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Review Article

Bioengineering of the Optimized Biosynthesis of Commercially Vital Carotenoids- Techno-Advanced Applications

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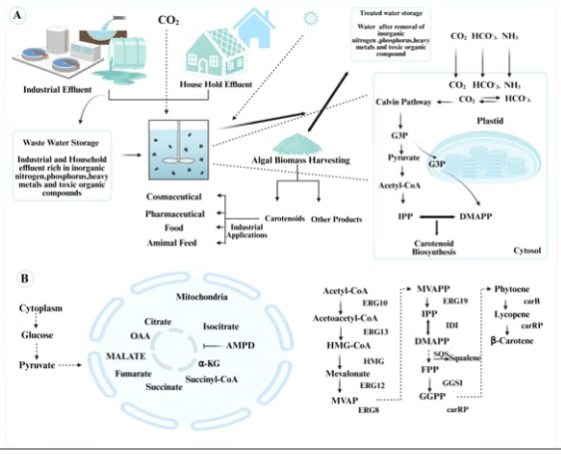
ABSTRACT

Beta-carotene, a carotenoid found in plants, fungi, and algae, is a crucial antioxidant and anti-cancer agent. It is primarily derived from plants, algae, and microbes, but this method has drawbacks like high costs and low productivity. The growing demand for carotenoids has led to large-scale industrial manufacturing. However, extracting and synthesizing these chemicals can be costly and technical. Microbial synthesis offers a cost-effective alternative. Synthetic biology and metabolic engineering technologies have been used in various studies for the optimization of pathways for the overproduction of carotenoids. Four metabolic components are involved in carotenoid biosynthesis, central carbon (C), isoprene supplement, and cofactor metabolism. Metabolic engineering is a potential solution to enhance β-carotene production. This article explores the biochemical routes, methods used by natural microbial species, and metabolic engineering potential of microbial organisms for β-carotenoids production. Currently, Escherichia coli, certain euglena and yeast species are the primary microorganisms used in metabolic engineering, offering minimal environmental impact, cost-effective manufacturing, and high yield.

GRAPHICAL ABSTRACT



INTRODUCTION

Carotenoids are essential for animal health, cellular machinery, and various industries. They exhibit pro-vitamin A, anticancer, and antibody properties. The growing demand for carotenoids has led to large-scale industrial manufacturing. This article explores the production of carotenoids by microbial consortia. This study aims to update and consolidate the fundamental aspects of carotenoid production from bacteria, algae, and

