

LAB REPORT

Investigating expression of the *flv3* gene in *Spirulina* to enhance photosynthetic efficiency

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The increasing concentration of carbon dioxide (CO_2) in the atmosphere is a major contributor to climate change. We hypothesize that the production of *flv3*, a flavodiiron protein that dissipates excess electrons during photosynthesis, in *Spirulina* will accelerate photosynthesis and reduce atmospheric CO_2 levels. The expression of the introduced *flv3* gene would result in the synthesis of flavodiiron proteins to balance electron flow in photosystem I. This would ultimately provide extra ATP and NADPH electron carriers necessary to drive the Calvin Cycle faster, thereby increasing photosynthetic rate. We have designed a plasmid based on the pDV044 backbone to contain the *flv3* gene from *Synechocystis nigrescens*, a model photosynthetic cyanobacterium. We identified the gene using NCBI BLAST (National Center for Biotechnology Information, 2018), then used the Benchling program to incorporate it into our plasmid and design primers for gene amplification. To measure the rate of photosynthesis, our procedure used changes in water pH to detect the efficiency of the light reactions during photosynthesis. This method ensured the system remained open, with gasses readily available to spirulina without risking damage or death. It used the principle that removing CO_2 from water raises the pH by reducing the formation of carbonic acid, making the water more basic and because the light reactions occur more efficiently. The hope was that, as the transformed and *flv3*-containing spirulina removes CO_2 faster than spirulina without the plasmid, the pH would rise faster. We selected spirulina for our study because of its global abundance and its potential for use in bioengineering efforts to reduce atmospheric CO_2 . Future work will focus on optimizing *flv3* expression and testing additional environmental conditions to directly assess whether this engineered spirulina is viable for improved photosynthetic efficiency and CO_2 reduction.

Keywords: Bacterial transformation, CO_2 removal, gene cloning, spirulina.



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SINCE the Industrial Revolution, CO_2 levels have climbed from roughly 280 ppm to over 420 ppm, a 50% spike that is directly driving climate change, ocean acidification, and increasingly devastating weather events worldwide (Lindsey, 2025). Most proposed solutions to mitigate high CO_2 levels are expensive, energy-intensive, and difficult to scale. Cyanobacteria have been fixing carbon through photosynthesis for billions of years. *Spirulina* (*Arthrospira platensis*) is an attractive organism to work with due to its fast growth, genetic accessibility, and well-

documented safety (Figure 1). However, under high light conditions, spirulina's photosynthetic efficiency is low. This is due to the buildup of NADPH that accumulates after photosystem I (Figure 2). Organisms such as *S. nigrescens* have developed pathways that can recycle NADPH to NADP^+ to prevent its build up. This work seeks to determine if the protein, *flv3*, that permits NADPH to NADP^+ recycling from *Synechocystis* will improve photosynthetic efficiency in *Spirulina*.

Our research pointed us toward flavodiiron proteins, specifically *flv3*, as a potential solution. In particular,



Figure 1. Photo of spirulina culture that we grew under a microscope.

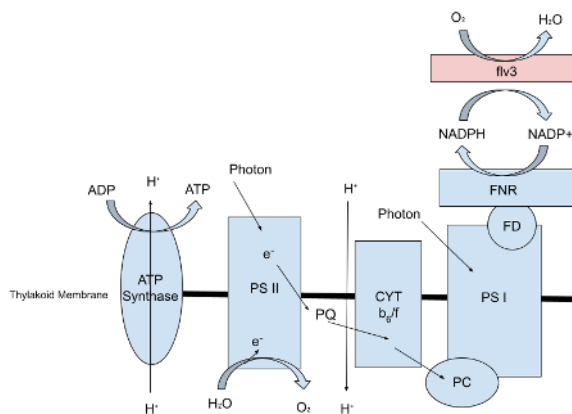


Figure 2. Diagram of the light reactions with *flv3* protein present.

flv3 acts as a drain for electrons, reducing the harmful buildup of ROS (Reactive Oxygen Species). Specifically, flv3 does this by forming water from oxygen, hydrogen, and electrons from NADPH buildup, protecting photosystem I, and keeping photosynthesis running efficiently (Allahverdiyeva et al., 2013; Hasunuma et al., 2014). We hypothesize that boosting *flv3* expression will meaningfully reduce photorespiration, thereby increasing CO₂ fixation. To test this, we identified the *flv3* gene from *Synechocystis* sp. PCC 6803 with the intent to introduce it into spirulina using the pDV044 plasmid backbone with the ultimate hope of engineering a fast-growing, low-cost biological carbon sink capable of contributing to real climate solutions.

