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# Carotenoids of Biotechnological Importance

**Gerhard Sandmann**

**Abstract** Carotenoids are natural pigments with antioxidative functions that protect against oxidative stress. They are essential for humans and must be supplied through the diet. Carotenoids are the precursors for the visual pigment rhodopsin, and lutein and zeaxanthin must be accumulated in the yellow eye spot to protect the retina from excess light and ultraviolet damage. There is a global market for carotenoids as food colorants, animal feed, and nutraceuticals. Some carotenoids are chemically synthesized, whereas others are from natural sources. Microbial mass production systems of industrial interest for carotenoids are in use, and new ones are being developed by metabolic pathway engineering of bacteria, fungi, and plants. Several examples will be highlighted in this chapter.

**Keywords** Carotenoid biosynthesis · Carotenoid market · Carotenoid production · Carotenoid sources · Metabolic pathway engineering

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# 1 Properties and Biosynthesis of Carotenoids

## *1.1 Carotenoid Structures and Chemical Features*

Carotenoids comprise a large group of terpenoid pigments found in pro- and eukaryotic organisms. They are initially synthesised with a chain of 30 or 40 carbon atoms from prenyl pyrophosphates, and they may be extended at a later stage up to 50 carbon atoms [28]. Carotenoids can also be cleaved to shorter metabolites, resulting in apo carotenoids such as crocetin (the red pigment of saffron) and bixin (from annatto seeds) [61]. They are lipophilic compounds and have to be stored in a lipophilic environment. In addition to a small number of acyclic structures, most carotenoids carry an ε- or a β-ionone terminal ring. The latter end group may carry oxo substituents, such as 2-HO, 3-HO, 4-keto, or 5,6-epoxy groups [11]. The most important structural feature of the carotenoid molecule is the conjugated polyene system. These Π-electrons determine the spectral properties with light absorbance in a range from approximately 400–500 nm which is responsible for their yellow via orange to red color and influences the chemical reactivity.

Cis/trans isomers occur at the double bonds of the polyene chain. In general, they are in the trans configuration, but during biosynthesis some can be inserted in a cis configuration. In natural β-carotene apart from all-E, prominent cis isomers are 9-, 13-, and 15-Z (Fig. 1). During handling and storage, trans double bonds can isomerize, especially under illumination. Some carotenoids modified at the ionone rings exist in different optical isomeric forms. For example, astaxanthin with 4-HO- and 4-keto groups at both ionone rings can exist with 3S,3′S, 3R,3′S, and 3R,3′R chirality (Fig. 2). The first enantiomer is present in most organisms that synthesize this carotenoid. The 3R,3′R isomer is the form found in astaxanthin from *Phaffia rhodozyma* (in the sexual state, named *Xanthophyllomyces dendrorhous*) [2]. Astaxanthin exists not only in its free form but also as fatty acid ester, such as in *Haematococcus pluvialis* [40].

## *1.2 Carotenoid Functions*

A universal function of carotenoids is protection at the cellular level against reactive oxygen species. This is highly important in organisms with photosynthesis, where carotenoids are essential. In the photosynthesis apparatus, specific carotenoids are integral components of pigment–protein complexes. In addition to their function in light harvesting, they quench the photosensitizer chlorophyll, preventing the formation of singlet oxygen and inactivate oxygen-derived radicals [36]. This antioxidative function is also exerted in nonphotosynthetic organisms, such as bacteria and fungi with photosensitizers other than chlorophyll. Carotenoids that are especially important in plants include 5,6-epoxy derivatives, which are the precursors for the synthesis of the phytohormone abscisic acid, which is an essential plant hormone regulating leaf

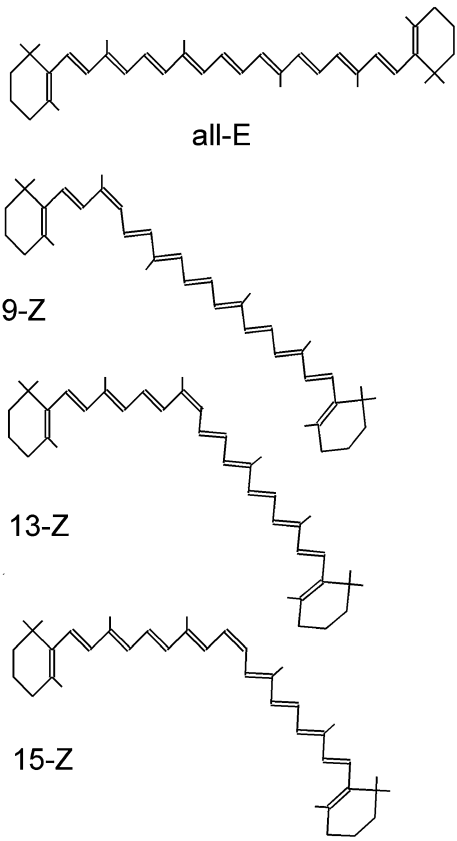


**Fig. 1** Prominent geometrical isomers of β-carotene

abscission, seed dormancy, and adaptation to drought stress [71]. In plants, flowers and fruit are pigmented by carotenoids to attract animals for pollination and seed dispersal.

