

Chapter 8

Carotenoids in Microalgae

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Abstract Carotenoids are a class of isoprenoids synthesized by all photosynthetic organisms as well as by some non-photosynthetic bacteria and fungi with broad applications in food, feed and cosmetics, and also in the nutraceutical and pharmaceutical industries. Microalgae represent an important source of high-value products, which include carotenoids, among others. Carotenoids play key roles in light harvesting and energy transfer during photosynthesis and in the protection of the photosynthetic apparatus against photooxidative damage. Carotenoids are generally divided into carotenes and xanthophyls, but accumulation in microalgae can also be classified as primary (essential for survival) and secondary (by exposure to specific stimuli).

In this chapter, we outline the high value carotenoids produced by commercially important microalgae, their production pathways, the improved production rates that can be achieved by genetic engineering as well as their biotechnological applications.

Keywords *Haematococcus pluvialis* • *Dunaliella salina* • Algae • Metabolic engineering • Cetocarotenoids • Astaxanthin

8.1 Introduction

Microscopic algae are thought to be among the oldest forms of life on earth. Microalgae comprise one of the most diverse kinds of plant life and they are the world largest group of photosynthetic organism which is capable of growing using CO₂ as the sole carbon source and light as energy (Gong et al. 2011; Leu and Boussiba 2014).

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Microalgae occupy the bottom of the food chain in aquatic ecosystems (Guedes et al. 2011). They contribute to approximately 50 % of the global photosynthetic activity, despite the fact that their photosynthetic biomass represents only about 0.2 % of that available on land (Parker et al. 2008). Microalgae are ubiquitous in nature. They have colonized nearly all of Earth's existing ecosystems, from the polar regions to deserts and hot springs (Cadoret et al. 2008). The estimated number of microalgae species that are known to exist ranges between 40,000 and 60,000, but estimations of the number of undescribed species range from hundreds of thousands to millions of species spread across the globe (Cadoret et al. 2012). Only a few thousands strains are kept in collections, a few hundred are investigated for chemical content and only a handful are currently of commercial significance (Olaizola 2003).

Microalgae have attained particular attention in recent years since they are an enormous biological resource, representing one of the most promising sources for new products and applications for commercialization (Pulz and Gross 2004). The application of microalgal biomass and/or its metabolites is an interesting and innovative approach for the development of healthier food products (Batista et al. 2011). Furthermore, some species of microalgae such as *Spirulina*, *Chlorella*, *Dunaliella*, *Haematococcus*, and *Schizochytrium* have been classified as food sources, falling into the GRAS (Generally Regarded as Safe) category by the U.S. Food and Drug Administration (Chacón-Lee and González-Mariño 2010). The potential of microalgal photosynthesis for the production of high-value compounds or for energetic use is well recognized because of their more efficient utilization of sunlight energy when compared with higher plants. Currently, algae are the main sustainable source of commercial carotenoids for the aquatic food chain (Dufosse et al. 2005; Borowitzka 2013). Microalgae are extremely efficient solar energy converters and they can generate a great variety of metabolites that can be harnessed for commercial use (Gong et al. 2011). Many microalgal species can naturally synthesize a wide range of metabolites, such as proteins, lipids, carbohydrates, carotenoids or vitamins for health, food and feed additives, cosmetics, pharmaceutical industries and for energy production (Chacón-Lee and González-Mariño 2010; Priyadarshani and Biswajit 2012).

Undoubtedly, carotenoids have received increased attention during the last decade due to their intrinsic antioxidant activity and potential function in preventing adverse health conditions in humans. Carotenoids are accessory pigments in the photosynthetic apparatus with a primary role in light harvesting. Carotenoids are accumulated up to 8–14 % of biomass in microalgae and are divided into carotenes, which are true hydrocarbons, and xanthophylls, which also contain oxygen atoms (Priyadarshani et al. 2012; Mulders et al. 2014). Carotenoids accumulated in microalgae can be classified as primary and secondary carotenoids. Primary carotenoids are essential for survival, acting as structural and functional components of the cellular photosynthetic apparatus. The secondary carotenes are only accumulated after exposure to specific environmental stimuli via carotenogenesis (Guedes et al. 2011). The carotenes include β -carotene and lycopene and the xanthophyll carotenoids include lutein and astaxanthin, among others (Fassett and Coombes 2011). In most green algae, carotenoids are synthesized within plastids

