Int Microbiol (2003) 6: 11–16
DOI 10.1007/s10123-003-0097-0

REVIEW ARTICLE

C. Sieiro · M. Poza · T. de Miguel · T.G.Villa

Genetic basis of microbial carotenogenesis

Received: 10 October 2002 / Accepted: 29 November 2002 / Published online: 12 February 2003

Abstract The synthesis of carotenoids begins with the formation of a phytoene from geranylgeranyl pyrophosphate, a well conserved step in all carotenogenic organisms and catalyzed by a phytoene synthase, an enzyme encoded by the *crtB* (*spy*) genes. The next step is the dehydrogenation of the phytoene, which is carried out by phytoene dehydrogenase. In organisms with oxygenic photosynthesis, this enzyme, which accomplishes two dehydrogenations, is encoded by the *crtP* genes. In organisms that lack oxygenic photosynthesis, dehydrogenation is carried out by an enzyme completely unrelated to the former one, which carries out four dehydrogenations and is encoded by the *crtI* genes. In organisms with oxygenic photosynthesis, dehydrogenation of the phytoene is accomplished by a ζ-carotene dehydrogenase encoded by the *crtQ* (*zds*) genes. In many carotenogenic organisms, the process is completed with the cyclization of lycopene. In organisms exhibiting oxygenic photosynthesis, this step is performed by a lycopene cyclase encoded by the *crtL* genes. In contrast, anoxygenic photosynthetic and non-photosynthetic organisms use a different lycopene cyclase, encoded by the *crtY* (*lyc*) genes. A third and unrelated type of lycopene β-cyclase has been described in certain bacteria and archaea. Fungi differ from the rest of non-photosynthetic organisms in that they have a bifunctional enzyme that displays both phytoene synthase and lycopene cyclase activity. Carotenoids can be modified by oxygen-containing functional groups, thus originating xanthophylls. Only two enzymes are necessary for the conversion of β-carotene into astaxanthin, using several ketocarotenoids as intermediates, in both prokaryotes and eukaryotes. These enzymes are a β-carotene hydroxylase (*crtZ* genes) and a β-carotene ketolase, encoded by the *crtW* (bacteria) or *bkt* (algae) genes.

Keywords Carotenes · Xanthophylls · Biosynthesis · Genetics

C. Sieiro · M. Poza · T. de Miguel · T.G. Villa (✉)
Departamento de Microbiología y Parasitología,
Facultad de Farmacia, Universidad de Santiago de Compostela,
15782 Santiago de Compostela, Spain
E-mail: mpvilla@usc.es
Tel.: +34-98-1592490
Fax: +34-98-1592490

Introduction

Carotenoids are natural pigments that are structurally very diverse—yet similar in their general chemical structure—and are widely distributed in nature, where they fulfill essential biological functions. These pigments act as protective agents against oxidative damage [5], are responsible for the color of many plants and animals [7] and are precursors of phytohormones [15]. Some related compounds of β-carotene, such as vitamin A, retinal and retinoic acid, have important roles in vision, nutrition and cellular growth and development [7]. Carotenoids are also important due to their potential antitumor properties [27] and because they are used as colorants in the food industry to pigment salmon, trout and poultry flesh, or to intensify the color of egg yolk [21]. Carotenoids typically consist of a C_{40} hydrocarbon backbone, in the case of carotenes, often modified by different oxygen-containing functional groups, to yield cyclic or acyclic xanthophylls. The absorption properties of each carotenoid depend on the degree of conjugation and isomerization state of the backbone polyene chromophore. Compounds with at least seven conjugated double bonds can absorb visible light [7]. In the microbial world, carotenoids are present in both anoxygenic and oxygenic photosynthetic bacteria and algae and in many fungi [2, 19, 26]. Carotenoids are essential for organisms with oxygenic photosynthesis (plants, algae, cyanobacteria) because of their protective role, which consists of both depleting the energy from chlorophyll and accepting it from other molecules, such as the reactive forms of oxygen. These pigments are not essential for other carotene-containing microorganisms, as is the