## *1.3 Biosynthesis*

Carotenoids are a branch of terpenoid biosynthesis from precursors. In fungi, these originate from the mevalonate pathway; in bacteria, algae, and plants, they are from the 1-deoxy-D-xylulose 5-phosphate pathway (alternatively named the 2-*C*-methyl-D-erythritol 4-phosphate pathway). The specific pathway exclusive to C40 carotenoids starts from two molecules of geranylgeranyl pyrophosphate, which are condensed to phytoene, the first carotene in the pathway with three conjugated double bonds [58]. In the following desaturation steps, double bonds are inserted adjacent to the existing conjugated ones, alternatively at each side. This integrates an isolated double bond into the conjugated system. In contrast to fungi and non-photosynthetic bacteria, desaturations in algae and plants proceed via poly cis

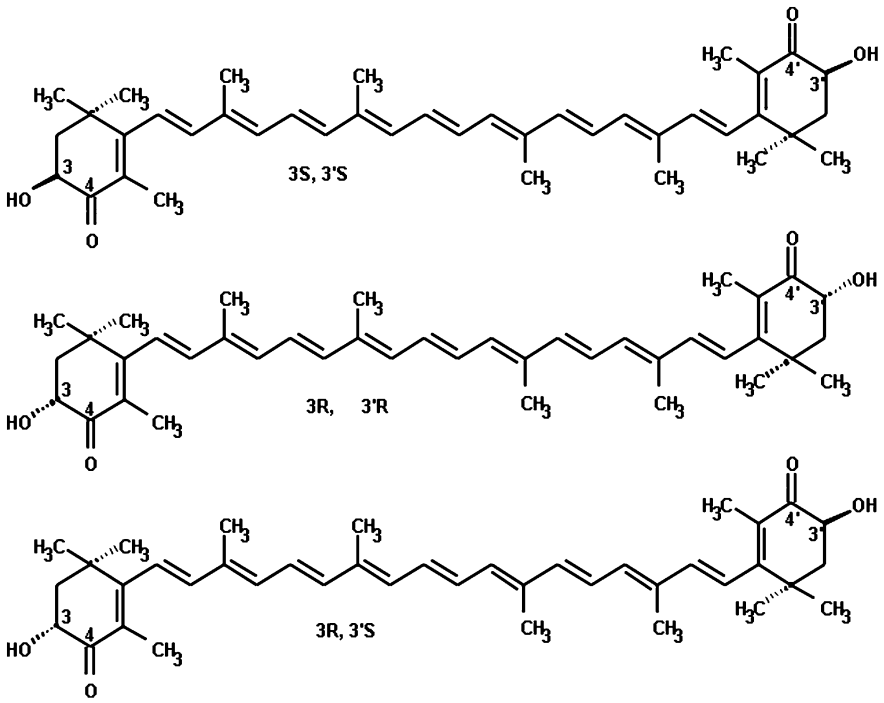


**Fig. 2** Stereoisomers of astaxanthin

carotenes, with a subsequent isomerization reaction yielding all-trans lycopene [10]. Most carotenoids are derived from lycopene by cyclization of both ends, forming either two β-ionone rings or one β- and one ε-ionone ring. These end groups are the primary targets for modifications. The most important oxo groups found in carotenoids are 3-hydroxy, 4-keto, and 5,6-epoxy. The pathway of commercially important carotenoids is shown in Fig. 3.

Carotenoids with less than 40 carbon atoms mostly originated from C40 carotenoids by cleavage. However, in a few bacteria, C30 carotenoids are synthesized directly from two molecules of C15 farnesyl pyrophosphate instead of C20 geranylgeranyl pyrophosphate. The subsequent metabolic reactions are similar to those of the C40 pathway [52]. In addition to C40 carotenoids, a few bacteria synthesize C45 and C50 carotenoids from lycopene by adding one or two C5 units to each end of the acyclic molecule [37].

## *1.4 Importance for Human Health*

Humans need carotenoids for their vision. The essential component of rhodopsin is the visual chromophore retinal. Precursors for retinal are carotenoids with an

