Quantitative transcriptome analysis reveals global regulation in response to CO$_2$ supplementation in oleaginous microalga *Coccomyxa subellipsoidea* C-169

Huifeng PENG, DongWEI*

*School of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, P.R. China*
Today, when we talk about renewable biofuels...

**Challenges: Energy Density**

<table>
<thead>
<tr>
<th>Fuel Type</th>
<th>Energy Density (Btu/gal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>76,330</td>
</tr>
<tr>
<td>Gasoline</td>
<td>116,090</td>
</tr>
<tr>
<td>Biodiesel</td>
<td>118,170</td>
</tr>
<tr>
<td>Diesel/Jet Fuel</td>
<td>128,545/135,000</td>
</tr>
</tbody>
</table>

**Oleaginous microalgae**

- quickly grow upon photosynthesis
- accumulate oil through assimilating CO₂
- gain high oil content and area yield
- renewable and environmentally friendly fuels

**Production Averages for Common Oil Crops**

<table>
<thead>
<tr>
<th>Plant</th>
<th>lb. oil/acre</th>
<th>Gallons of biodiesel/acre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae</td>
<td>6,757</td>
<td>700</td>
</tr>
<tr>
<td>Coconut</td>
<td>2,070</td>
<td>285</td>
</tr>
<tr>
<td>Jatropha</td>
<td>1,460</td>
<td>201</td>
</tr>
<tr>
<td>Rapseed</td>
<td>915</td>
<td>126</td>
</tr>
<tr>
<td>Peanut</td>
<td>815</td>
<td>112</td>
</tr>
<tr>
<td>Sunflower</td>
<td>720</td>
<td>99</td>
</tr>
<tr>
<td>Soybean</td>
<td>450</td>
<td>62</td>
</tr>
</tbody>
</table>
DOE, National Algae Biofuel Technology Roadmap 2010

Exhibit 10.5 The dynamic coupling and interdependencies across the algal biofuels and co-products supply chain

Market Externalities: Cost of Energy, Cost of Petroleum & Conventional Fuels, Demand & Price for Co-Products vs. their alternatives, etc.
Oleaginous microalgae

<table>
<thead>
<tr>
<th>Microalga</th>
<th>Oil content (% dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botryococcus braunii</td>
<td>25–75</td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>28–32</td>
</tr>
<tr>
<td>Cryptecodinium cohnii</td>
<td>20</td>
</tr>
<tr>
<td>Cylindrotheca sp.</td>
<td>16–37</td>
</tr>
<tr>
<td>Dunaliella primolecta</td>
<td>23</td>
</tr>
<tr>
<td>Isochrysis sp.</td>
<td>25–33</td>
</tr>
<tr>
<td>Monallanthus salina</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Nannochloris sp.</td>
<td>20–35</td>
</tr>
<tr>
<td>Nannochloropsis sp.</td>
<td>31–68</td>
</tr>
<tr>
<td>Neochloris oleoabundans</td>
<td>35–54</td>
</tr>
<tr>
<td>Nitzschia sp.</td>
<td>45–47</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>20–30</td>
</tr>
<tr>
<td>Schizochytrium sp.</td>
<td>50–77</td>
</tr>
<tr>
<td>Tetraselmis sueica</td>
<td>15–23</td>
</tr>
</tbody>
</table>
**Coccomyxa subellipsoidea C-169**

- Non-motile unicellular green alga, approximately 3 to 9 µm, and thin cell walls
- Isolated in the polar summer of 1959/60 at Marble Point, Antarctica, from dried algal peat
- DOE completed genome sequencing in 2012
  
  http://genome.jgi.doe.gov/Coc_C169_1/Coc_C169_1.home.html

- Be realized as a new model bioresource for algal oil production since it enables to accumulate triglycerides (TAGs) up to 58% DW under optimal condition

*Guillaume Blanc, et al. The genome of the polar eukaryotic microalga Coccomyxa subellipsoidea reveals traits of cold adaptation*
**Outline**

