frontiers | Frontiers in Marine Science

TYPE Mini Review
PUBLISHED 07 October 2022
DOI 10.3389/fmars.2022.1015419

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Marine Biotechnology and Bioproducts, a section of the journal Frontiers in Marine Science

RECEIVED 02 September 2022
ACCEPTED 27 September 2022
PUBLISHED 07 October 2022

CITATION
Patel A, Rova U, Christakopoulos P and Matsakas L (2022) Microalgal lutein biosynthesis: Recent trends and challenges to enhance the lutein content in microalgal cell factories. *Front. Mar. Sci.* 9:1015419. doi: 10.3389/fmars.2022.1015419

Microalgal lutein biosynthesis: Recent trends and challenges to enhance the lutein content in microalgal cell factories

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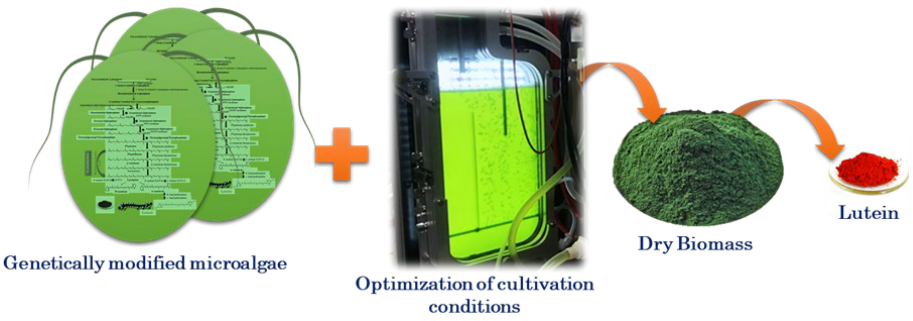
Photosynthetic organisms such as eukaryotic microalgae and prokaryotic cyanobacteria synthesize a wide range of valuable chemicals. They are predicted to become efficient and renewable sources for valuable products in the future due to their high biomass synthesis using CO_2 and solar energy. Microalgae are producers of several carotenoids including lutein, which is a xanthophyll carotenoid with several health advantages, including the prevention of age-related macular degeneration. Currently, it is extracted on commercial scale from marigold flower petals, however, production from plant sources is highly affected by seasonal variations, requires arable land, and has high production cost. Microalgae, on the other hand, are an ideal alternative for lutein synthesis due to their rapid growth and high biomass and lutein yield. It is, however, necessary to further improve lutein productivity, for a successful transition to commercial production. This article describes lutein biosynthesis in microalgae by using their native biochemical pathways, as well as possible target genes for genetic engineering to enhance lutein production. Understanding the processes behind lipid droplet synthesis in chloroplasts, as well as carotenoid transport across chloroplast membranes and carotenoid esterification, might lead to novel ways to boost lutein levels in microalgae.

KEYWORDS

microalgae, carotenoids, lutein, genetic engineering, fermentation



GRAPHICAL ABSTRACT

Introduction

Carotenoids are a group of terpenoid pigments having a C40 backbone that is beneficial to human health. Over 1100 carotenoids have been identified so far, and are found in a variety of animals, fruits, and green vegetables (Mussagy et al., 2021). Terpenoids are one of the most diverse classes of compounds found in nature, and their biological and pharmacological actions are making them essential for the food and pharmaceutical industries (Ma et al., 2021). Microalgae efficiently synthesize various bio-pigments intracellularly such as β-carotene, lutein, prasinoxanthin, siphonaxanthin, fucoxanthin, zeaxanthin, diadinoxanthin, lycopene, astaxanthin, chlorophyll, phycoerythrin and phycobiliproteins etc. (Begum et al., 2016). Since some carotenoids offer protection from reactive oxygen species and intense light, they are crucial for the survival of microalgae (Praveen et al., 2022). These pigments are synthesized by various microalgal species but the concentration in each microalga is dependent on the particular strain and their cultivation condition e.g. *Dunaliella salina* preferentially synthesize β-carotene and *Hematococcus pluvialis* predominantly synthesize astaxanthin (Khoo et al., 2019; Wolf et al., 2021).