and accumulate therein only. But in other microalgae, such as *Haematococcus pluvialis* sp., the secondary xanthophyll astaxanthin accumulates in lipid vesicles in the cytoplasm (Guedes et al. 2011). The most important source of carotenoids belongs to the Chlorophyceae family, which includes *Chlorella*, *Chlamydomonas*, *Dunaliella*, *Muriellopsis* and *Haematococcus* spp. (Pulz and Gross 2004). They have the biggest diversity of pigments and are the only group that overproduces secondary carotenoids in response to adverse growth conditions, when they have photoprotective roles (Mulders et al. 2014). Only a few microalgae strains are currently being commercially exploited for the production of β -carotene, astaxanthin or lutein, but research is seeking to optimize the production of these compounds as well as other commercially important carotenoids. Natural sources of these compounds studied to date are mainly achieved by *Dunaliella salina* (orange pigment β -carotene), *Haematococcus pluvialis* (red pigment astaxanthin) and *Scenedesmus* sp. and *Chlorella* sp. (lutein pigment). Additionally, some species of microalgae can be efficiently transformed, which makes it possible to enhance the productivity of natural compounds through metabolic engineering (Leon et al. 2007; Cordero et al. 2011). This chapter focuses on the high value carotenoids produced by these commercially important microalgae, their production pathways, improvement of production by genetic engineering and the biotechnological applications of these compounds.

8.2 Carotenogenesis Pathways Among Algae

The large groups of secondary metabolites called carotenoids are synthesized within the chloroplast but are catalyzed by enzymes encoded by nuclear genes. These enzymes are synthesized as precursor polypeptides with a transit peptide at the amino-terminus that targets them to the chloroplast (Leon et al. 2007). The most abundant naturally occurring carotenoids are hydrophobic tetraterpenoids that contain a C40 methyl branched hydrocarbon backbone. The polyene chains of carotenoids, consisting of conjugated double bonds, are responsible for the pigmentation of carotenoids and their ability to absorb photons in visible wavelengths (Baroli and Niyogi 2000; Leon et al. 2007). Carotenoids are synthesized by all photosynthetic organisms as well as by many non-photosynthetic bacteria and fungi. There are two main classes of naturally occurring carotenoids: carotenes, which are hydrocarbons that are either linear or cyclized at one or both ends of the molecule, such as β -carotene, and xanthophylls, that are oxygenated derivatives of carotenes such as astaxanthin (Fig. 8.1). All xanthophylls produced by higher plants, for example violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, and lutein, are also synthesized by green microalgae. However, in contrast to land plants, specific green algae species possess additional xanthophylls such as loroxanthin (Baroli and Niyogi 2000), astaxanthin and canthaxanthin (Grünewald et al. 2001). In addition, diatoxanthin, diadinoxanthin, and fucoxanthin are produced by brown algae or diatoms (Lohr and Wilhelm 1999; Lohr and Wilhelm 2001).

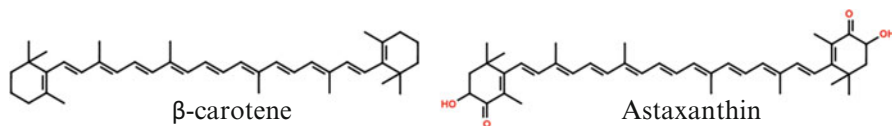


Fig. 8.1 Chemical structures of β -carotene and astaxanthin found in microalgae

Carotenoids play major roles in oxygenic photosynthesis, where they function in light harvesting and protect the photosynthetic apparatus from excess light by energy dissipation (Frank and Cogdell 1996; Baroli and Niyogi 2000; Telfer 2005; Leon et al. 2007). Carotenoids that fulfill these processes are commonly referred as primary carotenoids and are structural and functional components of the photosynthetic apparatus of the cell and therefore essential for cellular survival. In contrast, secondary carotenoids are defined functionally as carotenoids that are not obligatory for photosynthesis and are not localized in the thylakoid membranes of the chloroplast. Nevertheless, in some green algae, secondary carotenoids are accumulated outside the plastid in cytoplasmic lipid vesicles (Krishna and Mohanty 1998; Lemoine and Schoefs 2010) in large quantities as a response to stresses (carotenogenesis) like nutrient starvation, high salinity, high light, etc. (Lemoine et al. 2008; Moulin et al. 2010).

Actually, a large number of green microalgae and their relatives display the capacity to accumulate secondary carotenoids under stress (Lemoine and Schoefs 2010), despite this, only algae, such as *Haematococcus pluvialis* (Camacho et al. 2013; Nunes et al. 2013; Chekanov et al. 2014; Giannelli et al. 2015), *Chlorella zofingiensis* (Wang et al. 2009; Li et al. 2009; Liu et al. 2014), *Chlorella phototheoides* (Wei et al. 2008; Li et al. 2013), *Dunaliella salina* (Coesel et al. 2008a; Chen et al. 2011; Prieto et al. 2011) and *Scenedesmus almeriensis* (Cerón et al. 2008; Sanchez et al. 2008; Macias-Sanchez et al. 2010) have been extensively studied. This is because they are used in the commercial production of astaxanthin and/or β -carotene and lutein in medium- and large scale cultures (for reviews, see García-González et al. 2005; Sun et al. 2014; Lamers et al. 2008; Ye et al. 2008; Guedes et al. 2011; Lopez et al. 2013; Han et al. 2013; Leu and Boussiba 2014; Ranga Rao et al. 2014; Wan et al. 2014; Hong et al. 2015).

