Microbial Production of Astaxanthin

Chi-Ching Lee

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Abstract

Astaxanthin is a carotenoid reddish-orange pigment with remarkable biological activities and benefits in human health. It also provides enormous commercial value in the application of functional food, aquaculture, and pharmaceutical business. Presently commercial production of astaxanthin includes chemical synthesis, extraction, and isolation from natural microbial sources such as bacteria *Agrobacterium aurantiacum* and *Paracoccus carotinifaciens*, green microalgae *Haematococcus pluvialis*, and yeast *Phaffia rhodozyma*. Microbial production of astaxanthin by metabolic engineered microorganisms has developed for decades and become the main alternative in industrial astaxanthin production. Because of the increasing demand and importance of astaxanthin, biotechnological astaxanthin production has been established, and its

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biosynthetic pathway also has been studied in the heterobasidiomycetous yeast *Xanthophyllomyces dendrorhous,* green microalgae *Haematococcus pluvialis, and* the teleomorphic state of *Phaffia rhodozyma*. The introduction of biosynthetic astaxanthin pathway into *Corynebacterium glutamicum*, *Escherichia coli,* and *Saccharomyces cerevisiae* is also used in heterologous productions of astaxanthin. This chapter will discuss biosynthetic pathways and regulation of astaxanthin, astaxanthin production in natural sources and biotechnical methods, and the influence of environmental factors in astaxanthin biosynthesis. At the end of the chapter, perspectives concerning the innovative strategies for further developing astaxanthin production and its trends to improve the metabolically engineering microorganisms will be discussed.

Keywords

Astaxanthin · Microbial production of astaxanthin · *Haematococcus pluvialis* · *Phaffia rhodozyma* · *Xanthophyllomyces dendrorhous*

Introduction

Astaxanthin is an orange-reddish keto-carotenoid that the human body cannot synthesize by itself. It is widely distributed in algae, bacteria, yeasts, plankton, salmon, flamingo, the shells of shrimp, crabs, and other shellfish. Astaxanthin was originally first utilized as an aquaculture pigment in 1938 and has been recognized as a food colorant since 1991. Astaxanthin has become an important source of pigmentation in aquaculture and the food industry owing to its safety and consistent orange-reddish color. The antioxidant activity of astaxanthin is approximately ten up to a thousand times higher than many other antioxidants, including vitamin E, coenzyme Q10, β-carotene, polyphenols, and lycopene. Except for high antioxidant activity, there are various biological activities of astaxanthin and promising human health benefits, such as antiaging, anticancer, anti-inflammatory, anti-tumor, antihypertensive, prevention of cardiovascular diseases and diabetes mellitus complications, skin protection, and eye health. Several studies also confirmed that astaxanthin could be used for the treatment of *Helicobacter pylori* infections and enhancement of sports performance and muscle recovery. Astaxanthin can be produced in numerous levels by various microalgae, including *Haematococcus pluvialis*, *Protosiphon botryoides*, *Scotiellopsis oocystiformis*, *Neochloris wimmeri*, *Chlorella zofingiensis*, *Scenedesmus* sp., *Chlorococcum* sp., *Eremosphaera viridis*, and so on. Because *Haematococcus pluvialis* has the unique capacity to accumulate high levels of astaxanthin under stress circumstances, this green microalga is considered one of the most potent producers of astaxanthin in nature.

Astaxanthin is in high demand in the food industry, pharmaceuticals, cosmetics, and animal feeds due to its possible therapeutic benefits. In 2014, both natural and synthetic astaxanthin is reported at 280 metric tons worth $447 million in the global market. In 2020, it will reach 670 metric tons with $1 billion. By 2025, its global

market is estimated to achieve $2.57 billion (Park et al. 2018). As a result, many methods for producing astaxanthin have been investigated, such as the cultivation of natural microbial producers like the green microalgae *Haematococcus pluvialis*, *bacterium Paracoccus* sp., and the red yeast *Xanthophyllomyces dendrorhous*. Besides, other approaches to astaxanthin production contain chemical synthesis and direct extraction of crustacean waste like the shell of shrimp, lobsters, crabs, crawfish, and krill. However, the disadvantages of cultivation of natural microbial producers and direction extraction of crustacean waste have relatively high production costs. Therefore, synthetic astaxanthin accounts for more than 95% of the astaxanthin on the market. Synthetic astaxanthin has been demonstrated that it has a lower antioxidant capacity than natural astaxanthin due to a racemic mixture including three isomers with the mixture ratio of 1:2:1 in 3R, 3′R-astaxanthin, 3R, 3′S- or 3S, 3′R-astaxanthin, and 3S, 3′S-astaxanthin (Koller et al. 2014). Furthermore, the application of synthetic astaxanthin for direct human intake raises safety issues because of the possibility of biosynthesis intermediates and by-products being carried over (Shah et al. 2016). As a result, the natural astaxanthin market has been expanding faster than synthetic astaxanthin in recent years because of the radical increase in natural astaxanthin demand. A number of studies have been reported on many non-native industrial host microorganisms, such as *Yarrowia lipolytica*, *Escherichia coli*, and *Saccharomyces cerevisiae*, for astaxanthin synthesis to enhance conventional production employing natural hosts. In this chapter, the chemical structure of astaxanthin will be interpreted for its stereoisomers. Biosynthesis, regulation, biosynthesis localization, and storage distribution in astaxanthin will also be mentioned. For microbial biosynthesis of astaxanthin, natural native hosts and the metabolic-engineered non-native microbial host will be broadly discussed in a variety of microorganisms.

Structure and Isomerism of Astaxanthin

Stereoisomers of Astaxanthin

Astaxanthin (3,3′-dihydroxy-*β*,*β*′-carotene-4,4′-dione; CAS no. 472–61-7) is comprised of a double-bonded 40-carbon long polyene chain with one hydroxyl group on each terminal ring. The molecular formula of astaxanthin $C_{40}H_{52}O_4$ and its molar mass is 596.84 g/mol. Astaxanthin comes in a variety of forms that may be categorized into stereoisomers, geometric isomers, and free or esterified forms. Because astaxanthin is a symmetrical molecule with two chiral centers and each isomer can occur in either the R or S form, it possesses four stereoisomeric forms on both β-ionone terminal rings of the long-chain structure, which has two asymmetric carbons located at the numbered 3 and 3′ position. Synthetic astaxanthin contains an all-*trans* configuration of astaxanthin that may present in a 1:2:1 mixture of three configurational stereoisomers: one enantiomer (3R, 3′R), optically inactive meso forms (3R, 3′S or 3S, 3′R), and the other enantiomer (3S, 3′S).

Geometric Isomers of Astaxanthin

For geometrical isomers of astaxanthin, its long linear polyene chain contains many double bonds, and each of them can occur in either the *E* or *Z* (*trans* or *cis*) form depending on the configuration of the double bonds. All-*trans* astaxanthin (all-*E*-astaxanthin) is the most thermodynamically favorable and dominant derivative of the molecule even though at least two *cis* isomers, which are 9-*cis* and 13-*cis,* also exist in nature. *Z* isomers have been found in nature at carbon positions C9, C13, and C15, either singly or in multi-combination. As a result, there are nine geometric isomers, including (all-*E*)-, (9*Z*)-, (13*Z*)-, (15*Z*)-, (9*Z*, 13*Z*)-, (9*Z*, 15*Z*)-, (13*Z*, 15*Z*)-, and (13*Z*, 15*Z*)-, and (9*Z*, 13*Z*, 15*Z*)-astaxanthin (Bernhard 1989). The majority of astaxanthin found in rainbow trout (*Oncorhynchus mykiss*) is all-*trans* astaxanthin (97%) followed by 9-*cis* (0.4%), 13-*cis* (1.5%), and various isomers (1.1%) (Østerlie et al. 1999). Other than all-*trans*, 13-*cis*, and 9-*cis* isomers, 15-*cis* and di-*cis* isomers were discovered in numerous wild and cultivated shrimps in China, including *Trachysalambria curvirostris*, *Penaeus monodon*, *Fenneropenaeus chinensis*, *Litopenaeus vannamei*, and *Exopalaemon carinicauda* (Su et al. 2018; Su and Liu 2019). In Fig. 1, the structural arrangements of astaxanthin configurational isomers are described.

Free and Esterified Astaxanthin

On each terminal β-ionone ring of astaxanthin, there are a total of two unreacted hydroxyl groups. These can be unreacted hydroxyls or can generate an ester form when they react with an acid like a fatty acid. The esterified end in the chemical structure becomes more hydrophobic when a fatty acid is added to synthesize an ester (Fig. 1). It is noticed that astaxanthin diesters have more hydrophobicity than astaxanthin monoesters, and unesterified astaxanthin has the most hydrophilicity.

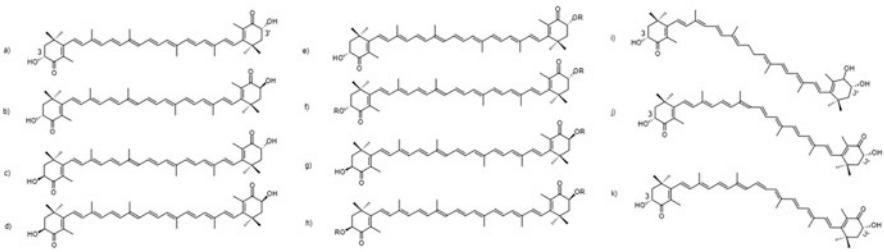

Fig. 1 Structural arrangement of astaxanthin configurational isomers: all-*E*-3R,3′R- astaxanthin (**a**), all-*E*-3R,3′S- astaxanthin (**b**), all-*E*-3S,3′R- astaxanthin (**c**), all-*E*-3R,3′R- astaxanthin (**d**), all-*E*-3R,3′R- astaxanthin monoester (**e**), all-*E*-3R,3′R- astaxanthin diester (**f**), all-*E*-3S,3′S-astaxanthin monoester (**g**), all-*E*-3S,3′S- astaxanthin diester (**h**), 9-cis-3R,3′R- astaxanthin (**i**), 13-*cis*-3R,3′R- astaxanthin (**j**), 1-*cis*-3R,3′R- astaxanthin (**k**)

Distribution of Different Isomers of Astaxanthin

The primary stereoisomer of astaxanthin discovered in a shrimp-like krill (*Euphausia superba*) is 3R, 3′R, with the majority of this occurring in the esterified form (Bernhard 1989). Besides, primary carotenoids as the 3R, 3′R stereoisomer, and the largely esterified astaxanthin also are observed in a basidiomycete red yeast *Xanthophyllomyces dendrorhous,* as formerly *Phaffia rhodozyma (*Golubev 1995*). On the other hand,* astaxanthin is found in wild salmon as the major 3S, 3′S isomer; in salmon meat, this stereoisomer is found as a free form of xanthophyll. In the green microalga *Haematococcus pluvialis*, astaxanthin is found as the 3S, 3′S stereoisomer as well (Bernhard 1989). Among all astaxanthin isomers in *Haematococcus pluvialis*, approximately 80% of astaxanthin monoesters and 15% of astaxanthin diesters were accumulated, with C18:1 and C20:0 being the most common fatty acids in the esters (Renstrøm and Liaaen-Jensen 1981).

