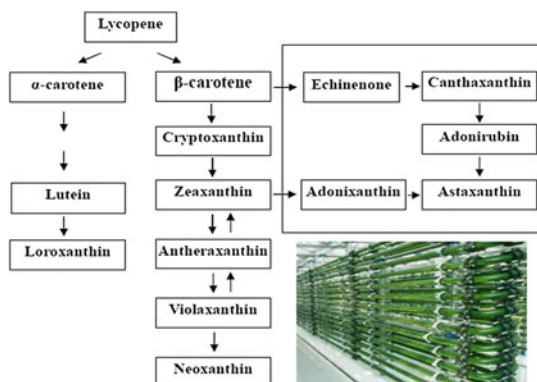


Microalgae as a Source of Lutein: Chemistry, Biosynthesis, and Carotenogenesis

Zheng Sun, Tao Li, Zhi-gang Zhou and Yue Jiang

Abstract Microalgae represent a sustainable source of natural products, and over 15,000 novel compounds originated from algal biomass have been identified. This chapter focuses on algae-derived lutein, a group of high-value products. Lutein belongs to carotenoids which have extensive applications in feed, food, nutraceutical, and pharmaceutical industries. The production of carotenoids has been one of the most successful activities in microalgal biotechnology. This chapter gives a mini review of microalgae-based lutein, where emphasis is placed on the biosynthetic pathway and the regulation of carotenogenesis.

Graphical Abstract



Z. Sun · Z. Zhou
College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China

T. Li
College of Basic Science, Tianjin Agricultural University, Tianjin 300384, China

Y. Jiang (✉)
Runke Bioengineering Co. Ltd., Zhangzhou, Fujian, China
e-mail: jiangy@gdrunke.com

Keywords Biosynthesis • Carotenogenesis • Health effects • Mass cultivation

Contents

| | | |
|---|--|----|
| 1 | Introduction | 38 |
| 2 | Structure | 39 |
| 3 | Bioactivities and Impact on Health | 39 |
| 4 | Distribution | 41 |
| 5 | Mode of Cultivation | 42 |
| | 5.1 Photoautotrophic Cultivation | 42 |
| | 5.2 Heterotrophic Cultivation | 43 |
| 6 | Biosynthesis | 46 |
| | 6.1 Formation of Isopentenyl Diphosphate (IPP) | 46 |
| | 6.2 Formation of Geranylgeranyl Pyrophosphate (GGPP) | 46 |
| | 6.3 Biosynthesis and Desaturation of Phytoene | 47 |
| | 6.4 Cyclization of Lycopene | 48 |
| | 6.5 Hydroxylation | 49 |
| 7 | Regulation of Carotenogenesis | 49 |
| | 7.1 Intercommunication of Cellular Organelles and Retrograde Regulation of Photosynthetic Genes | 49 |
| | 7.2 Stimulation of Carotenogenesis by Oxidative Stress | 51 |
| 8 | Conclusion and Future Perspectives | 52 |
| 9 | Acknowledgments | 53 |
| | References | 53 |

1 Introduction

Carotenoids are a group of organic pigments synthesized *de novo* in higher plants and some other photosynthetic organisms including algae, some types of fungus, and bacteria. They are widely distributed in nature and responsible for a broad variety of colors, such as brilliant yellow, orange, and red of fruits, leaves, and aquatic animals [1]. Thus far, over 700 carotenoids have been identified [2] in which lutein has achieved noticeable attention in both academic and industry research areas. As a natural colorant, lutein has been widely applied in the food industry, especially after the notorious “Sudan red” event [3]. It is also used for coloring fish and poultry, drugs, and cosmetics. In addition, lutein and zeaxanthin are the only carotenoids reported to be present in the eye retina and lens [4]. As these organs are susceptible to oxidant damage, lutein exerts antioxidant action to protect the eyes. These important properties make lutein a highly valuable chemical. According to the report from financial organizations, the global market of lutein is expected to grow to \$309 million by 2018 with a compound annual growth rate of 3.6 % (<http://www.companiesandmarkets.com/Market/Food-and-Drink/Market-Research/The-Global-Market-for-Carotenoids/RPT988273>).

Microalgae are a group of small photosynthetic organisms commonly found in aquatic environments. A number of microalgal species accumulate high contents of carotenoids (including lutein) as a part of their biomass and therefore represent an outstanding natural source of carotenoids. Production of lutein from microalgae is widely studied, for example, *Chlorella protothecoides* [5, 6], *Dunaliella salina* [7], *Scenedesmus almeriensis* [8], and *Galdieria sulphuraria* [9], which has been one of the most successful activities in microalgal biotechnology.

2 Structure

Carotenoids are of isoprenoid origin derived from a 40-carbon polyene chain backbone structure with cyclic groups [10]. There are two types of carotenoids: the hydrocarbon ones are known as carotenes (e.g., α -carotene, β -carotene, and lycopene), and the oxygenated derivatives are named xanthophylls. In the latter, oxygen may be present as OH groups (e.g., lutein), as oxo-groups (e.g., canthaxanthin), or in a combination of both (e.g., astaxanthin). Elements other than carbon, hydrogen, and oxygen are not directly attached to the carbon skeleton in naturally occurring carotenoids [11]. The molecular formula of lutein is $C_{40}H_{52}O_2$, as shown in Fig. 1. The most typical feature of lutein as well as other carotenoids is the long polyene chain. Such a conjugated double-bond system determines the photochemical properties and chemical reactivity that give the basic biological functions of carotenoids, such as antioxidant activities [12].

3 Bioactivities and Impact on Health

The most notable bioactivity of lutein is its antioxidant property. Lutein could be rapidly oxidized by a series of oxidants, which greatly reduces the availability of free radicals to react with other cellular components, such as unsaturated lipids, protein and DNA [13]. Similar to other carotenoid members, the antioxidative power of lutein is attributed to its conjugated carbon double-bond system. This structure allows the quenching of singlet oxygen and scavenging of free radicals. The singlet oxygen-quenching activity includes physical and chemical quenching,

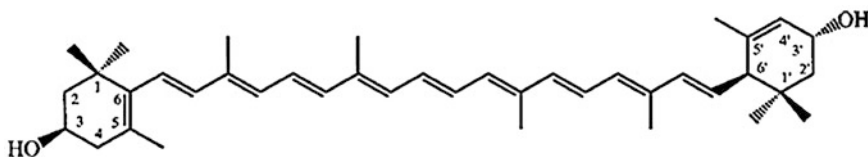


Fig. 1 Structure of lutein

Table 1 Scavenging capacity of carotenoids

| Carotenoids | Scavenging capacity ^a | | | |
|---------------|----------------------------------|-------------|-------------------|-----------------|
| | ROO [•] | HOCl | ONOO ⁻ | HO [•] |
| lutein | 0.6 | 0.97 | 4.81 | 0.78 |
| zeaxanthin | 0.56 | 1.41 | 3.87 | 0.77 |
| β-carotene | 0.14 | 0.71 | NA ^b | 1.02 |
| lycopene | 0.08 | 0.35 | 0.4 | 0.31 |
| fucoxanthin | 0.43 | 1.18 | 6.26 | NA |
| canthaxanthin | 0.04 | 0.28 | 0.1 | NA |
| astaxanthin | 0.64 | 1.66 | 9.4 | 0.73 |
| α-tocopherol | 0.48 | 1.77 | NA | 0.37 |
| quercetin | 0.84 | 1.42 | 5.63 | 0.97 |
| trolox | 1.00 | 1.00 | NA | NA |
| ascorbic acid | NA | NA | 0.41 | 1.00 |
| cysteine | 0.04 | NA | 1.00 | 0.02 |

Peroxyl radical (ROO[•]), hydroxyl radical (HO[•]), hypochlorous acid (HOCl), and peroxynitrite anion (ONOO⁻)

Source Adapted from Ref. [14]

^aThe scavenging capacity was calculated considering as reference: trolox for ROO[•] and HO[•], cysteine for HOCl, and ascorbic acid for ONOO⁻

^bNA: no activity was found within the tested concentrations

both of which require very close interaction between lutein and singlet oxygen. The energy transfer reaction absorbs the excited energy of singlet oxygen onto the carotenoid chain, which results in the formation of the carotenoid triplet state. This triplet state can readily return to the stable ground state by releasing the excess energy of singlet oxygen as heat. Rodrigues et al. [14] conducted a membrane-mimicking system to evaluate the scavenging activity of carotenoids against various reactive species based on the fluorescence loss of a fluorescent lipid (C11-BODIPY^{581/591}). The results are shown in Table 1.

