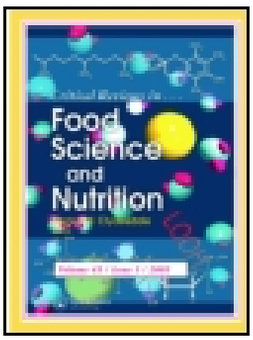

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Carotenoids biosynthesis and cleavage related genes from bacteria to plants

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Abstract

Carotenoids are essential for photosynthesis and photoprotection in photosynthetic organisms and beneficial for human health. Apocarotenoids derived from carotenoid degradation can serve critical functions including hormones, volatiles, and signals. They have been used commercially as food colorants, animal feed supplements, and nutraceuticals for cosmetic and pharmaceutical purposes. This review focuses on the molecular evolution of carotenogenic enzymes and carotenoid cleavage oxygenases (CCOs) from bacteria, fungi, cyanobacteria, algae, and plants. The diversity of carotenoids and apocarotenoids as well as their complicated biosynthetic

pathway in different species can shed light on the history of early molecular evolution. Some carotenogenic genes (such as phytoene synthases) have high protein sequence similarity from bacteria to land plants, but some (such as phytoene desaturases, lycopene cyclases, carotenoid hydroxylases and CCOs) have low similarity. The broad diversity of apocarotenoid volatile compounds can be attributed to large numbers of carotenoid precursors and the various cleavage sites catalyzed by CCOs enzymes. A variety of carotenogenic enzymes and CCOs indicate the functional diversification of carotenoids and apocrotenoids in different species. New carotenoids, new apocarotenoids, new carotenogenic enzymes, new CCOs, and new pathways still need to be explored.

Keywords

carotenoid biosynthesis; carotenogenic genes; phytoene desaturases; lycopene cyclases; carotenoid cleavage oxygenases (CCOs).

1. Introduction

Carotenoids are important natural pigments produced from bacteria, fungi, algae, and plants. Based on the structural features, carotenoids can be classified into two main groups: carotenes and xanthophylls (which contain oxygen), and the oxygenated xanthophylls are dominant. Carotenes are linear or cyclized hydrocarbons, such as lycopene, α-carotene and β-carotene. Xanthophylls are oxygenated carotenes, which contain hydroxyl, epoxy or keto groups, such as lutein, zeaxanthin, violaxanthin and astaxanthin. In addition, carotenoids can be formed in different stereoisomers (*E*/*Z*, i.e. *cis*/*trans*) and optical isomers (*R*/*S*) (Lerfall 2016).

Carotenoids play essential roles in light harvesting and protecting the photosynthetic apparatus from photooxidative damage under excess light conditions in photosynthetic organisms. In plants, they are precursors for the biosynthesis of the phytohormones, such as abscisic acid (ABA) and strigolactones, which are key regulators for plant development and stress response (Cazzonelli and Pogson 2010, Al-Babili and Bouwmeester 2015). A number of important biological functions of carotenoids are associated with their antioxidant properties and their function as precursors of vitamin A. Provitamin A carotenoids (α-carotene, β-carotene and β-cryptoxanthin) play essential roles in animals as precursors for the synthesis of retinoid, retinol (vitamin A), retinal (main visual pigment), and retinoic acid (Von Lintig 2010). Animals cannot synthesize carotenoids *de novo*, and they rely on dietary sources to provide sufficient levels of carotenoids. Carotenoids are beneficial for human health, serving as antioxidants in lipophilic environments and decreasing the risk of various diseases, particularly certain cancers and eye diseases (Krinsky and Johnson 2005). Carotenoids have been used commercially as food

colorants, animal feed supplements, and nutraceuticals for cosmetic and pharmaceutical purposes.

Apocarotenoids are carotenoid-derived compounds through the oxidative cleavage of carbon--carbon double bonds in carotenoid backbones in all kinds of organisms (Beltran and Stange 2016). They have important functions such as growth simulators and inhibitors, signaling molecules, volatile aromatic compounds, defense against pathogens and herbivores, as well as the phytohormones ABA and strigolactones (Hou *et al.* 2016). The broad diversity of apocarotenoids can be attributed to large numbers of carotenoid precursors and the various cleavage sites catalyzed by carotenoid cleavage oxygenases (CCOs) enzymes.

The diversity of carotenoids and apocarotenoids as well as their complicated biosynthetic pathway in different species can throw light on the history of early molecular evolution. This review focuses on the molecular evolution of carotenogenic enzymes and carotenoid cleavage oxygenases (CCOs) from bacteria, fungi, cyanobacteria, algae and plants. Phylogenetic analysis of genes/enzymes from the carotenoid and apocarotenoid biosynthetic pathways in these species can help researchers better understand the functions and evolution of these structurally diverse compounds with a common backbone. A variety of carotenogenic enzymes and CCOs evidences the functional diversification of carotenoids and apocarotenoids in different species.

2. Carotenoid components and their pathways of biosynthesis and cleavage

2.1 Carotenoid biosynthesis in bacteria and fungi

2.1.1 Carotenoids produced by bacteria and fungi

Bacteria can synthesize C_{40}-derivatives of β-carotene and γ-carotene, and acyclic carotenoids, such as spheroidene and spirilloxanthin (Figure 1A) (STEIGER *et al.* 2000). C_{30}-carotenoids, such as diaponeurosporene, can be synthesized only in some non- phototrophic bacteria, which are able to condense two C_{15} isoprenoids (Wieland *et al.* 1994, Schweiggert and Carle 2016). A variety of C_{45}- and C_{50}- carotenoids (such as decaprenoxanthin, Figure 1A) was found in some Gram- positive Eubacteria and Archaea (Krubasik *et al.* 2001, Yang *et al.* 2015, Schweiggert and Carle 2016). A web server called ProCarDB website (http://bioinfo.imtech.res.in/servers/procardb/) provides a valuable resource for researchers about the distribution of prokaryotic carotenoids and the genes/enzymes involved in the carotenoid biosynthetic pathways in bacteria (Nupur *et al.* 2016).

In yeasts and other fungi, monocyclic and carboxylic carotenoids like torulene and torularhodin (Figure 1A) are frequently encountered (Schweiggert and Carle 2016). *Blakeslea trispora*, a filamentous fungus, intensively used for the commercial production of β-carotene and lycopene. In *Rhodotorula* species, the biosynthetic monocyclization of lycopene results in the formation of γ- carotene, which is subsequently desaturated to torulene. In the yeast *Xanthophyllomyces dendrorhous* (previously *Phaffia rhodozyma*), γ- carotene is transformed to β- carotene and then to astaxanthin via other intermediates (Wöstemeyer *et al.* 2005).

2.1.2 Carotenoid biosynthetic pathway in bacteria and fungi

Bacteria and fungi derive C_5 isoprenoid precursors, i.e. isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) mainly via the mevalonate (MVA) pathway. A few bacterial *Streptomyces* species generate isoprenoid precursors using both the MVA and the 2-C-methyl-Derythritol-4-phosphate (MEP) pathways (Walter and Strack 2011, Moise *et al.* 2013). The MVA and the MEP pathways have been described in detail in a previous review (Moise *et al.* 2013). IPP is condensed with its isomer DMAPP to generate C_{10}-geranyl pyrophosphate (GPP) and elongate to C_{15}-farnesyl pyrophosphate (FPP) and C_{20}-geranylgeranyl diphosphate (GGPP) (Figure 2). In bacteria, the early step of carotenogenic pathway is the biosynthesis of the first carotene, phytoene, catalyzed by phytoene synthase (CrtB) from two GGPP molecules. Phytoene is desaturated by phytoene desaturase (CrtI) to form the linear, all-*trans* lycopene. The cyclation of lycopene (Figure 3) by lycopene β-cyclase (CrtY) introduces β-ionone end groups to form β-carotene. Hydroxylation of the carotene rings of β-carotene generates zeaxanthin by β-carotene hydroxylase (CrtZ).

In the bacterium *Erwinia uredovora* (also named as *Pantoea ananatis*), the carotenogenic gene cluster consists of *crtE*, *crtX* (encoding zeaxanthin β-glucosidase), *crtY*, *crtI*, *crtB*, and *crtZ* (Misawa *et al.* 1990, Misawa *et al.* 1995). Expression of these carotenogenic genes in *Escherichia coli* can result in carotenoid production. Plasmids pACCRT-EB, pACCRT-EIB, pACCAR16Δ*crtX*, and pACCAR25Δ*crtX* have been constructed and expressed in *E. coli* resulting in phytoene, lycopene, β-carotene, and zeaxanthin, respectively (Misawa *et al.* 1995). In the marine bacterium *Agrobacterium aurantiacum*, CrtZ and CrtW (carotene ketolase) (Figure 3) could mediate the oxygenation reactions from β-carotene to astaxanthin via multiple

intermediates, such as echinenone, canthaxanthin, adonixanthin, and adonirubin (Misawa *et al.* 1995). In some bacteria, C_{30}-carotenoids are synthesized via an independent route from two molecules of C_{15}-FPP condensed to dehydrosqualene by 4,4'-diapophytoene synthase (CrtM), and then 4,4′-diapophytoene desaturase (CrtN) catalyzes a three- or four-step reaction to form the yellow carotenoid 4,4’-diaponeurosporene or the red carotenoid 4,4’-diapolycopene in the C_{30}-carotenoid pathway (Figure 2) (Wieland *et al.* 1994, Umeno *et al.* 2002).

In fungi, enzymatic activities of phytoene synthase and lycopene cyclase are encoded by one fusion gene, *CrtYB*. So three enzymes CrtE, CrtI and CrtYB are involved in the synthesis of γ-carotene or β-carotene. Lycopene is the major branch point of the carotenogenic pathway, where it acts as precursor of cyclic carotenoids such as γ-carotene, β-carotene, torulene, torularhodin and astaxanthin. The *CrtI* gene from the fungus *Neurospora crassa* participated in a five-step desaturation to synthesize 3,4-didehydrolycopene (Figure 2), which can be converted to the monocyclic carotenoid torulene (Hausmann and Sandmann 2000). The single desaturase *CrtI* gene from red yeast *Sporidiobolus pararoseus* can be responsible for both four- and five-step dehydrogenation of phytoene, resulting in the production of lycopene (fourth step product) and 3,4-didehydrolycopene (fifth step product) (Li *et al.* 2016). In red yeast *X. dendrorhous*, astaxanthin synthase (CrtS), a cytochrome P450 enzyme, in combination with its cytochrome P450 reductase, are responsible for astaxanthin biosynthesis (Alcaíno *et al.* 2012).

2.2 Carotenoid biosynthesis in cyanobacteria, algae and plants

2.2.1 Carotenoids produced by cyanobacteria, algae and plants

Many cyanobacteria contain β-carotene, zeaxanthin, echinenone and myxoxanthophyll, and some cyanobacterial species contain additional carotenoids, such as canthaxanthin and nostoxanthin (Figure 1A) (Takaichi and Mochimaru 2007).

Microalgae species belonging to *Chlorella*, *Haematococcus*, and *Dunaliella*, are considered as the key producers of commercially vital carotenoids. *Chlorella* and *Haematococcus* species can produce astaxanthin, lutein, and β-carotene. *Haematococcus pluvialis* can naturally accumulate astaxanthin up to 3~5% of dry cell weight (DCW). *Dunaliella* species can accumulate up to 10% of β-carotene by DCW at certain stress conditions (Liang *et al.* 2015, Saini and Keum 2017). Some carotenoids are found only in some algal species. In algae, the distribution of α-carotene and its derivatives, such as lutein, loroxanthin and siphonaxanthin (Figure 1A), are limited to divisions of Rhodophyta (macrophytic type), Euglenophyta, Cryptophyta, Chlorarachniophyta and Chlorophyta (Takaichi 2011). C_{40}-skeletal fucoxanthin only can be found in brown algae and diatoms; C_{37}-skeletal peridinin only in Dinoflagellates, and the acetylenic carotenoids diadinoxanthin and diatoxanthin in Heterokontophyta, Haptophyta, Dinophyta and Euglenophyta (Takaichi 2011).

In land plants, carotenoids can accumulate in the plastids of fruits, flowers, leaves, roots and seed in higher plants (Howitt and Pogson 2006). The major carotenoids are β-carotene, lutein, violaxanthin, and 9'-*cis* neoxanthin (Figure 1A), which are derivatives of β-carotene and α-carotene. Ripe tomato fruits accumulate large amounts of the red linear carotene, lycopene.

Capsanthin and capsorubin are found mainly in flowers of lilies and matured fruits of red peppers (Jeknić *et al.* 2012). Flowers of *Adonis aestivalis*, as a special case of higher plants have a blood-red color derived from astaxanthin (Cunningham and Gantt 2011).