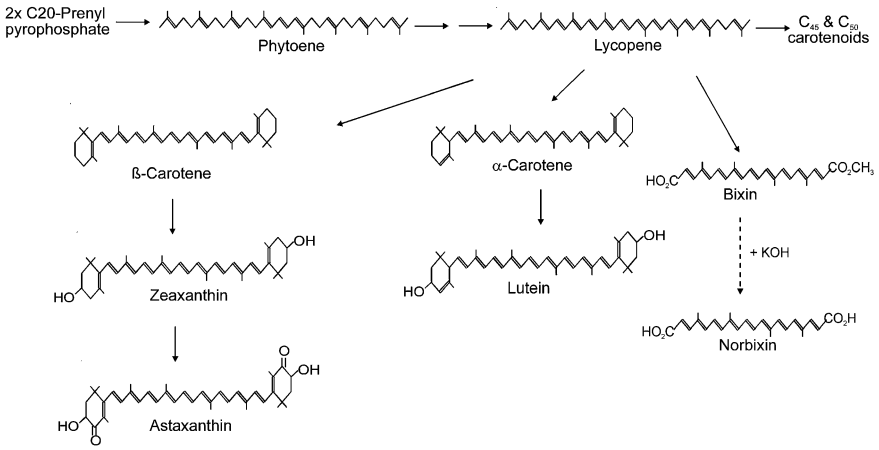


**Fig. 3** Structures and biosynthesis of commercially interesting carotenoids. *Dotted arrow* indicates chemical conversion

unsubstituted β-ionone ring, which are all called provitamin A. Because humans are devoid of synthesizing carotenoids, a deficiency of provitamin A carotenoids leads to blindness. Vitamin A deficiency is the leading cause of blindness in children, which is a severe problem in developing countries [7]. It is a big challenge to counteract this problem by supplementation and food fortification.

In addition to carotenoid-related rhodopsin acting as a visual pigment, two carotenoids—lutein and zeaxanthin—are located in the eye in the macula lutea (the yellow eye spot). There, they protect the retina from excess light and ultraviolet (UV) damage, thus preventing cataracts and age-related macular degeneration, which leads to blindness [38, 57]. Like the provitamin A carotenoids, zeaxanthin and lutein have to be provided in the human diet because low levels of intake increase the risk of age-related macular degeneration.

Other health-beneficial effects of carotenoids are attributed to carotenoids. They include protection against chronic diseases, anti-inflammatory actions, and enhancement of the immune response. Carotenoids are also regarded as potential treatment for some types of cancer. Some of these functions may be due to the antioxidative properties of the carotenoids.

## 2 Sources, Uses, and Production

Carotenoids are essential pigments for all photosynthetic organisms. In addition, they are also found in nonphotosynthetic prokaryotes and eukaryotes or nonphotosynthetic plant tissues. Carotenogenic plant tissues include leaves, roots, flowers, and fruit. In addition to natural carotenoids, several carotenoids are chemically synthesized in bulk quantities.

## 2.1 The Market for Carotenoids

There is a global market for carotenoids, which was valued at approximately US $1 billion in 2010, with good growth potential [4]. The dominating carotenoid was β-carotene, with a 25 % market share, followed by astaxanthin and lutein with 23 % each. The latter carotenoid is regarded to have the highest growth potential. The carotenoid with the highest market price was astaxanthin, at around $2,000 per kg for the synthetic compound and about $7,000 per kg for natural astaxanthin [64]. However, due to new suppliers for chemically synthesized astaxanthin, its price has recently dropped considerably. The structures of commercially important carotenoids are shown in Fig. 3.

Several carotenoids are approved and widely used as food colorants [44]. As a lipophilic compound, β-carotene provides a yellow pigmentation for food (e.g. butter) or liquids (e.g. soft drinks) after appropriate formulation. For the yellow pigmentation of dairy products, water-soluble norbixin (annatto) is used, which is derived from bixin by alkaline hydrolysis of the methyl ester group [33]. For red pigmentation in food and beverages, canthaxanthin or lycopene are appropriate.

There are two major applications for carotenoids as feed additives. In salmon and trout farming, the addition of either astaxanthin or canthaxanthin is essential to obtain the pink coloration of the flesh. In poultry, carotenoids are responsible for the color of the skin and egg yolk [67]. According to the different market demands for pigmentation, a broad range of synthetic or natural carotenoids are applied to the feed. Natural carotenoids in use are plant extracts or powder, such as those from the marigold as a source for yellow lutein or from red pepper as a source of reddish capsanthin.

The carotenoids astaxanthin and colorless phytoene are now used as ingredients in cosmetic skin products marketed by AstaReal and IBR, respectively. Phytoene, a colorless and relatively light-stable carotenoid, is used in dietary nutricosmetics accumulating in the skin and for topical skin application to protect against oxidative skin damage (http://www.cosmeticsandtoiletries.com/research/techtransfer/38701807.html).

## 2.2 Production of Carotenoids

For a few carotenoids, including β-carotene, astaxanthin, canthaxanthin, lycopene, and (to a certain extent) zeaxanthin, chemical synthesis is established on an industrial scale (see [18] for description of the synthesis processes). The main producers are BASF and DSM. These chemically synthesized carotenoids dominate the market, although they are also available as natural products to a small extent. For other commercially important carotenoids, such as lutein, only natural carotenoids are available. Considerable attempts have been made to replace chemically synthesized carotenoids at least partially by natural ones from renewable resources.