Biosynthesis and Regulation of Astaxanthin

There is not only metabolic engineering production for astaxanthin synthesis but also astaxanthin biosynthesis in natural hosts. Astaxanthin is present in many creatures in nature, including marine animals such as salmons, shrimps, lobsters, crabs, and krills, as well as birds such as flamingos and quails. These animals, on the other hand, do not generate astaxanthin and instead obtain it through their diet, which contains natural astaxanthin sources. Green microalgae (*Haematococcus pluvialis* and *Chlorella zofingiensis*) (Mendes-Pinto et al. 2001; Sarada et al. 2006), fungus (*Xanthophyllomyces dendrorhous*, anamorph *Phaffia rhodozyma*) (Verdoes et al. 2003), and bacteria (*Paracoccus* spp. and *Brevundimonas* spp.) are all-natural producers of astaxanthin (Ide et al. 2012). Furthermore, albeit at low levels, plants (*Adonis aestivalis and Adonis annua*) and protists (*Aurantiochytrium* sp.) generate astaxanthin from their flowers (Watanabe et al. 2018). Astaxanthin is a kind of xanthophyll that is frequently utilized in aquaculture as a pigment. In nature, the all-trans isomer is found alongside minor quantities of the 9-*cis* and 13-*cis* isomers. Canthaxanthin and astaxanthin can be produced by the addition of keto groups at the C4 and C4′ positions with or without hydroxylation at the C3 and C3′ positions (Bhosale and Bernstein 2005). In microalgae, yeasts, and non-photosynthetic bacteria, the production of keto carotenoids from β-carotene via echinenone and canthaxanthin is well developed by researchers Fraser et al. in 1997 and Britton in 2004.

Terpenoid astaxanthin production begins in the abovementioned natural producers with the two typical terpenoid precursors, five-carbon isomers isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Both IPP and DMAPP are produced naturally by two distinct biosynthetic pathways, including the mevalonic acid (MVA) pathway in eukaryotic microorganisms utilizing acetyl-CoA as a precursor and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, also known as the non-mevalonate or 1-deoxy-D-xylulose 5-phosphate (DXP)

pathway in prokaryotic microorganisms and plant plastids using glyceraldehyde-3-phosphate and pyruvate as precursors (Eisenreich et al. 2004). Acetyl-CoA acetyltransferase (AACT) condenses two acetyl-CoA molecules to generate acetoacetyl-CoA in the MVA pathway, which is then condensed with another acetyl-CoA molecule by hydroxymethylglutaryl-CoA synthase (HMGS) to produce 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) (Wang et al. 2019b). The mevalonate is then formed as a result of a reduction step by 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR). Following that, IPP is synthesized in three steps mevalonate kinase (MK), phosphomevalonate kinase (PMK), and mevalonate diphosphodecarboxylase (MVD). The generated IPP is subsequently isomerized to DMAPP by isopentenyl diphosphate isomerase (IDI). The enzyme geranyl pyrophosphate synthase (GPS) converts IPP and DMAPP produced through MEP or MVA pathways to ten-carbon geranyl pyrophosphate (GPP), which is then converted to fifteen-carbon farnesyl pyrophosphate (FPP) by the action of farnesyl diphosphate synthase (FFPS), and then to twenty-carbon geranylgeranyl pyrophosphate (GGPP) by the action of GGPP synthase (GGPPS) (Wang et al. 2019b). Therefore, two synthases of FPP and GGPP combine IPP and DMAPP to form geranylgeranyl pyrophosphate (GGPP). GGPP is subsequently converted by phytoene synthase (PSY or CrtB) to form the first forty-carbon carotenoid phytoene, which is then turned into lycopene by several desaturases. Phytoene is desaturated and converted to lycopene by phytoene desaturase (PDS) and zeta-carotene desaturase (ZDS), respectively. Lycopene is then converted into β-carotene by bacterial lycopene β-cyclase (LCY, CrtY, CrtYB, CarRRP, or LCYB). Then, through enzymatic activities that are reliant on the generating organisms, β-carotene is transformed into astaxanthin (Martín et al. 2008). In Fig. 2, possible biosynthetic pathways for β-carotene formation are described.

The biosynthetic pathway is sequential and substantially the same in various species until β-carotene. Nonetheless, beginning with β-carotene, the biosynthetic pathway differs among species (Figs. 3 and 4). The β-ionone ring of β-carotene can be converted into 4-keto intermediate product and 3-hydroxyl intermediate products oxidized by β-carotene ketolases and hydroxylases in bacteria, fungi, and algae. These two hydroxylation reactions and two ketolation reactions can take place consecutively or nonconsecutively. Two carbonyl groups and two hydroxyl groups are added to the 4, 4′ carbon and 3, 3′ carbon positions of the β-ionone ring accordingly, resulting in the conversion of β-carotene to astaxanthin (Fig. 3). Depending on the substrate preferences of the various enzymes, the hydroxylation and ketolation processes may occur alternately, causing the buildup of multiple intermediates (Fraser et al. 1998). In bacteria, fungi, and algae, β-carotene can be converted into astaxanthin via seven intermediates including β-cryptoxanthin, echinenone, canthaxanthin, zeaxanthin, 3-hydroxyechinenone, adonixanthin, and adonirubin (Zhang et al. 2018). In algae, the β-carotene ketolases are encoded by genes *crtO* (or *bkt*), and β-carotene hydroxylase is encoded by genes *crtR-B* (or *chyb*) (Han et al. 2013). In bacteria, the β-carotene ketolases are encoded by gene *crtW* and β-carotene hydrolyase encoded by gene *crtZ* (Breitenbach et al. 1996). In Fig. 3,

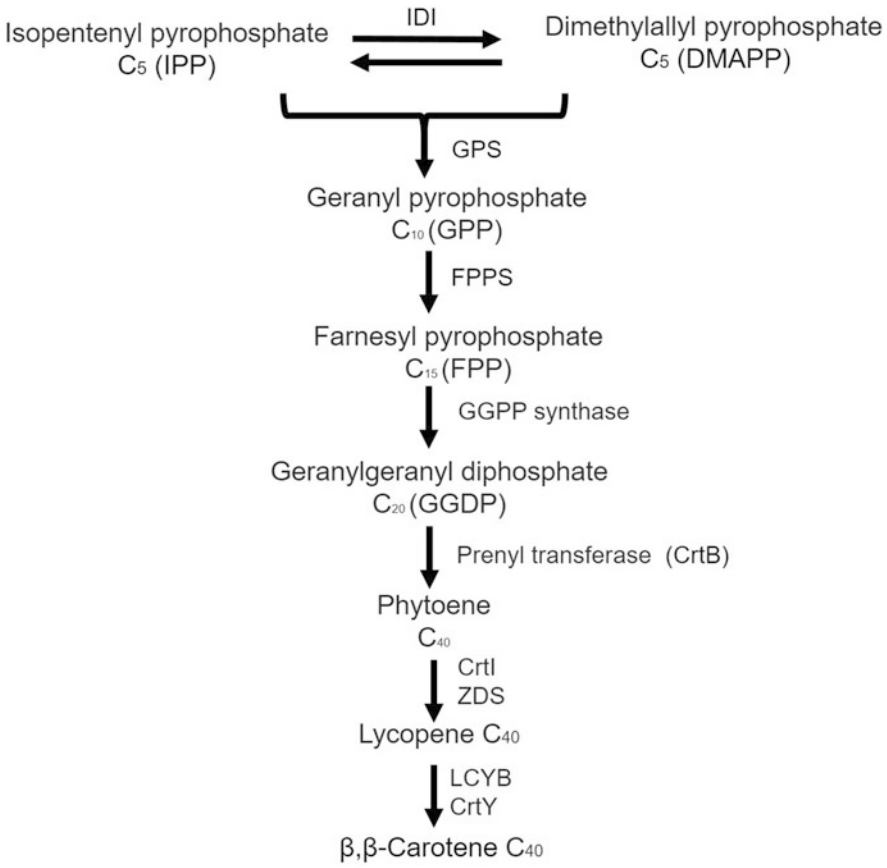

Fig. 2 Possible biosynthetic pathways for β-carotene formation. IDI, isopentenyl diphosphate isomerase; GPS, geranyl pyrophosphate synthase; FPPS, farnesyl pyrophosphate synthase; CrtB, bacterial phytoene synthase; crtI, bacterial phytoene desaturase; ZDS, zeta-carotene desaturase; CrtY, bacterial lycopene β-cyclase; LCYB, plant lycopene β-cyclase (Bhosale and Bernstein 2005; Zhang et al. 2020)

possible biosynthetic pathways for astaxanthin formation are described in bacteria, fungi, and algae.

