

Genetic Engineering of Cyanobacteria as Biodiesel Feedstock



LABORATORY DIRECTED RESEARCH & DEVELOPMENT

Early Career R&D Program

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Problem

Photosynthetic microorganisms offer a promising solution to current energy challenges, while at the same time, addressing growing environmental concerns through CO₂ mitigation. However, natural 'oil' production by eukaryotic algae cannot economically compete with current petroleum feedstocks.

Limiting factors:

- Productivity
- Oil extraction

Table 1: Natural oil productivity of eukaryotic algae

Eukaryotic Algal Species	Doubling time (h)	Oil content (% dry wt)
<i>Chlamydomonas reinhardtii</i>	8	15-25
<i>Chlorella vulgaris</i>	8	28-35
<i>Dunaliella salina</i>	10-20	16-44
<i>Nannochloropsis salina</i>	23	31-68
<i>Botryococcus braunii</i>	72-96	25-75

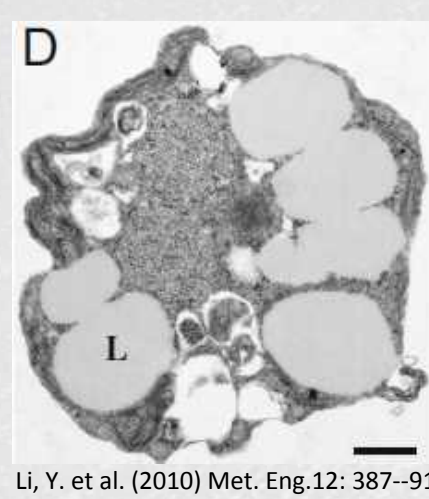


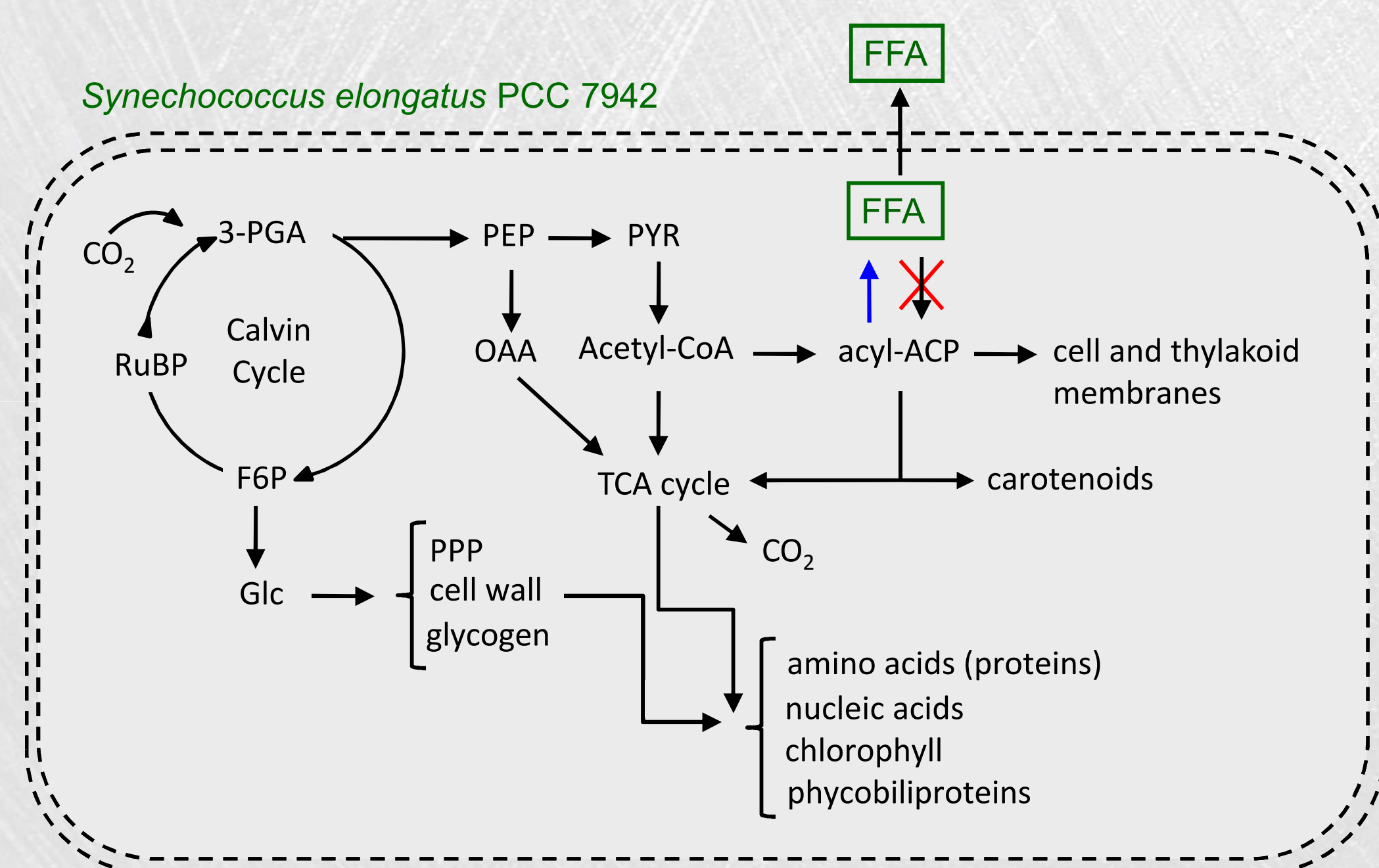
Figure 1: Accumulation of oil (L) in eukaryotic algae