To date, most of the carotenoids produced by plants and microorganisms cannot compete because they are less cost-efficient to produce. One obstacle is the low yield of biological systems. Nevertheless, some carotenoid products from plant raw materials are on the market, including lutein from the flowers of *Tagetes erecta*, lycopene from tomato fruit, β-carotene from carrot roots and the fruit of the oil palm tree, capsanthin from red pepper, and bixin from *Bixa orellana* seeds.

### 2.2.1 Plants

The best plant source for β-carotene is red palm oil from the African palm (*Elaeis guineensi*). The oil is produced from the outer mesocarp of the fruit covering the seeds. It contains about 40 mg/g β-carotene and about half this amount of α-carotene together with other minor carotenes [48]. World production of palm oil in 2006 was 14 million tons. The largest producers are Malaysia and Indonesia (http://www.soyatech.com/Palm_Oil_Facts.htm).

The tomato fruit has one of the richest lycopene tissues. In commercial varieties, one can find more than 100 μg/g fresh weight [34]. Natural lycopene from tomato competes with synthetic lycopene. Tomato lycopene is used as a nutraceutical and as red food colorant. The product Tomat-O-Red from LycoRed contains tomato lycopene extracted from oleoresin in a crystalline formulation to deliver a fine dispersion in water.

Lutein is the most abundant plant carotenoid. Marigold (*Tagetes erecta*) flowers are a commercially important lutein source. In the petals, lutein fatty acid esters accumulate to concentrations as high as 3–6 mg/g [51]. Marigold is grown on commercial plantations; oleoresin powder or extracts from the flowers are used as chicken feed to improve the color intensity of the egg yolk [67]. Lutein esters purified from the oleoresin or free lutein are also available as nutraceuticals.

### 2.2.2 Microbial Mass Production Systems for β-carotene and Astaxanthin of Industrial Interest

Two organisms have been developed for the industrial production of β-carotene: the mucor fungus *Blakesleea trispora* [13] and the alga *Dunaliella salina* (or *D. bardawil*) [47]. *D. salina* is a halophilic unicellular alga that accumulates high levels of β-carotene as a stress response. β-Carotene production depends on high salinity, high temperatures, and high light conditions [53]. Typically, production sites for β-carotene from *D. salina* are located in hot areas with high solar radiation and a cheap salt source nearby. However, optimum salinity for carotenoid production is beyond 27 % NaCl, whereas maximum cell growth is in the range of 18–22 % NaCl [8]. The extreme environment in which *D. salina* grows allows for economically favorable outdoor large-scale cultivation with low risk of contamination. Compared to open ponds, paddlewheel-driven raceway ponds yield substantially higher biomass. The global production of *D. salina* is estimated at

about 1,200 tons per year (http://www.oilgae.com/non_fuel_products/betacarotene.html). The expected cellular β-carotene concentrations are in the range of 100 mg/g dry matter, or even higher [5]. Products on the market are *D. salina* powder as a β-carotene-rich supplement for human health and animal feed or extracted β-carotene in vegetable oil in concentrations of 1–20 %.

Alternatively, production processes have been developed with the fungus *B. trispora* by co-cultivation of (+) and (–) sexual mating types simultaneously in large-scale fermenters with plant materials as substrates, such as molasses [13]. Higher yields were obtained with improved mutant strains and with intersexual heterokaryons, which contain nuclei of opposite sex [43]. The typical concentration of β-carotene in fermenter-grown *B. trispora* mycelium is around 5 % [45]. From the cultured material, β-carotene is extracted and processed into a pure beta-carotene crystal. β-Carotene products in the market include CaroPure (DSM), with a purity of >97 %, and Lyc-O-Beta (LycoRed Company). This type of β-carotene material is approved and safe for use in dietary supplements and as food colorant. Because lycopene is the acyclic precursor of β-carotene, the industrial process of β-carotene formation with *B. trispora* can be turned into a lycopene-producing process by the application of lycopene cyclase inhibitors, such as nicotine [41].

Another unicellular algal commercially relevant carotenoid producer is *Haematococcus pluvialis,* which as a chlorophyte is related to *D. salina* [40]. Under light and nutritional stress, it produces astaxanthin during encystment into a dormant state. Most of the astaxanthin is esterified with fatty acids via its 3- and 3'-hydroxy groups. The majority (∼70 %) exists as a monoester with 16:0, 18:1, and 18:2 fatty acids, and another 25 % represents diesters (Dore and Cysewski, Cyanotech Corporation at http://www.ruscom.com/cyan/web02/pdfs/naturose/nrtl09.pdf).

The production of astaxanthin containing *H. pluvialis* is a two-step process. In the first phase, cells are grown under conditions for optimal biomass production. In the second phase, *H. pluvialis* is stressed by deprivation of nutritional minerals and/or increased illumination [9]. Concentrations of astaxanthin and esters can reach up to 3 % of cell dry mass. Either bioreactors or outdoor systems are in use. Before the alga can be applied directly as feed, the dried encysted cells have to be cracked by milling to ensure the astaxanthin bioavailability. Some companies extract the astaxanthin derivatives and convert them to free astaxanthin by enzymatic hydrolysis. Products in the market include AstaREAL (Fuji Chemical Industry), AstaPure (Algatechnologies), and BioAstin (Cyanotech) [17].

