II.E.1 Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Overall Objectives

- Minimize, or truncate, the chlorophyll antenna size in green algae, and the phycobilisome antenna size in cyanobacteria to maximize culture photobiological solar energy conversion efficiency and H_2 production.
- Demonstrate that a truncated light-harvesting antenna (TLA) minimizes absorption and wasteful dissipation of bright sunlight by individual cells, resulting in better light utilization efficiency and greater photosynthetic productivity in high-density mass cultures.

Fiscal Year (FY) 2014 Objectives

Apply the TLA concept in cyanobacteria and test for improved culture productivity.

Technical Barriers

The project addresses the following technical barriers from the Biological Hydrogen Production section of the Production section of the Fuel Cell Technologies Office Multi-Year Research, Development, and Demonstration Plan:

(AN) Light Utilization Efficiency

Technical Targets

The Fuel Cell Technologies Office Multi-Year Plan technical target for this project was to apply the TLA concept in cyanobacteria and to test for the premise of improved culture productivity. The cyanobacterial project was competed on schedule. The technical targets for this project are listed in Table 1.

FY 2014 Accomplishments (TLA effort in cyanobacteria)

- Work on the application of the TLA concept in cyanobacteria was completed. The work provided first-time direct evidence of the applicability of the TLA concept in cyanobacteria, entailing substantial improvements in the photosynthetic efficiency and

TABLE 1. Microalgal Technical Targets, Milestones and Progress (Sunlight utilization efficiency is shown as percent of incident solar energy. Maximum possible based on PAR=30%. Maximum possible based on e-PAR=40%.)

	2000	2003	2005	2007	2008	2010	2011	2012	2015
Targets (Light utilization efficiency)	3%	10%				15%			20%
Tla strain with the highest efficiency identified	3% (WT)	10% TLA1	15% TLA2		25% TLA3				
Gene cloning from the TLA strains				TLA1: Mov34 MPN			TLA2: FTSY	TLA3: SRP43	

productivity of mass cultures upon minimizing the phycobilisome light-harvesting antenna size.

- A patent application was filed.
- A peer-reviewed paper was published with the following citation:
  - Kirst, H., Formighieri, C., Melis, A. (2014) Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size. Biochimica et Biophysica Acta, Bioenergetics DOI: 10.1016/j.bbabio.2014.07.009

INTRODUCTION

The goal of the research is to generate green algal and cyanobacterial strains with enhanced photosynthetic productivity and H_2 production under mass culture conditions. To achieve this goal, it is necessary to optimize the light absorption and utilization properties of the cells [1]. A cost-effective way to achieve this goal is to reduce the number of chlorophyll (Chl) molecules (green microalgae) or phycobilins (cyanobacteria) that function in the apparatus of photosynthesis.

The rationale for this work is that a truncated light-harvesting antenna size in green algae or cyanobacteria will prevent individual cells at the surface of a high-density culture from over-absorbing sunlight and wastefully dissipating most of it (Figure 1). A truncated antenna size will permit sunlight to penetrate deeper into the culture, thus enabling many more cells to contribute to useful photosynthesis and H_2 production (Figure 2). It has been shown that a truncated Chl antenna size will enable about 3-4 times greater solar energy conversion efficiency and photosynthetic productivity than could be achieved with fully pigmented green microalgal cells [2].

APPROACH

A phycocyanin-deletion mutant of *Synechocystis* (cyanobacteria) was generated upon replacement of the *CPC*-operon with a kanamycin resistance cassette.

