Chapter 2

Microbial Carotenoids

Preejith Vachali, Prakash Bhosale, and Paul S. Bernstein

Abstract

Carotenoids are among the most widely distributed pigments in nature, and they are exclusively synthesized by plants and microorganisms. These compounds may serve a protective role against many chronic diseases such as cancers, age-related macular degeneration, and cardiovascular diseases and also act as an excellent antioxidant system within cells. Recent advances in the microbial genome sequences and increased understanding about the genes involved in the carotenoid biosynthetic pathways will assist industrial microbiologists in their exploration of novel microbial carotenoid production strategies. Here we present an overview of microbial carotenogenesis from biochemical, proteomic, and biotechnological points of view.

Key words: Carotenoids, Carotene, Xanthophylls, Biosynthesis, Carotenogenesis, *Crt* genes

1. Introduction

Carotenoids are naturally occurring terpenoid pigments, consisting of isoprene residues and a polyene chain of conjugated double bonds. These pigments are responsible for the wide variety of orange-red colors seen in nature that absorb light in the wavelength range of 300–600 nm. The absorbance is directly related to the number of conjugated double bonds and functional groups present in the structure. Structurally and functionally, carotenoids can be broadly classified into hydrocarbons (HC, carotenes) and oxygenated derivatives (xanthophylls). These molecules are formed by the head to head condensation of two geranylgeranyl diphosphate molecules (GGDP) (C20 HC), which results in a basic symmetrical acyclic C40 HC structure called phytoene ($C_{40}H_{56}$) (1). The remainder of the natural carotenoids is derived from this basic molecule by a variety of biochemical reactions, mainly mediated by enzymes in plants and microorganisms. Animals are usually the dietary recipients at the other end of the food chain and

José-Luis Barredo (ed.), *Microbial Carotenoids From Fungi: Methods and Protocols*, Methods in Molecular Biology, vol. 898,
DOI 10.1007/978-1-61779-918-1_2,

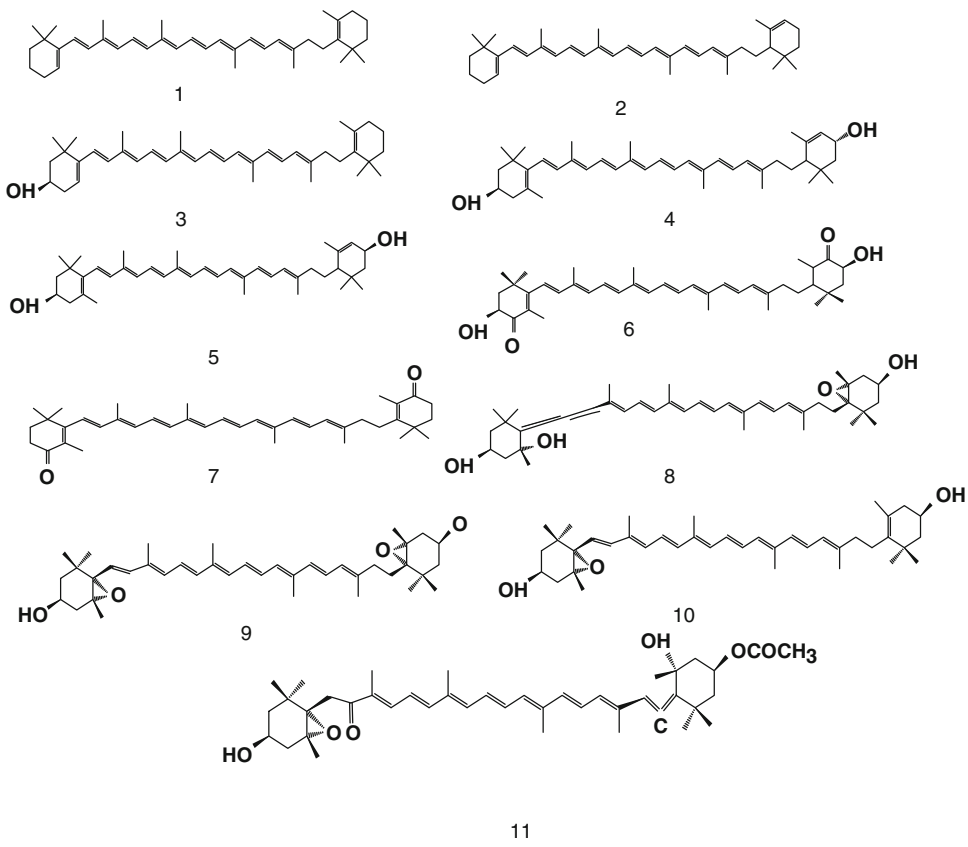

Fig. 1. Structures of important carotenoids produced by microorganisms. (1) β-Carotene, (2) α-Carotene, (3) β-Cryptoxanthin, (4) Lutein, (5) Zeaxanthin, (6) Astaxanthin, (7) Canthaxanthin, (8) Neoxanthin, (9) Violaxanthin, (10) Antheraxanthin, and (11) Fucoxanthin.

have limited ability to metabolically transform carotenoids (2, 3). Figure 1 shows the chemical structures of important carotenoids.

Carotenoid biosynthesis likely originated in ancient anoxygenic photosynthetic microorganisms. As these microbes evolved, carotenoid biosynthetic pathways have also branched out, resulting in structurally different carotenoids. The earliest record of oxygenic microbial carotenoid biosynthesis is attributed to cyanobacteria, which can be traced back 3.5 billion years based on fossil and molecular evidences (4). Prochlorophytes, another group of oxygenic photosynthetic bacteria, are reported to have carotenoid pigments similar to algae and eukaryotic plants, suggesting evolutionary relations with these groups (3). Single or multiple endosymbiotic associations with cyanobacteria or purple photosynthetic bacteria might be responsible for the chloroplast and pigment diversity in higher organisms (5). The change in the earth's atmosphere from anaerobic to aerobic condition is a major environmental factor responsible for the biosynthesis of structurally diverse carotenoids by oxygen-dependent enzymes (3).

Carotenoids are molecules of great interest in many scientific disciplines because of their unique properties, wide distribution and diverse functions (6). In photosynthetic organisms, carotenoids serve as light harvesting pigments. In many organisms, their major role is to act as an antioxidant by neutralizing free radicals and thereby preventing potential oxidative damage to the cells (3, 4, 7–15). Liu et al. demonstrated that a *Staphylococcus aureus* mutant with disrupted carotenoid biosynthesis is more susceptible to oxidant killing, indicating that carotenoids could act as a virulence factor (16). Carotenoids could reduce the penetration of singlet oxygen by decreasing membrane fluidity (17). Recent reports by Kamila et al. showed that polar carotenoids such as zeaxanthin could mediate transmembrane proton transfer in vivo (18).

The health benefits of carotenoids are becoming increasingly evident. Carotenoids play an important role as pro-vitamin A compounds. Of the more than 600 carotenoids that have been identified, approximately 30–50 are believed to have vitamin A activity. The most well-known compounds of this group are β-carotene and α-carotene. Beyond their pro-vitamin A role, epidemiological evidence and experimental results suggest antioxidant functions of dietary carotenoids that can prevent onset of many diseases such as arteriosclerosis, cataracts, age-related macular degeneration, multiple sclerosis, bone abnormalities, and most importantly, cancers, each of which may be initiated by free radical damage (19–24). Currently, a majority of the carotenoids available on the global market is produced by multi-step chemical synthesis or by solvent-based chemical extraction from their nonmicrobial natural sources (25–27).