## 2.3 *Concepts for Improvement of Biological Carotenogenic Systems*

Crop plants have been improved by conventional breeding and selection of useful traits. One of the targets is increased carotenoid content in carotenogenic tissue. For crossing and selection for higher pigmentation, the gene pools from elite lines, wild species, and interspecific hybrids can be used [34]. Identification of quantitative

trait loci related to carotenogenesis facilitated the selection of the progenies. Successful attempts to breed crops with increased carotenoid content have been reported for tomato [35], potato [12], and cassava [14].

Screening of different varieties of a crop for a certain carotenoid may also be successful. This is the case for orange pepper fruit, which is enriched with zeaxanthin to concentrations of up to 17 μg/g fw [50]. Breeding marigold plants to shift the flower carotenoids from lutein to zeaxanthin has also been achieved.

In microorganisms, the conventional approach to strain improvement is random mutagenesis (chemical or UV-initiated) and selection for the desired phenotype. Although this is easier for heterotrophically growing organisms, the mutagenesis approach was successful for *H. pluvialis*, generating mutants with a 30 % higher astaxanthin production [29]. Great efforts have been made to increase the astaxanthin content in *X. dendrorhous* by classical strain development. Chemical mutagenesis resulted in high-yield astaxanthin mutants of up to 5 mg/g dw from wild-type strains with less than one tenth of this amount [55, 64]). An alternative approach to mutagenesis is the construction of carotenoid-hyperproducing hybrids by protoplast fusion. With this procedure, *X. dendrorhous* strains with 1.6 mg/g dw of total carotenoid were obtained [16]. High-carotene mutants were also obtained with *B. trispora* after chemical mutagenesis [43]. In these mutants, β-carotene production was about 100-fold higher than in the wild-type strain.

Considerable progress has been made in targeted genetic modification of carotenoid biosynthesis by engineering this pathway in plants and microorganisms. This was facilitated by a deeper understanding of the carotenoid biosynthesis pathway and its interaction with other terpenoid pathways and the development of appropriate molecular genetic tools, including the cloning of useful genes from this pathway [61]. Several strategies can be followed by engineering a carotenoid pathway [59]. One is the enforcement of the formation of an already existing carotenoid. For this approach, the limiting step(s) of the biosynthesis pathway have to be recognized and the gene for the corresponding enzyme has to be overexpressed. In carotenogenesis, these limiting enzymes are phytoene synthase [24], catalyzing the step leading to the first carotenoid and 1-deoxy-d-xylulose-5-phosphate synthase, the initial enzyme for the synthesis of prenyl pyrophosphates in bacteria and plant plastidic terpenoid biosynthesis [1, 19]. Other goals are accumulation of an intermediate that is present at low level or the extension of an existing pathway to a novel product. Especially in microorganisms, it is possible to construct entire carotenoid pathways in noncarotenogenic hosts.

### 2.3.1 Engineering of Carotenoid Composition in Edible Crop Plants

When carotenoids are desired for nutritional supplementation, they can be produced in a suitable organism and added during food processing. However, it is more straightforward to engineer the synthesis in a staple crop for direct consumption. Pioneering work has been carried out with the tomato for higher lycopene content

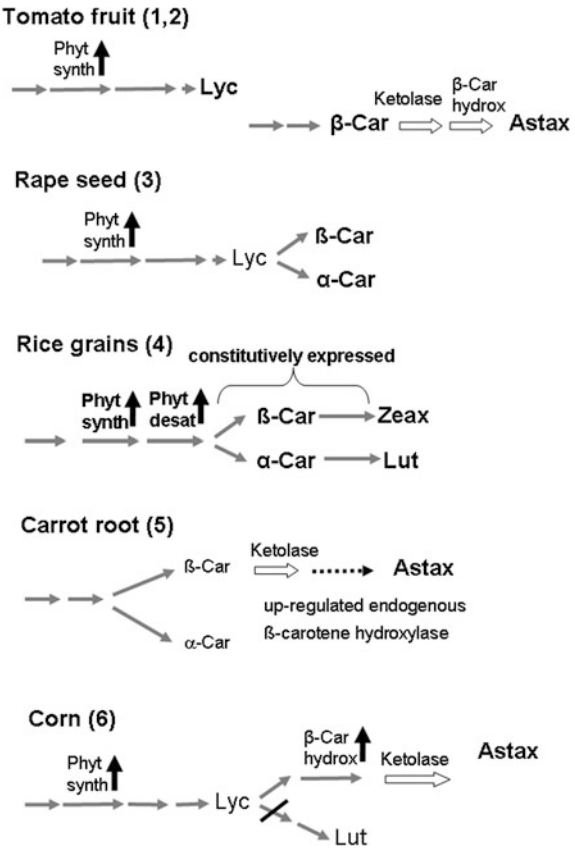


**Fig. 4** Genetic engineering of carotenoid biosynthesis in plants. Vertical arrows indicate over-expression of endogenous genes, open arrows indicate pathway extension by expression of foreign genes. References: *1* [24], *2* [31], *3* Shewmaker et al. 1999, *4* [73], *5* [32], *6* [74] and [20]. *Lyc* lycopene; *β-Car* β-carotene; *α-Car* α-carotene; *Lut* lutein; *Zeax* zeaxanthin; *Astax* astaxanthin

