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NEWSLETTER

EUROCAROTEN

EUROPEAN NETWORK TO ADVANCE CAROTENOID RESEARCH AND APPLICATIONS IN AGRO-FOOD AND HEALTH

CAROTENOID BIOSYNTHESIS IN MICROORGANISM AND PLANTS

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Carotenoids origin and structures

Carotenoids are isoprenoid metabolites synthesized by plants, algae, cyanobacteria and some fungi, archaea, bacteria, and animals[1]. In photosynthetic organisms, carotenoids participate in light harvesting and they are essential for photo protection of the respective organism. In non-photosynthetic tissues and organisms, carotenoids play a role as pigments, showing colors from yellow to red, and serve different functions such as phototaxis, photo protection, camouflage, antioxidants, and signaling such as breeding color. They are responsible for the autumn colors of many leaves (unmasked when the chlorophylls are degraded), and also for the colors of many flowers and fruits such as the yellow color of marigold, the orange color of pumpkin, carrots, strawberry tree and oranges, and the red color of tomato, pepper and watermelon, among others. In general, animals do not synthesize carotenoids de novo, and those found in animals are either directly accumulated from food or partly modified through metabolic reactions, which allow to maintain a healthy immune system and making certain vitamins, as vitamin A. Some insects as aphids, can produce carotenoids, which allow them to capture sunlight and use the energy for metabolic purposes[2].

All carotenoids and isoprenoids derive from common five-carbon (C5) building units and share common metabolic precursors in two independent pathways for the synthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP): the mevalonate (MVA) pathway, and the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway (**Figure 1**). Recent phylogenetic analyses have already confirmed the MEP pathway is restricted to bacteria and plastid-bearing eukaryotes. And in the three domains of life, the following preference have been reported: the MEP pathway in bacteria, the classical MVA pathway in eukaryotes, and the alternative MVA pathway in archaea[3].

Carotenoids in plants

In plants, carotenoids are composed of eight condensed C5 isoprene precursors that generate a C40 linear backbone. The carotenoidsynthesis takes place in plastids by the condensation of the universal C5 isoprenoid isomers IPP and DMAPP by using the MEP pathway. Some groups of algae retained both MEP and MVA pathways, while green algae (Chlorophyta) use only the MEP route. Among red algae there are examples for both the presence and absence of the MVA pathway4. The MEP pathway uses glyceraldehyde 3-phosphate and pyruvate as initial substrates to form

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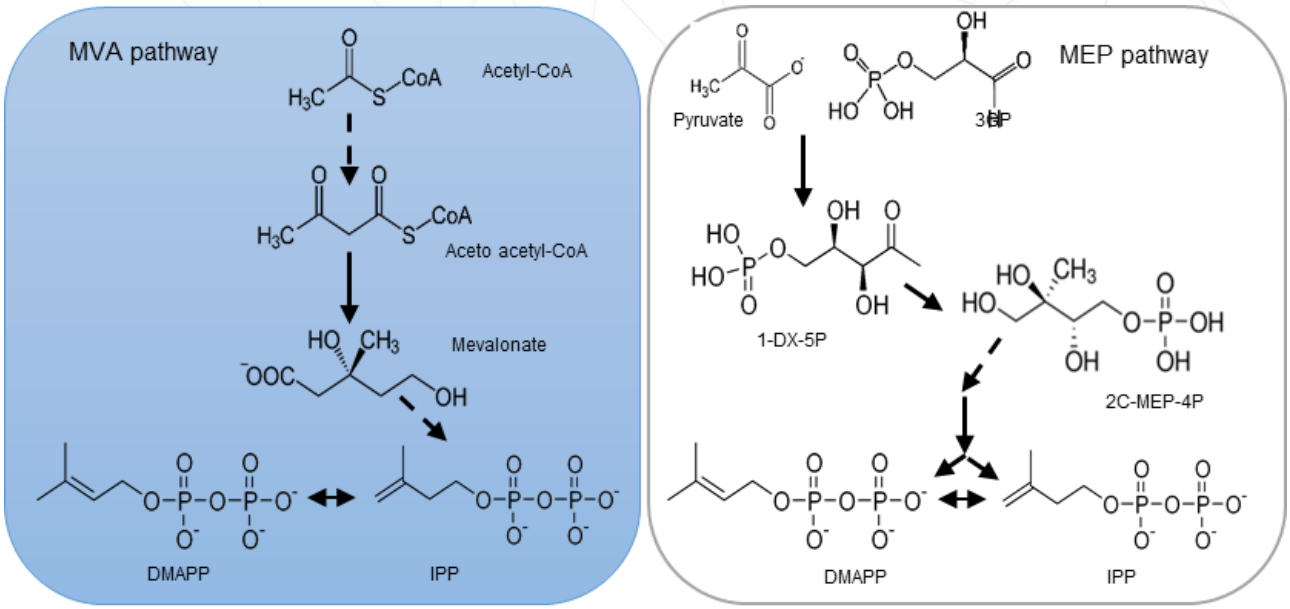