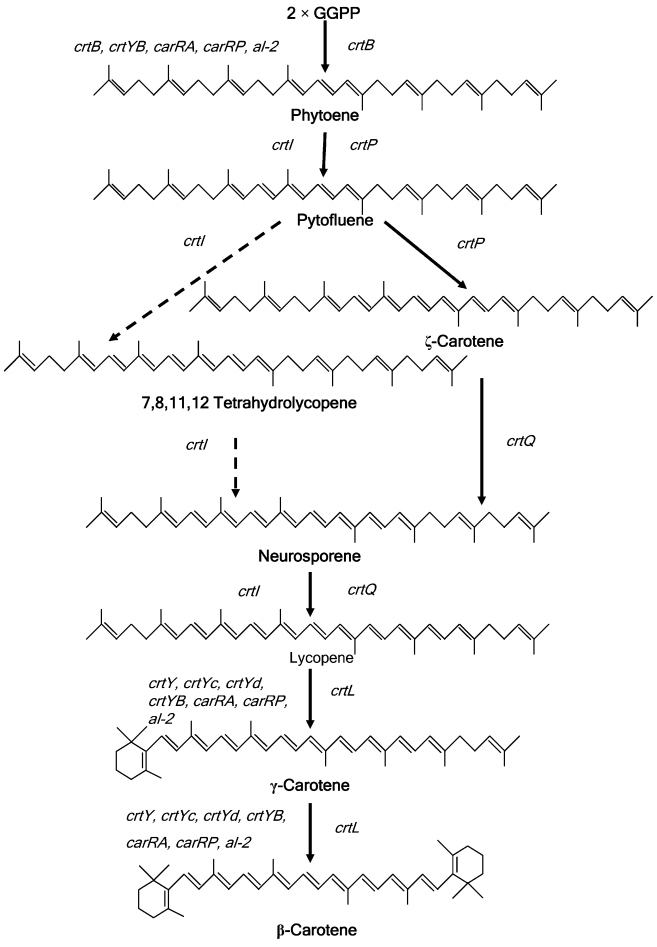

Fig. 1 Conversion of phytoene into β-carotene. *crt* genes required for specific carotenoid biosynthetic reactions *are shown either on the left* for non-photosynthetic and anoxygenic photosynthetic microorganisms *or on the right* for oxygenic photosynthetic microorganisms. The corresponding enzymes are listed in Table 1. *GGPP* Geranylgeranyl pyrophosphate

case for fungi, but they are indeed very important due to their ability to act as antioxidant agents [7, 14]. The most important advances in research on carotenoid biosynthesis have been made in the past few years and are summarized here.

Synthesis of phytoene

The synthesis of carotenoids derives from the biosynthetic pathway of isoprenoids [6, 31]. The formation of phytoene is the first specific and crucial step of the carotenoid pathway (Fig. 1). The union of two molecules of geranylgeranyl pyrophosphate (GGPP) produces phytoene, a still colorless C_{40} carotenoid with only three conjugated double bonds. Phytoene synthases—conserved among the carotenoid-producing organisms, from bacteria to plants—are the enzymes responsible for carrying out this step. These enzymes are encoded by the *crtB* genes in bacteria and by the *psy* genes in plants, algae and cyanobacteria. They are membrane-associated proteins, with a molecular mass of 35–39kDa, monomeric, dependent on divalent cations and have several conserved regions [8, 11, 29]. The phytoene synthases of plants and algae show small differences in the amino-terminus region due to the presence of a signal peptide responsible for the localization of these enzymes in chloroplasts and chromoplasts [9]. The known phytoene synthases have homology with the diapophytoene synthases and the squalene synthases, which condense two molecules of farnesyl pyrophosphate to form diapophytoene and squalene respectively, sharing some of the consensus regions with them [2, 9].

