Engineering of *Nannochloropsis* for triacylglycerol biosynthesis

Biochemical engineering approach vs. Genetic engineering approach

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Why *Nannochloropsis*?

- Large scale cultivation
- Effluent cultivation
- Flue gas
- Available genetic tools
- Fast growth
- High lipid productivity
- Successful transformation
Biochemical engineering approaches to improve TAG accumulation in *Nannochloropsis*
Ultrastructure of *Nannochloropsis* under different conditions

LD, lipid droplets; N, nucleus; ST, stacks of thylakoids; V, vacuoles. Bars, 0.5 μm.

What happened?
A two-stage cultivation system was employed with biomass production in the first stage followed by lipid accumulation in the second stage.

High light (HL) and nitrogen deficiency (−N) were applied individually or in combination to *N. oculata* to investigate the underlying mechanism for stress-associated TAG synthesis.
Growth of *N. oculata* in response to stress conditions

**Graph a:**
- **LL+N**
- **LL-N**
- **HL+N**
- **HL-N**

**Graph b:**
- **LL+N**
- **LL-N**
- **HL+N**
- **HL-N**

**Graph c:**
- **LL+N**
- **LL-N**
- **HL+N**
- **HL-N**

**Graph d:**
- **LL+N**
- **LL-N**
- **HL+N**
- **HL-N**
Lipid accumulation of *N. oculata* in response to stress conditions

(a) Neutral lipid (mg/g DW) over time (days)
(b) TAG (mg/g DW) over time (days)
(c) Polar lipids (mg/g DW) for different treatments
(d) TAG sn-2 FA (%) for different fatty acids
C14:0, C16:0, C16:1 and C20:5 were the main fatty acids and together accounted for about 80% of total fatty acids.

HL–N gave rise to the highest level of NL derived fatty acid.
Biochemical activities of some key enzymes

Biochemical engineering factors (nitrogen and irradiicace) significantly influenced the TAG synthesis.

Stresses might promote the lipid biosynthetic pathways by pushing carbon flux to fatty acid synthesis and pulling fatty acids linked to TAGs for storage, thereby leading to enhanced lipid production.
Genetic engineering approach to improve TAG accumulation in *Nannochloropsis*
RNAi mediated silencing of PDK promoted TAG synthesis

- Acetyl-CoA is a carbon precursor channeled into *de novo* fatty acid synthesis.

- Pyruvate dehydrogenase kinase is a negative regulator of pyruvate dehydrogenase complex (PDHC).
Knockdown vector construction and transformation of *N. salina*

- Endogenous promoter β-tubulin
- Genomic PCR detection of bleomycin resistant fragment (464 bp), GUS region (997 bp) and 18S rDNA fragment (1029 bp) from transgenic *N. salina*. 
Transcript abundance of PDK, total lipid content and enzyme activity
No difference between transgenic *N. salina* Kd1-9 and control (Empty vector introduced cells)
Protein and carbohydrate content

- Protein content **decreased slightly** in transgenic *N. salina* Kd1-9
- **No difference** in carbohydrate content
TFA, TAG and polar lipid content

(a) Total fatty acid (pg/cell)
- Control
- Kd1-9

(b) TAG (pg/cell)
- Control
- Kd1-9

(c) Polar lipids (pg/cell)
- Control
- Kd1-9

Legend:
- MGDGDGTS
- PE
- DGDG
- PG
- SQDG
- PI

Significance:
- * p < 0.05
- ** p < 0.01
C14:0, C16:0, C16:1 and C20:5 were the main fatty acids and together accounted for about 80% of total fatty acids.

C14:0, C16:0, and C16:1 were the main fatty acids in TAG.

Knockdown of PDK led to an increase in C16:0 and decrease in C14:0, C16:1 and C20:5.
Transcriptional regulation of key genes
## Conclusions

<table>
<thead>
<tr>
<th>Nannochloropsis</th>
<th>Biochemical engineering</th>
<th>Genetic engineering</th>
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<tbody>
<tr>
<td>TAG accumulation</td>
<td>20-fold increase (HL-N)</td>
<td>Double</td>
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<tr>
<td>Cell proliferation</td>
<td>Impaired growth</td>
<td>Similar growth</td>
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<tr>
<td>Membrane lipids</td>
<td>Significantly decrease</td>
<td>Decrease differentially</td>
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<tr>
<td>Controllability</td>
<td>Easy-controlled</td>
<td>Random integration</td>
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<tr>
<td>Stability</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Applications and perspective</td>
<td>A better understanding of TAG biosynthetic pathway</td>
<td>Exploring industrial strains capable of producing high level of lipid at high cell densities</td>
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Thanks for your attention!