



# Molecular challenges in microalgae towards cost-effective production of quality biodiesel



Young-Soo Chung<sup>a</sup>, Jin-Woo Lee<sup>b</sup>, Chung-Han Chung<sup>b,c,\*</sup>

<sup>a</sup> Department of Genetic Engineering, Dong-A University, Busan 604-714, South Korea

<sup>b</sup> Department of Biotechnology, Dong-A University, Busan 604-714, South Korea

<sup>c</sup> ReSEAT Program, Korea Institute of Science and Technology Information, Seoul 130-741, South Korea

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## ABSTRACT

Based on their environmental benefits, microalgae are currently the most favorable renewable biofeedstock materials for biodiesel production. However, the possibility of an economically viable production system using microalgae is still technology-driven, not yet market-driven due to its higher production cost. Accordingly, to establish industrial manufacturing systems for microalgal biodiesel, it is critical to develop technology for its cost-effective production. Here, we propose some novel molecular strategies, which have not been attempted for microalgal biodiesel production and are conducive to cost-effective production of biodiesel from microalgae. These include genetic manipulation strategies for higher biomass yield and extracellular production of free fatty acids, triacylglycerol, and fatty acid ethyl ester (biodiesel) with high quality, which could be exploited as a breakthrough technology for the cost-efficient production of microalgal biodiesel.

## 1. Introduction

### 1.1. Significance of microalgae in bioenergy sustainability and production

It is generally understood that net greenhouse gas savings and protection of biodiversity are the core elements in the concept of bioenergy sustainability. Based on this concept, the European Union (EU) has recently set stringent sustainability criteria for biofuels (for transportation vehicles) and bioliquids (for electricity and heating). The criteria ensure that such bioenergies cannot be produced from raw materials that are obtained from land with high biodiversity (EU Sustainability criteria). This sustainability issue suggests that the use of terrestrial plants as a biomass source for bioenergy production could be strictly restricted. As an alternative to terrestrial plant biomass, microalgae could be an excellent candidate for production of bioenergy, such as biodiesel, because microalgal biomass not only meets the sustainability criteria, but also has higher productivity in comparison with other feedstock materials. The additional benefit of microalgae in use as a biofuel feedstock is to address concerns over food security and competition for arable cropland, which are currently critical topic in the bioenergy manufacturing industry. Due to these benefits of microalgae, they have been exploited in various industrial sectors such as biofuel, food and feed [1–3]. The principal aim of this review is to

provide some novel molecular strategies conducive to cost-effective production of quality biodiesel from microalgae.

In view of the carbon footprint of biofuel production, microalgae are more efficient than terrestrial plant sources mainly due to their higher capability of fixing CO<sub>2</sub> emitted from carbon sources such as industry-sourced CO<sub>2</sub> and flue gases from fossil-fuel power plants [4–7]. Furthermore, microalgae can be used as a feedstock for production of value-added coproducts such as pharmaceuticals and food additives [8], and cultivated more sustainably in relation to water usage because the use of wastewaters derived from various sources and water reuse are possible depending on their cultivation system [9–11]. In particular, microalgae as feedstocks for biodiesel production have superior advantage over terrestrial plant biomass because of their higher capability of producing neutral carbon-rich lipids such as triacylglycerol (TAG) by utilizing solar energy and CO<sub>2</sub>. In addition, microalgae have rapid growth rates and very short harvesting cycles (~ 10 days), so they have very high biomass productivity and lipid accumulation [12]. At present, harnessing microalgae as a raw feedstock for economically viable production of biofuels is still technology-driven, not yet market-driven. However, in terms of their environmental benefits, it cannot be ruled out that in the near future microalgae will become one of the most promising source for producing green fuels [1,13].

\* Corresponding author at: ReSEAT Program, Korea Institute of Science and Technology Information, Seoul 130-741, South Korea.  
E-mail address: [chung@dau.ac.kr](mailto:chung@dau.ac.kr) (C.-H. Chung).

## 1.2. Strategic targets for cost-effective production of biodiesel from microalgae

Almost all transportation vehicles use petroleum-based fuels such as gasoline, diesel fuel, and liquefied petroleum gas (LPG). As an alternative to petroleum-based fuels, biodiesel, which is the product obtained by the catalytic conversion of TAG to its corresponding fatty esters, has environmentally acceptable properties including mitigation of exhaust emissions such as CO<sub>2</sub>, CO, sulfate, and others. Nonetheless, its commercial production is relatively small compared to other fuels, mainly due to its higher price [14]. A variety of technology developments to overcome the economic barrier to producing biodiesel have focused on some major cost-contributing factors such as feedstock availability, synthetic processing of biodiesel, and product extraction/recovery systems [14,15]. In particular, microalgal biodiesel has high potential as a cleaner biofuel, but the main bottleneck of its commercialization is its higher production cost compared with other feedstock-based biodiesel.