Dehydrogenation of phytoene

The dehydrogenation of phytoene is the second step in the biosynthetic pathway of carotenoids (Fig. 1). This involves the introduction of a new double bond into the molecule, using the enzyme phytoene dehydrogenase, also associated with the membrane. Usually, the dehydrogenation process is repeated 2–4 times and at each step the number of double bonds duplicates. Regarding this second step, organisms that synthesize carotenoids may be divided in two groups: those with oxygenic photosynthesis (cyanobacteria, algae, plants) and those lacking it (all other eubacteria, archaea, fungi). In organisms with oxygenic photosynthesis, the *crtP* or the *pds* genes code for phytoene dehydrogenase. This enzyme only carries out two dehydrogenations and ζ-carotene is the final product. In contrast, in the case of organisms lacking this type of photosynthesis, the *crtI* genes encode phytoene dehydrogenase. This enzyme is able to carry out four consecutive dehydrogenations, producing lycopene as a final product. *Rhodobacter capsulatus* and *R. sphaeroides*, where this enzyme only carries out three dehydrogenations and generates neurosporene as the main product [2], should be mentioned as exceptions, together with *Neurospora crassa*, where the enzyme carries out five dehydrogenations to generate 3,4-dihydrolycopene as an intermediate step in the formation of torulene [16]. It is clear that the enzyme encoded by the *crtI* genes has less specificity for the substrate than the enzyme codified by the *crtP* genes. Phytoene dehydrogenases are enzymes with a molecular mass of 53–69kDa. The larger ones correspond to fungi (CrtI), with a size that varies over 62–69kDa. The two types of enzyme (Crtp, CrtI) do not show any homology [35, 36] and their differences are not restricted to the number of dehydrogenations that each may undergo. Also, they differ in terms of their preference for cofactors and in their sensitivity to inhibitors. Those of the CrtP-type require NAD(P) and are inhibited by herbicides, such as norflurazon, whilst those of type CrtI normally use FAD and are sensitive to diphenylamine [16]. They are enzymes with different characteristics but with the same function; and in this regard they can be considered as a good example of convergent evolution [33].

As mentioned above, the product of the *crtP* gene has higher specificity for the substrate and can only carry out two dehydrogenations from phytoene. Therefore, in the case of organisms with oxygenic photosynthesis, in order to obtain lycopene additional enzymes must be used. These are the ζ-carotene dehydrogenases encoded by the *crtQ* or the *zds* genes. The ζ-carotene dehydrogenases have a very similar size to those of the dehydrogenases encoded by the *crtP*-type genes and are phylogenetically related to them [9]. Only one exception has been described: the ζ-carotene dehydrogenase of *Anabaena* PCC7120, which is related to the dehydrogenases of the CrtI-type but not with those of the CrtP-type [24].

The closest similarity between the two types of phytoene dehydrogenases described is found at the amino-terminus, where a dinucleotide-binding sequence is located [33]. Another sequence has also been found close to the carboxy-terminus (PROSITE PS00982), which is typical of the CrtI-type phytoene dehydrogenases. This sequence is also found in other enzymes involved in carotenogenesis, such as the methoxyneurosporene dehydrogenases (*crtD* gene). Besides the proteins encoded by the *crtD* and *zds* genes of *Anabaena*, there is a third type of protein, different to the others in function, that is related to the CrtI-type phytoene dehydrogenases. This protein is the product of the *crtO* gene, a β-carotene ketolase of *Synechocystis* PCC6803 [12]. Also, a group of 22 amino acids of the CrtP-type enzymes has been suggested as the carotenoid-binding region [33]. These amino acids are located close to the area corresponding to that occupied by the sequence mentioned for the CrtI proteins.

Cyclization of lycopene

Although not universal, the cyclization of lycopene is a common step in the synthesis of carotenoids (Fig. 1). Following the dehydrogenation of phytoene from ζ-carotene, neurosporene or lycopene, the biosynthesis of carotenoids can take different pathways, depending on the organism involved. Neurosporene can be hydroxylated to produce spheroidene and spheroidenone in the genus *Rhodobacter* (the conversion being carried out by the *crtC* gene), whilst in the majority of organisms the substrate for the next enzymatic step is lycopene. The cyclization of lycopene consists of the introduction, in a sequential manner, of a ring (β- or ε-type) at both ends of the molecule, thus varying the total number of double bonds but not the number of conjugated double bonds. The most common ring is the β-type since the ε-type is only found in plants and certain algae [9]. The enzymes that carry out this reaction are called lycopene β-cyclases and lycopene ε-cyclases, respectively.

As with the dehydrogenation of phytoene, the cyclization of lycopene is another case of convergent evolution. There are several types of lycopene β-cyclases that are unrelated phylogenetically. In organisms with oxygenic photosynthesis, the lycopene β-cyclases are encoded by the *crtL* genes and, in organisms without oxygenic photosynthesis, by the *crtY* or *lyc* genes. Both types of enzyme sequentially introduce two rings for the formation of β-carotene (through γ-carotene) or one ring if they use δ-carotene as substrate. The CrtY-type proteins have a size of around 43kDa, while those of the CrtL-type are about 46–56 kDa. The largest ones are those of plants, since they bear a signal peptide for translocation into chloroplasts and chromoplasts.