There are very few carotenoid-based products on the market that originate from microbial sources. The two most prominent microalgal sources of carotenoids which have sustained competition from synthetic manufactures are β-carotene from *Dunaliella salina* and astaxanthin from *Haematococcus pluvialis* (28–32). There has been escalating interest in the microbial sources of other carotenoids with significant health benefits, which could be attributed to consumer preferences for natural additives over synthetic sources and also to the potential cost effectiveness of production by industrial microbial biotechnology (2, 6, 14, 33–35).

2. Microbial Carotenogenesis

Microbial carotenoid biosynthesis is a well-regulated mechanism which is dependent on the biochemical makeup of the microorganism's environmental conditions and cultural stress incurred during growth (6, 36). Several microbial biosynthetic pathways have been proposed and experimentally confirmed in the last five decades by well-known carotenoid researchers (36–46).

The evolution of carotenoid biosynthetic pathways is a continuous process and extends beyond designated carotenoid biosynthetic pathways (47). Generally, carotenoid biosynthesis starts with the bioprecursor called isopentenyl pyrophosphate (IPP) (48–50). The biosynthetic pathway to IPP known as the mevalonic acid (MVA) pathway starts from the key precursor acetyl-CoA. The first step in the MVA pathway involves the conversion of acetyl-CoA to 3-hydroxy-3-methyl glutaryl CoA (HMG-CoA) catalyzed by HMG-CoA synthase. HMG-CoA gets converted into a C6 compound MVA. MVA is then converted into a C5 IPP by a succession of reactions involving phosphorylation by MVA kinase followed by decarboxylation (51, 52).

The condensation of one molecule of dimethylallyl diphosphate (DMADP) and three molecules of IPP by pyrenyltransferase produces a C20 diterpene GGDP compound. Two molecules of GGDP condense head to head to form the first colorless carotenoid, phytoene (15-*cis*-7, 8, 11, 12, 7′, 8′, 11′, 12′-octahydro-ψ, ψ-carotene). Subsequent desaturation of phytoene results in the formation of C40 acyclic carotenoids such as neurosporene or lycopene. The desaturation process is a multistep process which varies between microbes. The most common of all is the four step desaturation process which leads to the formation of lycopene via intermediate steps of phytofluene and 3,4-didehydrolycopene. However, in some purple photosynthetic bacteria such as the *Rhodobacter* species, neurosporene, spheroidene, and hydroxyspheroidene are produced as final products of the desaturation process (53).

All *trans*-lycopene acts as the precursor for many commercially important acyclic and cyclic carotenoids and oxygenated carotenoids, which are also called xanthophylls. The formation of carotene(s) such as β-carotene is considered to be the most common step in microbial carotenogenesis. Desaturated lycopene gets cyclized at both ends and forms a β-carotene or α-carotene molecule. These reactions are catalyzed by a β- or ε-cyclase in some of the green alga (54). The formation of xanthophylls involves sequential oxidations of post-carotene molecules yielding -hydroxy, -epoxy, and -oxo groups. The introduction of hydroxyl (–OH) groups at the positions of C3 and C3′ of the ionone rings leads to the formation of zeaxanthin and lutein, which are C3, C3′-dihydroxy derivatives of β,β-carotene and β,ε-carotene, respectively (55). A monohydroxy carotenoid, β-cryptoxanthin, acts as an intermediate in the biosynthesis of the dihydroxy carotenoids such as zeaxanthin. The carotenoids with keto (C=O) functional groups such as canthaxanthin and astaxanthin are formed by the introduction of keto groups at C4 and C4′ with or without hydroxylation at C3 and C3′. The formation of keto carotenoids from β-carotene has been well studied in algae, yeast, and nonphotosynthetic bacteria (55, 56). Violaxanthin, neoxanthin, and fucoxanthin were formed by

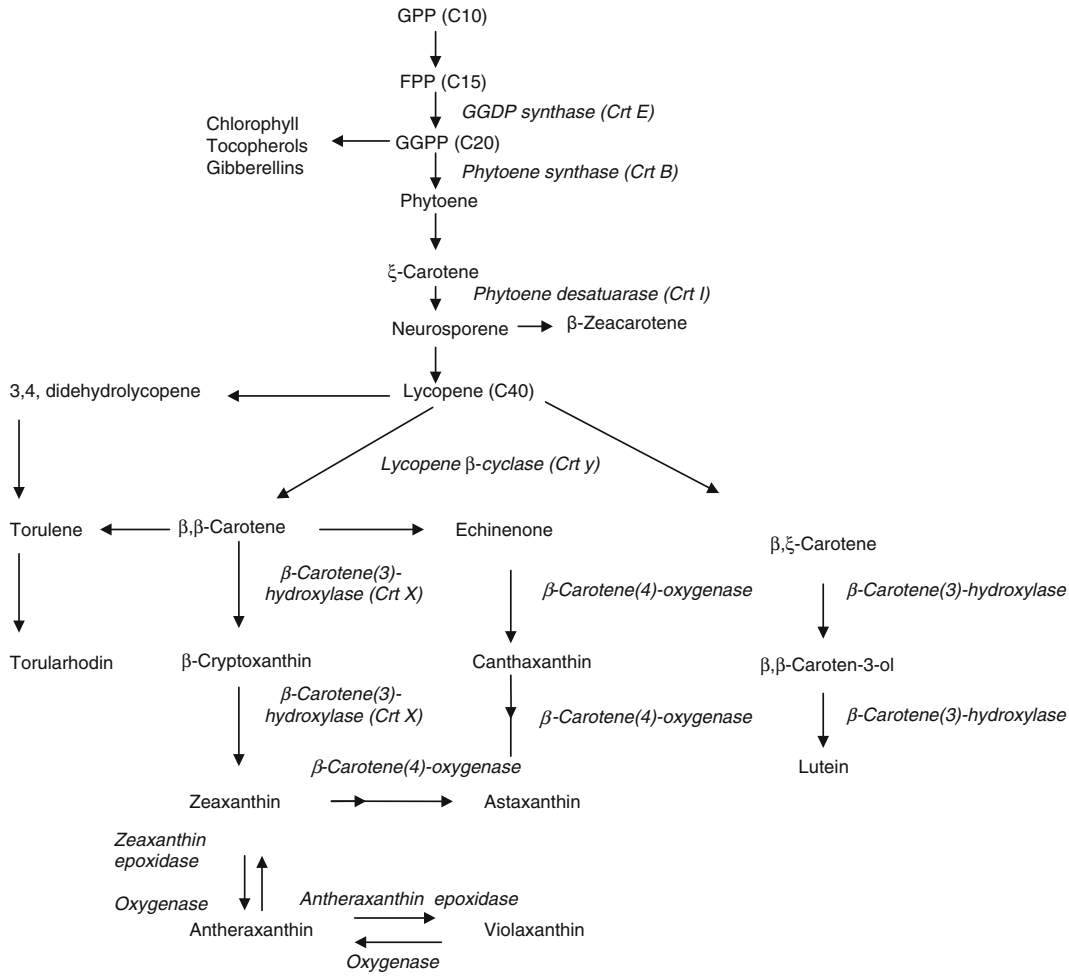

Fig. 2. Carotenoid biochemical pathway compiled from several reports on microbial production (1, 2, 6, 36, 38, 39, 50, 61, 65, 101, 104).

epoxidation at the 5, 6 and 5′, 6′ positions (55). Antia et al. and Yamamoto et al. studied the interconversion of violaxanthin, antheraxanthin, and zeaxanthin in green algae through epoxidation (2, 57, 58). Figure 2 represents the schematics of microbial carotenogenesis.

