Production of recombinant proteins in microalgae at pilot greenhouse scale

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Expression of recombinant proteins in algae chloroplasts

- Chloroplast expression machinery is adapted for high rate of translation and accumulation of proteins (e.g., D1 and RuBisCO)
- Gene silencing and position effects do not affect expression
- Recombinant protein accumulation of up to 70% TSP has been achieved in plant plastids
- Chloroplasts can also properly fold proteins with disulfide bonds
- Elimination of selection markers from the plastid genome is very easy
Algae chloroplast expression optimization

• In *Chlamydomonas reinhardtii*, maximum recombinant protein accumulation has been obtained with the *psbA* promoter/5’UTR (up to 10% TSP)

• This can only be achieved in a *psbA* deletion strain (non-photosynthetic)

• This has allowed us to express functional:
  - Immunostimulants
  - Cytokines
  - Hormones
  - Antibody-toxins
  - Malaria vaccines
  - Industrial enzymes

• **Limited scalability** because of acetate requirement and culture contamination
Photosynthesis reconstitution of a \textit{psbA} deletion strain expressing a recombinant protein

- Manuell et al., 2007 has previously shown that photosynthesis could be restored by a \textit{psbA} coding sequence driven by the \textit{psbD} promoter, while maintaining a reduced level of recombinant protein accumulation (Milk Amyloid A, MAA)

\textit{Chlamydomonas reinhardtii} chloroplast genomes
Unfortunately, MAA expression in the psbD-psbA reconstituted strain is not constitutive, even under laboratory growth conditions.

Recombinant protein accumulation is highly dependent upon dark-to-light shifts for induction, which is unviable for outdoor growth.

We optimized this strategy by reconstituting photosynthesis in the MAA strain with 40 combinations of promoters, 5’ UTRs and psbA CDS from 8 green algae, 1 diatom, 1 plant, 1 cyanobacteria, and 1 cyanophage.

Only 6 complemented strains became photosynthetic and they achieved different levels of MAA accumulation.
## Photosynthetic MAA-expressing strains

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Promoter 5’ UTR</th>
<th>psbA Coding Sequence</th>
<th>PSII Quantum yield</th>
<th>Growth Rate [day⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS1</td>
<td>psbA C. reinhardtii</td>
<td>T. pseudonanana</td>
<td>0.65±0.01</td>
<td>1.38±0.04</td>
</tr>
<tr>
<td>Syne1Optᵃ</td>
<td>psbA C. reinhardtii</td>
<td>S. elongatus</td>
<td>0.69±0.01</td>
<td>1.56±0.07</td>
</tr>
<tr>
<td>Duna</td>
<td>psbA D. salina</td>
<td>C. reinhardtii</td>
<td>0.66±0.00</td>
<td>1.46±0.02</td>
</tr>
<tr>
<td>Scene</td>
<td>psbA S. obliquus</td>
<td>C. reinhardtii</td>
<td>0.68±0.00</td>
<td>1.49±0.02</td>
</tr>
<tr>
<td>Chlamy atpA</td>
<td>atpA C. reinhardtii</td>
<td>C. reinhardtii</td>
<td>0.57±0.00</td>
<td>1.18±0.02</td>
</tr>
<tr>
<td>Chlamy tufA</td>
<td>tufA C. reinhardtii</td>
<td>C. reinhardtii</td>
<td>0.50±0.01</td>
<td>1.15±0.03</td>
</tr>
<tr>
<td>Chlamy psbDᵇ</td>
<td>psbD C. reinhardtii</td>
<td>C. reinhardtii</td>
<td>0.71±0.00</td>
<td>1.53±0.03</td>
</tr>
<tr>
<td>Chlamy psbAᵇ</td>
<td>psbA C. reinhardtii</td>
<td>C. reinhardtii</td>
<td>0.73±0.00</td>
<td>1.70±0.04</td>
</tr>
<tr>
<td>WT</td>
<td>psbA C. reinhardtii</td>
<td>C. reinhardtii</td>
<td>0.74±0.01</td>
<td>1.73±0.03</td>
</tr>
<tr>
<td>KOᵇ</td>
<td>psbA deficient</td>
<td>psbA deficient</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

ᵃContains a codon optimized version of the heterologous psbA coding sequence
ᵇOriginally reported in Manuell et al. (2007)
Anti-AtpB, D1 and MAA Western blots. AtpB was used as a loading control.

