# Chapter 1
# Carotenoid Distribution in Nature

**Jennifer Alcaíno, Marcelo Baeza, and Víctor Cifuentes**

**Abstract** Carotenoids are naturally occurring red, orange and yellow pigments that are synthesized by plants and some microorganisms and fulfill many important physiological functions. This chapter describes the distribution of carotenoid in microorganisms, including bacteria, archaea, microalgae, filamentous fungi and yeasts. We will also focus on their functional aspects and applications, such as their nutritional value, their benefits for human and animal health and their potential protection against free radicals. The central metabolic pathway leading to the synthesis of carotenoids is described as the three following principal steps: (i) the synthesis of isopentenyl pyrophosphate and the formation of dimethylallyl pyrophosphate, (ii) the synthesis of geranylgeranyl pyrophosphate and (iii) the synthesis of carotenoids *per se*, highlighting the differences that have been found in several carotenogenic organisms and providing an evolutionary perspective. Finally, as an example, the synthesis of the xanthophyll astaxanthin is discussed.

**Keywords** Carotenogenesis • Microbial carotenoids • Astaxanthin

## 1.1 Introduction

Carotenoids are red, orange and yellow natural pigments that are synthesized by plants and some microorganisms fulfilling important physiological functions. For example, in photosynthetic organisms, carotenoids are essential for photosynthesis and photoprotection, whereas in non-photosynthetic organisms; they participate in alleviating photooxidative damage. Considering their properties, carotenoids have various industrial applications as dyes, and due to their various beneficial effects for health, they have been exploited by the food and nutraceutical industries and recently, by the pharmacological industry, with an estimated annual production greater than 100 million tons (Fraser and Bramley 2004). For these reasons, there

J. Alcaíno (✉) • M. Baeza • V. Cifuentes
Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago 7800003, Chile
e-mail: jalcainog@u.uchile.cl; mbaeza@u.uchile.cl; vcifuentes@uchile.cl



C. Stange (ed.), *Carotenoids in Nature*, Subcellular Biochemistry 79,
DOI 10.1007/978-3-319-39126-7_1

has been an increasing interest in finding new sources of carotenoids and optimizing the existing production systems.

Carotenoids are a large class of pigments of which more than 750 different chemical structures have been determined to date (Takaichi 2011). Carotenoids normally contain a hydrocarbon backbone of 40 carbon atoms, consisting of 8 isoprene units. The great diversity of these pigments generally derives from an acyclic $C_{40}H_{56}$ structure that has a long central light-absorbing conjugated polyene compound constituting the chromophoric system, which absorbs light from part of the visible region of the electromagnetic spectrum (400–500 nm). This central structure may have additional chemical modifications, such as variations in the level of desaturation, cyclization of one or both ends and the addition of oxygen-containing functional groups, including hydroxy, epoxy, and/or oxo groups (Britton 1995). According to the last type of modification, carotenoids are classified as carotenes or xanthophylls, with only the latter containing oxygen-containing functional groups (Bhosale and Bernstein 2005). The oxygen-containing functional groups affect the solubility of carotenoids, making xanthophylls more polar than carotenes, thus allowing their separation using many types of chromatography.

In plants, algae and phototrophic bacteria, carotenoids (mainly xanthophylls) are located in specific pigment-protein complexes, serving as accessory pigments to harvest light for photosynthesis and constituting the basic structural units of the photosynthetic apparatus. In this regard, the contribution of carotenoids to harvesting light is even greater in organisms that live in environments that have less available light (Britton 2008b). In addition to light harvesting, carotenoids also act as photoprotectors by quenching the energy excess under high-light stress and preventing the formation of the highly reactive singlet oxygen, which has damaging and potentially lethal effects. Additionally, the carotenoids of plants also play a vital ecological role, which is important for the survival and propagation of the species, because they contribute to the color of fruits and flowers, which is an important signal to attract insects and animals for pollination and seed dispersal (Lu and Li 2008).

In contrast, animals (with the exception of some aphids that naturally produce the carotenoid torulene (Moran and Jarvik 2010)) are unable to synthesize these important nutritional molecules *de novo* and thus rely on their diet to supply them (Goodwin 1984). In animals, these pigments accumulate in certain tissues, giving characteristic colors to many birds, marine invertebrates and fishes. Additionally, among marine invertebrates, it is common for carotenoids to form complexes with proteins (carotenoproteins), which extends the color range of carotenoids to green, purple and blue. A typical case is the color change from a bluish hue to red when lobsters are cooked. Under this condition, the color of the free xanthophyll (which is astaxanthin) is revealed as it is liberated from the carotenoid-protein complex during the denaturation of the carotenoprotein complex (Schiedt 1998). Moreover, carotenoids have essential functions and provide many important health benefits. For example, β-carotene, also known as pro-vitamin A, is the main source of vitamin A, a deficiency of which is one of the most damaging micronutrient deficiencies in the world. Vitamin A deficiency can lead to blindness, decreased immune function

and even death, affecting approximately 250 million preschool children according to the World Health Organization (World Health Organization 2014). It is not only vitamin A that is essential for ocular health, other carotenoids are also important. This is the case for the xanthophylls zeaxanthin and lutein, which are the pigmented components of the macula, a yellow spot at the center of the human retina. These xanthophylls provide protection against age-related macular degeneration, which can also cause blindness (Abdel-Aal et al. 2013). In addition, given the antioxidant properties of carotenoids, a higher ingestion of carotenoids is related to a lower risk of chronic diseases, including cardiovascular diseases, cataract development and some types of cancer. Among the carotenoids, the xanthophyll astaxanthin stands out due to its strong antioxidant properties, which have been proven to be stronger than those of other recognized antioxidants, such as β-carotene or even α-tocopherol (Miki 1991).

Carotenoids are also important membrane components because they are lipid-soluble pigments that are generally located deep within the hydrophobic lipid core and are oriented parallel to the membrane surface (in the case of nonpolar carotenoids such as β-carotene) or span the membrane bilayer (in the case of polar carotenoids such as astaxanthin) (Britton 2008b). In this last case, the oxygen-containing functional groups on the ionone-rings contact the polar head of the membrane phospholipids, whereas the chromophore is located within the hydrophobic core of the membrane. Thus, in addition to providing photoprotection to prevent membrane damage, carotenoids also have effects on the structure and dynamics of membranes. For example, polar carotenoids restrict the molecular motion of lipids and increase the rigidity of the membrane in its liquid crystalline state; thus, they modulate the membrane fluidity (Gruszecki and Strzalka 2005). Moreover, the electron-rich polyene chain of carotenoids is susceptible to enzymatic or non-enzymatic oxidative cleavage and the generated products, known as apocarotenoids, also have important functions. Indeed, vitamin A is an example of an apocarotenoid because it is the product of the symmetrical oxidative cleavage of β-carotene (Britton 2008a). In plants, apocarotenoids are involved in development, serve as antifungals and contribute to the flavor and aroma of fruits and flowers (Lu and Li 2008). Among the plant apocarotenoids, abscisic acid is a well-known phytohormone that is involved in a wide range of biological processes, including plant development and growth, integrating various stress signals and regulating stress responses (Tuteja 2007).

## 1.2 Carotenoid Distribution in Microorganisms

Microorganisms that produce and accumulate carotenoid pigments are widely found in the three domains of life. As in plants and animals, it is proposed that pigments play important physiological roles in microbial cells, mainly those associated with the stress response. Nevertheless, studies of the cellular functions of pigments in microbes are scarce, mainly because the objectives of the research

in this field are focused on the use of microorganisms as sources of economically important pigments. Our knowledge of the effects of culture conditions on microbial pigment production comes mainly from studies related to improving pigment productivity under controlled conditions, because microorganisms in their natural environments generally provide insufficient yields for commercialization. Several criteria for industrial carotenoid production by microbes have been evaluated, including the ability to use a wide range of (preferably inexpensive) carbon and nitrogen sources, tolerance to pH, temperature requirement, necessary mineral concentration, tolerated oxygenation level and required light conditions. The interest in discovering new microorganisms that produce a particular pigment or that produce undescribed pigments with novel characteristics applicable in commercial areas has increased significantly during the last few decades. Below are several examples of microorganisms that produce carotenoid pigments, with an emphasis on the novel compounds, and some examples of the cellular functions of carotenoids are reviewed.

### *1.2.1 Carotenoids in Bacteria, Microalgae and Archaea*

Astaxanthin is a red-orange carotenoid that is one of the most commercially important carotenoids. The global market for astaxanthin is the third largest of the total global market for carotenoids (after the markets for lutein and β-carotene, which hold the second and first place, respectively), which in 2010 was estimated at nearly US$225 million and is expected to exceed US$250 million by 2018 (BCC-Research 2011). Astaxanthin is best known for its use in aquaculture for salmon-flesh pigmentation, which has a considerable economic impact on this industry. This xanthophyll is also used in chicken feed to enhance the pigmentation of the yolk and flesh. Furthermore, astaxanthin has strong antioxidant properties and there is growing interest in this compound due to recent findings of its beneficial roles in degenerative diseases and other potential benefits for human health (Wang et al. 2000; Higuera-Ciapara et al. 2006; Park et al. 2010; Yasui et al. 2011). This interest has led to a considerable increase in the effort to find new sources of this ketocarotenoid; however, there are few reports of microbial astaxanthin production to date.

One of the most promising biosources of astaxanthin is the microalgae *Haematococcus pluvialis*, which has been reviewed elsewhere (Guerin et al. 2003; Lemoine and Schoefs 2010). In recent studies, it was found that the microalgal species *Dunaliella salina*, *Tetraselmis suecica*, *Isochrysis galbana* and *Pavlova salina*, which were isolated from coastal or brackish water in Australia, produced 4.7–6.9 mg/g dry weight of carotenoids. In the same report, it was stated that *T. suecica* and *Nannochloropsis* sp. BR2 produced astaxanthin, which represented 39 % (2,261 mg/g dry weight) and 16 % (321 mg/g dry weight) of the total carotenoids, respectively (Ahmed et al. 2014). The production of carotenoids by the microalgal specie *Chlorella protothecoides* is induced under nutritional, luminosity

and salinity stresses, as demonstrated by the shift in coloration from green to orange-red. However, a minor amount of free astaxanthin is produced, with 7 % of the total carotenoid content (0.8 % w/w) being canthaxanthin (23 %) and echinenone (15 %), the main carotenoids produced (Campenni' et al. 2013). In the case of the mixotrophic microalgae *Chromochloris zofingiensis*, an increase in astaxanthin production was observed as a response to increased concentrations of iron in the culture media. The production of astaxanthin was doubled at 0.2 mM $Fe^{2+}$, an effect that was not observed when different concentrations of $Mn^{2+}$ and $Mg^{2+}$ were tested (Wang et al. 2013). Regarding bacteria, two species belonging to the *Paracoccus* genus have been reported to produce astaxanthin, including *P. haeundaensis* sp. nov., isolated from the Haeundae Coast of Korea (Lee et al. 2004), and *P. carotinifacien*, which was reported to be effective for coloring the flesh of Coho salmon, Atlantic salmon and rainbow trout (Bories et al. 2007). A flagellated Gram-negative strictly aerobic bacterium that developed reddish-orange colonies on marine agar was isolated from coastal surface seawater near the Taichung harbor of Taiwan, which based on molecular and chemotaxonomic characterization, was proposed to be a novel species within the *Sphingomicrobium* genus. The isolate was named *Sphingomicrobium astaxanthinifaciens* sp. nov. and the type strain CC-AMO-30BT synthesized approximately 40 mg/g dry weight of astaxanthin (Shahina et al. 2013). In addition, some marine bacterial strains produce astaxanthin derivatives such as (3S,3′S)-astaxanthin-beta-D-glucoside and (3S,3′R)-adonixanthin-beta-D-glucoside, which are produced by *Paracoccus* sp. (formerly *Agrobacterium aurantiacum*) (Yokoyama et al. 1995) and 2-hydroxyastaxanthin, which is synthesized by *Brevundimonas* sp. (Yokoyama et al. 1996).

It has long been known that prokaryotic microorganisms produce other carotenoid pigments; at present, it is estimated that 180 prokaryotic microorganisms produce potential carotenoids (Prokaryotic Carotenoid DataBase 2014). A method for the isolation and purification of the purple pigment spirilloxanthin from *Spirillum rubrum* Esmarch was published in 1935; later the name of bacterium was changed to *Rhodospirillum rubrum*, and in 1958, the spirilloxanthin synthesis pathway was described (Jensen et al. 1958). In studies of spheroidenone and spirilloxanthin in sediments from Little Round Lake, Ontario Canada, it was proposed that *Rhodopseudomonas* bacterial species were responsible for these *fossil pigments* (Brown 1968). Currently, the phototrophic purple non-sulfur bacterium *R. rubrum* is known to produce as many as eighteen different carotenoids (including phytoene, lycopene and neurosporene), isoprenoid-quinones, bacteriopheophytin and different phosphatidylglycerol species during microaerophilic growth (Bona-Lovasz et al. 2013). The xanthophyll zeaxanthin is commonly found as the major carotenoid produced by several members of the *Flavobacteriaceae* family, such as *Zeaxanthinibacter enoshimensis* (Asker et al. 2007a), *Flavobacterium multivorum* (Bhosale and Bernstein 2004), *Muricauda lutaonensis* (Hameed et al. 2011) and *Mesoflavibacter zeaxanthinifaciens* (Asker et al. 2007b). Two probable novel species of the genus *Muricauda* isolated from sandy beaches of the southwestern coast of India accumulated high amounts of zeaxanthin (1.2–1.5 mg/g) in media supplemented with glutamic acid, and when compared to commercial zeaxanthin,

the bacterial zeaxanthin showed higher *in vitro* antioxidant activity (Prabhu et al. 2013). Zeaxanthin is also the main carotenoid produced by a strain of the microalga *Chlorella saccharophila* isolated from the marine waters of New Zealand. The yield of zeaxanthin reached 11 mg/g when the microalga was cultured in media containing glycerol as the carbon source and this organism also produced 5 mg/g of β-carotene (Singh et al. 2013). Another example of a microorganism that produces several carotenoid derivatives is the thermophilic bacterium *Thermus filiformis*, which produces zeaxanthin and zeaxanthin derivatives, including zeaxanthin monoglucoside, thermozeaxanthins and thermobiszeaxanthins (Mandelli et al. 2012).