The antioxidative properties make lutein a class of important nutrients in health promotion, and there has been ever-increasing evidence supporting their protective effects in preventing or delaying chronic diseases (Table 2). Previous studies indicated that lutein inhibited the oxidation of low-density lipoprotein (LDL), which suppressed the progression of cardiovascular diseases [15, 16]. Dwyer et al. [17] reported that the dietary intake of lutein effectively prevented the occurrence of early atherosclerosis. Seddon et al. [18] reported that there was a direct relationship between carotenoid intake and a decreased risk of age-related macular degeneration (AMD). Among those selected carotenoids, lutein and zeaxanthin were found to exhibit the strongest protective effects. Lutein is also well known to ameliorate the onset or progression of cataracts [19]. Oxidative damage to lens cell membranes is considered to be mainly responsible for the initiation and aggravation of age-related cataracts [20]. Recently, our studies have revealed a novel physiological function of lutein: the antiglycoxidative effect. Glycoxidation, also known as the Maillard

Table 2 Health benefits of lutein

| Therapeutic indications | References | Model |
|-------------------------|------------|---|
| AMD ^a | [24, 25] | EIU ^b in Lewis rats |
| Atherosclerosis | [26, 27] | CNV ^c in C57BL/6 J mice induced by laser photocoagulation and b-End3, RAW264.7, and ARPE-19 cell lines |
| Retinal neural damage | [28, 29] | |
| Ultraviolet radiation | [30, 31] | |

^aAMD, age-related macular degeneration

^bEIU, endotoxin-induced uveitis

^cCNV, choroidal neovascularization

reaction, is a process in which reducing sugars react spontaneously with amino acids and amino groups of proteins. This reaction is a key factor contributing to the pathogenesis of diabetic complications [21]. We evaluated over 20 microalgal species and found that the extract of several *Chlorella* exhibited a potent antiglycoxidative effect, where lutein was identified as a major effective ingredient [22]. In cultured human-derived retinal pigment epithelial ARPE-19 cells, lutein inhibited the formation of endogenous advanced glycation end products (AGEs) through the suppression of intracellular oxidative stress, for example, nitric oxide, reactive oxygen species (ROS), and lipid peroxidation [23]. These results strongly suggested the significant potential of lutein in the management of diabetic retinopathy.

4 Distribution

Lutein is widely distributed among fruits, vegetables, and flowers, in which marigold flowers are by far the most abundant natural source of commercial lutein. However, a large amount of lutein present in marigold flowers is esterified with half of the weight corresponding to fatty acids [32], and therefore, chemical saponification is necessary in the manufacture of lutein products. In addition, the production of lutein from marigold is also limited by seasons, planting area, and the high manpower cost [33].

Microalgae have long been regarded as an excellent alternative to conventional plant sources as algae-derived lutein is in the free nonesterified form. Green algae *Chlorella* are good examples: *Chlorella pyrenoidosa* can synthesize lutein and other xanthophylls under heterotrophic cultivation using glucose as the carbon source [34]; a high content of lutein was produced by *C. protothecoides* grown in the nitrogen-limited fed-batch culture [5]; and in *Chlorella zofingiensis*, astaxanthin and lutein were biosynthesized in divergent pathways using the common lycopene precursor [35]. Several species of marine microalgae such as *Dunaliella bardawil* and *D. salina*, which have been commonly applied in the production of β -carotene

Table 3 Distribution of lutein in some representative microalgae

| Microalgae | Quantity (mg g ⁻¹) | Reference |
|---------------------------------|--------------------------------|-----------|
| <i>Chlorella protothecoides</i> | 4.6 | [6] |
| <i>Chlorella protothecoides</i> | 5.4 | [5] |
| <i>Dunaliella salina</i> | 6.6 ± 0.9 | [7] |
| <i>Scenedesmus almeriensis</i> | 5.3 | [8] |
| <i>Galdieria sulphuraria</i> | 0.4 ± 0.1 | [9] |

and phytoene, were also reported to accumulate lutein under specified conditions [36, 37]. Distribution of lutein in some representative algae is summarized in Table 3.

5 Mode of Cultivation

5.1 Photoautotrophic Cultivation

There are two major cultivation modes for microalgae: photoautotrophic and heterotrophic cultures (if a strain is able to grow heterotrophically, a mixotrophic mode may also be developed). Due to the presence of chloroplast, most microalgae grow photoautotrophically through photosynthesis. The idea of photoautotrophic cultivation was first proposed in Germany in the early 1940s, in which CO₂ and light energy are used as the carbon and energy sources, respectively. In the 1990s, Australian researchers proposed the method of “extensive cultivation,” which is easy to operate and economical [38]. It consists of large open ponds without CO₂ addition and with minimal control. Facilities are usually located in places where solar irradiance is maximal, cloudiness is minimal, and the climate is warm. The photoautotrophic mode has been commonly employed in the culture of *Dunaliella* for β-carotene production, because this alga can survive under extreme conditions (e.g., high salinity and high temperature) and its cultivation is easily maintained. To reduce the contamination of plankton, hypersaline water is usually used. A two-step approach, namely “intensive cultivation” has also been employed for the large-scale production of *Dunaliella* β-carotene. The aim of stage 1 is to optimize biomass production of cells with a low β-carotene-to-chlorophyll ratio; and in stage 2, the culture is diluted to about one-third to increase the light availability to cells, and carotenogenesis is further enhanced by nitrogen deficiency [39].

Although photoautotrophic cultivation is easily maintained, a high growth rate is barely achieved due to the hostile or unsteady environment. In addition, both light intensity and photosynthesis efficiency are low inside the cultures because of the absorption and scattering of light [40]. As a result, algal growth is largely limited by such inhomogeneous distribution of light. For example, the cell mass production of *Chlamydomonas reinhardtii* only reached 0.57 g cells L⁻¹ under photoautotrophic mode with optimized culture conditions [41]. The biomass of *C. zofingiensis*

cultivated photoautotrophically at $68 \mu\text{mol m}^{-2} \text{s}^{-1}$ was confined to 0.72 g L^{-1} [42]. To solve the problems, another culture system, heterotrophic cultivation, has been developed.

5.2 Heterotrophic Cultivation

Heterotrophic cultivation refers to the organism's use of organic carbon substances as carbon and energy sources rather than CO_2 and light to support the cell growth and other physiological events [43, 44]. Due to the elimination of light restriction, cells grow quickly and a high cell mass production can be achieved easily. Thus far, the maximum biomass content achieved in photoautotrophic cultures is 40 g L^{-1} of cell dry weight [45], much lower than that in heterotrophic cultures, where 100 g L^{-1} to greater than 150 g L^{-1} is available [46].