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fungus, unlike previous studies that focused on specific aspects [1]. Photosynthetic organisms, including plants, microalgae, bacteria, and some fungal ones, produce carotenoids through geranylgeranyl diphosphate and phytoene synthase enzymes. Most carotenoids come from phytoene, with some synthesized through enzymatic reactions [2]. Light-dependent species have photosynthetic organelles like chlorophylls, bacteriochlorophylls, and carotenoids, which serve as photoprotection agents, absorbing excess energy and protecting against light and stress damage [3]. Microalgae are potential sources of carotenoids used in various industries, including nutritional supplements, cosmetics, personal care products, foodstuffs, and aquaculture pigmentations [4]. The production process involves upstream and downstream stages, influenced by factors like light exposure, carbon source, aeration, metal ions, salts, and temperature. Studies have explored the economic feasibility of using yeast Phaffia rhodozyma for astaxanthin production and investigated metabolic pathway engineering to enhance carbon flow and carotenogenesis in yeast and bacterial cells [5]. Carotenoids are health-promoting compounds with antioxidants, provitamin-A, anti-aging, and lipid peroxidation properties. They are beneficial for bone and skin health, sports nutrition, vision, immune response, cancer protection, cardiovascular diseases, neuronal disorders, and digestive issues [6]. Carotenoids are added to supplemental meals to enhance skin coloration and reproductive capabilities of animals. Aquaculture feed is the primary industry generating revenue from carotenoids. The carotenoid products market is rapidly growing [7]. Carotenoids, in all their forms, function as inhibitors of multiple processes linked to atherosclerosis, including lipid peroxidation triggered by ROS, the generation of superoxide induced by lipopolysaccharide (LPS) and ROS, cytotoxicity resulting from peroxide, LDL - oxidation (LDL; low-density lipoprotein), and 12-15 levels Hydroxy F.A. (fatty acids) in plasma [8]. Reactive oxygen species (ROS), which tend to include OH (hydroxyl radical), O2– (superoxide), and singlet oxygen, can significantly impact on the degenerative disorders including cancer, aging, cataract, neurological diseases, and cardiovascular disease [9]. Xanthophyll carotenoids like canthaxanthin and astaxanthin are effective antioxidants and reactive oxygen species (ROS) scavengers due to their terminal carbonyl groups connected to the polyene backbone. These carbonyl groups stabilize carbon-centered radicals more efficiently than the polyene backbone alone. Astaxanthin is more hydrophilic than other carotenoids due to its ketone and α-hydroxy groups on each ring. Its polar rings prevent lipid peroxidation [10]. Research shows that consuming adequate carotenoids content can protect against degenerative diseases. Lutein reduces neuroinflammation in BV-2 microglia by limiting NF-κB factors for nuclear transcription activation [11]. Lycopene inhibits tumor metastasis by decelerating cell cycle progression, upregulating β-catenin and E-cadherin expression, and downregulating insulin-like growth factor-1, structural metalloproteinase, and PCNA. It has been found to inhibit the growth of various cancer cell lines, including colon, breast, lymphocytes, and prostate [12]. The potent hypolipidemic effects of lycopene are achieved through molecular mechanisms distinct from cholesterol regulation induced by statins. Additionally, lycopene reduced inflammatory marker's expression, which reduced PCSK-9 production [13]. Astaxanthin enhances immune system and oxidative DNA damage in healthy females, reducing age-related macular degeneration, cataracts, and other visual abnormalities by inhibiting RNS and ROS (reactive nitrogen, and reactive oxygen species) induced oxidative stress [14, 15]. β-carotene, and capsanthin decreases ROS production in epithelial cells of rat liver treated with H2O2, and they also help to mitigate the negative impacts of H2O2-induced suppression of gap junction intercellular communication. In recent work, the xanthophyll carotenoids 9-(Z) neoxanthin were the most effective in reducing the survival rate of cervical (HeLa) and lung (A549) cancer cells. Consuming neoxanthin regularly may help reduce the occurrence of lung and cervical cancer [16, 17]. Synthetic carotenoids are a popular source of carotenoids in commercial products, particularly in feed and beauty. They are more cost-effective than natural carotenoids, which are recommended for human consumption [17, 18]. In China, astaxanthin production costs are higher than in the USA, but using raceway ponds and low-cost photobioreactors can be cheaper. Labor costs in the USA could increase astaxanthin prices to over 600 per Kg. Photobioreactors could reduce production costs by up to 4.5 times. Raceway ponds can reduce operational and facility establishment expenses by 95%, but open-air raceway ponds face pollution issues [19, 20]. Current study explores the microbial synthesis of carotenoids and subsequent bioengineering of metabolic pathways to enhance the production. It discusses the carotenoids biosynthesis by utilizing bioengineering of metabolic pathways involved in the production of beta-carotenoids involving the microbial synthetic pathways engineered by the photosynthetic, and non-photosynthetic microbes. It also discusses the applications, advertising potential, and cost of carotenoids production. It will also discuss the chemistry, role of photosynthetic and non-photosynthetic microbes which may further enhance the commercialization potential of beta-carotenoids.

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Microbial Assisted Biosynthesis of Beta-carotenoids