Figure 2: *Synechococcus elongatus* PCC 7942 (a model cyanobacterium)

Advantages of cyanobacteria:

- Established tools/methods for genetic engineering
- Secretion of free fatty acids (FFA)

Approach



7942: Wild type
 SE01: Knockout of acyl-ACP synthetase
 SE02: Knockout of acyl-ACP synthetase; expression of thioesterase (*tesA* from *E. coli*)

1. Genetically engineer *Synechococcus elongatus* PCC 7942 for FFA production and characterize the engineered strains.

- Eliminate FFA metabolism
- Remove feedback inhibition of FA pathway
- Characterize growth, FFA production, photosynthetic pigments, photosynthetic activity, and membrane composition

2. Investigate physiological changes during FFA production using RNA-seq

- What native genes contribute to high FFA production?
- Are there any active transporters involved in FFA secretion?
- What changes in gene expression accompany the physiological changes observed during FFA production?

3. Improve FFA production in *S. elongatus* using genes from the FA biosynthetic pathway in the green alga *Chlamydomonas reinhardtii*

- Acyl-ACP thioesterase (Fat1)
- Chloroplast-associated acetyl-CoA carboxylase

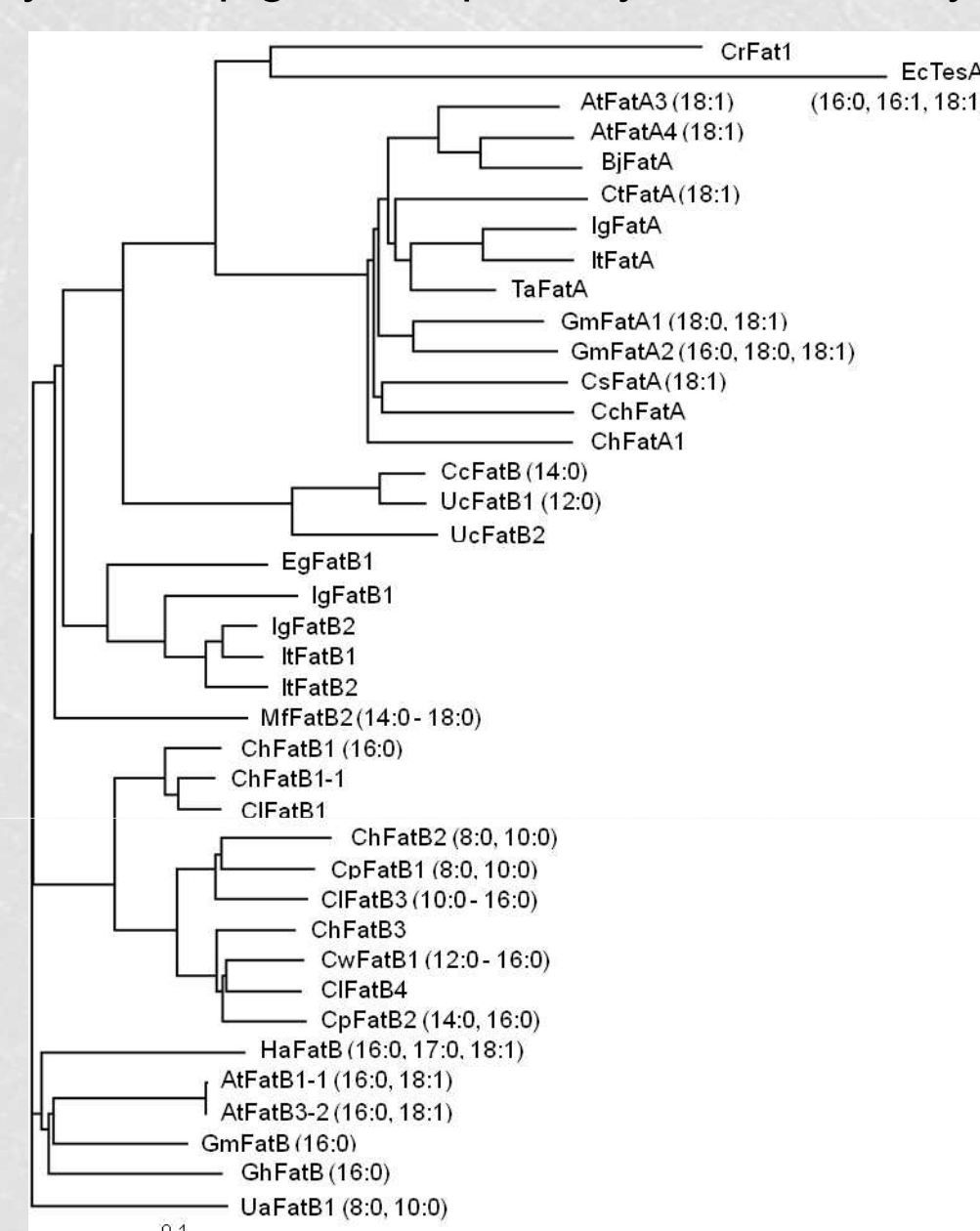


Figure 3: Phylogenetic comparison of acyl-ACP thioesterases

Results