Given the high costs of heterotrophic cultivation, this mode is generally used for the production of value-added compounds from microalgae, such as lutein and astaxanthin (Table 4). When *C. protothecoides* was heterotrophically cultivated, the algal cell dry weight and lutein yield reached 19.6 g L^{-1} and 83.8 mg L^{-1} , respectively, in the fermentor, and even achieved as high as 46.9 g L^{-1} and 225.3 mg L^{-1} using the fed-batch culture strategy [5, 6]. Wu and Shi [51] conducted a heterotrophic cultivation using another *Chlorella* species, *C. pyrenoidosa*; with the artificial neural network (ANN) model employed as the optimization strategy, the highest biomass concentration and the maximum productivity achieved 104.9 g L^{-1} dry cell weight and $0.613 \text{ g L}^{-1} \text{ h}^{-1}$, respectively. The high cell density can be achieved by the employment of fed-batch, continuous, and perfusion culture strategies that are commonly used in the fermentation of bacteria or yeasts. Fed-batch culture is an effective way to minimize substrate inhibition because a high initial concentration of substrates (e.g., sugars) may lead to growth inhibition. On the other hand, the fed-batch culture cannot overcome the inhibition caused by the toxic metabolites generated during the algal culture. They may accumulate as the cells build up and prevent further enhancement of cell density. Such metabolite-driven inhibition can be eliminated by continuous or semicontinuous cultivation systems, in which the fresh medium is continuously added to a well-mixed culture, while cells or products are simultaneously removed to keep a constant volume. Perfusion culture is a technique combining the advantages of fed-batch and continuous culture systems. Cells are physically retained by a retention device, whereas the spent medium is removed. Meanwhile, fresh medium is fed into the bioreactor to maintain a sufficient nutrient supply. Park et al. [52] established a two-stage mixotrophic culture system for *Haematococcus pluvialis*. By the perfusion process, the biomass density achieved was 3.09 and 1.67 times higher than batch and fed-batch processes, respectively. Zhang et al. [53] established an "attached cultivation" using the immobilized biofilm. Under the optimized condition, the maximum astaxanthin productivity reached $160 \text{ mg m}^{-2} \text{ d}^{-1}$.

Table 4 Heterotrophic cultivation of microalgae for the production of lutein and astaxanthin

| Carotenoids | Strain | Device | Culture mode | Cell concentration (g L ⁻¹) | Productivity (mg L ⁻¹ d ⁻¹) | Yield (mg L ⁻¹) | Reference |
|-------------|---------------------------------|-----------|--------------|---|--|-----------------------------|-----------|
| Astaxanthin | <i>Chlorella zofingiensis</i> | Flask | Batch | 10.2 | 0.7 | 10.3 | [47] |
| | <i>Chlorella zofingiensis</i> | Flask | Batch | 7.5 | 1.3 | 11.8 | [48] |
| | <i>Chlorella zofingiensis</i> | Flask | Batch | 7.3 | 1.4 | 12.6 | [49] |
| | <i>Haematococcus pluvialis</i> | Flask | Batch | NA | 0.8 | 9.0 | [50] |
| Lutein | <i>Chlorella protothecoides</i> | Fermentor | Batch | 19.6 | 11.3 | 83.8 | [6] |
| | <i>Chlorella protothecoides</i> | Fermentor | Fed-batch | 46.9 | 22.7 | 225.3 | [5] |

Source Adapted from Ref. [46]

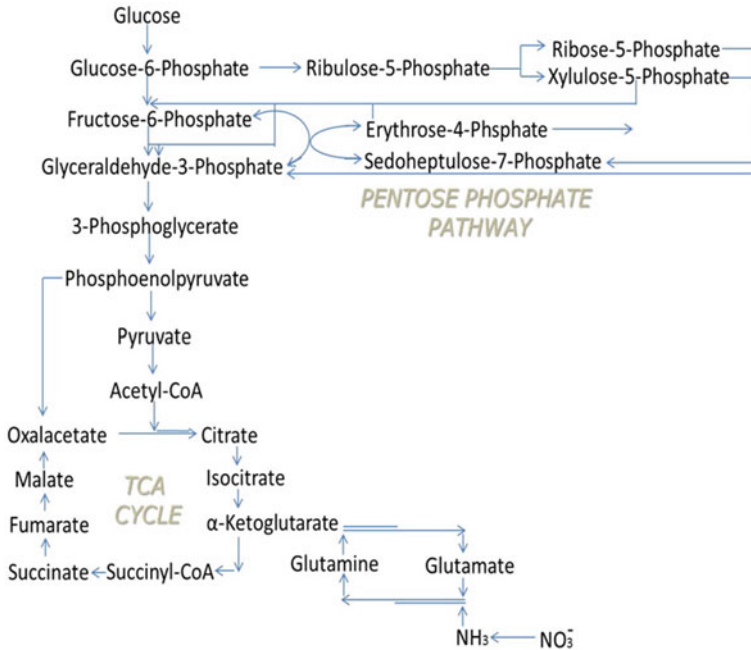


Fig. 2 Heterotrophic metabolism in microalgae (modified from Ref. [55])

The known carbon sources include glucose, glycerol, acetate, fructose, ethanol, and some low-cost media formulations with molasses or carob pulp syrup, as well as wastewater from the sugar or milk processing industries [54]. Among them, glucose is the most superior candidate, stimulating much higher growth rates of microalgae than other carbon sources. Through the comparison between heterotrophic and photoautotrophic modes, it was found that the ATP generated from the former using glucose as the energy supplier exceeded more than 600 % over the latter in which energy was supplied by light [55]. As shown in Fig. 2, in contrast to light conditions where the Embden–Meyerhof–Parnas (EMP) pathway acts as the predominant glycolysis, under darkness, glucose is mainly metabolized via the pentose phosphate (PP) pathway [56]. For example, in alga *Chlorella pyrenoidosa*, the PP pathway accounts for over 90 % of glucose metabolic flux distribution [55].

Heterotrophic cultivation also has some other advantages such as a high degree of process control, low costs for harvesting biomass because of the higher cell densities achieved, and so on [57]. Nevertheless, this is not to say that this cultivation mode is without drawbacks: the number of microalgal species able to grow heterotrophically is limited; the formation of light-induced metabolites will be diminished; contamination and competition with other microorganisms could take place, and so on. Table 5 shows the comparison between photoautotrophic and heterotrophic production of microalgae-derived products.

Table 5 Comparison between photoautotrophic and heterotrophic production of microalgae-derived products

| | Photoautotrophic mode | Heterotrophic mode |
|-------------------------|-----------------------|--------------------|
| Available algal species | A lot | Limited |
| Cell density | Low | High |
| Cost of production | Low | Highs |
| Product contents | Low | High |
| Productivity | Low | High |
| Scaleup | Easy | Difficult |

6 Biosynthesis

Major advances have been made in the elucidation of the carotenoid biosynthetic pathway in bacteria, algae, and higher plants, and it is believed that in these organisms, the biosynthesis of primary carotenoids follows a similar pathway. The biosynthesis process of β -carotene and α -carotene is outlined in Fig. 3, which can be divided into the following steps.

6.1 Formation of Isopentenyl Diphosphate (IPP)

Glyceraldehyde-3-phosphate (GAP) and pyruvate act as the first two precursors. They are utilized to produce 1-deoxy-D-xylulose-5-phosphate (DXP) which is then transformed to IPP through a series of reactions in the 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway present in the plastids of plants, cyanobacteria, algae, and certain bacteria [59].

6.2 Formation of Geranylgeranyl Pyrophosphate (GGPP)

GGPP is the first immediate precursor in the lutein biosynthesis pathway, which is synthesized from IPP. At first, IPP is isomerized to its allylic isomer, dimethylallyl diphosphate (DMAPP). Geranyl pyrophosphate (GPP) is then produced by the condensation reaction of IPP and DMAPP. Elongation of GPP by the addition of IPP results in the formation of farnesyl pyrophosphate (FPP) to which is further added another IPP to produce GGPP [60]. These condensation reactions are catalyzed by a single enzyme called GGPP synthase, which was isolated as a soluble and functional homodimer (74 kDa) from the chromoplasts of *Capsicum* [61].

