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Review

Current Advances in Carotenoid Production by *Rhodotorula* sp.

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Abstract: Microbial carotenoids are pigments of lipophilic nature; they are considered promising substitutes for chemically synthesized carotenoids in the food industry. Their benefits for human health have been demonstrated due to their antioxidant capacity. Yeasts of the genus *Rhodotorula* have genotypic characteristics that allow them to accumulate high concentrations of carotenes under certain stress conditions. The present review includes recent information covering different aspects of carotenoid production in *Rhodotorula* sp. fermentation. This review focuses on fermentation carotenoid production strategies, describing various economic raw materials as sources of carbon and nitrogen, the capacity for tolerance to heavy metals, and the effect of light, pH, and salts on the accumulation of carotenoids. Genetic modification strategies used to obtain strains with increased carotenoid production are described. Furthermore, using magnetic nanoparticles in the fermentation system, which could be a stress factor that increases pigment production, is considered for the first time. *Rhodotorula* is a potential source of high-value carotenoids with applications in the cosmetics, pharmaceutical, and food industries.

Keywords: *Rhodotorula*; carotenoids; fermentation

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1. Introduction

Carotenoids are a group of compounds extensively used in the chemical, pharmaceutical, and food industries. Their market size has been expanding, from 1.5 billion in 2019 to 2.0 billion dollars in 2026, at a rate of 4.2% per year [1]. Carotenoids, a precursor of vitamin A, can enhance immune response, and they have antioxidant capacity because they can scavenge oxygen radicals [2]. An in vivo effect of carotenoids suppressing tumor activity has also been demonstrated [3]. Many species of yeasts classified under *Basidiomycota*, known as red yeast due to their growth in pigmented colonies, can produce intracellularly accumulated carotenoids [4,5]. The genus *Rhodotorula* sp. (teleomorph is *Rhodosporidium*) is one of the most studied strains. It is a promising source of pigments such as β-carotene, γ-carotene, lycopene, torulene, and torularhodin [6–8]. Colonies can have an orange, salmon, pink, or red color depending on the type and concentration of pigments. β-carotene is the principal carotenoid, accounting for up to 70% of the total carotenoid content [9]. Another

important property of these yeasts is their capability to synthesize carotenoids while using low-cost substrates as growth substrates, such as molasses [10], raw glycerol [11], wastewater [12], and hydrolyzed lignocellulosic [13]. In addition, they have a high tolerance to inhibitors produced during the hydrolysis processes of lignocellulosic biomass, allowing the production of carotenoids in these biomasses [14,15]. Despite these advantages, the mechanisms of carotenoid biosynthesis and the relevant fermentation conditions remain poorly studied.

Cost-effective production of carotenoids using microorganisms requires selecting appropriate methods and metabolically and genetically robust strains, as well as designing and optimizing culture conditions (e.g., metal ions, pH, light, temperature, and oxygen accessibility) for large-scale fermentation in bioreactors (e.g., batch, fed batch, and continuous fermentation). The carotenoids produced by *Rhodotorula* yeast are secondary metabolites; therefore, metabolic stress conditions are necessary for the accumulation of these pigments [16]. This review describes the metabolic mechanisms of carotenoid biosynthesis by *Rhodotorula* sp., and the influence of environmental factors in strategies to increase carotenoid production. Studies of genetic modification strategies are presented. For the first time, using magnetic nanoparticles in fermentation is addressed as a strategy for accumulating secondary metabolites.

2. Types of Carotenoids and Mechanism of Biosynthesis in *Rhodotorula*

In the *Rhodotorula* carotenoid biosynthetic pathway, β-carotene ($C_{40}H_{56}$) is one of the best-known pigments in pharmaceutical formulations, food additives, cosmetics, eye care products, and other applications. Carotenes (α-, β- and γ-isomers) are synthesized from carotenoids by the enzyme β-carotene 15,15′-monooxygenase. β-Carotene is formed by two β-ionone rings connected by a polyene chain containing nine conjugated double bonds. It is a potent antioxidant, known for protecting cells from damage caused by light and oxygen. It is the most effective provitamin, because one molecule of β-carotene can be converted into two molecules of vitamin A [2]. This orange-red pigment is soluble in ethanol, ethers, chloroform, benzene, and oil, has a melting point of 176–180 °C, and is the most stable and effective form of carotene [17].