Recently, many genes responsible for carotenoid production, particularly related to astaxanthin biosynthesis, have been cloned and characterized in *H. pluvialis* (Cui et al. 2012; Gao et al. 2012; Kathiresan et al. 2015), *Chlorella zofingiensis* (Huang et al. 2008; Cordero et al. 2010; Liu et al. 2010, 2014) and *Chlamydomonas reinhardtii* (Cordero et al. 2011; Couso et al. 2011; Liu et al. 2013; Zheng et al. 2014). This has provided the opportunity to study the pathways and regulation of carotenoid biosynthesis and to further understand the biological role of astaxanthin in the stress response.

Isopentenyl pyrophosphate (IPP) is the precursor for carotenoid synthesis (Lichtenthaler 1999). Two distinct pathways for IPP biosynthesis have been found in higher plants: the mevalonate pathway in the cytosol and the non-mevalonate

1-deoxy-D-xylulose-5-phosphate pathway in the chloroplast (DOXP pathway or MEP pathway) (Lichtenthaler et al. 1997). In unicellular green microalgae such as *H. pluvialis* and *Chlamydomonas reinhardtii*, IPP is thought to be synthesized solely from the non-mevalonate DOXP pathway (Disch et al. 1998). Subsequently, the isopentenyl pyrophosphate isomerase (IPI) catalyzes the isomerization of IPP to dimethylallyl diphosphate (Lichtenthaler 1999; Rohdich et al. 2003; Rohmer 2007). In the unicellular green microalgae *H. pluvialis* and *Chlamydomonas reinhardtii*, IPP and its allylic isomer dimethylallylpyrophosphate (DMAPP) are synthesized along the non-mevalonate pathway in plastids (Disch et al. 1998; Ladygin 2000; Rohmer 2010). Two cDNAs of IPI, *IPI1* and *IPI2* have been cloned and characterized in *H. pluvialis* (Sun et al. 1998). Transcripts of both *IPI* genes increased in response to oxidative stress, but only *IPI2* was up-regulated at the translational level. Moreover, only the *IPI2* protein was detected in the mature red cysts in which astaxanthin was accumulated, suggesting that *IPI2* is responsible for synthesis of the secondary carotenoids, whereas the *IPI1* is responsible for primary carotenoid synthesis in the chloroplast of *H. pluvialis* (Sun et al. 1998).

Phytoene synthase (PSY) catalyzes the first committed step for carotenoid biosynthesis through condensation of two 20-carbon geranylgeranyl pyrophosphate (GGPP) molecules to form a 40-carbon phytoene, the precursor for all other carotenoids (Cunningham and Gantt 1998). Two classes of PSYs were found in certain green algae like *Ostreococcus* and *Micromonas*, while some other green algae like *C. reinhardtii* and *C. vulgaris* only possess one class of PSYs (Tran et al. 2009). One copy of the *PSY* gene has been cloned and characterized from a number of microorganisms including *H. pluvialis* (Steinbrenner and Linden 2001) and *C. zoofingensis* (Cordero et al. 2011).

As shown in Fig. 8.2, the successive desaturation reactions of phytoene synthase (PSY) are catalyzed by two structurally similar enzymes, phytoene desaturase (PDS) and ζ -carotene desaturase (ZDS), converting the colorless phytoene into red lycopene. Specifically, PDS catalyzes the first two dehydrogenation reactions to form phytofluene and ζ -carotene, whereas ZDS catalyzes two further reactions converting ζ -carotene to neurosporene and lycopene (Cunningham and Gantt 1998). These dehydrogenation reactions extend the conjugated carbon-carbon double bonds to form the chromophore of carotenoids (Lemoine and Schoefs 2010).

These FAD-containing enzymes require PTOX (Plastid Terminal Oxidase) and plastoquinone (PQ) as electron acceptors (Wu et al. 1999; Houille-Vernes et al. 2011). One *pds* and two *ptox* genes (i.e., *ptox1* and *ptox2*) have been cloned and characterized in *H. pluvialis* (Grünewald and Hagen 2000; Wang et al. 2009; Li et al. 2010). High light illumination and nitrogen deprivation increase the number of transcripts of *PDS* and *PTOX* simultaneously in *H. pluvialis*, suggesting that *PDS* and *PTOX* may act in concert to dehydrogenate phytoene and remove excess electrons under stress, thereby preventing over-reduction of the photosynthetic electron transport chain and the formation of excess ROS (Reactive Oxygen Species) (Grünewald and Hagen 2000; Wang et al. 2009; Li et al. 2010).

