Metabolic Engineering Cyanobacteria for Hydrocarbon Biofuels

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Abstract

With the global climate changes and the hike in prices at the fuel pump more groups have been looking at the feasibility of an alternative fuel source. To reduce the reliance on petroleum based fuels, a thorough investigation into biofuels has been a key concept in the last decade. The photosynthetic organism cyanobacteria is an interesting study into producing hydrocarbon chemicals such as a “drop-in” jet propulsion fuel. If successfully engineered, the cyanobacteria will be a photosynthetic micro factory for producing various high value chemicals. This goal of using a prokaryotic microalgae is already the source of some biofuel molecules utilized by a company called Algenol. They advertise inputs of sunlight, water, and CO2 which produce a byproduct ethanol for use in biofuels. The aim of this research is to utilize the already existing biochemical pathways in cyanobacteria to excrete a C-30 molecule, squalene into solution.

Introduction and Objectives

To metabolically engineer cyanobacteria for squalene overexpression, the non-mevalonate pathway(MEP) was targeted. The pathway includes a squalene synthase gene(SQS) and an upstream molecule, farsensyl diphosphate gene (FPS). The first two construct designs utilized an overexpression synthase gene acquired from a contributing lab with the PI. These two genes were also sequenced to express a protein that would act as a tail attached to the genes. The third construct consisted of a docker protein (CBM3). The purpose of CBM3 would be to bind the tail ends of each SQS and FPS gene so they would be in close proximity to each other to over express squalene production. Figure 1 is an illustrated model of this design and the hypothesis of the research.

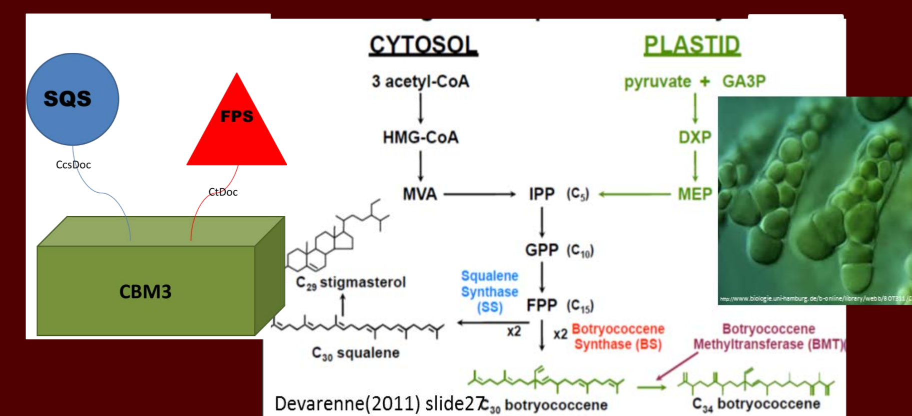

Figure 1 Construct design for overexpressing squalene in cyanobacteria

Methods

With the pSyn_1 vector, specific digestion sites were designed by Life Technologies to facilitate cloning into this vector. The digestion enzymes used in the first construct, utilized BamHI-HF followed by KPNI in the SQS design. By digesting both the vector and the target gene with these enzymes ligation with T4 Ligation enzyme was able to connect the sticky ends together. The pSyn_1 vector has no ribosome binding site(RBS) therefore when designing primers it is essential to express the desired protein that a RBS is included. The target genes for each construct were digested and ligated into the plasmid vector pSyn_1 using this same method which is illustrated in Figure 2. The plasmid also has a spectinomycin resistant gene in the genetic sequence. By adding spectinomycin to the LB media with our Mach1© chemically competent E. coli cells we were able to screen for transgenic organisms. Using the solution 1-2-3 plasmid extraction method we were then able to screen via gel electrophoresis the DNA band length of those plasmid vectors. This transformed vector was cloned into the E. Coli cells. Once gel electrophoresis and gene sequencing confirmed that the SQS gene was successfully transformed into E. Coli, a glycerol stock was made and the vector was ready to be transformed into cyanobacteria. GeneArt© protocol was used to transform the cyanobacteria. Using BG-11 Seawater with spectinomycin to screen for a positive colony is an effective technique to point out transformed cyanobacteria. Cyanobacteria will transform by mixing the e. coli extracted plasmid together and incubate the organism for a growth time of 5-7 days. This method is fundamentally quicker than the model plant Arabidopsis which has a growth time of 4-5 months.