Carotenoids are primarily synthesized during bacterial and fungal metabolic pathways, but commercially significant carotenoids are being manufactured using microalgal platforms. High-carotene microalgae like Lutein, Echinenone, and H. pluvialis are used, while various microorganisms, including bacteria, yeast species, fungus B. trispora, and carotenoid Decaprenoxanthin, are involved in large-scale production. Non-carotenogenic bacteria have also undergone genetic modification to produce essential carotenoids for industry [17]. Genes have been inserted into heterologous systems or genetically altered to produce large quantities of carotenoid pigments for industrial use. These pathways include MVA (Mevalonate) and MEP pathways, carotenoids biosynthesis, and ketocarotenoids apocarotenoids biosynthesis [21]. The efficiency and cost of synthesizing carotenoid using heterologous systems have been investigated. Strategies to enhance E. coli carotenoids production include increasing the availability of MEP pathway components, upregulating isoprenoid genes, and suppressing primary pathways that assimilate precursors of carotenoid [22]. The fourth strategy involves boosting ATP, NADPH production by overexpressing key genes in the tricarboxylic acid cycle and ATP synthase, ensuring adequate energy for biosynthetic activities [23]. Glycerol was used to optimize factors in the mevalonate pathway, leading to a significant increase in β-carotene production in E. coli DH5α. Glycerol, produced as a byproduct of biodiesel manufacturing, offers a cost-effective and environmentally friendly option for large-scale carotenoids production. The MAGE approach was used to enhance the MEP pathway in Escherichia coli, resulting in a fivefold increase in lycopene production within three days [24]. Methylerythritol phosphate (MEP) pathways supply precursors for the synthesis of natural carotenoids have been pictorially represented in Figure 1. The study focuses on the development of a consolidated bioprocessing organism (CBP) to convert biomass into lycopene. The organism incorporates a synthetic ten-gene pathway, including Xylose converter, xylitol oxidase, and D-xylulokinase, and five genes from the zeaxanthin biosynthetic pathway. The highest level of zeaxanthin synthesis from xylan was achieved at 0.74mg per liter. Five crucial pathways were manipulated to enhance the availability of precursors and cofactors. The study found that the whole mechanism responsible for β-carotene production in E. coli was enhanced, with a 3.5-fold increase in β-carotene accumulation when the MEP pathways (Dxs and Idi) were modified [25, 26]. The study found that modulating TCA and PPP modules together led to increased β-carotene production, but not when modulating TCA and ATP modules [27]. Enhancing NADPH accessibility was found to be more advantageous than ATP for enhancing β-carotene production. Adaptive evolution led to 300% more β-carotene production from mutant strains resistant to oxidative stress. Enhancing substrate channeling of β-carotene 3-hydroxylase and CLY β- in E. coli resulted in larger zeaxanthin production compared to D. salina [26, 28]. Table 1 presents carotenoid synthesis by using various bacterial species.

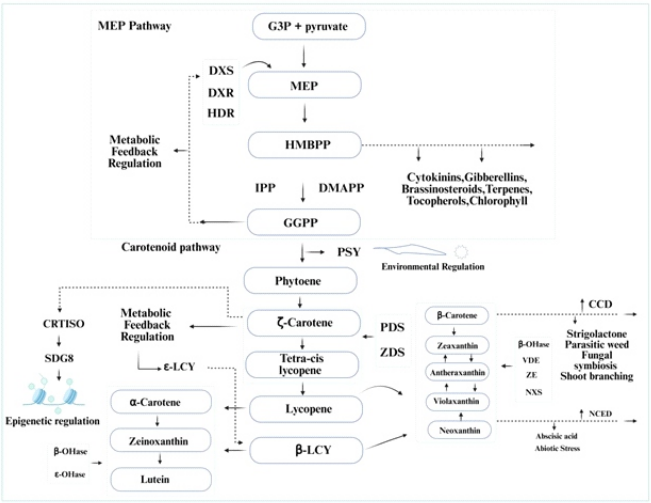

Figure 1: Methylerythritol phosphate (MEP) pathways supply precursors for the synthesis of natural carotenoids

Substrate channeling prevents the accumulation of intermediates along the pathway, enhancing the synthesis of zeaxanthin. Tunable intergenic regions (TIGR) were found to be superior to protein-mediated substrate channeling in facilitating the synchronized expression of these enzymes for zeaxanthin production. CrtZ from Pantoea ananatis had superior performance in facilitating the rate-limiting stage of zeaxanthin production [29].

Table 1: Overview of significant works on carotenoids synthesis optimization by using bacteria

Vitamin A	Process	Host Organism	State of culture	Production	References
Lycopene	The deletion of genes encoding zeaxanthin glucosyltransferase and lycopene-cyclase led to alterations in the NADPH expression and ATP-supplying enzymes.	*Escherichia coli*	Batch fermentation	3.52 gram per liter or 50.6 milligram per gram	[30]
Retinol	The simultaneous expression of β-cyclase (lycopene) and cleavage dioxygenase (carotenoids).	*Escherichia coli*	Flask culture	33 milligram per liter	[31]