Torulene ($C_{40}H_{54}$) and torularhodin ($C_{40}H_{52}O_2$) are two main carotenoids often found in red yeasts. Torulene contains a β-ionone ring attached to a polyene chain and has 13 double bonds, which gives it more potent antioxidant properties than β-carotene [18] (Figure 1). Torularhodin is more oxidative than torulene due to hydroxylation and oxidation. This molecule acts as a potent antioxidant, stabilizing cell membranes under stressful conditions. Torularhodin is more effective than β-carotene and torulene at quenching singlet oxygen. Furthermore, it can eliminate free radicals and inhibit the formation of lipid peroxides, which contributes to cell protection, including resistance to tumors [19]. Like lycopene, torulene and torularhodin have caused tumor cells to undergo apoptosis, inhibiting prostate cancer development, in anticancer tests [20]. Both substances exhibit anti-inflammatory [21,22] qualities. These two carotenoids' antibacterial properties have made them helpful in preventing infections, especially in goods, implants, and medicinal preparations that call for natural antimicrobials [23].

Analyses of the carotenoid synthesis metabolic pathways in several *Rhodotorula* species revealed that there are just three stages in a conserved or similar carotenoid biosynthesis process, shown in Figure 2. Hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase) catalyzes the conversion of acetyl-CoA to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). The first specific precursor in the terpenoid biosynthesis pathway, C6 mevalonic acid (MVA), is produced by HMG-CoA conversion. Subsequently, a sequence of events led by specific kinases and diphosphomevalonate decarboxylase transforms MVA into isopentenyl pyrophosphate (IPP). Eventually, three IPP molecules are added to DMAPP, one after the other, until IPP is isomerized to dimethylallyl pyrophosphate (DMAPP). These processes result in the C20 geranylgeranyl pyrophosphate molecule (GGPP), categorized as a prenyltransferase reaction. Phytoene synthase (CRTYB) joins two molecules, leading to

the formation of phytoene. The following reactions are classified as phytoene desaturase (CRTI). The participation of phytoene desaturase forms neurosporene. Neurosporene is converted to lycopene or β-zeacarotene. The γ-carotene synthesis is prioritized by the lycopene cycle or the dehydrogenation of β-zecarotene. The γ-carotene is the precursor of the β-carotene and torulene, while torulene produces torularhodin by hydroxylation and oxidation [16].

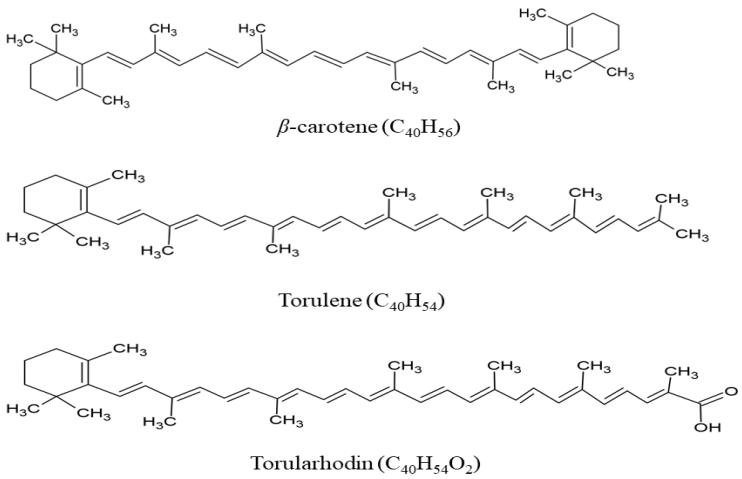

Figure 1. Structural formulas of several different kinds of carotenoids in the yeast genus *Rhodotorula*.