2.2.2 Carotenoid biosynthetic pathway in cyanobacteria, algae and plants

Photosynthetic cyanobacteria, algae, and plants can utilize the MEP pathway to synthesize C5 isoprenoid precursors, while algae and plants can also generate isoprenoid precursors via the MVA pathways (Walter and Strack 2011, Moise *et al.* 2013). In higher plants and algae, the early step of carotenogenic pathway (Camara *et al.* 1992, Liang *et al.* 2015, Nisar *et al.* 2015) (Figure 2) is the biosynthesis of phytoene from two GGPP molecules catalyzed by phytoene synthase (PSY). Then phytoene is desaturated by phytoene desaturases (PDS) and ζ-carotene desaturases (ZDS) and isomerized by 15-cis-ζ-carotene isomerase (ZISO) and carotenoid isomerase (CRTISO) to form the linear all *trans*-lycopene (ψ,ψ-carotene). Lycopene cyclases (Figure 3) catalyze cyclization reactions of lycopene. Lycopene β-cyclase (LCYb) is responsible for formation of two β-rings from lycopene to β-carotene (β,β-carotene) via γ-carotene (β,ψ-carotene). α-Carotene (β,ε- carotene) can be synthesized from lycopene via γ-carotene (β,ψ-carotene) or δ-carotene (ψ,ε- carotene) by LCYb and lycopene ε-cyclase (LCYe). LCYe can also introduce two ε-rings to synthesize ε-carotene (ε,ε- carotene). However, carotenes with two ε-rings are not generally found in plants, because LCYe enzymes from most plants tend to form only one ε-ring. It was reported that the LCYe from lettuce plants and the liverwort *Marchantia polymorpha* can convert lycopene to ε-carotene (Cunningham and Gantt 2001, Takemura *et al.* 2014).

β-Carotene is hydroxylated mainly by the non-heme di-iron β--carotene hydroxylase (BCH), which is converted into β--cryptoxanthin and then zeaxanthin. While α-carotene is mainly hydroxylated by cytochrome P450-type enzymes (CYP97A mainly catalyzes hydroxylation of the β-ring of α-carotene, and CYP97C catalyzes hydroxylation of the ε-ring of α-carotene), which can convert α-carotene into zeinoxanthin or α-cryptoxanthin, and then into the yellow pigment lutein. Recently, it was reported that red algal CYP97B29 has both β- and ε- ring activities, suggesting that the *CYP97A*, CYP97*B*, and CYP97*C* subfamilies originated before the divergence of higher plants and green algae lineages (Yang *et al.* 2014).

Zeaxanthin is converted by zeaxanthin epoxidase (ZEP) to antheraxanthin by epoxidation of one β- ring, and then epoxidation of the second β- ring leads to formation of violaxanthin. Violaxanthin de-epoxidase (VDE) reverses these reactions in intense light. Violaxanthin is converted to neoxanthin by neoxanthin synthase (NXS). In algae, two different hypotheses regarding the biosynthetic pathways of diadinoxanthin cycle carotenoids have been proposed (Figure 3). Violaxanthin or neoxanthin may be used as precursors of diadinoxanthin and fucoxanthin (Bertrand 2010, Dambek *et al.* 2012, Mikami and Hosokawa 2013). Some enzymes involved in these carotenoid biosynthetic pathways are still unknown. Diadinoxanthin can be converted into diatoxanthin by diadinoxanthin de-epoxidase (DDE), and this reaction can be reversible by diatoxanthin epoxidase (DEP) in low light conditions.

In the green alga *H. pluvialis*, β-carotene ketolase (BKT) not only converts β-carotene to canthaxanthin via echinenone but also converts zeaxanthin to astaxanthin via adonixanthin (Kajiwara *et al.* 1995). *Adonis aestivalis* as a special case of higher plants, can accumulate

astaxanthin in petals (Cunningham and Gantt 2011). Two astaxanthin synthesis related enzymes, carotenoid 4-hydroxy-β-ring 4-dehydrogenase (HBFD) and carotenoid β-ring 4-dehydrogenase (CBFD) can convert β-carotene to astaxanthin in *Adonis aestivalis* (Figure 3) (Cunningham and Gantt 2011). Firstly, the β- ring of β- carotene is hydroxylated at C4 by CBFD. Then, the hydroxyl group is dehydrogenated by HBFD to form a keto group. Finally, a second hydroxylation reaction at C3 by the same CBFD enzyme is carried out to synthesize astaxanthin with single keto and hydroxyl groups of the two β- rings.

In cyanobacteria, the lycopene biosynthetic pathway needs three enzymes (CrtP, CrtQ, CrtH), in which CrtP-type phytoene desaturase and CrtQ-type ζ-carotene desaturase catalyze two sequential desaturation steps respectively, and CrtH-type isomerase carries out the isomerization reaction. The cyclization reactions of lycopene to β-carotene can be catalyzed by CrtL- or CruA-type cyclases. β-Carotene can be hydroxylated by CrtR-type hydroxylase and ketonized by CrtW- or CrtO-type ketolase (Liang *et al.* 2006, Takaichi and Mochimaru 2007). Bacteria seldom produce α-carotene, but the genus *Prochlorococcus* belonging to cyanobacteria share the presence of high amounts of α- and β-carotenes with green algae and higher plants (Stickforth *et al.* 2003). And two lycopene cyclases have been found in *Prochlorococcus*: one is the typical lycopene β-cyclase; another is the bifunctional enzyme exhibiting lycopene β-cyclase activity but also catalyzing the formation of ε-ionone end groups (Stickforth *et al.* 2003).

2.3 Carotenoid degradation in all kinds of organisms

The broad diversity of apocarotenoid volatile compounds can be attributed to large numbers of carotenoid precursors and the various cleavage sites catalyzed by CCOs (Walter and Strack

2011). But little is known about the function exerted by carotenoid cleavage products in living systems.

In cyanobacteria, apocarotenoids act as photoprotective and accessory pigments. Besides, cyanobacteria can be responsible for undesired odor in water and fish from aquiculture. In fungi, there are mainly three kinds of apocarotenoid compounds: retinal, neurosporaxanthin, and trisporic acids (the sexual hormones) (Figure 1B) (Ahrazem *et al.* 2016a). In bacteria, fungi and animals, CCOs are essential for the biosynthesis of retinal (Ahrazem *et al.* 2016a).

In plants, the oxidative cleavage of carotenoids leads to the production of a range of apocarotenoids compounds that serve critical functions including photoprotection, photosynthesis, pigmentation, and signaling (Hou *et al.* 2016). The apocarotenoids include derivatives of β-ionone, which can exhibit pleasant scent and aroma in many flowers, and two important signaling molecules ABA and strigolactones (Figure 1B).

3. Enzymes involved in carotenoid biosynthetic pathway

3.1 GGPP to phytoene: phytoene synthase (PSY)

The C_{40}-carotenoid, 15-*cis* phytoene is synthesized by the condensation of two molecules of GGPP catalyzed by phytoene synthase (CrtB, PSY, EC 2.5.1.32). In some non- phototrophic bacteria, the C_{30}-carotenoid dehydrosqualene is synthesized by the condensation of two molecules of C_{15}-FPP by 4,4'-diapophytoene synthase (CrtM, EC 2.5.1.96) (Wieland *et al.* 1994, Umeno *et al.* 2002). Plant, algal and cyanobacterial PSY protein sequences are similar to the homologous bacterial and fungal CrtB enzymes. The difference is that more than one PSY

isoform can be existed in higher plants and some algae, such as *Ostreococcus* and *Micromonas* (Tran *et al.* 2009, Dibari *et al.* 2012). In monocots, the PSY gene family is characterized by three paralogous genes, annotated as PSY1, PSY2 and PSY3; whereas, in eudicots, the presence of PSY1 and PSY2 homologs was reported (Dibari *et al.* 2012). But there is only one *PSY* gene in *Arabidopsis*. The *Arabidopsis PSY* gene is expressed virtually in all tissues, including both photosynthetic and non-photosynthetic tissues (Welsch *et al.* 2003). Some PSY isoforms participate in the carotenoids biosynthesis in chloroplast-containing photosynthetic tissues, such as the leaf (tomato PSY2) (Bartley and Scolnik 1993), whereas others are involved in the carotenoids biosynthesis in non-photosynthetic tissues, such as the fruit (tomato PSY1) (Fraser *et al.* 2002), the seed endosperm (maize PSY1) (Li *et al.* 2008b), or the root (cassava, maize and rice PSY3) (Li *et al.* 2008a, Welsch *et al.* 2008, Arango *et al.* 2010). It was reported that PSY3 plays a specialized role in abiotic stress-induced ABA formation (Welsch *et al.* 2008).

3.2 Phytoene to lycopene: desaturases and isomerases

3.2.1 CrtI-type phytoene desaturases

CrtI-type phytoene desaturases commonly exist in many bacteria and fungi (Verdoes *et al.* 1999b, Harada *et al.* 2001). One CrtI-type bacterial desaturase (EC 1.3.99.28) from *Rhodobacter capsulatus* participated in a carotenogenic pathway to all-*trans* neurosporene in a three-step desaturation of phytoene (Raisig *et al.* 1996). Another CrtI-type bacterial desaturase (EC 1.3.99.31) from *Erwinia uredovora* involved in four desaturation steps to form all-*trans* lycopene (Fraser *et al.* 1992). The third CrtI-type phytoene desaturase (EC 1.3.99.30) from the fungus *Neurospora crassa* took part in a five-step desaturation to synthesize 3,4-dehydrolycopene (Hausmann and Sandmann 2000). Recently, it was reported that a single CrtI-type phytoene

desaturase gene from red yeast *Sporidiobolus pararoseus* CGMCC 2.5280 is responsible for both four- and five-step dehydrogenation of phytoene, leading to the conversion of phytoene to lycopene (four-step product) and 3,4-didehydrolycopene (fifth-step product) (Li *et al.* 2016). Fourthly, 2-step CrtIa-type phytoene desaturase (EC 1.3.99.29) and CrtIb-type *all-trans*-ζ-carotene desaturase (EC 1.3.99.26) performed the four desaturation steps that convert phytoene to lycopene (Iniesta *et al.* 2007). Additionally, the 4,4′-diapophytoene desaturase (CrtN, EC 1.3.8.2) from *Staphylococcus aureus* catalyzed a three-step reaction to form *all-trans*-4,4'-diaponeurosporene in the C_{30}-carotenoid pathway (Raisig and Sandmann 1999). Heliobacteria can accumulate 4,4′-diaponeurosporene as the main pigment and trace amounts of diapolycopene, diapo-ζ-carotene and diapophytofluene (Takaichi *et al.* 1997).

Although 15-*cis* phytoene isomer is used as substrate catalyzed by CrtI-type desaturases, all subsequent products like phytofluene, ζ-carotene, neurosporene and lycopene are formed as all-*trans* isomers (Figure 2) (Sandmann 2009). The CRTI enzyme is shown to require flavin adenine dinucleotide (FAD) as a cofactor and oxygen (aerobic) or quinones (anaerobic) as final electron acceptors (Schaub *et al.* 2012). The above desaturases all belong to a CrtI gene family together with the CrtN genes in a C_{30}-carotenoid pathway. The C_{40} four-step desaturases are closer related to the three-step C_{40} desaturases than to the CrtN desaturases (Figure 4).

Moreover, C-3,4 desaturase (CrtD, EC 1.3.99.27) can catalyze the addition of one double bond between C-3 and C-4. CrtD from marine bacterium strain P99-3 of *Flavobacteriaceae* plays a role in the synthesis of myxol (Teramoto *et al.* 2004). CrtD from *Synechocystis* sp. strain PCC 6803 is involved in myxoxanthophyll biosynthesis (Mohamed and Vermaas 2004).

Myxoxanthophyll and myxol have a double bond at the C-3,4 position. CrtD is structurally related to bacterial CrtI-phytoene desaturase, CrtN and CrtH (Figure 4). Substrates for these desaturases are quite different: diapophytoene for CrtN, hydroxyneurosporene or neurosporene for CrtD, ζ-carotene for CrtQ and phytoene for other bacterial desaturases.

3.2.2 PDS/CrtP phytoene desaturase and ZDS/CrtQ-type ζ-carotene desaturase

PDS/CrtP-type phytoene desaturase (EC 1.3.5.5) can transform 15-*cis* phytoene into 15,9'-di-*cis*-phytofluene, and eventually 9,15,9'-tri-*cis*-ζ-carotene (Figure 2). The cyanobacterial CrtP and plant PDS sequences were totally different to CrtI-type desaturase with a different phylogenic origin (Figure 4) (Sandmann 2009). In plants and algae, PDS and ZDS require the transfer of two electrons to oxidized quinones by plastid terminal oxidase (PTOX) using O_2 as a terminal acceptor (Carol and Kuntz 2001).