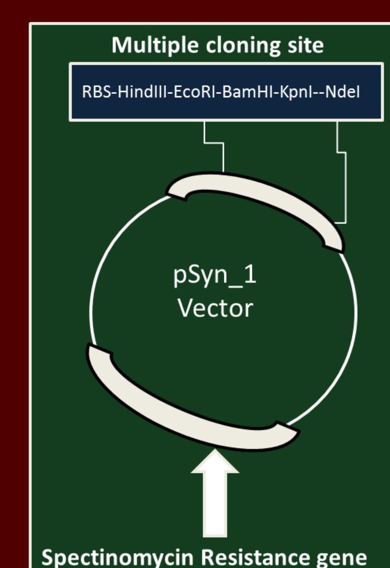

Figure 2 Vector design for cloning cyanobacteria

Results

Each of the metabolic designs that were set out in the objectives were fully designed for transformation. The independent SQS construct was successfully cloned into E. Coli and further transformed into cyanobacteria. Further analysis of the solution should be investigated with GC/MS data to quantify squalene production. This was unachievable due to a contaminated wild type cyanobacteria with a competing microorganism in the media it was able to out compete with the cyanobacteria leading to the inability to gain quantifiable data. The FPS and CBM3 constructs were digested and ligated with the pSYN_1 vector but no positive E. Coli colonies could be identified by gel electrophoresis. Further colonies should be screened to identify a transformed E. Coli colony. It is unclear whether the squalene is held inside the cell or on the surface and further research could help shed some light into this topic. The wild type cyanobacteria has been inoculated again and once the constructs are able to be transformed into cyanobacteria there will be data to suggest whether the experiments were a success.

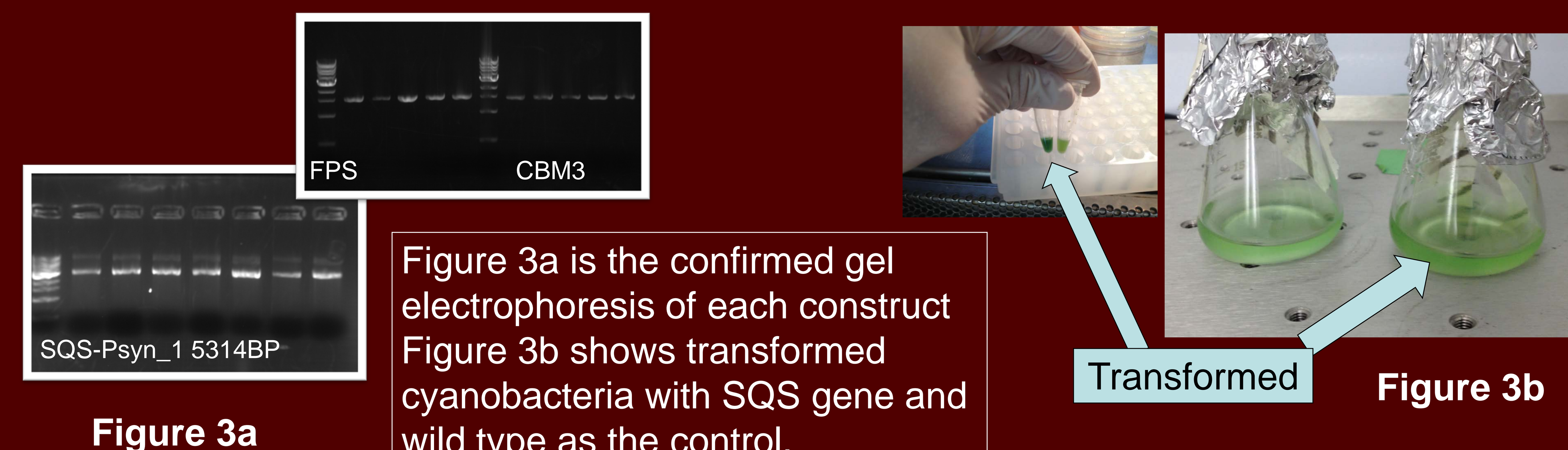

Figure 3a **Figure 3b**

Figure 3a is the confirmed gel electrophoresis of each construct Figure 3b shows transformed cyanobacteria with SQS gene and wild type as the control.

Conclusion

- Cyanobacteria is a preferred microorganism for biofuel production for various reasons:
  - Prokaryotic organisms are easily studied for metabolic engineering due to the lack of complexity in their genetic design and rapid growth cycles.
  - Many cyanobacteria species are capable of thriving in less than ideal conditions such as high thermal and high salinity environments.
- A hydrocarbon biofuel is a possibly economically feasible achievement through studying cyanobacteria further.
- Researching cyanobacteria and working in this lab has really made me excited for a career in microorganism research.

References

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