Both CrtY-type and CrtL-type lycopene cyclases are very different in their primary structure. However, three apparently conserved small sequences have been detected: (1) the sequence for binding to cofactors ($DX_4GXGXAX_4A$), (2) the consensus sequence I ($FXYX_4SX_6EXT$) and (3) the consensus sequence II ($GX_2AX_3HPX_2GY$); and there is also a particular hydropathic profile showing areas of probable transmembrane nature [10, 17, 34]. These similarities are the result of a convergent evolution of different types of enzyme which need certain domains to carry out essential functions.

The known *crtY* and *crtL* genes encode lycopene cyclases in eubacteria, algae and plants, but none responsible for encoding a lycopene cyclase in fungi has been described. In 1999, in the basidiomycete *Xanthophyllomyces dendrorhous*, Verdoes et al. [38] described a new enzyme for the biosynthesis of carotenoids encoded by the *crtYB* gene, which is a bifunctional protein with phytoene synthase and lycopene cyclase activity. Subsequently, this bifunctional protein was described in the zygomycete *Mucor circinelloides* (encoded by the *carRP* gene) [37], in the ascomycete *Neurospora crassa* (encoded by the *al-2* gene) [4] and in *Phycomyces blakesleeanus* (encoded by the *carRA* gene) [3]. Since all fungi (basidiomycetes and ascomycetes included) evolved from mucorales, the presence of this gene in *M. circinelloides* is very interesting. The products of the *crtYB*, *al-2*, *carRP* and *carRA* genes share two well defined domains. One is responsible for the phytoene synthase activity and is located in the carboxy-terminus region; and the other is responsible for lycopene cyclase activity and is located in the amino-terminus region. Sequence comparison suggests that the domain responsible for phytoene synthase activity is similar to that of the phytoene and squalene synthases of the other carotenogenic organisms. In contrast, the domain responsible for the activity of lycopene cyclase has no homology with the proteins CrtY and CrtL but does have homology with the two proteins responsible for this activity in *Brevibacterium linens*. The activity of lycopene cyclase in this bacterium is the result of the combined action of the *crtYc* and *crtYd* gene products. These genes are contiguous, related in their sequence and probably originated by gene duplication [23]. Furthermore, the domain responsible for lycopene cyclase activity in fungi and the genes that encode this activity in *B. linens* seem to be related both to the ORF7 and the ORF8 of the carotenogenesis

cluster of *Myxococcus xanthus* and to the first ORF that appears after the *crtB* gene in *Mycobacterium marinum* [37].

Very recently, Peck et al. [32] identified a gene (*crtY*) that converts lycopene into β-carotene in the archaeal *Halobacterium salinarium*. This gene encodes a membrane protein similar to the lycopene cyclases of *B. linens* and *Mycobacterium aurum* [23, 32, 37] and those of fungi. This protein has two domains that are similar to the proteins of *B. linens* involved in the cyclization of the lycopene, linked by an additional transmembrane segment that allows the topology of each domain to be maintained. All these data gradually led to the establishment of the possible evolutionary relationships between the lycopene cyclases of bacteria and fungi. In this sense, the *crtYc* and *crtYd* genes of *B. linens* could have appeared through the duplication of a gene that encodes a homodimeric lycopene cyclase. As previously mentioned, the *crtY* gene of carotenogenic archaea could have been formed from the genes *crtYc* and *crtYd* of *B. linens* and encodes an enzyme with a bacterial/fungal intermediate-type structural organization. Finally, the *crtYB* gene of fungi could have originated from the fusion of an archaeal-like gene for lycopene cyclase with a *crtB* gene for phytoene synthase that would have originated from a common ancestor of all the phytoene and squalene synthases [3, 32, 37, 38].