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Zeaxanthin	The activity of CrtY and -carotene 3-hydroxylase is synchronized.	*Escherichia coli*	Flask culture	11.95 milligram per gram	[32]
Zeaxanthin	Pantoea ananatis has a genetic makeup consisting of five genes for zeaxanthin production and three from E. coli for the MEP route.	*Pseudomonas putida*	Flask-culture	51 milligram per liter 7 milligram per gram	[33]
α-ionone	Coordination of LCY (lycopene-cyclase) along with the CrtY expression	*Escherichia coli*	Fed-batch fermentation	480 milligram per liter	[34]
β- ionone	CrtY and CCD1 expression is coordination.	*E. coli*	Bioreactor	500 milligram per liter	[35]
β-carotene	MVA pathways from Enterococcus faecalis and Streptococcus pneumonia, respectively, at the top and bottom.	*Escherichia coli*	Flask culture	465 milligram per liter	[36]
β-carotene	MEP and metabolic modules (three central) of ATP production, TCA cycle, and PPP are among the five main modules.	*E. coli 1*	Fermentation (Fed-batch)	60 milligram per gram / 2.1 gram per liter	[37]
Vitamin A	Glycerol serves as the carbon supply and accommodates the entire MVA route.	*E. coli*	medium; Glycerol	465 milligram per liter	[38,39]
//	Crt, dxs, idi, sucAB, sdhABCD, and talB overexpression	*E. coli 1*	LB medium	2.1 gram per liter	[40]
Canthaxanthin	TCA intermediate, Ketoglutarate, oxaloacetate and succinate 9.69 mM, 8.68 mM, and 8.51mM, respective supplementation	*Dietzia natronolimnaea* [2]	-	13.1 milligram per liter	[41]
Zeaxanthin	The Gulf has been the site of the isolation of Gram-negative short rod/ cocci bacteria, which are commonly associated with sponges.	*Shingomonas (Blastomonas) natatoria*	-	0.62 milligram per liter	[42]

PPP: Pentose Phosphate Pathway; 1 CAR005; 2 HS-1

Ketoconazole, an inhibitor of ergosterol synthesis, significantly increased β-carotene concentration in genetically engineered S. cerevisiae, demonstrating its role in carotenoid production. Microalgae species from dunaliella, haematococcus, and scenedesmus are studied for their potential in producing carotenoids. Marigold flower petals are considered inferior to microalgae from Muriellopsis, Scenedesmus, chlorella, chlorococcum, and neospongiococcus, making them economically viable for lutein production. Optimizing substrate content, stress, cultivation variables, inhibitors, and metabolic precursors is essential for cost-effective and efficient synthesis of specific carotenoids [43]. Genetic modification techniques have shown promising results in increasing carotenoids production in microorganisms. However, carotenoids' water-repelling properties can cause degradation by bacterial cells. To increase carotenoids production, cellular stress regulators must be altered, as large-scale and economically viable synthesis remains challenging. Various fungus species have been used for carotenoid synthesis, but their hydrophobic nature strains bacterial cells and cellular oxidants can deteriorate carotenoids.

Table 2: Overview of significant works on carotenoids synthesis optimization by using fungi

Types of Vitamin A	Process	Host Organism	Production	References
Canthaxanthin	The gene for -carotene ketolase from Paracoccus sp.	*Mucor circinelloides*	200 µg/g	[44]
Lycopene	The concentration of vitamin A acetate is 1000 ppm, with a piperidine inhibitor at 500 ppm.	*Blakeslea trispora*	775 mg/L	[45]
β-carotene	Surfactant [1] supplementation with vitamin A acetate [2]	*Blakeslea trispora*	830 mg/l	[46]

1 Span 20 @ 0.2 percent; 21000 ppm

X. dendrorhous was cultivated using a semi-industrial illuminated fermentation method, achieving a high concentration of

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astaxanthin synthesis. Another study achieved the highest production of carotenoids using fed-batch fermentation in a bioreactor with controlled pH and aeration rate. Surfactants, specifically Span 20 at a concentration of 0.2%, facilitate the spread of B. *trispora,* leading to increased β-carotene synthesis and enhanced permeability of yeast cells [47]. Table 3 entails the carotenoid synthesis by using various yeast organisms. Inhibitors that hinder lycopene cyclase can increase microbial lycopene production by obstructing the β-carotene biosynthesis pathway. Studies show that these inhibitors can increase lycopene creation to 100% of total carotenoid content [47, 48]. Agro-industrial waste, like beetroot and sugarcane molasses, can be used as cost-effective substitutes for nitrogen and carbon in microbial carotenoid synthesis. Stress-induced conversion of secondary carotenoids like astaxanthin in haematococcus reduces feedback inhibition and enhances taxane sequestration [49, 50].