(see [22] for a review) and with rape (*Brassica napus*) to increase carotenoid synthesis in seeds [65]. The cloning strategies followed with different plants for targeting the production of different carotenoids are outlined in Fig. 4.

During tomato fruit ripening, lycopene is accumulated due to an upregulation of phytoene synthase, making this enzyme a prominent target for genetic manipulation [24]. The first attempts to overexpress the phytoene synthase gene worked, but they severely affected the synthesis of terpenoid-related phytohormons in the chloroplast, which reduced the transgenic plants in stature. This problem was later avoided by the use of a fruit-specific promoter for the overexpression of the phytoene synthase gene [23]. Normal growing transgenic tomato lines were obtained with doubled fruit lycopene content of up to 5 mg/g dw. In an attempt to convert tomato lycopene into β-carotene, plastome transformation with a plant lycopene β-cyclase resulted in a β-carotene yield of 1 mg/g dw [3].

For the enrichment of β-carotene in rape seed oil, a similar strategy as for tomato was followed. A phytoene synthase gene was overexpressed in rape under a seed-specific promoter (Shewmaker et al. 1999). This led to a 50-fold increase in α-carotene and β-carotene, both with provitamin A activity. The rape seed oil

obtained from these transformants showed a β-carotene concentration of 0.1–0.15 %, together with up to 0.1 % α-carotene. Further engineering attempts with tomato have been reviewed [22].

Golden rice has gained considerable attention. Rice grains are colorless and devoid of carotenoids. Nevertheless, a part of the pathway already exists, but the initial steps in the carotenogenesis are absent [63]. This limitation could be overcome by expressing the genes of a phytoene synthase and a phytoene desaturase in rice endosperm [73]. This genetic modification is sufficient to complete the biosynthesis pathway for the synthesis of α- and β-carotene and their hydroxylation products lutein and zeaxanthin (Fig. 4). The next generation of transgenic rice improved by gene optimization reached a maximum 37 mg/g of carotenoids with a high proportion of β-carotene [49].

Only a few plants accumulate zeaxanthin. Typically, it is an intermediate in the synthesis pathway to the epoxy carotenoid violaxanthin. Tuber-specific antisense inactivation or co-suppression of the zeaxanthin epoxidase in potato inhibited violaxanthin formation and resulted in many lines with higher levels of zeaxanthin [56]. The best tubers accumulated zeaxanthin up to 40 μg/g dw, which corresponds to a 130-fold increase of this carotenoid.

Carotenoid pathway modification and extension for improved animal feed has been carried out with maize. A combinatorial transformation technique has been developed for genome insertion of a combination of genes simultaneously. It was successfully employed for engineering of carotenoid biosynthesis. This led to a variety of corn transformants with high content of β-carotene and other yellow carotenoids or pathway extensions ([74]). One goal was the extension of the enhanced endogenous synthesis capacity to the formation of astaxanthin by overexpression of limiting phytoene synthase and β-carotene hydroxylase, the inactivation of the lycopene ε-cylase, and the expression of foreign β-carotene ketolase (Fig. 4). This finally led to an astaxanthin yield of up to 20 μg/g dw in the seeds [21]. In addition, a maize transformant was generated with improved nutritional value, with not only a multifold higher β-carotene content but also with an increased level of the other vitamins ascorbate and folate [46].

In carrot root, the carotenoid pathway ends by accumulation of α- and β-carotene. Engineering of a ketolase gene was sufficient to extend the pathway to astaxanthin, although an additional hydroxylase is necessary [32]. As a response to the expressed ketolase, an endogenous β-carotene hydroxylase was upregulated to complete the biosynthesis pathway from β-carotene to astaxanthin (Fig. 4). In the best resulting plants, the roots contained 90 μg/g fw astaxanthin. Also, tomato has been used to engineer astaxanthin synthesis [31]. Genetic modification was carried out with a yellow variety, which accumulates β-carotene instead of lycopene. Transformation with a ketolase and a hydroxylase gene resulted in the accumulation of astaxanthin (Fig. 4). The astaxanthin content in the transgenic tomato fruits was up to 16 mg/g dw, which is more than 80 % of total carotenoids. This concentration is about 1.8-fold higher than in the engineered carrot and is suitable for the direct use of freeze-dried tomato powder as feed additive for trout and salmon. Table 1 lists the highest yields of major carotenoids obtained by plant transformation.