Synthesis of xanthophylls

In many organisms, the pathway of the carotenoid biosynthesis ends with β-carotene. In other cases, some carotenoids are modified with several functional groups that contain oxygen, thus originating the xanthophylls (Fig. 2). With a few exceptions, as in the case of species of the genus *Rhodobacter*, which synthesize acyclic xanthophylls from neurosporene, most organisms produce xanthophylls from β-carotene. Species of the genus *Rhodobacter* essentially synthesize spheroidene and spheroidenone from neurosporene (Fig. 2A). These conversions are carried out by the *crtC*, *crtD*, *crtF* and *crtA* genes [2]. The functions of these genes are listed in Table 1. Among microorganisms, the main producers of cyclic xanthophylls are species of the genus *Erwinia* [2], which basically synthesize β-cryptoxanthin and zeaxanthin or their glucosides (Fig. 2B), the marine bacteria *Agrobacterium aurantiacum* and *Alcaligenes* sp. strain PC1 [2, 30], which produce above all adonixanthin and astaxanthin (Fig. 2B). Also within this group are the yeast *X. dendrorhous* [1] and the alga *Haematococcus pluvialis* [26], which specifically synthesize astaxanthin, or astaxanthin and cantaxanthin, respectively (Fig. 2B).

Despite the considerable advances made concerning our knowledge of the biosynthetic pathway of carotenoids in recent years, there are few reports on the genes or enzymes involved in the metabolism of the xanthophylls. To date, only two genes (*crtZ*, *crtW*/*bkt*)

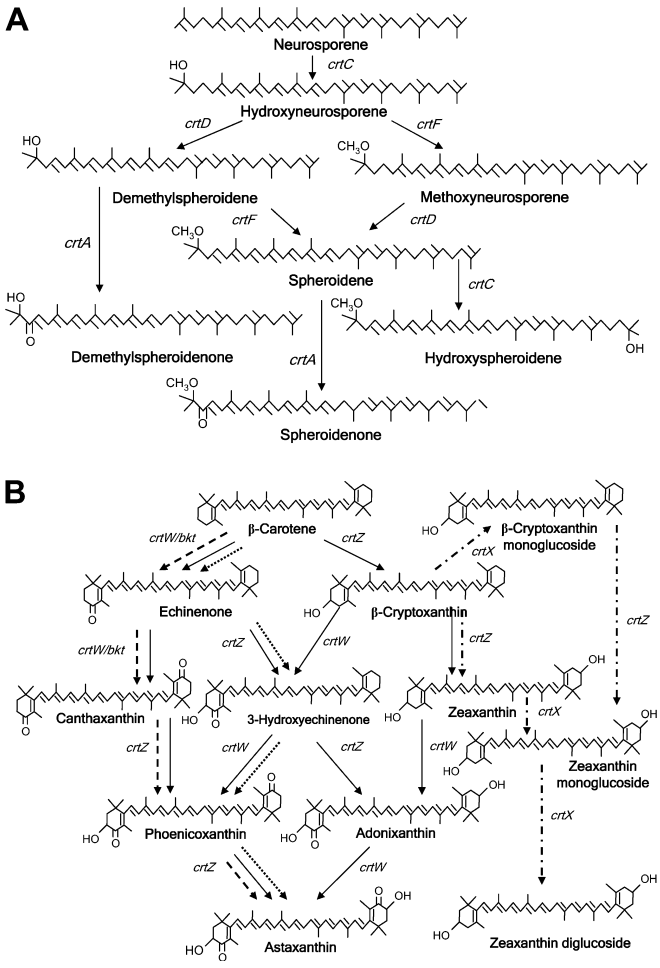

Fig. 2A, B Proposed biosynthesis pathways of xanthophylls. **A** Acyclic xanthophylls synthesized by *Rhodobacter* species. **B** Cyclic xanthophylls synthesized by *Erwinia* species (*dot*/*dash lines*), *Agrobacterium aurantiacum* (*solid lines*), *Haematococcus pluvialis* (*dashed lines*) and *Xanthophyllomyces dendrorhous* (*dotted lines*). *crt* genes required for the carotenoid biosynthetic reaction *are shown* and the corresponding enzymes are listed in Table 1

have been described and these appear to be sufficient for the transformation of β-carotene into astaxanthin for both prokaryotes and eukaryotes, using different ketocarotenoids as intermediates, depending on the microorganisms [18, 22, 25, 28, 30] (Fig. 2). The product of the *crtZ* gene (β-carotene hydroxylase), which has been isolated from species of *Erwinia* and marine bacteria, carries out the hydroxylation of β-carotene at positions 3 and 3′ of the β-ionone ring, producing zeaxanthin via β-cryptoxanthin. Moreover, β-C-4-ketolase, encoded by the *crtW* gene in marine bacteria and by the *bkt* gene of *H. pluvialis*, carries out the direct conversion of methylene into keto- groups at positions 4 and 4′ of the β-ionone ring, producing cantaxanthin via echinenone. On the basis of studies carried out in marine bacteria [30] and green algae [26] and also taking into account the intermediates detected [20] and the accumulated carotenoids in several mutants or in the presence of carotenogenesis inhibitors [13], a biosynthetic pathway for

