A Cooperative Relationship between Cyanobacteria & *E. coli* for the Production of Biofuels


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Cyanobacteria

Cyanobacteria use photosynthesis to provide energy and carbon skeletons for anaerobic processes. During the day excess fixed carbon can be converted to carbohydrates, glycogen and stored for later use. To achieve this goal we will engineer Synechocystis to do the following:

**Goals:**

1. **Increase sucrose production** by preventing glycogen synthesis. This will be accomplished by knocking out the ADP-glucose-pyrophosphorylase (apg) gene in Synechocystis PCC 6803.
2. **Convert sucrose to glucose and fructose** by transforming Synechocystis with the Z. mobilis invertase (inv) gene.
3. **Aid hexose transport** out of the cell by transforming Synechocystis with a *Z. mobilis* glucose facilitator transport (GLF) gene.

**Results:**

- DNA fragments for the assembly of both the AGP KO/inv and the THI KO/GLF open reading frames were amplified and isolated.
- Construction of both the THI KO/GLF open and AGP KO/inv open by Gibson assembly and SLIC were unsuccessful and could not be transformed into Synechocystis as planned.

*Synechocystis Transformation Techniques*

**Red** = germline sequences to facilitate homologous recombination into Synechocystis genome
**Blue** = Selectable marker for positive transfections
**Green** = promoter to drive expression of transparency

**Introduction**

In light of the growing energy crisis, research has been devoted to finding economical means of producing renewable fuels. Traditional methods for obtaining biofuels have relied mainly on the fermentation of agricultural crops; however reduction in cropland and low levels of CO2 biofixation relative to biomass are both major concerns. Alternatively, genetically modified *E. coli* producing biofuels could be an alternative. Unfortunately, production cost for *E. coli* media are expensive, ranging from 30-40% of total costs. Our project will surround these high production costs by engineering cyanobacteria, Synechocystis PCC 6803, to secrete large quantities of glucose that will feed our biofuel-producing *E. coli*. Cyanobacteria and *E. coli* will be co-cultivated in an apparatus that allows for the transfer of glucose from cyanobacteria to *E. coli* without the needs for a carbon source, but it will also create a novel cooperative system between bacterial species that may have further industrial applications.

**Cyanobacteria**

- **Generating Red**
- **Generating Yellow**
- **Generating Blue**
- **Generating Green**

**E. coli**

1. **To produce medium chain fatty acids**, the raw material for biofuel using the Bay Laurel thioesterase (BTE) generator.
2. **To produce ethanol using a Pyruvate Decarboxylase and Alcohol Dehydrogenase (PDC/ADH) generator.**

**E. coli Transformation Constructs**

- **Fatty Acid Generator**
- **Ethanol Generator**

**Results:**

- Medium chain fatty acids were produced up to 25 fold over background using the BTE generator in various *E. coli* strains.
- Acetolactate, the substrate for ethanol production, was produced at higher levels in NDB *E. coli* cells, based on the plate assay. 0.02% (v/v) ethanol was also produced in these cells; these results show that ethanol production had no effect on ethanol production suggesting that something is limiting the conversion of acetolactate to ethanol.

**Synechocystis**

**Co-Cultivation System**

- **Transformed**
- **Improved**
- **Reaching**
- **Community**

**Parts Submitted:**

- **BBa_K558006**
- **BBa_K558005**

**Goals:**

1. **Determine if E. coli** can grow in media required for cyanobacteria cultivation (BG-11).
2. **If E. coli** cannot grow in BG-11, find nutrients that will foster its growth.
3. **Find the minimum concentration of glucose that cyanobacteria must release to media to adequately feed E. coli.**

**Results:**

- **Common lab strains of E. coli** cannot grow in BG-11 unless supplemented with amino acids.
- **Casamino acids** provide these amino acids without compromising their studies, as they do not contain detectable levels of sugar.
- The minimum concentration of glucose needed is 2.5 mM.

**Apparatus**

We designed a co-cultivation apparatus that allows free exchange of growth media components, but prevents *E. coli* from shading out the cyanobacterium and decrease photosynthetic efficiency. The E. coli (right chamber) will be sent into the cyanobacteria chamber (left outer chamber) by circulating the E. coli through the dialysis tubing. The dialysis tubing allows glucose to be transferred through a channel from the cyanobacteria chamber to E. coli, providing E. coli with a carbon source necessary for survival.

**Conclusion**

The Nevada iGEM team set out to create a self-sustaining system where cyanobacteria, a photosynthetic bacteria, and *E. coli* could be capable of converting glucose to feed *E. coli* and save 30-40% on the cost of producing genetically engineered products in *E. coli*. A co-cultivation system was designed where transformed *E. coli* and cyanobacteria could grow in the same media and *E. coli* could produce genetically engineered products, such as biofuels.