Among them, lutein is categorised in the xanthophyll family which is the 2nd most prominent carotenoid having its valuable health benefits including prevention of acute and chronic coronary syndrome, and age-related macular degeneration, as well as assisting in maintaining normal vision (Zheng et al., 2022). Lutein, widely known as the "eye vitamin," protects the eye tissues from UV and oxidative damage and age-related macular degeneration (AMD) due to having antioxidants properties (Roberts et al., 2009). Oxidation or clouding of natural lens responsible of focusing of light on retina is major cause of cataracts. Lutein is one of the most significant carotenoids present in human milk responsible for infant visual and cognitive development (Fitzpatrick and Dhawan, 2014; Eggersdorfer and Wyss, 2018). It takes almost 66 to 77% of total carotenoids responsible for brain development in the infant (Vishwanathan et al., 2014).

Commercial extraction of lutein is carried out from marigold flower petals and its concentration varies from 17 to 570 mg/100g between the different species (Piccaglia et al., 1998). Lutein is found in its esterified form in the marigold flower, where half of its weight consists of fatty acids that need to be removed by saponification to obtain pure lutein (Zhao et al., 2021). However, commercial production of lutein from plant sources is hindered by several factors such as the limitation of production in certain periods of the year that the plant can be harvested and tedious petal separation (Lin et al., 2015). Other sources such as corn, leafy green vegetables and egg yolk have also drawbacks, owing to the low lutein concentration and bioavailability (Zhao et al., 2021). Those challenges can hinder the viable commercial production of lutein, making photosynthetic eukaryotic and prokaryotic microorganisms a promising alternative source. Under optimized cultivation conditions, microalgae can accumulate significant amounts of biomass and lutein (Ren et al., 2021). Lutein is an antenna pigment in the light-harvesting complex (LHC) of microalgal photosynthetic apparatus that can prevent oxidative damage of cells by non-photochemical quenching under high light intensity (Fernández-Sevilla et al., 2010).

Microalgae display a variety of metabolic pathways resulting in the accumulation of various metabolites. The role of these metabolites in carbon partitioning in microalgae having variable cell compositions depending on various cultivation modes (autotrophic, heterotrophic and mixotrophic) including light intensity, pH of the medium, dissolved O_2 and nutrient-limited conditions (Vuppaladadiyam et al., 2018). Primary metabolites are produced when microalgae are cultivated in sufficient nutrient and light conditions, while adverse conditions, including the nutrient deficit, high light intensity, and salt stress are critical elements which activate defence systems in microalga cells that lead to the accumulation of secondary metabolites (Vuppaladadiyam et al., 2018). Algae-

derived lutein is in free, non-esterified form, and has long been thought to be a good alternative to traditional plant sources. Harvesting costs, extraction of crude lutein, purification and bioavailability analyses have all been studied concerning microalgae to replace marigold plants in the production of lutein. Many microalgal genera, such as *Scenedesmus*, *Chlorella*, *Coccomyxa*, *Parachlorella*, and *Tetraselmis*, have been studied for lutein synthesis thus far (Xie et al., 2021). In this review article, we provide an overview of recent developments in the production of lutein from microalgae by employing high yield strains and genetic modification techniques to boost the lutein content.

Biosynthesis of lutein by microalgae

Several genera of microalgae have been studied for lutein production in the literature. The production yield varies based on the selected strains, the medium composition used for their growth and other cultivation conditions. As a result, optimizing cultivation conditions has been intensively studied with the objective of boosting growth performance and high lutein productivity. Microalgae can be cultivated under different mode of nutrition such as photoautotrophic, heterotrophic or mixotrophic for lutein production. Pigments are usually synthesized under photoautotrophic growth, but the cell density is usually low under these conditions. On the other hand, heterotrophic growth results in high biomass formation but with lower amount of pigments. The low growth rate in photoautotrophic conditions and low pigment concentration in heterotrophic conditions can be resolved by using mixotrophic cultivation. For example, *C. sorokiniana* MB-1-M12 synthesized lutein up to 50.6 mg/L under mixotrophic cultivation, by using 6 g/L of sodium acetate as organic carbon with replacement of medium under semi-continuous mode (Chen et al., 2019). In addition to this, other researchers used two-stage cultivation strategies to enhance lutein content. For example, *Chlorella sorokiniana* FZU60 was initially cultivated under mixotrophic conditions in fed-batch mode and then shifted to photoautotrophic mode for enhanced lutein production (Xie et al., 2020). Similarly, *Scenedesmus incrassatulus* was firstly cultivated under heterotrophic mode by using glucose as a carbon source for high cell density, and thereafter the shifted to a photobioreactor for lutein production (Flórez-Miranda et al., 2017).