The cyclization of lycopene catalyzed by lycopene β -cyclase (LCY-b) and lycopene ϵ -cyclase (LCY-e) is a branching point in carotenoid biosynthesis

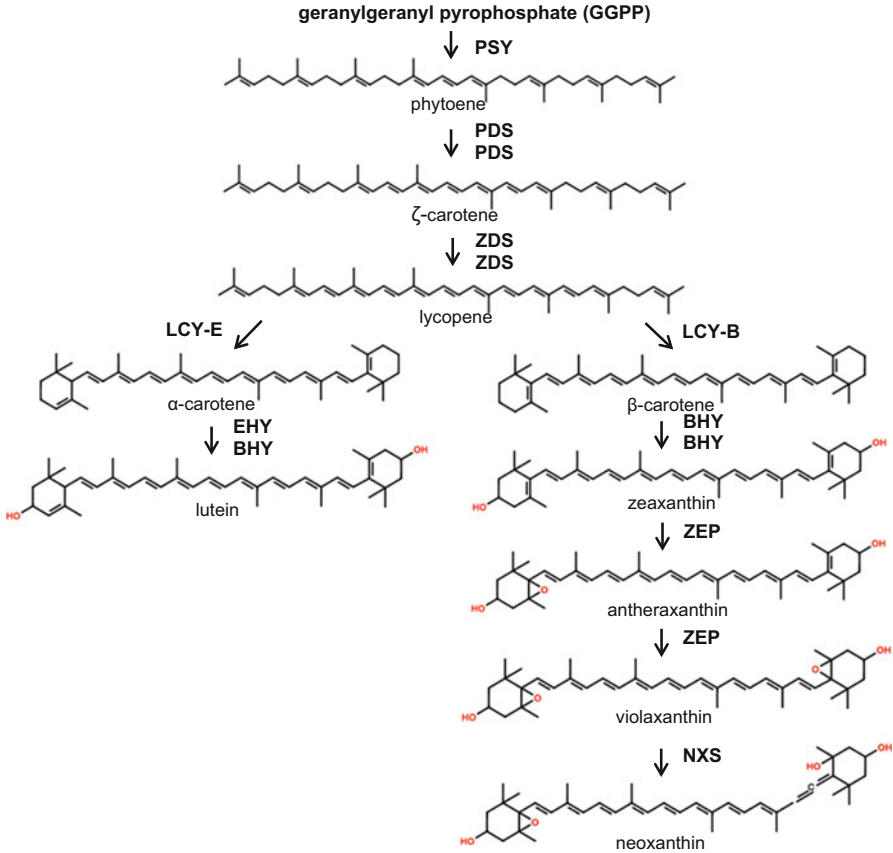


Fig. 8.2 A hypothetical pathway for carotenogenesis in microalgae. Enzymes for the relative conversions are in *bold*. *PSY* Phytoene Synthase, *PDS* Phytoene Desaturase, *ZDS* ζ-carotene Desaturase, *LYC-E* Lycopene ε-cyclase, *LYC-B* lycopene β-cyclase, *EHY* ε-carotene Hydroxylase, *BHY* β-carotene Hydroxylase, *ZEP* Zeaxanthin Epoxidase, *NXS* Neoxanthin Synthase (Modified from Ye et al. 2008)

(Cunningham and Gantt 1998). Lycopene is cyclized on both ends by the enzyme lycopene β-cyclase (LCY-b) to form β-carotene. The two beta rings of β-carotene are subjected to identical hydroxylation reactions to yield zeaxanthin, which in turn is epoxidated once to form antheraxanthin and twice to form violaxanthin. Neoxanthin is derived from violaxanthin by an additional rearrangement (Hirschberg 2001; Naik et al. 2003; Ye et al. 2008; Sandmann 2009). Higher plants and green algae have additional carotenoids, α-carotene derivatives (β, ε-carotene), which are also derived from lycopene by the action of LCY-b and LCY-e, for example hydroxylation of the β-ring and ε-ring of α-carotene forms lutein (Pogson et al. 1996, 1998). During xanthophyll formation (Fig. 8.2) the carotenoid structures are modified such that the end product pigments are often species-specific (Grossman et al. 2004; Sandmann