β-carotene is another carotenoid with commercial appeal because it is used as a food colorant and by the pharmaceutical and cosmetic industries. There are few reports of natural β-carotene-producing bacteria; two examples of such organisms are the carotenoid-accumulating *Sphingomonas* sp. that produce 1.7 mg/g dry weight of total carotenoids (29 % β-carotene and 36 % nostoxanthin) (Silva et al. 2004) and *Serratia marcescens*, which produces 2.5 μg/ml of β-carotene (Wang et al. 2012). The extremely halophilic archaeon *Halorubrum* sp., which was isolated from Urmia Lake, produces 11,280 μg/l of total carotenoids, with bacterioruberin being the most predominant compound, followed by lycopene and β-carotene (Naziri et al. 2014). A promising natural source of β-carotene is the green unicellular algae *Dunaliella salina*, in which β-carotene can comprise up to 10 % of its dry weight under stressful conditions, such as high salinity, nutrient deprivation, extreme temperatures and high light intensity (Lamers et al. 2010, 2012; Wichuk et al. 2014). Other microalgae that produce β-carotene are *Eustigmatos magnus, Eustigmatos polyphem, Eustigmatos vischeri, Vischeria helvetica, Vischeria punctata* and *Vischeria stellate*. All of these eustigmatophytes produce β-carotene as more than 50 % of their total carotenoids when cultured in a bubble-column photobioreactor, with *V. stellata* being the major producer of β-carotene, which can account for up to 5.9 % of its dry weight (Li et al. 2012).

Many groups have sought novel bioactive molecules with antioxidant, anticancer, antiproliferative and apoptosis-inducing properties. In this regard, a halophilic archaea that forms red-orange colonies, most likely *Halobacterium halobium*, was isolated from the brine of a local crystallizer pond of a solar saltern in Tunisia. Its carotenoid content was as high as 7.6 mg/l, consisting mainly of a bacterioruberin-like carotenoid, and the carotenoid extract exhibited significant antiproliferative activity against HepG2 human cancer cell lines (Abbes et al. 2013). Shindo and Misawa (2014) evaluated a collection of rare and novel marine bacteria that produce orange or red pigments as sources of new carotenoids. They reported the production of diapolycopenedioic acid xylosylesters A–C by *Rubritalea squalenifaciens*, methyl 5-glucosyl-5,6-dihydro-apo-4,4′-lycopenoate by *Planococcus maritimus* and (3R)-saproxanthin and (3R,2'S)-myxol, which had strong antioxidant properties, by novel species of the family *Flavobacteriaceae* (Shindo and Misawa 2014). Based on assays of lipid peroxidation in rat brain homogenates, the rare carotenoid saproxanthin showed high antioxidant activity, which was even stronger than those of β-carotene and zeaxanthin (Shindo et al. 2007). A

yellow-orange bacterium that produces the saproxanthin derivative (3R, 2'S)-2'-isopentenylsaproxanthin was isolated from a seaweed collected in Nabeta Bay in Shizuoka, Japan. The carotenoid production rate of this bacterium, most likely *Jejuia pallidilutea* according to 16S rDNA analysis, was high in an alkaline medium (pH 9.2), reaching 1.1 mg/g of cell dry weight or 1.2 μg/ml (Takatani et al. 2014). Another ketocarotenoid with a strong ability to scavenge reactive oxygen species is deinoxanthin, which is the main pigment produced by species of the *Deinococcus* genus. This xanthophyll may contribute to the high resistance to gamma and UV radiation shown by members of this genus, because *D. radiodurans* was isolated from irradiated canned meat and was classified as the second most radiation-resistant organism after *Thermococcus gammaloteran* (Lemee et al. 1997). The genome of a *Deinococcus xibeiensis* strain isolated from radiation-contaminated soils was assembled and several carotenogenic genes were found. These findings will facilitate the understanding of the biosynthetic pathway of deinoxanthin and the eventual identification of key genes as modification targets to increase the pigment yield (Hu et al. 2013). Another pigment identified as a main factor that protects cells against the lethal effects of oxidative DNA-damaging agents is the red pigment bacterioruberin, found in *Halobacterium salinarium* (Shahmohammadi et al. 1998). Bacterioruberin produced by the extremely halophilic archaeon *Haloarcula japonica* showed much higher antioxidant capacity than that of β-carotene (Yatsunami et al. 2014). The halobacterium *Haloferax mediterranei* requires 20–25 % NaCl for optimal growth and lyses at salt concentrations below 10 %, and it increased the production of carotenoids, including bacterioruberin, in response to stress caused by a low concentration of NaCl (<20 %) (D'Souza et al. 1997). Bacterial survival in low-temperature environments is also enhanced by the presence of bacterioruberin, which plays important roles in regulating membrane fluidity, which affects the water-barrier property of membranes and the permeability of oxygen and other molecules (Fong et al. 2001).

From our anthropocentric point of view, the production of carotenoids by microorganisms is an adaptive response to certain environmental and stressor conditions. In this regard, the production of carotenoids in microbial strains isolated from Antarctic environments would be pivotal for resistance to freeze-thaw cycles and solar radiation. For example, carotenoid-pigmented bacterial strains showed higher survival rates compared with those of non-pigmented isolates when exposed to solar radiation, 61 % versus 0.01 % (Dieser et al. 2010). Additionally, the response to solar radiation has been exploited for the adaptive laboratory-based evolution of species of the microalga *Dunaliella* to select economically valuable traits; in *D. salina*, an enhancement of lutein and β-carotene accumulation was achieved in the presence of blue light (Wichuk et al. 2014).

Unlike most eukaryotes and bacteria, the response of the archaea *Sulfolobus solfataricus* and *Sulfolobus acidocaldarius* to DNA-damaging agents in nature do not involve the induction of DNA-repair proteins. Instead, the up-regulated expression of β-carotene biosynthetic enzymes, which have UV-protective properties, was observed (Gotz et al. 2007). In addition, carotenoids play an important role in bacterial aggregation and biofilm formation because a correlation between the

accumulation of carotenoids and aggregation/biofilm formation in *Rhodococcus* sp. SD-74 was recently described (Zheng et al. 2013). This analysis was performed using label-free Raman microspectroscopy, a noninvasive technique that allows for measurement of the intracellular concentration of carotenoids. It has been proposed that using carotenoids as biomarkers in conjunction with resonance Raman spectroscopy will facilitate the exploration for life beyond earth (Marshall et al. 2007).

### *1.2.2 Carotenoids in Fungi*

Although fungi (filamentous fungi and yeasts) are a broad group of organisms that are widely distributed in nature, it is estimated that few species synthesize carotenoids mainly because, in contrast to plants, they are non-photosynthetic organisms. Fungi produce a large variety of carotenoids; however, the most predominant of these are carotenes, and xanthophylls are produced to a lesser extent. In the 1970s, a possible taxonomic relationship between fungi and carotenoids was proposed, suggesting that some of these pigments could be used as good taxonomic markers (Valadon 1976). In this regard, the presence of carotenoids and balistoconidias in basidiomycetes was used as a criterion to classify species in a particular genus. For this purpose, the carotenoid production was related to the 18S rRNA molecular-marker data, but the results of these studies did not support the phylogenetic relationship among basidiomycetous yeasts, indicating that carotenoids were a less significant taxonomic criterion than was originally believed (Nakase et al. 1993).

With respect to carotenes, in 1972, Goodwin reported that the most abundant carotenoid in nature, β-carotene, was present in a significant number of ascomycete fungi, among them *Calycella citrina*, *Heliotum citrinus*, *Mitrula paludosa*, *Spathularia flavida* and others from the Helotiales order and *Aleuria rhenana*, *Leucoscypha rutilans*, *Pithya vulgaris*, *Pulvinula constellatio*, *Sowerbyella radiculata* and others from the Pezizales order (Goodwin 1972). Other ascomycetes, such as *Protomyces pachydermus* and *P. inouyei*, produce β- and γ-carotene, as well as lycopene (Valadon 1964). Moreover, these carotenoids are also distributed among the basidiomycetes, such as *Ustilago scabiosae*, *Gymnosporangium juniperi-virginianae*, *Calocera viscosa*, *Dacrymyces ellisii*, *Cantharellus cibarius*, *Phyllotopsis nidulans*, *Cryptococcus flavescens*, *C. laurentii*, *C. luteolus*, *Rhodotorula aurea*, *R. flava* and *R. penaus*. Among the zygomycetes, specifically those in the Mucorales order, which comprises approximately 205 species, three species stand out for their ability to produce significant amounts of β-carotene as the major pigment, including *Phycomyces blakesleeanus*, *Mucor circinelloides* and *Blakeslea trispora*. Carotenogenesis in these organisms has been extensively studied due to its biological functions and potential biotechnological applications in the industrial production of natural β-carotene (Tisch and Schmoll 2010; Avalos and Limón 2014). Other β-carotene-producing species within the Mucorales order have been

reported, including *Mucor flavus*, *M. hiemalis*, *Choanephora circinans*, *Mortierella remanniana*, *Pilaria anomala* and *Pilobolus crystallinus*, among others.

The production of γ-carotene has been identified in a variety of fungi, such as the zygomycetes *Cladochytrium replicatum*, *Rhizophlyctis rosea*, *Allomyces arbuscula*, *A. macrogynus* and *A. moniliformis*, among others (Goodwin 1972) and the ascomycetes *Penicillium multicolor*, *Microglossum olivaceum*, *Aleuria aurantia*, *Cheilymenia crucipila*, *Scutellinia ampulosa*, *S. arenosa*, *S. scutellata*, *S. superba*, *S. umbrarum* and *S. trechispora*, the latter of which also produces β-zeacarotene. Some basidiomycetes have been reported produce to γ-carotene, including *Ustilago scabiosae*, *Clitocybe venustissimus* and *Sphaerobolus stellatus*, among others; to produce torulene, such as *Rhodotorula gracilis*, *R. infirmominata*, *R. pallida*, *R. sannieri*; and to produce torularhodine, such as the species *Rhodotorula mucilaginosa*, *R. minuta*, *R. rubra*, *Sporidiobolus johnsonii*, *Sporobolomyces roseus*, *S. ruberrimus* and *S. salmonicolor*. In 2002, Echavarri-Erasun and Johnson described the most representative carotenoids produced by different phylogenetic groups of fungi. For example, 3,4-didehydrolycopene, neurosporaxanthin, γ-carotene and β-carotene are produced by Myxomycetes; γ-carotene, lycopene and β-carotene by Chytridiomycetes; β-carotene by Zygomycetes; γ-carotene, lycopene, β-carotene, torulene, lycoxanthin and neurosporaxanthin by Pyrenomycetes and β-carotene, γ-carotene, 3,4-didehydrolycopene, torulene, phillipsiaxanthin, aleuriaxanthin and plectaniaxanthin by Discomycetes, being both Ascomycota; β-carotene, HDCO, echinenone, astaxanthin by the Basidiomycetous yeast *X. dendrorhous* (Echavarri-Erasun and Johnson 2002). Among fungi, members belonging to genus *Rhodotorula*, *Sporobolomyces* and *Xanthophyllomyces* may be prominent biotechnological sources of a variety of carotenoids (Mata-Gomez et al. 2014). As in many microorganisms, the carotenoid production and composition varies according to the culture conditions and carbon source and among strains belonging to different species within the *Rhodototula* genus (Frengova and Beshkova 2009). In order to use yeast as commercial sources of pigments, studies to identify inexpensive carbon sources and culture media and to determine suitable physiological and environmental conditions to increase the production are required. Several factors, including the carbon source, light, temperature, oxygen, salts, metals and chemicals, such as ethanol, among others, affect carotenoid production in yeasts, which has recently been reviewed (Mata-Gomez et al. 2014).