**Table 1** Engineered carotenoid synthesis in plants with the highest carotenoid yields

| Plant | Carotenoid | Content (mg/g dw) | Engineering | Reference |
|---|---|---|---|---|
| Tomato | Lycopene | 5 | Phytoene synthase | [23] |
| Tomato | β-Carotene | 1 | Lycopene β-cyclase | [3] |
| Tomato | Astaxanthin | 16 | β-Carotene hydroxylase, β-Carotene ketolase | [31] |
| Potato | Zeaxanthin | 0.04 | Inactivation of zeaxanthin epoxidase | [56] |

Details on carotenoid genetic pathway engineering of plants and resulting carotenoid yields have been reviewed elsewhere [27, 61].

### 2.3.2 Genetic Pathway Engineering with Microorganisms

Successful attempts have been made to engineer carotenogenesis in microorganisms, either by modification and enhancement of an existing pathway or by implementation of a carotenoid pathway into a noncarotenogenic host. The latter approach is much more problematic because of metabolic competition for precursors and interference with other well-balanced pathways. In addition, lipophilic sequestering systems must be present for substantial storage of carotenoids.

#### Bacteria

*Escherichia coli* was one of the first hosts for heterologous carotenoid synthesis. Initially, this bacterium was used for combinatorial synthesis of various and novel carotenoid structures by combining genes from different organisms with different carotenoid biosynthesis branches [60] to be tested as efficient lipophilic antioxidants. Recently, tremendous progress in the synthesis of carotenoids has been made by integration of carotenogenic genes into the *E. coli* chromosome. Concentrations of 6.2 mg/g dw of β-carotene, 1.4 mg/g dw astaxanthin [39], and approximately 30 mg/g dw of lycopene [15] have been obtained.

Some species of cyanobacteria synthesize zeaxanthin as the end product of their carotenoid biosynthesis. By overexpression of either the phytoene synthase gene or the β-carotene hydroxylase gene, zeaxanthin concentrations of 1.9 mg/g dw were reached [30].

#### Fungi

The biotechnological potential of fungi for the heterologous production of important carotenoids has already been reviewed [62]. The target carotenoids are mainly

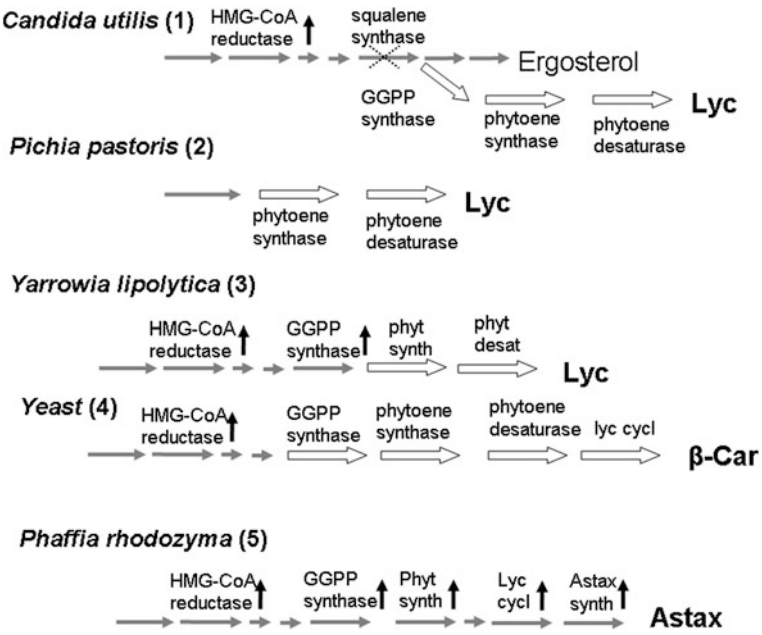


**Fig. 5** Genetic engineering of carotenoid biosynthesis in fungi. Vertical arrows indicate overexpression of endogenous genes, open arrows indicate pathway extension by expression of foreign genes. References: *1* [66], *2* [6], *3* [42], *4* [69], *5* [25]. *Lyc* lycopene; *β-Car*, β-carotene; *Astax* astaxanthin