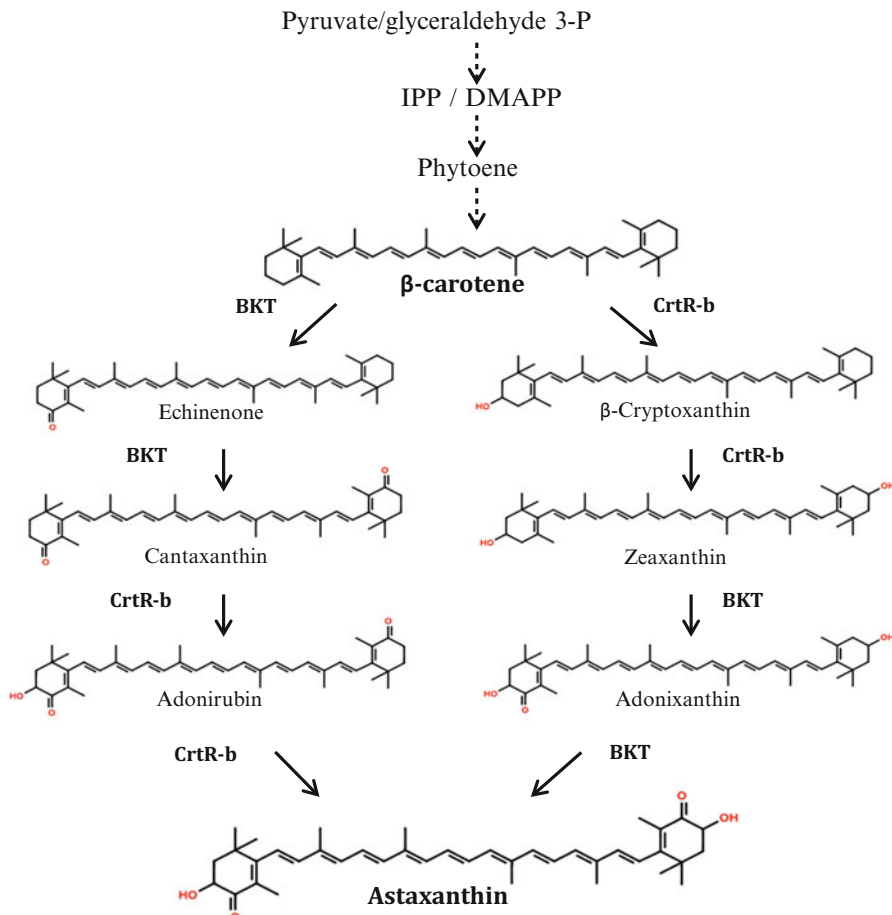


Fig. 8.3 *H. pluvialis* pathway of secondary carotenoid synthesis. The enzymes catalyzing the late enzymatic steps, namely β -carotene oxygenase (*CRTO*, β -carotene ketolase, BKT) and the β -carotene hydroxylase (*CrtR-B*) are indicated (Modified from Grünewald et al. 2001)

et al. 2006; Giuliano et al. 2008; Vidhyavathi et al. 2008; Ye et al. 2008; Lemoine and Schoefs 2010; Couso et al. 2011; Camacho et al. 2013). In *Haematococcus*, astaxanthin accumulation occurs in extra-plastidic lipid globules as a secondary carotenoid (Grünewald et al. 2001). Figure 8.3 shown the formation of astaxanthin at the expense of β -carotene, which requires the introduction of two hydroxyl groups at C3 and C3' by the β -carotene hydroxylase, CrtR-b (synonymous Chy/CrtZ) gene product (3,3'-hydroxylase) and two keto groups at C4 and C4' by β -carotene oxygenase, the CrtO gene product (synonymous bkt) (4,4'-ketolase), (Grünewald et al. 2001; Vidhyavathi et al. 2008; Lemoine and Schoefs 2010).

In contrast to *H. pluvialis*, *C. zoofingensis* may synthesize astaxanthin through the zeaxanthin pathway (Huang et al. 2006; Li et al. 2008). This xanthophyll is formed

through the catalytic action of violaxanthin deepoxidation (Moulin et al. 2010). Recently, the genes involved in the biosynthesis of astaxanthin in this species have been cloned and characterized, including *PSY* (Cordero et al. 2011), *PDS* (Huang et al. 2008), *LCY-b* (Cordero et al. 2010), *LCY-e* (Cordero et al. 2012), *BKT* (*CrtO*) (Huang et al. 2006), *CrtR-b* (*Chy-b*) (Li et al. 2008). Under high light conditions, the *PSY*, *PDS*, *BKT*, *CrtR-b* genes were up-regulated, whereas the mRNA levels of *LCY-b* and *LCY-e* remained constant, leading to formation of secondary carotenoids (Li et al. 2009; Cordero et al. 2012). Functional analysis of *C. zoofingiensis* BKT demonstrated that this enzyme did not only convert β -carotene to canthaxanthin via echinenone, but it also exhibited high enzymatic activity in converting zeaxanthin to astaxanthin via adonixanthin (Huang et al. 2006).