Chlorella sp. is the most studied genus for lutein production. The cold-tolerant microalga *Chlorella vulgaris* UTEX 265 was used in a coiled tubular tree photobioreactor where light intensity and period and temperature were optimized toward high lutein productivity. Under optimal conditions of lighting for 14 h per day with 25 µmol photons m^{-2} s^{-1} and growth at 22 °C, it synthesized 9.82 mg/g of lutein (Gong and Bassi, 2017). Chen et al. (2016) investigated the extraction of lutein from *Chlorella sorokiniana* MB-1 under semi-batch mixotrophic conditions, by studying various factors such as biomass pretreatment, extraction pressure, temperature and duration, and solvent type. They found that by using a French press for biomass pretreatment and tetrahydrofuran (THF) as solvent resulted as the extraction solvent, nearly 100% of lutein recovery was achieved in 40 minutes at 850 mbar and 25°C (Chen et al., 2016). In another study *C. sorokiniana* MB-1 was cultivated under a semi-batch-integrated two-stage cultivation mode with 80% of medium replacement, achieving the lutein productivity of 7.62 mg/L/d (Chen and Liu, 2018). Chen et al. (2021) cultivated *C. sorokiniana* AK-1 on untreated piggery wastewater where it synthesized 4.56 mg/g of lutein, while efficiently removing COD, BOD, TN and TP content from the wastewater (Chen et al., 2021). In another study, 0-200 mM sodium bicarbonate was used as an inorganic carbon source to cultivate *Chlorella pyrenoidosa* (Sampathkumar and Gothandam, 2019). When 100 mM of sodium bicarbonate was used, under photoautotrophic conditions with a light intensity of 42 µmol m^{-2} s^{-1} (16:8 light/dark cycles) at 27 °C, lutein content reached 4.84 mg/g (Sampathkumar and Gothandam, 2019). Asker and Awad (2019) isolated the novel marine strain *Auxenochlorella* sp. LEU27A, which is capable of accumulating up to 996.6 ± 98 µg/g of lutein under heterotrophic cultivation on marine broth 2216 (contains per L: yeast extract, 8 g; peptone, 5 g) (Asker and Awad, 2019). Lutein synthesis in *C. vulgaris* was optimized with the low light intensity of 160 µmolm^{-2} s^{-1} and the addition of nitrate in 5 L photobioreactor, resulting in 10.4 ± 5.5 mg/g which was more than double compared to the medium without nitrate addition (4.3 ± 2.9 mg/g) (McClure et al., 2019). The lutein concentration was further enhanced to up to 15 - 20 mg/L when the cultivation was performed in 50 L bubble column photobioreactor under semi-continuous mode (McClure et al., 2019).

Scenedesmus incrassatulus CLHE-S01 was cultivated in a fed-batch airlift photobioreactor under photoautotrophic conditions with three different nitrogen concentrations, synthesizing 1.88 ± 0.08 mgL^{-1} day^{-1} of lutein under high nitrogen concentration (García-Cañedo et al., 2016). In another study *S. incrassatulus* CLHE-Si01 was cultivated under a sequential two-stage, where initially it was cultivated heterotrophically in stirred tank bioreactor with glucose, followed by transfer to an airlift photobioreactor for light induction of lutein, which accumulates up to 1.49 ± 0.03 mg/g when supplemented with urea + vitamins (Flórez-Miranda et al., 2017). *Scenedesmus* sp. CAS-173 was cultivated under the mixotrophic conditions in an airlift photobioreactor using glycerol obtained from a biodiesel production unit for lutein production, resulting in maximum productivity of lutein of 3.59 mg/L/d with 6 g/L of crude glycerol (Rajendran et al., 2020). In another study, light intensity was optimized for lutein production from *Scenedesmus obliquus* FSP-3, showing maximum lutein productivity of 4.08 mg/L/d when cultivated

under a light intensity of 300 µmol m^{-2} s^{-1} by using a TL5 fluorescent lamp (Ho et al., 2014).