3. Proteomics of Microbial Carotenogenesis

Most of the enzymes involved in the MVA pathway are soluble proteins, whereas the enzymes involved in the later steps of the carotenogenic pathway are mostly membrane-bound proteins. The lipophilic products of carotenoid pathways partition to cytoplasmic

or organelle membranes (59). Bacteria and yeast contain many isoprenoid compounds such as dolichols, quinones, and ergosterols (60, 61). However, farnesyl pyrophosphate (FPP) was identified as the first substrate encoded by the *crt* genes cluster, which is a common precursor for many carotenoid biosynthetic pathways (62, 63). Misawa et al. suggested that by incorporating carotenogenic genes into these systems, it might be possible to partially direct the carbon flux for the biosynthesis of these isoprenoid compounds to the pathway for carotenoid production (63). They also proposed that elucidation of *crt* gene structure and function would help carotenoid researchers to explore the biosynthesis of the main carotenoids. This could be achieved by the appropriate combinations of the *Erwinia crt* genes using FPP as the precursor in microbial systems (63).

Much later, Markus et al. 2002 (64) cloned and characterized the genes coding for all the enzymes involved in the conversion of acetyl-CoA to farnesyl diphosphate (FPP) in the zeaxanthin-producing bacterium *Paracoccus zeaxanthinifaciens*. They identified two genes encoding enzymes catalyzing the condensation of two acetyl-CoA molecules to acetoacetyl-CoA. The gene cluster named the mevanolate operon regulates the six enzymes involved in the conversion of acetyl-CoA and acetoacetyl-CoA to isopentenyl diphosphate (IPP) and DMAPP. The genes encoding the enzymes catalyzing two consecutive condensations, IPP and DMAPP to geranyl diphosphate (GPP) and IPP and GPP to FPP, were also identified. It was reported that these genes were not clustered with any other genes encoding an enzyme of the isoprenoid pathway.

In the carotenoid biosynthetic pathway, phytoene synthase (*crtB*), phytoene desaturase (*crtI*), and lycopene cyclase (*crtY*) are the three essential enzymes responsible for the biosynthesis of both acyclic and cyclic carotenoids. In the early 1990s, Sandmann's group identified and mapped the major *Erwinia herbicola* genes responsible for the cyclization and hydroxylation of carotenoids (36). Much later, Phadwal reviewed the molecular phylogenies of the *crt* genes involved early in the carotenoid pathway (65). The work summarized the phylogenetic evolution of phytoene synthase (*crtB*), phytoene desaturase (*crtI*), and lycopene cyclase (*crtY*) among bacteria and their functional significance in microbial carotenogenesis. Acyclic hydroxyl carotenoids from the carotene hydratase (*crtC*) and carotene 3,4-desaturase (*crtD*) genes of *Rhodobacter* were also summarized by Steiger et al. (66). Several enzymes, such as carotene hydroxylases (*crtZ*), carotene oxygenase (*crtW*), carotene isomerase (*crtH*), and so on, have been linked with bacterial carotenogenesis. However, recent work shows that carotene hydroxylases (*crtZ*) and its cDNA sequence were also isolated from green alga such as *H. pluvialis*. The carotene hydroxylase isolated from flavobacterim was pursued for the biosynthesis of unique hydroxyl carotenoids (67). The hydroxylation reaction

among carotenoid producing microbes is considered common phenomena, but ketolation is quite limited, and is characteristic of selected microbes. β-Carotene ketolase enzymes (*crtW* and *crtO*) have been identified from methylotrophic bacterium such as *Methylomans*. The incorporation of the genes encoding these enzymes has resulted in a higher percentage of canthaxanthin in these strains (68). Recombinant DNA technology has enabled biotechnologists to make new carotenoids for a variety of uses (69).

4. Biotechnology of Microbial Carotenoids

Several studies have been conducted highlighting the importance of carotenoids in healthcare as well as the nutraceutical and food industries (2, 8, 34, 70–72). The market demand for carotenoids continue to rise as more and more clinical research studies surface, revealing the various health benefits of carotenoids (33, 34, 73–76). Of the 600 naturally occurring carotenoids, only a few have proven useful in human- and animal-based industries, and these have primarily received focus on their abilities to act as antioxidants and light-screening ingredients.

The carotenoid market is expected to increase to $919 million by 2015 with an annual growth rate of 2.3% (77). Most of these carotenoids are available from synthetic sources; however, synthetic pigments have been perceived to cause hazardous effects to human health at high dose ranges and have been subsequently warned by the Food and Drug Administration (78). This resulted in the hunt for a process of pigment production by alternative natural sources (79). There is a growing demand for microbial sources of pigments as an alternative.

Currently, β-carotene and astaxanthin are industrially produced from microbial sources and are widely used in food and feed industries (2, 80, 81). The major limitation on the use of microbial systems for commercial production is the low yield, slow growth, and high production cost compared with chemical synthesis. However, strain improvement strategies such as optimization of growth conditions and preparation of mutants in conjunction with metabolic engineering techniques could improve carotenoid productivity. In the following section, we discuss the major utilities and available microbial sources of some commercially important carotenoids.

4.1. Astaxanthin (3S,3S′-Dihydroxy-β,β-carotene-4,4′-dione)

Astaxanthin is the most commonly occurring red carotenoid in marine and aquatic animals (82). It is responsible for the pink color of salmon flesh and also gives coloration to crustacean shells. It is known to scavenge free radicals and quench singlet oxygen (83, 84). It can enhance the immune system (85) protect skin from radiation

injury, cancer (86, 87) and block reactions induced by other chemicals and toxins. It is widely used in the pharmaceutical, cosmetic, and nutraceutical industries (88). Two major microorganisms, which have been commercially exploited for astaxanthin production, are microalga *H. pluvialis*, and heterobasidiomycetous yeast *Xanthophyllomyces dendrorhous* (2, 89–91).

Recently, de la Fuente et al. reported an improved semi-industrial process for astaxanthin production by the fermentation of *X. dendrorhous*. A volumetric yield of 350 mg/L astaxanthin was reported with 800-L scale (92). Although *X. dendrorhous* has been studied by various researchers for the past three decades, it still attracts interest in various biotechnological industries (89, 92, 93).

Alternately, the freshwater unicellular alga, *H. pluvialis*, accumulates astaxanthin in its aplanospores under stressful conductions and is considered to be one of the richest sources of this carotenoid. Ranjbar et al. reported photoautotrophic conditions in a bubble column with the fed-batch addition of nutrients for the production of astaxanthin by *H. pluvialis*. A combination of the fed-batch addition of nutrients and dilution of broth for nutrient deficiency was proposed as the most promising method for attainment of high cell and astaxanthin concentrations in a bubble column photo-bioreactor. The final concentration of astaxanthin was reported to be 390 mg/L which was several times higher than anything ever previously reported (94). Sandesh Kamath et al. reported a 23–59% increase in the total carotenoid and astaxanthin contents by implementing a strain improvement strategy for *H. pluvialis* with chemical and UV mutation (35).

Apart from these two major carotenoid producing microorganisms, *Brevibacterium linens* (95) and *Agrobacterium aurantiacum* (96), the marine bacterium *Paracoccus haeundaensis* (97) and *Mycobacterium lacticola* (98) are also reported to produce astaxanthin, but are not considered commercially significant sources.

4.2. β-Carotene (β, β-Carotene)

β-Carotene is an important compound because of its role as an antioxidant, and as precursor of vitamin A in food and feed products (99, 100). In 2004, the worldwide market value of β-carotene was US $242 million and was proposed to reach US $253 million by 2009 (77, 101). Currently, more than 90% of commercialized β-carotene is produced through chemical synthesis (102).

β-Carotene is produced primarily by microalgae, fungi and yeasts, as well as some species of bacteria and lichens (103, 104). Commercially available β-carotene is produced mainly from the genus *Dunaliella* (101, 105). Since 1980, *Dunaliella* powder and extracts (yielding dried biomass and natural β-carotene) have been available in Israel, China, the USA, Australia, and Mexico (3, 101, 106).