ZDS/CrtQ-type ζ-Carotene desaturase (EC 1.3.5.6), mainly in cyanobacteria, algae and plants, catalyzes two desaturation steps from 9,9'-di-*cis*-ζ-carotene to 7,9,7',9'-tetra-*cis*-lycopene via 7,9,9'-tri-*cis*-neurosporene (Figure 2). CrtQa from *Anabaena* sp. PCC 7120 (also named as *Nostoc* sp. PCC7120) was functionally identified to convert ζ-carotene to lycopene (Linden *et al.* 1994), while CrtQb can be found in *Synechocystis* PCC 6803 (Breitenbach *et al.* 1998). *Gloeobacter violaceous* PCC 7421 was the only cyanobacterium which retained a CrtI-type phytoene desaturase (Tsuchiya *et al.* 2005). CrtQa shares little sequence similarity to the CrtP-type phytoene desaturase, but it has considerable conserved with the CrtI-type desaturase and CrtH. It is possible that the cyanobacterial CrtQa has an evolutionary link between the original CrtI phytoene desaturase and the CrtH isomerase. Additionally, CrtQb shows a high similarity to the CrtP-type desaturases (Takaichi and Mochimaru 2007). After desaturation by

CrtQb, CrtH or CRTISO catalyzes the isomerization to form all-*trans* lycopene (Masamoto *et al.* 2001, Isaacson *et al.* 2004). Light is also effective for their photoisomerization to all-*trans* forms (Bartley *et al.* 1999). In contrast to CrtQb, the reaction product of CrtQa is all-*trans* lycopene.

3.2.3 15-*cis*-ζ-carotene isomerase (ZISO) and carotenoid isomerase (CrtH/CRTISO)

Plant PDS produces 9,15,9′-tri-*cis*-ζ-carotene, which must be isomerized to form 9,9'-di-*cis*-ζ-carotene, the substrate of a second desaturase, ZDS (Breitenbach and Sandmann 2005). ZISO (15-*cis*-ζ-carotene isomerase, EC 5.2.1.12) catalyzes the *cis*- to *trans*-conversion of the 15-*cis*-bond in 9,15,9'-tri-*cis*-ζ-carotene to form 9,9'-di-*cis*-ζ-carotene (Figure 2). In the chloroplasts of photosynthetic tissues, this isomerization can occur in the presence of light via photoisomerization, but an enzymatic isomerization by ZISO is required in etiolated leaves and roots in the absence of light (Breitenbach and Sandmann 2005, Li *et al.* 2007). ZISO homologues can be found in plants and algae. ZISO shows no sequence homology to any known carotenogenic enzyme (Figure 4). It was reported that ZISO evolved from an ancestor related to nitrite and nitric oxide reductase U (NnrU) from denitrifying bacteria (Chen *et al.* 2010). Recently, it was demonstrating that ZISO catalyzes isomerization through a unique mechanism requiring a redox-regulated heme cofactor (Beltrán *et al.* 2015).

Carotenoid isomerase enzyme (EC 5.2.1.13) is known as CRTISO in plants (Isaacson *et al.* 2002, Park *et al.* 2002) and as CrtH in cyanobacteria (Breitenbach *et al.* 2001, Masamoto *et al.* 2001) that converts 7,9,7',9'-tetra-*cis*-lycopene (prolycopene) to all *trans*-lycopene (Figure 2). CrtH/CRTISO is structurally related to CrtI-type desaturase, and shows some sequence similarities to plant desaturases (PDS and ZDS) (Figure 4). It was suggested that CrtI might be the ancestor to evolve into CRTISO by losing the desaturase activity but retaining the function

for *cis* to *trans* isomerization (Sandmann 2009). The CRTISO activity can be partially substituted by light in green tissues via photoisomerization (Isaacson *et al.* 2002, Isaacson *et al.* 2004). In contrast to green tissues, CRTISO activity cannot be replaced by light in nonphotosynthetic tissues (Isaacson *et al.* 2002). CRTISO activity requires the presence of membranes and an enzyme-bound FAD cofactor in a reduced form (Yu *et al.* 2011). An epigenetic mechanism contributes to the regulation of carotenoid isomerization (Cazzonelli *et al.* 2009, Cazzonelli *et al.* 2010). The Set Domain Group 8 (SDG8) is a chromatin-modifying histone methyltransferase targeting the CRTISO promoter. CRTISO expression requires SDG8 activity during seedling development in *Arabidopsis* (Cazzonelli *et al.* 2010).

3.3 Lycopene to cyclic carotenoids: lycopene cyclases

3.3.1 The monomeric lycopene cyclases from bacteria, algae and plants

The CrtY-type lycopene cyclases can be found in many bacteria, such as *Erwinia uredovora* (Misawa *et al.* 1990) and *Agrobacterium aurantiacum* (Misawa *et al.* 1995), which may be the ancestor of CrtL-type lycopene cyclase in cyanobacteria and LCY-type in plants (Figure 5). CrtL-type cyanobacterial lycopene cyclase was known from *Synechococcus* sp. PCC 7942 (Cunningham *et al.* 1994), which, in turn, may have given rise to plant lycopene β- and ε-cyclase (LCYb, EC 5.5.1.19; and LCYe, EC 5.5.1.18) (Cunningham *et al.* 1996, Cunningham and Gantt 2001). And two lycopene cyclases have been found in *Prochlorococcus*: one is the typical CrtL cyclase; another is the bifunctional CrtLe cyclase exhibiting lycopene β-cyclase activity but also catalyzing the formation of ε-ionone end groups (Stickforth *et al.* 2003).

Carotenes with two ε-rings are not generally found in plants, because LCYe enzymes from most plants tend to form only one ε-ring. It was reported that the LCYe from lettuce plants and the liverwort *Marchantia polymorpha* can convert lycopene to ε-carotene (Cunningham and Gantt 2001, Takemura *et al.* 2014). LCYb and LCYe share significant similarities in their amino acid sequences, suggesting that they have originated from a common ancestor through gene duplication (Figure 5). CrtL- and CrtY-type cyclases do not share much similarity; however, they contain conserved sequence patterns indicating evolutionary relatedness (Krubasik and Sandmann 2000b).

The enzyme capsanthin-capsorubin synthase (CCS), known from *Capsicum annuum* and *Lilium lancifolium* (Bouvier *et al.* 1994, Hugueney *et al.* 1995, Jeknić *et al.* 2012), belongs to another member of the CrtL family. The CCS enzyme converts a six- carbon epoxy ring of antheraxanthin and violaxanthin to a κ- ring containing five carbons and one hydroxy group (Figure 3). CCS is highly homologous to LCYB. It also shows cyclization activity, but converts lycopene into β- carotene with much lower efficiency. Additionally, the tentative neoxanthin synthase (NSY) was shown to represent a chromoplast-specific lycopene β-cyclase (CycB) in tomato (Ronen *et al.* 2000). NSY is structurally similar to two carotenogenic enzymes, lycopene cyclase and CCS (Figure 5) (Bouvier *et al.* 2000).

3.3.2 The heterodimeric lycopene cyclases

The heterodimeric lycopene cyclases, CrtYc and CrtYd can be found mainly in the Gram-positive bacteria, such as *Brevibacterium linens* (Krubasik and Sandmann 2000a) and *Mycobacterium aurum* (Viveiros *et al.* 2000). These two different genes *CrtYc* and *CrtYd* encoded two small polypeptides (subunits), which interacted as a heterodimer to convert

lycopene into β-carotene. Moreover, the fusion-type lycopene cyclase encoded by one gene was found in archae *Halobacterium salinarum* (Peck *et al.* 2002) and *Sulfolobus solfataricus* (Hemmi *et al.* 2003). The N- and C-terminal halves of this fusion gene were homologous to the subunits of the bacterial heterodimeric enzymes, respectively (Figure 5).

3.3.3 The bifunctional lycopene cyclase/phytoene synthase in fungi

The bifunctional lycopene cyclase/phytoene synthase, designated as CrtYB (or CarRA), can be found in fungi, such as *Xanthophyllomyces dendrorhous* (Verdoes *et al.* 1999a), *Rhodosporidium diobovatum* (Guo *et al.* 2014), *Mucor circinelloides* (Velayos *et al.* 2000) and *Phycomyces blakesleeanus* (Arrach *et al.* 2001). CrtYB is expressed as a fusion protein with lycopene cyclase and phytoene synthase activities. Its C-terminal (about 400 amino acids) is homologous to phytoene synthases and has phytoene synthase activity; and the residual N-terminal region (about 200 amino acids), which is homologous to the heterodimeric lycopene cyclases CrtYc and CrtYd, has the catalytic domain of lycopene cyclase (Figure 5).

3.3.4 CruA-type lycopene cyclase

The novel type of lycopene cyclase, CruA, was firstly found in the photosynthetic green sulfur bacterium *Chlorobium tepidum* (Maresca *et al.* 2007). Two homologs of CruA, defined as CruA and CruP, also can be found in some cyanobacteria lacking CrtL-type lycopene cyclase, such as *Synechococcus* sp. PCC 7002, and both were shown to have lycopene cyclase activity (Maresca *et al.* 2007). However, heterologous expression of CruA from *Synechococcus* sp. PCC 7002 or *Synechocystis* sp. PCC 6803 in *E. coli* strains that could synthesize either lycopene or γ-carotene failed to detect the content of either γ-carotene or β-carotene, respectively (Maresca *et al.* 2007, Xiong *et al.* 2016). Recently, it was demonstrated that CruA has lycopene cyclase activity and to

reveal why CruA is inactive when expressed in *E. coli*; since CruA requires a binding of chlorophyll a molecule for cyclase activity (Xiong *et al.* 2016). Interestingly, CruP from *Synechocystis* sp. PCC 6803 did not have lycopene cyclase activity. In addition, it was found that CruP from *Arabidopsis thaliana* has a function other than lycopene cyclization, but plays a role in reducing reactive oxygen species (ROS) levels in oxygenic photosynthetic organisms under photoinhibitory stress (Bradbury *et al.* 2012). Moreover, the CruA- and CruP-type carotenoid cyclases are members of the FixC dehydrogenase superfamily and are distantly related to CrtL- and CrtY-type lycopene cyclases (Figure 5) (Maresca *et al.* 2007).

3.4 Cyclic carotenoids to xanthophylls: hydroxylases, ketolases and epoxidases

3.4.1 Carotenoid hydroxylases (CHYs)

Two classes of structurally unrelated enzymes of CHYs have been found in all biological organisms: one is non-heme di-iron β-carotene hydroxylase enzyme (EC 1.14.13.129), i.e. CrtZ-type in bacteria, CrtR-type in cyanobacteria, and BCH-type in plants and algae (Misawa *et al.* 1995, Lagarde and Vermaas 1999, Linden 1999, Kim *et al.* 2009); another is the heme-containing cytochrome P450 enzyme (P450-type), i.e. CYP175A1 from bacteria, CrtS (Asy) from fungi, and CYP97-type in plants and algae (Blasco *et al.* 2004, Álvarez *et al.* 2006, Kim *et al.* 2009).

3.4.1.1 BCH/CrtR/CrtZ-type β-carotene hydroxylase

BCH, CrtR, and CrtZ encode for β-carotene 3,3′-hydroxylase (or carotenoid 3,3′-hydroxylase; 3,3′-β-hydroxylase). CrtR from *Synechocystis* sp. PCC 6803 was involved not only in zeaxanthin synthesis but also in myxoxanthophyll synthesis (Lagarde and Vermaas 1999). BCH from

Haematococcus pluvialis was able to catalyze not only the conversion of β-carotene to zeaxanthin, but also the conversion of canthaxanthin to astaxanthin (Linden 1999). Plant BCHs mainly catalyze the conversion of β-carotene to zeaxanthin, and they share ~30% sequence identities with CrtZ and possibly have a common origin with CrtZ (Figure 6A) (Sun *et al.* 1996, Kim *et al.* 2009). Interestingly, cyanobacterial CrtR shows similarity with CrtW carotene ketolases from marine bacteria and BKT from *H. pluvialis* rather than other carotene hydroxylases (Figure 6B) (Lagarde and Vermaas 1999).

Moreover, a novel enzyme, 2,2'-β-hydroxylase (CrtG) has been identified from *Brevundimonas* sp. Strain SD212 (a marine bacterium) (Komemushi *et al.* 2005) and *Thermosynechococcus elongatus* Strain BP-1 (a thermophilic cyanobacterium) (Iwai *et al.* 2008), which synthesized 2-hydroxylated carotenoids such as caloxanthin ((2*R*,3*R*,3′*R*)-β,β-carotene-2,3,3′-triol) and nostoxanthin ((2*R*,3*R*,2′*R*,3′*R*)-β,β-carotene-2,3,2′,3′-tetrol). CrtG shares similarity with bacterial CrtZ (Figure 6A).

3.4.1.2 P450-type carotenoid hydroxylases

The heme enzyme CYP175A1 has been identified from the bacterium *Thermus thermophilus* HB27 and shown to have the same function as the above-mentioned non-heme β-carotene hydroxylase; however, CYP175A1 did not show any sequence similarity with bacterial CrtZ (Figure 6A) (Blasco *et al.* 2004).

In red yeast *X. dendrorhous*, a cytochrome P450 enzyme, astaxanthin synthase (CrtS or Asy), in combination with its cytochrome P450 reductase, are responsible for astaxanthin

biosynthesis (Álvarez *et al.* 2006, Ojima *et al.* 2006, Alcaíno *et al.* 2012). However, CrtS shows no significant identity with carotenoid hydroxylases from bacteria, algae, and plants (Figure 6A).

