The Influences of Nitrogen and Carbon source on Oil Accumulation and the Analysis of Metabolic Pathway in Microalgae

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Abstract: It is an inexorable trend that the depletion of nonrenewable fossil fuels is replaced by renewable and sustainable biodiesel. Some microalgae can accumulate considerable amounts of lipids under different nutrient-deficient conditions so that they are ideal feedstock for biofuel producer. This review focuses on the effects of nitrogen stress and carbon source changes on oil accumulation and the analysis of function and expressional regulation of key genes in the relevant metabolic pathways to provide references for oil producing microalgae genetic engineering modification.

With the development of global economy, the worldwide demand for energy is increasing day by day. Energy crisis has become a key problem facing the whole world. The development of renewable and environmentally friendly alternative fuels has become one of the main bottlenecks in the field of energy engineering. As pollution-free and renewable energy resources, bio-energy has attracted more and more attention, which can not only alleviate the energy crisis, but also achieve sustainable economic development [1].

Chlorella is an important raw material for the preparation of biodiesel due to its wide ecological distribution, easy cultivation, fast growth rate and rich oil content [2]. In this paper, chlorella was taken as an example to illustrate the effects of nutrient elements on oil accumulation in chlorella and the regulation mechanism, to provide reference for future study.

1. Nitrogen starvation promoted oil accumulation in chlorella

As the most abundant element in cells, nitrogen is the most important factor affecting the accumulation of microalgae oil, and nitrogen deficiency will lead to the oil accumulation [3,4]. Jianhua Fan et al. [5] reported that there was no significant difference in the growth rate of nitrogen removal *Chlorella Pyrenoidosa* the first 3 days. However, from the 4th day, the growth was obviously hindered and the contents of total and neutral fatty acids accumulated sharply. The lack of nitrogen caused a sharp decrease of chlorophyll B and light harvesting complex protein, which led to the reduction of photosynthetic efficiency significantly. The expression 1,5 bisphosphoribulose carboxylase (RuBisCO), phosphoenolpyruvate carboxylase (PEPC), malic enzyme (ME) were down-regulated, but Acetyl CoA carboxylase (ACC) and diacylglyceryl transferase (DGAT) decreased significantly were up-regulated.

In summary, nitrogen deficiency triggers the degradation of some proteins to provide nitrogen source for maintaining the necessary metabolism. Therefore, in the initial stage of nitrogen deficiency, algal cells can continue to grow, but the growth rate is slowed down significantly. The carbon used for cell construction originally is transferred to lipid synthesis through EMP pathway, TCA cycle, pyruvate metabolism and fatty acid synthesis pathway. Acetyl CoA plays a very important role in providing carbon framework and energy for the synthesis of fatty acids [6,7]. Pyruvate dehydrogenase, which catalyzes pyruvate to produce acetyl-CoA, links glycolysis to the TCA cycle. ME is a major donor of NADPH for the synthesis of fatty acids, providing both NADPH and pyruvate which is the

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precursor of acetyl-CoA [8,9]. These enzymes are closely related to lipid accumulation, and the encoding genes are up-regulated when nitrogen is limited.

2. Carbon source affects lipid accumulation in microalgae

2.1 The addition of carbon source promoted the oil accumulation in chlorella

The cell concentration and oil content of chlorella were significantly increased in the dark with adding carbon source [10,11]. The carbon sources that chlorella can use is extensive, including glucose, acid and acid salts, glycerin, starch, etc. (Table 1). Andruleviciute et al. [12] added glycerol and glucose for chlorella culture and oil production, and found that the growth rate and oil production increased significantly. C. Protothecoides can be heterotrophic and mixed cultured not only with glycerol or glucose substrate, but also with acetate substrate, and all grow better than photoautotrophs [19].

