS)