DOCTORAL THESIS

Characterization of lutein biosynthesis in green alga chlorella pyrenoidosa under heterotrophic condition

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Characterization of Lutein Biosynthesis in Green Alga *Chlorella pyrenoidosa* under Heterotrophic Condition

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Abstract

Lutein as a naturally occurring pigment has been widely applied in food industry due to its safety as well as properties beneficial to human health. At present the production of lutein from plant sources, such as marigold, is however limited by planting area, weather and high manpower costs. In contrast, green alga *Chlorella pyrenoidosa* (*C. pyrenoidosa*) which also contains lutein can survive under heterotrophic conditions has been regarded as an alternative producer of lutein due to its potential for large-scale industrial production in stainless-steel fermentors. The lutein content in *C. pyrenoidosa* under heterotrophic conditions however, is very low and decreases gradually with the elongation of culture time and the loss of light stimulation. In this study I investigated the characteristics of lutein biosynthesis and its relationship with chlorophyll biosynthesis *C. pyrenoidosa* grown in the dark. Moreover, I studied also the preliminary mechanism for the retrograde regulation of lutein biosynthesis by mitochondria under heterotrophic conditions. In addition, I also experimented with different ways to enhance lutein production in *C. pyrenoidosa* grown in the dark.

My results showed that glucose assimilation favored the cell biomass production, but not cellular lutein accumulation. Up-regulated lipid production induced by nitrogen deficiency was possibly the real reason for the glucose bleaching. The biosynthesis of lutein and chlorophyll was not strictly synchronized in *C. pyrenoidosa* under heterotrophic conditions. The proteomic analysis showed that chlorophyll deficiency had a great influence on the abundance of minor antenna protein CP26, CP29 in the chloroplast of *C. pyrenoidosa*. The major antenna protein LhcbM3 of light-harvesting complex Ⅱ is likely to have a close relationship with the accumulation of lutein and was not

greatly influenced by the presence of chlorophyll.

I further investigated the effect of some metabolic stimulators and intermediates on lutein production in *C. pyrenoidosa* grown in the dark. Vitamin A showed profound effect in stimulating lutein biosynthesis, with an increase of 70.9% of lutein content when 4 mM vitamin A was added at the beginning of the cultivation. β-carotene and zeaxanthin were also found to have positive effect on lutein accumulation, lutein content increased by 27.1% and 19.8% respectively with the addition of 0.05 mg/mL of zeaxanthin and β-carotene into the medium.

In addition, I also investigated the influence of different types of abiotic stress on lutein biosynthesis in *C. pyrenoidosa.* It was found that externally-introduced oxidative stress couldn't stimulate the lutein production in *C. pyrenoidosa* grown in the dark, but improved the lutein accumulation in photoautotrophically cultivated algal cells. In addition, cold stress showed significant effect on stimulating lutein production in mutant 15-2, a chlorophyll deficient *C. pyrenoidosa*, under heterotrophic conditions.

Finally, the role of mitochondrial respiratory electron transport chain (mRET) in retrograde regulation of lutein, chlorophyll biosynthesis and chloroplast development in *C. pyrenoidosa* grown in the dark was also investigated through mitochondrial dysfunction and proteomic analysis. My results showed that inhibition of alternative oxidase of mRET significantly enhanced lutein and chlorophyll biosyntheses in *C. pyrenoidosa* grown in the dark. The proteomic study indicated that mRET could indeed retrograde regulate the expression of chloroplast proteins, such as key enzymes involved in lutein and chlorophyll biosyntheses pathways.

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