Three different BKTs have been confirmed to be demonstrated in *Haematococcus pluvialis* in response to various environmental stress conditions. The degree of expression of these proteins varies depending on the stressful circumstances. In the *Haematococcus pluvialis* genome, six copies of the *bkt* genes (*bkt1a*, *bkt1b*, *bkt1c*, *bkt2a*, *bkt2b*, and *bkt3*) were discovered and thought to be the consequence of genetic recombination during genome development (Luo et al. 2019). Besides, two distinct β-carotene hydroxylase genes (crtR-B1 and crtR-B2) were identified in the genome of *Haematococcus pluvialis* (Huang et al. 2021). The products of these genes are thought to be confined to distinct cellular compartments except for the

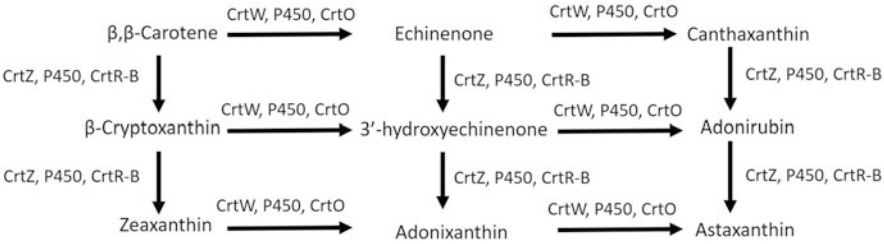

Fig. 3 Possible biosynthetic pathways for astaxanthin formation in bacteria, fungi, and algae. CrtW, bacterial β-carotene ketolase; P450, yeast astaxanthin synthase (CrtS), which depends on CrtR, the four-domain cytochrome P450 reductase; crtO, alga β-carotene ketolase (bkt); CrtZ, bacterial β- carotene hydroxylase; CrtR-B, alga β-carotene hydroxylase (Zhang et al. 2020)

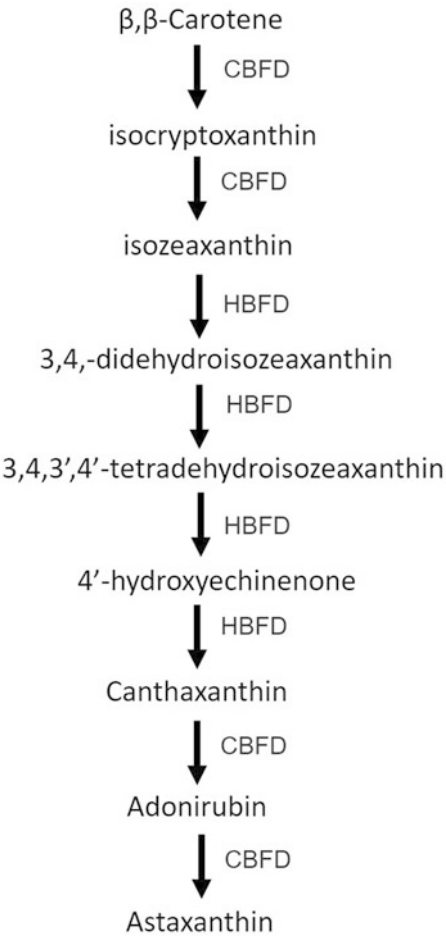

Fig. 4 Possible biosynthetic pathways for astaxanthin formation in the plants *Adonis aestivalis* and *Adonis annua*. CBFD, carotenoid β-ring 4-dehydrogenase; HBFD, carotenoid 4- hydroxy-β-ring 4-dehydrogenase (Cunningham Jr and Gantt 2011; Zhang et al. 2020)

various copies nature of these genes in *Haematococcus pluvialis*. These results might clarify why *Haematococcus pluvialis* produces significantly more astaxanthin than other sources. Likewise, in the red yeast *Xanthophyllomyces dendrorhous*, the β-carotene ketolase and hydroxylase are encoded by genes *crtS,* and these two enzymes (or called astaxanthin synthases) are cytochrome P450 oxygenases functioning with a cytochrome P450 reductase encoded by genes *crtR* (Álvarez et al. 2006). A bi-functional cytochrome P450 astaxanthin synthase (CrtS) in this red yeast is assumed to catalyze the conversion of β-carotene to astaxanthin (Fig. 3)**.** The redox partner cytochrome P450 reductase, CrtR, is required for astaxanthin production. In this case, expressing *crtS* gene alone in a β-carotene producing *Saccharomyces cerevisiae* did not result in astaxanthin accumulation until *crtR* was also expressed (Fuentealba et al. 2012). In addition, the astaxanthin biosynthetic pathway in the flowers of *Adonis aestivalis* and *Adonis annua* plants was proposed by Cunningham Jr. and Gantt in 2011 (Fig. 4). β-carotene is converted into isocryptoxanthin and sequentially isozeaxanthin by two carotenoid β-ring 4-dehydrogenases (CBFD) encoded by two cDNA gene *cbdf1* and *cbdf2*, formerly known as AdKeto1 and *AdKeto2* (Cunningham Jr and Gantt 2005). Successively, isozeaxanthin is converted into canthaxanthin via 3,4,-tetradehydroisozeaxanthin, 3,4,3′,4′-tetradehydroisozeaxanthin, 4′-hydroxyechinenone by two carotenoid 4-hydroxy-β-ring 4-dehydrogenases (HBDF), encoded by gene *hbdf1* and *hbdf2*. Lastly, astaxanthin is formed from canthaxanthin through adonirubin by CBFD. Overall, five intermediates exist between β-carotene and astaxanthin in algae, bacteria, and fungi. Nevertheless, there are seven distinct intermediates. Most species produce 3S, 3′S-astaxanthin in contrast to *Xanthophyllomyces dendrorhous*, which can naturally produce the 3R, 3′R-astaxanthin isomer. In Fig. 5, the chemical structures of carotenoids involved in the astaxanthin biosynthetic pathway are described.

Astaxanthin Biosynthesis Localization and Storage Distribution

The discovery of the subcellular location of astaxanthin production and storage is crucial for establishing astaxanthin overproducers, but just a few investigations have been undertaken in natural astaxanthin producers. Biosynthesis localization may occur at several subcellular sites because astaxanthin biosynthesis is a series of complex reaction involving numerous enzymes. In *Haematococcus pluvialis*, astaxanthin biosynthesis is assumed to be split between the chloroplast and the endoplasmic reticulum, with β-carotene being generated in the chloroplast and then transferred to the endoplasmic reticulum to be converted to astaxanthin before being esterified. The bioactive compound phycocyanin can be also commonly observed in blue-green algae (Ashaolu et al. 2021). Carotenogenesis in algae emerges from the MEP pathway, which initiates in the chloroplast. Multiple enzymes involved in β-carotene biosynthesis have also been found in the chloroplast. Endoplasmic reticulum-containing fractions are related to β-carotene conversion to astaxanthin based on a study utilizing different fractions of *Haematococcus*

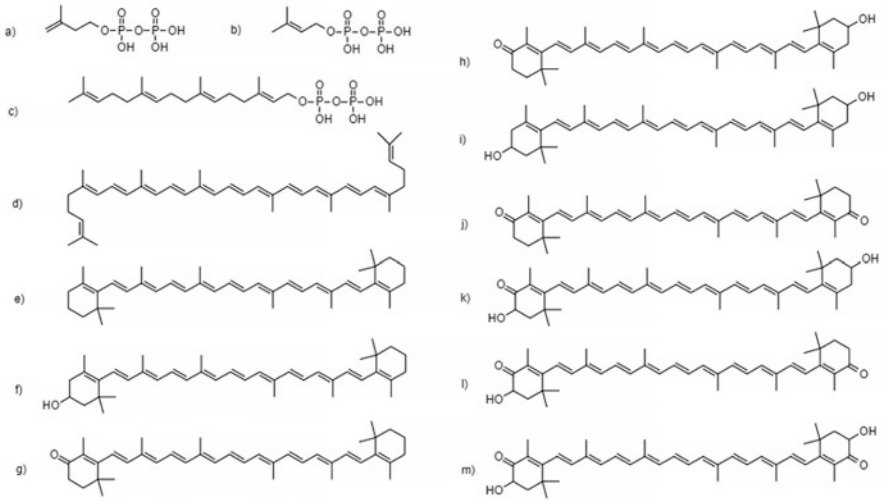

Fig. 5 Chemical structures of carotenoids: isopentenyl pyrophosphate (IPP) (**a**), dimethylallyl pyrophosphate (DMAPP) (**b**), geranylgeranyl pyrophosphate (GGPP) (**c**), lycopene (**d**), β-carotene (**e**), β-cryptoxanthin (**f**), echinenone (**g**), 3′-hydroxyechinenone (**h**), zeaxanthin (**i**), canthaxanthin (**j**), Adonixanthin (**k**), adonirubin (**l**), astaxanthin (**m**)

pluvialis cell lysates in vitro (Chen et al. 2015). The presence of β-carotene hydroxylase in lipid bodies and chloroplast membranes has been reported. Ketolase was exclusively found in lipid bodies instead (Grünewald et al. 2001). In eukaryotes, lipid bodies are known to be formed from the endoplasmic reticulum, and it may also explain why astaxanthin metabolic behavior was identified in the endoplasmic reticulum-containing fractions (Chen et al. 2015). The red yeast *Xanthophyllomyces dendrorhous* has restricted studies on biosynthetic activities and storage localization, in contrast to the widely researched *Haematococcus pluvialis*. For astaxanthin synthesis in this yeast, a membrane-enclosed cartoenogenic complex has been mentioned, and carotenogenesis may be localized to the endoplasmic reticulum (Verdoes et al. 2003). Furthermore, a bi-functional p450 monooxygenase (CrtS), which is presumed to be localized to the endoplasmic reticulum, is involved in astaxanthin production from β-carotene (Ojima et al. 2006; Fuentealba et al. 2012).

Because of the high hydrophobicity in carotenoids, cell membranes and lipid bodies with high lipid content are favorable environments for astaxanthin storage. Nevertheless, an esterified or free form of astaxanthin might impact astaxanthin dispersion in cells. Most of the astaxanthin is in esterified form and stored in thylakoid membranes and lipid bodies (Chen et al. 2015). Additionally, the free form content of astaxanthin in thylakoid membranes was substantially higher than that of the esterified astaxanthin, and more than half of the free form content was found in the thylakoid membrane. The result showed that the free form of astaxanthin has a higher affinity in the membrane than in lipid bodies (Mascia et al. 2017). But another study also assumed that this result might be questionable

because the cell lysates containing the thylakoid membranes might be contaminated with lipid bodies (Ota et al. 2018). On the contrary, most astaxanthin in *Xanthophyllomyces dendrorhous* is a free form in its natural state. The integration of astaxanthin in the plasma membrane and the accumulation of the carotenoids in lipid bodies of *Xanthophyllomyces dendrorhous* membranes were confirmed by electron paramagnetic resonance spectroscopy and laser confocal fluorescence microscopy (Blasko et al. 2008; An et al. 1991). Nonpolar carotenoids like β-carotene are localized in the triglyceride-filled core of the lipid bodies, and polar carotenoids like zeaxanthin are primarily observed in the esterified sterols-enclosed phospholipid monolayer of the lipid bodies. The assimilation of carotenoids in membranes is exhibited to be cytotoxic to cells, resulting in cell membrane degradation, oxidative stress, stimulation of drug stress responses, and a reduction in plasma permeability (Liu et al. 2016). Carotenoids are embedded in membranes in a variety of configurations depending on their polarity, with nonpolar carotenoids perpendicular to membrane lipid chains and polar carotenoids in parallel configurations. As a result, the polar carotenoid significantly influences membrane fluidity (Blasko et al. 2008).