Table 1 Carotenoid biosynthetic genes and enzymes

Gene	Enzymatic function	Organism [reference]
Synthesis of phytoene		
crtB	Phytoene synthase	*Agrobacterium aurantiacum*, *Erwinia herbicola*, *E. uredovora*, *Flavobacterium* sp., *Myxococcus xanthus*, *Rhodobacter capsulatus*, *R. sphaeroides*, *Streptomyces griseus*, *Synechococcus* sp., *Synechocystis* sp., *Thermus thermophilus* [2]
crtYB	Phytoene synthase	*Xanthophyllomyces dendrorhous* [38]
carRP	Phytoene synthase	*Mucor circinelloides* [37]
carRA	Phytoene synthase	*Phycomyces blakesleeanus* [3]
al-2	Phytoene synthase	*Neurospora crassa* [4]
Dehydrogenation of phytoene		
crtI	Phytoene dehydrogenase	*A. aurantiacum*, *E. herbicola*, *E. uredovora*, *Flavobacterium* sp., *M. xanthus*, *R. capsulatus*, *R. sphaeroides*, *S. griseus* [2]
crtP	Phytoene dehydrogenase	*Synechococcus* sp., *Synechocystis* sp. [2]
crtQ	ζ-Carotene dehydrogenase	*Anabaena* sp. [2]
Lycopene cyclization		
crtY	Lycopene cyclase	*A. aurantiacum*, *E. herbicola*, *E. uredovora*, *Flavobacterium* sp., *S. griseus* [2]
crtL	Lycopene cyclase	*Synechococcus* sp. [2]
crtYc, *crtYd*	Lycopene cyclase	*Brevibacterium linens* [23]
crtY	Lycopene cyclase	*Halobacterium salinarium* [32]
crtYB	Lycopene cyclase	*X. dendrorhous* [38]
carRP	Lycopene cyclase	*Mucor circinelloides* [37]
carRA	Lycopene cyclase	*P. blakesleeanus* [3]
al-2	Lycopene cyclase	*N. crassa* [4]
Formation of acyclic xanthophylls		
crtC	Hydroxyneurosporene synthase	*Rhodobacter* [2]
crtD	Methoxyneurosporene desaturase	*Rhodobacter* [2]
crtF	Hydroxyneurosporene-O-methyltransferase	*Rhodobacter* [2]
crtA	Spheroidene monooxygenase	*Rhodobacter* [2]
Formation of cyclic xanthophylls		
crtX	Zeaxanthin glucosylase	*E. herbicola*, *E. uredovora* [2]
crtZ	β-Carotene hydroxylase	*A. aurantiacum*, *Alcaligenes*, *E. herbicola*, *E. uredovora*, *Flavobacterium* sp. [2]
crtW	β-C-4-oxygenase	*A. aurantiacum*, *Alcaligenes* [2]
bkt	β-C-4-Oxygenase	*Haematococcus pluvialis* [26]

xanthophylls has been proposed for *X. dendrorhous* (Fig. 2B). Comparison of the amino acid sequences for the proteins encoded by the above-mentioned genes revealed a 90% identity between the *crtZ* genes of *A. aurantiacum* and *Alcaligenes* PC1. These enzymes also showed 54% identity with those of the species of *Erwinia*. In contrast, the CrtW proteins of marine bacteria share 75% identity amongst themselves and 37% with the BKT protein of *H. pluvialis*. Despite this and bearing in mind the large number of ketocarotenoid intermediates between β-carotene and astaxanthin synthesized by *A. aurantiacum* (Fig. 2B) in comparison with those synthesized by *H. pluvialis* or *X. dendrorhous*, it appears that the *crtZ* and *crtW* genes of *A. aurantiacum* have lower specificity for the substrate than the enzymes encoded by the same genes in *H. pluvialis*. Furthermore, it seems that the enzymes involved in xanthophyll metabolism are well conserved from bacteria up to higher eukaryotes, given the homology found between the CrtW/BKT proteins of bacteria and algae. The identification and characterization of the *crtZ* gene in green algae and of the *crtZ* and *crtW* genes in *X. dendrorhous* and in plants would be necessary in order to confirm this hypothesis.