In plants and algae, the CYP97 family functions in xanthophyll biosynthesis, and the three subfamilies CYP97A, CYP97B, and CYP97C are conserved from algae to land plants (Figure 6A). CYP97A3 (LUT5) and CYP97C1 (LUT1, EC 1.14.99.45) enzymes in *Arabidopsis* are primarily responsible for catalyzing hydroxylation of the β- and ε-rings of α-carotene, respectively (Tian *et al.* 2004, Kim and DellaPenna 2006). It has been showed that P450- type carotenoid hydroxylase (PuCHY1) from *Porphyra umbilicalis* share no significant sequence similarity with CYP175A1 or CrtS, the two P450- type carotenoid hydroxylases from bacteria and fungi, respectively, which do not belong to the CYP97 family (Yang *et al.* 2014).

3.4.2 CrtW/BKT/CrtO-type carotene ketolases

In bacteria, astaxanthin can be synthesized from β-carotene with the introduction of keto groups at the C-4,4′ positions and hydroxyl groups at the C-3,3′ positions into the β-ionone rings by CrtW (β-carotene 4,4′-ketolase; carotenoid 4,4′-oxygenase; 4,4′-β-oxygenase) and CrtZ via many hydroxylated or ketolated carotenoid intermediates (Misawa *et al.* 1995). CrtW enzymes show homology to the CrtR-type β-hydroxylase from cyanobacteria (Figure 6B) (Lagarde and Vermaas 1999). In green algae, BKT from *H. pluvialis* can convert β-carotene to canthaxanthin via echinenone and convert zeaxanthin to astaxanthin (Kajiwara *et al.* 1995). In cyanobacteria, a novel β-carotene ketolase called CrtO from *Synechocystis* sp. 6803 can introduce an asymmetric keto group into the β-ionone ring resulting in the formation of the monoketolated derivative echinenone from β-carotene (Fernández-González *et al.* 1997). CrtO is structurally related to the CrtI-type phytoene hydrogenases (Figure 6B) (Fernández-González *et al.* 1997).

3.4.3 Epoxidases and de-epoxidases

In plants and algae, two enzymes, ZEP and VDE, catalyze reversible reactions of epoxidation/de- epoxidation involved in the xanthophylls cycle (violaxanthin cycle) (Figure 3). Violaxanthin is converted to neoxanthin by NXS. Violaxanthin and neoxanthin can be isomerized to 9- *cis*- violaxanthin and 9′- *cis*- neoxanthin, respectively, which are the precursors for ABA biosynthesis. Several algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle (Lohr and Wilhelm 1999). The diadinoxanthin cycle consists of the conversion of diadinoxanthin to diatoxanthin by DDE in high light and the reverse reaction by DEP in low light.

4. Enzymes involved in carotenoid cleavage pathway

4.1 CCOs from plants

4.1.1 NCEDs from plants

In plants, carotenoid cleavage dioxygenases (CCDs, EC 1.14.99.n4) and 9-*cis*-epoxycarotenoid dioxygenases (NCEDs, EC 1.13.11.51) constitute the carotenoid cleavage oxygenase (CCO) family that catalyze the cleavage of carotenoids at specific double bonds (Rosas-Saavedra and Stange 2016). VP14 (*viviparous14*) from maize, which cleaves the 11,12 double bond of 9-*cis*-epoxycarotenoids (9-*cis*-violaxanthin or 9'-*cis*-neoxanthin) to produce the precursor of ABA, C_{15}-xanthoxin and a C_{25}-aldehyde (Figure 7A), is the first identified member of this enzyme family (Schwartz *et al.* 1997). Based on the substrate specificity, VP14 and its orthologs involved in ABA biosynthesis have been termed NCEDs.

ABA is a plant hormone that can control several important plant processes including embryo maturation, seed dormancy, germination, and responses to biotic and abiotic stresses (Finkelstein 2013). Besides higher plants, ABA can be also present in cyanobacteria, fungi, algae, moss, and lichens (Hartung 2010). Under stress conditions, the amount of endogenous ABA both in algae and higher plants, increases substantially. Green algae, such as *Chlorella* sp., *D. salina*, and *H. pluvialis*, can synthesize ABA, but *NCED*-homologous genes appear to be lacking in these algae. Algae, cyanobacteria and fungi that lack 9-*cis*-epoxycarotenoids or NCED enzymes synthesize ABA directly via C_{15}-FPP (Hartung 2010, Kiseleva *et al.* 2012), whereas higher plants synthesize ABA through a C_{40}-carotenoid-derived indirect pathway (Nambara and Marion-Poll 2005).

4.1.2 CCDs from plants

CCDs are distantly related to the NCEDs (Figure 8). Plant CCDs can be divided into five members, CCD1, CCD2, CCD4, CCD7 and CCD8, depending on their substrate specificities and cleavage sites (Schwartz *et al.* 2001, Tan *et al.* 2003, Auldridge *et al.* 2006, Frusciante *et al.* 2014). CCDs can cleave multiple carotenoid substrates to produce various apocarotenoids at different double bounds on the carotenoid backbone (Auldridge *et al.* 2006, Beltran and Stange 2016).

CCD1 enzymes can be involved in the cleavage of the 5,6 (5',6') (Vogel *et al.* 2008), 7,8 (7',8') (Ilg *et al.* 2009) and 9,10 (9',10') (Schwartz *et al.* 2001) double bonds to produce a variety of volatiles in different plant species (Figure 7B). In most instances, the plant CCD1 was able to cleave a variety of carotenoids at the 9,10 (9',10') positions symmetrically to produce a C_{14}-dialdehyde and two C_{13}-products such as pseudoionone, β-ionone and 3-hydroxy-β-ionone,

which vary depending on the carotenoid substrates (Schwartz *et al.* 2001, Simkin *et al.* 2004). It was reported that the *Arabidopsis*, maize, and tomato CCD1 enzymes also act on the 5,6 (5',6') double bonds of lycopene, generating the C_8-volatile 6-methyl-5-hepten-2-one (MHO) (Vogel *et al.* 2008). Additionally, rice CCD1 can cleave lycopene into C_{10}-geranial at the 7,8 (7',8') double bonds, which constitute a novel cleavage site for the CCD1 plant subfamily (Ilg *et al.* 2009). CCD1 distinguishes itself from other reported CCDs, as CCD1 is localized to the cytosol, but also known to associate with the cytoplasm-facing outer chloroplast membrane (Hou *et al.* 2016).

The CCD2 enzyme, very related to the CCD1 family (Figure 8), has been found in the stigmas of *Crocus* species as the key enzyme involved in the synthesis of saffron apocarotenoids, i.e. crocetin, crocins, picrocrocin, and safranal (Frusciante *et al.* 2014). CCD2 can cleave sequentially the 7,8 and 7',8' double bonds adjacent to a 3-hydroxy-β-ionone ring converting zeaxanthin to crocetin dialdehyde (Figure 7C) (Frusciante *et al.* 2014). No CCD2 homologues have been identified in other plants due to the very limited presence of crocetin and its derivatives in plants (Ahrazem *et al.* 2016b). CCD2 is localized in plastids, which is a major difference from the CCD1 subfamily (Ahrazem *et al.* 2016b).

The CCD4 family is only present in flowering plants and located in the plastids. Similar to CCD1, CCD4 is involved in the formation of volatile compounds and carotenoid turnover at the 9,10 (9',10') or the 7,8 (7',8') positions (Figure 7D) (Huang *et al.* 2009, Rodrigo *et al.* 2013). CCD4s produce β-ionone by cleaving β-carotene, β-apo-8'-carotenal, β-cryptoxanthin, or zeaxanthin at the 9,10 and/or 9',10' double bond (Rubio *et al.* 2008, Huang *et al.* 2009, Bruno *et*

al. 2015), and also asymmetrically cleave β-carotene, β-cryptoxanthin or zeaxanthin at the 7, 8 or 7′, 8′ double bond, leading to the pigments β-apo-8'-carotenal or 3-hydroxy-β-apo-8'-carotenal (β-citraurin) (Ma *et al.* 2013, Rodrigo *et al.* 2013, Rubio-Moraga *et al.* 2014).

Generally, CCD7 and CCD8 (EC 1.13.11.70) act sequentially to contribute to strigolactone synthesis (Figure 7E) and are localized in the plastids. Strigolactone has been considered as a new plant hormone involved in the inhibition of shoot branching, as important plant signals for the establishment of arbuscular mycorrhizal (AM) symbiosis, and as seed germination stimulants of the parasitic plants (Al-Babili and Bouwmeester 2015). CCD7 has been reported to cleave only asymmetrically and catalyze the 9,10 cleavage of β-carotene to produce one β-ionone and the C_{27} β-apo-10'-carotenal (Schwartz *et al.* 2004, Auldridge *et al.* 2006). Then CCD8 converts β-apo-10'-carotenal into C_{18} β-apo-13-carotenone (the precursor for strigolactone biosynthesis) and an acyclic dialdehyde at the 13,14 double bond (Vogel *et al.* 2010, Kohlen *et al.* 2012, Seto *et al.* 2014). It was reported that canonical CCD7 and CCD8 seem to be lacking in green algae and a majority of Chlorophyta including *C. reinhardtii* did not contain strigolactones (Delaux *et al.* 2012). While *Marchantia polymorpha* (liverwort) and Charophyte green algae (such as *Nitella hyalina*, *Nitella pseudoflabellata* and *Chara corallina*), which produce strigolactones, could lack CCD8, suggesting the presence of another strigolactones biosynthesis pathway (Delaux *et al.* 2012). Charales have been considered to be the closest relatives to land plants (Karol *et al.* 2001, Turmel *et al.* 2006).

The seeds of *Bixa orellana* are the only natural source of industrially produced high contents of bixin, an orange-red apocarotenoid. Three enzymes in *B. orellana*, i.e. lycopene

cleavage dioxygenase (BoLCD), bixin aldehyde dehydrogenase (BoBADH), and norbixin methyltransferase (BonBMT), catalyze the sequential conversion of lycopene into bixin. BoLCD is the first committed step in the biosynthesis of bixin to cleave all-*trans*-lycopene at the 5,6 (5′,6′) positions (Figure 7F). Introduction of these three genes in *E. coli*/pACCRT-EIB engineered to produce lycopene can induce bixin synthesis (Bouvier *et al.* 2003). BoLCD falls into the same clade as CCD4 (Figure 8).

4.2 CCOs from bacteria

Several mycobacterial species are known to synthesize carotenoids, interestingly, *Mycobacterium tuberculosis* does not contain carotenogenic genes, but has CCO enzymes. MtCCO from *M. tuberculosis* cleaves β-carotene at 15,15' double bond to form C_{20}-retinal, and cleaves β-carotene at the 13,14 double bond, leading to the formation of β-apo-14'-carotenal (C_{22}) and β-apo-13-carotenone (C_{18}) (Table 1) (Scherzinger *et al.* 2010). Apo-carotenoid 13,14-dioxygenase from *Novosphingobium aromaticivorans* (NACOX) can convert β-apo-8'-carotenal (C_{30}) to β-apo-13-carotenone (C_{18}) (Kim *et al.* 2012). Lignostilbene-α,β-dioxygenase (LSD, EC 1.13.11.43) from *Pseudomonas paucimobilis* TMYI009 is the key enzyme in the metabolic pathway of the C_α-C_β cleavage of dimeric lignin model compounds (Kamoda and Saburi 1993), and is significantly homologous to the bacterial and cyanobacterial CCDs (Figure 8).

In cyanobacteria, apo-carotenoid oxygenase (SynACO, or Diox1) from *Synechocystis* sp. PCC 6803 and NosACO (also known as NSC2) from *Nostoc* sp. PCC 7120 can convert *β*-apo-carotenals instead of *β*-carotene into retinal (β-apo-15′-carotenal) at the 15,15' position (Table 1) (Ruch *et al.* 2005, Scherzinger *et al.* 2006). Besides NSC2, another two CCO

homologs, NSC1 (also designated as NosCCD) and NSC3, were also identified in *Nostoc* sp. PCC 7120 (Marasco *et al.* 2006). NSC1 can symmetrically cleave bicyclic xanthophylls at the 9,10 (9'10') position, and shares 44% homology with AtCCD1 from *Arabidopsis thaliana*, which was considered as an ortholog of plant CCD1 enzymes (Figure 8). NSC1 also can asymmetrically cleave monocyclic substrates at the 9,10 and 7',8' double bonds (Scherzinger and Al-Babili 2008a). NSC3 was found to act only on apocarotenoid substrates and cleave at the 9,10 bond (Marasco *et al.* 2006). Recently, it was reported that NSC3 cleaved β-apo-8'-carotenal at 3 positions, i.e. C13 = C14, C15 = C15', and C13' = C14', revealing a unique cleavage pattern (Heo *et al.* 2013).

4.3 CCOs from fungi

Fungi also can produce retinal from C_{40} carotenoid substrates. The carotenoid oxygenase enzyme CarX was reported to be the first retinal-synthesizing enzyme in the fungi (Table 1). CarX from the ascomycete *Fusarium fujikuroi* cleaved β-carotene, γ-carotene, torulene, and β-apo-8'-carotenal (at least one β-ionone ring in the substrates) at the 15,15' double bond to produce retinal (Prado-Cabrero *et al.* 2007b). Furthermore, Cco1 (also called CarX) from the basidiomycete *Ustilago maydis* catalyzed the symmetrical cleavage of β-carotene to yield two molecules of retinal (Estrada *et al.* 2009). The other one NcCAO1 from *Neurospora crassa* clusters with the CarX enzyme of *F. fujikuroi* (Saelices *et al.* 2007), which was also shown to cleave β-carotene into retinal.