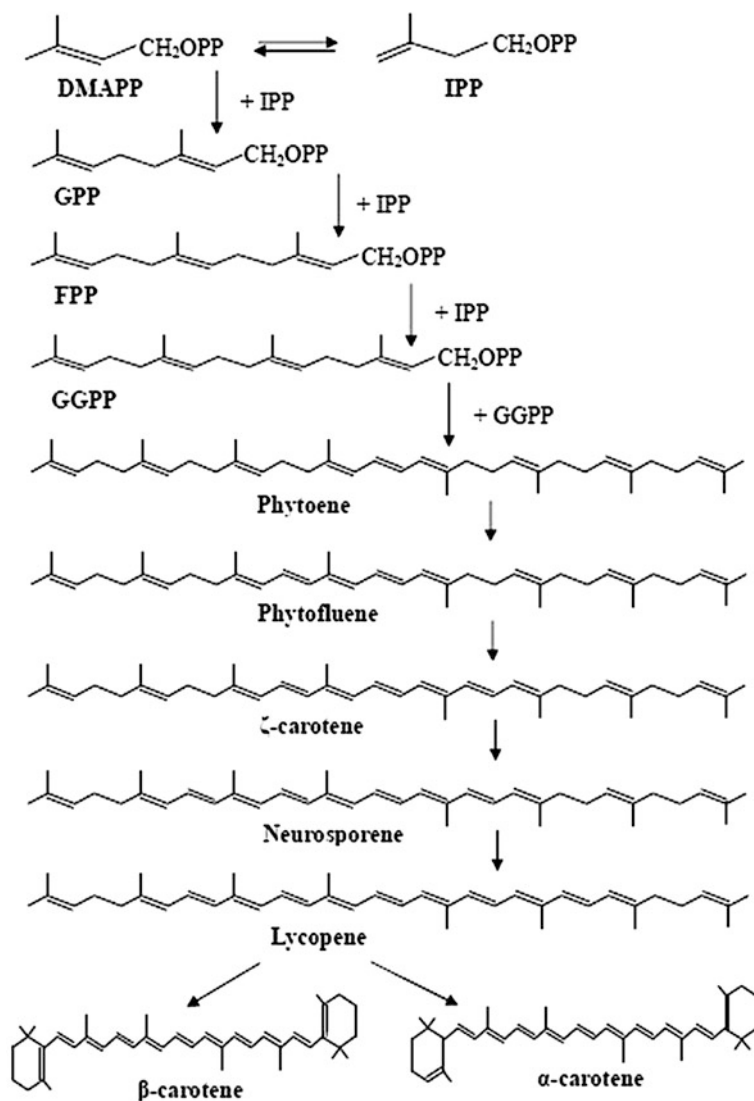


Fig. 3 Carotenoid biosynthetic pathway to form β -carotene and α -carotene (adapted from Ref. [58]). Abbreviations: *DMAPP* dimethylallyl diphosphate; *IPP* isopentenyl diphosphate; *GPP* geranyl diphosphate; *FPP* farnesyl diphosphate; *GGPP* geranylgeranyl diphosphate

6.3 Biosynthesis and Desaturation of Phytoene

Phytoene is synthesized through condensation of two GGPP molecules under the catalysis of phytoene synthase (PSY), a membrane-associated enzyme [62]. PSY has been reported to be a rate-limiting enzyme of carotenoid biosynthesis in

different plant organs, tissues, and locations, such as in canola seeds, ripening tomato fruits, and in marigold flowers [63–65]. In addition, PSY is highly conserved among archaea, bacteria, and eukaryotes [66]. Lycopene is subsequently formed by the conversion of phytoene, which is catalyzed by two desaturases, namely phytoene desaturase (PDS) and zeta-carotene desaturase (ZDS) through a series of reactions [67].

6.4 Cyclization of Lycopene

Carotenoid cyclization refers to the formation of a six-membered ring at one or both ends of lycopene and an important branching point of the carotenoid biosynthesis pathway in photosynthetic organisms. One branch leads to the formation of β -carotene and its derivative xanthophylls, such as zeaxanthin and astaxanthin, and the other one leads to α -carotene and lutein in plants and microalgae [68]. The formation of β and ϵ rings at both ends of lycopene results in the yield of α -carotene under the catalysis of β -cyclase (LCY-b) and ϵ -cyclase (LCY-e), respectively, whereas the formation of two β -rings at the two ends of lycopene gives rise to β -carotene catalyzed by LCY-b alone [67].

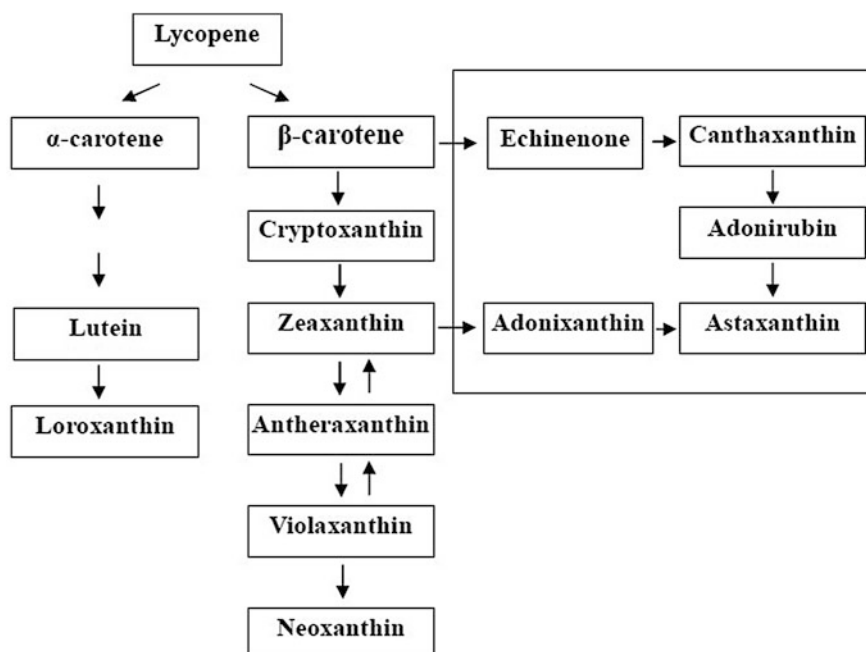


Fig. 4 Schematic diagram of pathway of xanthophyll biosynthesis. In the box is the astaxanthin biosynthetic pathway present in certain algae. Two possible ways are indicated (adapted from Ref. [69])

6.5 Hydroxylation

In higher plants and algae, xanthophylls are enzymically formed through the oxidation of α - and β -carotene. Hydroxylation at the C-3 and C-3' positions of α -carotene by β - and ϵ -carotene hydroxylase encoded by genes *CrtR-b* and *CrtR-e* lead to the formation of lutein, whereas hydroxylation of β -carotene by β -carotene hydroxylase alone results in the formation of zeaxanthin [67]. The subsequent epoxidation of zeaxanthin leads to the production of violaxanthin, which is further converted to neoxanthin. Meanwhile, in some algae, additional xanthophyll biosynthetic pathways could also be present. For example, oxygenation and hydroxylation of β -carotene bring on the synthesis of astaxanthin (Fig. 4).