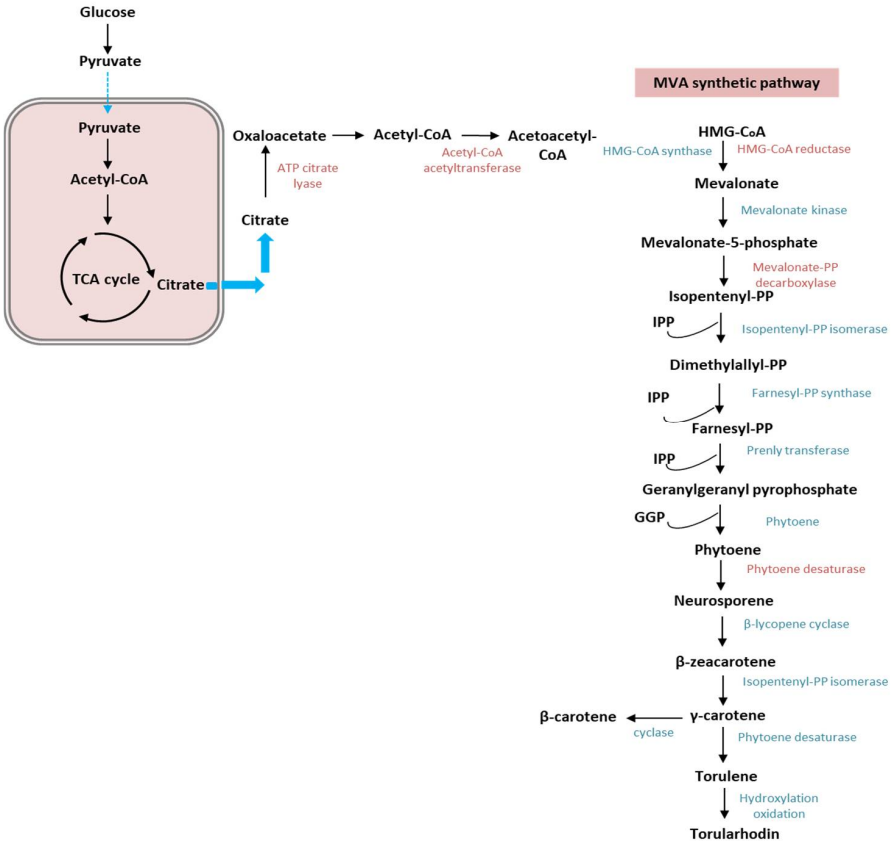

Figure 2. Metabolic pathways related to carotenoid biosynthesis in *Rhodotorula*. Acetyl-CoA, synthesized in the tricarboxylic acid (TCA) cycle, serves as the metabolic intermediary for the biosynthesis of carotenoids through the MVA pathway. The substrates and metabolites are connected by arrows, while the enzymes that catalyze the reaction are indicated in blue and red.

3. Fermentation Factors Influence the Production of Carotenoids

Rhodotorula has certain advantages in obtaining biomolecules of interest, such as carotenes. It grows rapidly and produces high cell densities with a high product content. It uses various economical and renewable substrates, such as lignocellulosic hydrolysates, organic industrial waste, and vegetable mandi waste. It has optimal growth at low pH, which makes it possible to reduce bacterial growth [1]. However, it is crucial to consider certain factors that can significantly influence the fermentation process (Figure 3). The selection of the type of bioreactors can influence the production process of biomolecules of industrial interest, such as carotene. Depending on the type and mode of operation, the temperature, pH, aeration, agitation, nutrient supply, etc., must be appropriately selected to ensure higher productivity with economical production [24].