Table 3: Overview of significant works on carotenoids synthesis optimization by using yeast

Types of Vitamin A	Process	Host Organism	Production	References
Vitamin A	Experiment with the heterologous pathway and find the ideal promoter combination for each of the genes under consideration.	*Y. lipolytica*	6.5 g/L	[47]
Astaxanthin	Illuminated fermentation processes	*Xanthophyllomyces dendrorhous*	3.0miligram per lite 119 milligram per liter	[48]
Total carotenoids	Efficient glycerol utilization for C/N (85) ratio, glycerol concentration of 9.5 percent, & surfactants (0.15%: Tween-20)	*Rhodotorula glutinis*	135.25 milligram per liter	[51]
Lycopene	With peroxisome target signal, phytoene synthase, phytoene desaturase, and GGPP (Geranylgeranyl diphosphate) Synthase were expressed.	*Pichia pastoris X-33*	4.6 milligram per gram 3.9 mg/L	[52]
β-carotene	Increased HMGCoA1-reductase activity, additionally to the incorporation of an ergosterol production blocker (100 milligram per liter ketoconazole),	*Saccharomyces cerevisiae*	6.29 milligram per gram	[53]

1 HMGCoA: 3-hydroxy-3-methylglutaryl coenzyme A

Enhancing biomass production is crucial for carotenoid synthesis from microalgal platforms. Challenges include the reliance on light for metabolism and low efficiency of photosynthesis in microalgae within photobioreactors. To improve the process, strategies such as structuring the light-harvesting antenna have been implemented [54]. Scientists have discovered methods to enhance biomass productivity by manipulating electron transport in photosynthesis, enhancing the Calvin-Benson cycle's energy and reductant storage capacity, and advancing Rubisco (i.e., Ribulose-1,5-bisphosphate oxygenase/carboxylase) technology. This, along with the production of synthetic chloroplast photorespiratory bypasses, can result in heightened productivity.

Bioengineering of Carotenoid Biosynthesis Pathways

Carotenoids are classified into provitamin-A and non-provitamin-A types, with non-provitamin A carotenoids like lutein, astaxanthin, and lycopene not being converted to retinal. C50 carotenoids and apocarotenoids, including decaprenoxanthin, are produced through enzymatic reactions by carotenoid cleavage dioxygenases (CCDs). Apocarotenoids include visual pigments, plant hormones, annatto pigments, and aromatic fragrance compounds. The production of glycosylated derivatives of decaprenoxanthin is exclusive to Corynebacterium glutamicum and other microorganisms [55]. Carotenoids are essential for photosynthesis by absorbing light and capturing energy. Primary and secondary forms like antheraxanthin, zeaxanthin, and violaxanthin are produced and stored to protect cells under abiotic stress, while canthaxanthin and astaxanthin are produced for protection. Mevalonate (MVA) and 2-C-methyl-D-erythritol 4-phosphate (MEP) are crucial carotenoid compounds produced by the MVA or MEP pathways [56]. The balance between β-carotene and α-carotene synthesis is influenced by the interplay between β- and ε-cyclases, which is genetically and physiologically regulated. Carotenoids with two rings are rare in nature, but β-carotene is formed when one LCY- enzyme adds two rings to lycopene. Multiple ketolase and hydrolase enzymes are involved in enzymatic synthesis of carotenoids [57]. Carotenoids are essential for various biological activities such as photoprotection, pigmentation, cell signaling, and photosynthesis. They produce resonance-stabilized carbon-centered peroxyl radicals, antioxidants, potent pigments, and PSII and PSI reaction centers in photosynthetic organisms. Apocarotenoids, such as strigolactone, dihydroactinidiolide, and ABA, play crucial physiological functions in microbial growth and development [58, 59]. The pathway involved in carotenoid biosynthesis directly impacts its yield. Whereas an inefficient biosynthesis leads to toxic IPP/DMAPP accumulation and lower carotenoids yield [60]. Strategies to engineer this module include selecting novel enzymes, engineering key enzymes, optimizing gene expression, and increasing storage [61]. The development of enzymes for carotenoid production requires careful selection of

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heterologous genes. Conventional cell hosts like S. cerevisiae and Escherichia coli lack certain genes needed for carotenoids production [62]. Therefore, it is crucial to select gene sources for carotenoid biosynthesis. Marine microorganisms serve as natural reservoirs for various carotenoids, such as β-carotene hydroxylase enzymes from Fulvimarina pelagi. Innovative bifunctional enzymes like phytoene synthase and lycopene cyclase have been studied and utilized in carotenoid biosynthesis. Fusion enzymes eliminate competition with precursors and undesired side reactions, resulting in high yields. Protein engineering and manipulation of expression levels have been devised to enhance carotenoid synthesis. Directed evolution techniques have been employed to enhance enzyme functionality and efficacy [63].