Another fungal xanthophylls are neurosporaxanthin, which was identified in the ascomycete fungus *Neurospora crassa* (Zalokar 1957), cordyxanthin, found in the mushroom *Cordyceps militaris* (Dong et al. 2013; Yang et al. 2014), canthaxanthin found in *Cantharellus cinnabarinus* (Haxo 1950) and astaxanthin, which is produced by the yeast *X. dendrorhous* (Miller et al. 1976; Andrewes and Starr 1976). Moreover, three basidiomycete carotenogenic yeast species, including *Dioszegia* sp., *Rhodotorula mucilaginosa* and *R. laryngis*, have been isolated from ice samples obtained from an Italian Alpine glacier. Of these, *Dioszegia* sp. produces a still unidentified xanthophyll that contains two hydroxyl groups (Amaretti et al. 2014).

Some fungal species have been used as model microorganisms in carotenogenesis studies. Among them are the zygomycetes *Phycomyces blakesleeanus*, *Blakeslea trispora* and *Mucor circinelloides* (Avalos et al. 2014), in which each step of the carotenoid biosynthetic pathway, the genes encoding the enzymes involved and their regulation are known. Regarding the above, the expression of the carotenogenic genes *carB* and *carRA* in *B. trispora* are induced during the sexual reproductive phase; that is, the transcription of these genes is mating-dependent (Schmidt et al. 2005). Moreover, β-carotene was reported to play an important role in the biology of the fungus in *P. blakesleeanus*, specifically in its sexual reproduction because its pheromones are derived from β-carotene. This process is controlled by the *carS* gene, which in addition to producing a gene product that cleaves β-carotene, regulates the carotenoid biosynthesis and sexual reproduction of this fungus (Tagua et al. 2012). The genes controlling the biosynthesis of carotenoids in *N. crassa* were identified using classical genetic techniques and after, relevant regulatory mechanisms were ellucidated. The *al*-1, *al*-2, *al*-3 and *ylo*-1 genes of *N. crassa* were isolated and their involvement in the neurosporaxanthin biosynthetic pathway was studied (Schmidhauser et al. 1990, 1994; Nelson et al. 1989; Estrada et al. 2008). The biosynthesis of carotenoids in this organism is induced by light with the participation of the White Collar photosystem (Nelson et al. 1989).

The basidiomycete yeast *X. dendrorhous* produces astaxanthin, a xanthophyll that is widely used in salmon farming. The carotenogenic genes from this organism have been isolated and characterized at the molecular level, and metabolic engineering approaches have been applied for the heterologous production of astaxanthin in *S. cerevisiae*, albeit at a minimal level (Ukibe et al. 2009). In general, the results indicated that the latter yeast was able to produce only β-carotene when the currently described carotenogenic genes of *X. dendrorhous* are introduced, suggesting that other genetic elements of *X. dendrorhous* are required to successfully transform β-carotene into astaxanthin in *S. cerevisiae*. In addition, comparative studies have been conducted for the biotechnological production of astaxanthin using various natural sources of this xanthophyll and chemical synthetic processes, focusing on the advantages of the yeast *X. dendrorhous* as a model producing organism and its potential industrial use (Schmidt et al. 2011).

## 1.3 Carotenoid Biosynthesis

Carotenoid pigments are one of the most representative groups of isoprenoids, and isoprenoids constitute one of the largest families of natural compounds produced by members of the three domains of life. Carotenoids are formed using the same $C_5$ building block, the isoprene ($C_5H_8$) unit, from which isopentenyl pyrophosphate (IPP) and its allylic isomer dimethylallyl pyrophosphate (DMAPP) are produced. In isoprenoid synthesis, three molecules of IPP are sequentially added to DMAPP by prenyl transferase enzymes to yield geranylgeranyl-pyrophosphate (GGPP, $C_{20}$). The later condensation of two molecules of GGPP yields phytoene ($C_{40}$), which is

the first carotenoid to be synthesized. Phytoene is a symmetrical colorless linear carotene with only three conjugated double bonds; the great diversity of carotenoids is derived through chemical modifications of this molecule (Ajikumar et al. 2008). Thus, it is possible to divide the synthetic pathway of carotenoids into three major stages as follows:

(i) The synthesis of IPP and the formation of DMAPP
(ii) The synthesis of GGPP
(iii) The synthesis of carotenoids *per se*

In different carotenoid-producing organisms, several downstream modifications of the carotenoid backbone, such as desaturation, cyclization, oxidization and further modifications, such as glycosylation and oxidative cleavage, may be performed. All of these modifications contribute to the enormous diversity of this type of compound in nature. As an example, the synthesis of astaxanthin is discussed.

### *1.3.1 The Synthesis of IPP and DMAPP*

The carotenoid precursors IPP and DMAPP are essential metabolites and the production of which varies among organisms and cellular compartments. IPP and DMAPP can be synthesized via the following two independent non-homologous metabolic pathways: the mevalonate (MVA) pathway (Miziorko 2011) and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, also referred to as the 1-deoxy-D-xylulose 5-phosphate (DXP) or the non-MVA pathway (Lichtenthaler 2000). The MEP pathway [KEGG: M00096] (Kanehisa and Goto 2000; Kanehisa et al. 2014) begins with the condensation of D-glyceraldehyde 3-phosphate and pyruvate by DXP synthase, which releases $CO_2$ and produces DXP (Fig. 1.1). DXP is then reduced into MEP by DXP isomeroreductase, which is subsequently converted into IPP via five catalytic steps that sequentially involve the following enzymes: 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase, 4-(Cytidine 5'-pyrophospho)-2-C-methyl-D-erythritol kinase, 2-C-methyl-D-erythritol 2,4-cyclopyrophosphate synthase, 4-hydroxy-3-methylbut-2-enyl-pyrophosphate synthase and 4-hydroxy-3-methylbut-2-enyl pyrophosphate reductase. Interesting, the last enzyme of this pathway is also known as IPP:DMAPP synthase because it is able to produce IPP and DMAPP simultaneously in an 85:15 IPP/DMAPP ratio (Tritsch et al. 2010). Thus, DMAPP can be synthesized by this enzyme via the MEP pathway and by an IPP-isomerase (see below). In the MVA pathway [KEGG: M00095] (Kanehisa and Goto 2000; Kanehisa et al. 2014), the synthesis of IPP involves 6 steps, starting with the condensation of two molecules of acetyl-coenzyme A (CoA) by acetoacetyl-CoA thiolase to give acetoacetyl-CoA (Fig. 1.1). Next, acetoacetyl-CoA is converted into 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by HMG synthase and afterward, HMG-CoA is reduced to MVA by HMG-CoA reductase. Then, two phosphorylation steps at the C-5 of MVA and a decarboxylation reaction by MVA kinase, phospho-MVA (MVA-P) kinase

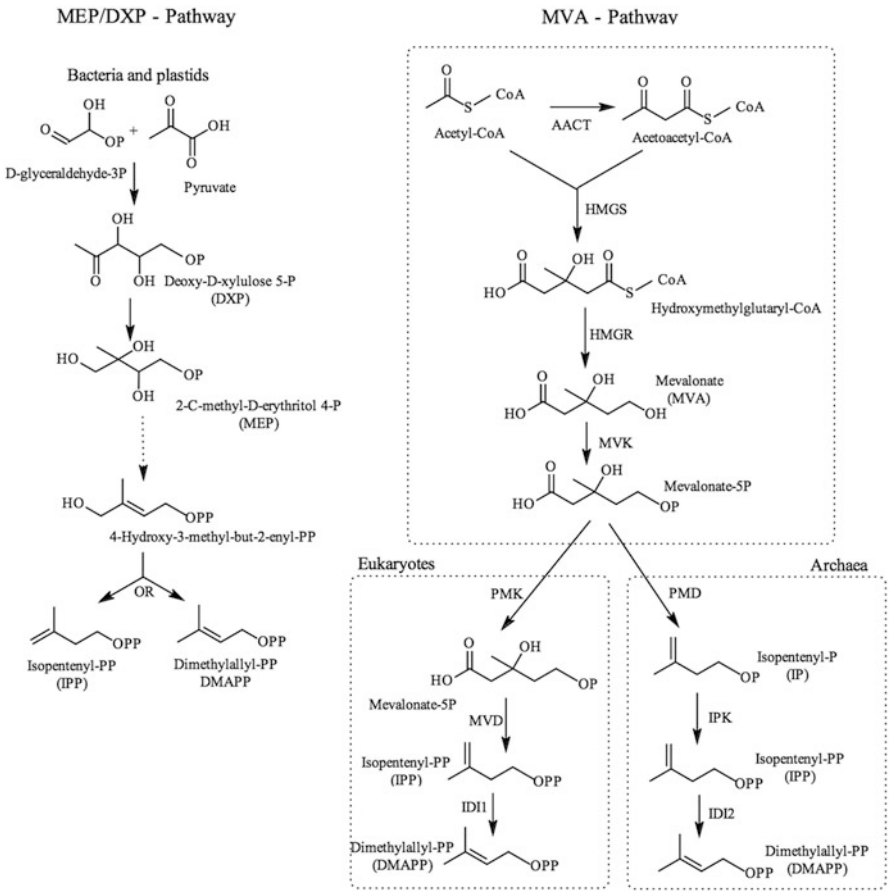


**Fig. 1.1** Biosynthesis of isopentenyl pyrophosphate and dimethylallyl pyrophosphate. Schematic representations of the synthesis of IPP and DMAPP through the MEP/DXP pathway in bacteria and in plant plastids (on the left, adapted from [KEGG: M00096] (Kanehisa and Goto 2000; Kanehisa et al. 2014)) and through the MVA pathway in eukaryotes and archaea (on the *right*, adapted from (Lombard and Moreira 2011)). The MVA pathways of eukaryotes and archaea are somewhat different; they share the only first four steps (*boxed*). Abbreviations: *MEP* (2-C-methyl-D-erythritol-4-P), *DXP* (Deoxy-D-xylulose-5-P), *MVA* (mevalonate), *IPP* (isopentenyl pyrophosphate), *DMAPP* (dimethylallyl pyrophosphate), *AACT* (acetoacetyl-CoA thiolase), *HMGS* (hydroxymethylglutaryl-CoA synthase), *HMGR* (hydroxymethylglutaryl-CoA reductase), *MVK* (mevalonate kinase), *PMK* (phosphomevalonate kinase), *MVD* (mevalonate pyrophosphate decarboxylase), *PMD* (phosphomevalonate decarboxylase), *IPK* (isopentenyl phosphate kinase), *IDI1* (isopentenyl pyrophosphate isomerase type 1) and *IDI2* (isopentenyl pyrophosphate isomerase type 2)

and MVA-PP decarboxylase, respectively, produce IPP. Finally, the interconversion between IPP and DMAPP is catalyzed by an IPP-isomerase.

With the exception of plants, most organisms only have one pathway for the synthesis of IPP and DMAPP. Most bacteria and cyanobacteria only have the MEP pathway, whereas in most eukaryotes and archaea; only the MVA pathway is present. Although plants have both pathways, the pathways have different cellular localizations; IPP is synthesized in the cytoplasm via the MVA pathway, whereas this metabolite is synthesized via the MEP pathway in plastids (Vranova et al. 2013; Misawa 2010). This strict compartmentalization of two alternative pathways for IPP synthesis in plants is consistent with the prokaryotic origin of chloroplasts, which are presumed to have derived from a cyanobacteria-like endosymbiont (Lange et al. 2000). Interestingly, the isoprenoids of the green algae *Chlorella fucsa*, *Chlamydomonas reinhardtii* and *Scenedesmus obliquus* are synthesized via the MEP pathway (Dobrowolska 2006) and no MVA-pathway encoding genes were found in the *C. reinhartdtii* genome (Vranova et al. 2013). These observations indicate that the green algae ancestor most likely never had the MVA pathway or that this metabolic route was lost in these organisms during evolution. In either case, efficient mechanisms for exporting isoprenoids and their intermediates from plastids to the cytoplasm for the synthesis of cytosolic isoprenoids, such as sterols, have been developed in green algae (Vranova et al. 2013). In contrast, the MVA pathway has been found in only a few bacteria, which was attributed to acquisition via horizontal gene transfer from eukaryotes or archaea (Lombard and Moreira 2011).

Even though the presence of the MVA pathway is characteristic of eukaryotes and archaea, there are differences between the MVA pathways of the two domains. Analyses of archaeal genomes revealed that some species lack the genes encoding the last enzymes of the MVA pathway including those encoding MVA-P kinase, MVA-PP decarboxylase and IPP isomerase (Lombard and Moreira 2011). To explain this situation, it has been proposed that non-homologous replacements of these genes occurred in archaea (Smit and Mushegian 2000). In support of this hypothesis, an enzyme that can phosphorylate isopentenyl phosphate (IP) to produce IPP (the isopentenyl phosphate kinase, IPK) has been found in some archaea (Grochowski et al. 2006), suggesting that the order of the last phosphorylation and decarboxylation steps in the MVA pathway in archaea might be switched. Related to this idea, a phosphomevalonate decarboxylase (PMD) was recently identified and characterized together with an IPK, both from *Haloferax volcanii*, clarifying the existence of an alternate mevalonate pathway in archaea and proving that the archaeon decarboxylation step occurs prior to the phosphorylation step (Vannice et al. 2014). In addition, an IPP isomerase that is non-homologous to the eukaryotic enzyme was first described in *Streptomyces* sp. (Kaneda et al. 2001) and later was found to be encoded by several archaea genomes (Barkley et al. 2004) (see below). Considering these observations, recent phylogenomic analyses of the available data for the bacterial, eukaryotic and archaeal MVA-pathway genes, strongly supported the hypothesis that the MVA pathway could have been an ancestral metabolic pathway in the three domains of life, so it was likely present in the last common ancestor of all organisms and was lost and replaced by the MEP pathway in

a variety of bacterial phyla (Lombard and Moreira 2011). Additionally, the last MVA-pathway genes in most archaeal species could have been modified by non-homologous gene replacement, excluding Sulfolobales because they have MVA-P kinase- and MVA-PP decarboxylase-encoding genes (Lombard and Moreira 2011).