The biosynthesis of neurosporaxanthin involves a different cleavage enzyme named CarT, which cleaves torulene at the 4′,5′ double bond to form β-apo-4'-carotenal, the corresponding aldehyde of neurosporaxanthin (β-apo-4′-carotenoic acid) in *Fusarium* carotenoid metabolism

(Table 1) (Prado-Cabrero *et al.* 2007a). NcCAO2 from *Neurospora crassa* was similar to CarT (Figure 8) and could catalyze torulene cleavage (Saelices *et al.* 2007).

Carotene cleavage is the necessary initial step in the biosynthesis of the C_{18}-derivative trisporic acid, the sexual signal in zygomycete fungi (Schimek and Wöstemeyer 2009). Two genes encoding putative CCOs (TSP3, TSP4) were identified from *Rhizopus oryzae* and *Blakeslea trispora*, suggesting that TSP3 might act in concert with TSP4 to generate C_{18}-trisporoids in a sequential cleavage mechanism comparable to the organization of CCOs (CCD7 and CCD8) for strigolactone biosynthesis in plants (Burmester *et al.* 2007). Recently, it was demonstrated that the first reactions in the apocarotenoid pathway of *Phycomyces blakesleeanus* are the cleavage of β-carotene at its 11',12' double bond to form β-apo-12'-carotenal (C_{25}) by CarS (also called TSP3) and the cleavage of the resulting C_{25}-fragment to generate β-apo-13-carotenone (C_{18}, the precursor for trisporic acids biosynthesis) at its 13,14 double bond by AcaA (Table 1) (Medina *et al.* 2011, Tagua *et al.* 2012).

Conclusion

Some carotenogenic genes have high similarity from bacteria to land plants, but some have low similarity. Phytoene synthases have high sequence similarity from bacteria to plants, respectively. In bacteria and fungi, only a single desaturase (CrtI) catalyzes a series of carotenoid desaturation steps, but in cyanobacteria, algae and plants, carotenoid desaturation is accomplished by two distinct desaturases and one or two carotenoid isomerases. The CrtY-type lycopene cyclase may be the ancestor of CrtL-type cyclase in cyanobacteria and LCY-type in

algae and plants. Two classes of structurally unrelated enzymes of CHYs can be found in all biological organisms: one is non-heme di-iron β-carotene hydroxylase enzyme; another is the heme-containing P450 type. Carotene ketolases only exist in bacteria, cyanobacteria, and some algae; land plants seem to be lacking of carotene ketolases.

Components of the biosynthetic pathways of some carotenoids vary in different species. Astaxanthin can be synthesized from β-carotene by CrtZ and CrtW/CrtO in bacteria, by CrtS in *Xanthophyllomyces* and *Phaffia* species, by BCH and BKT in *Haematococcus* species, and by CBFD and HBFD in *Adonis* species. Some carotenoids are only found in some species, such as fucoxanthin in brown algae and diatoms, capsanthin and capsorubin in peppers and *Lilium* species, C_{30}-, C_{45}- and C_{50}- carotenoids in some bacteria. Some unusual carotenogenic genes may exist and are involved in these special carotenoids biosynthetic pathways; however, some enzymes involved in the biosynthesis of diadinoxanthin and fucoxanthin or the isomerization to 9- *cis*- epoxycarotenoids remain to be identified. Much more newly identified carotenoids, new carotenogenic genes/enzymes, and new pathways still need to be explored.

Carotenoids are important precursors of a variety of apocarotenoids: the C_{20}-retinoids, C_{18}-trisporic acids, C_{15}-ABA, C_{18}-strigolactones and C_{13}-ionone, and so on. The broad diversity of apocarotenoid volatile compounds can be attributed to large numbers of carotenoid precursors and the various cleavage sites catalyzed by CCOs enzymes. Some CCOs like apocarotenoid oxygenase (ACO) from cyanobacteria are reserved for the apocarotenoid specific oxygenases. Some CCOs can act on both carotenoids and apocarotenoids symmetrically or asymmetrically. Some CCOs can act on multiple cleavage sites of double bond of carotenoids. Some CCOs can

not only catalyze the oxidative cleavage of carotenoids, but also catalyze the isomerization reactions. In addition, the catalytic mechanisms by different CCOs are still unknown, and much more efforts are still needed to reveal the newly identified CCOs involved in the metabolic conversions of carotenoids and apocarotenoids. Moreover, determining the biological functions of new apocarotenoids in all living organisms will be a future challenge.

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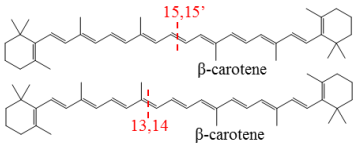
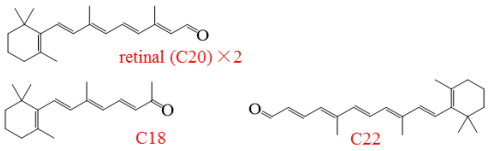
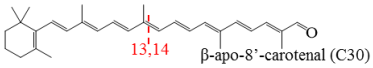
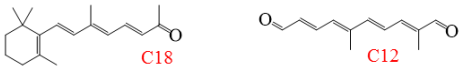
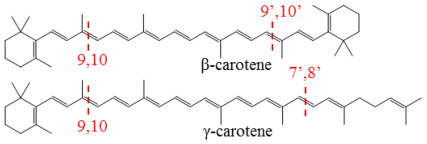
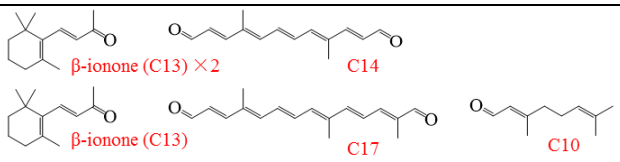
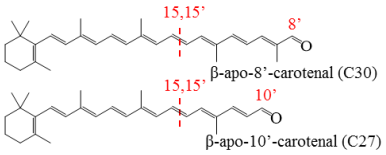
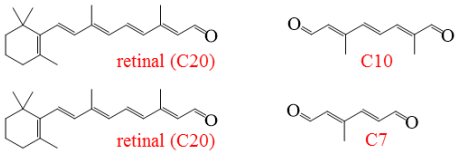
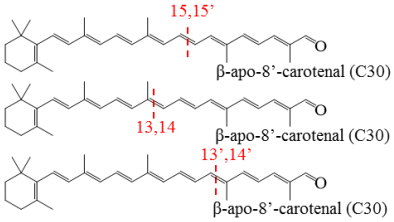
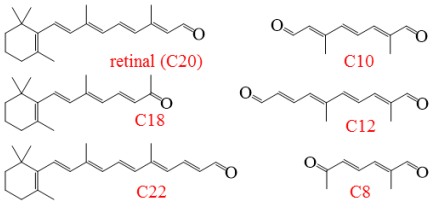
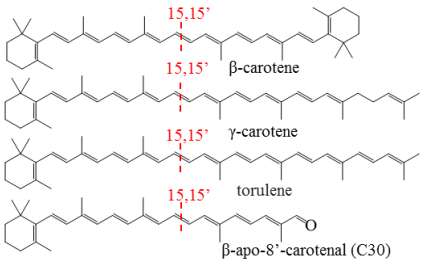
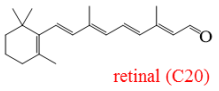
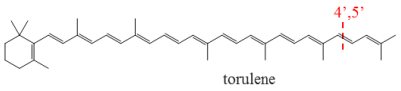
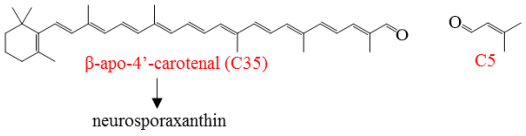
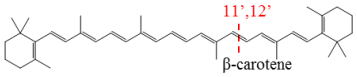
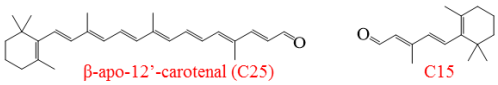
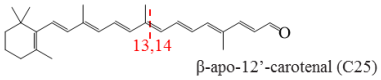
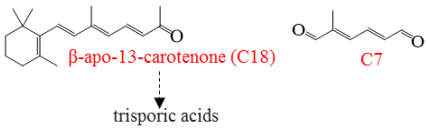
Table 1 Enzymatic activity and substrate specificity of different carotenoid cleavage oxygenases in bacteria, fungi and animals.

Substrates	Oxygenases	Products	References
Bacteria			
	MtCCO		(Scherzinger *et al.* 2010)
	NACOX		(Kim *et al.* 2012)
	NSC1 (NosCCD)		(Scherzinger and Al-Babili 2008a)
	NSC2 (NosACO); Diox1 (SynACO)		(Ruch *et al.* 2005, Scherzinger *et al.* 2006)
	NSC3		(Heo *et al.* 2013)
Fungi			

	CarX (CAO1)		(Prado-Cabrero *et al.* 2007b)
	CarT (CAO2)		(Prado-Cabrero *et al.* 2007a)
	CarS (TSP3)		(Medina *et al.* 2011, Tagua *et al.* 2012)
	AcaA		(Medina *et al.* 2011, Tagua *et al.* 2012)

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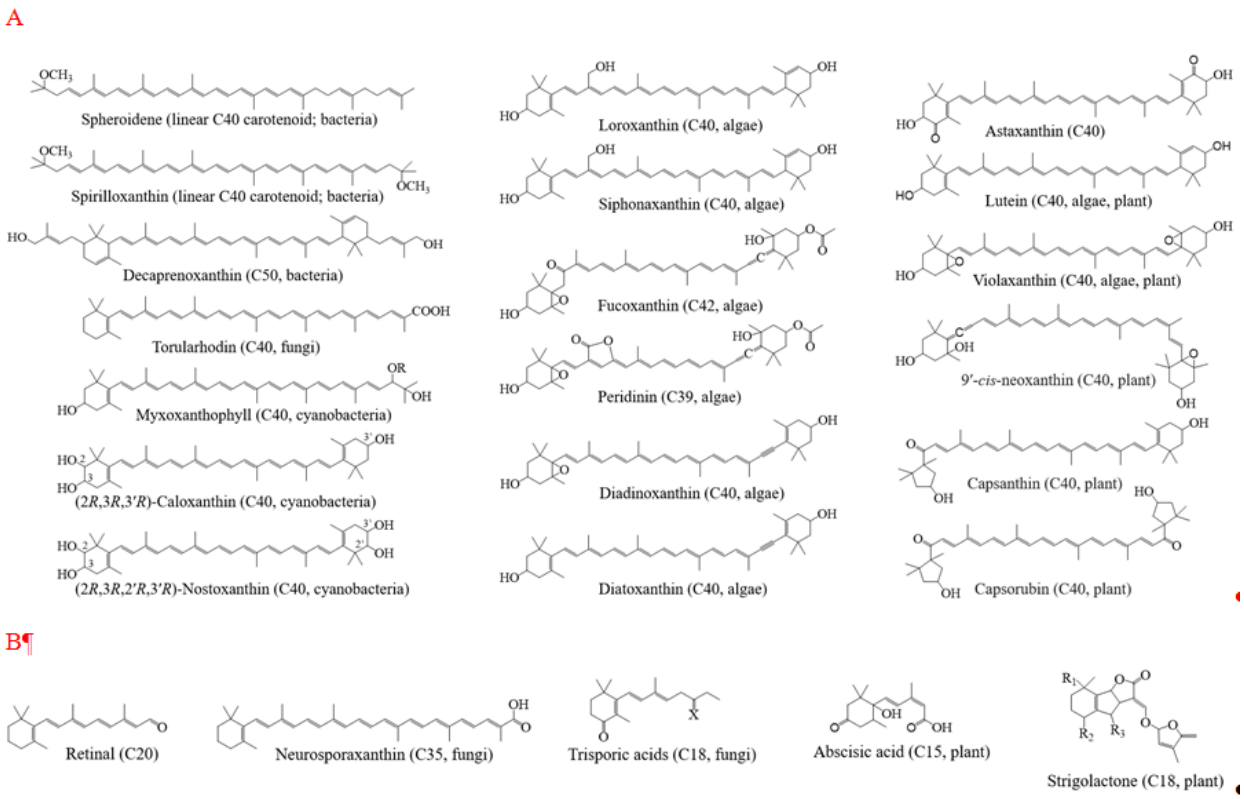


Figure 1 Structural diversity of selected carotenoids and apocarotenoids from various organisms.

A, chemical structures of selected carotenoids; B, chemical structures of selected apocarotenoids.

