



Review

Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches

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ABSTRACT

This paper compares three possible strategies for enhanced lipid overproduction in microalgae: the biochemical engineering (BE) approaches, the genetic engineering (GE) approaches, and the transcription factor engineering (TFE) approaches. The BE strategy relies on creating a physiological stress such as nutrient-starvation or high salinity to channel metabolic fluxes to lipid accumulation. The GE strategy exploits our understanding to the lipid metabolic pathway, especially the rate-limiting enzymes, to create a channelling of metabolites to lipid biosynthesis by overexpressing one or more key enzymes in recombinant microalgal strains. The TFE strategy is an emerging technology aiming at enhancing the production of a particular metabolite by means of overexpressing TFs regulating the metabolic pathways involved in the accumulation of target metabolites. Currently, BE approaches are the most established in microalgal lipid production. The TFE is a very promising strategy because it may avoid the inhibitive effects of the BE approaches and the limitation of “secondary bottlenecks” as commonly observed in the GE approaches. However, it is still a novel concept to be investigated systematically.

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1. Introduction

Biodiesel is one of the most promising renewable transportation fuels that have achieved remarkable success worldwide. According to a World Bank report (2008), 6.5 billion litres of biodiesel was produced worldwide in 2006, 75% of which by the European Union and 13% by the USA. The current contribution of biodiesel to global transportation fuel consumption is, however, only 0.14% and the favourable policies of major countries in the world are expected to increase this contribution by 5 times by 2020. It is therefore predictable that massive global demand on renewable energy will continue to drive the rapid growth of biodiesel production in an unprecedented scale. Nevertheless, current increase of food prices worldwide had brought about public awareness and concerns regarding the competition for agricultural resources between the food industry and the energy sector. Development of sustainable and cost-effective alternatives to the traditional agricultural and forestry crops is therefore of urgent need for sustainable biofuel production.

Oil-rich microalgae have been demonstrated to be a promising alternative source of lipids for biodiesel production (Chisti, 2007; Li et al., 2008b; Song et al., 2008; Walker et al., 2005b; Wang et al., 2008). There seems to be little doubt that fast growing microalgae should be able to provide enough renewable biofuels for the replacement of fossil transportation fuels (Li et al., 2008b). An integrated strategy was proposed to enhance the economical cost-effectiveness and environmental sustainability by combining the benefits of biofuel production, CO₂ mitigation, waste heat utilization, wastewater treatment and novel bioproduct production using the microalgal cultivation processes (Li et al., 2008b; Wang et al., 2008). Nevertheless, significant challenges remain in the economics of microalgal biodiesel production and extensive studies have been carried out to cope with these challenges. In this minireview, we focus on the progress, challenges, and future perspectives of lipid overproduction using microalgae by different approaches, including the BE, GE, and the emerging TFE approaches.

2. Biochemical engineering approaches

The BE approach here refers to the strategy of enhancing lipid production of microalgae by controlling the nutritional or cultivation conditions (e.g., temperature, pH, and salinity) to channel metabolic flux generated in photobiosynthesis into lipid biosynthesis. Nutrient-starvation has so far been the most commonly employed approach for directing metabolic fluxes to lipid biosynthesis of microalgae. In this scenario, microalgae accumulate lipids as a means of storage under nutrient limitation when energy source (i.e., light) and carbon source (i.e., CO₂) are abundantly available and when the cellular mechanisms for the photobiosynthesis are active. While a number of nutrients such as phosphorus and iron deficiency have been reported as being able to cause cell growth cessation and channel metabolic flux to lipid/fatty acid biosynthesis, nitrogen is the most commonly reported nutritional limiting factor triggering lipid accumulation in microalgae.

Nitrogen-starvation has been observed to lead to lipid accumulation in a number of microalgal species. For instance, *Chlorella* usually accumulates starch as storage material. However, it was

observed by Illman et al. (2000) that *C. emersonii*, *C. minutissima*, *C. vulgaris*, and *C. pyrenoidosa* could accumulate lipids of up to 63%, 57%, 40%, and 23% of their cells on a dry weight basis, respectively, in low-N medium. *Neochloris oleoabundans* was reported, under nitrogen deficient conditions, to be able to accumulate 35–54% lipids of its cell dry weight and its TAGs comprised 80% of the total lipids (Kawata et al., 1998; Tornabene et al., 1983). It was also observed (Yamaberi et al., 1998) that the TAGs accumulated in *Nannochloris* sp. cells could be 2.2 times as that in the cells in nitrogen sufficient cultures. Our studies (Li et al., 2008a) showed that sodium nitrate was the most favourable nitrogen source for cell growth and lipid production of *N. oleoabundans* among the three tested nitrogen-containing compounds, i.e., sodium nitrate, urea, and ammonium bicarbonate. It was observed that lipid cell contents decreased with the increase of sodium nitrate in the medium in the range of 3–20 mM. The trend that lower nitrogen source concentration in medium led to higher lipid cell content was hypothetically explained by the fact that nitrogen would have exhausted earlier at low cell density when the initial concentration of nitrogen source in medium was low. As a result, cells started to accumulate lipid when light had good penetration (at low cell density), when individual cells were exposed to a large quantity of light energy, resulting in more metabolic flux generated from photosynthesis to be channelled to lipid accumulation on an unit biomass basis.

Phosphate limitation was also observed to cause enhancement of lipid accumulation of *Monodus subterraneus* (Khozin-Goldberg and Cohen, 2006). With decreasing phosphate availability from 175 to 52.5, 17.5 and 0 μM (K₂HPO₄), the cellular total lipid content of starved cells increased, mainly due to the drastic increase in TAG levels. In the absence of phosphate, the proportion of phospholipids was reduced from 8.3% to 1.4% of total lipids, and the proportion of TAG increased from 6.5% up to 39.3% of total lipids. Furthermore, iron deficiency has also been reported to stimulate lipid accumulation in microalgae *Chlorella vulgaris*, which accumulated up to 56.6% lipid of biomass by dry weight under the optimal condition (1.2×10^{-5} mol FeCl₃) (Liu et al., 2008).

In addition to nutrient-starvation, other stress conditions may also cause enhanced accumulation of lipids in microalgae. For instance, Takagi et al. (2006) observed that TAG content increased in *Dunaliella*, a marine alga, under high salinity conditions. In that research, an initial NaCl concentration higher than 1.5 M was found to markedly inhibit cell growth. However, when the initial NaCl concentration increased from 0.5 (equal to seawater) to 1.0 M, it resulted in a higher intracellular lipid content (67%) in comparison with 60% for the salt concentration of 0.5 M. Addition of 0.5 or 1.0 M NaCl at mid-log phase or the end of log phase during cultivation with initial NaCl concentration of 1.0 M further increased the lipid content to 70%.

An inherited disadvantage of the BE strategy is, however, nutrient-starvation or the physiological stress required for accumulating high lipid content in cells is associated with reduced cell division (Ratledge, 2002). Since lipids are intracellular products, the overall lipid productivity is the product of cell lipid content multiplied by biomass productivity. The overall lipid/energy productivity will therefore be compromised due to the lowered biomass productivity. For instance, Scragg et al. (2002) studied the energy recovery

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