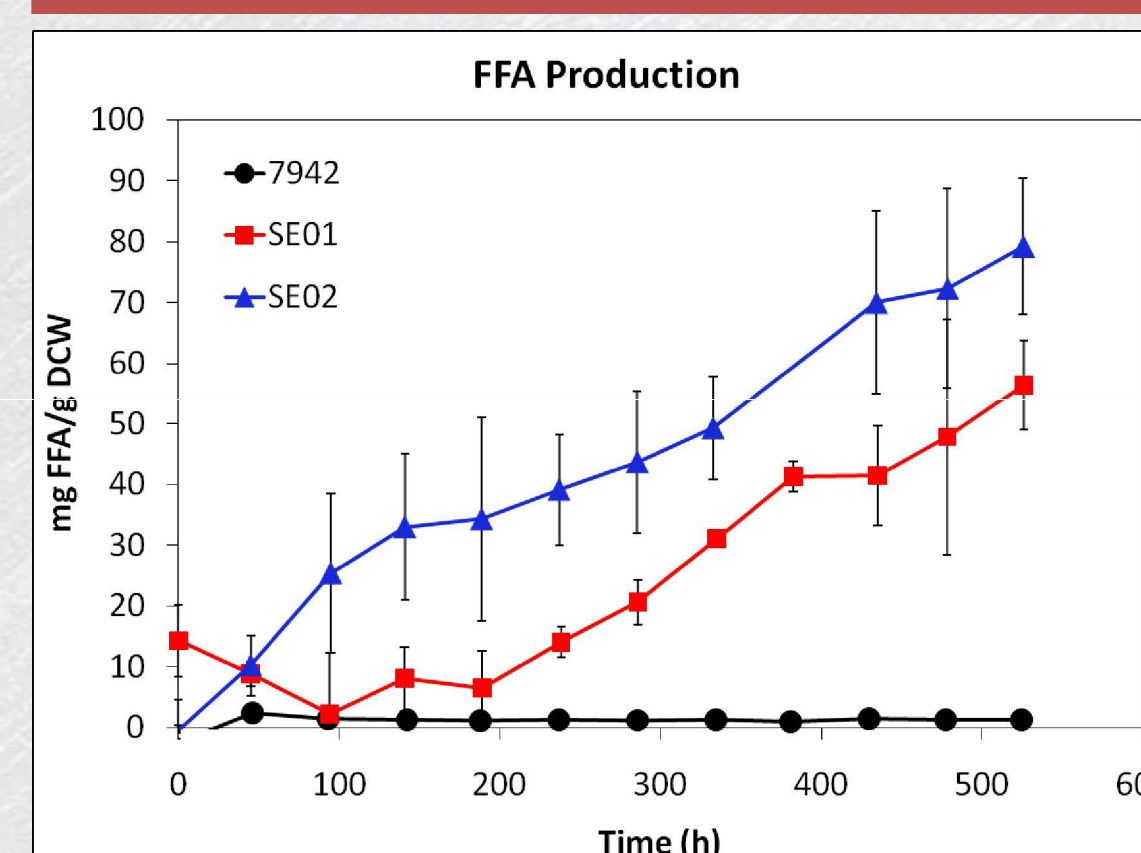


Figure 4: Secreted FFA on a dry cell weight basis

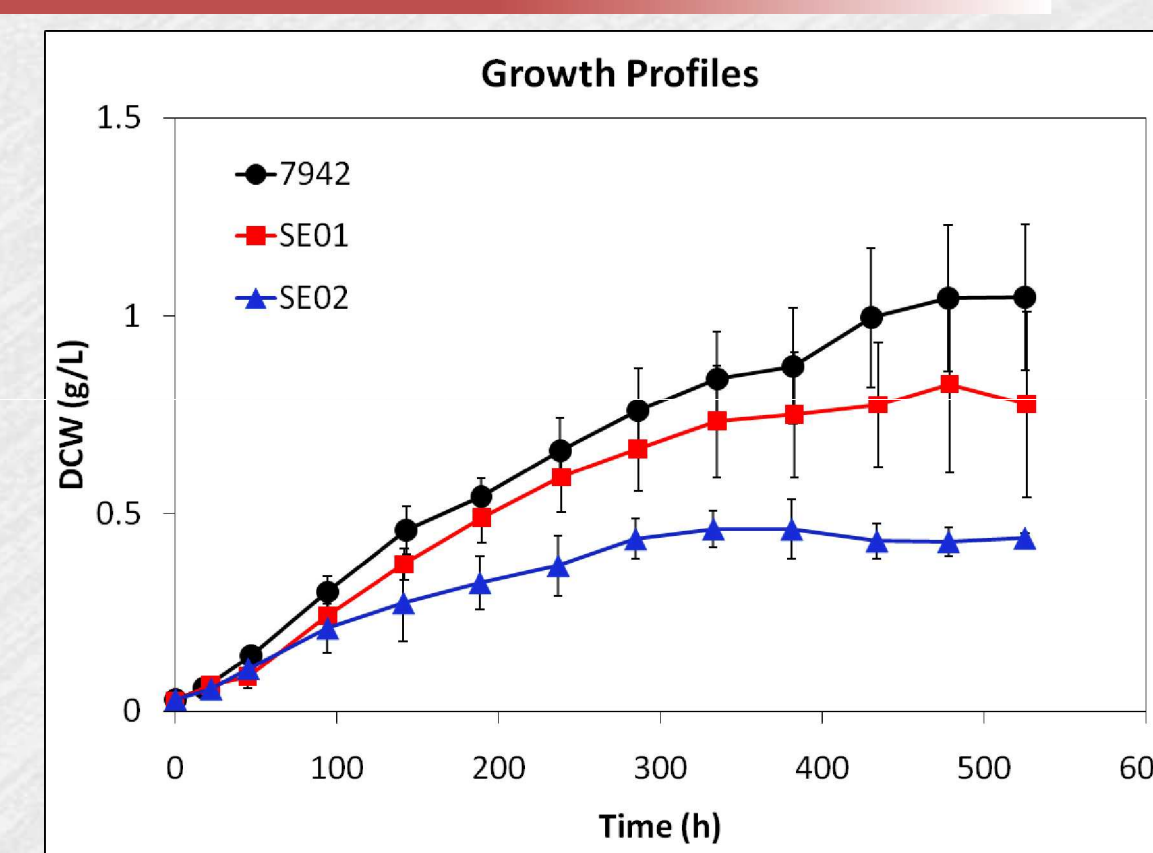


Figure 5: Cell growth of wild type and engineered strains

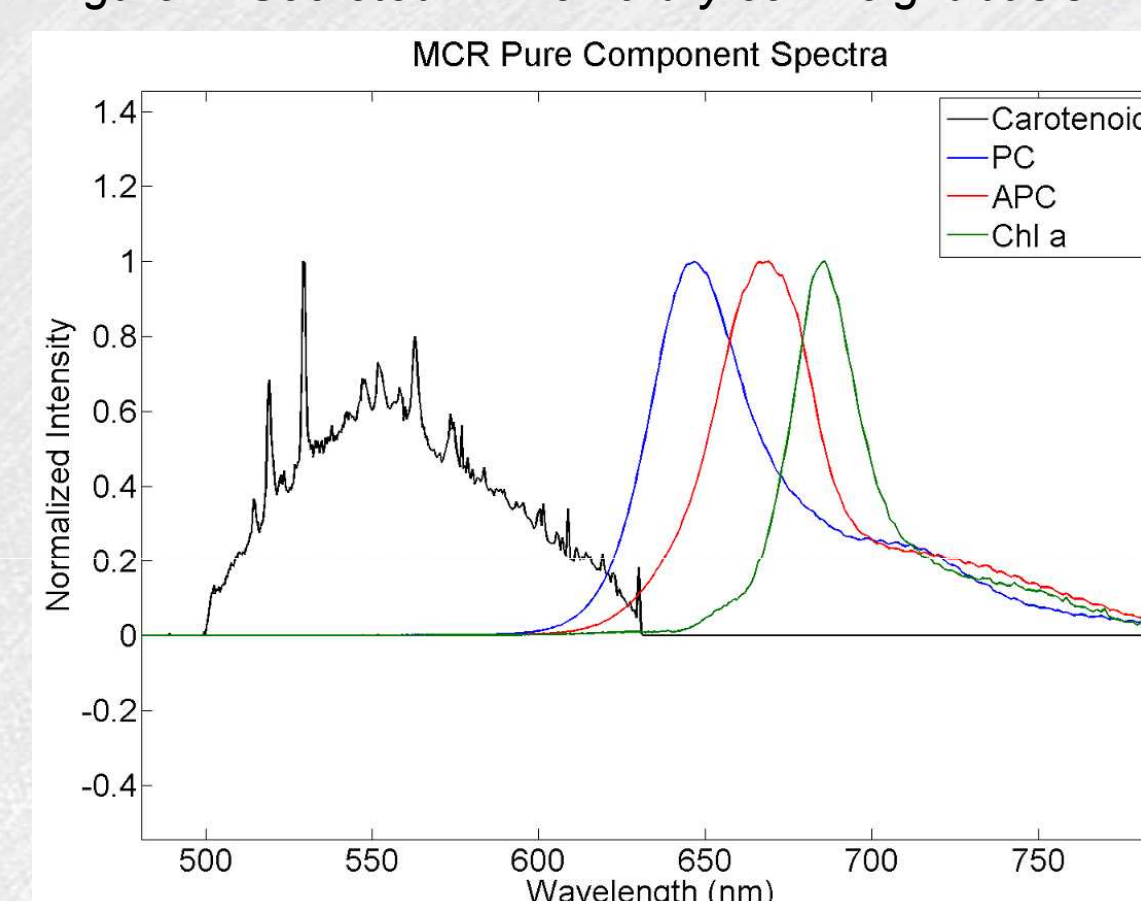


Figure 6 (above): Fluorescence emission spectra of pure components from data collected using hyperspectral confocal fluorescence imaging and multivariate curve resolution analysis.

