Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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University of California - Berkeley
Thursday, 16 May 2013

Project ID # PD036

This presentation does not contain any proprietary, confidential, or otherwise restricted information
Overview

Timeline

- Start: 01-Dec-2004
- End: 31-Jan-2014
- Completion: 95%

Budget

- Total Project Funding
  - DOE: $1.74M
  - UCB: $675K
  - Swedish Res Council: $55.2K (FY11)

- Funding received in FY12: $150K
- Planned funding for FY13: $150K

Barriers

- Low Light Utilization Efficiency in Photobiological Hydrogen Production due to a Large Photosystem Chlorophyll Antenna Size (Barrier AN).

Partners

- NREL
- Swedish Research Council
The TLA concept
(TLA = Truncated Light-harvesting Antenna):
Minimize the light-harvesting antenna size of the photosystems to prevent the early light-saturation of photosynthesis and the associated wasteful dissipation of absorbed sunlight.

Relevance

Improve the sunlight-utilization efficiency of photosynthesis in microalgae by up to 300%, which will improve H₂ or fuels production in microalgae and cyanobacteria by about the same percentage.

The work links with effort both at NREL and at the J. Craig Venter Institute, where H₂-production technologies in microalgae and cyanobacteria are being developed.
Bright Sunlight

Heat dissipation

Fully pigmented cells over-absorb and wastefully dissipate bright sunlight.
Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture.
Objectives and Approach

Chlorophyll a and b antenna size in microalgae

Large Chl Antenna Size
(600 Chl a and b)

Genetic determinants

Truncated Chl Antenna Size
(130 Chl a)

Wild type

TLA

Identify genes and molecular mechanisms to enable a truncated antenna size in microalgae and cyanobacteria.

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Objectives and Approach

Objectives:

Identify genes and associated molecular mechanisms that confer a Truncated Light-harvesting Antenna (TLA property) in the tla3 strains of Chlamydomonas reinhardtii.

Develop protocols for the targeted truncation of the light-harvesting antenna size in cyanobacteria.

Approach:

(a) Cloning of the genes responsible for the TLA3 phenotype in Chlamydomonas reinhardtii. (b) Identification of genes to be interrupted or deleted in cyanobacteria. (c) Functional analysis of the transformants (Berkeley expertise).
PART 1

Green Microalgae
# Microalgae Accomplishments and Progress

## Sunlight Utilization Efficiency, % of Incident Solar Energy

(maximum possible = 30%)

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</thead>
<tbody>
<tr>
<td><strong>Targets</strong></td>
<td>3%</td>
<td>10%</td>
<td></td>
<td></td>
<td></td>
<td>15%</td>
<td></td>
<td></td>
<td>20%</td>
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<tr>
<td>(Light utilization efficiency)</td>
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<tr>
<td><strong>Tla strain with the highest efficiency identified</strong></td>
<td>3% (WT)</td>
<td>10% <em>TLA1</em></td>
<td>15% <em>TLA2</em></td>
<td>25% <em>TLA3</em></td>
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<td><strong>Gene cloning from the TLA strains</strong></td>
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<tr>
<td></td>
<td>TLA1: Mov34 MPN</td>
<td>TLA2: FTSY</td>
<td>TLA3: SRP43</td>
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</table>
Microalgae Accomplishments and Progress
Mechanism of TLA2 and TLA3 Function in LHC-protein assembly
PART 2

Cyanobacteria
Photosystem stoichiometry and phycobilisome-chlorophyll antenna organization in the thylakoid of cyanobacteria. Cyanobacteria may possess up to 850 phycocyanin (PC), allophycocyanin (AP), and chlorophyll (Chl) molecules per unit photosynthetic apparatus.

Phycobilisome (PBS) schematic adapted from Glazer and Melis 1987.
Cyanobacterial Approach
Unit photosynthetic apparatus and cyanobacterial TLA organization
The model shows the anticipated antenna size in TLA cyanobacteria

Go/No-Go Decision: Present evidence at the molecular and biochemical levels to demonstrate proof-of-concept and capability of gene replacement in cyanobacteria, as the method of choice for the generation of TLA mutants in these photosynthetic microorganisms.
Cyanobacterial Approach
Replacement of the phycocyanin-encoding operon (Go/No-Go Decision)

Wild type *Synechocystis* genomic DNA

WT cpc replacement

Expected PCR product size:

<table>
<thead>
<tr>
<th></th>
<th>WT [bp]</th>
<th>Δcpc [bp]</th>
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<tbody>
<tr>
<td>F3R3</td>
<td>4683</td>
<td>2126</td>
</tr>
<tr>
<td>F1R5</td>
<td>4973</td>
<td>2416</td>
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</tbody>
</table>

Kanamycin resistance

Δcpc replacement

Expected PCR product size:

F3 F1 F3 F1 R3 R5 R3 R5

kb

5 4 3 2
Cyanobacterial Approach

Molecular and Physiological Evidence of Transformation (Go/No-Go Decision)

Wild type *Synechocystis*
- Blue-green phenotype due to PC and Chl pigments

Δ*cpc* transformants
- Green phenotype indicating loss of PC
Cyanobacterial Approach
Biochemical Evidence of Transformation (Go/No-Go Decision)

Absorbance

WT
Δcpc

PC  APC  Chl

Synechocystis

Wavelength, nm
Evidence is presented at the genetic and molecular levels to demonstrate successful double homologous recombination for replacement of the \textit{cpc} DNA operon in cyanobacteria.

