

Synechococcus sp. PCC 7002: A Superior Host for Converting Carbon Dioxide into Free Fatty Acids

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Background

Cyanobacteria are excellent hosts for renewable biofuel production, as they naturally fix carbon dioxide to form complex hydrocarbon molecules. The potential for cyanobacterial-based biofuel production has been demonstrated by the genetic engineering of cyanobacteria for the production of a variety of fuels and fuel precursors, including ethanol, butanol, isoprene, free fatty acids (FFAs), and alkanes. While these biofuels have been successfully produced by cyanobacterial hosts, the high titers required for economical production of these low-value commodities have yet to be achieved. Cyanobacterial host tolerance of the target fuel is often a limiting factor, as demonstrated in the production of FFAs by *Synechococcus elongatus* PCC 7942.¹ (Figure 1)

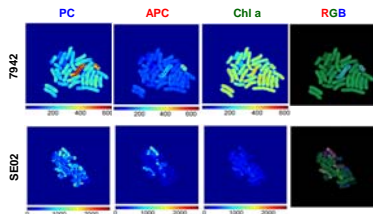


Figure 1: Changes in photosynthetic pigments due to FFA production in *S. elongatus* PCC 7942 are illustrated in these hyperspectral confocal fluorescence images comparing the wild type (7942) and FFA-producing strain (SE02). PC: phycocyanin; APC: allophycocyanin; Chl a: chlorophyll-a; RGB: composite red-green-blue image.

Synechococcus sp. PCC 7002 has many characteristics that make this strain ideal for biofuel production:

1. Large-scale biofuel production systems will be more economical if the algal strain can grow in marine and other non-freshwater sources. Moreover, evaporative losses in algal ponds will further increase the salt concentration. *Synechococcus* sp. PCC 7002 is a marine strain that can tolerate salt concentrations higher than 1.5M.²
 2. Algal ponds and PBRs in biofuel production systems will also be exposed to high light intensities during peak sunlight hours. *Synechococcus* sp. PCC 7002 displays extreme high light tolerance, surviving under light intensities as high as 2 x peak sunlight (4.5 mE m⁻² s⁻¹).³
 3. Lastly, ponds and PBRs are also exposed to large temperature swings, with high temperatures in PBRs being particularly problematic. *Synechococcus* sp. PCC 7002 has a wide temperature range of growth and can survive at temperatures as high as 47°C.²
- Due to these beneficial host characteristics, *Synechococcus* sp. PCC 7002 was investigated as a potential host for FFA production.

Approach

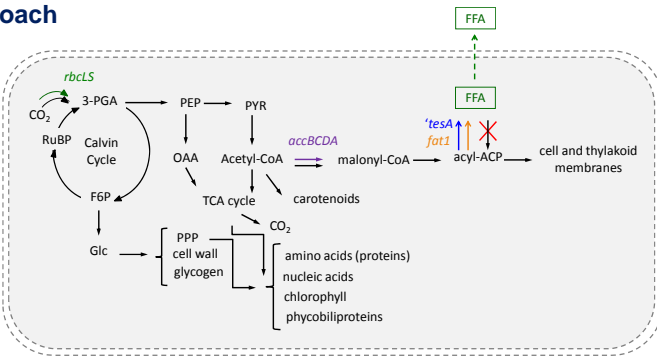


Figure 2: Schematic of the metabolism of *Synechococcus* sp. PCC 7002, with target genetic modifications highlighted in color. X indicates gene knockout while arrows indicate gene overexpression targets. Genes: *accBCDA* – acetyl-coA carboxylase genes from *Chlamydomonas reinhardtii*, *fat1* – acyl-ACP thioesterase from *C. reinhardtii*, *rbcLS* – RubisCO large and small subunits (native), *tesA* – truncated thioesterase from *Escherichia coli*.

Genetic Targets

- Gene knockout of *fadD*: long-chain-fatty-acid-CoA ligase
- Expression of a thioesterase for fatty acid release
- Overexpression of RuBisCO
- Overexpression of acetyl-CoA carboxylase (ACCase)

Gene Expression Optimization

- Inducible promoters: P_{Trc} and P_{UlaC01}
- Strong constitutive promoters: P_{psbA1}

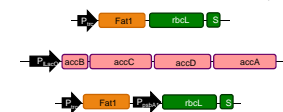


Table 1: Engineered, FFA-producing strains of *S. elongatus* PCC 7942 (constructed previously)¹ and *Synechococcus* sp. PCC 7002 (constructed in this study).

Genetic modifications	<i>S. elongatus</i> 7942	<i>Synechococcus</i> sp. 7002
<i>Δaas/fadD</i>	SE01	S01
<i>Δaas/fadD, tesA</i>	SE02	S02
<i>Δaas/fadD, Fat1</i>	SE03	S03
<i>Δaas, Fat1</i> (SE04) or <i>tesA</i> (S05), <i>rbcLS</i>	SE04	S05
<i>Δaas, Fat1, rbcLS, accBCDA</i>	SE05	S06
<i>Δaas, Fat1</i> (SE06) or <i>tesA</i> (S07), P _{psbA1} <i>rbcLS</i>	SE06	S07

Results

FFA Production: *S. elongatus* PCC 7942 vs. *Synechococcus* sp. PCC 7002

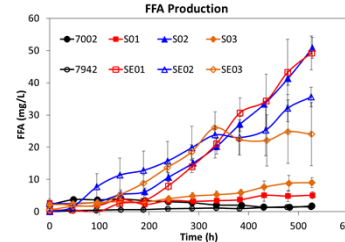


Figure 3: Excreted FFA concentrations in cultures of engineered cyanobacteria. Data are averages of 3 biological replicates.