Chlamydomonas reinhardtii is considered a model microalga for carotenoid production with fast growth rate but limited biomass production. Light intensity is a major factor for lutein synthesis in this microalga. For example, when *C. reinhardtii* was cultivated under constant lighting of 149 µmol m^{-2} s^{-1} intensity for 10 days, it produced 0.87 mg/g lutein (El-Mekkawi et al., 2019). In another study, *Chlamydomonas acidophila* was illuminated with PAR light of 240 µmol m^{-2} s^{-1} and synthesized 20.2 ± 0.5 mg/L of lutein while increment of light intensity to 1000 µmol m^{-2} s^{-1} did not enhance lutein production (Garbayo et al., 2008). On the other hand, lutein content was enhanced under UV-A radiation or heated at 40 °C (Garbayo et al., 2008). The cultivation temperature was optimized for lutein production in *Chlamydomonas* sp. JSC4, achieved a maximum lutein productivity of 3.27 mg/L/d at 35°C while the maximum lutein content was achieved at 20 °C and reached 3.82 mg/g (Ma et al., 2020).

Biosynthesis of lutein in microalgae by native pathway

Carotenoids have a C40 methyl branched hydrophobic backbone with conjugated double bonds, which is necessary for pigmentation and visible wavelength photon absorption (Velmurugan and Kodiveri Muthukaliannan, 2022). Based on their distribution in the intracellular compartments and their role in cellular metabolisms, they can be divided into carotenes and xanthophylls, which can be primary or secondary. Primary carotenoids are mainly structural and functional photosystem metabolites and are synthesized in chloroplasts, whereas secondary carotenoids are synthesized in cytoplasmic lipid vesicles, and become more abundant in response to stress (Huang et al., 2017). Lutein is confined to several taxa of microalgae, but it is mainly found in Chlorophyta classes.

Elucidation of carotenoids production in bacteria, algae, protist (Patel et al., 2022) and higher plants has progressed significantly, and it is expected that primary carotenoids biosynthesis in these species follows a similar process. Figure 1 depicts the lutein synthesis route in green algae (Chlorophyta), which is subdivided into the different stages (Huang et al., 2021; Kato et al., 2021; Lou et al., 2021; Xie et al., 2021; Song et al., 2022; Velmurugan and Kodiveri Muthukaliannan, 2022). The major metabolic pathway includes the synthesis of isopentenyl diphosphate (IPP), geranylgeranyl pyrophosphate, phytoene, and desaturation of phytoene into lycopene, followed by cyclisation and hydroxylation of lycopene to lutein.