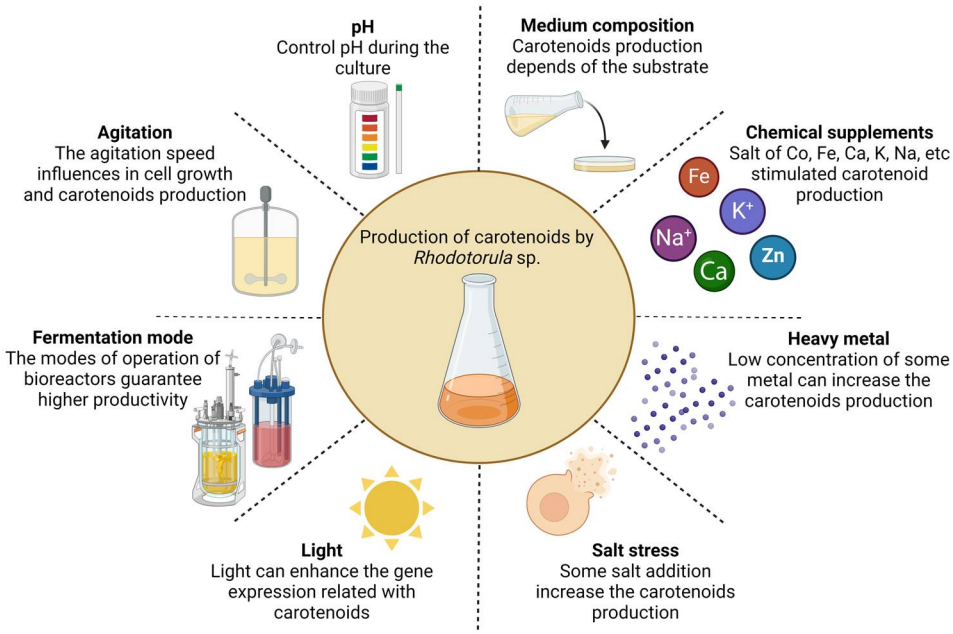

Figure 3. Factors that influence the production of carotenoids by *Rhodotorula* sp.

3.1. Medium Composition

The type and composition of substrate directly impact pigment production and the cost of biotechnological processes. Substrates composed of sucrose and glucose are the most reported carbon sources in the bioproduction of carotenoids [25]. The carotenoid formation in this oleaginous yeast is stimulated in with a low C/N ratio; otherwise, a high C/N ratio causes higher lipid production than carotenoids [26]. In *R. toruloides*, when the C/N ratio increases, cell proliferation decreases due to the gradual depletion of the nitrogen source [27]. Cells convert β-carotene into other carotenoids at high C/N ratios. Lopes et al. [28] reported the change from C/N = 80 to C/N = 120 of β-carotene, torularhodin, and torulene produced by *R. toruloides*. *R. kratochvilovae* Y-42 produced up to 2.59 mg/L of carotenoids in a glucose-based medium at C/N = 80 [29]. The production capacity of torularhodin (63%, 50.5 3.0 g/g) from *R. mucilaginosa* was evaluated in a media containing xylose and glycerol. In this case, the highest carotenoid content was 121.3 µg/g [30]. *R. glutinis* JMT 21978 accumulated 1.6 mg/g of cellular carotenoid with 30% torulene when it was cultured in a medium containing glucose and yeast extract (C/N ratio 50:1) [31]. Mata-Gómez et al. [32] showed that when the C/N ratio is increased up to a relation of 17:1, both the growth and production of carotenoids in *R. glutinis* P4M422 is improved. The highest carotenoid yield depends on the optimal C/N ratio, the *Rhodotorula* sp. strain, and the types of carbon and nitrogen sources.

Recent research has shown that diverse agro-industrial wastes can be used to produce low-cost carotenoids in red yeasts as part of waste valorization efforts. Singh et al. [33]

found that *R. toruloides* can produce up to 62 mg/L β-carotene from a vegetable market waste (Mandi) medium including glucose, xylose, and glycerol. Ghilardi et al. [34] found that using olive mill waste (Alperujo) water as a growth medium for *R. mucilaginosa* resulted in total carotenoid production of 7.3 mg/L, with the majority being torulene and torularhodin. Rodrigues et al. [35] demonstrated that fed-batch fermentation of *R. mucilaginosa* CCT 7688 in sugar cane molasses and corn steep liquor using various feeding techniques resulted in β-carotene production (3.7 mg/L). Various raw materials obtained from the agro-food industry have been classified as promising substrates to grow red yeasts and produce carotenoids, considering their high nutrient availability, low cost, and feasibility. Summarized information is available in Table 1.

Table 1. Carotenoids produced from agroindustry waste of different strains of *Rhodotorula*.