Beside *Dunaliella*, the greatest yields have been obtained by the mating of (+) and (−) strains of *Blakeslea trispora* (103) resulting in yields that are comparable to those of chemical processing (107).

The US Department of Agriculture's process reported a yield of 17 mg of β-carotene per gram of mycelium and recently improved to about 30 mg of β-carotene per gram of mycelium and about 3 g/L (107). *Phycomyces* is used as a model system by many researchers to study the regulation of the biosynthesis of the pigment β-carotene in fungi as it accumulates β-carotene, at a lower extent than *B. trispora*, in the lipid globules of their mycelia (108). Sang-Hwal Yoon et al. reported a novel approach by the combinatorial expression of the whole bacterial mevalonate pathway for the production of β-carotene in *Escherichia coli*. The recombinant *E. coli* DH5α harboring the whole MVA pathway and β-carotene synthesis genes produced a β-carotene yield of 465 mg/L at a glycerol concentration of 2% (w/v) (102). However, the regulatory concern surrounding the use of recombinant strains is still a major roadblock for the success of microbial biotechnology.

4.3. β-Cryptoxanthin (3R-β, β-Caroten-3-ol)

β-Cryptoxanthin is a xanthophyll carotenoid with the potential to act as provitamin A and has been reported to improve bone health. A study conducted in experimental rats demonstrated that β-cryptoxanthin also stimulates unique anabolic bone calcification (109). Yamaguchi et al. (75) claimed that β-cryptoxanthin, which may promote osteogenesis, could be of value as an active ingredient in the treatment of bone diseases. Recent studies also indicate that β-cryptoxanthin is highly preventative against prostate cancer (76, 110), lung cancer (111–113), colon cancer (114), and rheumatoid arthritis (115).

While there is currently little to no commercial demand for microbial β-cryptoxanthin, it holds great potential for future investigation, production, and use. Information on microbial production of β-cryptoxanthin is very limited, mainly due to the lack of proper microbial sources and feasible culture conditions (2, 116). *B. linens*, which is traditionally known for its cheese ripening process, accumulates β-cryptoxanthin in low amounts (117). *Flavobacterium lutescens* and *Flavobacterium multivorum* are reported to produce β-cryptoxanthin under optimized media conditions (116, 118). However, these microbial sources are not yet competitive in comparison to the naturally occurring citrus and capsicum based sources of β-cryptoxanthin (119).

4.4. Canthaxanthin (4,4′ Diketo-β-carotene)

Canthaxanthin is a diketo-carotenoid, which was first isolated from edible mushrooms (3). It is widely used as a colorant in food, feed additives for egg yolk, fish, and crustacean farms and also in the cosmetic industry as a tanning agent for human skin (2, 120). Canthaxanthin is reported to prevent UV-induced immune suppression in mice, protect against skin cancer in experimental animals and also to be useful in the treatment of skin diseases such as photodermatosis (2). In vitro studies demonstrated that canthaxanthin has greater antioxidant activity than its non-oxygenated

analog such as β-carotene due to the presence of keto groups at the 4 and 4′ positions in the β-ionone ring (121). Only a few microbial sources of canthaxanthin have been reported. Askar et al. (122) identified an extremely halophilic bacteria, *Haloferax alexandrinus*, with a 0.69 mg/g cellular accumulation of canthaxanthin. Other bacterial producers of canthaxanthin include *Corynebacterium michiganense*, *Micrococcus roseus*, *Brevibacterium* sp. strain KY 4313, *Gordonia jacobaea*, and *Dietzia natronolimnaea* HS-1 (123). It was also discovered that various green microalgae such as *Chlorella pyrenoidosa*, *Chlorella zofingiensis*, *Chlorella emersonii*, and *Dictyococcus cinnabarinus* produce canthaxanthin under various growth conditions (123). Recently, it was reported that under submerged fermentation, a mutant strain of *Aspergillus carbonarius* produces canthaxanthin with a yield of 32 mg/g (124). The current market demand for canthaxanthin is fulfilled by chemical synthesis (125). However, much attention is being devoted by researchers to discovering a microbial source for canthaxanthin and developing novel production strategies for promising microbial strains (123, 126, 127).

4.5. Fucoxanthin (3′-(Acetyloxy)-6′, 7′-didehydro-5, 6-epoxy-5,5′,6,6′,7, 8-hexahydro-3, 5′-dihydroxy-8-oxo-β, β-carotene)

Fucoxanthin is a naturally occurring xanthophyll carotenoid found in brown algae and edible brown seaweeds that has fascinated the nutraceutical and food industries recently due to its unique health benefits such as antiobesity, antidiabetes, etc., which have not been reported with other carotenoids (74). Fucoxanthin intake has been shown to promote fat metabolism, particularly around the abdominal area (33, 128, 129). An antidiabetic effect of fucoxanthin was also reported; a 0.2% fucoxanthin supplementation decreased the blood glucose and plasma insulin concentrations in experimental mice (130). A study conducted in KKAy mice, a model for obese/type II diabetes, showed that fucoxanthin could enhance the amount of DHA in the liver of mice fed with soybean oil without direct fish oil supplementation (131). Fucoxanthin is shown to have anticancer properties (132, 133). Fucoxanthin isolated from brown algae has also been found to act as an anticoagulant (71), anti-inflammatory (34), antioxidant (73), and antimicrobial (134).

Undaria pinnatifida and *Lamaria* sp. are the most popular edible seaweeds in Japan and many other Southeast Asian countries, and fucoxanthin accounts for >10% of the total carotenoids in these seaweeds (74). Other brown sea weeds that have been reported to produce fucoxanthin include *Hijikia fusiformis*, *Ecklonia stolonifera*, and *Sargassum siliquastrum* (135–137).

4.6. Lutein [(3R,3′R,6′R)-β, ε-Carotene-3,3′-diol]

Lutein is one of the fastest growing carotenoids on the market (77). Currently, the natural commercial source of lutein is the solvent extract of marigold (*Tagetes erecta*) petals (125, 138). However, the lutein content of marigold petals is low, 0.03% dry wt., and it contains several esters with similar polarity, making them difficult to separate from each other (139).

In recent years, several microalgae have been studied as potential lutein sources, such as *Chlamydomonas reinhardtii* (140), *Muriellopsis* sp. (141), *Chlorella protothecoides* (142), and *Scenedesmus almeriensis* (139); however, microbial sources still lack commercial potential mainly due to lack of studies involving strain improvement and high-volume bioreactors.

4.7. Zeaxanthin [(3R,3′R)-β, β-Carotene-3,3′-diol]

Zeaxanthin is an isomer of lutein, and its commercial demand exists in parallel to lutein mainly in the ocular health market. Zeaxanthin coexists with its several optical isomers in natural sources such as corn, alfalfa, yellow peppers, egg yolks, and marigold flowers (143, 144). Among the microbial sources, marine bacterium *Flavobacterium* species are well documented for their zeaxanthin production (107, 145–148). Unlike lutein, which is typically present in photosynthetic microorganisms, zeaxanthin occurs in cyanobacteria (149) and also in some non-photosynthetic bacteria (143). In the non-photosynthetic bacteria, zeaxanthin sometimes presents in the form of glycoside esters (143, 150). Other microbial sources include *Dunaliella* sp., which produces zeaxanthin under various stress and gene manipulation conditions (151, 152) and *Microcystis aeruginosa* (153).