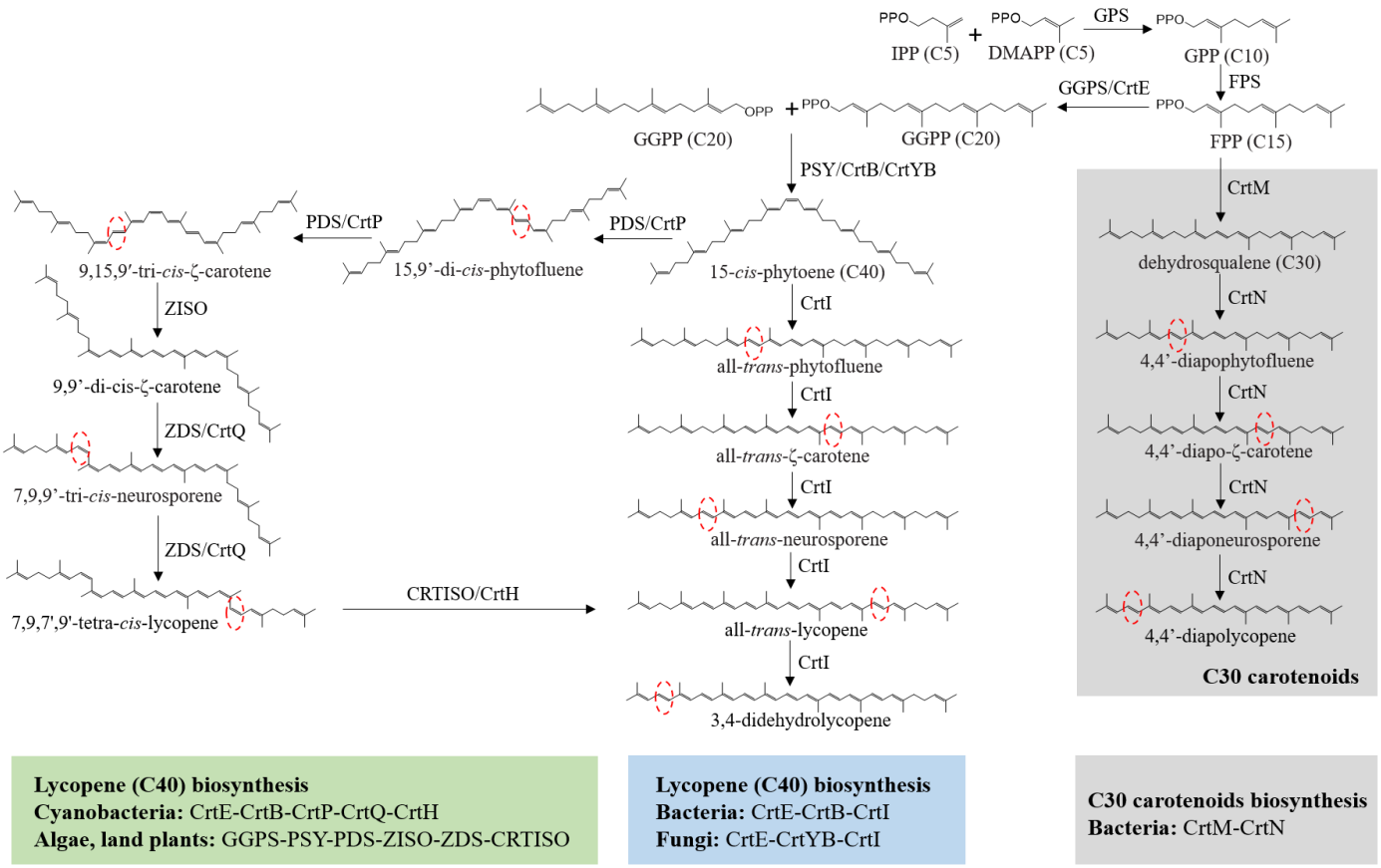


Figure 2 Biosynthesis of acyclic carotenoids in bacteria, fungi, cyanobacteria, algae and plants. Bacteria can synthesize C_{30}- and C_{40} carotenoids via different biosynthetic pathways. Bacterial CrtI can catalyze the three-step or four-step desaturations; while fungal CrtI can catalyze the four-step or five-step desaturations.

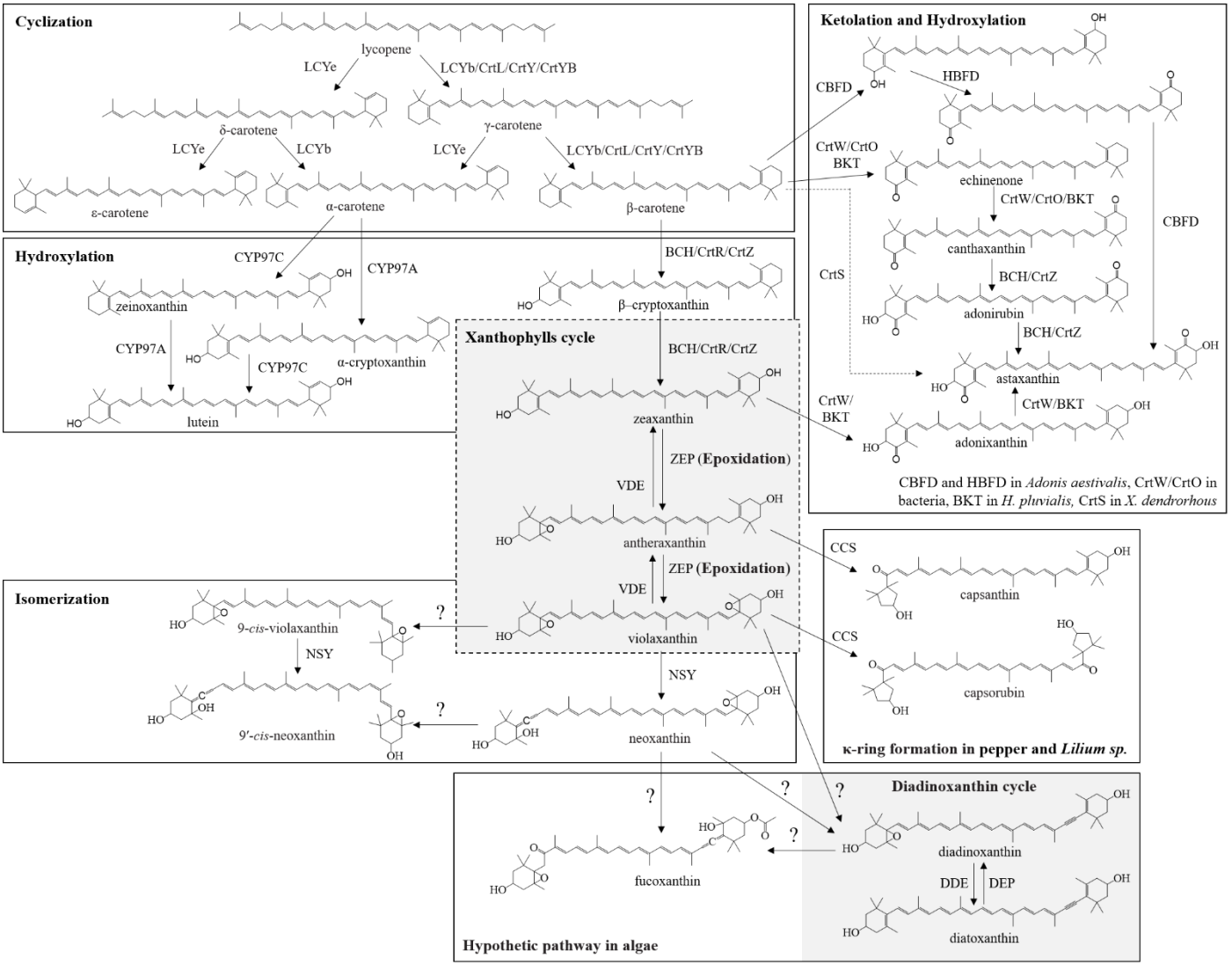
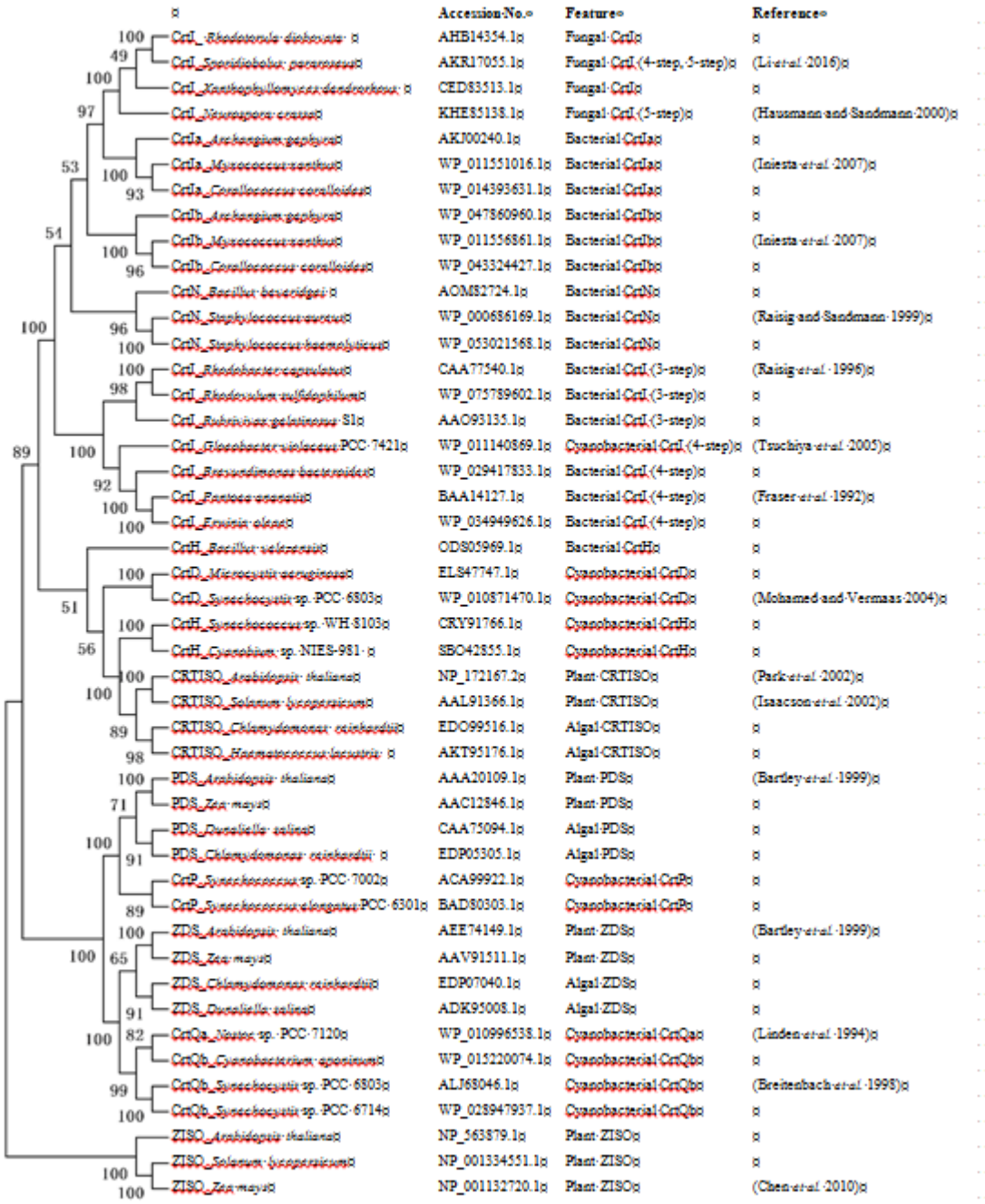


Figure 3 Biosynthesis of cyclic carotenoids in bacteria, fungi, cyanobacteria, algae and plants. Carotenogenic enzymes in different kinds of organisms may not the same. Some enzymes participated in some reactions are still unknown.