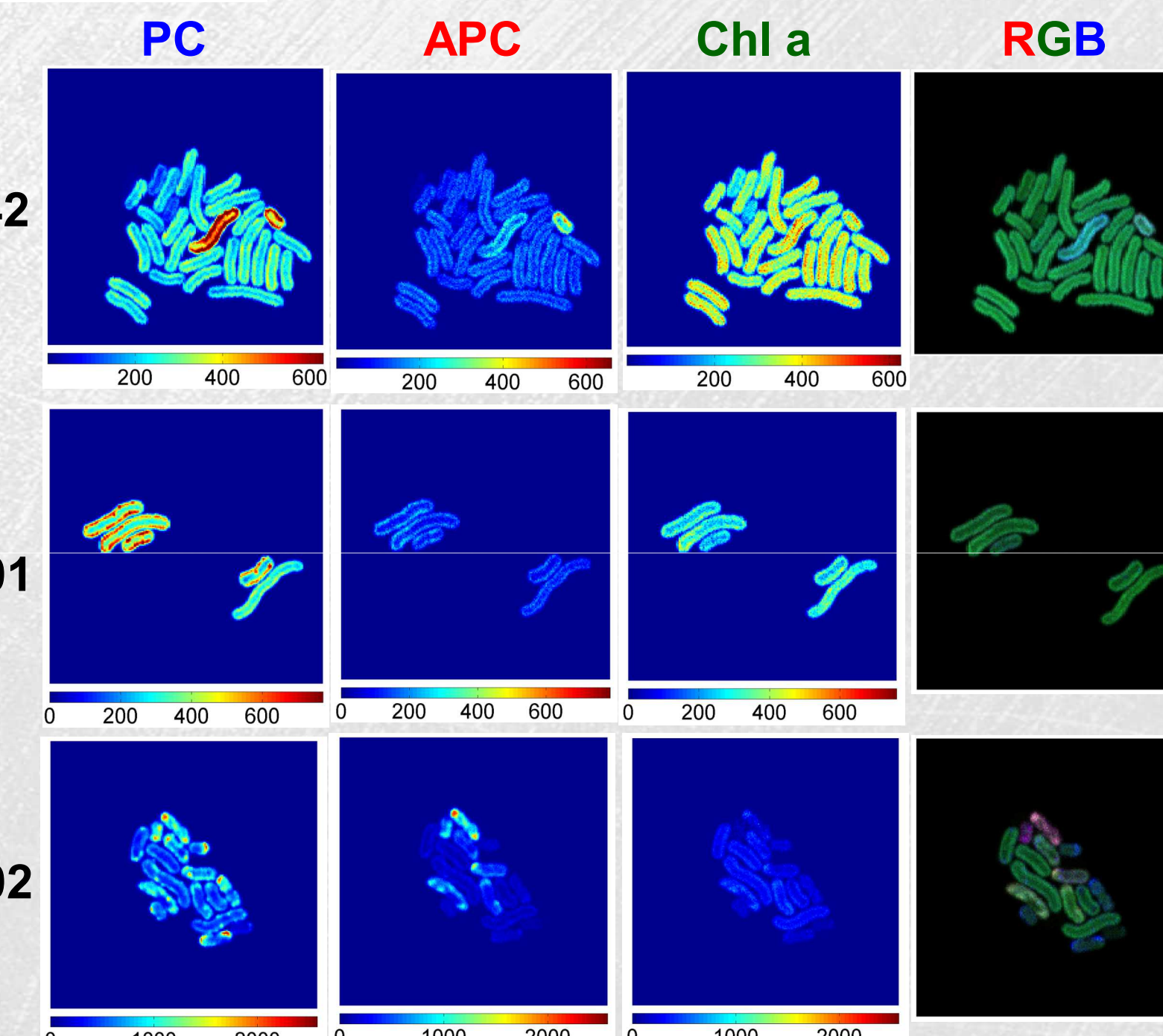


Figure 7 (right): Concentration plots and RGB images of photosynthetic pigments in wild type (7942) and engineered strains (SE01 and SE02). PC: phycocyanin (blue); APC: allophycocyanin (red); Chl a: Chlorophyll a (green).