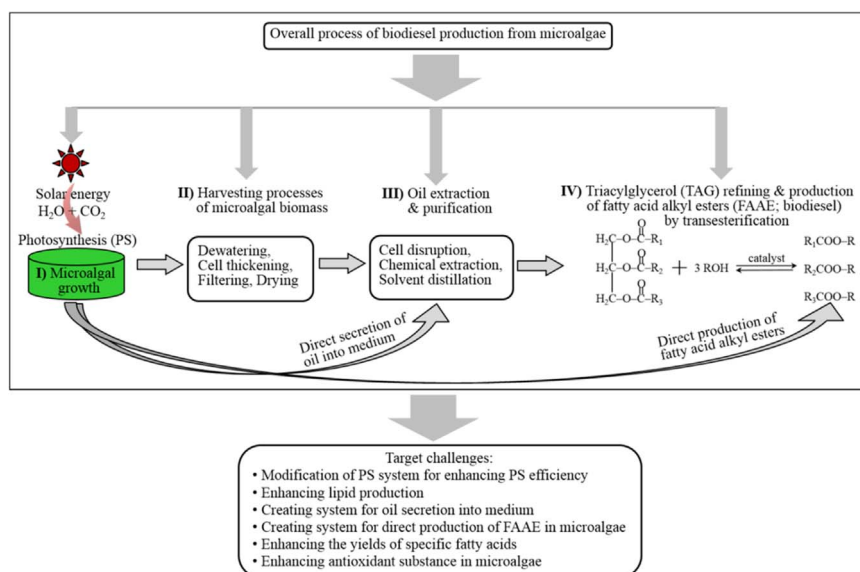
To cost-effectively produce biodiesel from microalgae, three pivotal cost-contributing factors are considered: i) availability of feedstock materials such as microalgal biomass and lipids, ii) synthetic processing/extraction procedure, and iii) production of quality microalgal biodiesel. These factors account for a large fraction of the total production cost of microalgal biodiesel (roughly > 80%) [14,16]. Accordingly, it is imperative that the majority of molecular efforts towards improving the economics of microalgal biodiesel are dedicated to these factors. To overcome these challenges, three key molecular schemes could be targeted: enhancing the yields of microalgal biomass and lipids as a feed substrate for biodiesel synthesis, minimizing synthetic processing/extracting processes of biodiesel products, and improving biodiesel quality for its engine performance. Because these processes contribute to high production cost of microalgal biodiesel, their elimination strategies could be the main genetic targets. In addition, some strategies to improve the biodiesel quality for diesel engine performance are also included in this article [17–19]. The molecular strategies for these challenges are outlined in Fig. 1 and their potential applications are addressed in the following sections.

## 2. Molecular strategies for improving microalgal cultures and lipid accumulation

As shown in Fig. 1, the prime molecular target of the process step I for promoting the cost-effective production of biodiesel from microalgae is to obtain improved microalgal cultures that are capable of producing high amounts of biomass and lipid feedstocks such as TAG for biodiesel production [20–22]. For genetically improving microalgae, a variety of bioengineering technologies have been introduced, such as RNAi gene silencing, homologous recombination, alteration of gene sequences, and multigene engineering. These technologies can be applied as a useful tool for genetically altering microdiesel [23–28].

The light condition in microalgal cultures could be an essential factor for the yield of increased biomass of microalgae because it directly affects their photosynthetic efficiency and thus it is a critical parameter for microalgal growth [2,5,29]. For example, under high light intensity of microalgal cultures, photodamage is frequently confronted due to excess excitation energy, which is derived from large size light-harvesting antenna complexes. These complexes resulted from an evolutionary adaptation of microalgae to maximize light absorption in order for microalgae to survive in low light environments. As a result, the damaged photosynthetic apparatus leads to reduced cell density of microalgae cultures due to insufficient light penetration into the inward parts of their culture medium.

To compensate for these problems in microalgal cultures, some RNAi tools have been employed to reduce the size of antenna complexes to enhance the photosynthetic efficiency, but their practical application is still in its infancy [20,30–32]. A new alternative challenge for improved photosynthetic efficiency in microalgae may be directed to overexpressing carbonic anhydrase (CA) in the CO<sub>2</sub>-concentrating mechanisms (CCMs) system because the enzyme is responsible for CO<sub>2</sub> accumulation around Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), which catalyzes CO<sub>2</sub> fixation in microalgae [33–35]. It is known that in microalgae, three types of CAs, α-, β and γ-CA, are present in specific locations. It is thought that carboxysomal α-Type CA may be responsible for microalgal CO<sub>2</sub> fixation because the CA plays a key role in the conversion reaction of bicarbonate to CO<sub>2</sub> in the microalgal CCMs system [36,37]. Based on this aspect, it may be possible to genetically manipulate the CA enzyme for enhancing photosynthetic efficiency, and thus could contribute to promoting microalgal cultures. A study has been attempted in the



**Fig. 1.** The synthesis processes of biodiesel from microalgae cultures and the molecular challenges for cost-effective production of biodiesel from microalgae. The biomass growth of photosynthetic microalgae is significantly affected by light conditions. Secretion of microalgal oils into their culture medium eliminates the harvesting step II, which is a costly process. Furthermore, the direct production of FAAE skips the intermediate steps II, III, and IV, thus significantly reducing the total production cost of biodiesel from microalgae.

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