	Accession No.	Feature	Reference
CrtI_Rhodotorula diobovata	AHB14354.1	Fungal CrtI	
CrtI_Sporidiobolus pararoseus	AKR17055.1	Fungal CrtI (4-step, 5-step)	(Li et al. 2016)
CrtI_Xanthophyllomyces dendrorhous	CED83513.1	Fungal CrtI	
CrtI_Neurospora crassa	KHE85138.1	Fungal CrtI (5-step)	(Hausmann and Sandmann 2000)
CrtIa_Archangium gephyra	AKJ00240.1	Bacterial CrtIa	
CrtIa_Myxococcus xanthus	WP_011551016.1	Bacterial CrtIa	(Iniesta et al. 2007)
CrtIa_Corallococcus coralloides	WP_014393631.1	Bacterial CrtIa	
CrtIb_Archangium gephyra	WP_047860960.1	Bacterial CrtIb	
CrtIb_Myxococcus xanthus	WP_011556861.1	Bacterial CrtIb	(Iniesta et al. 2007)
CrtIb_Corallococcus coralloides	WP_043324427.1	Bacterial CrtIb	
CrtN_Bacillus beveridgei	AOM82724.1	Bacterial CrtN	
CrtN_Staphylococcus aureus	WP_000686169.1	Bacterial CrtN	(Raisig and Sandmann 1999)
CrtN_Staphylococcus haemolyticus	WP_053021568.1	Bacterial CrtN	
CrtI_Rhodobacter capsulatus	CAA77540.1	Bacterial CrtI (3-step)	(Raisig et al. 1996)
CrtI_Rhodovulum sulfidophilum	WP_075789602.1	Bacterial CrtI (3-step)	
CrtI_Rubrivivax gelatinosus S1	AAO93135.1	Bacterial CrtI (3-step)	
CrtI_Gloeobacter violaceus PCC 7421	WP_011140869.1	Cyanobacterial CrtI (4-step)	(Tsuchiya et al. 2005)
CrtI_Brevundimonas bacteroides	WP_029417833.1	Bacterial CrtI (4-step)	
CrtI_Pantoea ananatis	BAA14127.1	Bacterial CrtI (4-step)	(Fraser et al. 1992)
CrtI_Erwinia uredovora	WP_034949626.1	Bacterial CrtI (4-step)	
CrtH_Bacillus selenitireducens	ODS05969.1	Bacterial CrtH	
CrtD_Micrococcus aeruginosus	ELS47747.1	Cyanobacterial CrtD	
CrtD_Synechocystis sp. PCC 6803	WP_010871470.1	Cyanobacterial CrtD	(Mohamed and Vermaas 2004)
CrtH_Synechococcus sp. WH 8103	CRY91766.1	Cyanobacterial CrtH	
CrtH_Cyanobium sp. NIES 981	SBO42855.1	Cyanobacterial CrtH	
CRTISO_Arabidopsis thaliana	NP_172167.2	Plant CRTISO	(Park et al. 2002)
CRTISO_Solanum lycopersicum	AAL91366.1	Plant CRTISO	(Isaacson et al. 2002)
CRTISO_Chlamydomonas reinhardtii	EDO99516.1	Algal CRTISO	
CRTISO_Haematococcus lacustris	AKT95176.1	Algal CRTISO	
PDS_Arabidopsis thaliana	AAA20109.1	Plant PDS	(Bartley et al. 1999)
PDS_Zea mays	AAC12846.1	Plant PDS	
PDS_Dunaliella salina	CAA75094.1	Algal PDS	
PDS_Chlamydomonas reinhardtii	EDP05305.1	Algal PDS	
CrtP_Synechococcus sp. PCC 7002	ACA99922.1	Cyanobacterial CrtP	
CrtP_Synechococcus elongatus PCC 6301	BAD80303.1	Cyanobacterial CrtP	
ZDS_Arabidopsis thaliana	AEE74149.1	Plant ZDS	(Bartley et al. 1999)
ZDS_Zea mays	AAV91511.1	Plant ZDS	
ZDS_Chlamydomonas reinhardtii	EDP07040.1	Algal ZDS	
ZDS_Dunaliella salina	ADK95008.1	Algal ZDS	
CrtQa_Nostoc sp. PCC 7120	WP_010996538.1	Cyanobacterial CrtQa	(Linden et al. 1994)
CrtQb_Cyanobacterium aponinum	WP_015220074.1	Cyanobacterial CrtQb	
CrtQb_Synechocystis sp. PCC 6803	ALJ68046.1	Cyanobacterial CrtQb	(Breitenbach et al. 1998)
CrtQb_Synechocystis sp. PCC 6714	WP_028947937.1	Cyanobacterial CrtQb	
ZISO_Arabidopsis thaliana	NP_563879.1	Plant ZISO	
ZISO_Solanum lycopersicum	NP_001334551.1	Plant ZISO	
ZISO_Zea mays	NP_001132720.1	Plant ZISO	(Chen et al. 2010)

Figure 4 Phylogenetic tree of enzymes related to phytoene desaturases from bacteria, fungi, cyanobacteria, algae, and plants. Phylogenetic tree was constructed using neighbor-joining methods of MEGA5 software. Numbers associated with the branches were the neighbor-joining

bootstrap values (n = 1000). The enzymes include CrtI, CrtIa, CrtIb, CrtN, and CrtH from bacteria, CrtI from fungi, CrtI, CrtD, CrtH, CrtP, CrtQa, and CrtQb from cyanobacteria, and PDS, ZISO, ZDS, and CRTISO from algae and plants.

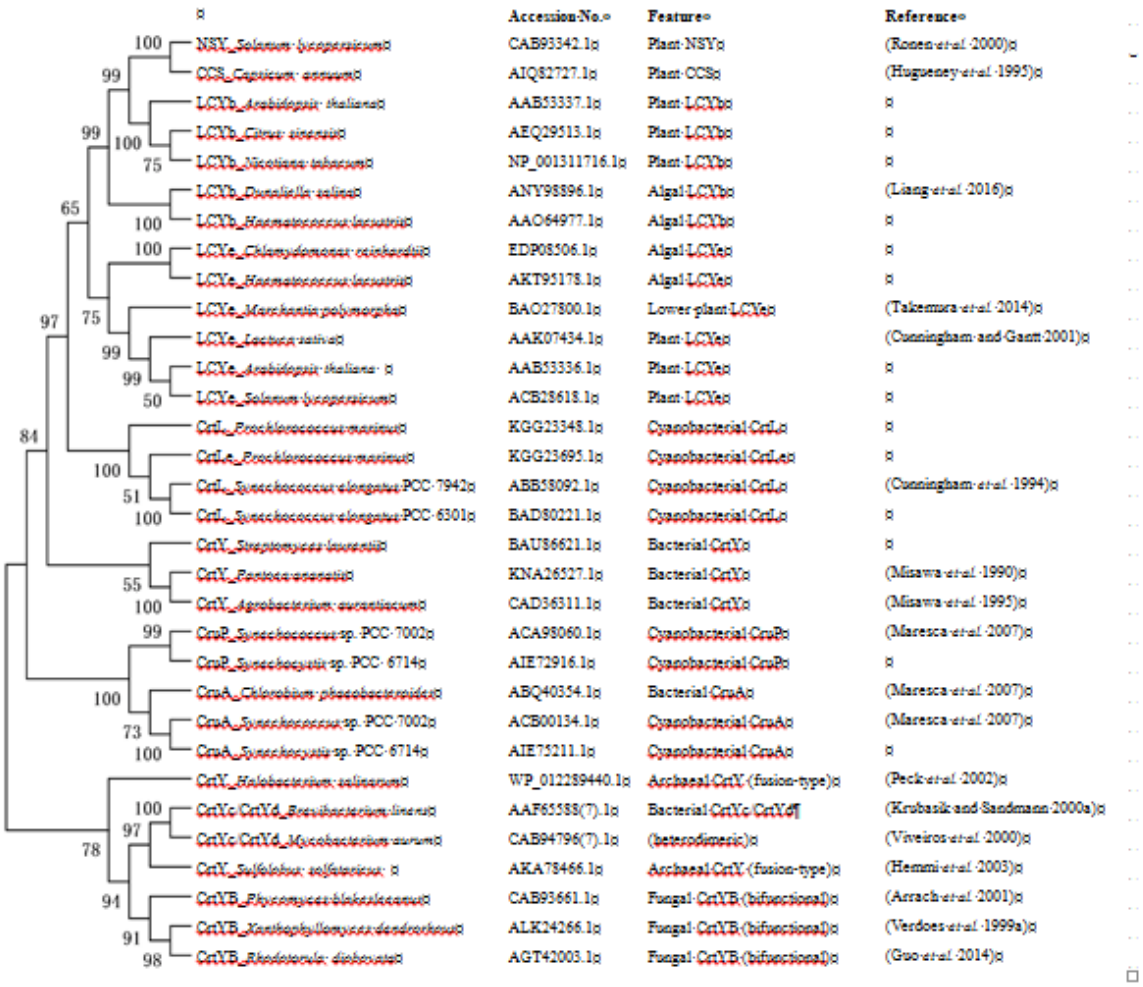
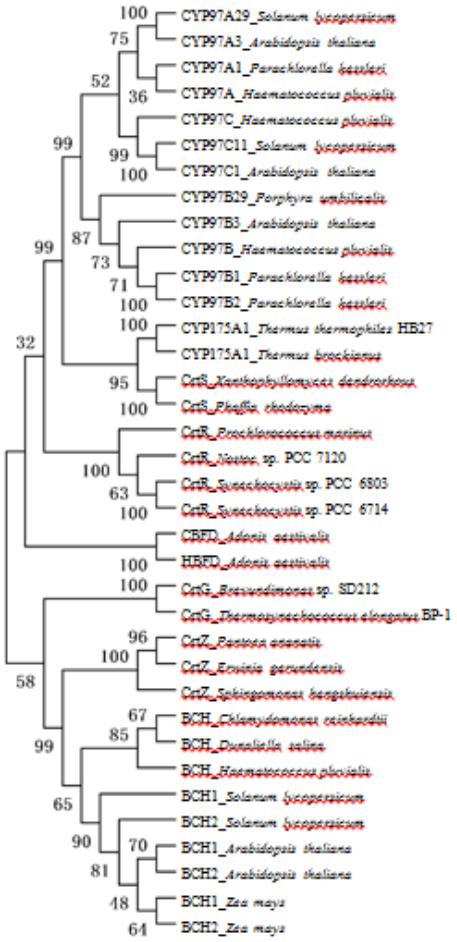


Figure 5 Phylogenetic tree of enzymes related to lycopene cyclases from bacteria, fungi, cyanobacteria, algae, and plants. Phylogenetic tree was constructed using neighbor-joining methods of MEGA5 software. Numbers associated with the branches were the neighbor-joining bootstrap values (n = 1000). The enzymes include CrtY, CrtYc/CrtYd, and CruA from bacteria, fusion-type CrtY from Archaea, bifunctional CrtYB from fungi, CrtL, CrtLe, CruA, and CruP from cyanobacteria, LCYb and LCYe from algae, and LCYb, LCYe, CCS and NSY from plants.

A



	Accession No.	Feature	Reference
CYP97A29_*Solanum lycopersicum*	NP_001234049.1	Plant CYP97A	
CYP97A3_*Arabidopsis thaliana*	NP_564384.1	Plant CYP97A	(Kim and DellaPenna 2006)
CYP97A1_*Parachlorella kessleri*	AGN27239.1	Algal CYP97A	
CYP97A_*Haematococcus pluvialis*	AFR31786.1	Algal CYP97A	
CYP97C_*Haematococcus pluvialis*	AFQ31612.1	Algal CYP97C	
CYP97C11_*Solanum lycopersicum*	NP_001234058.2	Plant CYP97C	
CYP97C1_*Arabidopsis thaliana*	NP_190881.2	Plant CYP97C	(Tian *et al.* 2004)
CYP97B29_*Porphyra umbilicalis*	AHA51695.1	Algal CYP97B	(Yang *et al.* 2014)
CYP97B3_*Arabidopsis thaliana*	AEE83557.1	Plant CYP97B	
CYP97B_*Haematococcus pluvialis*	AFR36909.1	Algal CYP97B	
CYP97B1_*Parachlorella kessleri*	AGK38421.1	Algal CYP97B	
CYP97B2_*Parachlorella kessleri*	AGK38422.1	Algal CYP97B	
CYP175A1_*Thermus thermophilus* HB27	BAW00444.1	Bacterial CYP175A1	(Blasco *et al.* 2004)
CYP175A1_*Thermus brockianus*	APD10551.1	Bacterial CYP175A1	
CrtS_*Xanthophyllomyces dendrorhous*	ALK24270.1	Fungal CrtS	(Alcaino *et al.* 2012)
CrtS_*Phaffia rhodozyma*	AII26675.1	Fungal CrtS	
CrtR_*Prochlorococcus marinus*	WP_036906182.1	Cyanobacterial CrtR	
CrtR_*Nostoc* sp. PCC 7120	WP_044522047.1	Cyanobacterial CrtR	
CrtR_*Synechocystis* sp. PCC 6803	ALJ67182.1	Cyanobacterial CrtR	(Lagarde and Vermaas 1999)
CrtR_*Synechocystis* sp. PCC 6714	AIE73765.1	Cyanobacterial CrtR	
CBFD_*Adonis aestivalis*	ABK41044.1	Plant CBFD	(Cunningham and Gantt 2011)
HBFD_*Adonis aestivalis*	ABK41045.1	Plant HBFD	(Cunningham and Gantt 2011)
CrtG_*Brevundimonas* sp. SD212	BAD99415.1	Bacterial CrtG	(Komemushi *et al.* 2005)
CrtG_*Thermosynechococcus elongatus* BP-1	BAC09469.1	Bacterial CrtG	(Iwai *et al.* 2008)
CrtZ_*Pantoea ananatis*	WP_013027996.1	Bacterial CrtZ	(Misawa *et al.* 1990)
CrtZ_*Erwinia persicinus*	CUU22453.1	Bacterial CrtZ	
CrtZ_*Sphingomonas baekryungensis*	AJP74675.1	Bacterial CrtZ	
BCH_*Chlamydomonas reinhardtii*	EDO99380.1	Algal BCH	
BCH_*Dunaliella salina*	AEN25578.1	Algal BCH	
BCH_*Haematococcus pluvialis*	AKQ20654.1	Algal BCH	(Linden 1999)
BCH1_*Solanum lycopersicum*	NP_001234348.1	Plant BCH	
BCH2_*Solanum lycopersicum*	NP_001265981.1	Plant BCH	
BCH1_*Arabidopsis thaliana*	AAF85797.1	Plant BCH	(Kim *et al.* 2009)
BCH2_*Arabidopsis thaliana*	BAA98075.1	Plant BCH	(Kim *et al.* 2009)
BCH1_*Zea mays*	NP_001105907.1	Plant BCH	
BCH2_*Zea mays*	NP_001148085.1	Plant BCH	