Lutein synthesis starts with the formation of 1-deoxy-D-xylulose-5-phosphate (DXP) from glyceraldehyde-3-phosphate (GAP) and pyruvate. MEP (2-C-methyl-d-erythritol 4-phosphate) pathway plays a major role to convert DXP into IIP through a series of reactions which is found in plants, microalgae, cyanobacteria, and a few bacterial species (Rohmer, 1999). Geranyl pyrophosphate (GPP) is synthesized by the condensation of IIP and its isomer dimethylallyl diphosphate (DMAPP) and is considered as the first intermediate precursor in the lutein biosynthetic route. GPP is converted into farnesyl pyrophosphate (FPP) through the condensation with IIP, followed by another IIP unit addition which is catalyzed by the GGPP synthase and results in the synthesis of Geranylgeranyl pyrophosphate (GGPP) (Fraser et al., 2000). Then a rate-limiting membrane-bound, phytoene synthase (PSY) enzyme catalyzes the condensation of two GGPP molecules to form Phytoene (Shaker et al., 2021). PSY is highly conserved in prokaryotes and eukaryotes. Phytoene is subsequently converted into lycopene by two distinct desaturases, namely phytoene desaturase (PDS) and zeta-carotene desaturase (ZDS), through various steps, in which PDS initially converts phytoene to phytofluene and thereafter to ζ-carotene, followed by its conversion to neurosporene by ζ-carotene desaturase (ZDS). PDS is membrane-bound oxidoreductase enzyme that resides in plastids where it performs photosynthesis and carotenoid synthesis (Narang et al., 2022). Finally, neurosporene is converted to lycopene by ζ-carotene desaturase (ZDS), ζ-carotene isomerase (Z-iso), and carotenoid isomerase (CRTISO) (Cunningham and Gantt, 1998; Liang et al., 2019). This process differs in cyanobacteria, where a single phytoene desaturase (CrtI) enzyme catalyses the phytoene into lycopene (Molina-Márquez et al., 2019). Lycopene is usually the bright color carotenoid found in vegetables such as tomatoes. In photosynthetic microorganisms, lycopene is further converted into carotene by cyclization of one or both lycopene ends which is a crucial branching point for carotenoids synthesis. Finally, α-carotene is formed by β-cyclase (LCY-b) and ϵ-cyclase (LCY-e) that is responsible for β and ϵ rings formation at both ends of lycopene and is mainly found in plants and microalgae (Molina-Márquez et al., 2019). On the other hand, β-carotene is formed by the action of LCY-b alone, which catalyzed the synthesis of two β-rings at both ends of lycopene. Xanthophylls are formed by the oxidation of α- and β-carotene in higher plants and microalgae. Lutein is formed by hydroxylation of α- carotene at C-3 and C-3′ positions by the action of β- ring and ϵ-ring carotene hydroxylase encoded by *CrtR-b* and *CrtR-e*, respectively (Quinlan et al., 2012).

Use of chemical inhibitors to enhance the lutein content in microalgae

Certain chemical inhibitors such as imidazole, pyridine, triethylamine, piperidine, nicotinic acid and nicotine, modify

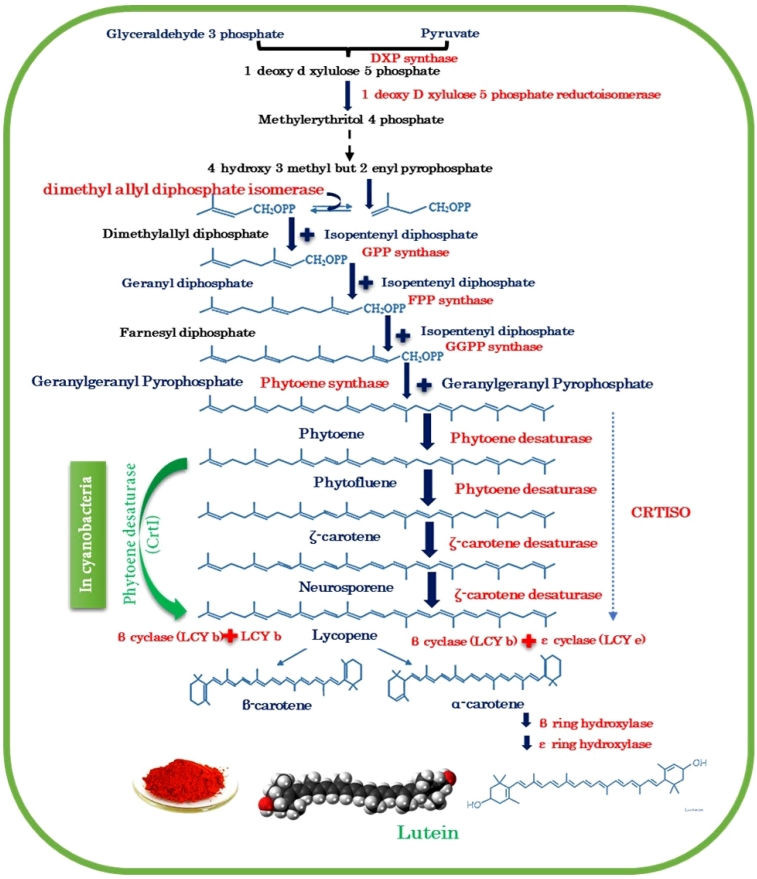


FIGURE 1
Biosynthetic pathway of lutein in microalgae; source references (Huang et al., 2021; Kato et al., 2021; Lou et al., 2021; Xie et al., 2021; Song et al., 2022; Velmurugan and Kodiveri Muthukaliannan, 2022).