7 Regulation of Carotenogenesis

7.1 Intercommunication of Cellular Organelles and Retrograde Regulation of Photosynthetic Genes

In higher plants, algae, and other photosynthetic eukaryotic cells, mitochondria, chloroplast, and nucleus are three essential organelles. These cellular components are not isolated or independent, but have interaction and communication with each other. In most cases, chloroplast and mitochondria have their own organelle genome, but these genomes only encode few amounts of proteins. Current proteomics and genomics studies show that these organelles comprise thousands of proteins. Studies in cyanobacterium indicated that most proteins (93–99 %) were encoded by nuclear genes and were transported into the organelles after translation and processing in cytoplasm [70, 71]. Therefore, there must be a mechanism regulating and coordinating the nucleus genome to encode and synthesize the most necessary organelle proteins during cell growth and development.

Anterograde regulation refers to the control of the biogenesis and function of organelles by the nuclear genome and has been widely studied in the past decades. Along with anterograde regulation, retrograde regulation refers to the communication between organelles and nucleus. The functional and developmental organelles send signals to the nucleus in order to activate the necessary genes to meet the particular need of the cell. The detailed retrograde regulation mechanisms in chloroplast and mitochondria remain unclear, and thus far, four organelle retrograde regulation mechanisms have been determined: (1) organellar gene expression (OGE), (2) tetrapyrrole biosynthesis, (3) organellar redox state, and (4) ROS [72].

Johanningmeier and Howell [73] studied the regulation of light-harvesting chlorophyll-binding protein (LHCP) mRNA accumulation in *Chlamydomonas reinhardtii*. With the block of late chlorophyll biosynthesis, the intermediate of chlorophyll synthesis, namely Mg-protoporphyrin IX monomethyl ester (Mg-ProtoMe), inhibited by a feedback mechanism the light induction of LHCP

mRNA accumulation. Treatments that presumably resulted in an accumulation of Mg-ProtoMe repressed the expression or transcription of chloroplast genes [74, 75]. Walker and Willows [76] reported the roles of Mg-chelatase in the chlorophyll synthesis pathway. Mg-chelatase is a three-component enzyme comprising subunits ChlD, ChlH, and ChlI, which catalyzes the insertion of Mg^{2+} into the porphyrin ring of Proto IX. Mochizuki et al. [77] observed that the plastid-to-nucleus signal transduction pathway was disrupted in two *Arabidopsis* CHIH mutants but not in ChlI mutants. Also, CHIH was found to be able to bind to deuteroporphyrin even in the absence of the other two chelatase subunits [78]. In this regard, Mochizuki et al. [77] proposed that CHIH may not only serve as the chelatase in tetrapyrrole biosynthesis, but also function as the tetrapyrrole sensor in a chloroplast-to-nucleus signaling pathway. Another possibility is that CHIH could directly participate in the plastid-to-nucleus retrograde signaling pathway by forming a CHIH-tetrapyrrole complex with other cofactors without affecting the tetrapyrrole transport [79, 80].

There is another chloroplast-to-nucleus retrograde regulation pathway derived from the redox and ROS signals in plants and green algae. For redox signal retrograde regulation, two possible sources have been proposed, the redox state of the PQ pool in the photorespiration electron transport chain and light-harvesting complex II (LHCII) kinase. Escoubas et al. [81] proposed that in *Dunaliella tertiolecta*, the redox state of PQ may influence the regulation of nuclear photosynthesis genes, and such retrograde regulation was possibly achieved via a chloroplast protein kinase. Similar regulation mechanisms also exist in higher plants [82]. On the other hand, some research groups also suggested that LHC II kinase, rather than the PQ pool, may serve as the retrograde regulation signal source. Pursiheimo et al. [83] demonstrated that the transcription of genes *Lhcb*, *rbcS*, and *psbA* was positively related to LHCII protein phosphorylation. Bonardi et al. [84] also found that STN7, a dual-function chloroplast thylakoid protein kinase, participated in the chloroplast redox signaling pathway in *Arabidopsis thaliana*. These results indicated that protein kinase may also function as a necessary component in the chloroplast redox signaling transduction pathway.

The retrograde regulation also exists in mitochondria. It is believed that the mitochondrion interacts closely with other cellular organelles, such as chloroplast and nucleus, and a wide range of signaling events in plant cells can be strongly influenced by changes in mitochondrial function. Such changes trigger altered nuclear gene expression by a process of mitochondrial retrograde regulation (MRR) [85]. MRR is manifested at mitochondria dysfunctions induced by mutations, chemical agents, or stresses. Previous studies indicated that plant mitochondrial dysfunction led to the induction of nucleus-located genes encoding proteins for the restoration of mitochondria function. Meanwhile, genes encoding antioxidant enzymes were also activated to detoxify the ROS and protect the cell organelle [85]. The most well-studied MRR thus far is on the expression of gene encoding alternative oxidase (AO) in mitochondria. Two possible signal transduction pathways have been proposed: one involves some intermediates of TCA

cycles such as organic acid 2-oxoglutarate, citrate, and malate, and the other is derived from the ROS produced in mitochondria [86]. MRR is still a poorly understood process and very little is known about the exact factors involved in the signal transduction pathway. Components able to react with ROS in mitochondria or cytosol (e.g., lipids, antioxidant enzymes, or small molecular weight compounds) should be considered as the candidates of primary or secondary signal molecules. Matsuo and Obokata [87] studied the MRR in *C. reinhardtii*. Under heterotrophic and mixotrophic conditions, nucleus-located photosynthetic genes were remote-controlled by a mitochondrial respiratory electron transport chain (RET). When algal cells were cultured under autotrophic and mixotrophic conditions, the controller became a photosynthetic electron transport (PET). These results suggested the cooperation between cellular organelles in algae and indicated the regulatory system of photosynthetic genes changes in response to shifts in the dominant energy source between photosynthesis and respiration.

7.2 Stimulation of Carotenogenesis by Oxidative Stress

7.2.1 Enhancement of Carotenoid Synthesis Induced by ROS

Jahnke [88] reported that the accumulation of carotenoids was one of the responses of microalgae to the oxidative stress induced by UV radiation. When *D. bardawil* was exposed to the UV-A radiation of $70 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 84 h, the contents of lutein and zeaxanthin within algal cells increased about threefold and fivefold, respectively [89].

Carotenogenesis in algae could also be stimulated in response to chemically generated oxidative stresses. Kobayashi et al. [90] indicated that Fe^{2+} may serve as an HO^\cdot generator via an iron-catalyzed Fenton reaction, and the generated ROS played an essential role in enhancing the carotenoid formation in *H. pluvialis*. This viewpoint was verified by Ip and Chen [49]. When the *C. zofingiensis* culture was supplemented with ROS generators Fe^{2+} (18 μM) and H_2O_2 (0.1 mM), the yield of astaxanthin increased from 9.9 to 12.58 mg L^{-1} . The production of lutein was also enhanced in *C. protothecoides* under heterotrophic cultivation. The lutein content increased from 1.75 to 1.98 mg g^{-1} when 0.01 mmol L^{-1} H_2O_2 and 0.5 mmol L^{-1} NaClO were added to generate $^1\text{O}_2$ [91]. Inactivation of antioxidant enzymes is another way to stimulate the formation of carotenoids as they act as nonenzymatic antioxidants. For example, sodium azide is known as an inhibitor of catalase (CAT) and superoxide dismutase (SOD), two important ROS scavengers. Shaish et al. [92] reported that when *D. bardawil* cells were treated with sodium azide, the content of β -carotene increased from 11.7 to 27.5 pg cell^{-1} .