Acknowledgements The authors wish to express their deepest appreciation to the PETRI program (grant PTR1995-0572-OP), to the Xunta de Galicia (grant PR404D 2001/4-0) and to the Ramón Areces Foundation for the financial support that made this work possible.

References

1. Andrews A, Phaff HJ, Starr MP (1976) Carotenoids of *Phaffia rhodozyma*, a red pigmented fermenting yeast. Phytochemistry 15:1003–1009
2. Armstrong G (1997) Genetics of eubacterial carotenoid biosynthesis: a colorful tale. Annu Rev Microbiol 51:629–659
3. Arrach N, Fenández-Martín R, Cerdá-Olmedo E, Avalos J (2001) A single gene for lycopene cyclase, phytoene synthase, and regulation of carotene biosynthesis in *Phycomyces*. Proc Natl Acad Sci USA 98:1687–1692
4. Arrach N, Schmidhauser TJ, Avalos J (2002) Mutants of the carotene cyclase domain of *al-2* from *Neurospora crassa*. Mol Genet Genomics 266:914–921
5. Bartley GE, Scolnik PA (1995) Plant carotenoids: pigments for photoprotection, visual attraction, and human heath. Plant Cell 7:1027–1038
6. Bauer CE, Bollivar DW, Suzuki JY (1993) Genetic analyses of photopigmented biosynthesis in eubacteria: a guiding light for algae and plants. J Bacteriol 175:3919–3925