Figure 8 (below): Changes in saturated fatty acid composition of cell and thylakoid membranes.

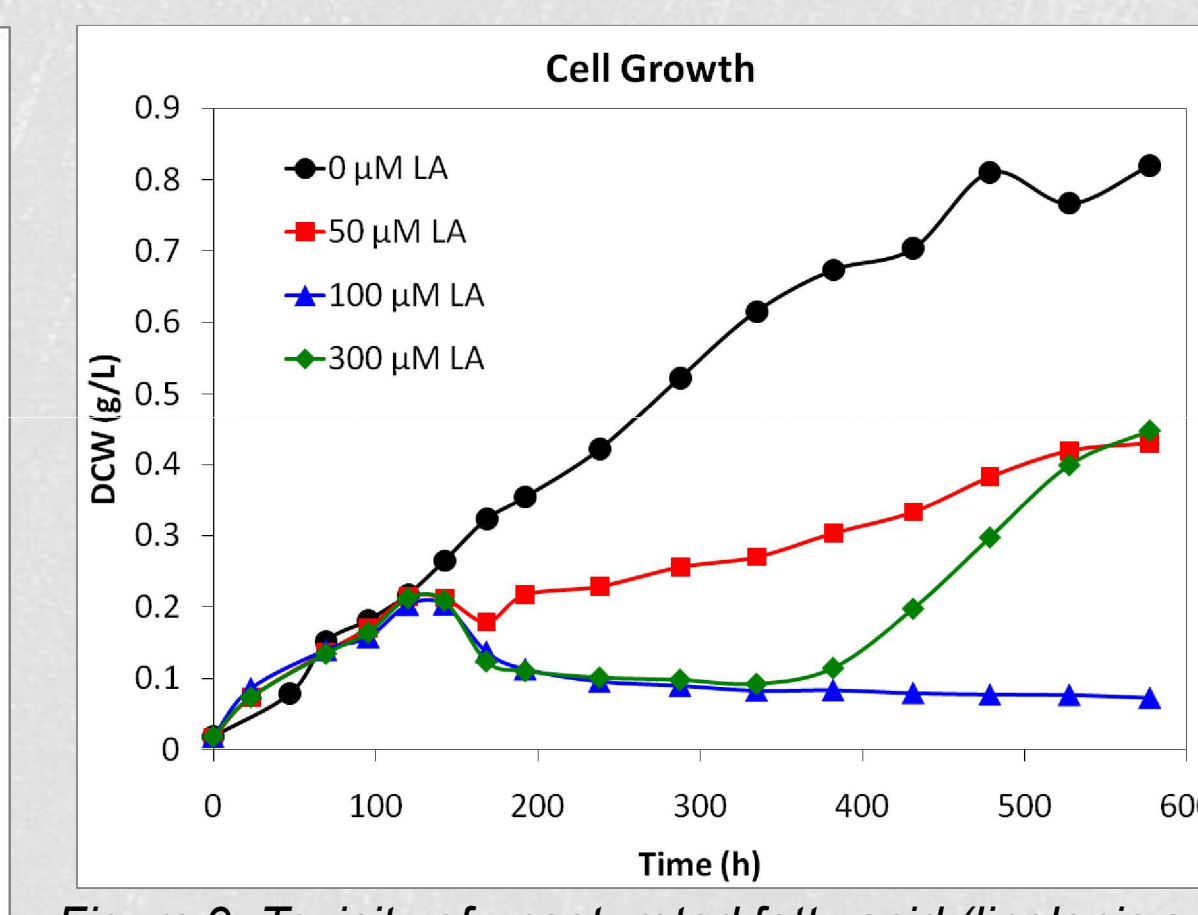
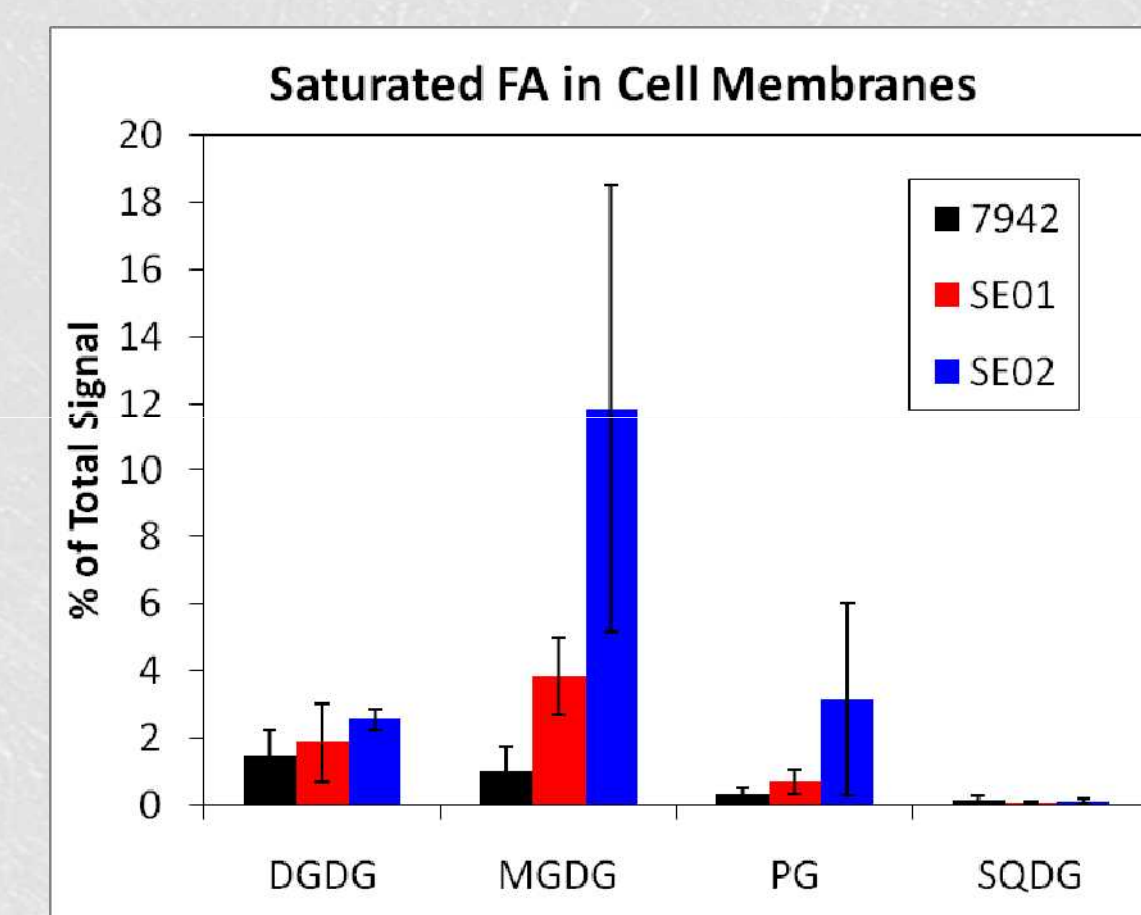


Figure 9: Toxicity of unsaturated fatty acid (linolenic acid)

Significance

1. Demonstrates that cyanobacteria can be engineered for production of a biodiesel precursor (FFA).
2. Identifies mechanisms limiting cell growth and FFA production:
 - Changes in composition of cell and photosynthetic membranes
 - Toxicity of unsaturated FFA
3. The changes in cell growth, photosynthesis, and membrane structure that accompany FFA synthesis must be addressed to allow for FFA production at levels that are economically feasible.