Lutein and zeaxanthin together have many potential uses in the pharmaceutical and nutraceutical industries. Various studies suggest that they play an important role in the prevention of cancer (154), age-related macular degeneration (AMD) (70, 155), and enhancement of immunity. They are also used as colorants in food and in the cosmetic industry (156).

Table 1 summarizes the major microbial producers of carotenoids vs. natural sources. In general, the microbial yield is quite low compared with other nonmicrobial sources such as plants or animals. Microbial biosynthesis has an economic niche for those carotenoids which have complex structures that make them difficult to synthesize chemically. With proper strain improvement strategies and fermentation technologies, it is possible to produce higher levels of pure and isomer-free carotenoids from microbial sources.

5. Conclusions

Microbial carotenogenesis is a well-studied phenomenon that has been, and will continue to be, researched for years for its regulation and functionality using several biochemical approaches. Over the years, numerous groups of microorganisms have been characterized by their specific compositions of carotenoids. The characterization of genes and proteins involved in the biochemical pathways coupled with the use of genetic and metabolic engineering tools to improve the selective pathways has directed the biotechnologist toward the

Table 1
Natural and microbial sources of carotenoids

Carotenoids	Natural sources[a]	Content[a]	Microbial sources	Yield[b]	References
Astaxanthin	Krill	120 mg/kg	*Xanthophyllomyces dendrorhous*	1,080 μg/g; 4.7 mg/g dry cell matter (420 mg/L)	(6, 92, 94)
	Arctic Shrimp	1.2 g/kg	*Haematococcus pluvialis*	350 mg/L	
	Adonis annua	300–500 mg/kg		390 mg/L	
				22.7 mg/g	
β-Carotene	Carrots	183 μg/kg	*Blakeslea trispora*	420 μg/g	(6)
	Mango	131 μg/kg	*Dunaliella salina*	10.35 mg/L	(143)
	Sweet potato	95 μg/kg	*Streptomyces chrestomcyeticus subsp. Rubescens*	NG	(104)
	Pumpkin	69 μg/kg	*Rhodotorula glutinis mutant 32*	250 mg/L	
	Apricots	26 μg/kg			
	Cantaloupe	16 μg/kg			
β-Cryptoxanthin	Red bell peppers	22,050 μg/kg	*Brevibacterium linens*	0.3 μg/mL	(2)
	Mango	15,500 μg/kg	*Flavobacterium lutescens*	770 mg/kg	(119)
	Papaya	2,250 μg/kg			(116)
	Cilantro	4,040 μg/kg			
	Oranges	3,240 μg/kg			
	Corn	1,190 μg/kg			
	Watermelon	1,030 μg/kg			

Fucoxanthin	Edible Brown Sea Vegetable (Kelp)	3.5 g/kg	*Undaria pinnatifida*	30 g/kg	(157)
			Sargassum fusiforme	79.5 g/kg	(157)
			Laminaria japonica	122.1 μg/g	(158)
Lutein	Corn	12,720 μg/kg	*Chlorella zofingiensis*	21 μg/mL	(2)
	Marigold flower	0.3 g/kg	*Chlorella protothecoides CS-41*	225 μg/mL	(27)
	Collard	78,250 μg/kg	*Muriellopsis sp.*	35 μg/mL	(138)
	Kale	156,250 μg/kg	*Scenedesmus almeriensis*	0.7 g/L	(139)
	Spinach	11,607 μg/kg			
Zeaxanthin	Corn	5,280 μg/kg	*Dunaliella salina*	6 mg/g	(2)
	Collard	2,660 μg/kg	*Phormidium laminosum*	5.9 mg/mg	(144)
	Persimmon Japanese	4,880 μg/kg	*Flavobacterium multivorum*	10.65 μg/mL	(138)
	Spinach raw	3,310 μg/kg	*Microcystis aeruginosa*	0.962 mg/g	(153)

NG not given

[a]Adapted from Database,1998 (159)

[b]Cellular accumulation and volumetric data are added as reported

hyperproduction of carotenoids from microbial processes. Although the developed processes for microbial production of selected carotenoids appears to be very promising, gaining a deeper understanding and further development of the fermentation process in future years will be necessary before microbial sources become a realistic alternative to synthetic carotenoids.

Acknowledgments

This work was supported by National Institute of Health Grant EY-11600. We thank Kelly Nelson for critical reading of the manuscript.

References

1. Sandmann G (1994) Carotenoid biosynthesis in microorganisms and plants. Eur J Biochem 223:7–24
2. Bhosale P, Bernstein PS (2005) Microbial xanthophylls. Appl Microbiol Biotechnol 68:445–455
3. Johnson E, Schroeder W (1996) Microbial carotenoids. In: Fiechter A (ed) Advances in biochemical engineering/biotechnology. Springer, Berlin, pp 119–178
4. Liang C et al (2006) Carotenoid biosynthesis in cyanobacteria: structural and evolutionary scenarios based on comparative genomics. Int J Biol Sci 2:197–207
5. Gray MW (1989) The evolutionary origins of organelles. Trends Genet 5:294–299
6. Bhosale P (2004) Environmental and cultural stimulants in the production of carotenoids from microorganisms. Appl Microbiol Biotechnol 63:351–361
7. Cantrell A et al (2003) Singlet oxygen quenching by dietary carotenoids in a model membrane environment. Arch Biochem Biophys 412:47–54
8. Tinkler JH et al (1994) Dietary carotenoids protect human cells from damage. J Photochem Photobiol B 26:283–285
9. Truscott TG et al (1995) The interaction of carotenoids with reactive oxy-species. Biochem Soc Trans 23:252S
10. Krinsky NI et al (2003) Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr 23:171–201
11. Bohm F et al (1998) Enhanced protection of human cells against ultraviolet light by antioxidant combinations involving dietary carotenoids. J Photochem Photobiol B 44:211–215
12. Altermann W, Kazmierczak J (2003) Archean microfossils: a reappraisal of early life on Earth. Res Microbiol 154:611–617
13. Schopf JW (1993) Microfossils of the Early Archean Apex chert: new evidence of the antiquity of life. Science 260:640–646
14. Edge R et al (1997) The carotenoids as antioxidants—a review. J Photochem Photobiol B 41:189–200
15. El-Agamey A et al (2004) Are dietary carotenoids beneficial? Reactions of carotenoids with oxy-radicals and singlet oxygen. Photochem Photobiol Sci 3:802–811
16. Liu GY et al (2005) Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. J Exp Med 202:209–215
17. Subczynski WK et al (1991) Effect of polar carotenoids on the oxygen diffusion-concentration product in lipid bilayers. An EPR spin label study. Biochim Biophys Acta 1068:68–72
18. Kamila K et al (2008) Can membrane-bound carotenoid pigment zeaxanthin carry out a transmembrane proton transfer? Biochim Biophys Acta 1778:2334–2340
19. Peto R et al (1981) Can dietary β-carotene materially reduce human cancer rates? Nature 290:201–208
20. Greenberg ER et al (1990) A clinical trial of beta-carotene to prevent basal-cell and squamous-cell cancers of the skin. N Engl J Med 323:789–795