Table 4: Overview of significant works on carotenoids synthesis optimization by using algae

Types of Vitamin A	Process	Host Organism	Production	References
Astaxanthin	An outdoor tube photobioreactor is operating under specific conditions, including a 0.7 L/day dilution rate, 0.5 mmol/g day nitrate level, and 2500 mE/m2 s irradiance.	*Haematococcus pluvialis*	8.0 mg/L per day	[64]
Canthaxanthin, astaxanthin, lutein, zeaxanthin, and echinenone	Salinity stress at 20 g/L NaCl	*Chlorella protothecoides*	Canthaxanthin echinenone free astaxanthin and lutein/ zeaxanthin (23.3%), (14.7%), (7.1%), and (4.1%), respectively. Optimum level of carotenoid was achieved as 0.8 percent.	[65]
Lutein	sodium nitrate as nitrogen source	*Desmodesmus sp.*	5.18 mg/g	[66]

Scientists have modified the N/C-terminal of enzymes to improve carotenoid production, and mutations of crucial enzymes have been explored. The impact of N/C-terminal fusion tags on protein solubility has been examined to enhance overall activity. A toolkit based on the Cas9 protein was used to modify enzymatic solubility in the synthesis of terpene taxadiene, leading to a significant fifteen-fold increase in taxadiene production. Genetically modified β-carotene ketolase enzymes have also been found to enhance production by 60% in Chlamydomonas reinhardtii [67]. Enzyme expression is associated with the effectiveness of a certain pathway, and overexpression of foreign or native proteins can be achieved through various factors. The modification of the promoter, fine-tuning the ribosome binding site (RBS), regulating gene expression, and controlling gene copy number might further augment carotenoid synthesis. Amplifying the gene copy numbers of crucial enzymes can also be used to achieve carotenoid overproduction [63]. The graphical representation of yields produced by various organisms has been given in Figure 2.

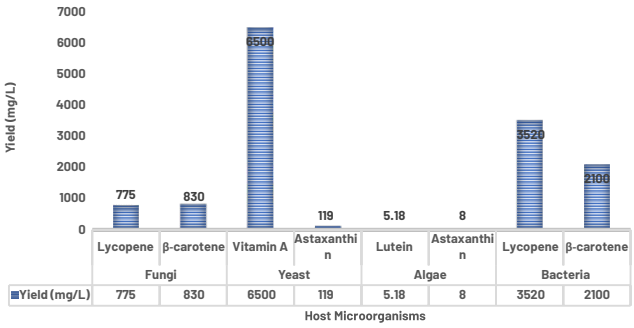

Figure 2: Carotenoid bio-synthesis by using various metabolic engineering techniques [55, 68-70]

Bio-carotenoids are produced by enhancing their accumulation capacity while minimizing their harmful impacts. Yarrowia lipolytica, a yeast with lipid body production and large storage potential, can be used for overproduction. Researchers have developed a new technique in E. coli that uses artificial membrane vesicles to release hydrophobic compounds, resulting in a 61% increase in beta-carotene production. Deletion of lipoprotein genes promotes the production of outer membrane vehicles, accelerating β-carotene excretion.

Designing the isoprene supplementation module is crucial for achieving significant microbial carotenoids production [71]. IPP (Isopentenyl diphosphate) is a crucial precursor for microbial carotenoids, produced through mevalonate and methylerythritol phosphate pathways. Excessive expression of these pathways can lead to harmful intermediate substances and feedback inhibition. Techniques like enhancing enzyme expression and controlling pathways have been used to improve IPP availability [72]. The mevalonate kinase (MK) is an essential enzyme in the mevalonate pathway, which plays a significant role in carotenoids production. However, enzyme HMG-CoA reductase indicating limited expression, can be addressed through overexpression, or substituting it with a more potent variant derived from Staphylococcus aureus. Techniques such as directed evolution, overexpression, and scaffold assembly have been used to enhance the production of IPP downstream products [73]. Figure 3 illustrates MVA and MEP pathways responsible for the biosynthesis of Lycopene. The HMGR1 enzyme, a coenzyme-A reductase, might be hindered by FPP (farnesyl pyrophosphate) and GPP, leading to a reduction in

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carotenoid synthesis. To mitigate this issue, researchers have identified and examined various MKs that are resistant to feedback inhibition and can be introduced into different microbes to enhance the production of carotenoids [74].