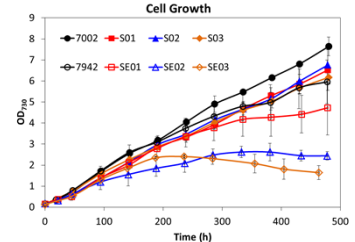


Figure 4: Cell growth (OD₇₃₀) in cultures of engineered cyanobacteria. Data are averages of 3 biological replicates.

- In *Synechococcus* sp. PCC 7002, the *E. coli* thioesterase *tesA* is required for high levels of FFA (Figure 3).
- Unlike *Synechococcus elongatus* PCC 7942, FFA-producing *Synechococcus* sp. PCC 7002 strains do not show a significant reduction in cell growth (Figure 4).

Effect of Temperature on FFA Production and Photosynthesis

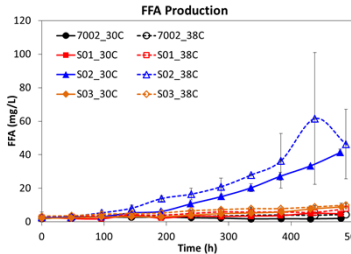


Figure 5: FFA production in engineered *Synechococcus* sp. PCC 7002 at 30°C and 38°C. Data are averages of 3 biological replicates.

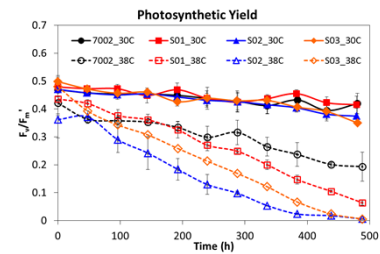


Figure 6: Photosynthetic yield measurements of engineered *Synechococcus* sp. PCC 7002 cultures at 30°C and 38°C. Data are averages of 3 biological replicates.

- Engineered *Synechococcus* sp. PCC 7002 has higher FFA production at 38°C (Figure 5) but reduced photosynthetic yields (Figure 6).
- Higher temperatures also led to reduced photosynthetic pigments (both phycobiliproteins and chlorophyll-a, Figure 7).

Effect of Gene Expression on FFA Production

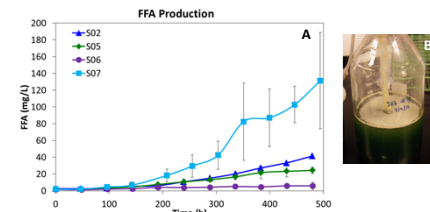


Figure 8: (A) FFA production in engineered *Synechococcus* sp. PCC 7002. Data are averages of 3 biological replicates. (B) Excreted FFAs in S07 precipitate and float to the top of the culture.

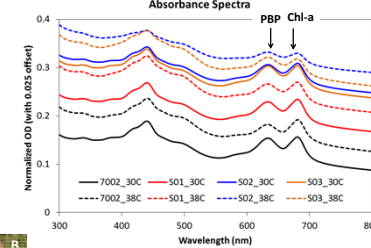


Figure 7: Absorbance spectra of engineered *Synechococcus* sp. PCC 7002 cultures at 30°C and 38°C. Data are averages of 3 biological replicates.

- Expression of RuBisCO with the *psbA1* promoter in S07 led to more than a 3-fold increase in FFA production (Figure 8A).
- High FFA production led to FFA precipitation (Figure 8B).

Conclusions

- Engineered *Synechococcus* sp. PCC 7002 synthesizes and excretes FFAs without detrimental effects on cell growth and photosynthesis.
- FFA tolerance of *Synechococcus* sp. PCC 7002 is temperature dependent.
- Gene expression is a key factor for high FFA yields in engineered *Synechococcus* sp. PCC 7002.

Reference & Acknowledgements

1. Ruffing A, Jones HDT. 2012. Physiological effects of free fatty acid production in genetically engineered *Synechococcus elongatus* PCC 7942. *Biotechnol. Bioeng.* 109:2190-2199.
2. Ludwig M, Bryant DA. 2012. *Synechococcus* sp. strain PCC 7002 transcriptome: Acclimation to temperature, salinity, oxidative stress, and mixotrophic growth conditions. *Frontiers in Microbiol.* 3:354.
3. Nomura CT, Sakamoto T, Bryant DA. 2006. Roles for heme-copper oxidases in extreme high-light and oxidative stress response in the cyanobacterium *Synechococcus* sp. PCC 7002. *Arch Microbiol.* 185: 471-479.

Funding: President Harry S. Truman Fellowship in National Security Science and Engineering.