Table 1 Carbon Source use to chlorella Cultivation

Microalgal specie	Result	Carbon source
Chlorella sorokiniana[11]	Biomass, Lipids↑	Glucose
Chlorella sp. [12]	Biomass↑ , oil↑	Glycerol and Glucose
Chlorella saccharophila[13]	Biomass↑ , oil↑	glucose and glycerol
Chlorella protothecoides[14]	Lipids ↑	Glucose
Chlorella protothecoides[15]	Lipids↑	Jerusalem artichoke hydrolysate
Chlorella protothecoides[16]	Biomass↑, Lipids ↑	Cassava starch hydrolysate
Chlorella protothecoides[17]	oil↑	Molasses hydrolysate
Chlorella pyrenoidosa[18]	Biomass↑	Food waste hydrolysate
Chlorella vulgaris[19]	lipid productivity ↑	Acetate and crude glycerol

2.2 Metabolic path analysis of carbon sources

The utilization of added carbon such as acetic acid, glucose, lactic acid need the synergistic action of multiple enzyme systems involved multiple metabolic pathways in chlorella, as shown in fig. 1 [20]. The utilization of carbohydrates is usually catalyzed by hexokinase. The glycerol is first phosphorylated and oxidized to glyceraldehyde 3-phosphate, which forms pyruvate through the EMP pathway, and then enters the TCA cycle. 3-phosphoglyceraldehyde can also be obtained by the reduction of 3-phosphoglycerate in the Calvin cycle. Lactic acid is reduced by D-lactate dehydrogenase to pyruvate. Acetyl-CoA is generated by acetyl-CoA synthase using one molecule of ATP, and then enters the glyoxylic acid cycle pathway. Compared with TCA cycle, glyoxylic acid cycle requires two special enzymes: malate synthase and isocitrate lyase. Syrett et al. found that glyoxylic acid cycle existed in chlorella Vulgaris, and the activities of malate synthase and isocitrate lyase were significantly increased when acetate was added to autotrophic cells under dark conditions [21]. Glucose and other related carbohydrates are metabolized through the pentose phosphate pathway (PPP) and glycolysis (EMP) pathways. The PPP pathway is important for carbon metabolism because most of the enzymes on this pathway are also involved in the Calvin cycle.

