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Theoretical lessons for increasing algal biofuel: Evolution of oil accumulation to avert carbon starvation in microalgae

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HIGHLIGHTS

- Oil accumulation by an alga that utilizes carbon and nitrate is modeled.
- Allocation ratio for the accumulation at ESS is determined.
- Direct trigger for accumulation is avoiding an increased death by carbon starvation.
- Nitrate limitation promotes the accumulation but is not a necessary condition.
- Strong carbon starvation and moderately limited nitrate will maximize total biofuel.

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ABSTRACT

Microalgae-derived oil is considered as a feasible alternative to fossil-derived oil. To produce more algal biomass, both algal population size and oil accumulation in algae must be maximized. Most of the previous studies have concentrated on only one of these issues, and relatively little attention has been devoted to considering the tradeoff between them. In this paper, we first theoretically investigated evolutionary reasons for oil accumulation and then by coupling population and evolutionary dynamics, we searched for conditions that may provide better yields. Using our model, we assume that algae allocate assimilated carbon to growth, maintenance, and carbon accumulation as biofuel and that the amount of essential materials (carbon and nitrate) are strongly linked in fixed proportions. Such stoichiometrically explicit models showed that (i) algae with more oil show slower population growth; therefore, the use of such algae results in lower total yields of biofuel and (ii) oil accumulation in algae is caused by carbon and not nitrate starvation. The latter can be interpreted as a strategy for avoiding the risk of increased death rate by carbon starvation. Our model also showed that both strong carbon starvation and moderately limited nitrate will promote total biofuel production. Our results highlight considering the life-history traits for a higher total yields of biofuel, which leads to insight into both establishing a prolonged culture and collection of desired strains from a natural environment.

1. Introduction

Proven oil reserves are estimated to be 1652.6 thousand million barrels and daily oil consumption is estimated to be 88,034 thousand barrels (Ruhl, 2012). If oil consumption continues at this rate, world oil reserves will be exhausted in 50 years. New sources of oil are continuously being searched for and found; therefore, it is unlikely that the world supply of fossil oil will be exhausted soon. However, oil is a finite resource and is certain to be exhausted at some point of time in the future. Thus, it is always important to find renewable oil to enable its continuous consumption by future generations. If renewable oil replaces fossil oil, it will also contribute to reduce the level of carbon dioxide, which is considered the main contributor for global warming (McCarthy, 2001; Solomon, 2007).

Producing renewable fuels is a task that requires immediate action in this century. Microalgae came into spotlight as fuel producers during the 1970s when the world faced an oil crisis (Regan and Gartside, 1983; Sheehan et al., 1998). Microalgae assimilate carbon by photosynthesis and accumulate carbon in the form of oils such as triacylglycerides. These accumulated oils yield biodiesel fuels (Georgianna and Mayfield, 2012) (hereafter, we will refer to this

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microalgal oil as biofuel). Algae possess numerous advantages over higher plants as being a source of biofuels, particularly for reducing the environmental load and are a competition for conventional agriculture (Chisti, 2008; Smith, 2010). However, in the 1970s, the prices of oil produced by algae were much higher than those of fossil-derived oils; therefore, the effort was abandoned (Haag, 2007). Thereafter, with the continuous rise in oil prices and the increasing global warming, “algae bloomed again” (Haag, 2007) and vast research and development resources are now being invested in studies seeking algae with high oil production efficiency (Georgianna and Mayfield, 2012).

Researchers looked for conditions leading to high biofuel accumulation within cells and found that deficiencies of nutrients such as nitrogen, phosphorus, and silicon were triggers for oil accumulation (e.g., Sheehan et al., 1998; Rodolfi et al., 2008; Griffiths and Harrison, 2009; Přibyl et al., 2014). Although the physiological/biochemical processes responsible for oil accumulation induced by nutrient deficiency are unclear (Přibyl et al., 2014), the underlying principle is that insufficient nutrients for protein synthesis necessary for growth channels would transfer the excess carbon produced during photosynthesis into storage molecules such as triacylglycerides (Rodolfi et al., 2008; Scott et al., 2010; Přibyl et al., 2014).

It was also found that nutrient deficiencies inhibited cell division, resulting in low population growth (Rodolfi et al., 2008; Scott et al., 2010). Because total biofuel yield depends both on the amount of biofuel in cells and the number of algae in a given population, maximizing the oil within cells does not necessarily maximize its total yield (Chisti, 2007; Rodolfi et al., 2008; Smith, 2010; Shurin et al., 2013).