Yeast Strain	Sustrate	Carotenoid	Fermentation	Reference
R. glutinis LOCKR13	Potato wastewater/glycerol	Total carotenoids (3.4–3.7 mg/L)	Batch 28 °C, 200 rpm, pH 4.0–7.072 h	[36]
R. toruloides ATCC 204091	Vegetable market waste (Mandi)-derived medium containing glucose, xylose, and glycerol.	β-carotene (62 mg/L)	Batch 100 h	[33]
R. toruloides NCYC 921	Carob pulp syrup	Total carotenoids (0.42 mg/g)	Fed-batch 30 °C	[37]
R. glutinis	Cassava wastewater	Total carotenoids (167 µg/g)	Batch 30 °C, 200 rpm 120 h	[38]
R. mucilaginosa	Sisal bagasse hydrolyzate	Total carotenoids (1.13 g/L)	Batch 22 °C, 200 rpm 96 h, pH 7	[39]
R. mucilaginosa CCT3892	Hydrolyzed sugarcane molasses at 40 g/L	Total carotenoids (53.0 µg/g)	Batch 30 °C, pH 6.49 200 rpm, 120 h	[10]
R. mucilaginosa	Olive mill waste (Alperujo)	Total carotenoids (7.3 mg/L)	Batch 30 °C, pH 5.15–5.39 150 rpm, 144 h	[34]
R. gracilis ATCC 10788	Potato wastewater/glycerol	Total carotenoid (6.24 mg/L) 47% β-carotene 51% torulene	Batch 20 °C/28 °C 120 h, 300 rpm	[11]
R. toruloides M18	Tea waste hydrolysate	β-carotene (10.08 mg/g)torularhodin (481.9 µg/g) torulene (501 µg/g)	Batch 30 °C pH 6.0, 200 rpm,120 h	[40]
R. mucilaginosa MTCC-1403	Onion peel powder and mung bean husks	Total carotenoids (819.2 µg/g)	Batch 25.8 °C, pH 6 119.6 rpm, 84 h	[9]
R. mucilaginosa	Sisal bagasse hydrolyzate	Total carotenoids (1.13 g/L)	Bach 22.0 °C pH 7.0	[39]
R. toruloides DSM 4444	Camelina sativa meal hydrolysates Flower of Calendula officinalis, Zea mays seed flour, potato seed flour	β-Carotene (5.5 mg/L, 12.6 mg/L, 16.0 mg/L)	Batch 30 °C, 160 rpm	[41]
R. mucilaginosa IST 390 *R. toruloides* PYCC 5615	Sugar beet pulp hydrolysates	Total carotenoids (1.4 mg/L) (5.4 mg/L)	Batch 30 °C, pH 5 250 rpm, 150 h	[42]

Table 1. *Cont.*

Yeast Strain	Sustrate	Carotenoid	Fermentation	Reference
R. mucilaginosa CCT7688	Sugarcane molasses/corn steep liquor	Total carotenoids (121.4 µg/g)	Batch/fed-batch 25 °C, pH 6.0 180 rpm, 144 h	[43]
R. toruloides NRRL Y-1091	Wheat straw hydrolysate	Total carotenoids (14.09 mg/L)	Batch 30 °C, 250 rpm, 72 h	[15]
R. toruloides ATCC 204091	Lignocellulosic waste hydrolysate	Total carotenoids (19 mg/L)	Batch 25 °C 120 rpm	[33]
R. mucilaginosa CCT 3892	Sisal bagasse hydrolyzate	Total carotenoids (223.5 µg/g)	Batch 28 °C, pH 6.0 150 rpm, 120 h	[44]
R. mucilaginosa URM 7409	Sugarcane molasses/cassava wastewater	Total carotenoids (168.08 µg/g)	Batch 25 °C, pH 6.0 130 rpm	[45]
R. mucilaginosa ANL-001L	Banana peel extract	Total carotenoids (317 µg/g)	Batch 28 °C 300 rpm, 144 h	[46]
R. glutinis CCT-2186	Sugarcane Bagasse Hydrolysate	β-carotene (118.6 mg/L)	Batch 72 h	[13]
R. glutinis	Manipueira	Total carotenoids (1410 µg/g)	Batch 35 °C, pH 5.0 150 rpm	[47]
R. toruloides *R. kratochvilovae*	Coffee oil/waste glycerol.	Total carotenoids (10.51 mg/g) (10.75 mg/g)	Fed-batch 25 °C, pH 6.5 60 rpm, 168 h	[48]
R. mucilaginosa	Artichoke Agro-industrial Waste	Total carotenoids (1228.53 µg/g)	Batch pH 5, 120 rpm and 30 °C for 72 h	[49]
R. glutinis	Olive mill wastewater	Total carotenoid (192.09 µg/g) torulene 85% torularhodin 80% β-carotene 39.45%	Batch	[12]
R. toruloides KP324973	Corn steep liquor	Total carotenoid (12.31 mg/g/h)	Batch/fed-batch 11.7 °C, pH 6.1	[50]
R. glutinis P4M422	Hydrolyzed goat milk whey	Total carotenoid (4075 µg/L)	Batch 30 °C, pH 4.5 200 rpm, 72 h	[32]
R. kratochvilovae Y-42	Cheese whey/fermented wheat bran	Total carotenoids (36.4 mg/L) 97% lycopene	Batch	[7]

3.2. Temperature

The incubation temperature is the main factor in producing biomass and carotenoids, and depends on the type of microorganism. Generally, the ideal temperature ranges for carotenogenesis differ from those for cell growth. Low temperature typically does not contribute to cell growth or carotenoid production, while high temperatures can denature the enzymes necessary for carotenogenesis and inhibit cell growth [51].