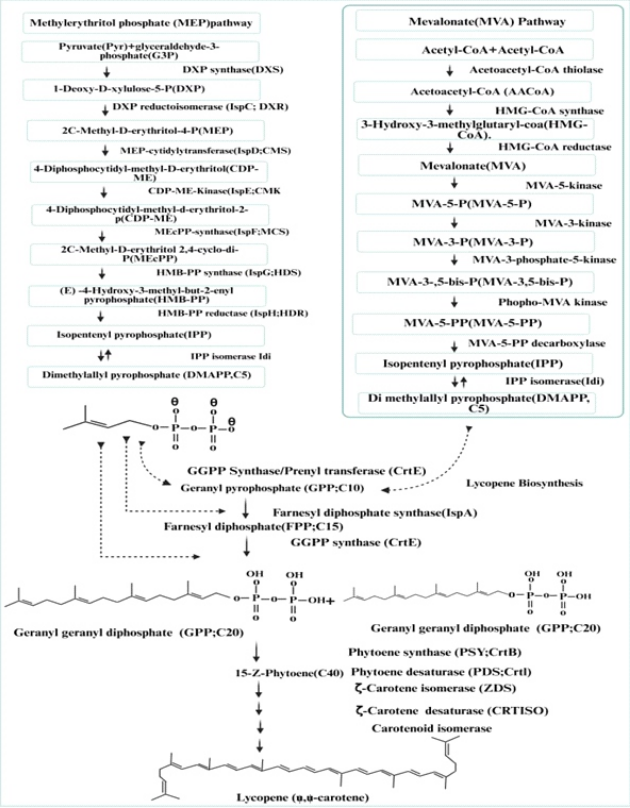

Figure 3: Mevalonate (MVA) and 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways responsible for the biosynthesis of Lycopene [38, 61]

The overexpression of the initial three genes involved in the MVA pathway can lead to an increase in HMG-CoA concentrations, which in turn can cause insufficient cellular development and reduced production of MVA. By manipulating their expression through the selection of promoters and controlling the number of gene copies, MVA production was enhanced by 700%. Additionally, enhancing the efficiency of the upper MVA pathway can be achieved through the use of tunable intergenic regions (TIGRs) that regulate mRNA fragment's stability using oligonucleotides [75]. Scaffold assembly ensures the proper arrangement of enzymes in the appropriate proportion, hence enhancing the production of the IPP precursor. Zeaxanthin production is enhanced by altering the expression of genes that regulate mevalonate MVA's lower pathway. Galactose-controlled promoters were inserted into the genes encoding the MVA system to enable controllable expression of the whole system, thereby lowering the synthesis of hazardous intermediate chemicals and increasing the availability of IPP [76]. Mitochondria, another cellular compartment, plays a significant role in generating substantial quantities of acetyl-CoA (A-CoA), which serves as MVA pathway's precursor. When the full MVA pathway is expressed on purpose within mitochondria, S. cerevisiae produces more A-CoA and significantly more isoprene, which is a downstream outcome of IPP [75]. To further enhance the flow of molecules along the MEP route, it is necessary to reduce the inhibitory effect caused by feedback regulation. IPP and DMAPP which are known as Isopentenyl pyrophosphate and dimethylallyl pyrophosphate, respectively, have been found to hinder the activity of Dxs enzyme, while FPP has been shown to impede the function of IspF enzyme. A method has been created to introduce the heterologous MVA pathway into bacteria, bypassing natural control mechanisms and counteracting the inhibitory effects in the MEP pathway, resulting in an increased concentration of IPP [77]. In order to effectively incorporate the MEP pathway into eukaryotes, it was necessary to address the bottleneck caused by the last two stages, which are catalyzed by IspG and IspH and enzymes. Another study involved the screening and characterization of orthologous genes, which encode for IspH, and IspG, together with flavodoxin (Fld); the redox partners, and FNR (Ferredoxin/Fld NADP+ reductase) in S. cerevisiae. Further investigations are required to delve into the MEP control system and the interconnection between MVA and MEP pathways [78].

Modification In Ipp Isomerization & Isopentenol Utilization Pathway (iup)- A Novel Method To Supplement Ipp Precursors

The Modification in Isopentenol Utilization Pathway (IUP) is a novel method to supplement IPP precursors and IPP isomerization. The efficacy of the MVA and MEP routes has been improved by various initiatives, but these efforts continue to depend on the original substrate for control [79]. A unique IUP has been built, which generated over 100 times more IPP than the MEP pathway in just 5 minutes. The majority of carbon, over 95%, in the taxadiene product is derived from the IUP pathway, whereas less than 5% comes from the MEP system. This indicates that the IUP pathway is an efficient source of IPP for carotenoid biosynthesis [80]. The enzyme isopentenyl diphosphate isomerase (Idi) plays a crucial role in the biosynthesis of carotenoids, but its functionality is limited by its diminished expression, enzymatic activity, brief effectiveness duration, and low substrate affinity [81]. Type 2 Idi, found in plants and archaea, is more effective in boosting carotenoid synthesis than type 1 Idi and is more effective in isoprenoid synthesis in E. coli. Directed evolution of the idi gene from S. cerevisiae led to a 1.8-fold increase in lycopene production in E. coli [82]. The mevalonate (MVA) pathway in E. coli involves the use of three acetyl-CoAs (A-CoAs), which are precursors for acetate, ethanol, and lactate. Carotenoid production can be increased