IPP isomerases are present in the living organisms all of kingdoms, and considering that the MEP pathway produces both IPP and DMAPP, whereas the MVA pathway yields only IPP, IPP isomerase is essential in organisms that possess only the MVA pathway. Based on their amino acid sequences and coenzyme requirements, IPP isomerases have been classified into two subfamilies, type 1 (IDI1) and type 2 (IDI2), which based on their gene sequence are most likely evolutionally independent. These two types of IPP-isomerases may be found in organisms that have either the MEP and/or MVA pathway (Laupitz et al. 2004). IDI1 has a wide distribution in nature, covering a large variety of organisms among eukaryotes, prokaryotes and archaea, and it was first discovered in crude extracts of *S. cerevisiae* in the late 1950s (Berthelot et al. 2012). This enzyme functions as a monomer in the presence of a divalent metal cation, such as $Mg^{+2}$ or $Mn^{+2}$. In contrast, IDI2 was more recently discovered (Kaneda et al. 2001) and functions as a homotetramer which, in addition requiring to a divalent metal cation for activity, requires FMN and NAD(P)H (Kuzuyama et al. 2010). IDI2 is found mainly in Gram-positive bacteria, proteobacteria, cyanobacteria and a few Achaea, with an intriguing prevalence in thermophilic species (Berthelot et al. 2012).

### *1.3.2 The Synthesis of GGPP*

As mentioned before, the biosynthesis of isoprenoids originates from a basic $C_5$ isoprene unit as its active forms IPP and DMAPP. First, IPP is isomerized to DMAPP by IPP-isomerase and then, isoprenyl pyrophosphate synthases successively add three molecules of IPP to allylic pyrophosphate molecules in a head-to-tail manner, beginning with DMAPP, resulting in geranyl pyrophosphate (GPP, $C_{10}$), farnesyl pyrophosphate (FPP, $C_{15}$) and finally, GGPP [KEGG: hsa_M00367] (Fig. 1.2, Kanehisa and Goto 2000; Kanehisa et al. 2014). These three last molecules are the precursors of most of the isoprenoid compounds. For example, the condensation of two units of FPP yields squalene, which is the precursor of sterols, whereas the union of two molecules of GGPP yields phytoene, the first carotenoid that is synthesized in carotenogenesis. Prenyltransferases are a class of enzymes that transfer allylic prenyl groups to acceptor molecules, including DMAPP, aromatic intermediates of quinones and specific proteins (Brandt et al. 2009). Based on the length of the final product and the stereochemistry of the double bonds that are formed during product elongation, prenyltransferases are classified as *trans*- or (*E*)-prenyltransferases and *cis*- or (*Z*)-prenyltransferases (Vandermoten et al. 2009). Short-chain prenyltransferase enzymes are *trans*-prenyltransferases, and GPP-synthase, FPP-synthase and GGPP-synthase, are included in this group.

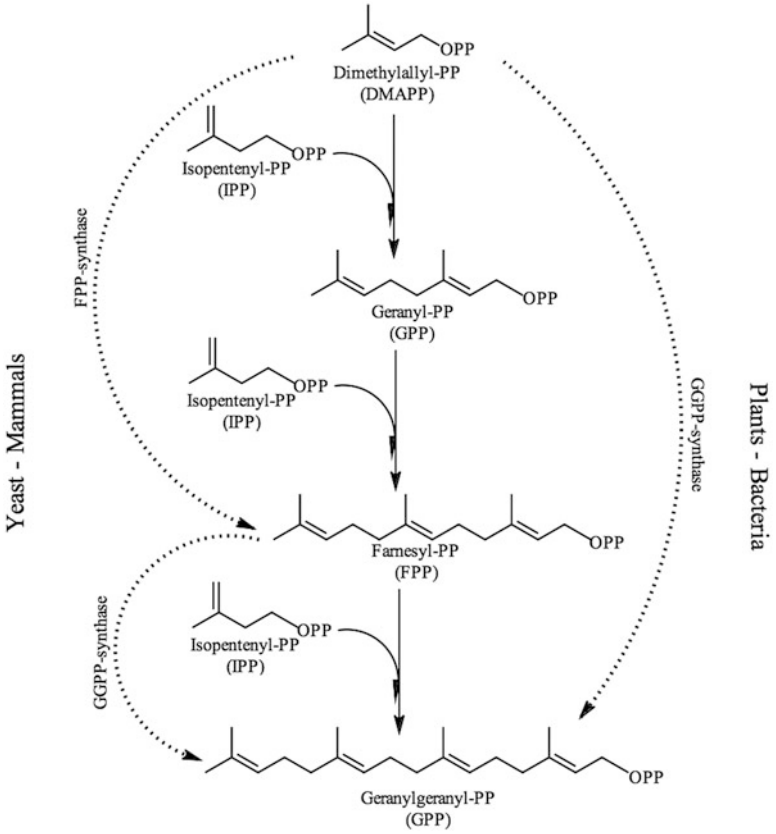


**Fig. 1.2** Biosynthesis of geranylgeranyl pyrophosphate. Geranylgeranyl pyrophosphate can be synthesized from isopentenyl pyrophosphate through two prenyl transferases, FPP-synthase and GGPP-synthase (*left*) or through one prenyl transferase, GGPP-synthase (*right*). Abbreviations: *DMAPP* (dimethylallyl pyrophosphate), *IPP* (isopentenyl pyrophosphate), *GPP* (geranyl pyrophosphate), *FPP* (farnesyl pyrophosphate) and *GGPP* (geranylgeranyl pyrophosphate)

The short-chain prenyltransferases of various organisms share many features, suggesting that they are derived from a common ancestor, and currently, seven conserved regions (I to VII) are recognized. Among these conserved regions, II and VI contain the First and the Second Aspartic acid-Rich Motifs (DDxxD), known as FARM and SARM, respectively. Mutagenesis studies showed that the aspartic residues within the FARM motif are involved in catalysis and the binding of the allylic substrate (Vandermoten et al. 2009). Additionally, a Chain-Length Determination (CLD) region involved in the determination of the product chain length was identified, localized upstream of the FARM motif (Ohnuma et al. 1997). The presence of at least one aromatic residue at the fourth and fifth positions before the FARM motif is conserved among the FPP synthases, whereas smaller residues are found at these positions in longer chain-producing homologs (Gao et al. 2012).

GPP-synthase catalyzes the condensation of DMAPP and IPP to produce GPP, the precursor of mono ($C_{10}$)-terpene biosynthesis (Fig. 1.2). Monoterpenes are the main constituents of the essential oils that are produced by plants and also function as aggregation and dispersion pheromones in insects, but only a few GPP-synthase genes have been described (Vandermoten et al. 2009). In contrast, much more is known about FPP- and GGPP-synthases. FPP-synthase catalyzes the condensation of IPP and DMAPP to produce GPP, which then receives a second molecule of IPP, producing FPP. FPP is the precursor of sesqui ($C_{15}$)- and tri ($C_{30}$)-terpenes, including sterols. GGPP-synthase produces GGPP, the precursor of di ($C_{20}$)-terpenes and of phytoene ($C_{40}$), which in turn is the precursor of most carotenoids. In plants and bacteria, GGPP-synthase sequentially adds IPP to the allylic co-substrates DMAPP, GPP and FPP to finally produce GGPP; whereas in yeast and mammals, GGPP-synthase uses only FPP as a co-substrate to produce GGPP (Vandermoten et al. 2009). Thus, GGPP may be synthesized from IPP and DMAPP via different systems involving one or two enzymes, an FPP-synthase and/or a GGPP-synthase. In the first system, which is common in plants and bacteria, only a GGPP-synthase that is able to produce GGPP from IPP and DMAPP is involved. In the second system, which is common in mammals and yeasts, two enzymes are involved; first FPP-synthase produces FPP from IPP and DMAPP in two condensation steps and then, GGPP-synthase produces GGPP from FPP and IPP (Alcaino et al. 2014). Considering that FPP is essential, it is interesting that even though their growth rate was lower than that of wild-type strains, *E. coli* mutants lacking the FPP-synthase gene were still viable and exhibited a low level of FPP-synthase activity (Saito et al. 2007), suggesting that there is a third system that combines the other two systems to simultaneously yield GGPP.

### *1.3.3 The Synthesis of Carotenoids per se*

A tree-like hierarchical structure has been proposed for the carotenoid biosynthetic pathways, with the trunk being the assembly of the carotenoid backbone from which several variable branches and sub-branches arise, generating the vast diversity of carotenoids that are produced in nature (Umeno et al. 2005). Several downstream modifications to the carotenoid backbone contribute to the broad diversity of carotenoids, including the introduction of additional conjugated double bonds, cyclization of one or both ends of the polyene carotenoid backbone, introduction of oxygen-containing functional groups and further modifications, such as glycosylation and oxidative cleavage.

Carotenoid synthases catalyze complex reactions similar to those of squalene synthase, which is the first enzyme involved in sterol biosynthesis. It is likely that these enzymes have a common evolutionary origin because they share certain conserved domains (Umeno et al. 2005). In general, the synthesis of carotenoids begins with the head-to-head condensation of two molecules of GGPP by the enzyme phytoene synthase, which yields phytoene ($C_{40}$), the first carotenoid to be

synthesized (Fig. 1.3). However, some bacteria produce unusual $C_{30}$ carotenoids that derive from diapophytoene, which is formed by the condensation of two molecules of FPP, instead of GGPP, by diapophytoene synthase (CrtM) (Misawa 2010). In both cases, the catalysis is performed in two steps. In first step, the pyrophosphate group is removed from one prenyl donor, which then undergoes condensation with a prenyl acceptor to form a stable cyclopropyl intermediate. Next, the cyclopropyl intermediate is rearranged, releasing the second pyrophosphate group (Umeno et al. 2005).

Phytoene synthase is encoded by the *crtB* gene in bacteria and by the *PSY* gene in plants, algae and cyanobacteria (Sieiro et al. 2003), but analyses of their sequences suggest that these genes share a common ancestor (Takaichi 2013). A bifunctional enzyme has been described in fungi, which in addition to having phytoene synthase activity, also has lycopene-cyclase activity to produce β-carotene from lycopene. For this reason, the fungal enzyme was named phytoene β-carotene synthase (PBS, encoded by the *crtYB* gene), in which the phytoene synthase activity resides in the C-terminus, whereas the lycopene cyclase activity is restricted to the N-terminus. This peculiarity was first demonstrated by heterologous complementation studies of the "supposed" lycopene cyclase-encoding gene of the carotenogenic basidiomycete yeast *X. dendrorhous* in recombinant *E. coli* strains bearing bacterial carotenogenic gene clusters (Verdoes et al. 1999). Although the deduced amino acid sequence of the *crtYB* product resembled that of bacterial and plant phytoene synthases, the fungal phytoene synthase domain has an approximately 300-residue extension at its C-terminus (Verdoes et al. 1999). Interesting, similar genes encoding this unique enzyme have been reported in other fungi, such as the ascomycete *N. crassa* (Schmidhauser et al. 1994) and the zygomycetes *M. circinelloides* (Velayos et al. 2000) and *P. blakesleeanus* (Arrach et al. 2001). Its wide distribution throughout the fungal kingdom suggests that this bifunctional enzyme was acquired early in the evolution of fungi (Krubasik and Sandmann 2000).