Figure 1. Compartmentalized biosynthetic pathways of isoprenoids: the cytosolic acid mevalonic (MVA; left), and the plastidic (MEP; right). Both pathways produce isopentenyl diphosphate (IPP) and its isomer DMAPP, the universal building block for the synthesis of all carotenoids.

deoxy-D-xylulose-5-phosphate (DXP). This reaction is catalyzed by DXP synthase (DXS). The activity of DXS, is highly regulated at multiple levels[5] and it is important in carotenoid flux regulation. Although abiotic and biotic factors may influence the availability of isoprenoid precursors for carotenoid production, light and circadian oscillations can alter the expression of nearly all MEP genes and several carotenoid biosynthesis encoding genes[6]. MEP is subsequently formed via an intramolecular rearrangement and reduction of DXP by the enzyme DXP reductoisomerase (DXR). IPP and DMAPP are formed after a number of subsequent steps and consequently undergo a sequential series of condensation reactions to yield the precursor of carotenoid biosynthesis, geranylgeranyl diphosphate (GGPP). Condensation of three IPP and one DMAPP molecules generates GGPP, the direct metabolic precursor not only for carotenoids but also for several other plastidial isoprenoids, with important functions in photosynthesis (chlorophylls, tocopherols, plastoquinones, phylloquinones), growth control and regulation (gibberellins), or environmental interactions (diterpenes), among others[7].

The first committed step of plant carotenoid biosynthesis is the condensation of two GGPP (C20 molecules) to form the first achromatic C40 carotenoid, 15-cis-phytoene. This step, catalyzed by the enzyme phytoene synthase (PSY), is generally accepted to be the main rate-determining reaction of the carotenoid pathway and to control the metabolic flux to carotenoids[8,9] (**Figure 2**). While there is only one PSY gene in the model plant Arabidopsis, PSY is typically encoded by small gene families that are differentially expressed in response of the developmental and environmental signals[10]. The reaction product, 15-cis-phytoene, is converted to all-trans-lycopene by sequential desaturation and isomerization reactions. Most of carotenoids in nature are actually in the all-trans configuration. However, a small but biologically relevant proportion must be in the cis configuration to be functional in the light-harvesting complex as well as in the formation of abscisic acid (ABA). The desaturations steps from phytoene are catalyzed by phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS). Both PDS and ZDS, which are important to control flux though the carotenoid pathway[11,12], require the operation of an electron