21. Stahl W, Sies H (1996) Lycopene: a biologically important carotenoid for humans? Arch Biochem Biophys 336:1–9
22. Hennekens CH (1997) Beta-carotene supplementation and cancer prevention. Nutrition 13:697–699
23. Moeller SM et al (2000) The potential role of dietary xanthophylls in cataract and age-related macular degeneration. J Am Coll Nutr 19:522S–527S
24. Bone RA et al (2000) Lutein and zeaxanthin in the eyes, serum and diet of human subjects. Exp Eye Res 71:239–245
25. Haigh GW (1994) High purity beta-carotene. US Patent 5,310,554
26. Khachik F (2009) Process for isolation, purification, and recrystallization of lutein from saponified marigold oleoresin and uses thereof. US Patent RE409,31
27. Ausich RL, Sanders DJ (1997) Process for the formation, isolation and purification of comestible xanthophyll crystals from plants. Patent 5648564
28. Boussiba S, Vonshak A (2000) Procedure for large-scale production of astaxanthin from *Haematococcus*. US Patent 602,270,1
29. Venkatesh NS et al (2005) Medium for the production of betacarotene and other carotenoids from *Dunaliella salina* (ARL 5) and a strain of *Dunaliella salina* for production of carotenes using the novel media. US patent 693,645,9
30. Park EK et al (2001) Effects of medium compositions for the growth and the astaxanthin production of *Haematococcus pluvialis*. Sanop Misaengmul Hakhoechi 29:227–233
31. Orosa M et al (2001) Comparison of the accumulation of astaxanthin in *Haematococcus pluvialis* and other green microalgae under N-starvation and high light conditions. Biotechnol Lett 23:1079–1085
32. Orset S, Young AJ (1999) Low-temperature-induced synthesis of α-carotene in the microalga *Dunaliella salina* (chlorophyta). J Phycol 35:520–527
33. Nakazawa Y et al (2009) Comparative evaluation of growth inhibitory effect of stereoisomers of fucoxanthin in human cancer cell lines. J Funct Foods 1:88–97
34. Heo SJ et al (2010) Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264.7 macrophages. Food Chem Toxicol 48:2045–2051
35. Sandesh KB et al (2008) Enhancement of carotenoids by mutation and stress induced carotenogenic genes in *Haematococcus pluvialis* mutants. Bioresour Technol 99:8667–8673
36. Sandmann G (1991) Biosynthesis of cyclic carotenoids: biochemistry and molecular genetics of the reaction sequence. Physiol Plant 83:186–193
37. Sandmann G (2001) Genetic manipulation of carotenoid biosynthesis: strategies, problems and achievements. Trends Plant Sci 6:14–17
38. Armstrong GA (1997) Genetics of eubacterial carotenoid biosynthesis: a colorful tale. Annu Rev Microbiol 51:629–659
39. Schmidt DC (2000) Engineering novel carotenoids in microorganisms. Curr Opin Biotechnol 11:255–261
40. Ducrey Sanpietro LM, Kula MR (1998) Studies of astaxanthin biosynthesis in *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*). Effect of inhibitors and low temperature. Yeast 14:1007–1016
41. Lee PC, Schmidt DC (2002) Metabolic engineering towards biotechnological production of carotenoids in microorganisms. Appl Microbiol Biotechnol 60:1–11
42. Chemler J et al (2006) Biosynthesis of isoprenoids, polyunsaturated fatty acids and flavonoids in *Saccharomyces cerevisiae*. Microb Cell Fact 5:20
43. Armstrong GA (1995) Genetic analysis and regulation of carotenoid biosynthesis: structure and function of the *crt* genes and gene products. In: Blankenship RE et al (eds) Advances in photosynthesis. Kluwer Academic, Dordrecht, pp 1135–1157
44. Chamovitz D et al (1993) Molecular and biochemical characterization of herbicide-resistant mutants of cyanobacteria reveals that phytoene desaturation is a rate-limiting step in carotenoid biosynthesis. J Biol Chem 268: 17348–17353
45. Hirschberg J, Chamovitz D (1994) Carotenoids in cyanobacteria. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer Academic, Dordrecht, pp 559–579
46. Hodgson DA, Murillo FJ (1993) Genetics of regulation and pathway of synthesis of carotenoids. In: Dworkin M, Kaiser D (eds) Myxobacteria II. American Society for Microbiology, Washington, DC, pp 157–181
47. Umeno D et al (2005) Diversifying carotenoid biosynthetic pathways by directed evolution. Microbiol Mol Biol Rev 69:51–78
48. Simpson KL et al (1964) Biosynthesis of yeast carotenoids. J Bacteriol 88:1688–1694
49. Goodwin TW (1980) Biosynthesis of carotenoids. In: Goodwin TW (ed) The biochemistry of the carotenoids. Chapman and Hall, London, pp 33–76
50. Goodwin TW (1993) Biosynthesis of carotenoids: an overview. In: Packer L (ed) Methods

in enzymology. Carotenoids, part B: metabolism, genetics and biosynthesis. Academic, San Diego, pp 330–340

51. Lynen F (1967) Biosynthetic pathways from acetate to natural products. Pure Appl Chem 14:137–167
52. Bloch KE (1983) Sterol structure and membrane function. CRC Crit Rev Biochem 14:47–92
53. Takaichi S (1999) Carotenoids and carotenogenesis in anoxygenic photosynthetic bacteria. In: Frank HA et al (eds) The photochemistry of carotenoids. Kluwer Academic, Dordrecht, pp 39–69
54. Shaish A et al (1992) Biosynthesis of β-carotene in Dunaliella. In: Lester P (ed) Methods in enzymology. Academic, New York, pp 439–444
55. Britton G (1998) Overview of carotenoid biosynthesis. In: Britton G et al (eds) Carotenoids: biosynthesis and metabolism. Birkhauser Verlag, Basel, pp 13–140
56. Fraser PD et al (1997) In vitro characterization of astaxanthin biosynthetic enzymes. J Biol Chem 272:6128–6135
57. Antia N, Cheng JY (1983) Evidence for anomalous xanthophyll composition in a clone of *Dunaliella tertiolecta* (Chlorophyceae). Phycology 22:235–242
58. Yamamoto HY et al (1999) Biochemistry and molecular biology of the xanthophyll cycle. In: Frank HA et al (eds) Advances in photosynthesis: the photochemistry of carotenoids. Kluwer Academic, Dordrecht, pp 293–303
59. Bramely PM (1985) The in vitro biosynthesis of carotenoids. Adv Lipid Res 21:243–219
60. Sherman MM et al (1989) Isolation and characterization of isoprene mutants of *Escherichia coli*. J Bacteriol 171:3619–3628
61. Misawa N, Shimada H (1997) Metabolic engineering for the production of carotenoids in non-carotenogenic bacteria and yeasts. J Biotechnol 59:169–181
62. Sandmann G, Misawa N (1992) New functional assignment of the carotenogenic genes *crtB* and *crtE* with constructs of these genes from *Erwinia species*. FEMS Microbiol Lett 69:253–257
63. Shimada H et al (1998) Increased carotenoid production by the food yeast *Candida utilis* through metabolic engineering of the isoprenoid pathway. Appl Environ Microbiol 64:2676–2680
64. Humbelin M et al (2002) Genetics of isoprenoid biosynthesis in *Paracoccus zeaxanthinifaciens*. Gene 297:129–139
65. Phadwal K (2005) Carotenoid biosynthetic pathway: molecular phylogenies and evolutionary behavior of *crt* genes in eubacteria. Gene 345:35–43
66. Steiger S et al (2002) Heterologous production of two unusual acyclic carotenoids, 1,1′-dihydroxy-3,4-didehydrolycopene and 1-hydroxy-3,4,3′,4′-tetradehydrolycopene by combination of the *crtC* and *crtD* genes from *Rhodobacter and Rubrivivax*. J Biotechnol 97:51–58
67. Rählert N et al (2009) A *crtA*-related gene from *Flavobacterium* P99-3 encodes a novel carotenoid 2-hydroxylase involved in myxol biosynthesis. FEBS Lett 10:1605–1610
68. Tang X et al (2007) Improvement of a *CrtO*-type of β-carotene ketolase for canthaxanthin production in *Methylomonas sp*. Metab Eng 9:348–354
69. Misawa N et al (1991) Production of beta-carotene in *Zymomonas mobilis* and *Agrobacterium tumefaciens* by introduction of the biosynthesis genes from *Erwinia uredovora*. Appl Environ Microbiol 57:1847–1849
70. Snodderly DM (1995) Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am J Clin Nutr 62:1448S–1461S
71. Athukorala Y et al (2007) Anticoagulant activity of marine green and brown algae collected from Jeju island in Korea. Bioresour Technol 98:1711–1716
72. Meléndez-Martínez AJ et al (2004) Nutritional importance of carotenoid pigments. Arch Latinoam Nutr 54:149–154
73. Je JY et al (2009) Antioxidant activity of enzymatic extracts from the brown seaweed *Undaria pinnatifida* by electron spin resonance spectroscopy. LWT-Food Sci Technol 42:874–878
74. Maeda H et al (2008) Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient. Asia Pac J Clin Nutr 17(Suppl 1):196–199
75. Yamaguchi M, Shizuoka JP (2006) Osteogenesis promoter containing beta-cryptoxanthin as the active ingredient. US Patent 200,601,061,15
76. Binns CW et al (2004) The relationship between dietary carotenoids and prostate cancer risk in Southeast Chinese men. Asia Pac J Clin Nutr 13:S117
77. Ulrich M (2008) The Global Market for Carotenoids, BBC Market Research
78. Klaui H, Bauerfeind CJ (1981) Carotenoids as food colors. In: Bauerfeind JC (ed) Carotenoids as colorants. Academic, New York, pp 48–292