Another carotenoid in high demand around the world is β -carotene (Ye et al. 2008). Some species of *Dunaliella*, especially *D. salina* and *D. bardawil*, have the ability to accumulate large amounts of carotenoids when exposed to specific extreme environmental conditions, such as high light intensity, high salinity, extreme temperatures and/or nutrient deprivation. *D. salina* can accumulate up to $\sim 10\%$ of dry algal biomass as β -carotene (García-González et al. 2005; Lamers et al. 2008).

Although the biochemistry and physiology of *Dunaliella* has been well investigated, molecular elucidation of the carotenogenic pathway of *Dunaliella* has only been conducted in recent years, and only some of the carotenogenic enzymes have been isolated (Yan et al. 2005; Sun et al. 2008a, b; Ramos et al. 2009). The carotenogenic pathway of *Dunaliella* is in accordance with that of higher plants (Ye and Jiang 2010). The first two enzymes specifically used in the carotenoid biosynthetic pathway are *PSY* and *PDS*, which together convert two geranylgeranyl diphosphate molecules into phytoene and ζ -carotene, respectively. These genes (*psy* and *pds*) are under transcriptional control in response to environmental stimuli and are considered to play a key role in the regulation of carotenogenesis (Rabbani et al. 1998; Sanchez-Estudillo et al. 2006; Coesel et al. 2008a, b). The pathway of carotenogenesis from geranylgeranyl pyrophosphate (GGPP) to β -carotene in *Dunaliella* is shown in Fig. 8.2.

Several microalgae have been proposed as potential producers of lutein, such as *Chlorella protothecoides* (Shi et al. 2002; Li et al. 2013) and *Scenedesmus almeriensis* (Sanchez et al. 2007, 2008). Lutein is one of the main photosynthetic pigments in the xanthophyllid family, and contains a large conjugated carbon system attached with hydroxyl or carbonyl groups (Ho et al. 2014). Lutein biosynthesis follows the general pathway of carotenoids, with *PDS* being the key enzyme involved in biosynthesis (Li et al. 2013). As shown in Fig. 8.2, in one branch the concentrated action of β - and ϵ -cyclases results in the formation of α -carotene, hydroxylation of which leads to the formation of lutein (Pogson et al. 1996, 1998; Li et al. 2013).

The most important factors that affect lutein content in microalgae are temperature, light, pH, the availability and source of nitrogen, salinity (or ionic strength) and the presence of oxidizing substances (or redox potential); however, specific growth rate also plays a crucial role in the biosynthesis of lutein (Wei et al. 2008; Guedes et al. 2011; Campenni et al. 2013).

8.3 Metabolic Engineering of Carotenoid Biosynthesis in Eukaryotic Microalgae

Despite the high value of carotenoids and the advantages of microalgal platforms, there are few efforts towards the optimization of carotenoid production through metabolic engineering of these organisms. Since most of the carotenogenic pathway occurs in the chloroplast of green algae, two genetic strategies can be undertaken for this purpose. Expression of inserted genes from the chloroplast genome ensures that the proteins will be localized in the right place, and it is generally regarded as the best strategy for high levels of protein accumulation (Barrera et al. 2014). On the other hand, nuclear expression requires that the proteins should be translocated to the chloroplast (as happens for all the endogenous carotenogenic enzymes). Expression from the nucleus is usually associated with lower expression levels due to silencing and position effects, but it is the only location where one can express proteins that require eukaryotic post-translational modifications for their function (Kempinski et al. 2015).

Chlamydomonas reinhardtii has served as the main model organism for studying the effects of genetic engineering in carotenoid accumulation. In the first report of this type, Fukusaki et al. (2003) successfully expressed an archeal gene for a heat-stable version of the geranylgeranyl-pyrophosphate (GGPP) synthase enzyme involved in the early steps of carotenoid biosynthesis in the chloroplast of *C. reinhardtii*. Unfortunately, there were no measurable effects on the isoprenoid profile of the algae. Three years later, another group attempted to produce keto-carotenoids (e.g. astaxanthin) in *C. reinhardtii* by nuclear overexpression of the β -carotene ketolases from *H. pluvialis* (*bkt3*) and *C. reinhardtii* itself (*CRBKT*). Following several efforts using different expression vectors, no keto-carotenoids could be detected (Wong 2006). In parallel, Leon et al. (2007) used an analogous approach, but using the *bkt1* gene from *H. pluvialis* instead. In this case a small peak of 4-ketolutein could be detected, which was not present in the parental strain. Unfortunately, no peak for astaxanthin was seen. RNA interference technology has also been used for altering the carotenoid profile of *C. reinhardtii*. By targeting the *pds* gene coding for the enzyme responsible for second step of carotenoid biosynthesis, its mRNA was reduced up to 93 % percent. Nonetheless, the carotenoid content of the algae did not change significantly, pointing towards the existence of additional rate-limiting processes (Vila et al. 2008). Additionally, the *psy* gene, which encodes the first step enzyme for carotenoid synthesis, has been transformed into the *C. reinhardtii* nucleus, causing an increase in carotenoid accumulation. Transformed strains overexpressing *psy* from *Dunaliella salina* and *Chlorella zofingiensis* stored 2.6 and 2.2-fold more lutein than the wild-type algae, respectively (Cordero et al. 2011; Couso et al. 2011). Recently, the *C. reinhardtii* nucleus has been transformed with a point mutant version of its endogenous *pds* gene. The mutant enzyme had a 27 % increase in its desaturase activity *in vitro*. The algae became resistant to the herbicide norflurazon and accumulated more lutein, β -carotene, zeaxanthin, and violaxanthin *in vivo* (Liu et al. 2013).