RESULTS

The *Δcpc* transformant strains (*Δcpc*) exhibited a green phenotype, compared to the blue-green of the wild type (WT), lacked the distinct phycocyanin absorbance at 625 nm, had a lower Chl per cell content, and a lower Photosystem I/Photosystem II reaction center ratio compared to the WT. Molecular and genetic analyses showed replacement of all WT copies of the *Synechocystis* DNA with the transgenic version, thereby achieving genomic DNA homoplasmy. Biochemical analyses showed absence of the phycocyanin α- and β-subunits, and overexpression of the kanamycin resistance NPTI protein in the *Δcpc*. Physiological analyses revealed a higher, by a factor of about 2, intensity for the saturation of photosynthesis in the *Δcpc* compared to the WT. Under limiting intensities of illumination, growth of the *Δcpc* was slower than that of the WT. This difference in the rate of cell duplication diminished gradually as growth irradiance increased. Identical rates of cell duplication of about 13 h for both WT and the *Δcpc* were observed at about 800 µmol photons m^{-2} s^{-1} or greater. (Note: Full sunlight intensity at sea level is about 2,200 mmol photons m^{-2} s^{-1}.) Culture productivity analyses under simulated bright sunlight and high cell-density conditions showed that biomass accumulation by the *Δcpc* was 1.57 times greater than that achieved by the WT. Results were published in Kirst et al. (2014) Biochim Biophys Acta DOI: 10.1016/j.bbabio.2014.07.009.

CONCLUSIONS

Cyanobacterial TLA Project

The work provided first-time direct evidence of substantial improvements in the biomass productivity of mass cultures upon minimizing the phycobilisome light-harvesting antenna size in cyanobacteria.

Green Microalgal TLA Project

The following green microalgal ***tla*** strains have been deposited and are available to the public from the Chalmydomonas Resource Center (<chlamycollection.org>).

CC-4473 tla3 mt+

CC-4474 tla4 mt+

CC-4475 tla5 mt+

CC-4472 tla2-ΔFtsY (cw15) mt+

CC-4476 tla2-ΔFtsY (CW15+) mt+

CC-4561 tla3-Δcpsrp43 (cw+) mt+

CC-4562 tla3-Δcpsrp43 (cw+) mt-

CC-4169 tla1 cw15 sr-u-2-60 mt+ Chromosome: 05Locus: TLA1

CC-4170 tla1 nr-u-2-1 mt- Chromosome: 05Locus: TLA1

e-PAR Project

In silico work was conducted to advance exploration of the "*extended Photosynthetically Active Radiation*" (e-PAR) concept. Proprietary preliminary information on the molecular genetic design was arrived at but not disclosed. Successful implementation of the e-PAR concept is a long-term project, one that could not be further pursued under the auspices of this contract.

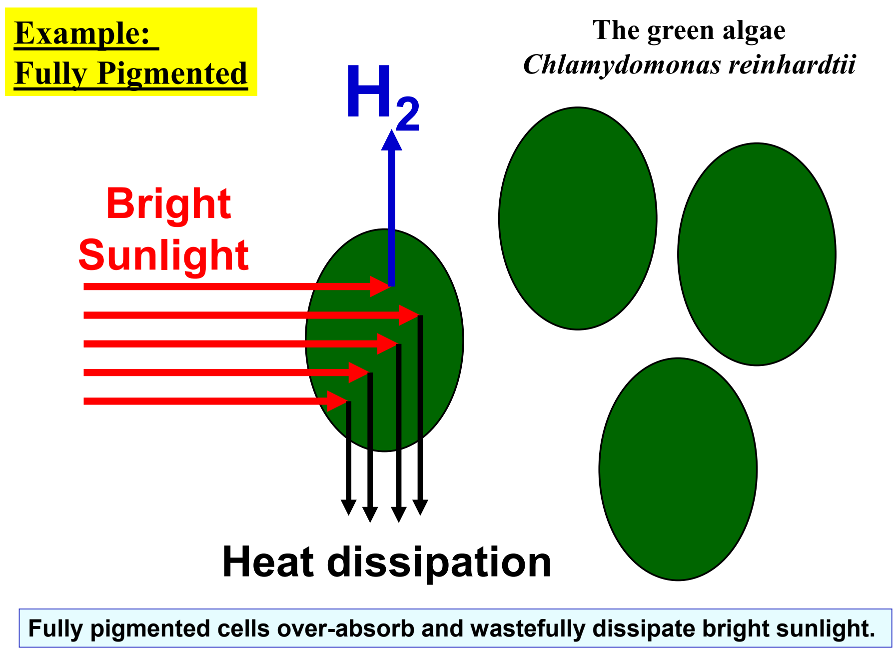

FIGURE 1. Schematic presentation of the fate of absorbed sunlight in fully pigmented (dark green) algae. Individual cells at the surface of the culture over-absorb incoming sunlight, i.e., they absorb more than can be utilized by photosynthesis, and 'heat dissipate' most of it. Note that a high probability of absorption by the first layer of cells would cause shading of cells deeper in the culture.