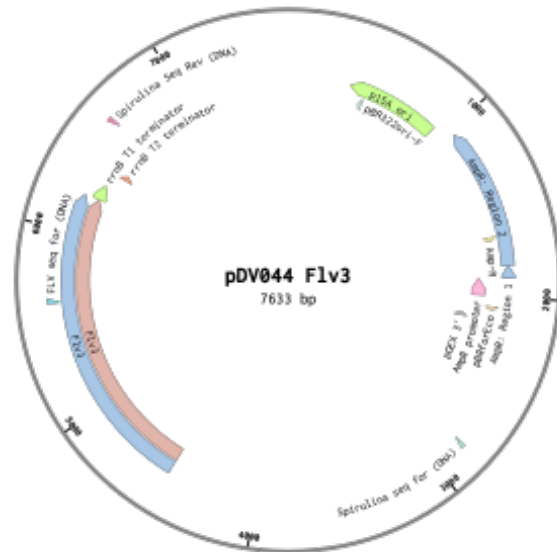


Figure 3. Map of the design plasmid with the gene of interest.

Materials and methods

We used a program called Benchling to design a unique plasmid that includes the pDV044 backbone with our gene of interest, *flv3*. We found the sequence of our plasmid backbone from Addgene and the sequence of *flv3* from NCBI. We designed our own primers to insert the gene of interest into the backbone and put them together in the Benchling program.

The gene of interest, *flv3*, was obtained from *S. nigrescens*. The *flv3* gene was amplified using polymerase chain reaction (PCR) with New England Biolabs (NEB) PCR Master Mix, Ipswich, Massachusetts, and specific primers. We attempted to isolate the plasmid backbone, pDV044, from *Escherichia coli* using the Monarch Spin Plasmid Miniprep kit (NEB, Ipswich, Massachusetts). We then ran a plasmid digest using the restriction enzyme BsaI.

Photosynthetic rate was measured by monitoring changes of pH, using Vernier pH probes (Vernier Science Education, Beaverton, Oregon), of the culture medium as an indirect indicator of carbon fixation (Measuring the Rate of Photosynthesis, 2024). Multiple cultures of wild-type *A. platensis* samples were exposed to identical environmental conditions with the exception of light presence. As a negative control, a sample was enclosed in a beaker covered with aluminum foil and kept away from light, so that the spirulina would not have access to light. The other sample was exposed to high intensity light. pH was recorded over time to measure change due to photosynthesis.

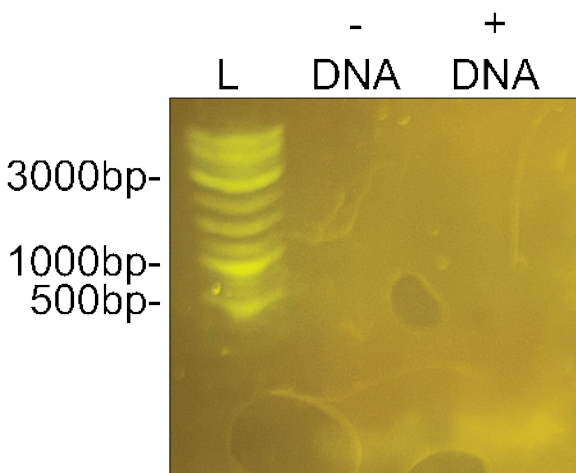


Figure 4. Gel electrophoresis of the attempted PCR of *flv3*.

Safety

All procedures were conducted in accordance with standard microbiological safety practices (Vanderbilt University Medical Center, n.d.). These include the use of protective equipment such as gloves and goggles, sterile tools and techniques to prevent contamination, and proper waste disposal. Both organisms used in experimentation, spirulina and synechocystis, are Bio-Safety Level 1 (BSL-1) organisms, widely recognized as safe (BacDive, n.d.).

Results

After running a PCR to isolate and amplify the *flv3* gene from *S. nigrescens*, the gel electrophoresis showed extremely faint bands far past the ladder and no other bands. Expected results were one band at 1752 bp, which would have been the gene and attached primers. The faint bands past the ladder are the primers that had no gene to attach themselves to. No other DNA was present, meaning the PCR to amplify the gene was unsuccessful.

After attempting to isolate the pDV044 plasmid backbone from *E. coli* and running the digest, the gel electrophoresis showed no bands at all. The expected results were a single band at about 5910 bp, which would have been the plasmid backbone. Because no DNA was present, the plasmid isolation was unsuccessful.