The best temperature for obtaining the highest biomass and carotenoid production is around 20–30 °C. Above 30 °C, the synthesis of carotenoids decreases, with 25 °C being the ideal temperature for the maximum synthesis of these pigments [39,52]. Kot et al. [53] reported a high increment in carotene biosynthesis by *R. gracilis* (360.4 µg/g), *R. glutinis* (280.3 µg/g), and *R. mucilaginosa* (150 µg/g) at 20 °C. Sharma & Ghoshal [9] optimized the production of carotenoids by *R. mucilaginosa* using agro-industrial wastes at 25.8 °C as the ideal temperature. Da Silva et al. [39] obtained a maximum carotenoid content

(1.13 g/L) with by *R. mucilaginosa* at 22 °C, while the lowest carotenoid (0.34 g/L) was obtained at 34 °C. Allahkarami et al. [44] indicated the increment in temperature (up to 28 °C) is accompanied by rising carotenoid formation (2.43 mg/L) by *R. mucilaginosa* while it was reduced above 35 °C (0.60 mg/L). Maia et al. [47] demonstrated that *R. glutinis* produced carotenoids at 35 °C. Temperature directly influences the carotenogenic pathways, thus guaranteeing their optimization by regulating the enzymatic activity and the concentration of the reactions they catalyze. However, this effect varies depending on the strain, the environmental parameters, and the composition of the medium.

3.3. The pH

The pH of the medium was changed according to the yeast growth and carotenoid production. During the synthesis of carotenoids in the fermentation processes, there is a natural change in pH in the culture medium, depending on the growth phase of the yeast. In general, pH decreases during the first 72 h; then, pH values increase because of the intensive carotenogenesis phase [51]. In general, *Rhodotorula* sp. prefers more acidic pH values; however, at shallow pH values, yeast growth is inhibited, resulting in the reduction of carotenoid production. Alkaline pH has been suggested to act as a stressor and alter metabolic rates and nutrient uptake, inducing glucose cellular metabolism and enhancing polysaccharide synthesis instead of carotenoids. Xie et al. [26] proposed a strategy to increase carotenoids using two stages of fermentation with changes in pH: a first stage with a pH that favors growth and a second stage with a decrease in pH that stimulates the biosynthesis of carotenoids. The highest production of carotenoids in Rhodotorulas sp. has been reported in the pH ranges 5.0–6.0. At low pH, an increase of catalase and superoxide dismutase enzymes has been demonstrated in response to the induction of oxidative stress [7]. Da Silva et al. [39] reported that yeast growth was slow at a pH of approximately 5.0 and that carotene production was favored at this pH. Allahkarami et al. [44] reported an optimal pH around 6.0 for highest carotenoid production (3.3 mg/L). Similar optimal pH values (pH 5.5) were reported for carotenoid production of β-carotene by Rhodotorula sp. RY1801, R. acheniorum, and R. mucilaginosa [52,54]. Dias et al. [55] determined the optimal pH values (pH 5.0) for carotenoid production by R. toruloides NCYC 921. Sharma & Ghoshal [9] reported that the maximum carotenoid yield (63.37 µg/g) was obtained at pH 6.1 in R. mucilaginosa. Keskin et al. [12] demonstrated that the total carotenoid yield (192.09 ± 0.16 µg/g) in R. glutinis increased significantly at low pH, high temperature, and lighting, favoring high production of β -carotene and torulene; however, torularhodin was produced at high pH and low temperature conditions. Each type of microorganism has an optimal pH value for growth, and carotenoid biosynthesis depends on this factor because it is a secondary metabolite [56].