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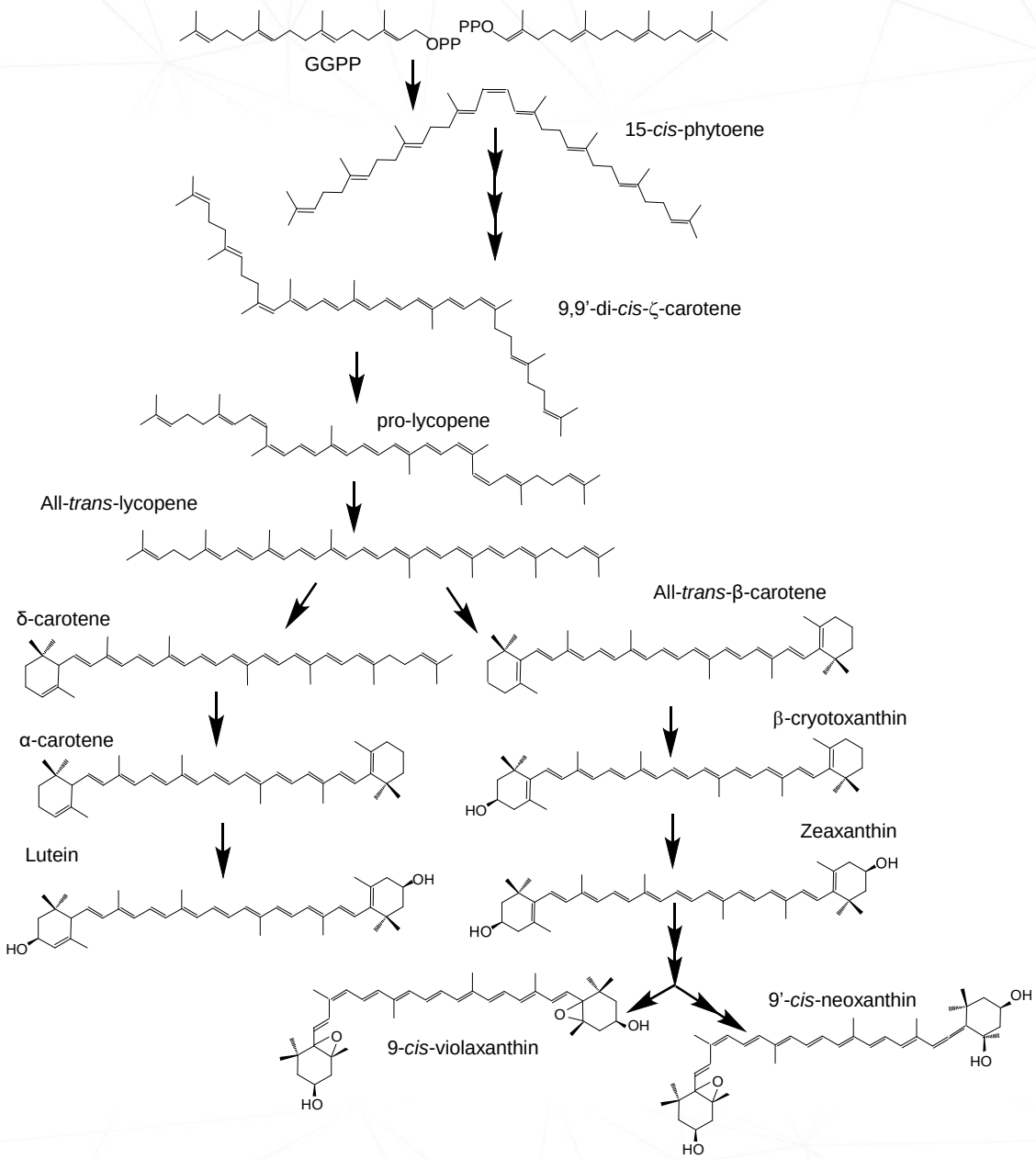

Figure 2. Biosynthetic pathways for carotenoids in plants.

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transfer process involving the plastidial terminal oxidase (PTOX) and plastoquinone. In concert with these steps, the action of ζ-carotene isomerase (Z-ISO) catalyzed the cis to trans conversion of the product of PDS, whereas carotenoid isomerase (CRTISO) transforms the four cis-bonds of poly-cis lycopene into trans-lycopene. CRTISO has emerged as a regulatory node in the pathway. Interestingly, the Z-ISO enzyme has been found to be similar to the product of *NnrU*, a bacterial gene (for nitrite and nitric oxide) required for denitrification, whereas CRTISO is thought to be evolved from bacterial-type desaturase (CrtI)[13,14]. Nonetheless, both CRTISO and Z-ISO have distinct roles in carotene isomerization, even although the activity of both of these enzymes can partially be compensated by photoisomerization of the upstream cis-carotenes to yield all-trans-lycopene in photosynthetic tissues.