the carotenoids pathway to form specific products in microalgae. Yildirim et al., 2017 suggested that lutein biosynthesis in microalga can be enhanced by adding native biosynthetic pathways inhibitors. In this regard, they used 2-methylimidazole (2MI) during the cultivation of *Dunaliella salina* to inhibit the cyclization reactions in the biosynthesis pathway carotenoids (Yildirim et al., 2017). The addition of 2MI at a concentration of 1 mM enhanced the lutein content by 1.7-folds with a simultaneous reduction in β-carotene content, hence proving that 2MI affects the activity of lycopene β-cyclase (*LCY-b*) that is responsible for α-carotene synthesis (Yildirim et al., 2017). They suggested that inhibitor 2 MI-1 mM might be more effective on lycopene β-cyclase than lycopene ϵ-cyclase, and it directs the pathway to the α-carotene branch followed by lutein synthesis (Yildirim et al., 2017). Similarly, DCMU [3–(3′,4′-dichlophenyl)-1,1-dimethylurea] was used as an inhibitor of lycopene β-cyclase in the culture of *Dunaliella bardawil* strain V-101 that enhanced the lutein content by 2 fold (Mysore Doddaiah et al., 2013). They suggested that reducing β-carotene content in DCMU-treated cells was due to inhibition of lycopene β-cyclase resulting in enhancing lutein content through lycopene ϵ-cyclase (*CrtR-e*) and β-and ϵ-carotene hydroxylase activities (Mysore Doddaiah et al., 2013). In a study it has been showed that the overexpression of lycopene ϵ-cyclase (*LCY-e*) gene in *C. reinhardtii* resulted into enhancing the conversion of lycopene to α-carotene and finally the lutein concentration (Tokunaga et al., 2021). Likewise, lutein content in *C. sorokiniana* was improved by random mutagenesis with N-methyl-N′-nitro-nitrosoguanidine (MNNG), resulting in a 2-fold increase in the concentration of lutein compared (42 mg/L) to the wild strain (Cordero et al., 2011b).

Genetically modified microalgae for enhanced production of lutein

Some examples of genetically modified microalgal strains for overproduction of lutein are listed in Table 1. In a study, the lutein content in *C. reinhardtii* was boosted by improving the biomass

TABLE 1 Genetically modified microalgal strains for enhanced lutein production.

Microalgal Strain	Strategies used to enhance lutein content	Lutein content	References
Chlamydomonas reinhardtii	Knocked out zaxanthin epoxidase (*ZEP*)	2.71 ± 0.04 to 3.08 ± 0.07 mg/g	(Baek et al., 2018)
Chlamydomonas reinhardtii	Heterologous expression of phytoene synthase (*CzPSY*) gene from *Chlorella zofingiensis*	2.2-fold higher	(Cordero et al., 2011a)
Chlamydomonas reinhardtii	Phytoene synthase (*PSY*) gene from *Dunaliella salina*	2.6-fold increment	(Couso et al., 2011)
Chlorella vulgaris NRF 13	Ethyl methanesulfonate (*EMS*) based random mutagenesis	40 mg/L, 5.4-fold higher than the wild-type	(Guardini et al., 2021)
Chlorella zofingiensis mutant (CZ-bkt1)	Chemical mutagenesis causes dysfunctional carotenoid ketolase responsible for conversion of zeaxanthin to violaxanthin or astaxanthin	13.81 ± 1.23 mg/g or 33.97 ± 2.61 mg/L	(Huang et al., 2018)
Chlamydomonas reinhardtii	Heterologous expression of lycopene epsilon-cyclase (*LCY-e*) gene from *Chlorella vulgaris*	2.3-fold higher than the wild-type strain	(Lou et al., 2021)
Chlamydomonas reinhardtii	Phytoene-β-carotene synthase (*CrtYB*) gene from the red yeast *Xanthophyllomyces dendrorhous*	8.9 mg/g	(Rathod et al., 2020)
Chlamydomonas reinhardtii	CRISPR–Cas9 RNP–mediated knock–out of zeaxanthin epoxidase (*ZEP*) and ADP–glucose pyrophosphorylase (*AGP*)	2.93 mg/g; almost 20% higher than with wild type strain	(Song et al., 2022)
Haematococcus pluvialis	Modified phytoene desaturase by site-directed mutagenesis	0.46 to 0.64 μg/g	(Steinbrenner and Sandmann, 2006)

