Construction of A Platform for Fatty Acid Secretion from Chlorella

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Abstract. Concerns about petroleum supplies, high energy costs, and environmental pollutions are drawing considerable attention to find renewable biofuels. Biodiesel is produced from renewable biomass by transesterification of triacylglycerols from plant oils, yielding monoalkyl esters of long-chain fatty acids with short-chain alcohols. Production of biodiesel by the transesterification process using alkali catalysts has been widely accepted for its high conversion and reaction rates. However, such a process has also raised serious concerns about its impact on environment. In contrast, enzymatic transesterification has recently become the method of choice for biodiesel production as it produces high-purity product and enables easy separation of glycerol from the byproducts. Hence, more attention has been focused on the application of genetically engineered microalgae in the production of biodiesel. However, there is an upper limit in the lipid content of the microalgae (~50%). In addition, an energy-consuming step is required to break down the microalgae to extract the intracellular lipids. Up to date, the price of biodiesel is still much higher than that of petroleum-based diesel fuel. Therefore, a new strategy for producing low-cost biodiesel is imperative. To this end, we constructed a highly efficient fatty acid (FA) secretion system in chlorella, a genus of single-celled green algae. This strategy can not only reduce the cost of biodiesel by skipping the breaking-down step, but also aid in the development of a consolidated bioprocess.

Keywords: chlorella, biodiesel, fatty acid secretion.

1. Introduction

1.1. Chlorella
The name Chlorella is derived from the Greek word chloros, meaning “green” and the Latin diminutive suffix ella, meaning "small". They are typically small (2 to 10 μm in diameter), unicellular, coccoid, and nonmotile. Chlorella harbors the green photosynthetic pigments in its chloroplast. It multiplies rapidly and requires only carbon dioxide, water, sunlight, and a small amount of minerals to reproduce.

1.2. Biodiesel
Biofuels are a variety of fuels derived from biomass. Biodiesels are fatty acid methyl esters (FAMEs) made from transesterification of plant oils or fats. It is a liquid, similar in composition to fossil/mineral diesel [1]. In addition, biodiesel is the most common biofuel because it can be used in any diesel engine in any ratio. There are several important steps in order to make biodiesel from algae, including culture, fatty acid extraction, and transesterification [2]. Transesterification, a frequently used organic chemistry conversion technology, is the process of exchanging the organic group R'' of an ester with the organic group R' of an alcohol. In other words, it is a chemical process that triglycerides are reacted with methanol to yield glycerol and fatty acid methyl esters (FAME) [3].

1.3. Fatty acid secretion platform
There are many undergoing studies on increasing the biomass and oil content [4, 5]. However, fatty acid extraction is still tough and needs to be improved. Regardless of using pressure or ultrasonic to extract fatty
acids, they consume a great deal of energy. So, a new way is needed to solve this problem. There is little information on how the fatty acids are secreted from *Chlorella*. In contrast, there are many studies on how plants release fatty acids to extracellular matrix. We summarized here four mechanisms by which fatty acids can be secreted from plants or microorganisms (Fig. 1). The first is a carrier protein, which can bind hydrophobic substance and move to or through cell membrane [6]. The second is a channel protein. This type of protein contains at least one trans-membrane domain and is embedded in the cell membrane, becoming a channel. Substrates can pass through the membrane from inside to outside by the channel [7]. The third is a pump protein, which is typically a protein complex embedded in the cell membrane. Only some specific substrates can bind to the pump protein and pass through the cell membrane [8]. The last is an as-yet-undefined system, which releases fatty acid not by carrier protein, channel protein, or pump protein. For example, knock-down of fatty acyl-CoA synthetase in *S. cerevisiae* increases oil content with fatty acids secreted in culture medium [9].

### 1.4. Summary

Little is known about fatty acid secretion in *Chlorella*. Our primary goal is to create a highly efficient fatty acid secretion platform in *Chlorella* using one of the four systems mentioned above. The target genes of interest were cloned in appropriate plasmids (Table 1), and the resulting constructs were transformed into *E. coli* or *chlorella* for functional assays. The intracellular lipids were assayed by Nile red fluorescent staining (Fig. 2), and the ratio of the fatty acids between intracellular and extracellular matrixes was further analyzed by GC (gas chromatography) (Fig. 3). The data showed that a carrier protein from *Synechocystis sp. 6803* and *A. thaliana* significantly increases fatty acid secretion from *E. coli*.

![Mechanisms of fatty acid secretion](image)

**Fig 1. Mechanisms of fatty acid secretion.** FABP (Fatty Acid Binding Protein) and Lipid transfer protein (LTP) are carrier proteins that accelerate the transfer of fatty acids and lipids to cell membrane or outside. CER5 is a channel protein that ships fatty acids or lipids form inside to outside [10].

| Carrier protein |  
|-----------------|---|
| *A. thaliana*    | FABP-A |
*A. thaliana*  
FABP-B

*Synechocystis sp 6803*  
FABP-C

*Synechocystis sp 6803*  
FABP-D

**Other**

*A. thaliana*  
Acyl-ACP thioesterase A (FatA)

*A. thaliana*  
Acyl-ACP thioesterase B (FatB)

*E. coli*  
Lysophopholipase (tesA)

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**Fig 2. Neil red fluorescent staining.** Plasmids that carry target genes of interest were transformed into *E. coli*. The transformants were stained with nile red and visualized under a fluorescence microscope.

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**Lipid Distribution**

<table>
<thead>
<tr>
<th></th>
<th>Extracellular</th>
<th>Intracellular</th>
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<tbody>
<tr>
<td>Vector</td>
<td>94.76</td>
<td>5.24</td>
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<tr>
<td>AtFatA</td>
<td>97.21</td>
<td>2.79</td>
</tr>
<tr>
<td>EctesA</td>
<td>95.88</td>
<td>4.12</td>
</tr>
<tr>
<td>SspFABP-D</td>
<td>89.03</td>
<td>10.97</td>
</tr>
<tr>
<td>AtFABP-A</td>
<td>94.79</td>
<td>5.21</td>
</tr>
<tr>
<td>AtFABP-B</td>
<td>88.17</td>
<td>11.83</td>
</tr>
</tbody>
</table>

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Fig 3. Lipid distribution analysis by gas chromatography. E. coli was transformed with various genes from Synechocystis sp 6803 and A. thaliana, and the lipid distribution in the transformants was examined by gas chromatography.

2. Acknowledgements

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3. References