The cyclization of trans-lycopene is a critical step for the synthesis of carotenoids since it is the first branching step of the pathway (**Figure 2**). The action of lycopene ε-cyclase (LCYE) or lycopene β-cyclase (LCYB) generates carotenoids with ε- or β-ionone rings, respectively. The cyclization of both ends of the linear lycopene molecule by LCYB generates β-carotene, while the coordinated action of LCYE and LCYB produces α-carotene. Cyclization of both ends of lycopene with ε rings is very uncommon in nature. The lettuce LCYE enzyme appears to be atypical in generating bicyclic ε,ε-carotene and its hydroxylated derivative lactucaxanthin[15]. Genes encoding LCYB and LCYE share significant sequence identity, suggesting that they may have originated by duplication of a common ancestor. The coordinated operation of LYCE and LYCB cyclases plays a major role in the regulation of the metabolic flux of the carotenoid pathway to either the β,β or the β,ε branch[10].

The cyclic carotenoids α-Carotene and β-carotene are further hydroxylated to produce xanthophylls (e.g. lutein and zeaxanthin) (**Figure 2**), which are among the main carotenoid pigments in the photosystems of plants. Xanthophyll formation requires ring-specific hydroxylation reactions. Thus, α-carotene suffers two sequential hydroxylations: hydroxylation of the β ring by β-carotene hydroxylase (CHYB) enzymes convert α-carotene into zeinoxanthin, which ε-carotene hydroxylase (CHYE) subsequently transforms into lutein, the most abundant carotenoid in green tissues. Alternatively, CHYE can first hydroxylate the ε ring of α-carotene to produce α-cryptoxanthin (which unlike zeinoxanthin retains an unsubstituted β ring and hence can be used as a vitamin A precursor) and then CHYB can hydroxylate the β ring of α-cryptoxanthin to produce lutein. These reactions are usually catalyzed by heme-containing cytochrome P450 enzymes that can hydroxylate both rings of α-carotene, even though CYP97A mostly acts as a CHYB and CYP97C mostly functions as a CHYE[10]. In the case of β-carotene, two sequential hydroxylation at both β-rings producing β-cryptoxanthin first and then zeaxanthin. Hydroxylation of β-carotene (BCH) is normally catalyzed by CHYB enzymes of the non-heme di-iron (BCH) type[9,10]. Zeaxanthin epoxidase (ZEP) introduces two epoxy groups in the rings of zeaxanthin, resulting in the sequential formation of antheraxanthin and violaxanthin. These reactions can be reverted by violaxanthin de-epoxidase (VDE). Interconversion of zeaxanthin and violaxanthin is known as the xanthophylls cycle and has a critical role in the dissipation of excess light energy and in the adaptation of plants to different light conditions[16]. The introduction of an allenic double bound in the molecule of violaxanthin produces neoxanthin in a step catalyzed by neoxanthin synthase (NSY). The identity of the enzyme and the mechanism of action, however, remain unclear.

Especially during ripening of fruits and senescing vegetables, chloroplasts are gradually transformed into chromoplasts, and at the same time, a gradual decrease of free carotenoid pigments with concomitant increase of xanthophyll esterification with fatty acids occurs. As a result, in most fruits and flowers, as well as in many seeds and some tubers, xanthophylls are mono- or diesterified with different fatty acids[17]. This modification increases their lipophilicity and stability. They are less water-soluble than their corresponding free xanthophylls and even than carotenes, providing a better integration into membrane structures, therefore reducing the susceptibility to adverse conditions in their environments[18].