A pH test on a culture of wild-type *A. plantensis* (spirulina) was run. When photosynthesis occurs, it is expected for the pH to increase because CO₂ levels decrease, and therefore carbonic acid, (CO₂ dissolved in water) decreases. This causes a decrease in acidity,

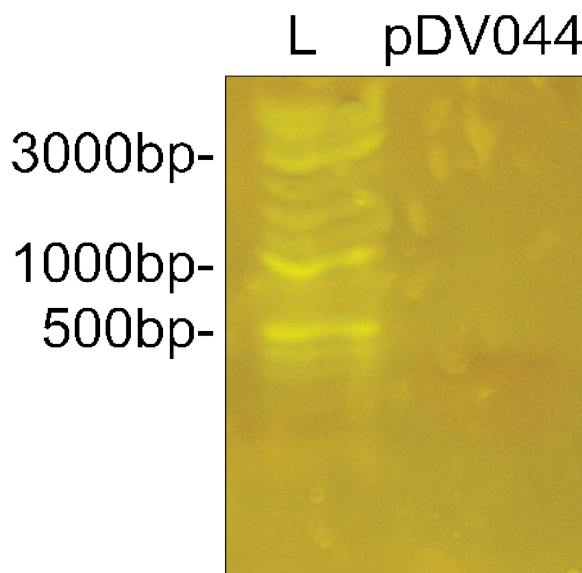


Figure 5. Gel electrophoresis of the attempted pDV044 isolation.

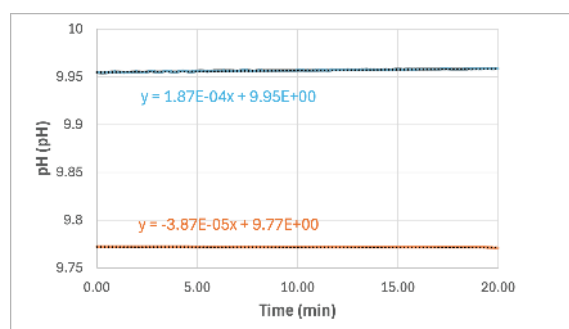


Figure 6. Graph of pH in the light and dark trials over time.

and an increase in pH levels. Results showed a successful proof of concept. pH in the culture of spirulina exposed to light had an increase in pH over time and the culture of spirulina in darkness had a decrease in pH over time.

Discussions

The absence of any full bands on the gel electrophoresis (Figure 4) illustrates a failure of our PCR. The faint bands past the ladder represented the primers, as they failed to attach to absent DNA. Because our PCR was unsuccessful, we believe the *flv3* gene sequence that we found online was not the same as the one in the bacteria we used for the PCR. We found the gene sequence for *Synechocystis* sp. PCC 6803, a slightly different version of synechocystis than the one we could afford. We designed our primers for the exact version

we found online. If the experimental gene we obtained and PCR-amplified had a different gene sequence, the PCR would have been unsuccessful. This leaves us with two options: either sequence the genome of *S. nigrescens* or purchase one of the three (very expensive) sequenced bacterial strains with *flv3* in order to successfully isolate the *flv3* gene.

Analysis of the plasmid isolation results suggests that a mistake was made (Figure 5). This error was most likely due to a procedural error during the miniprep protocol rather than flaws in the experiment design. This mistake caused variation in DNA recovery and led to the absence of detectable plasmid DNA. This outcome highlights the complexity of plasmid isolation protocols and the importance of avoiding even small deviations in technique. In future experiments, more careful attention to protocol steps and improved handling during the miniprep procedure will be implemented to increase the likelihood of a successful isolation.

The pH test results (Figure 6) show that our concept is functional and ready for future testing. The initial pH testing shows that pH can be used to indicate changes in photosynthetic rates. This means that this test could be used to determine increases in photosynthetic rate of transformed bacteria in future trials.

Next steps

Our team aims to obtain a strain of *synechocystis* with a sequenced genome including *flv3*. Once, we will isolate and amplify the *flv3* gene through PCR. The amplified gene will then be inserted into a plasmid backbone using Golden Gate cloning with the BsaI restriction enzyme and ligase (NEB, Ipswich, Massachusetts). The recombinant plasmid will be transformed into competent *E. coli*, and successful insertion will be verified through restriction digest analysis. Verified plasmids containing the gene of interest will then be introduced into *A. platensis* cells, which will be cultured under controlled conditions to allow the stable expression of our gene (Tabakh et al., 2023). If transformation is successful, pH testing will be used to determine success of photosynthetic improvements.

Author contributions

ZP led the pH data collection, monitoring and analysis. EC led everything regarding the isolation of the plasmid and the cloning of the gene of interest. EC, MR, and ZP were responsible for drafting and finalizing the abstract. All authors collaborated on the research regarding the *Spirulina* cultivation together. The entire team contributed to the completion of our labs, which include PCR and transformation, as well as the design

and assembly of a final presentation poster and this paper

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