With the aim of overcoming the tradeoff between growth rate and fuel accumulation by identifying the metabolic processes involved in fuel accumulation with the aid of genetic engineering, the mainstream of this research area has shifted to physiology and biochemistry (Sheehan et al., 1998; Shurin et al., 2013). Despite the lack of knowledge pertaining to the metabolic processes associated with nutrient deficiencies, considerable effort has been made to identify genes involved in higher growth rates and/or increased biofuel accumulation (Hu, 2008; Beer et al., 2009; Miller et al., 2010; Radakovits et al., 2010, 2012; Rismani-Yazdi et al., 2012; Tanaka et al., 2015). If genes with higher performance are identified, would it then be possible that a strain with a high growth rate and high biofuel accumulation in cells could be used to establish a culture system for high biofuel yield? This task may not be so easy.

Cell division and fuel accumulation require certain resources. In microalgae, these resources assimilate carbon by photosynthesis and nutrient uptake. Because available resources are always limited, it is natural that microalgae will allocate these resources in a manner to maximize their fitness (Maynard-Smith and Price, 1973). Thus, although “good” genes are incorporated into a microalga, these genes may not function well unless the resources are allocated for gene expression. Together with these considerations, to achieve higher biofuel yield from microalgal culture, it is important to consider the adaptation underlying the microalgal allocation of resources (Bull and Collins, 2012; Shurin et al., 2013).

On a time scale much longer than the average lifetime of algae, biofuel accumulation may be acquired through an evolutionary process. What are the function and the adaptive advantages of oil accumulation in algae? It is natural to consider that its main function is the storage of energy and carbon (but see Solovchenko, 2012). Intuitively, because nutrient deprivation inhibits cell division, carbon assimilated by photosynthesis and not used for cell division (the portion of carbon not used for cell growth is termed “surplus carbon” throughout this paper) is expected to be translocated to organelles for storage as oils (e.g., Roessler, 1990; Rodolfi et al., 2008; Scott et al., 2010; Přibyl et al., 2014). This verbal argument is commonly accepted as conventional wisdom by the microalgal biochemistry community and leads to the following speculation: the adaptive purpose of oil accumulation is that surplus carbon resulting from nutrient deprivation would be used for storage (Solovchenko, 2012). However, this speculation is not so straightforward, given that this surplus carbon can be used for other purposes such as maintaining life by ATP synthesis. Algae that do not accumulate these fuels probably allocate this surplus carbon to maintain life. Thus, oil accumulation is an adaptive strategy for a specific environment, wherein these algae have higher fitness. Accordingly, it is important to identify the types of selection pressures directed towards the evolution of accumulation depending on environmental conditions.

Previous studies based on theoretical evolutionary ecology have shown the possibility that food deprivation promoted the evolution of energy storage. For example, Shertzer and Ellner (2002) analyzed alga-rotifer (prey-predator) dynamics, and showed that energy storage of rotifers would evolve to overcome periodically or chaotically fluctuating algal density. Kooi and Troost (2006) analyzed the similar dynamic but assumed an external periodic supply of algae that are non-vital. However, these studies considered binary allocation: to store or to consume immediately. Alga uses carbon and nitrate as essential resources. It is considered that the amount of these two resources are dependently interacted each other since alga is composed of carbon and nitrate brought together in a nonaribitrary proportion (C/N ratio). Therefore, ecological-stoichiometry model that explicitly incorporates the fixed proportion and the conservation of mass in chemical reactions (Elser et al., 2012) is suited for describing dynamics in alga. With incorporating the usage of two resources into population dynamics, optimal life-history trait responsible for accumulation is little known. Although, using such stoichiometric models, several studies have investigated how organisms use multiple resources to maximize their growth rates (e.g., Klausmeier et al., 2007; Litchman et al., 2007; Bonachela et al., 2013), energy storage as a risk-hedging strategy to avert future food deprivation in the presence of multiple resources has not been considered.

In this study, we considered the allocation of two resources (carbon and nitrate) and sought an evolutionary optimum. We also aimed to identify a condition leading to the maximum cultivation of oil from algae. For this purpose, not only the amount of stored oil within an alga but also the number of algal individuals must be considered because the total yield of oils is multiple of these two.

By artificially strengthening selection pressure, we can generally select algae with increased accumulations of biofuel. Although we cannot predict the direction in which this evolution will occur, it is natural to expect that it will occur in the direction of rapid growth if there are sufficient resources in the culture environment (Bull and Collins, 2012), i.e., natural selection favors individuals that allocate far more resources to cell division than to oil storage. Establishing a culture system that prevents microalgal strains from evolving in this undesired direction would be required.

Here we theoretically model physiological responses in microalgae and investigate conditions that can lead to the evolution of increased biofuel accumulation. Our model explicitly includes a tradeoff for a resource allocated for growth, fuel accumulation, and the maintenance of life. Based on the analyses of population dynamics, we investigated the optimum balance of allocations for both growth and accumulation such that accumulation could be maximized. We proposed a hypothesis for the adaptive reasons that explained the evolution of biofuel accumulation.

2. Modeling

We used a simple resource allocation model whose simplest case has been well illustrated by Gurney and Nisbet (1998). We modeled (1) algal population dynamics and (2) carbon dynamics within an alga. These two models were coupled and followed by the