Microbial Biosynthesis of Astaxanthin

Many recent studies demonstrate several approaches to biotechnological development and metabolic engineering in microbial astaxanthin production. To further understand and compare these approaches, there are native and non-native astaxanthin-producing microorganisms. However, the modified astaxanthin, such as astaxanthin esters, is produced by the native host, and the modified natural products are challenging to monitor and utilize. Besides, biocatalytic development and untargeted strategies like random mutagenesis are typical options for native hosts due to the difficulty of targeted genetic modification. As a result, metabolic-engineered microorganisms as non-native hosts have the controllable and modifiable feature of genetic engineering techniques. However, biosynthesis complexity with multiple genes and inefficient production with heterologous enzymes are the difficulties in metabolic engineered production in non-host. Besides, there are abundant intermediate carotenoids with similar chemical structures or stereoisomers during biosynthetic production. To overcome these issues, a number of innovative metabolic engineering strategies and biomimetic technologies have been established.

Astaxanthin Production in Native Microbes

Haematococcus pluvialis

Compared to other microorganisms, *Haematococcus pluvialis* is the most prolific producer of natural astaxanthin. As a result, it is critical to comprehend the astaxanthin production of *Haematococcus pluvialis* from the cultivation stage through downstream astaxanthin synthesis. The lifecycle of *Haematococcus*

pluvialis contains a green vegetative (macrozooid) motile stage and a red hematocyst (aplanospore) non-motile stage. The biomass rises with the growth of vegetative cells in the green vegetative motile stage, but no astaxanthin accumulates. Astaxanthin starts to deposit in the membrane of hematocysts in the red non-motile stage. The astaxanthin extraction and bio-availability to animals are impeded by the sporopollenin-containing thick cell wall of the cysts. Environmental stresses cause the transition between the green vegetative motile stage and the red hematocyst non-motile stage of *Haematococcus pluvialis*, such as excessive salinity, nutritional nitrogen, phosphorus deficiency, temperature gradient, or a mixture of various stress influences. The traditional astaxanthin production methods include solvent extraction, mechanical disruption, and direct extraction using vegetable oils. As well, the innovative astaxanthin production strategies contain ionic liquids extraction, magnetic-assisted extraction, supramolecular solvent extraction, and supercritical CO_2 extraction. Because of the requirements for this two-stage cultivation strategy, the production period is always extended, and the final biomass is frequently low. Genome enhancement heavily depends on more traditional procedures such as random mutagenesis and screening because of the absence of engineering simulation and modeling. As a result, numerous studies have been reported on optimizing abiotic conditions and biosynthesis establishment (Zhang et al. 2020). To achieve a high astaxanthin concentration and cell population, the vegetative growth phase must be divided from the hematocyst phase which astaxanthin accumulation occurs because the optimum of vegetative cell growth differs from those for astaxanthin biosynthesis. As a result, commercial production of astaxanthin from *Haematococcus pluvialis* is often performed in two phases. Under low-stress environments, the number of flagellate green motile-type cells and immature latent non-motile-type cells increases in the first green vegetative growth phase. In the second red hematocyst phase, these two distinct green cells are subjected to severe stress, halting cell multiplication. Nonetheless single-cell mass starts to rise due to morphological metamorphosis in encystment from green cells to red aplanospore cysts for astaxanthin production under carotenogenesis (Fábregas et al. 2003; Hong et al. 2015).

Domesticated *Haematococcus pluvialis* was used to enhance dry biomass weight and astaxanthin yield under mutant screening with nuclear irradiation and 15% CO_2 treatment. After ten generations under 15% CO_2 domestication, the biomass production of the mutant improved to 1.3 times in comparison with air. The dry biomass weight was significantly enhanced by 62% when nitrogen and phosphorus ratios were optimized. The astaxanthin output was triggered by 15% CO_2 and high light of 135 μmol photons per square meter and second climbed to 87.4 mg/L, which was six times higher than the output triggered with the same high light in air. It can improve astaxanthin yield and decrease the flue gas cost in coal-fired power plants (Cheng et al. 2016). Under the photoautotrophic environment, high temperatures between 30 and 36 °C reduced astaxanthin accumulation in *Haematococcus pluvialis*. While outdoor cultivation during summer, heat stress had a detrimental impact on autotrophic astaxanthin biosynthesis. The level of intracellular reactive oxygen species increases in the high-temperature environment when carotenogenesis is decreased.

Under summer temperatures, vegetative cell proliferation in the green stage was severely slowed, and the production of astaxanthin was somewhat hampered in the red stage. The heat stress-induced inefficient astaxanthin synthesis was significantly increased by the iron-catalyzed Haber–Weiss reaction to enhance reactive oxygen species and lipid peroxidation. As a consequence, after 18 days of photoautotrophic induction, the astaxanthin content of cells cultivated at high temperatures in the presence of 450 μM iron solution improved by 75% at 30 °C and 133% at 36 °C as compared to the high heat stress cells without iron treatment. For commercial astaxanthin production, the heat stress-induced Haber–Weiss reaction will be profitable by increasing photoautotrophic astaxanthin biosynthesis with low-cost energy. Especially, it can be used in outdoor photo-induction *Haematococcus pluvialis* manufacturing due to natural heat and light from solar radiation (Hong et al. 2015). Another similar study is to operate a closed system like photobioreactor during the summer for *Haematococcus* culture by inoculating the red cyst cells to enhance heat-stress-induced unproductive vegetative cell growth in the green stage and supplementing 50 μM iron (II) to trigger the Haber–Weiss reaction induced by high heat in the red stage. The astaxanthin production under summer temperatures between 23.4 and 33.5 °C increased 147% up to 5.53 mg/L daily compared to that of the cell cultivation under spring lower temperatures between 17.5 and 27.3 °C. These findings disclosed that maintaining moderate intracellular carotenoid concentrations during photoautotrophic growth is a critical component for astaxanthin production in *Haematococcus pluvialis* at high temperatures (Hong et al. 2016).

The enhancement of *Haematococcus pluvialis* resistance to nitrogen starvation and high light was confirmed by the addition of melatonin. During 13-day cultivation, the astaxanthin concentration was 14.36 mg per gram sample in the control group. However, the astaxanthin concentration significantly increased to 32.37 mg per gram sample under 10 μM melatonin treatment. It demonstrated a 2.25-fold increase in astaxanthin production after induction with melatonin (Ding et al. 2018). An economical and efficient two-stage approach for producing natural astaxanthin from *Haematococcus pluvialis* was reported by avoiding the separation and media replacement step between the first induction stage and the second accumulation stage. In the first green stage, *Haematococcus pluvialis* was cultivated under low illumination until it achieved its maximum biomass. In the second red stage, cells were converted to astaxanthin accumulation by using a combination of high illumination and high carbon dioxide levels at 5 or 15% for 4 days. Without replacing the medium, CO_2 affected the carbon and nitrogen ratio balance, resulting in a nutritional shortage of cells. The astaxanthin production was 2–3 times higher than other single-stress treatments in control conditions. The culture under 15% CO_2 balanced with air and exposed to intense light illumination caused astaxanthin accumulation of up to 36 mg/g dry cell weight. The advantages of this strategy contain a short production period of fewer than 4 days and no change-out of cultural media (Christian et al. 2018). The astaxanthin production of a new *Haematococcus pluvialis* JNU35 isolate with high biomass and astaxanthin yield was evaluated in an mBBM medium under a combined treatment of the light path (inner diameter of 3 cm or 6 cm) and illumination mode (unilateral or bilateral high light illumination)

in column photobioreactors. The result indicated that the *Haematococcus pluvialis* JNU35 isolate could produce 18.1 mg/L astaxanthin daily and an ultrahigh biomass concentration of 20.1 g/L under an optimal photo-induction combination which are 6 cm light path in the first green stage and 3 cm light path in the second red stage with bilateral high light illumination for a total of 30 days. An optimal phase of green vegetative cells transferred into the red inductive stage was established by the substitution of fresh media to increase biomass concentration to 27.3 g/L. Besides, under the optimal conditions mentioned above, *Haematococcus pluvialis* JNU35 isolate acquired a higher astaxanthin level in a flat-plate photobioreactor with a substantial amount of 5.6% (or 56 mg/g dry cell weight) of astaxanthin and a higher 3.0 g/L of biomass content. This study presented a novel strategy for increasing biomass production in industrial applications and proficient photo-induction of *Haematococcus pluvialis* cultures (Wang et al. 2019a).

To accelerate the rupture of the encysted cells of *Haematococcus pluvialis*, a variety of chemical and physical methods were reported. The productivity of these methods was measured in terms of astaxanthin recovery, which was determined by measuring the amount of astaxanthin released into an extractant. A variety of treatments were used, including NaOH 0.1 M, 15 min and 30 min; HCl 0.1 M, 15 min and 30 min; autoclave 30 min, 121 °C, 1 atm; spray drying, inlet 180 C and outlet 115 °C. An enzymatic mixture of 0.1% protease K and 0.5% driselase in a phosphate buffer was used with pH 5.8 at 30 °C for 1 h. A mechanical disruptor with a cell homogenizer was also designed for cell rupture. The result exhibited that the most efficient extraction and availability methods were autoclave treatment and mechanical cell homogenization with acid pretreatment. Both methods were in 2 ml acetone solvent for 16 h at 20 °C with 30-minute acetone pretreatment. Astaxanthin yields under autoclave and homogenization treatment were 18 mg/g and 19 mg/g, respectively, and astaxanthin recovery of these two treatments is more than 85% (Mendes-Pinto et al. 2001). The efficacy of ethanol on cellular growth and astaxanthin production was evaluated in *Haematococcus pluvialis*. The results revealed that 0.4% ethanol could stimulate astaxanthin biosynthesis during the earlier cultivation phase within 4 days and significantly increased the cellular astaxanthin level to 4.23% of dry cell weight, which was 5.87-fold higher than the control. Besides, 95% of astaxanthin accumulation was esterified in the form of astaxanthin monoesters, which was substantiated by increased monounsaturated fatty acid concentration. Morphological, ultrastructural examination displayed that ethanol can alter physiological characteristics and cell wall structure by inducing methyl jasmonate biosynthesis on the intricate carotenogenic pathway (Liu et al. 2019).

Chromochloris zofingiensis

Heterotrophic *Chromochloris zofingiensis* is recognized to be a potential way to produce astaxanthin. The astaxanthin level in heterotrophic cells is typically lower than that of photoautotrophic cells. The influences of glucose on astaxanthin production were examined. Within 24 h, the addition of glucose boosted astaxanthin biosynthesis by promoting the gene expression of *PSY*, *LCYb*, *CHYb*, and *BKT*.