7.2.2 Expression Variation of Genes Encoding Enzymes Involved in Carotenoid Biosynthesis After Oxidative Stress Treatment

When the microalgae are under stress treatments such as high light and high salt stress, the expression of genes encoding enzymes involved in carotenoid biosynthesis is upregulated, which subsequently increases the accumulation of carotenoids. According to Steinbrenner and Linden [93], in *H. pluvialis*, all four genes encoding PSY, lycopene cyclase, PDS, and carotenoid hydroxylase showed higher transcript levels in response to increased illumination. Similar research was conducted by Ramos et al. [94] using *D. salina*. The highest steady-state mRNA level of *Lcy-β* (encoding lycopene β -cyclase) was observed in algal cells exposed to high light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) or low light ($45 \mu\text{mol m}^{-2} \text{s}^{-1}$) combined with nutrient depletion for 24 and 48 h, respectively. As a result, the cellular β -carotene content increased faster in the case of exposure to high light conditions at 24 h.

7.2.3 ROS Sensing Signaling Cascade Involved in Stimulating Carotenogenesis

Due to the high cytotoxic and reactive feature of ROS, their concentration must be tightly controlled. Higher plants and algae can sense the ROS signals and make appropriate cellular responses. The redox-sensitive proteins that reversibly become oxidized or reduced depending on the cellular redox state are essential in the response process [19]. Pfannschmidt et al. [82] indicated that heterotrimeric G-proteins participated in the signaling pathway mediated by ROS. Protein phosphorylation controlled by protein Tyr phosphatases and specific mitogen-activated protein (MAP) kinases are two main well-studied molecular mechanisms of redox-sensitive regulation of protein function in cells [19, 95]. In microalgae, a gene encoding a MAP kinase was isolated from *D. salina* and its expression was found to be affected by changes in temperature and salinity conditions that also influenced the accumulation of β -carotene [96]. Eom et al. [97] detected 16 (11 %) differently expressed genes from *H. pluvialis* that were involved or potentially involved in defense or stress responses. Nedelcu [98] indicated that tumor suppressor protein p53, which plays a major role in cellular response to stress, was also present and mediated cellular responses to stress in two algae, *Volvox carteri* and *C. reinhardtii*.

8 Conclusion and Future Perspectives

Lutein production has been one of the most successful activities in the microalgal industry. As the world's market demand is growing, there are still substantial challenges to be addressed to enhance the production capacity and economics of microalgae-based lutein, which urgently requires the development of microalgal

biotechnology. For example, genetic engineering is a feasible approach to improve the biosynthetic pathway of lutein in microalgae and heighten its cellular accumulation. Another important issue to consider is the cost. As mentioned earlier, although the heterotrophic cultivation allows a higher cell density and lutein productivity, the high costs of glucose hinder its commercial application. It is logical to develop low-cost and effective carbon sources from industrial or agricultural wastes. Finally, the production of lutein may be combined with other algal-based metabolisms (such as oil) and coupled with wastewater treatment and biofixation of carbon dioxide to develop an integrated industrial chain that may increase the process economy and bring environmental benefits as well.

9 Acknowledgments

The authors acknowledge the Shanghai Pujiang Program (13PJ1403500), “Chen Guang” project supported by the Shanghai Municipal Education Commission and Shanghai Education Development Foundation (13CG52), Doctoral Fund of Ministry of Education of China (20133104120004), and Shanghai Ocean University Doctoral Start-up Grant (A-0209-13-0105389) for providing financial support for this work.

References

1. Lorenz RT, Cysewski GR (2000) Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. *Trends Biotechnol* 18:160–167
2. Zhang J, Sun Z, Sun P, Chen T, Chen F (2014) Microalgal carotenoids: beneficial effects and potential in human health. *Food Funct* 5:413–425
3. Wang C, Kim JH, Kim SW (2014) Synthetic biology and metabolic engineering for marine carotenoids: new opportunities and future prospects. *Mar Drugs* 12:4810–4832
4. Khachik F, de Moura FF, Zhao DY, Aebischer CP, Bernstein PS (2002) Transformations of selected carotenoids in plasma, liver, and ocular tissues of humans and in nonprimate animal models. *Invest Ophthalmol Vis Sci* 43:3383–3392
5. Shi XM, Jiang Y, Chen F (2002) High-yield production of lutein by the green microalga *Chlorella protothecoides* in heterotrophic fed-batch culture. *Biotechnol Prog* 18:723–727
6. Shi X, Zhang X, Chen F (2000) Heterotrophic production of biomass and lutein by *Chlorella protothecoides* on various nitrogen sources. *Enzyme Microb Technol* 27:312–318
7. González S, Astner S, An W, Goukassian D, Pathak MA (2003) Dietary lutein/zeaxanthin decreases ultraviolet B-induced epidermal hyperproliferation and acute inflammation in hairless mice. *J Invest Dermatol* 121:399–405
8. Sánchez JF, Fernández JM, Ación FG, Rueda A, Pérez-Parra J (2008) Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis*. *Process Biochem* 43:398–405
9. Graziani G, Schiavo S, Nicolai MA, Buono S, Fogliano V et al (2013) Microalgae as human food: chemical and nutritional characteristics of the thermo-acidophilic microalga *Galdieria sulphuraria*. *Food Funct* 4:144–152

10. Croteau R, Kutchan TM, Lewis NG (2000) Natural products (secondary metabolites). In: Buchanan BB, Gruissem W, Jones RL (eds) *Biochemistry and molecular biology of plants*. American Society of Plant Physiologists. Rockville, MD, pp 1250–1317
11. Liaaen-Jensen S (2004) Basic carotenoid chemistry. In: Mayne ST, Krinsky NI, Sies H (eds) *Carotenoids in health and disease*. Marcel Dekker Press, New York, pp 1–30
12. Britton G (1995) Structure and properties of carotenoids in relation to function. *FASEB* 9:1551–1558
13. Woodall AA, Lee SW, Weesie RJ, Jackson MJ, Britton G (1997) Oxidation of carotenoids by free radicals: relationship between structure and reactivity. *Acta Bioch Bioph* 1336:33–42
14. Rodrigues E, Mariutti LR, Mercadante AZ (2012) Scavenging capacity of marine carotenoids against reactive oxygen and nitrogen species in a membrane-mimicking system. *Mar Drugs* 10:1784–1798
15. Chopra M, Willson RL, Thurnham DI (1993) Free radical scavenging of lutein in vitro. *Ann NY Acad Ssc* 691:246–249
16. Chopra M, Thurnham DI (1994) Effect of lutein on oxidation of low-density lipoprotein (LDL) in vitro. *P Nutr Soc* 53:1993 #18A
17. Dwyer JH, Navab M, Dwyer KM, Hassan K, Sun P, Shircore A et al (2001) Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles atherosclerosis study. *Circulation* 103:2922–2927
18. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC et al (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA* 272:1413–1420
19. Shao HB, Chu LY, Lu ZH, Kang CM (2011) Primary antioxidant free radical scavenging and redox signaling pathway in higher plant cells. *Int J Biol Sci* 4:8–14
20. Bron AJ, Vrensen GF, Koretz J, Maraini G, Harding JJ (2000) The ageing lens. *Ophthalmologica* 214:86–104
21. Ahmed N (2005) Advanced glycation endproducts: role in pathology of diabetic complications. *Diabetes Res Clin Pract* 67:3–21
22. Sun Z, Peng XF, Liu J, Fan KW, Wang M, Chen F (2010) Inhibitory effects of microalgal extracts on the formation of advanced glycation endproducts (AGEs). *Food Chem* 120:261–267
23. Sun Z, Liu J, Zeng X, Huangfu J, Jiang Y, Wang M, Chen F (2011) Protective actions of microalgae against endogenous and exogenous advanced glycation endproducts (AGEs) in human retinal pigment epithelial cells. *Food Funct* 2:251–258
24. Bian Q, Gao S, Zhou J, Qin J, Taylor A (2012) Lutein and zeaxanthin supplementation reduces photooxidative damage and modulates the expression of inflammation-related genes in retinal pigment epithelial cells. *Free Radic Biol Med* 53:1298–1307
25. Xu XR, Zou ZY, Xiao X, Huang YM, Wang X (2013) Effects of lutein supplement on serum inflammatory cytokines, ApoE and lipid profiles in early atherosclerosis population. *J Atheroscler Thromb* 20:170–177
26. Kim JE, Leite JO, DeOgburn R, Smyth JA, Clark RM, Fernandez ML (2011) A lutein-enriched diet prevents cholesterol accumulation and decreases oxidized LDL and inflammatory cytokines in the aorta of guinea pigs. *J Nutr* 141:1458–1463
27. Sasaki M, Ozawa Y, Kurihara T, Noda K, Imamura Y (2009) Neuroprotective effect of an antioxidant, lutein, during retinal inflammation. *Invest Ophthalmol Vis Sci* 50:1433–1439
28. Li SY, Fung FK, Fu ZJ, Wong D, Chan HH, Lo AC (2012) Anti-inflammatory effects of lutein in retinal ischemic/hypoxic injury: in vivo and in vitro studies. *Invest Ophthalmol Vis Sci* 53:5976–5984
29. González S, Astner S, An W, Goukassian D, Pathak MA (2003) Dietary lutein/zeaxanthin decreases ultraviolet B-induced epidermal hyperproliferation and acute inflammation in hairless mice. *J Invest Dermatol* 121:399–405