3.4. Agitation

Agitation speed influences cell growth and carotenoid production by enhancing the mass transfer of oxygen and other nutrients to aerobic microbial cells, stimulating enzymes such as phytoene desaturase, β-carotene hydrolase, and lycopene cyclase [57]. A high aeration rate increases yeast's specific growth and total carotenoid production [24]. *R. mucilaginosa* MTCC-1403 produced the highest carotenoids in aerobic fermentation conditions (819.23 mg/g), compared to control (717.35 mg/g) [9]. Ribeiro et al. [58] demonstrated that the highest carotenoid yields were achieved at high agitation rates (700 rpm) by *R. toruloides*. Aeration produces a concentration gradient, which may play an important role in fermentation efficiency. The increased diffusion method makes it easier for yeast cells to absorb sources in their medium while also removing gasses and other byproducts throughout the cultivation process.

3.5. Light

Light also influences carotenoid production. Until now, a two-phase theory of photoinduction in carotenoid production has been described. The first phase relates to the

stimulation effect light induces on microbial growth. The second considers the accumulation of carotenoids in the cell as associated with the increase in the carotenogenic enzyme activity [51]. Carotenoids act as antioxidants that protect cells from light and other environmental stresses. Under light pressure, *Rhodotorula* also increase carotenoid production, which is considered a photoprotective response mechanism [59].

As such, the carotenoid content produced by *Rhodotorula* sp. grown under light irradiation has been more significant than those cultivated in dark conditions. Light-emitting diodes (LED) have been used to improve the production of target compounds, such as carotenoids. Cells grown under white-light LEDs produced more carotenoids than cells grown without LEDs [60]. Kot et al. [53] demonstrated that white-light irradiation stimulated the biosynthesis of torularhodin in *R. glutinis* and *R. mucilaginosa*; however, it increased torulene synthesis in *R. gracilis*. Pinheiro et al. [61] reported that light irradiation produced a carotenoid yield greater than 70% from *R. toruloides*. Another study demonstrated that *R. mucilaginosa* AJB01 increased carotenoid production (118.3 μg/g) when exposed to UV radiation for 1.5 min and a photoperiod of 18:6 h light:dark [62]. Rodrigues et al. [63] demonstrated that LEDs increased the production of carotenoids (β-carotene and astaxanthin) in *R. mucilaginosa* CCT 7688. In this research, authors confirmed that green LEDs (3473 μg/L) and red LEDs (3497 μg/L) increased carotenoid production by 93.5 and 94.9%, respectively. Gao et al. [64] reported that *R. toruloides* Z11 showed higher carotenoid content (1.29 mg/g) when exposed to light for 12 h daily. Production was increased by 1.98 times compared to dark cultivation. Transcriptome profiling demonstrated that light stress may effectively boost the gene expression levels of GGPS1 and AL1 in the carotenoid biosynthesis pathway. It has been shown that carotenoid biosynthesis is regulated by irradiation at the transcriptional level. Transcription levels of the carotenogenic genes *CAR1* and *CAR2* in cultured cells under light conditions increased 2–3 times more than those of cells cultured in dark conditions [65]. However, the effect of light on carotenoid production is a complex network mechanism that includes several regulatory pathways, and more studies are needed to elucidate this mechanism at the genetic level to and optimize the carotenoid production process in yeasts.

3.6. Heavy Metal Ions and Salts

Some heavy metal ions have been studied for their effect on carotenoid biosynthesis in *Rhodotorula* species [4]. In general, they can limit the development of yeast by increasing intracellular oxidation levels [66]. However, studies conducted on *R. toruloides* MH023518 in the presence of ZnO nanoparticles demonstrated increased carotenes production (297.8 ± 0.8 mg/L). The authors showed an increase in the enzymatic activity of catalase and superoxide dismutase in the presence of the nanoparticles [67]. Studies have also showed that a low concentration of Cd^{2+} (5.0 μmol/L) improves yeast growth, increasing the production of carotenoids [26]. *R. mucilaginosa* in the presence of Pb increased its growth and production of carotenes, specifically β-carotene [68]. Carotenoids, as part of a cell's non-enzymatic protection system, collaborate with the enzymatic protection system to maintain the balance of intracellular components and redox status when organisms are exposed to oxidative stress [69].