Conversely, the expression of those genes in the glucose group generally decreased after 24 h. Instead, astaxanthin production in the control group increased after 48 h. The astaxanthin concentration in the control group was 1.26 times at 96 h and 1.27 times at 192 h in comparison with those in the glucose group. A slight enhancement in total fatty acid concentration and modifications in cellular lipid structure were also triggered by glucose. These results revealed that glucose might modulate astaxanthin synthesis by modifying cellular morphology (Zhang et al. 2019). Induction of astaxanthin accumulation in *Chlorella zofingiensis* was confirmed in glucose-fed culture under high illumination and nitrogen deprivation. As a result, a two-step cultivation method was implemented, which comprised high biomass output fermentation and induction using an energy-free rotating floating photobioreactor outdoors. The greatest cell density of 98.4 g/L and astaxanthin production of 73.3 mg/L were attained during the fermentation. The astaxanthin production was enhanced by 1.5-fold during the outdoor induction, resulting in the greatest astaxanthin productivity of 5.26 mg/L daily at an optimal dilution of five-fold (Zhang et al. 2017). The effects of starvation of nitrogen, phosphorus, and sulfur on astaxanthin accumulation were studied in photoautotrophic *Chlorella zofingiensis*. Stresses significantly boosted astaxanthin levels, which reached 3.9 mg/g dry weight by nitrogen starvation. The maximum triacylglycerol productivity (52.4 mg/L per day) was achieved by sulfur starvation, whereas the highest astaxanthin productivity (0.624 mg/L per day) was achieved by nitrogen starvation. Besides, astaxanthin was mostly found in the form of monoesters and diesters. Under various conditions, genes associated with astaxanthin synthesis and triacylglycerol presented distinct transcriptional patterns (Mao et al. 2018). The influence of glucose, mannose, fructose, sucrose, galactose, and lactose on astaxanthin production was investigated. The most effective carbon sources for algal development in the dark were glucose and mannose, as evidenced by relatively high specific growth rates (0.03 per hour) and high cell dry densities (10 g/L sample). Additionally, algal cells treated with glucose and mannose produced the most astaxanthin (1.0 mg/g), demonstrating a link between cellular metabolism and astaxanthin synthesis. The maximum concentration of dry cell weight (53 g/L) and astaxanthin production (32 mg/L) were achieved utilizing glucose fed-batch fermentation (Sun et al. 2008).

Xanthophyllomyces dendrorhous

In yeast *Xanthophyllomyces dendrorhous* (teleomorph of *Phaffia rhodozyma*), the biosynthesis route for astaxanthin, has been characterized (Ducrey Sanpietro and Kula 1998; Verdoes et al. 2003). There are four major enzyme activities to accelerate the conversion of common isoprenoid precursors to β-carotene. The first geranylgeranyl pyrophosphate (GGPP) synthase is encoded by the *crtE* gene, which converts farnesyl pyrophosphate and isopentenyl pyrophosphate to GGPP. The second is phytoene synthase encoded by the *crtYB* gene, which combines two molecules of GGPP to produce phytoene. The third is phytoene desaturase encoded by the *crtI* gene, which adds four double bonds to the phytoene to produce lycopene. The fourth is lycopene cyclase encoded by the *crtYB* gene, which transforms the ψ acyclic ends of lycopene to β rings to produce β-carotene and γ-carotene accordingly

(Martín et al. 2008). Xanthophylls are now obtained mostly using extraction processes with various solvents from natural sources or chemical synthesis (Bhosale and Bernstein 2005). Synthetic xanthophylls, including zeaxanthin, canthaxanthin, and astaxanthin, are currently manufactured on a large scale industrially instead of extraction from natural sources. The astaxanthin biosynthetic genes *crtE*, *crtI*, and *crtYB* from *Xanthophyllomyces dendrorhous* have been cloned, but no direct link between their gene transcript and carotenoid production was discovered (Verdoes et al. 1999; Visser et al. 2003; Lodato et al. 2004). There are two enzymes in bacteria to convert β-carotene into astaxanthin via various biosynthetic intermediates. One enzymatic activity is ketolase, which integrates two keto groups at C-4 and C-4′ in the molecule of β-carotene. The other enzymatic activity is hydroxylase, which introduces two hydroxyl groups at C-3 and C-3′ (Martín et al. 2008). The presence of a monocyclic route that diverges from the bicyclic pathway with neurosporene and continues via β-zeacarotene, 3,4-didehydrolycopene, torulene, and 3-hydroxy-3′,4′- didehydro-β,ψ-carotene-4-one to the end product 3,3′-dihydroxy-b,w-carotene-4,4′-dione (An et al. 1999; Visser et al. 2003). The red yeast *Xanthophyllomyces dendrorhous* grows more rapidly than microalgae like *Haematococcus pluvialis*, is ready to expand up in bioreactors, and is usually prone to genetic modification. An increase in astaxanthin production in this red yeast has been reported by a number of factors, including the media composition modification such as different phosphate concentrations, carbon sources, carbon and nitrogen ratio, and abiotic variables such as oxygen levels, temperature, and pH (Schmidt et al. 2011). An efficient flow cytometry method was designed to examine *Xanthophyllomyces dendrorhous* mutants for astaxanthin overproducing. Even though the fluorescence emission peak of astaxanthin dissolved in acetone solution was at 570 nm, intracellular astaxanthin concentration was associated better with emission at approximately 675 nm. Cell mutagenesis was screened with ethyl methanesulfonate using emission wavelength at 675 nm resulting in identified mutants effectively that expressed 1.5 to 3.8-fold more astaxanthin production than wild-type cells (Ukibe et al. 2008).

Metabolic engineering approaches have been confined to overexpression of carotenogenesis genes such as the bifunctional phytoene synthase/lycopene cyclase from gene *crtYB* and phytoene synthase from gene *crtI* because of an absence of effective molecular biology techniques. Most astaxanthin production can only reach 3–4 mg/g dry cell weight (Verdoes et al. 2003; Visser et al. 2003). A two-step technique *Xanthophyllomyces dendrorhous* was implemented. Random mutagenesis was initially used, resulting in colonies with much higher astaxanthin concentrations. Afterward, the resultant strain was then genetically modified to improve astaxanthin production by targeted restrictive processes. The maximum astaxanthin levels recorded for this yeast were obtained by using this two-step method combined with an adequate growing medium. After 216 h in a fermenter culture, the highest values of astaxanthin production were 9 times higher (3.9 mg/g dry weight) in mutant AXJ-20 and 22 times higher (9.7 mg/g dry weight) in transformant AXJ-20/crtYB in comparison with that of the wild type (0.438 mg/g dry weight). The study suggested that throughout fermenter culture, the mutational and engineering impacts are both stable and promising (Gassel et al. 2013). The astaxanthin

production was expressively enhanced by white light (4.0 mg/g dry cell weight) and ultraviolet (4.4 mg/g dry cell weight) at the small-scale flask. When the fermentation scale was expanded to 10-L with 80 W white light and 800-L with 324 W white light in the fermenters under glucose feeding for 240 h, astaxanthin production can achieve 4.7 mg/g and 4.1 mg/g, respectively. The purity of the astaxanthin was approximately 84% on both scales (de la Fuente et al. 2010). The medium composition was investigated for astaxanthin production in *Xanthophyllomyces dendrorhous* mutant JH1. For the highest astaxanthin production, the optimal medium composition contained glucose 3.89%, yeast extract 0.29%, KH_2PO_4 0.25%, $MgSO_4$ 0.05%, $MnSO_4$ 0.02%, and $CaCl_2$ 0.01%. Besides, the most crucial ingredients are glucose and yeast extract. After 7 days of cultivation, the maximum value of astaxanthin production was 36.06 mg/L (Kim et al. 2005). Both traditional mutagenesis and genetic engineering methods were combined to optimize precursors in carotenogenesis and increase metabolism and conversion of intermediates for desirable astaxanthin as a final product. Furthermore, the transformation plasmids were constructed for sequential gene expression. The results suggested that the AXG-13 and AXJ-20 mutants most probably altered astaxanthin production upregulation with a 15-fold higher astaxanthin concentration than the wild type, and pathway engineering might enhance output by another sixfold. Shaking cultures yielded a maximum astaxanthin concentration of about 9 mg/g cell dry weight (Gassel et al. 2014).

Paracoccus Sp.

The astaxanthin production of *Paracoccus haeundaensis* was evaluated in different medium conditions with lactic acid bacteria under the co-culture system. Besides, *Lactobacillus fermentum* was selected as the most suitable strain under co-cultivation. PMF medium containing 0.1% Fe^{2+} was also confirmed as the optimum culture condition for the highest astaxanthin production. The dried cell mass and astaxanthin contents were 1.84 g/L and 0.821 mg/g dry cell weight, respectively. It was 2.5 times higher than that of this strain growth in the Luria-Bertani broth medium (Choi et al. 2021). Among bacterial astaxanthin providers, the marine bacterium *Paracoccus carotinifaciens* has been recognized and studied. The productivity of astaxanthin in *Paracoccus* sp. strain N-81106 was enhanced by a combined strategy containing random mutagenesis and overexpression and cloning genes in the astaxanthin-biosynthetic pathway. Astaxanthin-overproducing NG5 and NG9 mutants were isolated after random mutagenesis of strain N-81106. Both mutants produced 17 times more astaxanthin than wild-type strain N-81106. A broad-host-range vector was utilized for cloning strain N-81106 genes in *Escherichia coli*, and these genes were transferred to strains N-81106, NG5, and NG9. After cultivation for 5 days, the recombinant NG5 isolate generated 58 mg/L astaxanthin, which was 56-fold higher than the parent strain. Fed-batch fermentation was used to enhance the astaxanthin production to 480 mg/L culture fluid (Ide et al. 2012). Because astaxanthin is produced in esterified or glycosylated forms, as are a number of other structurally correlated carotenoids, it might be difficult to achieve high purity free astaxanthin in native hosts. As numerous previous studies have

proven, free astaxanthin with high purity may be obtained in non-native microorganisms using metabolic engineering and synthetic biology technologies. In Table 1, major native microorganisms for astaxanthin production were compared and summarized.

Astaxanthin Production in Metabolic Engineered Non-native Microbial Host

Microorganisms were broadly utilized as the producer of biologically active compounds under metabolic engineering technology and synthetic biochemistry over the last decade. Several strategies have been developed under different metabolic-engineered methods and biosynthetic techniques. Expanding raw material utilization includes sugars, lignocellulose (Zhang et al. 2019), food and industrial wastes, and natural gas and CO_2 (Clomburg et al. 2017). In addition, product lines are developed beyond basic chemicals, such as specialty chemicals, consumer chemicals, liquid fuels, and others (Liao et al. 2016). Moreover, highly efficient titers, rates, and yields can be obtained and exceed the limit of theoretical productivity (Meadows et al. 2016). Industrial microorganisms that do not generate carotenoids have been genetically modified to produce astaxanthin owing to these accomplishments. Several microbes, including *Escherichia coli*, the oleaginous yeast *Yarrowia lipolytica*, and the budding yeast *Saccharomyces cerevisiae*, have all made significant advances recently.