H. pluvialis, *D. salina* and *Chlorella* are also highly desirable candidates for carotenoid metabolic engineering given their commercial relevance. In the first attempt of stable nuclear transformation, *H. pluvialis* was engineered with a mutated *pds* gene that conferred resistance to norflurazon. Transgenic strains accumulated up to 26 % more astaxanthin than the *wild-type* control after 48 h of high light induction (Steinbrenner and Sandmann 2006). RNA interference constructs have been used to reduce the mRNA accumulation of the *D. salina pds* gene by up to 72 %. Intriguingly, the carotenoid content of these strains was not reported (Sun et al. 2008a, b). The most recent metabolic engineering effort involves the development of a nuclear transformation method for *Chlorella zofingiensis*. A mutant version of the endogenous *pds* gene was transformed, conferring resistance to norflurazon. The mutant PDS enzyme had a 33 % higher *in vitro* desaturation activity. Transformed *C. zofingiensis* strains accumulated up to 32.1 % more total carotenoids and 54.1 % more *in vivo* astaxanthin (Liu et al. 2014).

Finally, it is worth mentioning that the efforts related to carotenoid metabolic engineering in eukaryotic microalgae will probably increase in the short-term, given the great amount of newly sequenced genomes, and the increasing efforts to develop efficient and stable transformation techniques for commercially relevant strains. Furthermore, terpenoids from microalgae are now also regarded as a highly desirable feedstock, as a basis for biofuels and for the production of specialty chemicals, which will further broaden the interest for research towards optimizing the production of these compounds (Davies et al. 2014; Heider et al. 2014).

8.4 Biological Functions and Applications

Nowadays, there is a growing interest in the production of secondary carotenoids by microalgae, because they are molecules of high commercial value, specifically for the pharmaceutical industry and nutritional applications. These compounds have pigmentation properties, which have extensive application in the food and feed industry (Lorenz and Cysewski 2000). They play key role in high-grade animal nutrition, from aquaculture to farm animals (García-Chavarría and Lara-Flores 2013) as well as providing protection due to their antioxidant activity in many organisms, including in human health (Guerin et al. 2003). The powerful anti-oxidative properties make carotenoids an important class of nutrients in health promotion. In aquaculture and animal farming they have positive effects on adequate growth and reproduction of commercially valuable species (Del Campo et al. 2007). Appropriate levels of intake prevent or delay chronic diseases in humans (Onogi et al. 1998; Ciccone et al. 2013; Fernández-Sevilla et al. 2010). The carotenoid market has grown exponentially in the last few years and this trend is projected to continue. According to the update of the BCC Research report FOD025C, The Global Market for Carotenoids published in 2008 (<http://www.reportlinker.com>), the worldwide market value of the commercial application of carotenoids was estimated at nearly \$1.2 billion in 2010, with a chance to grow to \$1.4 billion in 2018 with a compound