7. Briton G (1983) The biochemistry of natural pigments. Cambridge University Press, Cambridge
8. Chamovitz D, Misawa N, Sandmann G, Hirschberg J (1992) Molecular cloning and expression in *Escherichia coli* of a cyanobacterial gene coding for phytoene synthase, a carotenoid biosynthesis enzyme. FEBS Lett 296:305–310
9. Cunningham FX Jr, Gantt E (1998) Genes and enzymes of carotenoid biosynthesis in plants. Annu Rev Plant Physiol Plant Mol Biol 49:557–583
10. Cunningham FX Jr, Sun Z, Chamovitz D, Hirschberg J, Gantt E (1994) Molecular structure and enzymatic function of lycopene cyclase from the cyanobacterium *Synechococcus* sp. strain PCC 7942. Plant Cell 6:1107–1121
11. Dogbo O, Laferriére A, D'Harlingue A, Camara B (1988) Carotenoid biosynthesis: isolation and characterization of a bifunctional enzyme catalyzing the synthesis of phytoene. Proc Natl Acad Sci USA 85:7054–7058
12. Fenández-González B, Sandmann G, Vioque A (1997) A new type of asymmetrically acting β-carotene ketolase is required for the synthesis of echinenone in the cyanobacterium *Synechocystis* sp. PCC 6803. J Biol Chem
13. Girard P, Falconnier B, Bricout J, Vladescu B (1994) β-Carotene producing mutants of *Phaffia rhodozyma*. Appl Microbiol Biotechnol 41–47
14. Goodwin TW (1986) Metabolism, nutrition, and function of carotenoids. Annu Rev Nutr 6:273–281
15. Hable WE, Oishi KK, Schumaker KS (1998) Viviparous-5 encodes phytoene desaturase, an enzyme essential for abscisic acid (ABA) accumulation and seed development in maize. Mol Gen Genet 257:167–176
16. Hausmann A, Sandman G (2000) A single five-step desaturase is involved in the carotenoid biosynthesis pathway to β-carotene and torulene in *Neurospora crassa*. Fungal Genet Biol 30:147–153
17. Hugueney P, Badillo A, Chen H-C, Klein A, Hirschberg J, Camara B, Kuntz M (1995) Metabolism of cyclic carotenoids: a model for the alteration of this biosynthetic pathway in *Capsicum annuum* chromoplasts. Plant J 8:417–424
18. Hundle BS, Alberti M, Nievelstein V, Beyer P, Kleinin H, Armstrong GA, Burke DH, Hearst JE (1994) Functional assignment of *Erwinia herbicola* Eho10 carotenoid genes expressed in *Escherichia coli*. Mol Gen Genet 254:406–416
19. Johnson EA, An G-H (1991) Astaxanthin from microbial sources. Crit Rev Biotechnol 11:297–306
20. Johnson EA, Lewis MJ (1979) Astaxanthin formation by the yeast *Phaffia rhodozyma*. J Gen Microbiol 115:173–183
21. Johnson EA, Schroeder WA (1995) Microbial carotenoids. Adv Biochem Eng Biotechnol 53:119–178
22. Kajiwara S, Kakizono T, Saito T, Kondo K, Ohtani T, Nishio N, Nagai S, Misawa N (1995) Isolation and functional identification of a novel cDNA for astaxanthin biosynthesis from *Haematococcus pluvialis* and astaxanthin synthesis in *Escherichia coli*. Plant Mol Biol 29:343–352
23. Krubasik P, Sandmann G (2000) A carotenogenic gene cluster from *Brevibacterium linens* with novel lycopene cyclase genes involved in the synthesis of aromatic carotenoids. Mol Gen Genet 263:423–432
24. Linden H, Vioque A, Sandmann G (1993) Isolation of a carotenoid biosynthesis gene coding for ζ-carotene desaturase from *Anabaena* PCC 7120 by heterologous complementation. FEMS Microbiol Lett 106:99–104
25. Lotan T, Hirschberg J (1995) Cloning and expression in *Escherichia coli* of the gene encoding β-C-4-oxygenase that converts β-carotene to the ketocarotenoid cantaxanthin in *Haematococcus pluvialis*. FEBS Lett 363:125–128
26. Margalith PZ (1999) Production of ketocarotenoids by microalgae. Appl Microbiol Biotechnol 51:431–438
27. Mayne ST (1996) Beta-carotene, carotenoids, and disease prevention in humans. FASEB J 10:690–701
28. Misawa N, Nakagawa M, Kobayashi K, Yamano S, Izawa Y, Nakamura K, Harashima K (1990) Elucidation of the *Erwinia uredovora* carotenoid biosynthetic pathway by functional analysis of the gene products expressed in *Escherichia coli*. J Bacteriol 172:6704–6712
29. Misawa N, Truesdale MR, Sandmann G, Fraser PD, Bird C, Schuch W, Bramley PM (1994) Expression of a tomato cDNA coding for phytoene synthase in *Escherichia coli*, phytoene formation in vivo and in vitro, and functional analysis of various truncated gene products. J Biochem 116: 980–985
30. Misawa N, Satoni Y, Kondo K, Yokoyama A, Kajiwara S, Saito T, Ohtani T, Miki W (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
31. Ourisson G, Rohmer M, Poralla K (1987) Prokaryotic hopanoids and other polyterpenoid sterol surrogates. Annu Rev Microbiol 41:301–333
32. Peck RF, Johnson EA, Krebs MP (2002) Identification of a lycopene β-cyclase required for bacteriorhodopsin biogenesis in the archaeon *Halobacterium salinarium*. J Bacteriol 184:2889–2897
33. Pecker I, Chamovitz D, Linden H, Sandmann G, Hirschberg J (1992) A single polypeptide catalyzing the conversion of phytoene to ζ-carotene is transcriptionally regulated during tomato fruit ripening. Proc Natl Acad Sci USA 89:4962–4966
34. Pecker I, Gabbay R, Cunningham FX Jr, Hirschberg J (1996) Cloning and characterization of the cDNA for lycopene β-cyclase from tomato reveals decrease in its expression during fruit ripening. Plant Mol Biol 30:807–819
35. Sandmann G (1994a) Phytoene desaturase: genes, enzymes and phylogenetic aspects. J Plant Physiol 143:444–447
36. Sandmann G (1994b) Carotenoid biosynthesis in microorganisms and plants. Eur J Biochem 223:7–24
37. 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
38. Verdoes JC, Krubasik P, Sandmann G, Ooyen AJJ van (1999) Isolation and functional characterization of a novel type of carotenoid biosynthetic gene from *Xanthophyllomyces dendrorhous*. Mol Gen Genet 262:453–461