Escherichia coli

The function of twelve β-carotene ketolase and four β-carotene hydroxylase genes isolated from five cyanobacterial species were examined for microbial astaxanthin production. Additionally, this in vivo assessment selects and implements the most promising genetic components inside a dual expression vector maintained in *Escherichia coli*. Within a β-carotene accumulating host, simultaneous overexpression of individual β-carotene ketolase and β-carotene hydroxylase genes results in containing more than 90% astaxanthin and a 23.5-fold increase in total carotenoid production over the parental strain. Astaxanthin biosynthesis is increased 7.9 (18.0) fold on a 1.99 mg/g (2.88 mg/L) basis to the parental strains (Scaife et al. 2009). Shiga toxin-producing *Escherichia coli* is related from many foodborne outbreaks on leafy greens such as spinach and lettuce (Lee et al. 2016). Surface-functionalized antimicrobials have been broadly established as effective antimicrobial nanoparticles against foodborne pathogens (Bahrami et al. 2022). A plasmid-free *Escherichia coli* strain containing biosynthetic genes was developed for astaxanthin production in vivo. The stable chromosomal insertion of heterologous genes allows the recombinant plasmid to be maintained without the need for selection makers. The plasmid-free *E. coli* BW-ASTA strain can produce 1.41 mg/g dry cell weight of astaxanthin. The biosynthetic genes were designed individually as single expression units inserted into the *Escherichia coli* genome. This methodology enables the regulation of particular gene expression levels, which is difficult to do

Table 1 Astaxanthin production in native microbial hosts

Microorganisms	Highest astaxanthin yield (mg/g dry cell weight, or mg/L sample)	Cultivation designs	Optimized conditions	Significant result	References
Haematococcus pluvialis	87.4 mg/L	The strain was cultivated in 500 mL volume glass tubular airlift photobioreactor with Bold's Basal Medium at 25 °C for 16 days	15% CO_2 at a rate of 120 mL/min continuously and high light of 135 μmol photons m^{-2} s^{-1} continuous illumination	The astaxanthin yield increased to 87.4 mg/L, which was 6 times higher than the yield triggered with the same high light intensity.	Cheng et al. (2016)
	21.8 mg/g of astaxanthin at 30 °C; 12.1 mg/g of astaxanthin at 36 °C	In the first green phase, grown in the NIES-C medium at pH 7.5, a weak light (20 μE m^{-2} s^{-1}) with a dark/light cycle of 12:12 h. In the second red phase, grown on nitrogen depletion NIES-N medium at pH 7.5 with continuous illumination of strong light intensity (200 μE m^{-2} s^{-1}) at 23 °C or high temperatures of 30 to 36 °C. All cultures were incubated in 500 mL Erlenmeyer flasks containing 300 mL culture volume in a photoincubator shaking at 150 rpm and aerated with 3% CO_2 mixed	450 μM ferrous sulfate	After 18 days of photoautotrophic induction, the astaxanthin content of cells cultivated at high temperatures in the presence of 450 μM iron solution improved by 75% at 30 °C and 133% at 36 °C as compared to the high heat stress cells without iron treatment	Hong et al. (2015)

(continued)

Table 1 (continued)

Microorganisms	Highest astaxanthin yield (mg/g dry cell weight, or mg/L sample)	Cultivation designs	Optimized conditions	Significant result	References
		gas at a rate of 65 mL/min for 18 days			
	32.37 mg/g	Bubbling column 3 liter photobioreactor with 2 L of Bold's Basal Medium was mixed with filtered air at a rate of 0.4 per unit volume of growth medium per minute for 13 days. The light intensity was 30 μmol m^{-2} s^{-1}	10 μM melatonin treatment	The astaxanthin concentration significantly increased to 32.37 mg per gram sample under 10 μM melatonin treatment, which is a 2.25-fold increase in astaxanthin production compared to 14.36 mg/g in the control group	Ding et al. (2018)
	36 mg/g	30 mL cultures in 50 mL flasks was maintained at room temperature in the MES-Volvox medium under continuous illumination of low light (80 μmol m^{-2} s^{-1}) and high light intensities (300 μmol m^{-2} s^{-1}) with the cool white full-spectrum fluorescent bulb for 4 days	In the first green stage with low illumination. In the second red stage, with high illumination and high CO_2 levels at 5 or 15%	The astaxanthin production was 2–3 times higher than other single-stress treatments. The culture under 15% CO_2 level and intense light illumination caused 36 mg/g of astaxanthin accumulation.	Christian et al. (2018)
	56 mg/g	Pure cultures were maintained in 250 mL flasks with 100 mL of the modified BG-11 medium at 25 °C	6 cm light path in the first green stage and 3 cm light path in the second red stage	The JNU35 isolate can produce 18.1 mg/L astaxanthin daily and an ultrahigh biomass	Wang et al. (2019a)

		under continuous illumination of 30–40 µmol photons m^{-2} s^{-1}. In the green stage, unilateral low light illumination (150 µmol photons m^{-2} s^{-1}) was used for 15 days. In the red stage, the nitrogen-free medium with unilateral or bilateral high light illumination (300 µmol photons/m^2/s) was used for an additional 15 days. All cultivation was under a 3 cm or 6 cm light path glass column photobioreactor and aerated with 1% CO_2 (*v*/v)	with bilateral high light illumination	concentration of 20.1 g/L. An optimal phase of green vegetative cells transferred into the red inductive stage was established by the substitution of fresh media to increase biomass concentration to 27.3 g/L. The optimized photoinduction has high astaxanthin content at 5.6%, or 56 mg/g dry cell weight after 30 days of cultivation	
	18 mg/g (autoclave); 19 mg/g (cell homogenization)	NaOH 0.1 M, 15 min and 30 min; HCl 0.1 M, 15 min and 30 min; autoclave 30 min, 121 °C, 1 atm; spray drying, inlet 180 C and outlet 115 °C. An enzymatic mixture of 0.1% protease K and 0.5% driselase in a phosphate buffer was used with pH 5.8 at 30 °C for one hour	Autoclave 30 min, 121 °C; mechanical disruptor with a cell homogenizer	Astaxanthin yields under autoclave and homogenization treatment were 18 mg/g and 19 mg/g, respectively, and astaxanthin recovery of these two treatments are more than 85%	Mendes-Pinto et al. (2001)
	4.23% of total dry cell weight	The culture was in 250 mL flasks with 200 mL Bold's Basal Medium in a 12 h:12 h light cycle with a continuous	0.4% ethanol	0.4% ethanol significantly increased the astaxanthin level on the fourth day to 4.23% of dry cell weight,	Liu et al. (2019)

(continued)

Table 1 (continued)

Microorganisms	Highest astaxanthin yield (mg/g dry cell weight, or mg/L sample)	Cultivation designs	Optimized conditions	Significant result	References
		illumination (80 μmol·m^{-2}·s^{-1}) at 25 °C. Cells were aerated with sterilized 15% CO_2 at a feed velocity of 7 mL/min. Microalgae in the late-log phase (2.23 × 10^5 cells/mL) was in Bold's Basal Medium for ethanol (0, 0.1, 0.2, 0.3, 0.4, and 0.5% *v*/*v*) treatment for 14 days		which was 5.87-fold higher than control. 95% of astaxanthin production was astaxanthin monoesters	
	5.53 mg/L/day	In the first green stage, cells were cultured under weak lights of 25–45 μE m^{-2} s^{-1} for 15 days in nitrogen replete. In the second red stage, the cells were cultured under high solar irradiance of 305–360 (spring) and 315–380 μE m^{-2} s^{-1} (summer) during 78 days of nitrogen depletion. Flue gas was 3–4% CO_2	Red cyst inoculation with the iron (Fe^{2+}) supplementation	The astaxanthin production (23.4–33.5 °C) increased 147% up to 5.53 mg/L daily in comparison with lower temperature (17.5–27.3 °C)	Hong et al. (2016)

Chromochloris zofingiensis	0.55% of total dry cell weight	The activated cells were suspended in 200 mL nitrogen-free Kuhl medium at an initial dry weight of 0.75 g L^{-1}, and cultured in the photobioreactor with CO_2 aeration (1.5% CO_2 mixed in air) and white light illumination (120 μmol m^{-2} s^{-1}) at 25 °C. Culture time was 192 h	5 g/L glucose	Within the first 24 h, glucose increased astaxanthin formation, but beyond 48 h, it impeded it. Glucose may affect lipid composition by enhancing total fatty acid content. Glucose may modulate astaxanthin production by modifying cellular morphology.	Zhang et al. (2019)
	73.3 mg/L (fermentation), 5.26 mg/L/day (outdoor induction)	This strain was maintained in darkness at 25 °C in 250-mL flasks containing 50 mL Kuhl medium. Algal cells were grown in heterotrophic batch culture; 5 g/L glucose was used as the carbon source. After 3-day cultivation, the late exponential phase cells were suspended at a cell density of 0.5 g/L	High light with glucose and nitrogen-free (350 μmol m^{-2} s^{-1} light with 5 g/L glucose without potassium nitrate)	Astaxanthin production was 73.3 mg/L. The astaxanthin content was enhanced by 1.5-fold during the outdoor induction. The astaxanthin productivity was up to 5.26 mg/L per day.	Zhang et al. (2017)

(continued)

Table 1 (continued)

Microorganisms	Highest astaxanthin yield (mg/g dry cell weight, or mg/L sample)	Cultivation designs	Optimized conditions	Significant result	References
	3.9 mg/g dry weight or productivity 0.624 mg/L/day	The algal cells were inoculated in 10 mL Kuhl medium in flasks at 25 °C for 4 days with orbital shaking at 150 rpm and illuminated with continuous light of 30 μE m^{-2} s^{-1}. Then the cells were inoculated into 250 mL columns illuminated with 80 μE m^{-2} s^{-1} and aerated with 1.5% CO_2 enriched air for 5 days	Nitrogen starvation	Astaxanthin production was 3.9 mg/g dry weight by nitrogen starvation. The maximum triacylglycerol productivity (52.4 mg/L per day) was achieved by sulfur starvation, whereas the highest astaxanthin productivity (0.624 mg/L per day) was achieved by nitrogen starvation.	Mao et al. (2018)
	1 mg/g; 32 mg/L (glucose fed-batch fermentation)	The alga was grown in 10 mL of CZ-M1 broth aerobically in flasks at 27 °C for 4 days with orbital shaking at 150 rpm and illuminated with continual fluorescence light at 60 μmol m^{-2} s^{-1}. A two-stage fed-batch culture was 2 L in a 3.7-L fermenter with pH 6.6; 25 °C; agitation 480 rpm; and dissolved oxygen concentration 50% saturation; 5–20 g/L glucose	Glucose and mannose	The most effective carbon sources for algal development in the dark were glucose and mannose. Cells treated with glucose and mannose produced the most astaxanthin (1 mg/g). The maximum cell dry weight and astaxanthin production were achieved by glucose fed-batch fermentation.	Sun et al. (2008)