79. Francis FJ (2000) Carotenoids as food colorants. Cereal Food World 45:198–203
80. Jacobson GK et al (2000) Astaxanthin overproducing strains of *Phaffia rhodozyma*. Method for their cultivation and their use in animal feeds. Patent 6,015,684
81. Lorenz RT, Cysewski GR (2000) Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends Biotechnol 18:160–167
82. Miao F et al (2006) Characterization of astaxanthin esters in *Haematococcus pluvialis* by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Anal Biochem 352:176–181
83. Mortensen A et al (1997) Comparative mechanisms and rates of free radical scavenging by carotenoid antioxidants. FEBS Lett 418:91–97
84. Miki W (1991) Biological functions and activities of animal carotenoids. Pure Appl Chem 63:141–146
85. Jyonouchi H et al (1993) Studies of immunomodulating actions of carotenoids. II. Astaxanthin enhances in vitro antibody production to T-dependent antigens without facilitating polyclonal B-cell activation. Nutr Cancer 19:269–280
86. Mayne ST (1996) Beta-carotene, carotenoids, and disease prevention in humans. FASEB J 10:690–701
87. Chew BP et al (1999) A comparison of the anticancer activities of dietary beta-carotene, canthaxanthin and astaxanthin in mice in vivo. Anticancer Res 19:1849–1853
88. Guerin M et al (2003) *Haematococcus* astaxanthin: applications for human health and nutrition. Trends Biotechnol 21:210–216
89. Johnson EA (2003) *Phaffia rhodozyma*: colorful odyssey. Int Microbiol 6:169–174
90. Bubrick P (1991) Production of astaxanthin from *Haematococcus*. Bioresour Technol 38:237–239
91. Zheng YG et al (2006) Large-scale production of astaxanthin by *Xanthophyllomyces dendrorhous*. Food Bioproducts Process 84:164–166
92. de la Fuente JL et al (2010) High-titer production of astaxanthin by the semi-industrial fermentation of *Xanthophyllomyces dendrorhous*. J Biotechnol 148:144–146
93. Lee JH et al (2008) Fermentation kinetic studies for production of carotenoids by *Xanthophyllomyces dendrorhous*. J Biotechnol 136:S732–S732
94. Ranjbar R et al (2008) High efficiency production of astaxanthin by autotrophic cultivation of *Haematococcus pluvialis* in a bubble column photobioreactor. Biochem Eng J 39:575–580
95. Krubasik P, Sandmann G (2000) A carotenogenic gene cluster from *Brevibacterium linens* with novel lycopene cyclase genes involved in the synthesis of aromatic carotenoids. Mol Gen Genet 263:423–432
96. Misawa N et al (1990) Elucidation of the *Erwinia uredovora* carotenoid biosynthetic pathway by functional analysis of gene products expressed in *Escherichia coli*. J Bacteriol 172:6704–6712
97. Lee JH et al (2003) Isolation of cDNAs for gonadotropin-II of flounder (*Paralichthys olivaceus*) and its expressions in adult tissues. J Microbiol Biotechnol 13:710–716
98. Fang TJ, Chiou TY (1996) Batch cultivation and astaxanthin production by a mutant of the red yeast *Phaffia rhodozyma* NCHU-FS501. J Ind Microbiol Biotechnol 16:175–181
99. Borowitzka LJ (1992) Beta-Carotene production using algal biotechnology. J Nutr Sci Vitaminol (Tokyo) 38:248–250
100. Paiva Sergio AR, Russell RM (1999) Beta-Carotene and other carotenoids as antioxidants. J Am Coll Nutr 18:426–433
101. Ye Z et al (2008) Biosynthesis and regulation of carotenoids in *Dunaliella*: progresses and prospects. Biotechnol Adv 26:352–360
102. Yoon SH et al (2009) Combinatorial expression of bacterial whole mevalonate pathway for the production of beta-carotene in *E. coli*. J Biotechnol 140:218–226
103. Ciegler A (1965) Microbial carotenogenesis. In: Wayne WU (ed) Advances in applied microbiology. Academic, New York, pp 1–34
104. Bhosale P, Gadre RV (2001) Production of β-carotene by a *Rhodotorula glutinis* mutant in sea water medium. Bioresour Technol 76:53–55
105. Raja R et al (2007) Exploitation of *Dunaliella* for β-carotene production. Appl Microbiol Biotechnol 74:517–523
106. Borowitzka MA (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 70:313–321
107. Ninet L, Renault J (1979) Carotenoids. In: Peppier HJ, Perlman D (eds) Microbiol technology, 2nd edn. Academic, New York, pp 529–253
108. Reyes P et al (1964) The mechanism of beta-ionone stimulation of carotenoid and ergosterol biosynthesis in *Phycomyces blakesleeanus*. Biochim Biophys Acta 90:578–592
109. Uchiyama S et al (2004) Anabolic effect of beta-cryptoxanthin on bone components in