In the next stage of carotenogenesis, phytoene is typically converted into lycopene through four desaturation reactions, sequentially giving phytofluene, ζ-carotene, neurosporene and lycopene (Fig. 1.3). In non-photosynthetic carotenogenic organisms, such as fungi and eubacteria, the formation of the four double bonds in phytoene that leads to lycopene is performed by only one phytoene desaturase enzyme encoded by the *crtI* gene. Nevertheless, the bacterial phytoene desaturase of *Rhodobacter capsulatus* produces neurosporene, which is synthesized from phytoene in three desaturation steps (Sandmann 2009). In addition, in the $C_{30}$-carotenogenic pathway, a diapophytoene desaturase that is encoded by the *crtN* gene, which is homologous to *crtI*, catalyzes a three-step desaturation (Misawa 2010). However, the $C_{40}$ four-step desaturases appear to be most closely related to the three-step $C_{40}$ desaturases than to the $C_{30}$ CrtN desaturases (Sandmann 2009). In contrast, in photosynthetic organisms, such as plants, algae and cyanobacteria, two closely related isomerases are involved in this stage. First, two double bonds are sequentially introduced into phytoene, giving ζ-carotene. This process is performed by a phytoene desaturase, which is encoded by the *PDS* gene in plants and algae and by the *crtP* gene in cyanobacteria (Sieiro et al. 2003). Then, a ζ-carotene desaturase, which is encoded by the *ZDS* gene in plants and algae and by the *crtQ* gene in

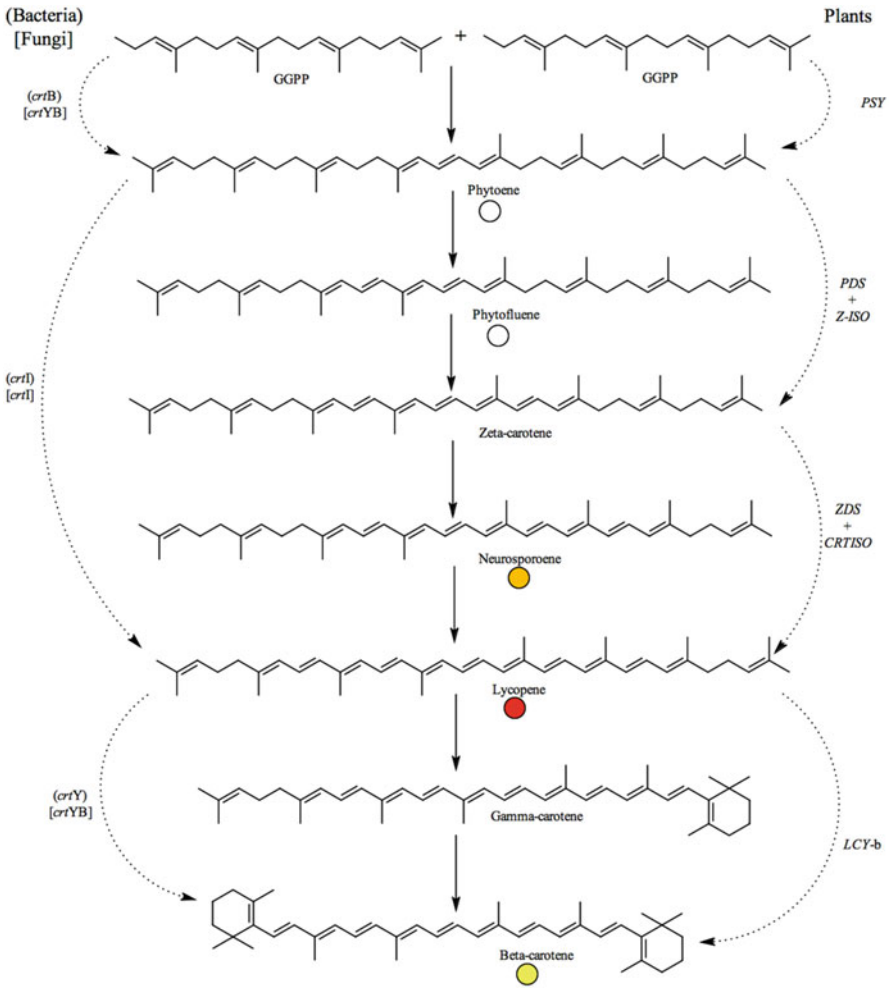


**Fig. 1.3** Biosynthesis of β-carotene (beta-carotene). Schematic representation of the synthesis of β-carotene in bacteria and fungi (on the left) and in plants (on the right). β-carotene is synthesized from two molecules of geranylgeranyl pyrophosphate (GGPP) through phytoene, phytofluene, ζ-carotene (zeta-carotene), neurosporene, lycopene and γ-carotene (gamma-carotene). The relevant bacterial genes (in parentheses) are *crtB* (phytoene synthase), *crtI* (phytoene desaturase) and *crtY* (lycopene cyclase); the relevant fungal genes (in square brackets) are *crtYB* (phytoene β-carotene synthase) and *crtI* (phytoene desaturase) and the relevant plant genes are *PSY* (phytoene synthase), *PDS* (phytoene desaturase), *Z-ISO* (ζ-carotene isomerase), *ZDS* (ζ-carotene desaturase), *CRTISO* (carotene isomerase) and *LCY*-b (lycopene β-cyclase). The metabolic pathways were adapted from [KEGG: M00097] (Kanehisa and Goto 2000; Kanehisa et al. 2014) and the carotenoid structures were based on those of (Britton et al. 2004)

cyanobacteria, introduces two additional double bonds to ζ-carotene, producing lycopene (Sieiro et al. 2003). Interesting, *Gloeobacter violaceus*, a primitive cyanobacterium, has a CrtI-type phytoene desaturase instead of a plant-related

one (Steiger et al. 2005). This situation suggests that in photosynthetic organisms, the desaturation steps at this stage of carotenogenesis evolved from a simpler one-enzyme process into a more complex process (Sandmann 2009). Moreover, most carotenoids exist in an all-*trans* configuration, but some are produced in *cis* configuration. In plants, two isomerases (a ζ-carotene isomerase or Z-ISO (Chen et al. 2010) and a carotene isomerase or CrtISO (Isaacson et al. 2004)), participate in producing the final all-*trans* form of lycopene. These isomerases are phylogenetically related to the CrtI-type phytoene desaturase, which appears to also have the property of catalyzing the *trans* conversion of carotenes (Sandmann 2009).

The cyclization of lycopene by lycopene cyclases is a frequent step in the biosynthesis of carotenoids, which can occur at one or both of its ends, generating mono- and bi-cyclic carotenoids, respectively. There are two major types of rings, the β-ionone and the α-ionone rings (β- and ε-rings, respectively) (Britton 1998). The β-ring is the most common form, and it is found in the carotenoids of the majority of photosynthetic organisms, including cyanobacteria, algae, and higher plants, as well as in non-photosynthetic bacteria, yeasts, and fungi. In contrast, the distribution of the ε-ring form is more restricted, and it is found in plants and cyanobacteria. Carotenoids generally have two β-rings, a ε-ring and a β-ring, or have only one β-ring. The introduction of a β-ring at both ends of lycopene gives β-carotene, through γ-carotene, which is one of the best-known carotenoids (Fig. 1.3). The introduction of a ε-ring followed by the formation of a β-ring in the other end of the carotene gives α-carotene through δ-carotene in higher plants (Misawa 2010).

The first lycopene β-cyclase-encoding gene, which was isolated from *Pantoea ananas* (formerly *Erwinia uredovora*) and was named *crtY* (Misawa 2010) is distributed among the eubacteria. In plants and cyanobacteria, the *crtL*-b (or *LCY*-b) gene encodes lycopene β-cyclase, whereas lycopene ε-cyclase is encoded by the *crtL*-e (or *LCY*-e) gene. Additionally, lycopene β-monocyclase-encoding genes (*crtYm*, *crtLm*) have been described in eubacteria. These cyclases, which have five conserved regions and contain an NAD(P)/FAD-binding motif, have been further divided in two families, the CrtY family and the CrtL family (Takaichi 2013). Other unrelated classes of lycopene cyclases have been described. For example, the actinomycete bacterium *Brevibacterium linens* has a heterodimeric lycopene cyclase that is responsible for the conversion of lycopene to β-carotene, which is formed by two polypeptides encoded by the *crtYc* and *crtYd* genes (Krubasik and Sandmann 2000). As mentioned above, a bifunctional phytoene synthase and lycopene cyclase (*crtYB* gene) has been identified in fungi, and the fungal lycopene cyclase domain appears to be related to the *crtYc* and *crtYd* gene products of *B. linens*. Thus, the bifunctional enzyme in fungi most likely developed through the recombination of these two genes and a phytoene synthase gene (Krubasik and Sandmann 2000). In addition, a third class of lycopene cyclase (*cruA* gene) was recently identified in the green sulfur bacterium *Chlorobium tepidum* through heterologous complementation in a lycopene-producing *E. coli* strain (Maresca et al. 2007). Two homologous genes (denoted *cruA* and *cruP*), the products of which showed lycopene cyclase activity, were found in the cyanobacterium *Synechococcus* sp. PCC 7002 and in other cyanobacterial genomes, demonstrating their wide

distribution among cyanobacteria (Maresca et al. 2007). However, even though the *Anabaena* sp. strain PCC 7120 and the *Synechocystis* sp. strain PCC 6803 contain *cruA* orthologous genes in their genomes, no lycopene cyclase activity related to these genes has been detected (Mochimaru et al. 2008). Considering that these organisms produce β-carotene and that in addition, no lycopene cyclase-encoding genes related to *crtY* or to *crtL* have been found in their available and complete sequenced genomes, the existence of another additional type of lycopene β-cyclase is suspected (Takaichi 2013).

The post-phytoene acyclic, monocyclic and dicyclic carotenes may be subjected to a series of downstream enzymatic modifications. In this regard, the wide diversity of xanthophylls results from the incorporation of oxygen-containing functional groups, such as hydroxy-, epoxy- and keto- groups, into carotenes, mainly α- and β-carotene. As mentioned before, one of the most studied xanthophylls is astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione), largely due to its numerous commercial applications. Currently, commercial astaxanthin is mainly produced via chemical synthesis and only a few organisms produce this xanthophyll naturally. However, astaxanthin is of special interest because its biosynthesis does not proceed in the same way in all of the astaxanthin-producing organisms; thus, these pathways represent good examples of the evolution of carotenogenesis.

### *1.3.4 The Synthesis of Astaxanthin*

To date, few organisms that naturally synthesize astaxanthin are known, which include the microalga *H. pluvialis* (Lemoine and Schoefs 2010), some marine bacteria, such as *Paracoccus haeundaensis* (Lee and Kim 2006) and *Brevudimonas* sp. (Yokoyama and Miki 1995), the basidiomycete yeast *X. dendrorhous* (Andrewes and Starr 1976) and the plant *Adonis aestivalis*, in which this pigment accumulates in the petals of the flowers (Renstrøm et al. 1981). However, the mechanisms involved in the biosynthesis of astaxanthin in these organisms are different. In general, astaxanthin is produced by the introduction of a hydroxyl- and a keto-group to the carbons at positions 3 and 4, respectively, in each β-ionone ring of β-carotene via eight possible intermediate xanthophylls (Fig. 1.4, echinenone,

**Fig. 1.4** (continued) Biosynthesis of astaxanthin. Schematic representation of the synthesis of astaxanthin from β-carotene, showing the genes that control each step, in the yeast *X. dendrorhous* (in square brackets, obtained from (Ojima et al. 2006)), the microalgae *H. pluvialis* (in parentheses, obtained from (Huang et al. 2012)), bacteria (no special indicators, obtained from (Tao et al. 2006)) and in the plant *A. aestivalis* (enclosed in an oval on the right, obtained from (Cunningham and Gantt 2011)). The astaxanthin biosynthetic pathway was summarized from [KEGG: map00906] (Kanehisa and Goto 2000; Kanehisa et al. 2014) and the carotenoid structures were based on those of (Britton et al. 2004). The corresponding gene products are astaxanthin synthase (*crtS*), bacterial β-carotene ketolase (*crtW*), bacterial β-carotene hydroxylase (*crtZ*), β-carotene ketolase (*bkt*), carotenoid hydroxylase (*chy*), carotenoid β-ring 4-dehydrogenase (*cbfd*) and hydroxy-β-ring 4-dehydrogenase (*hbfd*)

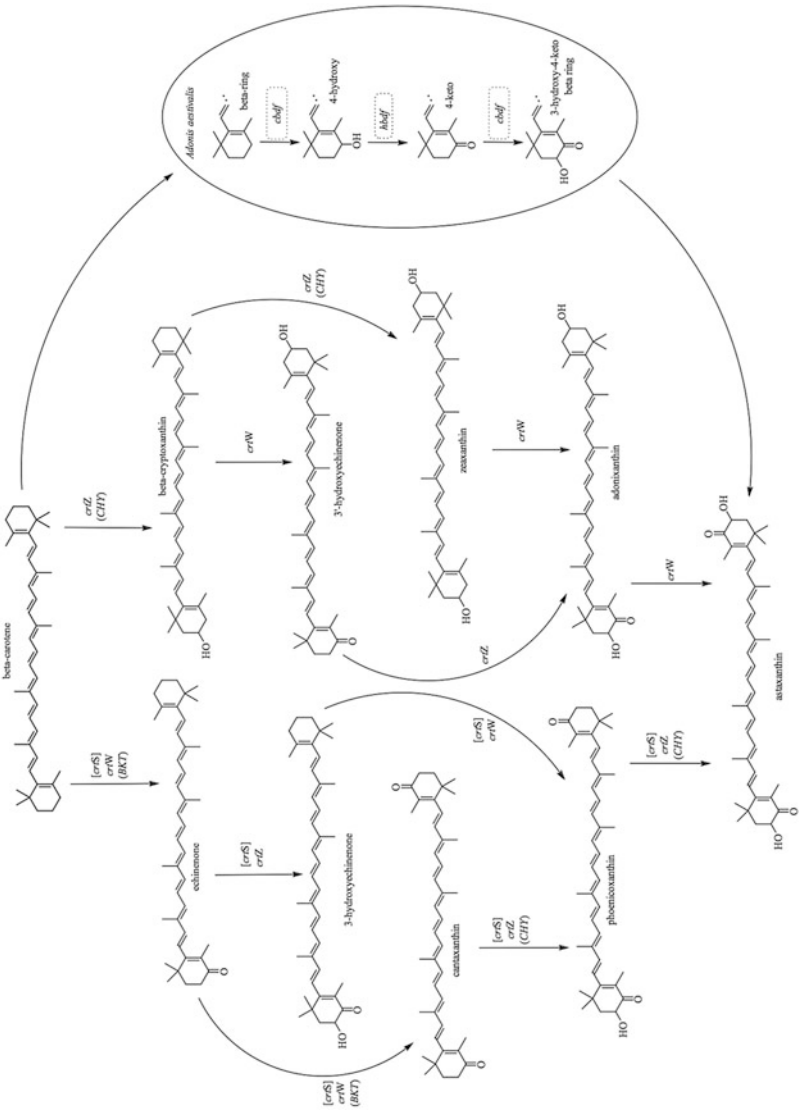


**Fig. 1.4** (continued)

3-hydroxyechinenone or 3′-hydroxyechinenone, β-cryptoxanthin, canthaxanthin, zeaxanthin, phoenicoxanthin and adonixanthin), which vary depending on the producing organism.

