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Review

Manipulating environmental stresses and stress tolerance of microalgae for enhanced production of lipids and value-added products–A review

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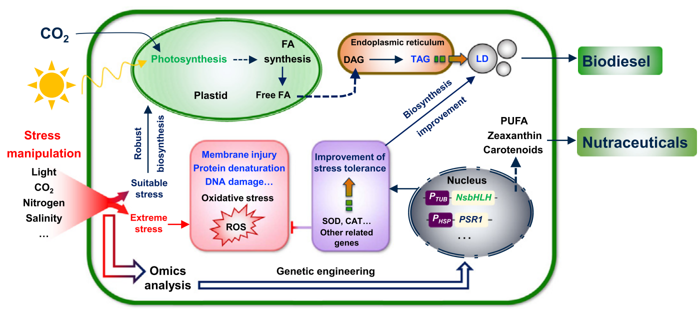
HIGHLIGHTS

- Stress modulation by process engineering to improve production level was reviewed.
- Molecular mechanisms underlying stress responsive lipid production was summarized.
- Metabolic engineering methods to improve microalgae biorefinery are discussed.

GRAPHICAL ABSTRACT

Enhanced production of valuable molecules in microalgae via deoxidization and genetic engineering.



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ABSTRACT

Microalgae have promising potential to produce lipids and a variety of high-value chemicals. Suitable stress conditions such as nitrogen starvation and high salinity could stimulate synthesis and accumulation of lipids and high-value products by microalgae, therefore, various stress-modification strategies were developed to manipulate and optimize cultivation processes to enhance bioproduction efficiency. On the other hand, advancements in omics-based technologies have boosted the research to globally understand microalgal gene regulation under stress conditions, which enable further improvement of production efficiency via genetic engineering. Moreover, integration of multi-omics data, synthetic biology design, and genetic engineering manipulations exhibits a tremendous potential in the betterment of microalgal biorefinery. This review discusses the process manipulation strategies and omics studies on understanding the regulation of metabolite biosynthesis under various stressful conditions, and proposes genetic engineering of microalgae to improve bioproduction via manipulating stress tolerance.

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Contents

1. Introduction

Biofuels are considered as a clean alternative to fossil fuels owing to their advantages of sustainability, non-toxicity, biodegradability and lower emission of greenhouse gas (GHG) (Man and Lee, 2012). The fact that first generation biofuels (from food and oil crops) and second generation biofuels (from non-food crop feedstocks, agriculture, and forest residues) are constrained by the competition with either food production or arable land has emphasized the importance of using alternative feedstocks, among which microalgae are attractive options (Brennan and Owende, 2010). Microalgae offer many superiorities over traditional oil crops to produce biofuels and high-value chemicals, such as hyper lipid productivities and photosynthetic efficiencies, robust environmental adaptability, no competition with food or arable land, rapid fixation of environmental carbon, cultivation on wastewater and year-round cultivation (Ho et al., 2014b). Nevertheless, high cost in microalgae-based biodiesel has constrained the economic competitiveness and significantly restricted their industrialization. Therefore, biofuels production associated with high-value products has been proposed for microalgal biorefinery (Su et al., 2017).

Microalgae-based biorefinery starts with strains cultivation, biomass harvesting, cell disruption and compound extraction, then fractionation and purification, aiming to separate and recover the desired molecules (e.g., lipids, carbohydrates, protein, and pigments) from the same batch of microalgal biomass and *ipso facto* reducing the production cost (Chew et al., 2017). For example, further fractionation of omega 3 fatty acids, carotenoids and astaxanthin are suggested to follow the separation of lipids, proteins, and carbohydrates after mild cell disruption (Vanthoor-Koopmans et al., 2013). However, there are still great challenges to achieve economic microalgal biorefinery.

Environmental stresses, such as nitrogen, pH, salinity and light, are commonly used to promote the accumulation of lipid and carbohydrates, as well as valuable compounds (i.e., carotenoids) (Cheng and He, 2014; Markou and Nerantzis, 2013; Minhas et al., 2016). For instance, neutral lipids (mainly triacylglycerides, TAG) in *Neochloris oleoabundans* HK-129 reached 51.6 mg L^{-1} d^{-1} under nitrogen limitation and high light intensity, a typical approach applied in the laboratory and pilot-scale for microalgal biorefinery (Sun et al., 2014). However, stressful conditions often adversely affect the microalgal growth and eventually lower the yield of desired products. It is demonstrated that 2-fold increase in biomass productivity contributes to 41–42% decrease of biodiesel price, which reflects the significance of manipulating environmental stresses to achieve both improved accumulation of target molecules and biomass (Nagarajan et al., 2013). Unraveling the stress tolerance mechanisms and reorient the metabolic pathways will contribute to sustainable development of microalgae biorefinery (Guihéneuf et al., 2016). However, so far most studies only focus on process optimization using stress manipulation strategy, there is a need to combine microalgal strain development and process integration for efficient bioproduction.

Recent advances in microalgal omics (genomics, transcriptomics, proteomics, and metabolomics) have deepened the understanding of genes regulation, proteins function, protein-protein interaction, and metabolites change involved in the biosynthesis of valuable molecules in microalgae (Guarnieri and Pienkos, 2015). Hence, omics analysis results benefit microalgal strain development to manipulate the carbon and nitrogen flux under stressful conditions to improve the productivity of desired biochemicals.

This review focuses on the recent trends to manipulating environmental stresses and stress tolerances of microalgae to produce various compounds. We propose that an enhanced efficiency of microalgae biorefinery may be achieved through innovative stress manipulation via integration of ambient stresses, omics technologies and genetic engineering for sustainable and cost-effective production using microalgae (Fig. 1).

2. Manipulating different stresses to improve microalgal lipid production

2.1. Manipulation of major nutrient stresses to enhance lipid production

Nitrogen is one of the most important nutrients affecting microalgal growth and lipid yield. Nitrogen starvation has been intensively studied to increase lipid content in microalgae. For instance, *Chlorella vulgaris* NIES-227 contained 89% fatty acid methyl esters in microalgal dry cell weight (DCW) under nitrogen-starved conditions even glucose feeding was mandatory (Shen et al., 2015), *Nannochloropsis* sp. F&M-M24 doubled its lipid content during nitrogen limitation and reached 60% (by DCW) phototrophically (Rodolfi et al., 2009), while *Acutodesmus dimorphus* accumulated 75% neutral lipid of total lipids under certain nitrogen-starvation conditions (Chokshi et al., 2017). Nitrogen deprivation can induce the biosynthesis of more TAGs and an overall decrease of polar lipids in *Nannochloropsis* sp. (Martin et al.,