Xanthophyllomyces dendrorhous	9.7 mg/g	Fermenter cultures were grown in 330 mL Villadsen medium in a 1.3-L Fed-Batch Pro Bioreactor at 21 °C, pH at 5.5, and air flow at 10 L/hr. Stirrer speed 300–1000 rpm, less than 2 g/L glucose for a total 216 h	Transformant AXJ-20/crtYB in Villadsen medium	Astaxanthin production was 22 times higher (9.7 mg/g dry weight) in transformant AXJ-20/crtYB than that of the wild type (0.438 mg/g dry weight).	Gassel et al. (2013)
	4.7 mg/g	Cultures were in 10-L and 800-L fermenters and illuminated under several light colors (white, blue, green, yellow, and ultraviolet) for 240 h	80 W white light and glucose feeding at the 10-L scale in borosilicate glass fermenters	The highest astaxanthin production was under white light and ultraviolet at the small scale; 10-L and 800-L with white light and glucose feeding at the industrial scale	de la Fuente et al. (2010)
	36.06 mg/L	The main culture was in the 30 mL different media in 250 mL baffled flask at 22 °C, pH at 7.0140 rpm for 7 days	The optimal medium contained glucose 3.89%, yeast extract 0.29%, KH_2PO_4 0.25%, $MgSO_4$ 0.05%, $MnSO_4$ 0.02%, and $CaCl_2$ 0.01%	Under the optimal medium composition for 7 days, astaxanthin production reached 36.06 mg/L. The most crucial ingredients are glucose and yeast extract.	Kim et al. (2005)
	9 mg/g	Shaking cultures were in 50 mL within 500 mL baffled Erlenmeyer flasks, 180 rpm, over 8 days at 20 °C. A modified GSM medium with a ten-fold increased $CuSO_4$ and a 4.8-fold increased biotin content was used in two mutants AXG-13 and AXJ-20	AXG-13 and AXJ-20 mutants in modified GSM medium under shaking at 180 rpm and 20 °C	AXG-13 and AXJ-20 mutants produced a 15-fold higher astaxanthin content than the wild type, and pathway engineering enhanced content by another sixfold.	Gassel et al. (2014)

(continued)

Table 1 (continued)

Microorganisms	Highest astaxanthin yield (mg/g dry cell weight, or mg/L sample)	Cultivation designs	Optimized conditions	Significant result	References
Paracoccus haeundaensis	0.821 mg/g	*Paracoccus haeundaensis* and *Lactobacillus fermentum* were co-cultured for 72 h at 25 °C in the different media	PMF medium containing 4 g tryptone, 0.2 g polypeptone, 1.0 g bacto soytone, 1.0 g proteose peptone, 3.5 g bacto yeast extract, 3% NaCl, 0.01% ferric citrate, and 1-L distilled water	Astaxanthin production was stimulated by *Lactobacillus fermentum*. PM medium containing 0.01% Fe^{2+} (PMF medium) was also confirmed as the optimum culture conditions.	Choi et al. (2021)
Paracoccus carotinifaciens	480 mg/L (fed-batch fermentation); 58 mg/L (recombinant isolate NG5)	1.2-L fed-batch fermentation was performed in medium at 22 °C at 600 rpm agitation speed, 1.8 L/min air flow rate, 5 g/L glucose, and pH 7.0–7.4	The recombinant NG5 and NG9 strains	Both NG5 and NG9 mutants produced 17 times more astaxanthin than wild-type strain N-81106. The recombinant NG5 isolate generated 58 mg/L astaxanthin. Fed-batch fermentation produced astaxanthin 480 mg/L culture fluid	Ide et al. (2012)

when the astaxanthin biosynthetic genes are clustered inside a single operon. The recombinant *Escherichia coli* strain effectively converted β-carotene into astaxanthin by modulating the *crtZ* gene expression content to enhance astaxanthin production (Lemuth et al. 2011).

A metabolically engineered *Escherichia coli* was designed to produce astaxanthin effectively in a fermenter. The gene expression in the upper astaxanthin biosynthetic pathway was examined by experimenting with alternative promoters and genetic configurations. Numerous fusion tags were also linked to the truncated BKT gene, a critical heterologous membrane protein from *Chlamydomonas reinhardtii*, to increase its stability and membrane binding. Under fed-batch astaxanthin fermentation, the *hok/sok* system to stabilize the modified strain along with gene overexpression targets in silico flux variability analysis was also investigated based on enforced objective flux. The result showed that overexpression of the *ispD* and *ispF* genes caused the highest astaxanthin production of 7.12 mg/g dry cell weight with a productivity of 9.62 mg/L per hour. Besides, in the *hok*/*sok* system, there was a high astaxanthin production of 6.98 mg/g dry cell weight with a productivity of 7.86 mg/L per hour (Park et al. 2018). The attachment and biofilm formation of a Shiga-like toxin-producing *Escherichia coli* vary in different water environments (Lee et al. 2015). Surface-functionalized antimicrobials have been broadly established for effective antimicrobial nanoparticles against foodborne pathogens (Bahrami et al. 2022). An efficient and scalable technique for simultaneously traversing the expression space of multiple proteins was evaluated. Protein abundance was regulated by many orders of magnitude by combining genes through a restricted collection of ribosome-binding sites. A synthetic operon including fluorescent proteins was used by traversing a three-dimension color space. A recombinant carotenoid biosynthesis pathway in *Escherichia coli* was operated to disclose a variety of phenotypes with specific characteristics of carotenoid production. By overexpressing a carotenoid pathway gene *dxs* with the addition of a ribosome-binding sites-modulated *dxs* gene to the synthetic operon, astaxanthin synthesis increased from 2.6 to 5.8 mg/g of dry cell weight under a single combinatorial assembly (Zelcbuch et al. 2013). The *Escherichia coli* strain CAR026 was designated as the starting strain for the astaxanthin synthesis because it had a balanced metabolic flux. The expression of β-carotene ketolase (CrtW) and β-carotene hydroxylase (CrtZ) was synchronized, and a limitation was overcome by increasing the copy number of *crtY* in strain CAR026. The astaxanthin productivity was also increased by regulating the groES-groEL chaperone genes. The optimal Gro-46 strain yielded the highest astaxanthin at 26 mg/L in shake flasks with a yield of 6.17 mg/g dry cell weight. Besides, 1.18 g/L of astaxanthin was produced after fermentation in fed-batch conditions for 60 h (Gong et al. 2020).

Saccharomyces cerevisiae

The innovative promotion strategy of astaxanthin production promotion was developed in *Saccharomyces cerevisiae* by integrating heterologous module engineering and mutagenesis by screening atmospheric and room temperature plasma. CrtZ from *Agrobacterium aurantiacum* raised astaxanthin yield by 1.78-fold and ratio from

66.6% to 88.7% when compared to the strain containing CrtZ from *Alcaligenes* sp. strain PC-1. Atmospheric and room temperature plasma mutagenesis enhanced astaxanthin production by 0.83-fold with 10.1 mg/g dry cell weight at the shake-flask level. In the large-scale fermentation, astaxanthin production was 13.8 mg/g dry cell weight (217.9 mg/L) and 89.4% of astaxanthin ratio without any inducers. Besides, the underlying molecular target *CSS1* was confirmed as a potential molecular target for astaxanthin production (Jin et al. 2018). To increase the synthesis and efficiency of rate-limiting enzymes for astaxanthin production in *Saccharomyces cerevisiae*, metabolic and protein engineering were combined. A mutant of geranylgeranyl diphosphate synthase (CrtE03M) was overexpressed with three additional rate-limiting enzymes to boost the critical precursor β-carotene for astaxanthin. The colorimeter can measure the color of the enzyme reactions by various optical analytical methods (Lee et al. 2020). A color screening technique was designed for the directed evolution of β-carotene ketolase (OBKT), resulting in a triple mutant OBKTM with 2.4-fold increased activity to speed up the conversion of β-carotene to astaxanthin. Subsequently, the diploid strain YastD-01 was developed by crossing two astaxanthin-producing haploid strains under the same carotenogenic pathway. In shake flask cultures, 8.10 mg/g dry cell weight (47.18 mg/L) of (3S, 3′S)-astaxanthin was produced by overexpression of OCrtZ and OBKTM in the YastD-01 strain (Zhou et al. 2017). Heterologous CrtZ and CrtW were transferred into *Saccharomyces cerevisiae* to establish the astaxanthin biosynthesis pathway in an existing high β-carotene producing strain. Through the combinatorial expression and screening of CrtZ and CrtW in various species, the optimal combination for astaxanthin production was found to be CrtW from *Brevundimonas vesicularis* DC263 and CrtZ from *Alcaligenes* sp. strain PC-1. Furthermore, enhancing the ratio of CrtZ to CrtW by boosting the *crtZ* promoter activity improved astaxanthin production by 30.4% (from 3.4 to 4.5 mg/g dry cell weight) in a culture medium containing 2% or 4% glucose. The astaxanthin yield in a 5-L bioreactor achieved 81.0 mg/L in high cell density fed-batch fermentation for 7 days with a carbon source limitation system (Wang et al. 2017).