β-carotene and astaxanthin. The first attempts to engineer a carotenoid pathway into a non-carotenogenic fungus were made with yeast for the production of lycopene [72]. However, *Candida utilis* was the first fungus in which the carotenoid pathway was systematically engineered by genetic modification for the high-yield production of the carotenoids lycopene, β-carotene, and astaxanthin [66]. In addition to the genes for the establishment of carotenogenesis, the gene encoding 3-hydroxy-3-methylglutaryl–coenzyme A reductase from *C. utilis* was overexpressed and the gene for squalene synthase was partially inactivated, redirecting parts of ergosterol biosynthesis into carotenoid biosynthesis (Fig. 5). The resulting lycopene concentration of 7.8 mg/g dw was set as a benchmark for all future engineering attempts. Furthermore, some of the engineering strategies developed for *C. utilis* were used in the following years for other fungi. When later the engineering carotenoid pathway in yeast was extended for the synthesis of β-carotene, optimization of precursor supply by simultaneous overexpression of the genes encoding 3-hydroxy-3-methylglutaryl–coenzyme A reductase and geranylgeranyl pyrophosphate synthase (GGPP) (Fig. 5) resulted in a β-carotene yield of 5.9 mg/g dw [69]. This value for β-carotene synthesis in yeast could be further increased to 7.4 mg/g dw by the insertion of controllable promoters for the carotenogenic genes inserted as duplicates with galactose induction and glucose repression of β-carotene synthesis [70].

**Table 2** Engineered carotenoid synthesis in microorganisms with the highest carotenoid yields

| Strain | Carotenoid | Content (mg/g dw)* | Engineering | Reference |
|---|---|---|---|---|
| *Escherichia coli* | β-Carotene | 6.2 | Whole pathway | [39] |
| | Astaxanthin | 1.4 | Whole pathway | [39] |
| | Lycopene | 30 | Whole pathway | [15] |
| *Synechococcus* | Zeaxanthin | 1.9 | Hydroxylation | [30] |
| *Candida utilis* | Lycopene | 7.8 | Whole pathway + inactivation of parallel pathway | [66] |
| *Pichia pastoris* | Lycopene | 4.6 | Whole pathway | [6] |
| *Yarrowia lipolytica* | Lycopene | 1.5 | Whole pathway | [42] |
| Yeast | β-Carotene | 7.4 | Whole pathway | [70] |
| *Phaffia rhodozyma* | Astaxanthin | 9 | Mutant + pathway optimization | [25] |

*In shake-flask cultures

A successful approach to increase β-carotene production in a genetically engineered yeast was by laboratory evolution [54]. Making use of the carotenoid antioxidative potential, the application of hydrogen peroxide as stressor resulted in β-carotene concentrations of 18 mg/g dw.

In *Pichia pastoris* engineered only for the synthesis of lycopene from GGPP without any further optimization of precursor supply, lycopene accumulated to 4.6 mg/g dw [6]. Another noncarotenogenic fungus used recently for carotenoid production is *Yarrowia lipolytica,* which is an oleaginous yeast that has a high storage capacity for lipophilic compounds, such as carotenoids. A strain already modified for high accumulation of lipid bodies was used for transformation with the heterologous genes. In addition to the genes for lycopene synthesis from GGPP, again the genes for 3-hydroxy-3-methylglutaryl–coenzyme A reductase and GGPP synthase were also overexpressed (Fig. 5). In laboratory cultures, synthesis of 1.5 mg/g dw of lycopene was achieved [42]. This accumulation could be increased to 16 mg/g dw under controlled fed-batch fermentation.

The only carotenogenic fungus optimized for carotenoid biosynthesis is the basidiomycetous yeast *P. rhodozyma,* which is one of the rare producers of astaxanthin; it can be genetically improved for carotenogenesis [68]. High-yield astaxanthin strains have been obtained by a combination of classical mutagenesis and genetic pathway engineering [26]. This approach started from chemically-induced mutants with a 15-fold higher astaxanthin content. The genes of limiting carotenogenic reactions were then expressed stepwise, resulting in an additional 6-fold increase and a final astaxanthin concentration of 6 mg/g dw [25].

## 3 Conclusion

Astaxanthin and β-carotene dominate the global market as feed additives and colorants. More than 90 % of these carotenoids are chemically synthesized. However, lutein is another feed additive that is progressing strongly in the market; it is mainly of plant origin. Other carotenoids, such as lycopene, zeaxanthin, and phytoene, are of minor economic importance.

It is a great challenge to develop biological systems for the production of astaxanthin and β-carotene, in particular, as well as other carotenoids. Although suitable producers exist, they currently serve only a niche market due to their inferior economic competitiveness compared to the synthetic products. Therefore, considerable attempts are being made to increase the carotenoid yields of various organisms. One challenge is the genetic pathway engineering of suitable hosts. This includes optimization of the entire pathway, from precursor supply to the enhancement of limiting carotenogenic reactions. Either noncarotenogenic organisms have been used for the establishment of the entire pathway, or carotenogenic organisms have been used to enforce their pathways or to extend them for novel carotenoids. To date, these engineering approaches have been especially successful with some bacteria and several fungi. Table 2 lists the best carotenoid yields that have been obtained due to genetic modifications in different organisms. These values were obtained with laboratory shake-flask cultures. With controlled fermenter cultivation, a 3- to 10-fold increase has been demonstrated to be possible compared to the shake-flask cultures [26, 42]. Therefore, optimized fermenter cultures of some of the genetically modified strains listed in Table 1 should be considered as the next step in developing cost-competitive bioprocesses.

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