Carotenoid biosynthesis in archaea and bacteria

Carotenoid biosynthesis in archaea and bacteria is more complicated than the one from plants, which is in accordance with the highest variability of carotenoids they produce (**Figure 3**). In contrast to plants that only produce C40 or C40-derived carotenoids, archaea and non-photosynthetic bacteria synthesize all C45 and most of the C30 and C50carotenoids found to date. The main final product of the carotenoid pathway in all archaea

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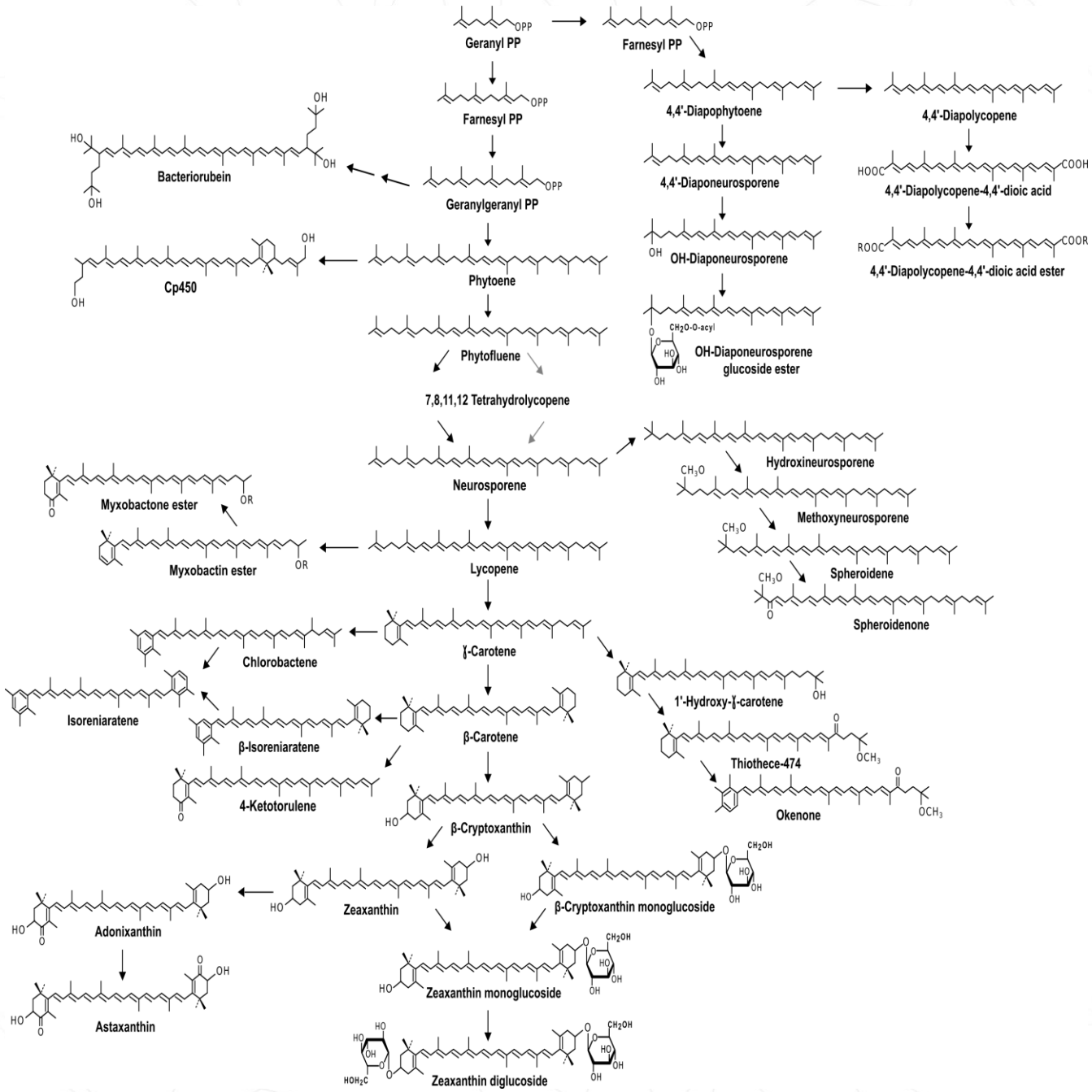

Figure 3. Biosynthetic pathways for carotenoids in bacteria. Figure modified from Rodriguez-Concepción *et al.* 2018[1].