In most astaxanthin-producing organisms, the oxidation of β-carotene is performed by ketolases and hydroxylases (Fig. 1.4). There are several organisms that produce keto-xanthophylls other than astaxanthin but, in general, plants do not produce ß-carotene-derived ketocarotenoids (Zhu et al. 2007). However, *Adonis* plants are able to produce the ketocarotenoid astaxanthin from β-carotene through three steps that are catalyzed by two enzymes, carotenoid-β-ring-4-dehydrogenase (CBFD) and 4-hydroxy-β-ring-4-dehydrogenase (HBFD). A first hydroxyl group is introduced to the carbon at position 4 of the β-ring by CBFD, which then is further dehydrogenated by HBFD to form a keto group at this position. Next, CBFD introduces another hydroxyl group to the carbon at position 3 to yield a 3-hydroxy-4-keto-β-ring (Cunningham and Gantt 2011).

A different mechanism has been proposed in *H. pluvialis*, which produces astaxanthin only under stressful conditions (Vidhyavathi et al. 2008). During its green vegetative phase, *H. pluvialis* produces zeaxanthin by the incorporation of a hydroxyl group at position 3 of both β-ionone rings via a hydroxylase enzyme (CHY). However, under stressful conditions, a keto group is introduced at position 4 of both β-ionone rings by a β-carotene ketolase (BKT), which is encoded by two paralogous genes, *bkt1* and *bkt2*. Nevertheless, it is unlikely that the β-carotene in *H. pluvialis* is converted into astaxanthin through an initial hydroxylation step because it has been shown that BKT can use only a non-substituted β-ionone ring as a substrate (Lemoine and Schoefs 2010). Thus, in *H. pluvialis*, astaxanthin should be produced from β-carotene primarily via echinenone, canthaxanthin and phoenicoxanthin, with β-carotene and echinenone being the substrates of BKT and canthaxanthin and phoenicoxanthin being substrates of CHY. In addition, the hydroxyl groups of astaxanthin are further esterified in *H. pluvialis*, leading to the production of mono- and di-esterified astaxanthins (Lemoine and Schoefs 2010). In contrast, bacterial ketolases (encoded by the *crtW* gene) can convert a non-substituted β-ionone ring as well as 3-hydroxylated β-ionone rings into their respective 4-keto forms. In the same manner, bacterial β-carotene hydroxylases (encoded by the *crtZ* gene) can use non-substituted and 4-ketolated β-ionone rings as substrates to produce their respective 3-hydroxylated forms (Misawa et al. 1995).

There are two main types of β-carotene hydroxylases, the non-heme di-iron (NH-di-iron) hydroxylases and the cytochrome P450 monooxygenases (P450s) (Martin et al. 2008). The first types are related to fatty-acid desaturases and require molecular oxygen, iron, ferredoxin, and ferredoxin oxido-reductase for their functions (Tian and DellaPenna 2004). In contrast, the P450s comprise a large superfamily of heme-containing monooxygenases that are distributed in organisms from all domains of life (Estabrook 2003; McLean et al. 2005). Cytochrome P450s are involved in the oxidative metabolism of a wide range of exogenous and endogenous substrates (Degtyarenko and Archakov 1993) and act as a terminal electron acceptor in multicomponent P450-dependent monooxygenation systems, which lead to the reductive activation of molecular oxygen followed by the insertion

of one oxygen atom into the substrate molecule and the reduction of the other to water (van den Brink et al. 1998). Two electrons are required for cytochrome P450 catalysis, which are transferred primarily from NADPH via a redox partner (van den Brink et al. 1998). Recently, the involvement of cytochrome P450s in carotene hydroxylation has been demonstrated (Inoue 2004), which was first described in the thermophilic bacterium *Thermus thermophiles* (CYP175A1) (Blasco et al. 2004) and in *Arabidopsis thaliana* (CYP97C1) (Tian et al. 2004). It was shown that CYP175A1 could introduce hydroxyl groups into both β-rings of β-carotene to produce zeaxanthin in a recombinant *E. coli* strain that carried carotenoid biosynthetic genes (Blasco et al. 2004). In contrast, in *A. thaliana*, CYP97C1 was shown to be involved in the synthesis of lutein, which is an α-xanthophyll. The synthesis of lutein from α-carotene (β, *ε*-carotene) requires the hydroxylation of one β- and one *ε*-ring, and it was demonstrated that CYP97C1 could achieve *ε*-ring hydroxylation as well as also contribute to β-carotene hydroxylase activity *in vivo* (Tian et al. 2004). However, it was later demonstrated that another P450 (CYP97A3) was also involved in the synthesis of lutein in *Arabidopsis* and in this case, the enzyme displayed β-carotene-hydroxylase activity (Kim and DellaPenna 2006). Thus, the synthesis of lutein from α-carotene in *Arabidopsis* involves two P450s that act sequentially, whereas in the synthesis of β-xanthophylls from β-carotene that requires two β-ring-hydroxylation reactions, two non-heme di-iron-type β-carotene hydroxylases are involved (Kim et al. 2009).

In *X. dendrorhous*, a single gene (*crtS*) controls the synthesis of astaxanthin from β-carotene. For this reason, the gene product was named astaxanthin synthase. The astaxanthin-synthase enzyme catalyzes the hydroxylation and ketolation of β-carotene to produce astaxanthin and interesting, this enzyme also belongs to the cytochrome P450 protein family (Alvarez et al. 2006; Ojima et al. 2006). Although two different functional groups are incorporated through the oxygenation of β-carotene to form astaxanthin, these are products of hydroxylations performed by astaxanthin synthase. The proposed synthetic mechanism (Ojima et al. 2006) involves a series of hydroxylations of allylic carbons, starting at the C4 of the β-ionone ring of β-carotene, which is hydroxylated twice, followed by the spontaneous elimination of a water molecule to form the keto group at this position. Next, the C3 in the C4-keto intermediate β-ring is hydroxylated. In this manner, the synthesis of astaxanthin proceeds from β-carotene through echinenone to phoenicoxanthin via canthaxanthin or hydroxy-echinenone, to yield astaxanthin.

To date, a P450 system involved in the synthesis of astaxanthin from β-carotene has been reported only in *X. dendrorhous*, suggesting that a unique P450 system evolved independently in this yeast and specialized in the synthesis of astaxanthin through a mechanism different from that employed in other astaxanthin-producing organisms. Furthermore, in the eukaryotic microsomal class II P450 systems, the P450 redox partner is generally the flavoprotein cytochrome P450 reductase (CPR) (McLean et al. 2005; van den Brink et al. 1998), and a *X. dendrorhous* mutant strain lacking the wild-type CPR-encoding gene accumulates β-carotene because it is unable to synthesize astaxanthin (Alcaino et al. 2008). In addition, in a recombinant *S. cerevisiae* strain that carried the *X. dendrorhous* carotenogenic genes, astaxanthin

production, although in a very small proportion, was achieved only in the presence of the *X. dendrorhous* CPR enzyme even though *S. cerevisiae* has an endogenous CPR and could heterologously express several functional cytochrome P450s (Ukibe et al. 2009). Furthermore, based on protein modeling and molecular dynamic simulations, the larger interfacial area of interaction and the higher number of hydrogen bonds and saline bridges formed at the interaction surface indicated that astaxanthin synthase preferentially interacts with the cognate CPR rather than with the CPR of *S. cerevisiae* (Alcaino et al. 2012). These observations suggested that astaxanthin synthase is a unique P450 enzyme that has a high specificity for its own CPR.

## 1.4 Concluding Remarks

Carotenoids are a large group of natural compounds that are broadly distributed in nature and that are responsible for the color of many animals, plants and microorganisms. These metabolites play essential and important biological roles as accessory light-harvesting pigments of photosynthetic systems, signals for pollination and seed dispersal, photoprotective antioxidants, regulators of membrane fluidity and contributors to ocular health and to the health of other animal and human bodily systems, among others. Due to their numerous properties and applications, carotenoids have also received particular attention from commercial enterprises. In this regard, the global market for commercially applied carotenoids was estimated to be approximately US$1.2 billion in 2010 and it is expected to grow to US$1.4 billion in 2018 through a compound annual growth rate of 2.3 % (BCC-Research 2011).

Carotenoids are found in many fungi and bacteria; however, not all of these microorganisms produce these pigments and no carotenogenic genes are found in their genomes. Interestingly, carotenoids are not essential for all of the carotenoid-producing organisms. Whereas carotenoids are essential for photosynthetic organisms, several mutant microorganisms unable to produce these pigments do not exhibit any special phenotypic modifications under laboratory-controlled conditions, except the evident deficiency of pigmentation. However, albino mutants obtained from several carotenoid-producing species have been shown to be less tolerant to oxidative or light stress. These findings suggest that even though carotenoids are not essential for the survival of some species, without doubt, the ability to produce these pigments provide a protective advantage to microorganisms.

**Acknowledgements** This study was supported by Fondecyt grant 11121200 and INACH grant RG_07-12 to JA, Fondecyt grant 1130333 to MB and Fondecyt grant 1140504 to VC.

## References


Abbes M, Baati H, Guermazi S et al (2013) Biological properties of carotenoids extracted from *Halobacterium halobium* isolated from a Tunisian solar saltern. BMC Complement Altern Med 13:255

Abdel-Aal E-SM, Akhtar H, Zaheer K et al (2013) Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. Nutrients 5:1169–1185

Ahmed F, Fanning K, Netzel M et al (2014) Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish waters. Food Chem 165:300–306

Ajikumar PK, Tyo K, Carlsen S et al (2008) Terpenoids: opportunities for biosynthesis of natural product drugs using engineered microorganisms. Mol Pharm 5:167–190

Alcaino J, Barahona S, Carmona M et al (2008) Cloning of the cytochrome p450 reductase (*crtR*) gene and its involvement in the astaxanthin biosynthesis of *Xanthophyllomyces dendrorhous*. BMC Microbiol 8:169

Alcaino J, Fuentealba M, Cabrera R et al (2012) Modeling the interfacial interactions between CrtS and CrtR from *Xanthophyllomyces dendrorhous*, a P450 system involved in astaxanthin production. J Agric Food Chem 60:8640–8647

Alcaino J, Romero I, Niklitschek M et al (2014) Functional characterization of the *Xanthophyllomyces dendrorhous* farnesyl pyrophosphate synthase and geranylgeranyl pyrophosphate synthase encoding genes that are involved in the synthesis of isoprenoid precursors. PLoS One 9:e96626

Alvarez V, Rodriguez-Saiz M, de la Fuente JL et al (2006) The *crtS* gene of *Xanthophyllomyces dendrorhous* encodes a novel cytochrome-P450 hydroxylase involved in the conversion of beta-carotene into astaxanthin and other xanthophylls. Fungal Genet Biol 43:261–272

Amaretti A, Simone M, Quarteri A et al (2014) Isolation of carotenoid-producing yeasts from an Alpine glacier. Chem Eng Trans 38:217–222

Andrewes AG, Starr MP (1976) (3R, 3′R)-astaxanthin from the yeast *Phaffia rhodozyma*. Phytochemistry 15:1009–1011

Arrach N, Fernandez-Martin R, Cerda-Olmedo E et al (2001) A single gene for lycopene cyclase, phytoene synthase, and regulation of carotene biosynthesis in *Phycomyces*. Proc Natl Acad Sci U S A 98:1687–1692

Asker D, Beppu T, Ueda K (2007a) *Zeaxanthinibacter enoshimensis* gen. nov., sp. nov., a novel zeaxanthin-producing marine bacterium of the family *Flavobacteriaceae*, isolated from seawater off Enoshima Island, Japan. Int J Syst Evol Microbiol 57:837–843

Asker D, Beppu T, Ueda K (2007b) *Mesoflavibacter zeaxanthinifaciens* gen. nov., sp. nov., a novel zeaxanthin-producing marine bacterium of the family Flavobacteriaceae. Syst Appl Microbiol 30:291–296

Avalos J, Díaz-Sánchez V, García-Martínez J et al (2014) Chapter 8: carotenoids. In: Martín JF, García-Estrada C, Zeilinger S (eds) Biosynthesis and molecular genetics of fungal secondary metabolites. Springer, New York, pp 149–185

Avalos J, Limón MC (2014) Biological roles of fungal carotenoids. Curr Genet. doi:10.1007/s00294. doi:10.1007/s00014