Yarrowia lipolytica

The oleaginous yeast *Yarrowia lipolytica* was used for new astaxanthin production by fermentation. Twelve *Yarrowia lipolytica isolates were screened for* β-carotene production. Phytoene desaturase (*crtI*) and bi-functional phytoene synthase/lycopene cyclase (*crtYB*) from *dendrorhous* were introduced into β-carotene biosynthesis. For the conversion from β-carotene into astaxanthin, gene *crtW* from *Paracoccus* sp. N81106 and gene *crtZ* from *Pantoea ananatis* were used to construct a strain that produced 10.4 mg/L of astaxanthin. In a microtiter plate culture, the copy numbers of gene *crtZ* and *crtW* were optimized to yield 3.5 mg/g dry cell weight (54.6 mg/L) of astaxanthin (Kildegaard et al. 2017). In another study, *Yarrowia lipolytica,* a yeast with oleaginous properties, was designed to generate large titers of astaxanthin in submerged fermentation. A constructed strain with an optimized β-carotene pathway was developed. The genes *crtW*, *crtZ,* and *bkt* (β-carotene ketolases encoded from *Haematococcus pluvialis*) were inserted into the constructed strain in varied copy

numbers. After screening all strains, the best strain, which contained algal β-ketolase and β-hydroxylase, produced 44 mg/L of astaxanthin. After the same strain was grown in controlled bioreactors for 7 days on fermented medium containing glucose, it can yield a titer of 285 mg/L of astaxanthin. The result exhibited the feasibility of *Yarrowia lipolytica* as an astaxanthin-producing cell factory (Tramontin et al. 2019). Three-dimensional gel complexes using oleogelators have been developed to stabilize the biochemical structure (Demirci et al. 2020). The expression of the astaxanthin biosynthetic pathway was also examined in the oleaginous yeast *Yarrowia lipolytica.* Both enzyme β-carotene ketolase and hydroxylase were merged to increase the conversation from β-carotene to astaxanthin, and it outperformed individual enzyme expression. The astaxanthin pathway was targeted to subcellular organelles by investigating the pathway singly and in the mixture of components of the peroxisome, lipid body, and endoplasmic reticulum. In fed-batch fermentation, tethering enzymes to these three organelles concurrently yielded 858 mg/L of astaxanthin which is the most significant improvement in astaxanthin production and a 141-fold increase over the control strain (Ma et al. 2021).

Rhodovulum sulfidophilum

The photosynthetic bacteria *Rhodovulum sulfidophilum* was used to produce astaxanthin because it has storage capacity and sufficient precursors for heterologous carotenoid synthesis. The predominant carotenoid (>90%) in green mutants was neurosporene or chloroxanthin, whereas phytoene accumulated in gray mutants. Two enzyme phytoene dehydrogenase (*crtI*) and lycopene cyclase (*crtY*) genes from *Erythrobacter longus* were combined with the other two enzymes, β-carotene oxygenase (*crtW*) and β-carotene hydroxylase (*crtZ*) genes from *Paracoccus* to construct an expression vector for astaxanthin synthesis. Therefore, 2 μg/g dry cell weight of astaxanthin can be produced by the transconjugant. In Table 2, major non-native microorganisms for astaxanthin production under different engineering strategies were compared and summarized.

Conclusions and Perspectives

The microbial production of astaxanthin has been well-discussed based on various current research reports and scientific studies. The astaxanthin production from the native host is relatively costly and has a low yield. For example, astaxanthin produced from green microalgae *Haematococcus pluvialis* costs $2500–7000/kg. Nowadays, synthetic astaxanthin manufacturing still dominates the commercial market because of its low cost (about $1000/kg), with BASF and Hoffman-La Roche as the leading manufacturers. However, human consumption of synthetic astaxanthin has yet to be approved (Koller et al. 2014). By using a combination of various metabolic engineering processes, the highest production of astaxanthin was reached in recombinant *Escherichia coli* at 1.18 g/L, which is massively higher than the most prevalent native microbial producers *Haematococcus pluvialis* and *Xanthophyllomyces dendrorhous* (Gong et al. 2020). In comparison to this

Table 2 Astaxanthin production in non-native microbial hosts

Microorganisms	Highest astaxanthin yield (mg/g dry cell weight, or mg/L sample)	Culture conditions	Methodology	Significant results	References
Escherichia coli	1.99 mg/g, or 2.9 mg/L	Cells were cultivated at 30 °C with continuous aeration shaking at 150 rpm in shake Erlenmeyer flask under the dark for 2 days.	Twelve β-carotene ketolase and four β-carotene hydroxylase genes isolated from five cyanobacterial species were examined and screened	Astaxanthin biosynthesis in overexpression of β-carotene ketolase and β-carotene hydroxylase genes contained >90% astaxanthin and increased 7.9 fold than the parental strains	Scaife et al. (2009)
	1.41 mg/g	*Escherichia coli* BW-CARO and DH5α were grown at 37 °C in LB medium for 2 days	The expression of *crtEBIY* along with the β-carotene-ketolase gene (*crtW148*) and the hydroxylase gene (*crtZ*) in *E. coli* BW-ASTA	The recombinant strain effectively converted β-carotene into astaxanthin by modulating the *crtZ* expression to enhance astaxanthin production.	Lemuth et al. (2011)
	7.12 mg/g or 432.82 mg/L	Cells were at 30 °C shaken at 220 rpm for 36 h in the bioreactors	Simultaneously overexpressing the *ispD* and *ispF* genes in 0.5 mM isopropyl β-D-1-thiogalactopyranoside	Overexpression of the *ispD* and *ispF* genes caused the highest astaxanthin production of 7.12 mg/g dry cell weight with a productivity of 9.62 mg/L per hour.	Park et al. (2018)

	5.8 mg/g	Selective clones were grown in shake flasks containing 100 mL of LB medium at 37 °C for 2 days	Combinatorial assembly with modulating operons in the ribosome-binding site. Overexpressing a carotenoid pathway gene *dxs* with the addition of a ribosome-binding sites-modulated *dxs* gene to the synthetic operon	Astaxanthin synthesis increased from 2.6 to 5.8 mg/g of dry cell weight under a single combinatorial assembly	Zelcbuch et al. (2013)
	26 mg/L or 6.17 mg/g in shake flasks; 1.18 g/L in fed-batch fermentation	The fermentation in a 5-L bioreactor was at 30 °C, pH at 7.0 with 5 M NH_4OH, and dissolved O_2 at 30% by agitation at 250 rpm for 60 h	The expression of CrtW and CrtZ was synchronized under genetic manipulation	A limitation was overcome by increasing the copy number of *crtY* in strain CAR026. The astaxanthin productivity was also increased by regulating the groES-groEL chaperone genes	Gong et al. (2020)
Saccharomyces cerevisiae	10.1 mg/g	The fermentation in a 5-L bioreactor was at 30 °C, pH at 5.8 with 6 M sodium hydroxide, 2 g/L glucose, and airflow at 1.5 per unit volume of growth medium per minute, and dissolved O_2 at 30% by agitation cascade from 400 to 800 rpm for 140 h.	The integration of heterologous module engineering and mutagenesis by screening atmospheric and room temperature plasma	Atmospheric and room temperature plasma mutagenesis enhanced the highest astaxanthin production by 0.83-fold at the shake-flask level	Jin et al. (2018)

(continued)

Table 2 (continued)

Microorganisms	Highest astaxanthin yield (mg/g dry cell weight, or mg/L sample)	Culture conditions	Methodology	Significant results	References
	8.10 mg/g or 47.18 mg/L	50 mL culture in the YPD medium with 0.52 mM Fe^2 in the shake flask by agitation at 220 rpm at 30 °C for 84 h	Combination directed evolution and transcriptional regulation of rate-limiting enzymes in biosynthetic pathway	(3S, 3′S)-astaxanthin was the most compound produced by overexpression of enzyme OCrtZ and a triple mutant OBKTM in the diploid YastD-01 strain	Zhou et al. (2017)
	4.5 mg/g in a shake flask, 81.0 mg/L in a 5-L bioreactor	2.5 L culture YP medium with 2% glucose in 5-L stirred-tank bioreactor controlled at 30 °C, pH 6, dissolved oxygen >30% and air flow 1.5 per unit volume of growth medium per minute for 7 days	Heterologous CrtZ and CrtW were introduced into this strain to establish the astaxanthin biosynthesis pathway. A combination of CrtZs and CrtWs were screened from diverse species	The optimal combination for astaxanthin production was found to be CrtW from *Brevundimonas vesicularis* DC263 and CrtZ from *Alcaligenes* sp. strain PC-1. The crtZ promoter activity was increased to enhance the ratio CrtZ/CrtW for improvement of astaxanthin production	Wang et al. (2017)
Yarrowia lipolytica	3.5 mg/g, or 54.6 mg/L	The microtiter plate culture was incubated at 30 °C with 300 rpm agitation with 500 μL YP + 8% glucose for 72 h	Beta-carotene overproduction and gene copy number modification	Two genes *crtW* and *crtZ* were used to construct a strain, and the copy numbers of gene *crtZ* and *crtW* were optimized	Kildegaard et al. (2017)

	285 mg/L, 6 mg/g dry cell weight in a bioreactor	Fermented culture in the bioreactor was at 28 °C, pH 5.5, aeration 2 per unit volume of growth medium per minute, agitation 500–1000 rpm, 5 g/L glucose, and dissolved oxygen >20% for 7 days	*GGPPs7* (from *Synechococcus* sp.), *bkt*, *crtZ* (from *Haematococcus pluvialis)* varied copy numbers	*HpBKT*, *HpcrtZ* were the optimal gene combination. The optimized strain produced about 0.3 g/L of astaxanthin In fed-batch culture	Tramontin et al. (2019)
	16.7 mg/g, 858 mg/L	Shake flask fermentation was in YPD medium at 30 °C and 230 rpm for 3 days	Subcellular organelles were examined singly or combined in components of the peroxisome, lipid body, and endoplasmic reticulum with β-carotene ketolase and hydroxylase	In fed-batch fermentation, tethering enzymes to all three organelles concurrently caused the highest astaxanthin production.	Ma et al. (2021)
Rhodovulum sulfidophilum	2 μg/g	The culture was in RCVB medium containing 1.5% NaCl with 20μE m^{-2} s^{-1} light intensity under anaerobic conditions at room temperature	Chemical mutagenesis was performed. *crtI*, *crtY*, *crtW*, and *crtZ* genes were used to construct an expression vector for astaxanthin synthesis	The transconjugant with constructed vector yield the highest astaxanthin content	Mukoyama et al. (2006)

non-native producer, native microbial producers like *Haematococcus pluvialis* and *Xanthophyllomyces dendrorhous* optimize astaxanthin production mostly by directed evolution and fermentation modification with chemical supplementation. Nonetheless, even though these approaches have been shown to elevate astaxanthin content, they are time-consuming and inefficient. Excepting the metabolic engineering methods outlined above, introducing non-native pathways to increase the availability of precursors could have a big impact on astaxanthin synthesis. Furthermore, comprehensive research of gene expression and modulation, such as epigenetic alterations, non-coding RNAs, and post-translational regulations, is required to enhance microbial production. Natural native microorganisms and non-conventional producers like *Yarrowia lipolytica* require exceedingly effective genetic modification techniques and methods. Astaxanthin production from metabolic engineered non-native microorganisms is starting to compete with traditional approaches for producing astaxanthin in terms of high titer, rate, and yield. Additionally, because these biosynthetic techniques are environmentally friendly and highly sustainable, microbial-derived astaxanthin will eventually overtake synthetic and natural-produced astaxanthin.

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