production after heterologous expression of carbonic anhydrase from *Mesorhizobium loti* (MlCA) and *Sulfurihydrogenibium yellowstonense* (SyCA) which increased CO_2 capture (Lin et al., 2022). This microalga synthesized maximum lutein of 21.32 mg/L (a 4-fold increase from the wild type strain) under the mixotrophic condition in a photobioreactor by using 5% CO_2 (Lin et al., 2022). When this microalga was genetically modified through heterologous expression of phytoene synthase gene (*CzPSY*) from *Chlorella zofingiensis*, it synthesized 2.2 folds higher lutein content than the one observed in untransformed cells (Cordero et al., 2011a). When another phytoene synthase gene isolated from *D. salina* was heterologously expressed, lutein content increased by 2.6 folds compared to the wild type cells (Couso et al., 2011). In the lutein synthesis pathway, lycopene epsilon-cyclase (*LCY-e*) is an important enzyme for the conversion of lycopene into δ-carotene, α-carotene and lutein. In this regard, *CvLCYE* gene from *C. vulgaris* was heterologously expressed in *C. reinhardtii* where it showed an enhanced lutein content by 2.3 folds compared to the wild type (Lou et al., 2021). Tokunaga et al. (2021) overexpressed the same *LCY-e* gene endogenously in *C. reinhardtii* resulting in an enhanced lutein production without affecting the total cell yields (Tokunaga et al., 2021). Rathod et al. (2020) further improved the lutein production in *C. reinhardtii* by heterologous expression of phytoene-β-carotene synthase gene (*CrtYB*) from the yeast *Xanthophyllomyces dendrorhous* (Rathod et al., 2020). This gene has a dual functional role in phytoene synthase (psy) and lycopene cyclization (Lcyb) in the carotenoid synthetic pathway (Rathod et al., 2020). Heterologous expression of this gene in *C. reinhardtii* boosted lutein content up to 8.9 mg/g which was 60% higher than the wild type under low light intensities of 75 μmol photons m^{-2} s^{-1} (Rathod et al., 2020). Recently, Song et al. (2022), used CRISPR-Cas9 RNP mediated knock out strategies to delete the zeaxanthin epoxidase (*ZEP*) gene along with the ADP–glucose pyrophosphorylase (*AGP*) gene in the *C. reinhardtii* that is responsible for xanthophyll and starch biosynthesis in this microalga (Song et al., 2022). This double knock-out mutant enabled to accumulate 2.93 mg/g of lutein under optimal cultivation conditions (Song et al., 2022).

Baek et al. (2018) suggested that genetically modified *C. reinhardtii* CC-4349 through knocking out the zeaxanthin epoxidase gene by preassembled DNA-free CRISPR-Cas9 ribonucleoproteins, showed a 56-fold increment in zeaxanthin content without affecting the lutein content significantly, which increased from 2.71 ± 0.04 mg/g to 3.08 ± 0.07 (Baek et al., 2018).

Conclusion and future perspective

The majority of lutein that is now available on the market is produced from marigold petals. Microbial fermentation employing engineered cell factories is an appealing new production path to fulfil the expanding market demand, which is estimated to reach 360 million USD in 2022 (Lutein Market - Global Forecast to 2022, 2018). The majority of microalgal studies are limited to certain metabolites with the most notable to be lipids, however, other secondary metabolites are explored in a few microalgae strains. Among several genera, *Chlorella* is widely employed for lutein production using various fermentation strategies while *C. reinhardtii* is extensively explored for genetic modifications through traditional engineering, due to its GRAS status (generally recognized as safe). As a result of the advancement of efficient gene-editing tools, target specific gene editing has grown increasingly popular

in recent years. In order to increase lutein content and productivity in microalgal strains, genetic engineering approaches are utilized to change the rate-limiting stage of the metabolic pathway (Saini et al., 2020). Even though the area of microalgal genetic engineering is still in its infancy, progress has been made in recent years utilizing this technique to boost the lutein content of microalgae. Our knowledge of the regulation of lutein metabolism in microalgal species has expanded with the introduction of novel molecular techniques like CRISPR/Cas9 genome editing and genome sequencing. (Baek et al., 2018; Patel et al., 2019).

