Genetic engineering augments the content of C-phycocyanin in a model and production strain of cyanobacteria.

Ryo Takeuchi, Hui Zhao, Margaret McCormick and James Roberts

Matrix Genetics LLC
Seattle WA
Matrix Genetics

Cyanobacteria

- Recombinant DNA
- Pigment
- Protein
- Lipid
Our missions

1. Seek for genetic modifications that increase the commercial values of cyanobacteria using a model strain (*Synechococcus elongatus* PCC 7942).

- One of the best studied strains
- Grows fast under laboratory conditions
- Naturally transformable
- Small chromosome (2,700 genes on 2.7 Mbp)

2. Translate new findings to production strains.

- Filamentous strain.
- Grows in an alkaline, high-salt medium.
- No genetic engineering approach established
- Larger chromosome (6,000 genes on 6.5 Mb)
Phycobilisome and C-phycocyanin

- Unique protein complex that harvests light energy for photosynthesis, and accounts for more than a half of total proteins.
- Composed of allophycocyanin (Apc), C-phycocyanin (Cpc), linker proteins and a cap protein.

- Major protein colored blue in phycobilisome.
- Has been used as a natural color and a fluorescent probe.
- Has been known to be an antioxidant and a reactive oxygen species scavenger.
C-phycocyanin (Cpc)

- Donut-shaped, protein-chromophore complex that is composed of 6 each of α- and β-subunits.
- These subunits are encoded by cpcA and cpcB, which are well conserved among cyanobacteria.
- One phycocyanobilin is linked to the α-subunit, and the two molecules are bound to the β-subunits.
Biosynthesis of C-phycocyanin (Cpc)

Introducing the third pair of \(cpcB\) and \(cpcA\)

Se PCC 7942 chromosome

\[\begin{array}{c}
\text{cpcB1} \quad \text{cpcA1} \\
\text{cpcB2} \quad \text{cpcA2}
\end{array}\]

\[\text{WT} + \text{cpcB} / \text{cpcA}\]

Growth

Se PCC 7942

Lysis and Centrifugation

The contents of Cpc and Apc
Genetic engineering of *Arthrospira platensis*

This gene knock-out causes loss of motility.
Increasing the copy number of \textit{cpcB}/\textit{cpcA} enhances accumulation of C-phycoerythrin in \textit{Spirulina}.
Increasing the copy number of \textit{cpcB/cpcA} enhances accumulation of C-phycocyanin in Spirulina.

<table>
<thead>
<tr>
<th>50 L x 4 photobioreactors</th>
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<tbody>
<tr>
<td>WT</td>
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<tr>
<td>+cpcB/cpcA</td>
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Cell lysis
↓
Ammonium sulfate precipitation
↓

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<tr>
<th>WT</th>
<th>+cpcB/cpcA</th>
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<tr>
<td>OD620/OD280: 1.8 for WT; 2.5 for +cpcB/cpcA</td>
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Summary

1. We have developed methods to genetically manipulate *Arthrospira platensis*, which increases the commercial values of this production strain.

2. Introducing an additional copy of *cpcB* and *cpcA* promotes Cpc accumulation, indicating that an expression of Cpc subunits is a limiting step of Cpc biosynthesis.

3. Overproduction of Cpc has little impact on the growth of the two different cyanobacterial strains.

These results illustrate that findings using model strains can be translated to production strains.
Thank you!

We are here!