Characterization of Growth and Hydrogen Production from Photoantennae Mutants of Cyanobacterium *Synechocystis* sp. PCC 6803

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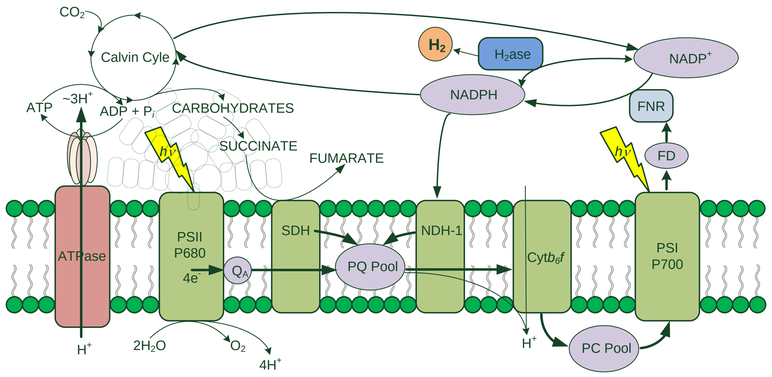
Subsurface Biosphere Initiative
Research and education focused on life below Earth's surface.

Introduction

Today, fossil fuels are the main source of power for the world. Not only are these fuels in limited supply, they contribute heavily to greenhouse gas emissions and global climate change. We currently use 13 terawatts (TW) and by 2050 it is predicted we will increase our power use to 28TW. An alternative energy source that can match the world's growing demands, while reducing pollution and greenhouse gas output, is needed. 89,000TW of sunlight reach the earth's surface, the question is how to capture it and convert it into usable energy. One possibility is using photosynthetic organisms, such as cyanobacteria, to produce hydrogen, which can be used in hydrogen fuel cells to produce heat and power.

Cyanobacteria are phototrophic organisms which can produce hydrogen under specific conditions. However, much work remains before they can make enough hydrogen to be a feasible energy source.