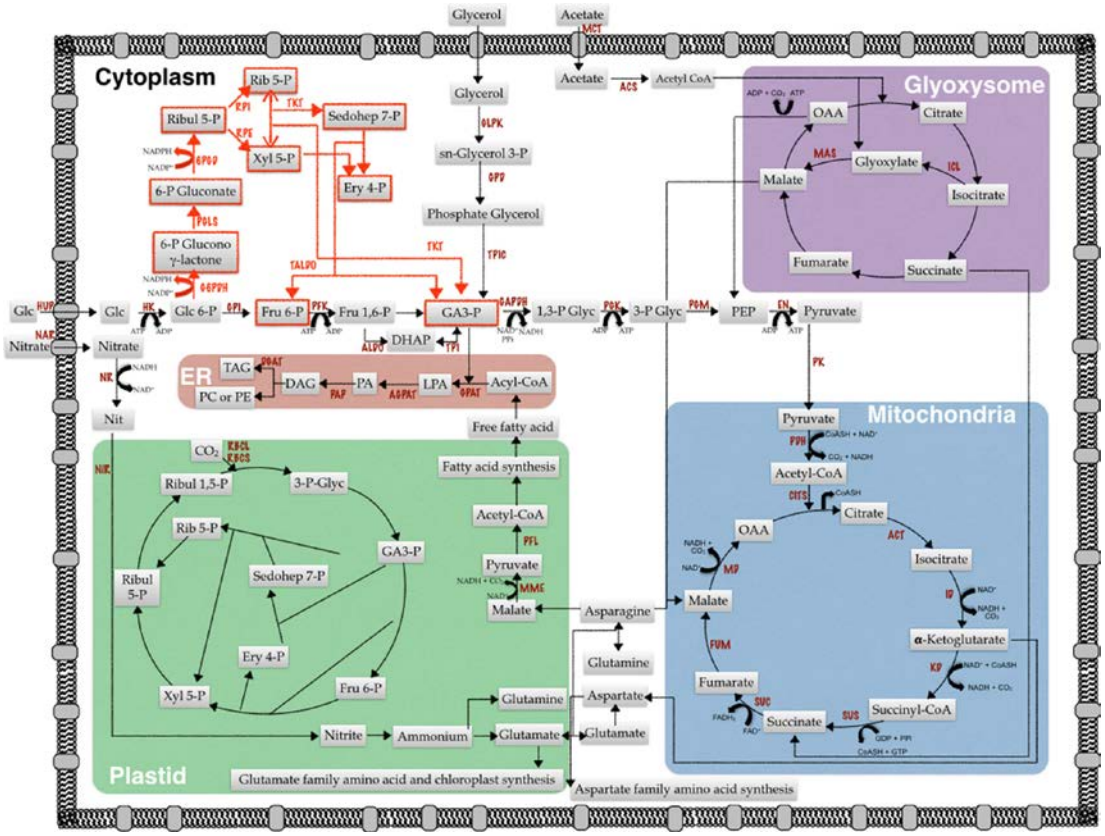

Fig. 1 Overview of the Metabolic Pathways of Heterotrophic Microalgae

2.3 Regulation of lipid synthesis under heterotrophic culture conditions

Gao et al. revealed the difference of carbon metabolism pathways between autotrophic and heterotrophic of *c. protothecoides* by the study of genome, transcriptome and proteome [22]. Under heterotrophic conditions, proteins and RNA involved in photosynthesis and carbon CO_2 were almost degraded, and glucose was transported to the cell by glucose carrier. Chlorella grown without influence, and large amounts of glucose were used for energy supply and fatty acid synthesis. Glucose was converted into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate through glycolysis pathway. Part of dihydroxyacetone phosphate was converted into pyruvate, and then flows into the synthesis of fatty acids. And the others were catalyzed by glycerol 3-phosphate dehydrogenase to produce glycerol 3-phosphate for triglyceride production. The upregulation of two isoenzymes of glycerol 3-phosphate dehydrogenase ensures the conversion of dihydroxyacetone phosphate to glycerol 3-phosphate for TAG synthesis. In order to meet the demand for dihydroxyacetone phosphate, microalgae adopted the mechanism of deactivation of triosephosphate isomerase (TPI) like in higher plants. The loss of TPI can increase the content of lipids in *Arabidopsis* root cells [23], which may be the key enzyme regulating carbon flow to fatty acid synthesis. Almost all enzyme involved glycolytic including speed limit enzymes are increased obviously in heterotrophic cells. And TCA cycle is also increased, which may lead to the higher glucolysis ability and oil accumulation in *C.protothecoides.* Acetyl-CoA carboxylase is also a key enzyme that regulates the carbon flow to fatty acid synthesis. In addition, most enzymes involved in fatty acid biosynthesis and subunits of fatty acid synthase catalyzing fatty acid chain lengthening were also up-regulated. The cell was prone to fatty acid synthesis, and lipid droplet proteins were significantly up-regulated.

3. Conclusions

In addition to nitrogen and carbon source, phosphorus light intensity could affect the oil accumulation of chlorella, but the degree of influence was differed among species. It was found that phosphorus deficiency affected the growth of chlorella, but the oil content increased slightly [25,26]. Lipid accumulation in microalgae cells involves many enzyme systems in multiple metabolic

pathways. It is not obvious to increase lipid accumulation by regulating the activity of single enzyme. Scholars proposed transcription factor engineering approaches (TFE) based on the whole cell level to regulate metabolism [27,28]. Transcription factors regulate the biosynthesis of target products by affecting a large number of genes in multiple metabolic pathways, which has been extensively studied in animal, plant, and microorganisms. Regulation of lipid accumulation by transcription factors will be an important approach in the future.

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