Barkley SJ, Cornish RM, Poulter CD (2004) Identification of an Archaeal Type II Isopentenyl Diphosphate Isomerase in *Methanothermobacter thermautotrophicus*. J Bacteriol 186:1811–1817

BCC-Research (2011) The global market for carotenoids. Report code: FOD025D. http://www.bccresearch.com/pressroom/fod/global-carotenoids-market-reach-$1.4-billion-2018. Accessed 19 May 2016

Berthelot K, Estevez Y, Deffieux A et al (2012) Isopentenyl diphosphate isomerase: a checkpoint to isoprenoid biosynthesis. Biochimie 94:1621–1634

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

Bhosale P, Bernstein PS (2005) Microbial xanthophylls. Appl Microbiol Biotechnol 68:445–455

Blasco F, Kauffmann I, Schmid RD (2004) CYP175A1 from *Thermus thermophilus* HB27, the first beta-carotene hydroxylase of the P450 superfamily. Appl Microbiol Biotechnol 64:671–674

Bona-Lovasz J, Bona A, Ederer M et al (2013) A rapid method for the extraction and analysis of carotenoids and other hydrophobic substances suitable for systems biology studies with photosynthetic bacteria. Metabolites 3:912–930

Bories G, Brantom P, Brufau de Barberà J et al (2007) Safety and efficacy of Panaferd-AX (red carotenoid-rich bacterium *Paracoccus carotinifaciens*) as feed additive for salmon and trout. Eur Food Saf Authority J 546:1–30

Brandt W, Brauer L, Gunnewich N et al (2009) Molecular and structural basis of metabolic diversity mediated by prenyldiphosphate converting enzymes. Phytochemistry 70:1758–1775

Britton G (1995) Structure and properties of carotenoids in relation to function. FASEB J 9:1551–1558

Britton G (1998) Chapter 2: overview of carotenoid biosynthesis. In: Britton G, Liaaen-Jensen S, Pfander H (eds) Carotenoids. Volume 3: biosynthesis and metabolism. Birkhäuser Verlag, Basel, pp 13–148

Britton G (2008a) Chapter 15: functions of carotenoids metabolites and breakdown products. In: Britton G, Liaaen-Jensen S, Pfander H (eds) Carotenoids. Volume 4: natural functions. Birkhäuser Verlag, Basel, pp 309–324

Britton G (2008b) Chapter 10: functions of intact carotenoids. In: Britton G, Pfander H, Liaaen-Jensen S (eds) Carotenoids. Volume 4: natural functions. Birkhäuser Verlag, Basel, pp 189–212

Britton G, Liaaen-Jensen S, Pfander H (2004) Carotenoids: handbook. Springer, Berlin

Brown SR (1968) Bacterial carotenoids from freshwater sediments. Limnol Oceanogr 13:233–241

Campenni' L, Nobre BP, Santos CA et al (2013) Carotenoid and lipid production by the autotrophic microalga *Chlorella protothecoides* under nutritional, salinity, and luminosity stress conditions. Appl Microbiol Biotechnol 97:1383–1393

Chen Y, Li F, Wurtzel ET (2010) Isolation and characterization of the Z-ISO gene encoding a missing component of carotenoid biosynthesis in plants. Plant Physiol 153:66–79

Cunningham FXJ, Gantt E (2011) Elucidation of the pathway to astaxanthin in the flowers of *Adonis aestivalis*. Plant Cell 23:3055–3069

D'Souza SE, Altekar W, D'Souza SF (1997) Adaptive response of *Haloferax mediterranei* to low concentrations of NaCl (<20 %) in the growth medium. Arch Microbiol 168:68–71

Degtyarenko KN, Archakov AI (1993) Molecular evolution of P450 superfamily and P450-containing monooxygenase systems. FEBS Lett 332:1–8

Dieser M, Greenwood M, Foreman CM (2010) Carotenoid pigmentation in Antarctic Heterotrophic Bacteria as a Strategy to Withstand Environmental Stresses. Arct Antarct Alp Res 42:396–405

Dobrowolska A (2006) Molecular view on the carotenogenesis in plants-a mini review. Pol J Food Nutr Sci 15:385

Dong JZ, Wang SH, Ai XR et al (2013) Composition and characterization of cordyxanthins from *Cordyceps militaris* fruit bodies. J Funct Food 5:1450–1455

Echavarri-Erasun C, Johnson EA (2002) Fungal carotenoids. In: (eds) 2. pp 45–85

Estabrook RW (2003) A passion for P450s (remembrances of the early history of research on cytochrome P450). Drug Metab Dispos 31:1461–1473

Estrada AF, Youssar L, Scherzinger D et al (2008) The *ylo*-1 gene encodes an aldehyde dehydrogenase responsible for the last reaction in the *Neurospora* carotenoid pathway. Mol Microbiol 69:1207–1220

Fong NJC, Burgess ML, Barrow KD et al (2001) Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilis* in response to thermal and salt stress. Appl Microbiol Biotechnol 56:750–756

Fraser PD, Bramley PM (2004) The biosynthesis and nutritional uses of carotenoids. Prog Lipid Res 43:228–265

Frengova GI, Beshkova DM (2009) Carotenoids from *Rhodotorula* and *Phaffia*: yeasts of biotechnological importance. J Ind Microbiol Biotechnol 36:163–180

Gao Y, Honzatko RB, Peters RJ (2012) Terpenoid synthase structures: a so far incomplete view of complex catalysis. Nat Prod Rep 29:1153–1175

Goodwin TW (1972) Carotenoids in fungi and non-photosynthetic bacteria. Prog Ind Microbiol 11:29–88

Goodwin TW (1984) The biochemistry of carotenoids, vol. II animals. Chapman Hall, New York

Gotz D, Paytubi S, Munro S et al (2007) Responses of hyperthermophilic crenarchaea to UV irradiation. Genome Biol 8:R220

Grochowski LL, Xu H, White RH (2006) *Methanocaldococcus jannaschii* uses a modified mevalonate pathway for biosynthesis of isopentenyl diphosphate. J Bacteriol 188:3192–3198

Gruszecki WI, Strzalka K (2005) Carotenoids as modulators of lipid membrane physical properties. Biochim Biophys Acta 1740:108–115

Guerin M, Huntley ME, Olaizola M (2003) *Haematococcus* astaxanthin: applications for human health and nutrition. Trends Biotechnol 21:210–216

Hameed A, Arun AB, Ho HP et al (2011) Supercritical carbon dioxide micronization of zeaxanthin from moderately thermophilic bacteria *Muricauda lutaonensis* CC-HSB-11 T. J Agric Food Chem 59:4119–4124

Haxo F (1950) Carotenoids of the mushroom *Cantharellus cinnabarinus*. Bot Gaz 112:228–232

Higuera-Ciapara I, Felix-Valenzuela L, Goycoolea FM (2006) Astaxanthin: a review of its chemistry and applications. Crit Rev Food Sci Nutr 46:185–196

Hu Y, Xu X, Song P et al (2013) Draft Genome Sequence of *Deinococcus xibeiensis* R13, a New Carotenoid-Producing Strain. Genome Announc 1:e00987-13

Huang J, Zhong Y, Sandmann G et al (2012) Cloning and selection of carotenoid ketolase genes for the engineering of high-yield astaxanthin in plants. Planta 236:691–699

Inoue K (2004) Carotenoid hydroxylation-P450 finally! Trends Plant Sci 9:515–517

Isaacson T, Ohad I, Beyer P et al (2004) Analysis in vitro of the enzyme CRTISO establishes a poly-cis-carotenoid biosynthesis pathway in plants. Plant Physiol 136:4246–4255

Jensen SL, Cohen-Bazire G, Nakayama TOM et al (1958) The path of carotenoid synthesis in a photosynthetic bacterium. Biochim Biophys Acta 29:477–498

Kaneda K, Kuzuyama T, Takagi M et al (2001) An unusual isopentenyl diphosphate isomerase found in the mevalonate pathway gene cluster from *Streptomyces* sp. strain CL190. Proc Natl Acad Sci U S A 98:932–937

Kanehisa M, Goto S, Sato Y et al (2014) Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res 42:D199–D205

Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30

Kim J, DellaPenna D (2006) Defining the primary route for lutein synthesis in plants: the role of *Arabidopsis* carotenoid beta-ring hydroxylase CYP97A3. Proc Natl Acad Sci U S A 103:3474–3479

Kim J, Smith JJ, Tian L et al (2009) The evolution and function of carotenoid hydroxylases in *Arabidopsis*. Plant Cell Physiol 50:463–479

Krubasik P, Sandmann G (2000) Molecular evolution of lycopene cyclases involved in the formation of carotenoids with ionone end groups. Biochem Soc Trans 28:806–809

Kuzuyama T, Hemmi H, Takahashi S (2010) Chapter 12: mevalonate pathway in bacteria and Archaea. In: Editors-In-Chief: Mander LL, H-W., Volume 1: edited by Townsend CE, Y (eds) Comprehensive natural products II: chemistry and biology. Volume 1: natural products structural diversity-I secondary metabolites: organization and biosynthesis. Elsevier Science, Oxford, pp 493–514

Lamers PP, Janssen M, De Vos RC et al (2012) Carotenoid and fatty acid metabolism in nitrogen-starved *Dunaliella salina*, a unicellular green microalga. J Biotechnol 162:21–27

Lamers PP, van de Laak CC, Kaasenbrood PS et al (2010) Carotenoid and fatty acid metabolism in light-stressed *Dunaliella salina*. Biotechnol Bioeng 106:638–648

Lange BM, Rujan T, Martin W et al (2000) Isoprenoid biosynthesis: the evolution of two ancient and distinct pathways across genomes. Proc Natl Acad Sci U S A 97:13172–13177

Laupitz R, Hecht S, Amslinger S et al (2004) Biochemical characterization of *Bacillus subtilis* type II isopentenyl diphosphate isomerase, and phylogenetic distribution of isoprenoid biosynthesis pathways. Eur J Biochem 271:2658–2669

Lee JH, Kim YS, Choi TJ et al (2004) *Paracoccus haeundaensis* sp. nov., a Gram-negative, halophilic, astaxanthin-producing bacterium. Int J Syst Evol Microbiol 54:1699–1702

Lee JH, Kim YT (2006) Cloning and characterization of the astaxanthin biosynthesis gene cluster from the marine bacterium *Paracoccus haeundaensis*. Gene 370:86–95

Lemee L, Peuchant E, Clerc M (1997) Deinoxanthin: A New Carotenoid Isolated from *Deinococcus radiodurans*. Tetrahedron 53:919–926

Lemoine Y, Schoefs B (2010) Secondary ketocarotenoid astaxanthin biosynthesis in algae: a multifunctional response to stress. Photosynth Res 106:155–177

Li Z, Sun M, Li Q et al (2012) Profiling of carotenoids in six microalgae (Eustigmatophyceae) and assessment of their beta-carotene productions in bubble column photobioreactor. Biotechnol Lett 34:2049–2053

Lichtenthaler HK (2000) Non-mevalonate isoprenoid biosynthesis: enzymes, genes and inhibitors. Biochem Soc Trans 28:785–789

Lombard J, Moreira D (2011) Origins and early evolution of the mevalonate pathway of isoprenoid biosynthesis in the three domains of life. Mol Biol Evol 28:87–99

Lu S, Li L (2008) Carotenoid metabolism: biosynthesis, regulation, and beyond. J Integr Plant Biol 50:778–785

Mandelli F, Miranda VS, Rodrigues E et al (2012) Identification of carotenoids with high antioxidant capacity produced by extremophile microorganisms. World J Microbiol Biotechnol 28:1781–1790

Maresca JA, Graham JE, Wu M et al (2007) Identification of a fourth family of lycopene cyclases in photosynthetic bacteria. Proc Natl Acad Sci U S A 104:11784–11789

Marshall CP, Leuko S, Coyle CM et al (2007) Carotenoid analysis of halophilic archaea by resonance Raman spectroscopy. Astrobiology 7:631–643

Martin JF, Gudina E, Barredo JL (2008) Conversion of beta-carotene into astaxanthin: Two separate enzymes or a bifunctional hydroxylase-ketolase protein? Microb Cell Fact 7:3

Mata-Gomez LC, Montanez JC, Mendez-Zavala A et al (2014) Biotechnological production of carotenoids by yeasts: an overview. Microb Cell Fact 13:12

McLean KJ, Sabri M, Marshall KR et al (2005) Biodiversity of cytochrome P450 redox systems. Biochem Soc Trans 33:796–801

Miki W (1991) Biological functions and activities of animal carotenoids. Pure Appl Chem 63:141–146

Miller MW, Yoneyam M, Soneda M (1976) *Phaffia*, a new yeast genus in the Deuteromycotina (Blastomycetes). Int J Syst Evol Microbiol 26:286–291

Misawa N, Satomi Y, Kondo K et al (1995) Structure and functional analysis of a marine bacterial carotenoid biosynthesis gene cluster and astaxanthin biosynthetic pathway proposed at the gene level. J Bacteriol 177:6575–6584

Misawa N (2010) Chapter 20: carotenoids. In: Editors-In-Chief: Mander LL, H-W, Volume 1: edited by Townsend CE, Y (eds) Comprehensive natural products II: chemistry and biology. Volume 1: natural products structural diversity-I secondary metabolites: organization and biosynthesis. Elsevier Science, Oxford, pp 733–752