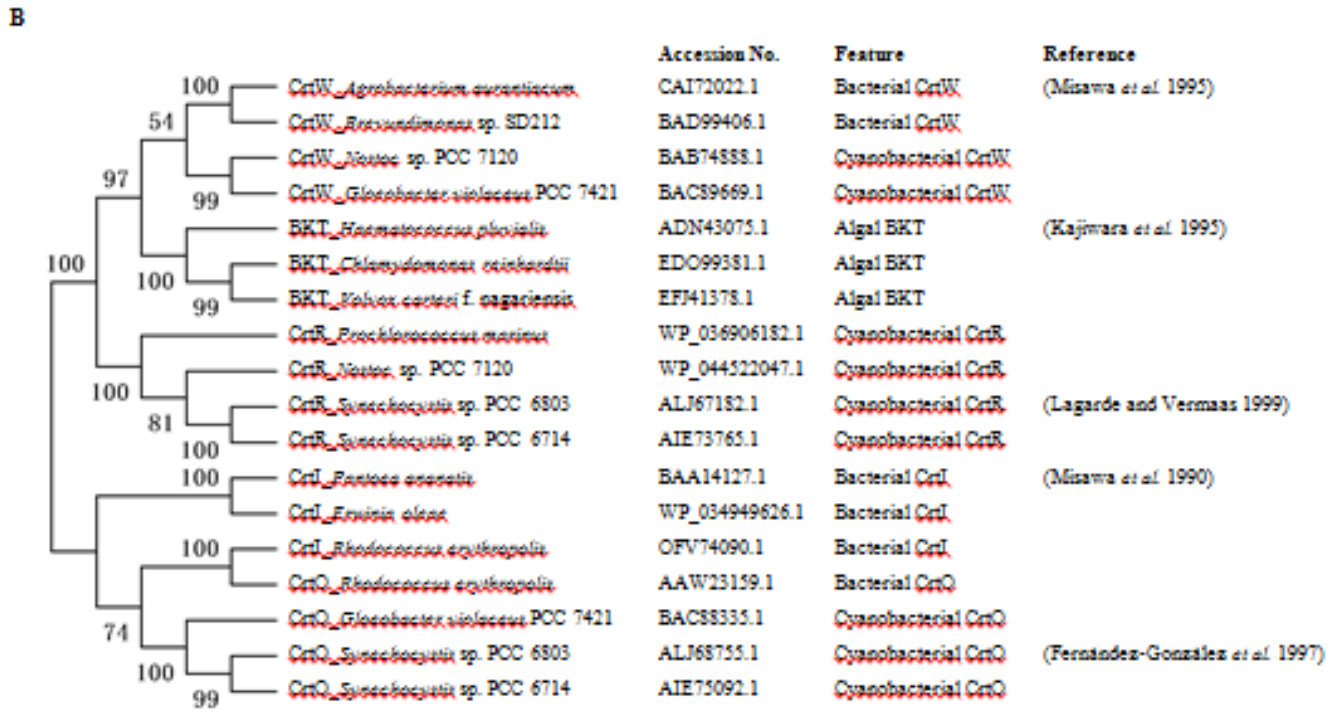


Figure 6 Phylogenetic tree of enzymes related to CHYs and carotene ketolases from bacteria, fungi, cyanobacteria, algae and plants. Phylogenetic tree was constructed using neighbor-joining methods of MEGA5 software. Numbers associated with the branches were the neighbor-joining bootstrap values (n = 1000). A, Phylogenetic tree of enzymes related to CHYs from bacteria, fungi, cyanobacteria and algae. The enzymes include CrtZ, CrtG and CYP175A1 in bacteria, CrtS in Fungi, CrtR in cyanobacteria, BCH and CYP97s in algae, and BCH, CYP97s, CBFD and HBFD in land plants. B, Phylogenetic tree of enzymes related to carotene ketolases from bacteria, fungi, cyanobacteria, algae and plants. The enzymes include CrtW, CrtO and CrtI in bacteria, CrtW, CrtO and CrtR in cyanobacteria, and BKT in algae.

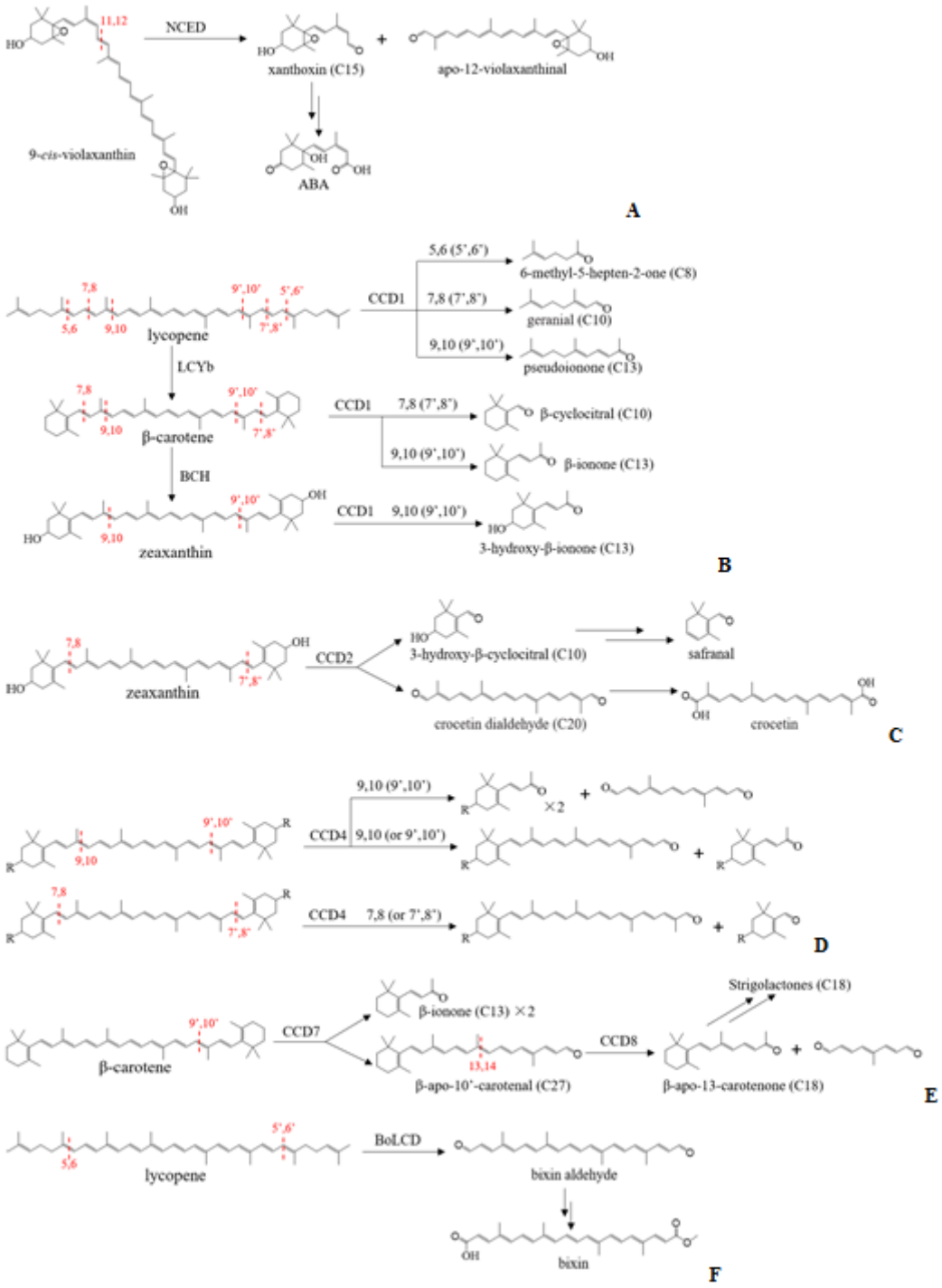


Figure 7 Specific enzymatic cleavage reactions of carotenoids or apocarotenoids catalyzed by various CCOs from plants. Cleavage sites in substrates are indicated by dotted red lines.

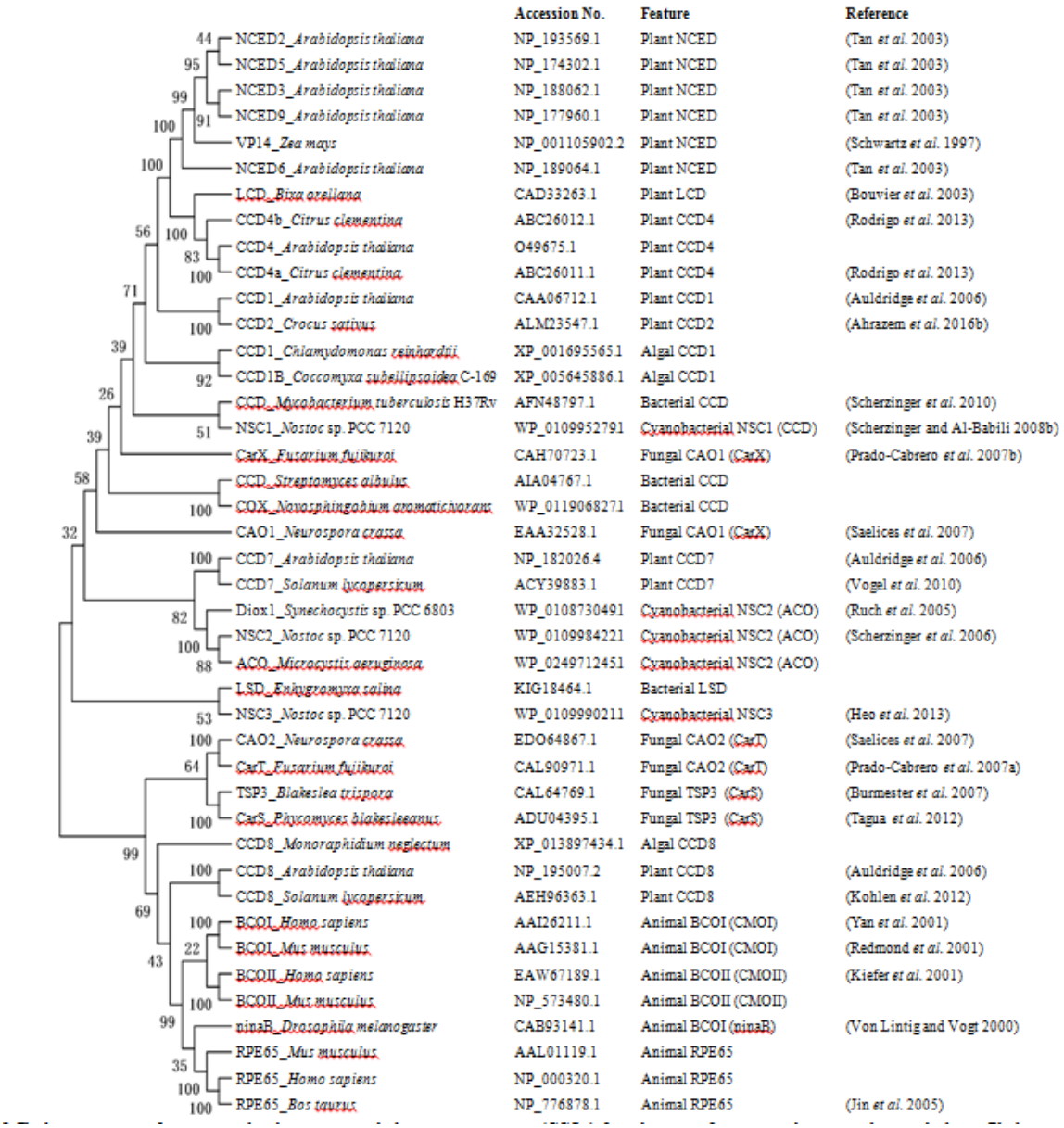


Figure 8 Phylogenetic tree of enzymes related to carotenoid cleavage oxygenases (CCOs) from bacteria, fungi, cyanobacteria, algae and plants. Phylogenetic tree was constructed using neighbor-joining methods of MEGA5 software. Numbers associated with the branches were the neighbor-joining bootstrap values (n = 1000). The enzymes include CCD and LSD from bacteria;

CAO1 (CarX), CAO2 (CarT), and TSP3 (CarS) from fungi; NSC1 (CCD), NSC2 (ACO), and NSC3 from cyanobacteria; CCD1, CCD8 from algae; CCD1, CCD2, CCD4, CCD7, CCD8, LCD and NCED from plants; BCOI (CMOI), BCOII (CMOII), and RPE65 from animals.