The numbers underneath represent the amount of total protein loaded relative to WT.

**a**: Equal amount of total protein loaded (10 µg per well).

**b**: Equal amount of culture volume loaded (equivalent to 75 µl of algae culture per well).
Growth of strain “Scene” at pilot greenhouse scale
Growth parameters and MAA accumulation

<table>
<thead>
<tr>
<th>Bag</th>
<th>Dry weight&lt;sup&gt;a&lt;/sup&gt; [g·L&lt;sup&gt;-1&lt;/sup&gt;]</th>
<th>Max. Productivity&lt;sup&gt;b&lt;/sup&gt; [g·L&lt;sup&gt;-1&lt;/sup&gt;·day&lt;sup&gt;-1&lt;/sup&gt;]</th>
<th>T°C&lt;sup&gt;c&lt;/sup&gt;</th>
<th>pH&lt;sup&gt;c&lt;/sup&gt;</th>
<th>MAA&lt;sup&gt;d&lt;/sup&gt; [mg·L&lt;sup&gt;-1&lt;/sup&gt;]</th>
<th>MAA&lt;sup&gt;d&lt;/sup&gt; % expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7</td>
<td>0.265</td>
<td>0.047 (11)</td>
<td>23.0±4.6</td>
<td>6.6±0.2</td>
<td>1.86±0.07</td>
<td>1.21±0.05%</td>
</tr>
<tr>
<td>D9</td>
<td>0.256</td>
<td>0.037 (11)</td>
<td>23.1±4.6</td>
<td>6.5±0.2</td>
<td>1.66±0.06</td>
<td>1.12±0.04%</td>
</tr>
<tr>
<td>D11</td>
<td>0.278</td>
<td>0.069 (9)</td>
<td>23.4±4.9</td>
<td>6.6±0.2</td>
<td>3.28±0.21</td>
<td>1.86±0.12%</td>
</tr>
<tr>
<td>Average&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.267±0.011</td>
<td>0.051±0.016</td>
<td>23.2±0.2</td>
<td>6.6±0.1</td>
<td>2.27±0.88</td>
<td>1.40±0.40</td>
</tr>
</tbody>
</table>

<sup>a</sup>Achieved on day 12 of the culture
<sup>b</sup>Parentheses show the day in which the maximum productivity was attained.
<sup>c</sup>The uncertainty (except for the “Average” row) corresponds to the standard deviation from all daily measurements.
<sup>d</sup>The uncertainty (except for the “Average” row) corresponds to the standard deviation from the triplicate wells in the ELISA plate.
<sup>e</sup>The uncertainty represents the standard deviation between the three bags without error propagation.
Future prospects

- MAA productivity could be further enhanced by growing at a warmer season and by reducing the optical path length of the culture.

- Large scale production of MAA is a potentially viable solution for reducing the risks of infectious diarrhea:
  - Direct anti-microbial properties
  - Prevents bacterial adhesion to the gut
  - Stimulates phagocytosis in macrophages

- At a given productivity cost of $100/kg dry weight algae, our system produces MAA sixty times cheaper than the main natural source, bovine milk colostrum.

<table>
<thead>
<tr>
<th></th>
<th>MAA Accumulation</th>
<th>Cost/Kg</th>
<th>MAA cost/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum</td>
<td>0.255 g/Kg</td>
<td>$138</td>
<td>$542.20</td>
</tr>
<tr>
<td>C. reinhardtii</td>
<td>11.799 g/Kg</td>
<td>$100</td>
<td>$8.48</td>
</tr>
</tbody>
</table>
Thank you!

- Steve Mayfield
- James Hyun
- Nathan Schoepp
- Rest of the Mayfield Lab
- AFOSR
- DOE
- CONICYT