Further evidence is presented at the biochemical level to show the ensuing absence of phycocyanin light-harvesting pigments from cyanobacteria (\textit{\Delta cpc} strains), seen by the green coloration of the latter, as opposed to the blue-green coloration of the wild type and by the absence of phycocyanin from the absorbance spectra of the \textit{\Delta cpc} strains.
Peer reviewed publications:


• Successfully completed the TLA3 project, deposited strains in the *Chlamydomonas* library

• Successfully applied the TLA concept to cyanobacteria.

• Potential of enhancing photosynthetic efficiencies and hydrogen production of cyanobacteria under mass culture conditions.
Collaborations, Applications, and Impact of the R&D

- The TLA concept is applied at:
  - NREL and by the Brisbane (Australia)-Bielefeld (Germany) groups in *Chlamydomonas* for H₂ production and by the U-Wageningen (The Netherlands) for biomass production, and by two companies in the private sector for commercial production of polyunsaturated fatty acids (PUFAs) in *Nannochloropsis*.

- TLA strains were requested from the *Chlamydomonas* library and acquired by universities (x29), industry (x14), government labs (x4), research institutions (5), and high schools (x6).

- (A total of 58 strains were shipped since 2010, most of them in 2012.)

- Collaboration was offered in the form of advising some of the above groups in the use of the TLA strains.
Proposed Future Work

Compete the analysis of the TLA cyanobacteria, including:
(i) Assessment of stability and fitness of the Δcpc transformants;
(ii) Organization of the photosynthetic apparatus (measurement of PS stoichiometry and functional antenna size in the Δcpc transformants);
(ii) Efficiency and productivity of photosynthesis measurements from the light-saturation curve); and
(iii) Measurements of Δcpc productivity under mass culture conditions. (Currently in progress.)

Advance exploration of the “extended photosynthetically active radiation” (ePAR) concept.
(Proprietary molecular genetic design not disclosed. Please see next slide for a concept explanation, to be further discussed during the presentation.)
Proposed Future Work

Extended photosynthetically active radiation” (ePAR) concept

![Graph showing Quantum irradiance vs Wavelength with data points for visible and near infrared wavelengths, indicating 45% of sunlight in the visible range and 20% in the near infrared range.]

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Technical Backup Slides
Additional Exploratory PCR Analyses
Undertaken for
Wild type and Δcpc transformants
### Expected product size

<table>
<thead>
<tr>
<th>Primer set</th>
<th>Length [bp]</th>
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<tr>
<td>F1R1</td>
<td>935</td>
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<tr>
<td>F2R1</td>
<td>819</td>
</tr>
<tr>
<td>F3R1</td>
<td>769</td>
</tr>
<tr>
<td>F1R2</td>
<td>1003</td>
</tr>
<tr>
<td>F2R2</td>
<td>887</td>
</tr>
<tr>
<td>F3R2</td>
<td>837</td>
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</tbody>
</table>

**Image**: Gel electrophoresis showing bands at 1000 bp and 850 bp.
WT gDNA

Δcpc transformant gDNA

Expected product size

<table>
<thead>
<tr>
<th>Primer set</th>
<th>Length [bp]</th>
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<tbody>
<tr>
<td>F4R3</td>
<td>825</td>
</tr>
<tr>
<td>F4R4</td>
<td>894</td>
</tr>
<tr>
<td>F4R5</td>
<td>949</td>
</tr>
<tr>
<td>F5R3</td>
<td>1008</td>
</tr>
<tr>
<td>F5R4</td>
<td>1077</td>
</tr>
<tr>
<td>F5R5</td>
<td>1132</td>
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</tbody>
</table>
Chl Antenna Size vs Light Utilization Efficiency

Utilization Efficiency of Absorbed Light Energy

- Wild type antenna size = **470 Chl molecules** (100%) (PSII=230; PSI=240)
  Photon use efficiency of WT photosynthesis = ~6-10%
  Utilization Efficiency of Absorbed Light Energy by WT: ~3-5%

- **tla1** antenna size = **275 Chl molecules** (59% of control) (PSII=115; PSI=160)
  Photon use efficiency of **tla1** photosynthesis = ~20%
  Utilization Efficiency of Absorbed Light Energy by **tla1**: ~10%

- **tla2** antenna size = **195 Chl molecules** (42% of control) (PSII=80; PSI=115)
  Photon use efficiency of **tla2** photosynthesis = ~30%
  Utilization Efficiency of Absorbed Light Energy by **tla2**: ~15%

- Long-term goal: 132 Chl molecules (28% of control) (PSII=37; PSI=95)
  Photon use efficiency of photosynthesis goal = ~60%
  Utilization Efficiency of Absorbed Light Energy goal: ~30%
Phycobilisome-Chlorophyll antenna size
In Cyanobacteria

- Large PBS-Chl Antenna Size
  (850 Pcy and Chl)

  Genetic determinants

  Truncated Antenna Size
  (130 Chl a)

Wild type

TLA