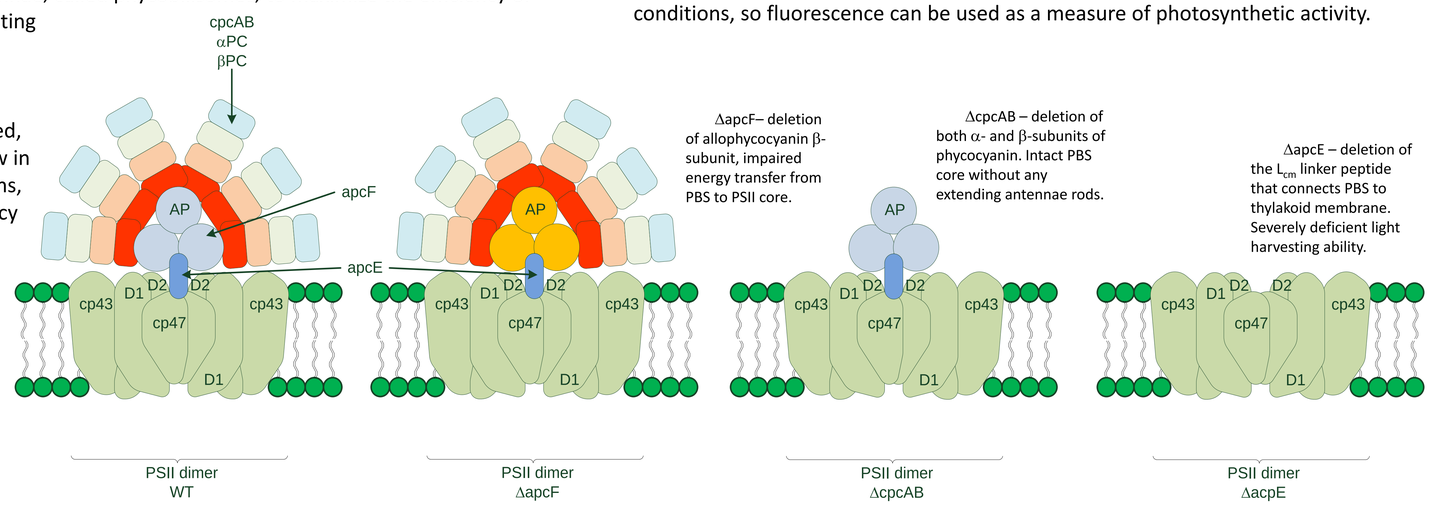


The figure above illustrates photosynthesis. Photosystems in the thylakoid membrane of the cell harvest light through their photoantennae. Photons absorbed by PSII drive the water splitting reaction, which oxidizes water into protons, electrons, and oxygen. Electrons are carried down the electron transport chain, by carrier proteins, to PSI. PSI absorbs more photons to make a strong reducing agent, NADPH, which the hydrogenase enzyme can use as a substrate to produce hydrogen.

The goal of this research is to explore conditions under which cyanobacteria produce more hydrogen. There has already been extensive research on the wild type cyanobacterium *Synechocystis* sp. PCC 6803, and research on three new mutants of this species began this summer. Optical density readings, hydrogen production analysis, and fluorescence measurements, were used to characterize the growth and health of these mutants with the objective of exploring their ability to produce hydrogen.

Antennae Mutants

These mutations were done to limit the amount of light the organism can absorb through its photoantennae, called phycobilisomes, to maximize the efficiency of their light use. By deleting portions of the phycobilisome, the organism's ability to harvest light is inhibited, which allows it to grow in brighter light conditions, increasing the efficiency of the system.



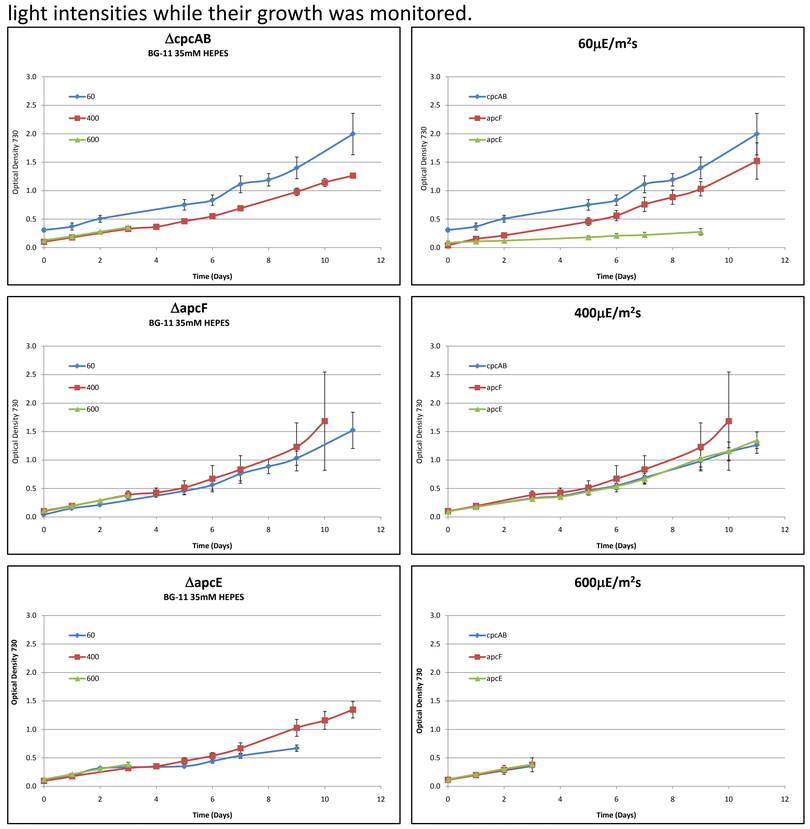
ΔapcF– deletion of allophycocyanin β-subunit, impaired energy transfer from PBS to PSII core.

ΔcpcAB – deletion of both α- and β-subunits of phycocyanin. Intact PBS core without any extending antennae rods.

ΔapcE – deletion of the L_{cm} linker peptide that connects PBS to thylakoid membrane. Severely deficient light harvesting ability.

Growth

Each of the three mutants were grown in triplicate flasks under three different light intensities while their growth was monitored.