Miziorko HM (2011) Enzymes of the mevalonate pathway of isoprenoid biosynthesis. Arch Biochem Biophys 505:131–143

Mochimaru M, Masukawa H, Maoka T et al (2008) Substrate specificities and availability of fucosyltransferase and beta-carotene hydroxylase for myxol 2'-fucoside synthesis in *Anabaena* sp. strain PCC 7120 compared with *Synechocystis* sp. strain PCC 6803. J Bacteriol 190:6726–6733

Moran NA, Jarvik T (2010) Lateral transfer of genes from fungi underlies carotenoid production in aphids. Science 328:624–627

Nakase T, Takematsu A, Yamada Y (1993) Molecular approaches to the taxonomy of ballistosporous yeasts based on the analysis of the partial nucleotide sequences of 18S ribosomal ribonucleic acids. J Gen Appl Microbiol 39:107–134

Naziri D, Hamidi M, Hassanzadeh S et al (2014) Analysis of Carotenoid Production by *Halorubrum* sp. TBZ126; an Extremely Halophilic Archeon from Urmia Lake. Adv Pharm Bull 4:61–67

Nelson MA, Morelli G, Carattoli A et al (1989) Molecular cloning of a *Neurospora crassa* carotenoid biosynthetic gene (albino-3) regulated by blue light and the products of the white collar genes. Mol Cell Biol 9:1271–1276

Ohnuma S, Hirooka K, Ohto C et al (1997) Conversion from Archaeal Geranylgeranyl Diphosphate Synthase to Farnesyl Diphosphate Synthase: Two Amino Acids Before the First Apartate-Rich Motif Solely Determine Eukaryotic Farnesyl Diphosphate Synthase Activity. J Biol Chem 272:5192–5198

Ojima K, Breitenbach J, Visser H et al (2006) Cloning of the astaxanthin synthase gene from *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*) and its assignment as a beta-carotene 3-hydroxylase/4-ketolase. Mol Genet Genomics 275:148–158

Park JS, Chyun JH, Kim YK et al (2010) Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. Nutr Metab (Lond) 7:18

Prabhu S, Rekha PD, Young CC et al (2013) Zeaxanthin production by novel marine isolates from coastal sand of India and its antioxidant properties. Appl Biochem Biotechnol 171:817–831

ProCarDB, PCD (2014) List of prokaryotic orgnanisms who are known to have carotenoids. http://bioinfo.imtech.res.in/servers/procardb/?c=aboutus&m=organismlist. Accessed 19 May 2016

Renstrøm B, Berger H, Liaaen-Jensen S (1981) Esterified, optical pure (3S, 3′S)-astaxanthin from flowers of *Adonis annua*. Biochem Syst Ecol 9:249–250

Saito K, Fujisaki S, Nishino T (2007) Short-chain prenyl diphosphate synthase that condenses isopentenyl diphosphate with dimethylallyl diphosphate in *ispA* null *Escherichia coli* strain lacking farnesyl diphosphate synthase. J Biosci Bioeng 103:575–577

Sandmann G (2009) Evolution of carotene desaturation: the complication of a simple pathway. Arch Biochem Biophys 483:169–174

Schiedt K (1998) Chapter 7: absorption and metabolism of carotenoids in birds, fish and crustaceans. In: Britton G, Liaaen-Jensen S, Pfander H (eds) Carotenoids. Volume 3: biosynthesis and metabolism. Birkhäuser Verlag, Basel, pp 285–358

Schmidhauser TJ, Lauter FR, Russo VE et al (1990) Cloning, sequence, and photoregulation of *al*-1, a carotenoid biosynthetic gene of *Neurospora crassa*. Mol Cell Biol 10:5064–5070

Schmidhauser TJ, Lauter FR, Schumacher M et al (1994) Characterization of *al-2*, the phytoene synthase gene of *Neurospora crassa*. Cloning, sequence analysis, and photoregulation. J Biol Chem 269:12060–12066

Schmidt AD, Heinekamp T, Matuschek M et al (2005) Analysis of mating-dependent transcription of *Blakeslea trispora* carotenoid biosynthesis genes *carB* and *carRA* by quantitative real-time PCR. Appl Microbiol Biotechnol 67:549–555

Schmidt I, Schewe H, Gassel S et al (2011) Biotechnological production of astaxanthin with *Phaffia rhodozyma*/*Xanthophyllomyces dendrorhous*. Appl Microbiol Biotechnol 89:555–571

Shahina M, Hameed A, Lin SY et al (2013) *Sphingomicrobium astaxanthinifaciens* sp. nov., an astaxanthin-producing glycolipid-rich bacterium isolated from surface seawater and emended description of the genus *Sphingomicrobium*. Int J Syst Evol Microbiol 63:3415–3422

Shahmohammadi HR, Asgarani E, Terato H et al (1998) Protective roles of bacterioruberin and intracellular KCl in the resistance of *Halobacterium salinarium* against DNA-damaging agents. J Radiat Res 39:251–262

Shindo K, Kikuta K, Suzuki A et al (2007) Rare carotenoids, (3R)-saproxanthin and (3R,2'S)-myxol, isolated from novel marine bacteria (*Flavobacteriaceae*) and their antioxidative activities. Appl Microbiol Biotechnol 74:1350–1357

Shindo K, Misawa N (2014) New and rare carotenoids isolated from marine bacteria and their antioxidant activities. Mar Drugs 12:1690–1698

Sieiro C, Poza M, de Miguel T et al (2003) Genetic basis of microbial carotenogenesis. Int Microbiol 6:11–16

Silva C, Cabral JMS, Van Keulen F (2004) Isolation of a β-carotene over-producing soil bacterium, *Sphingomonas* sp. Biotechnol Lett 26:257–262

Singh D, Puri M, Wilkens S et al (2013) Characterization of a new zeaxanthin producing strain of *Chlorella saccharophila* isolated from New Zealand marine waters. Bioresour Technol 143:308–314

Smit A, Mushegian A (2000) Biosynthesis of Isoprenoids via Mevalonate in Archaea: The Lost Pathway. Genome Res 10:1468–1484

Steiger S, Jackisch Y, Sandmann G (2005) Carotenoid biosynthesis in *Gloeobacter violaceus* PCC4721 involves a single *crtI*-type phytoene desaturase instead of typical cyanobacterial enzymes. Arch Microbiol 184:207–214

Tagua VG, Medina HR, Martín-Domínguez R et al (2012) A gene for carotene cleavage required for pheromone biosynthesis and carotene regulation in the fungus *Phycomyces blakesleeanus*. Fungal Genet Biol 49:398–404

Takaichi S (2011) Carotenoids in algae: distributions, biosyntheses and functions. Mar Drugs 9:1101–1118

Takaichi S (2013) Tetraterpenes: carotenoids. In: Ramawat KG, Mérillon J-M (eds) Natural products. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp 3251–3283

Takatani N, Nishida K, Sawabe T et al (2014) Identification of a novel carotenoid, 2'-isopentenylsaproxanthin, by J*ejuia pallidilutea* strain 11shimoA1 and its increased production under alkaline condition. Appl Microbiol Biotechnol 98:6633–6640

Tao L, Wilczek J, Odom JM et al (2006) Engineering a β-carotene ketolase for astaxanthin production. Metab Eng 8:523–531

Tian L, DellaPenna D (2004) Progress in understanding the origin and functions of carotenoid hydroxylases in plants. Arch Biochem Biophys 430:22–29

Tian L, Musetti V, Kim J et al (2004) The *Arabidopsis LUT1* locus encodes a member of the cytochrome p450 family that is required for carotenoid epsilon-ring hydroxylation activity. Proc Natl Acad Sci U S A 101:402–407

Tisch D, Schmoll M (2010) Light regulation of metabolic pathways in fungi. Appl Microbiol Biotechnol 85:1259–1277

Tritsch D, Hemmerlin A, Bach TJ et al (2010) Plant isoprenoid biosynthesis via the MEP pathway: in vivo IPP/DMAPP ratio produced by (E)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase in tobacco BY-2 cell cultures. FEBS Lett 584:129–134

Tuteja N (2007) Abscisic Acid and abiotic stress signaling. Plant Signal Behav 2:135–138

Ukibe K, Hashida K, Yoshida N et al (2009) Metabolic engineering of *Saccharomyces cerevisiae* for astaxanthin production and oxidative stress tolerance. Appl Environ Microbiol 75:7205–7211

Umeno D, Tobias AV, Arnold FH (2005) Diversifying carotenoid biosynthetic pathways by directed evolution. Microbiol Mol Biol Rev 69:51–78

Valadon LRG (1964) Carotenoid pigments of some lower Ascomycetes. J Exp Bot 15:219–224

Valadon LRG (1976) Carotenoids as additional taxonomic characters in fungi: a review. Trans Br Mycol Soc 67:1–15

van den Brink HM, van Gorcom RF, van den Hondel CA et al (1998) Cytochrome P450 enzyme systems in fungi. Fungal Genet Biol 23:1–17

Vandermoten S, Haubruge E, Cusson M (2009) New insights into short-chain prenyltransferases: structural features, evolutionary history and potential for selective inhibition. Cell Mol Life Sci 66:3685–3695

Vannice JC, Skaff DA, Keightley A et al (2014) Identification in *Haloferax volcanii* of phosphomevalonate decarboxylase and isopentenyl phosphate kinase as catalysts of the terminal enzyme reactions in an archaeal alternate mevalonate pathway. J Bacteriol 196:1055–1063

Velayos A, Eslava AP, Iturriaga EA (2000) A bifunctional enzyme with lycopene cyclase and phytoene synthase activities is encoded by the *carRP* gene of *Mucor circinelloides*. Eur J Biochem 267:5509–5519

Verdoes JC, Krubasik KP, Sandmann G et al (1999) Isolation and functional characterisation of a novel type of carotenoid biosynthetic gene from *Xanthophyllomyces dendrorhous*. Mol Gen Genet 262:453–461

Vidhyavathi R, Venkatachalam L, Sarada R et al (2008) Regulation of carotenoid biosynthetic genes expression and carotenoid accumulation in the green alga *Haematococcus pluvialis* under nutrient stress conditions. J Exp Bot 59:1409–1418

Vranova E, Coman D, Gruissem W (2013) Network analysis of the MVA and MEP pathways for isoprenoid synthesis. Annu Rev Plant Biol 64:665–700

Wang B, Lin L, Lu L et al (2012) Optimization of β-carotene production by a newly isolated *Serratia marcescens* strain. Electron J Biotechnol 15:3

Wang X, Willen R, Wadstrom T (2000) Astaxanthin-rich algal meal and vitamin C inhibit *Helicobacter pylori* infection in BALB/cA mice. Antimicrob Agents Chemother 44:2452–2457

Wang Y, Liu Z, Qin S (2013) Effects of iron on fatty acid and astaxanthin accumulation in mixotrophic *Chromochloris zofingiensis*. Biotechnol Lett 35:351–357

Wichuk K, Brynjolfsson S, Fu W (2014) Biotechnological production of value-added carotenoids from microalgae: emerging technology and prospects. Bioengineered 5:204–208

World Health Organization, WHO (2014) Nutrition: micronutrient deficiencies. http://www.who.int/nutrition/topics/vad/en/. Accessed 19 May 2016

Yang T, Sun J, Lian T et al (2014) Process optimization for extraction of carotenoids from medicinal caterpillar fungus, *Cordyceps militaris* (Ascomycetes). Int J Med Mushrooms 16:125–135

Yasui Y, Hosokawa M, Mikami N et al (2011) Dietary astaxanthin inhibits colitis and colitis-associated colon carcinogenesis in mice via modulation of the inflammatory cytokines. Chem Biol Interact 193:79–87

Yatsunami R, Ando A, Yang Y et al (2014) Identification of carotenoids from the extremely halophilic archaeon *Haloarcula japonica*. Front Microbiol 5:100

Yokoyama A, Adachi K, Shizuri Y (1995) New carotenoid glucosides, astaxanthin glucoside and adonixanthin glucoside, isolated from the astaxanthin-producing marine bacterium, *Agrobacterium aurantiacum*. J Nat Prod 58:1929–1933

Yokoyama A, Miki W (1995) Composition and presumed biosynthetic pathway of carotenoids in the astaxanthin-producing bacterium *Agrobacterium aurantiacum*. FEMS Microbiol Lett 128:139–144

Yokoyama A, Miki W, Izumida H et al (1996) New trihydroxy-keto-carotenoids isolated from an astaxanthin-producing marine bacterium. Biosci Biotechnol Biochem 60:200–203

Zalokar M (1957) Isolation of an acidic pigment in *Neurospora*. Arch Biochem Biophys 70:568–571

Zheng YT, Toyofuku M, Nomura N et al (2013) Correlation of carotenoid accumulation with aggregation and biofilm development in *Rhodococcus* sp. SD-74. Anal Chem 85:7295–7301

Zhu C, Gerjets T, Sandmann G (2007) *Nicotiana glauca* engineered for the production of ketocarotenoids in flowers and leaves by expressing the cyanobacterial *crtO* ketolase gene. Transgenic Res 16:813–821