the femoral tissues of aged rats in vivo and in vitro. J Health Sci 50:491–496

110. Giovannucci E et al (1995) Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst 87:1767–1776
111. Yuan JM et al (2001) Prediagnostic levels of serum β-cryptoxanthin and retinol predict smoking-related lung cancer risk in Shanghai, China. Cancer Epidemiol Biomarkers Prev 10:767–773
112. Yuan JM et al (2003) Dietary cryptoxanthin and reduced risk of lung cancer: the Singapore Chinese Health Study. Cancer Epidemiol Biomarkers prev 12:890–898
113. Kohno H et al (2001) Inhibitory effect of mandarin juice rich in beta-cryptoxanthin and hesperidin on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced pulmonary tumorigenesis in mice. Cancer Lett 174: 141–150
114. Tanaka T et al (2000) Suppression of azoxymethane-induced colon carcinogenesis in male F344 rats by mandarin juices rich in beta-cryptoxanthin and hesperidin. Int J Cancer 88:146–150
115. Pattison DJ et al (2004) The role of diet in susceptibility to rheumatoid arthritis: a systematic review. J Rheumatol 31:1310–1319
116. Serrato JO et al (2006) Production of β-cryptoxanthin, a provitamin-A precursor, by *Flavobacterium lutescens*. J Food Sci 71:E314–E319
117. Guyomarc'h F et al (2000) Production of carotenoids by *Brevibacterium linens*: variation among strains, kinetic aspects and HPLC profiles. J Ind Microbiol Biotechnol 24:64–70
118. Bhosale P, Bernstein PS (2004) Beta-carotene production by *Flavobacterium multivorum* in the presence of inorganic salts and urea. J Ind Microbiol Biotechnol 31:565–571
119. Khachik F et al (2007) Partial synthesis of (3R,6′R)-alpha-cryptoxanthin and (3R)-beta-cryptoxanthin from (3R,3′R,6′R)-lutein. J Nat Prod 70:220–226
120. Baker Rémi TM (2001) Canthaxanthin in aquafeed applications: is there any risk? Trends Food Sci Technol 12:240–243
121. Palozza P, Krinsky NI (1992) Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. Arch Biochem Biophys 297:291–295
122. Asker O (2002) Production of canthaxanthin by *Haloferax alexandrinus* under non-aseptic conditions and a simple, rapid method for its extraction. Appl Microbiol Biotechnol 58: 743–750
123. Nasri N et al (2010) Use of response surface methodology in a fed-batch process for optimization of tricarboxylic acid cycle intermediates to achieve high levels of canthaxanthin from *Dietzia natronolimnaea* HS-1. J Biosci Bioeng 109:361–368
124. Krupa D et al (2010) Extraction, purification and concentration of partially saturated canthaxanthin from *Aspergillus carbonarius*. Bioresour Technol 101:7598–7604
125. Ausich RL (1997) Commercial opportunities for carotenoid production by biotechnology. Pure Appl Chem 69:2169–2173
126. Khodaiyan F et al (2008) Optimization of canthaxanthin production by *Dietzia natronolimnaea* HS-1 from cheese whey using statistical experimental methods. Biochem Eng J 40:415–422
127. Lotan T, Hirschberg J (1995) Cloning and expression in *Escherichia coli* of the gene encoding *beta-C-4-oxygenase*, that converts beta-carotene to the ketocarotenoid canthaxanthin in *Haematococcus pluvialis*. FEBS Lett 364:125–128
128. Maeda H et al (2005) Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. Biochem Biophys Res Commun 332:392–397
129. Maeda H et al (2007) Effect of medium-chain triacylglycerols on anti-obesity effect of fucoxanthin. J Oleo Sci 56:615–621
130. Maeda H et al (2007) Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-Ay mice. J Agric Food Chem 55:7701–7706
131. Tsukui T et al (2007) Fucoxanthin and fucoxanthinol enhance the amount of docosahexaenoic acid in the liver of KKAy obese/diabetic mice. J Agric Food Chem 55: 5025–5029
132. Hosokawa M et al (2004) Fucoxanthin induces apoptosis and enhances the antiproliferative effect of the PPARgamma ligand, troglitazone, on colon cancer cells. Biochim Biophys Acta 1675:113–119
133. Das SK et al (2005) Fucoxanthin induces cell cycle arrest at G0/G1 phase in human colon carcinoma cells through up-regulation of p21WAF1/Cip1. Biochim Biophys Acta 1726:328–335
134. Yun SM et al (2007) Isolation and identification of an antibacterial substance from sea mustard, *Undaria pinnatifida*, for *Streptococcus mutans*. Korean Soc Food Sci Nutr 36:149–154

135. Cahyana AH et al (1992) Pyropheophytin A as an antioxidative substance from the marine alga, Arame (*Eicenia bicyclis*). Biosci Biotechnol Agrochem 18:1533–1535
136. Yan X et al (1999) Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. Biosci Biotechnol Biochem 63:605–607
137. Kang HS et al (2003) A new phlorotannins from the brown alga *Ecklonia stolonifera*. Chem Pharm Bull 51:1012–1014
138. Khachik F (2005) Process for extraction and purification of lutein, zeaxanthin and rare carotenoids from marigold flowers and plants. US Patent 6,262,284
139. Sánchez JF et al (2008) Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis*. Process Biochem 43:398–405
140. Francis GW et al (1975) Variations in the carotenoid content of *Chlamydomonas reinhardii* throughout the cell cycle. Arch Microbiol 104:249–254
141. Del Campo JA et al (2001) Lutein production by *Muriellopsis sp.* in an outdoor tubular photobioreactor. J Biotechnol 85:289–295
142. Shi XM et al (2000) Heterotrophic production of biomass and lutein by *Chlorella protothecoides* on various nitrogen sources. Enzyme Microb Technol 27:312–318
143. Nelis HJ, De Leenheer AP (1991) Microbial sources of carotenoid pigments used in foods and feeds. J Appl Microbiol 70:181–191
144. Khachik F et al (1989) Separation, identification, and quantification of the major carotenoids in extracts of apricots, peaches, cantaloupe, and pink grapefruit by liquid chromatography. J Agric Food Chem 37:1465–1473
145. McDERMOTT CB et al (1973) Effect of inhibitors on zeaxanthin synthesis in a *Flavobacterium*. J Gen Microbiol 77: 161–171
146. Alcantara S, Sanchez S (1999) Influence of carbon and nitrogen sources on *Flavobacterium* growth and zeaxanthin biosynthesis. J Ind Microbiol Biotechnol 23:697–700
147. Masetto A et al (2001) Application of a complete factorial design for the production of zeaxanthin by *Flavobacterium sp.* J Biosci Bioeng 92:55–58
148. Sajilata MG et al (2010) Development of efficient supercritical carbon dioxide extraction methodology for zeaxanthin from dried biomass of *Paracoccus zeaxanthinifaciens*. Sep Purif Technol 71:173–177
149. Fresnedo O et al (1991) Carotenoid composition in the cyanobacterium *Phormidium laminosum*. Effect of nitrogen starvation. FEBS Lett 282:300–304
150. Yokoyama T et al (1995) Thermozeaxanthins, new carotenoid-glycoside-esters from thermophilic eubacterium *Thermus thermophilus*. Tetrahedron Lett 36:4901–4904
151. Salguero A et al (2005) UV-A mediated induction of carotenoid accumulation in *Dunaliella bardawil* with retention of cell viability. Appl Microbiol Biotechnol 66:506–511
152. Jin ES et al (2003) A mutant of the green alga *Dunaliella salina* constitutively accumulates zeaxanthin under all growth conditions. Biotechnol Bioeng 81:115–124
153. Chen F et al (2005) Isolation and purification of the bioactive carotenoid zeaxanthin from the microalga *Microcystis aeruginosa* by high-speed counter-current chromatography. J Chromatogr A 1064:183–186
154. Ziegler RG et al (1996) Importance of α-carotene, β-carotene, and other phytochemicals in the etiology of lung cancer. J Natl Cancer Inst 88:612–615
155. Beatty S et al (2004) Macular pigment optical density and its relationship with serum and dietary levels of lutein and zeaxanthin. Arch Biochem Biophys 430:70–76
156. Hadden WL et al (1999) Carotenoid composition of marigold (*Tagetes erecta*) flower extract used as nutritional supplement. J Agric Food Chem 47:4189–4194
157. Li Y, Li L (2010) Method for producing fucoxanthin. US Patent 201,001,522,86
158. Wang WJ et al (2005) Isolation of fucoxanthin from the Rhizoid of *Laminaria japonica*. J Integr Plant Biol 47:1009–1015
159. Holden JM et al (1999) Carotenoid content of US foods: an update of the database. J Food Compost Anal 12:169–196

http://www.springer.com/978-1-61779-917-4

Microbial Carotenoids From Fungi
Methods and Protocols
Barredo, J.-L. (Ed.)
2012, XI, 290 p. 51 illus., 9 illus. in color., Hardcover
ISBN: 978-1-61779-917-4
A product of Humana Press