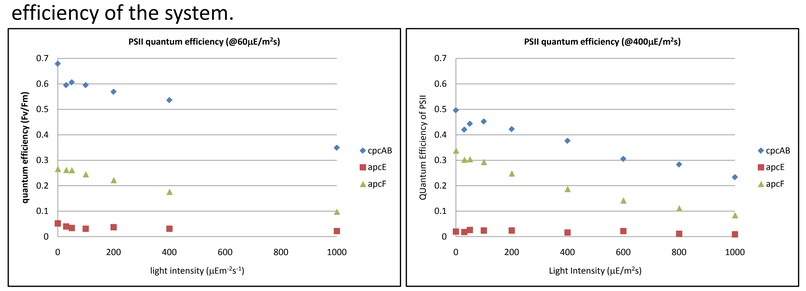
Grouped by mutation. Grouped by light intensity.

All three mutants grow faster with increased light intensity. This is indicated by the doubling time of the culture. Under 60 $\mu Em^{-2}s^{-1}$ the doubling time ranges between 3.5 and 7 days, at 400 $\mu Em^{-2}s^{-1}$ it is 3.5 to 4 days, and at 600 $\mu Em^{-2}s^{-1}$ the doubling time ranges between 2 and 2.5 days.

Fluorescence

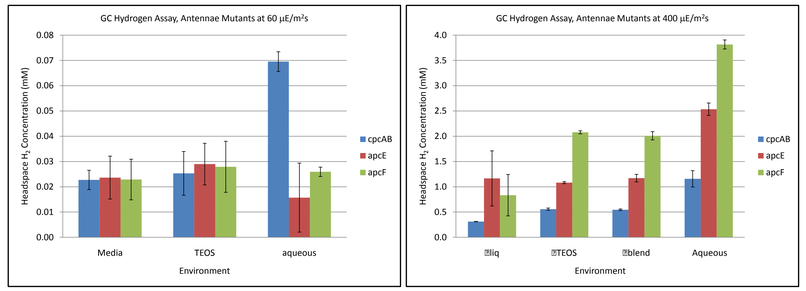
Fluorescence is one of the ways photosynthetic organisms release excess energy. When a photon is absorbed it has one of three fates: photochemistry, heat, or fluorescence. Heat release from cyanobacteria is considered negligible under most conditions, so fluorescence can be used as a measure of photosynthetic activity.

The mutations cause changes in fluorescence signals, suggesting these mutants are able to more efficiently utilize light that is absorbed. This allows light that would not be used for photosynthesis to pass and be used by adjacent cells, increasing the efficiency of the system.



Hydrogen Production

Cyanobacteria will only produce hydrogen under certain conditions. They produce increased amounts of hydrogen when they are first conditioned in the EHB-1 media, which encourages them to store large amounts of glycogen by reducing the amount of available sulfur and nitrogen. After conditioning them for several days they are encapsulated in silica sol-gel and placed in a nitrogen environment for 2 hours to deprive them of oxygen, accelerating them to an anaerobic condition. They are then stored in the dark for 48 hours, during which time they produce hydrogen through fermentation of stored glycogen.



Above are two plots showing the hydrogen production of the antennae mutants under different light conditions. Cultures conditioned under 400 $\mu Em^{-2}s^{-1}$ (right) produced as much as 150 times more hydrogen than the mutants conditioned 60 $\mu Em^{-2}s^{-1}$ (left).

Conclusions

The mutants of *Synechocystis* sp. PCC 6803, ΔcpcAB, ΔapcF, and ΔapcE, are capable of growing more quickly and of producing significantly more hydrogen under high light conditions. During this study they produced the highest levels of hydrogen observed to date in this lab. While research is still in its early stages, hydrogen from cyanobacteria could contribute greatly to the future of energy resources.

Future Work

- Continue to explore conditions in which these mutants of *Synechocystis* sp. PCC 6803, ΔcpcAB, ΔapcF, and ΔapcE produce more hydrogen.
- Continue investigation of growth under increasing light intensity.
- Optimize a system of layering the mutants and the wild-type in silica sol-gel that would improve efficiency of light use, and possibly increase hydrogen production.

Acknowledgements

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