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by eliminating these byproducts. Researchers have used redirecting the PP pathways and converting pyruvate directly into A-CoA to enhance its levels, stimulating carotenoid synthesis [83, 84]. Glucose is the primary carbon source for acetyl-CoA, G3P, and pyruvate, and boosting carotenoid synthesis can be achieved by increasing glucose absorption and improving transport through the MEP pathway [85]. Xylose enhances the rate of ethanol production by facilitating the conversion of acetyl-CoA and serves as an extra source for carotenoid biosynthesis. Novel artificial cofactor systems, including a modified ED route, can improve the production of NADPH and ATP supplements. Multi-module engineering can improve carotenoid biosynthesis flow by separating modules within microorganisms, allowing for specialized cellular machinery for each module, preventing strain on a single host cell [86, 87]. Microorganisms produce native carotenoids, but only 5% of studies confirm their pathways. Understanding genetic and metabolic factors is crucial for optimizing carotenoids synthesis. High astaxanthin concentration in microalgae makes commercial production feasible, and agro-industrial wastes can be used as an affordable and environmentally sustainable carbon source. Metabolic engineering techniques, machine learning, and multi-omics analysis can improve carotenoid biosynthesis and reduce metabolic strain [88, 89]. Excessive carotenoids production can significantly impact cellular metabolism. Improving the isoprenol utilization pathways is crucial due to its toxic properties. More efforts are needed to understand carotenoid biosynthesis and advance microbial engineering for increased carotenoid production.

CONCLUSIONS

Microorganisms produce native carotenoids, but only five percent of the studies have confirmed their pathways. Understanding the genetic and metabolic factors of carotenoid accumulations is crucial for optimizing carotenoids synthesis. High astaxanthin concentration in microalgae has made commercial production feasible, and agro-industrial wastes can be used as an affordable and environmentally sustainable carbon source. Microalgae store astaxanthin effectively through cytoplasmic lipid droplets. Metabolic engineering techniques like protein engineering, control, and enzyme and gene screening have significantly improved carotenoids production in microorganisms. However, only a small number of genetically modified microbial strains are widely used. Machine learning can facilitate enzyme production and enhance protein performance. Multi-omics analysis can improve carotenoid biosynthesis and encouraging the Isoprenoid Utilization Pathway (IUP) can reduce metabolic strain. Metabolic engineering techniques have improved carotenoids synthesis in microorganisms through enzyme and gene screening, protein engineering, and control. However, their use in industry is limited due to genetic instability, as most bioprocesses favor chromosomal genes and rely on plasmids for gene regulation. To address this, various methodologies, such as marker-free genetic integration techniques like the CRISPR-Cas system, have been applied.

FUTURE PERSPECTIVES

Carotenoids, as secondary metabolites, can significantly impact cellular metabolism when produced excessively. Improving the Isoprenoid Utilization Pathway (IUP) due to the poisonous nature of isoprenol is necessary to enhance carotenoids production. Machine learning can assist in novel enzyme screening and protein evolution, leading to the acquisition of highly efficient enzymes. The introduction of unnatural base pairs and amino acids can enhance protein evolution diversity. Despite the completion of many omics analyses in carotenoid overproduction strains, there is still a need for a more comprehensive understanding of the connection between pathway regulation and metabolite intake. Multi-omics studies, including transcriptomics, proteomics, and metabolomics, can enhance our comprehension of carotenoid biosynthesis, identify bottlenecks, and improve carotenoid production. Further efforts, including various strategies, will be necessary to enhance understanding of carotenoid biosynthesis and advance microbial engineering for increasing carotenoid output. More study is needed to advance microbial engineering's goal of increasing carotenoid production and improve our understanding of carotenoid biosynthesis.

Authors Contribution

Conceptualization: IP, NA, SM, YS, SN, QS, SHA
Writing, review and editing: BB, FA, IP, SN, RE, QS, SHA, SR
All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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