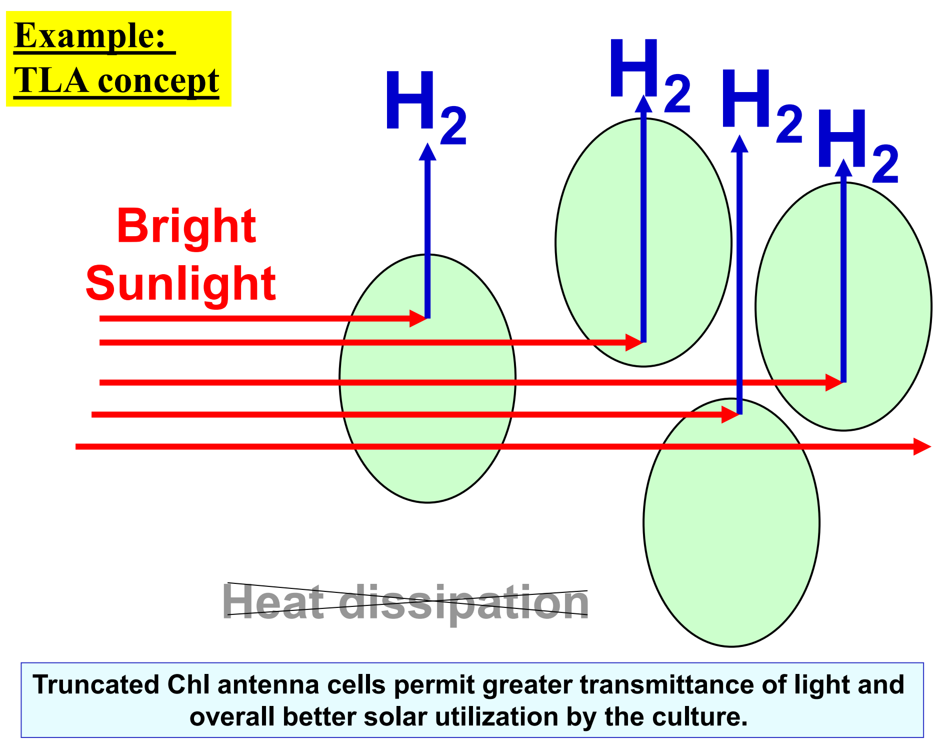

FIGURE 2. Schematic of sunlight penetration through cells with a truncated chlorophyll antenna size. Individual cells have a diminished probability of absorbing sunlight, thereby permitting penetration of irradiance and H2 production by cells deeper in the culture.

FUTURE DIRECTIONS

This project has achieved all its objectives and is about to be officially terminated at the end of September 2014.

FY 2014 PUBLICATIONS

1. Kirst H, Melis A (2014) The chloroplast *Signal Recognition Particle* pathway (CpSRP) as a tool to minimize chlorophyll antenna size and maximize photosynthetic productivity. Biotechnology Advances 32: 66–72 DOI: 10.1016/j.biotechadv.2013.08.018.

2. Kirst H, Formighieri C, Melis A (2014) Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size. Biochim Biophys Acta - Bioenergetics DOI: 10.1016/j.bbabio.2014.07.009 in press.

REFERENCES

1. Kok B (1953) Experiments on photosynthesis by *Chlorella* in flashing light. In: Burlew JS (ed), Algal culture: from laboratory to pilot plant. Carnegie Institution of Washington, Washington D.C., pp 63-75.

2. Melis A, Neidhardt J, Benemann JR (1999) *Dunaliella salina* (Chlorophyta) with small chlorophyll antenna sizes exhibit higher photosynthetic productivities and photon use efficiencies than normally pigmented cells. J. appl. Phycol. 10: 515-525