30. Lee EH, Faulhaber D, Hanson KM, Ding W, Peters S, Kodali S et al (2004) Dietary lutein reduces ultraviolet radiation-induced inflammation and immunosuppression. *J Invest Dermatol* 122:510–517
31. Jin XH, Ohgami K, Shiratori K, Suzuki Y, Hirano T, Koyama Y et al (2006) Inhibitory effects of lutein on endotoxin-induced uveitis in Lewis rats. *Invest Ophthalmol Vis Sci* 47:2562–2568
32. Del Campo JA, García-González M, Guerrero MG (2007) Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Appl Microbiol Biotechnol* 74:1163–1174
33. Tsao R, Yang R, Young JC, Zhu H, Manolis T (2004) Separation of geometric isomers of native lutein diesters in marigold (*Tagetes erecta* L.) by high-performance liquid chromatography mass spectrometry. *J Chromatogr A* 1045:65–70
34. Theriault RJ (1965) Heterotrophic growth and production of xanthophylls by *Chlorella pyrenoidosa*. *Appl Microbiol* 13:402–416
35. Liu J, Sun Z, Gerken H, Liu Z, Jiang Y, Chen F (2014) *Chlorella zofingiensis* as alternative microalgal producer of astaxanthin: biology and industrial potential. *Mar Drugs* 12:3487–3515
36. García-González M, Moreno J, Manzano JC, Florencio FJ, Guerrero MG (2004) Production of *Dunaliella salina* biomass rich in 9-cis- β -carotene and lutein in a closed tubular photobioreactor. *J Biotechnol* 115:81–90
37. León R, Vila M, Hernández D, Vilchez C (2005) Production of phytoene by herbicide-Treated microalgae *Dunaliella bardawil* in two-phase systems. *Biotechnol Bioeng* 92:695–701
38. Borowitzka M, Borowitzka L (1988) *Dunaliella*. In: Borowitzka M, Borowitzka L (eds) *Micro-algal Biotechnology*. Cambridge University Press, Cambridge, pp 27–58
39. Ben-Amotz A (1995) New mode of *Dunaliella* biotechnology: two-phase growth for β -carotene production. *J Appl Phycol* 7:65–68
40. Ogbonna JC, Tanaka H (2000) Light requirement and photosynthetic cell cultivation-developments of processes for efficient light utilization in photobioreactors. *J Appl Phycol* 12:207–218
41. Akimoto M, Yamada H, Ohtaguchi K, Koide K (1997) Photoautotrophic cultivation of the green alga *Chlamydomonas reinhardtii* as a method for carbon dioxide fixation and α -linolenic acid production. *J Am Oil Chem Soc* 74:181–183
42. Orosa M, Torres E, Fidalgo P, Abald J (2000) Production and analysis of secondary carotenoids in green algae. *J Appl Phycol* 12:553–556
43. Kaplan D, Richmond AE, Dubinsky Z, Aaronson S (1986) Algal nutrition. In: Richmond A (ed) *Handbook of microalgal mass culture*. CRC Press, Boca Raton, pp 147–199
44. Chen F (1996) High cell density culture of microalgae in heterotrophic growth. *Trends Biotechnol* 14:421–426
45. Doucha J, Lívanský K (2006) Productivity, CO₂/O₂ exchange and hydraulics in outdoor open high density microalgal (*Chlorella* sp.) photobioreactors operated in a Middle and Southern European climate. *J Appl Phycol* 18:811–826
46. de Swaaf ME, Pronk JT, Sijtsma L (2003) High-cell-density fed-batch cultivation of the docosahexaenoic acid producing marine alga *Cryptocodinium cohnii*. *Biotechnol Bioeng* 81:666–672
47. Ip PF, Chen F (2005) Production of astaxanthin by the green microalga *Chlorella zofingiensis* in the dark. *Process Biochem* 40:733–738
48. Ip PF, Chen F (2005) Peroxynitrite and nitryl chloride enhance astaxanthin production by the green microalga *Chlorella zofingiensis* in heterotrophic culture. *Proc Biochem* 40:3595–3599
49. Ip PF, Chen F (2005) Employment of reactive oxygen species to enhance astaxanthin formation in *Chlorella zofingiensis* in heterotrophic culture. *Process Biochem* 40:3491–3496
50. Kobayashi M, Kurimura Y, Tsuji Y (1997) Light-independent, astaxanthin production by the green microalga *Haematococcus pluvialis* under salt stress. *Biotechnol Lett* 19:507–509
51. Wu ZY, Shi XM (2006) Optimization for high-density cultivation of heterotrophic *Chlorella* based on a hybrid neural network model. *Lett Appl Microbiol* 44:13–18