It is very difficult to produce lutein in non-photosynthetic microorganisms through heterologous expression due to challenges involved in lycopene asymmetric cyclization. However, some studies have succeeded in the heterologous biosynthesis in non-photosynthetic microorganisms such as *Escherichia coli* (Takemura et al., 2021) and *Saccharomyces cerevisiae* (Bian et al., 2021). Integration of several genes including isopentenyl diphosphate isomerase (*IDI*), lycopene ϵ-cyclase (*LCY-e*), lycopene β-cyclase (*LCY-b*) and cytochrome P450 97C (CYP97C) in *E. coli* leads to the synthesis of 11 mg/L of lutein (Takemura et al., 2021). In the other approach, combinatorial engineering in *S. cerevisiae* provides lutein content up to 438 $\mu g/g_{CDW}$ (Bian et al., 2021). The strategy used for the efficient heterologous lutein biosynthesis in yeast revealed that temporospatial pathway control may be used to solve intra-pathway competitions in both photosynthetic and non-photosynthetic strains as well, and it could also be used to promote the biosynthesis of other natural products.

In conclusion, lutein production from microalgae has been carried out significantly at the lab scale and is slowly approaching towards commercialization of microalgal-based lutein. However, the level of lutein in microalgal candidates is still very low in comparison with other carotenoids such as β-carotene (up to 14%) and astaxanthin (up to 5%) in *Dunaliella* spp. (Pourkarimi et al., 2020) and *Haematococcus* spp., respectively (Shah et al., 2016). It is already discussed by Xie et al. (2021), that lutein sequestration is a critical issue for its high content production in the intracellular compartment in microalgae. Lutein is synthesized in the inner membrane of the chloroplast that accumulates over there and can't float in a hydrophilic environment of chloroplast due to lipophilic unsaturated polyene chain and the hydrophobic hydroxyl group of rings. This results in feedback inhibition of lutein synthesis or degradation by reactive oxygen species (ROS) or carotenoid cleaveage dioxygenases (CCDs) if appropriate sequestration is not achieved. As a result, lutein sequestration is required for lutein accumulation, and lutein storage capacity may influence the maximum lutein level in microorganisms (Xie et al., 2021).

Other challenges of lutein production from microalgae are its costly and time-consuming downstream processing that requires harvesting of microalgal biomass, extraction and purification of lutein. Due to the low cell density of large-scale microalgal cultures, energy-intensive harvesting and dewatering techniques are needed. To produce lutein of optimum quality, the microalgal slurry must be concentrated at least ten times before being processed for lutein extraction. Harvesting of lutein-rich microalgal biomass is usually carried out by several physical and chemical methods. Physical methods include gravimetric sedimentation, centrifugation, and filtration of biomass whereas chemical methods involve floatation and flocculation. Some effective techniques such as centrifugation can be costly, time-consuming or there is a chance of lutein degradation. Extraction is usually carried out after the proper disruption of biomass by physical, non-mechanical or enzymatic methods, which can be energy intensive due to the hard cell wall of microalgae that require significant pressures in order to rupture. Therefore, the implementation of microalgal lutein production depends on the advance of technology for processing microalgal biomass that is affordable, energy-efficient, and eco-friendly.

Author contributions

AP drafted the manuscript. UR, LM and PC, conceived the study, and discussed the content of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

Authors would like to thank the Swedish Research Council (FORMAS) Sweden for supporting this work as part of the projects 'Green and sustainable approach to valorise high saline and oily fish processing effluents for the production of nutraceuticals' (INVENTION; 2020-01028).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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