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is the acyclic polyhydroxylated C50 carotenoid bacterioruberin[19]. This unusual carotenoid is not exclusive of archaea, as it has also been found in bacteria. Biosynthesis of C30 carotenoids can take place in two different ways: symmetric C30 carotenoids, also called 4,4´-diapocarotenoids, are synthesized by head-to-head condensation of two molecules of farnesyl diphosphate (C15), whereas asymmetric C30 carotenoids, like apo-8`-carotenoids, are formed accordingly by condensation of geranyl diphosphate (C10) and GGPP (C20)[20]. Furthermore, unusual carotenoids, like C35 carotenoids, are built from farnesyl diphosphate (FPPC15) and GGPP (C20)[21]. C40 and C50 carotenoids share the red chromophore lycopene as a common precursor. C50 carotenoids are formed by elongation of lycopene through the addition of two C5 prenyl units and subsequent hydroxylation[22]. Due to the presence of multiple conjugated double bounds and hydroxyl groups, C50 carotenoids exhibit shifted light absorption and strong antioxidative and reactive oxygen-quenching properties.

The condensation of two molecules of GGPP to produce phytoene is catalyzed by well conserved enzymes among archaea, bacteria and eukaryotic organisms. The identification of the first prokaryotic genes for carotenoid biosynthesis settled a terminology (crt) that has been maintained to refer to homologous genes in many other bacteria. The genes encoding GGPP synthase (CrtE), phytoene synthase (CrtB), phytoene and □-carotene desaturases (CrtI and CrtQ), and lycopene cyclase (CrtY) are conserved between non-photosynthetic and photosynthetic bacteria. These genes for bacterial carotenoid biosynthesis, and also those for regulation, are usually organized as operons. Further modifications like hydroxylations and glycosylations are carried out by CrtZ and CrtX, respectively. In the majority of bacteria, the colorless phytoene is dehydrogenized by the CrtI-type phytoene desaturase via three, four, five or even six consecutive steps to form neurosporene, lycopene, 3,4-didehydrolycopene or 3,4,3',4'-tetradehydrolycopene, respectively[23].

In photosynthetic bacteria most C40 carotenoids are acyclic and contain sulfates, methoxy, carbonyl, carboxyl, glycosides, and hydroxyl groups[24,25]. GGPP is formed by CrtE, which catalyzes the condensation of farnesyl pyrophosphate with an isopentyl pyrophosphate moiety. The second step catalyzed by CrtB is the formation of phytoene from the head-to-head condensation of two molecules of GGPP. Subsequent dehydrogenations catalyzed by the phytoene desaturase CrtI convert the phytoene to neurosporene in three desaturation steps or to lycopene in four steps. After the action of these three enzymes (CrtE, CrtB, and CrtI), the biosynthetic pathways diverge depending on the species leading to the accumulation of various different carotenoids. Five main carotenogenesis pathways have been proposed for anaerobic photosynthetic bacteria:

i. Spirilloxanthin pathway that is divided in normal spirilloxanthin, unusual spirilloxanthin, spheroidene, and carotenal pathways
ii. Isorenieratene pathway, that also includes that producing chlorobactene
iii. Okenone pathway, that includes the okenone and the R.g-keto carotenoid pathways
iv. γ- and β-carotene pathway
v. diapocarotene pathway

There are additional pathways leading to carotenoid glucosides and carotenoid glucoside fatty acid esters. In the case of aerobic photosynthetic bacteria, most of them use the spirilloxanthin pathway[26].

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