52. Park JC, Choi SP, Hong ME, Sim SJ (2014) Enhanced astaxanthin production from microalga, *Haematococcus pluvialis* by two stage perfusion culture with stepwise light irradiation. *Bioprocess Biosyst Eng* 37:2039–2047
53. Zhang W, Wang J, Wang J, Liu T (2014) Attached cultivation of *Haematococcus pluvialis* for astaxanthin production. *Bioresour Technol* 158:329–335
54. Bumbak F, Cook S, Zachleder V, Hauser S, Kovar K (2011) Best practices in heterotrophic high-cell-density microalgal processes: achievements, potential and possible limitations. *Appl Microbiol Biotechnol* 91:31–46
55. Yang C, Hua Q, Shimizu K (2000) Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light-autotrophic/dark-heterotrophic conditions. *Biochem Eng J* 6:87–102
56. Perez-Garcia O, Escalante FME, de-Bashan LE, Bashan Y (2011) Heterotrophic cultures of microalgae: metabolism and potential products. *Water Res* 45:11–36
57. Barclay WR, Meager KM, Abril JR (1994) Heterotrophic production of long-chain omega-3 fatty acids utilizing algae and algae-like microorganisms. *J Appl Phycol* 6:123–129
58. Sandmann G (2002) Molecular evolution of carotenoid biosynthesis from bacteria to plants. *Physiol Plant* 116:431–440
59. Rohmer M (1999) The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. *Nat Prod Rep* 16:565–574
60. Fraser PD, Schuch W, Bramley PM (2000) Phytoene synthase from tomato (*Lycopersicon esculentum*) chloroplasts-partial purification and biochemical properties. *Planta* 211:361–369
61. Dogbo O, Camara B (1987) Purification of isopentenyl pyrophosphate isomerase and geranylgeranyl pyrophosphate synthase from *Capsicum chromoplasts* by affinity chromatography. *Biochemica Biophysica Acta* 920:140–148
62. Ladygin VG (2000) Biosynthesis of carotenoids in the chloroplasts of algae and higher plants. *Russ J Plant Physiol* 47:796–814
63. Fraser PD, Truesdale MR, Bird CR, Schuch W, Bramley PM (1994) Carotenoid biosynthesis during tomato fruit development. *Plant Physiol* 105:405–413
64. Shewmaker CK, Sheehy JA, Daley M, Colburn S, Ke DY (1999) Seed specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. *Plant J* 20:401–412
65. Moehs CP, Tian L, Osteryoung KW, DellaPenna D (2001) Analysis of carotenoids biosynthetic gene expression during marigold petal development. *Plant Mol Biol* 45:281–293
66. Sandmann G (2001) Carotenoid biosynthesis and biotechnological application. *Arch Biochem Biophys* 385:4–12
67. Fraser PD, Bramley PM (2004) The biosynthesis and nutritional uses of carotenoids. *Prog Lipid Res* 43:228–265
68. Cunningham FX Jr, Pogson B, Sun Z, McDonald KA, DellaPenna D, Gantt E (1996) Functional analysis of the B and E lycopene cyclase enzymes of Arabidopsis reveals a mechanism for control of cyclic carotenoid formation. *Plant Cell* 8:1613–1626
69. Jin E, Polle JE, Lee HK, Hyun SM, Chang M (2003) Xanthophylls in microalgae: from biosynthesis to biotechnological mass production and application. *J Microbiol Biotechnol* 13:165–174
70. Adersson SG, Karlberg O, Canback B, Kurland CG (2003) On the origin of mitochondria: genomis perspective. *Philos Trans R Soc Lond B Biol Sci* 358:165–177
71. Woodson JD, Chory J (2008) Coordination of gene expression between organellar and nuclear genomes. *Nat Rev Genet* 9:383–395
72. Pesaresi P, Schneider A, Kleine T, Leiseter D (2007) Interorganellar communication. *Curr Opin Plant Biol* 10:600–606
73. Johanningmeier U, Howell SH (1984) Regulation of lightharvesting chlorophyll-binding protein mRNA accumulation in *Chlamydomonas reinhardtii*: possible involvement of chlorophyll synthesis precursors. *J Biol Chem* 259:13541–13549

74. Oster U, Brunner H, Rudiger W (1996) The greening process in cress seedlings. V. Possible interference of chlorophyll precursors, accumulated after thujaplicin treatment, with light-regulated expression of Lhc genes. *J Photochem Photobiol* 36:255–261
75. Zavgorodnyaya A, Papenbrock J, Grimm B (1997) Yeast-aminolevulinic acid synthase provides additional chlorophyll precursor in transgenic tobacco. *Plant J* 12:169–178
76. Walker CJ, Willows RD (1997) Mechanism and regulation of Mg-chelatase. *Biochem J* 327:321–333
77. Mochizuki N, Brusslan JA, Larkin R, Nagatani A, Chory J (2001) *Arabidopsis* genomes uncoupled 5 (GUN5) mutant reveals the involvement of Mg-chelatase H subunit in plastid-to-nucleus signal transduction. *Proc Natl Acad Sci* 98:2053–2058
78. Karger GA, Reid JD, Hunter CN (2001) Characterization of the binding of deuteroporphyrin IX to the magnesium chelatase H subunit and spectroscopic properties of the complex. *Biochemistry* 40:9291–9299
79. Moller SG, Kunkel T, Chua NH (2001) A plastidic ABC protein involved in intercompartmental communication of light signaling. *Genes Dev* 15:90–103
80. Surpin M, Larkin RM, Chory J (2002) Signal transduction between the chloroplast and the nucleus. *Plant Cell* S327–S338
81. Escoubas JM, Lomas M, LaRoche J, Falkowski PG (1995) Light intensity regulation of cab gene transcription is signaled by the redox state of the plastoquinone pool. *PNAS* 92:10237–10241
82. Pfanschmidt T, Schütze K, Brost M, Oelmüller R (2001) A novel mechanism of nuclear photosynthesis gene regulation by redox signals from the chloroplast during photosystem stoichiometry adjustment. *J Biol Chem* 276:36125–36130
83. Pursiheimo S, Mulo P, Rintamäki E, Aro EM (2001) Coregulation of light-harvesting complex II phosphorylation and Lhcb accumulation in winter rye. *Plant J* 26:317–327
84. Bonardi V, Pessaresi P, Becker T, Schieff E, Wagner R, Pfanschmidt T et al (2005) Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. *Nature* 437:1179–1182
85. Rhoads DM, Subbiah CC (2007) Mitochondrial retrograde regulation in plants. *Mitochondrion* 7:177–194
86. Gray GR, Maxwell DP, Villarimo AR, McIntosh L (2004) Mitochondria/nuclear signaling of alternative oxidase expression occurs through distinct pathways involving organic acids and reactive oxygen species. *Plant Cell Rep* 23:497–503
87. Matsuo M, Obokata J (2006) Remote control of photosynthetic genes by the mitochondrial respiratory chain. *Plant J* 47:873–882
88. Jahnke LS (1999) Massive carotenoid accumulation in *Dunaliella bardawil* induced by ultraviolet-A radiation. *J Photochem Photobiol B* 48:68–74
89. Salguero A, León R, Mariotti A, Morena B, Vega JM, Vilchez C (2005) UV-A mediated induction of carotenoid accumulation in *Dunaliella bardawil* with retention of cell viability. *Appl Microbiol Biotechnol* 66:506–511
90. Kobayashi M, Kakizono T, Nagai S (1993) Enhanced carotenoid biosynthesis by oxidative stress in acetate-induced cyst cells of a green unicellular alga, *Haematococcus pluvialis*. *Appl Environ Microb* 59:867–873
91. Xiong W, Li X, Xiang J, Wu Q (2008) High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production. *Appl Microbiol Biotechnol* 78:29–36
92. Shaish A, Avron M, Pick U, Ben-Amotz A (1993) Are active oxygen species involved in induction of β -carotene in *Dunaliella bardawil*? *Planta* 190:363–368
93. Steinbrener J, Linden H (2003) Light induction of carotenoid biosynthesis genes in the green alga *Haematococcus pluvialis*: regulation by photosynthetic redox control. *Plant Mol Biol* 52:343–356
94. Ramos A, Coesel S, Marques A, Rodrigues M, Baumgartner A, Noronha J et al (2008) Isolation and characterization of a stress-inducible *Dunaliella salina* Lcy- β gene encoding a functional lycopene β -cyclase. *Appl Microbiol Biotechnol* 79:819–828

95. Kiffin R, Bandyopadhyay U, Cuervo AM (2006) Oxidative stress and autophagy. *Antioxid Redox Sign* 8:152–162
96. Lei GP, Qiao DR, Bai LH, Xu H, Cao Y (2008) Isolation and characterization of a mitogen activated protein kinase gene in the halotolerant alga *Dunaliella salina*. *J Appl Phycol* 20:13–18
97. Eom H, Lee CG, Jin E (2006) Gene expression profiling analysis in astaxanthin-induced *Haematococcus pluvialis* using a cDNA microarray. *Planta* 223:1231–1242
98. Nedelcu AM (2006) Evidence for p53-like-mediated stress responses in green algae. *FEBS Lett* 580:3013–3017



<http://www.springer.com/978-3-319-23807-4>

Microalgae Biotechnology

Posten, C.; Feng Chen, S. (Eds.)

2016, XIX, 188 p., Hardcover

ISBN: 978-3-319-23807-4