*Coccomyxa C-169 cultured with/without CO2*

Physiological study
- Cell growth
- Neutral lipid content
- Chlorophyll content
- Fatty acid profile

Transcriptomic analysis
- DEGs identification
- GO enrichment
- Pathway analysis
- Key regulatory nodes
Physiological study

Cell growth was prompted by CO$_2$ supplementation. 2% CO$_2$ seemed to enhance C-169 growth, and stimulate lipid accumulation.
Neutral lipid and chlorophyll content

- Lipid accumulation was boosted after the 4th day
- Chl a auto-fluorescence decreased roughly after the 4th day

Flow cytometry/Nile Red

Confocal laser scanning microscope/Nile Red
The maximal FA content with 2% CO₂ reached 48.5% DW on the 12th day;
The total C16 and C18 accounted for over 97% of total FA;
Oleic acid (C18:1) content was the most remarkable change as a result of CO₂
Cells from the 4th day cultured with 0.04% CO\textsubscript{2} (control group) and 2% CO\textsubscript{2} (treatment group) were subjected to mRNA extraction and Digital Gene Expression (DGE) analysis.

- Three biological replicates for each group;
- High correlation (Pearson’s r>0.9)
1737 differentially expressed genes (|log2 fold change| >1 and FDR<0.001) were identified, with 871 up-regulated and 866 down-regulated.
GO enrichment

DEGs were primarily associated with:

oxidation-reduction process
metabolic process
transmembrane transport
protein phosphorylation
ATP hydrolysis coupled proton transport

…
Several central pathways were significantly affected by CO$_2$ supplementation:

- Glycolysis/gluconeogenesis
- Citric acid cycle (TCA cycle)
- Oxidative phosphorylation
- Pentose phosphate pathway
- Fatty acid degradation
- Nitrogen metabolism
- CO$_2$ fixation

Pathway analysis
Enhanced pathways (up-regulated):

Glycolysis (1): two rate-limiting enzymes (PFK) and (PK)

TCA cycle (2)
Pentose phosphate pathway (3)
CO₂ fixation (4)
Represed modules (down-regulated):
Fatty acid degradation
Photosynthetic system I and II
Light-harvesting protein
Regulatory profile of metabolic pathways in response to CO₂ supplementation in C-169

Enhanced pathways guarantee adequate energy, reducing equivalent and precursors for anabolism with abundance of carbon source;

Some genes were down-regulated to improve the utility of nitrogen source and preserve the TAG storage.
Key nodes findings:

Phosphoenolpyruvate carboxylase (PEPCase): catalyzes the addition of bicarbonate (HCO$_3$-) to PEP, which can replenish oxaloacetate in the TCA cycle and subsequently is beneficial to the synthesis of FA and other biomolecules.

Argininosuccinate lyase (ASL): coordinates carbon and nitrogen metabolism; HCO$_3^-$, NH$_4^+$, and ornithine, with the involvement of ASL subsequently can be oriented to TCA cycle via aspartate-argininosuccinate shunt. It might be an important enzyme under high C/N ratio environment.

Vacuolar H$^+$-ATPase (V-ATPase): It is associated with cytoplasmic pH homeostasis; the subunits of V-ATPase were significantly over-expressed; it may be crucial for the adaption for high CO$_2$ concentration.
• The present study confirmed the potential of C-169 as a candidate for lipid production with CO\textsubscript{2} supplementation under optimal condition.

• Transcriptomic analysis showed that the C-169 adopted a fine-tuned strategy to sustain rapid cell growth and lipid production.

• Key metabolic nodes were found and might be bio-engineering targets for strain improvement.
Acknowledgements

National Science Foundation of China (NSFC, Grant No. 20876058, 2014-2017)
The Major State Basic Research Development Program of China (973 Project) (Grant No.

Huifeng Peng (PhD student)