annual growth rate of 2.3 %. Growth of this market has been led by Europe followed by North America. The U.S. carotenoid market not only dominates North America but also the global market, but the Asia-Pacific region is projected to be the fastest-growing carotenoid market for the period under consideration (Carotenoids Market by Type, Source, Application, & by Region - Global Trends & Forecasts to 2019, <http://www.reportlinker.com>). The major carotenoids with commercial interest are β -carotene followed by the xanthophylls - astaxanthin and lutein. The β -carotene from *Dunaliella salina* was the first carotenoid from algae to be commercialized. *Dunaliella salina* is a unicellular, bi-flagellate and naked green alga with no cell wall. This halotolerant microalga is the richest natural source of the carotenoid β -carotene when exposed to stress conditions such as high light intensity or nutrient starvation (over 10 %) (Emeish 2012). Like all other carotenoids, β -carotene is an antioxidant, protects the body from damaging free radicals and is a source of photosynthetic dye pigments (Oren 2005). β -Carotene is a provitamin that is converted to vitamin A, which is needed to form rhodopsin in the outer segment of eye rod cells. Vitamin A is required for good vision and eye health, for a strong immune system, and for healthy skin and mucous membranes (Perusek and Maeda 2013). β -Carotene is also used as a food coloring (the yellow color in margarine), as a food additive to enhance the color of the flesh of fish and the yolk of eggs, and to improve the health and fertility of grain-fed cattle (Borowitzka and Borowitzka 1987). Research studies by the National Cancer Institute have shown that β -carotene is anti-carcinogenic; other studies have found that is effective in controlling cholesterol and in reducing the risk of heart disease as well (Priyadarshani and Biswajit 2012). Moreover, β -carotene is a valuable nutraceutical, used as a vitamin C supplement. Lastly, *D. salina* is commercially produced in several countries, including Israel, Australia and USA (Borowitzka 2013).

Haematococcus pluvialis is a ubiquitous freshwater green microalga of major economic interest, known to synthesize and accumulate astaxanthin under specific natural and artificial culture conditions (Boussiba 2000). Astaxanthin has received extensive attention because it is a strong antioxidant and a natural colorant with high market value (Leu and Boussiba 2014). It can be found in the encysted cells of *Haematococcus* as a symmetric ketocarotenoid (3.3 % dihydroxy- β , β -carotene 4.4 % dione) responsible for the red-pink color of many freshwater and marine fish as well as other aquatic organisms. Astaxanthin is one of the most abundant carotenoids in nature (Breithaupt 2007). Since the color of the muscle is an important quality parameter, xanthophylls are used for pigmentation in the aquaculture industry, also offering strong antioxidant and provitamin A activity to fish (Matsuno 2001; Miki 1991). Astaxanthin has terminal carbonyl groups that are conjugated to a polyene backbone and it is a more potent antioxidant and scavenger of free radicals than carotenoids such as β -carotene (Fassett and Coombes 2011). Thus, dietary supplementation with astaxanthin could potentially provide antioxidant protection of cells, including protection of the skin from the effects of UV radiation, amelioration of macular degeneration, protection against chemically induced cancers, atherosclerotic cardiovascular disease and enhancement of immune system (Lorenz and Cysewski

2000; Ranga Rao et al. 2014). Astaxanthin is a high value (\$15,000/Kg pigment) nutraceutical antioxidant, which has been extensively studied and produced worldwide by several companies in Chile, Israel and China (Leu and Boussiba 2014). Indeed, the unicellular green algae *Haematococcus pluvialis* accumulates astaxanthin esters mainly in cytoplasmic lipid vesicles at up to 5% of its total cellular dry weight when exposed to unfavorable growth conditions (Leu and Boussiba 2014).

At present, no commercial production of lutein is obtained from microalgae, although several species have been viewed as a promising alternative feedstock for production of the xanthophyll lutein (3R,30R,60R- β -carotene-3,30-diol). The most natural source of commercial lutein is from marigold flowers but this lutein is esterified with half of the weight corresponding to fatty acids and thus, chemical saponification is needed for purification (Lin et al. 2014). In this regard, microalgae such as *Muriellopsis sp.*, *Chlorella zofingensis*, *Scenedesmus sp.* and *Chlorella protothecoides* appear to be potential lutein producers capable of accumulating a much higher content: 0.5–1.2% dry weight (Sun et al. 2014; Chana et al. 2013). Pilot scale outdoor production has already been set up for lutein-rich cells of strains of *Muriellopsis sp.* and *Scenedesmus sp.* (Yaakob et al. 2014).

Lutein is often consumed as an additive used for flavor and color in foods, drugs and cosmetics (Yaakob et al. 2014). Furthermore it is employed as feed additive to brighten the colors of poultry feathers and deepen the yellow of egg yolk (Lin et al. 2014). Moreover, lutein is considered as an effective functional nutrient, providing beneficial properties to human health by ameliorating cardiovascular diseases (Dwyer et al. 2001), cancers (Ho et al. 2014), and preventing the development of cataracts and also preventing blindness or decrease in vision caused by age-related macular degeneration (Yaakob et al. 2014; Chiu and Taylor 2007).

8.5 Concluding Remarks

As described in this chapter, microalgae have attained particular attention since they are an enormous potential biological resource of a wide range of metabolites, especially the carotenoids, which represent an important group of structurally diverse terpenoid pigments with broad application in the feed, food, nutraceutical and pharmaceutical industries. Carotenoids also have intrinsic antioxidant activity and a potential role in preventing degenerative diseases and health conditions in humans. To significantly improve the carotenoid production, future research should be focused on metabolic engineering strategies in combination with cultivation optimization.

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