The effect of salts on carotenoid biosynthesis has been studied. It has been shown that Na^+, K^+, Zn^{2+}, Al^{3+}, etc., stimulate carotenoid production. Elfeky et al. [31] reported that the addition of 0.7 mM $Al_2(SO_4)_3$ to an optimized medium increased the total carotenoid content of *R. glutinis* (2.2 mg/L and 212.9 μg/g), producing around 2.16 mg/L torulene (98%). Supplementing *R. toruloides* cultures with 0.1 M K+ and 1.0 M Na+ increased β-carotene production by 60% [70]. In the study conducted by Li et al. [71], it was reported that *R. glutinis* ZHK exhibited an increase in total carotenoids when subjected to 0.5 M (3.91 mg/L) and 0.75 M (5.41 mg/L) NaCl treatments, and an increase in torulene and torularhodin production was identified. Transcriptome profiling demonstrated that salt stress efficiently promotes the gene expression of CAR1 and CRTYB, which are respon-

sible for the coding of phytoene dehydrogenase and lycopene cyclase like CAR1 and CAR2, respectively.

4. Genetic Engineering to Improve Carotenoid Production

Genetic engineering and molecular biology strategies can improve carotenoid production in yeast by manipulating gene expression in metabolic pathways (Table 2). Among these strategies is redesigning the metabolic pathways to produce key intermediates for specific carotenoid compounds and blocking the synthesis of an unwanted secondary product [72]. Physical and chemical factors mediate mutagenesis to introduce random mutations into the genome. They are genetic manipulation tools used to increase yeast pigment production. The disadvantage of random mutagenesis is that the mutants are unstable, and, in some cases, targeted mutagenesis also does not produce mutants desirable to overproduce a specific carotenoid component [24]. Some authors have combined the use of physical mutagenesis mediated by atmospheric room temperature plasma (ARTP) followed by chemical mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine (NTG) to improve the production of total carotenoids from *R. toruloides* NP11 [73].

Genetic modification and metabolic pathways that lead to the formation of products are among the most used tools of recombinant DNA technology. This technique has several advantages, including producing carotenogenic pathways in rapidly multiplying non-carotenogenic strains [24]. However, the key challenge is to have stable strains that can be easily selected over the wild type. An agrobacterium-mediated transformation method (AMT) was developed to improve transformation efficiency in *R. toruloides*. This method allows multiple genes to be integrated into the *R. toruloides* chromosome simultaneously, using genetic elements such as strong promoters (e.g., GPDp and PGKp), plasmid vectors, and antibiotic resistance markers [74].

Lin et al. [75] used the genetic transformation of *Agrobacterium tumefaciens* to randomly insert the transfer DNA (T-DNA) containing hygromycin-resistant cassettes into the genome of *R. toruloides* NP11. The authors reported a 2.4-fold increase in gamma-carotene, a 9-fold increase in torulene, and a 1.7-fold increase in beta-carotene compared to the wild-type strain.

Table 2. Yeast strains genetically modified to improve carotenoid production.

Yeast Strains	Genetic Modification	Carotenoid	Reference
R. gracilis	Genomic mutagenesis (Zeocin as a mutagen)	Carotenoids	[76]
R. mucilaginosa C2.5t1	T-DNA insertional mutagenesis	Carotenoids	[77]
R. toruloides	Genetic manipulation methods based on Agrobacterium-mediated transformation (ATMT)	Torularhodin and β-carotene	[78]
R. toruloides	Ultraviolet (UV) and gamma irradiation mutagenesis	Astaxanthin	[79]
R. glutinis P4-10-9–63Y-14B	Overexpression of truncated HMG1 (tHMG1) from *K. marxianus*	β-carotene	[80]
R. mucilaginosa KC8	Plasma-induced mutagenesis Metabolic engineering	Carotenoids	[81]
R. glutinis	UV irradiation	Carotenoids	[82]
R. toruloides M18	ARTP mutagenesis	β-carotene, Torulene Torularhodin	[40]
R. mucilaginosa A734	UV irradiation	Carotenoids	[83]
R. mucilaginosa JH-R23	New Generation Manned Spacecraft Test Ship	Carotenoids	[84]
R. glutinis NCIM 3353	UV irradiation	β-carotene	[85]
R. toruloides CCT 7815	light irradiation and adaptive laboratory evolution (ALE)	Carotenoids	[61]
R. toruloides CCT 0783	Golden Gate DNA assembly system (RtGGA).	Carotenoids	[86]

5. Submerged Fermentation Strategies

Batch operation improves the control of substrate concentration and therefore minimizes substrate inhibition. The most practical application of fed-batch operation to produce carotenoids is maintaining a constant feeding rate, which is simple and aligns well with the nature of carotenoid production in cells. Carotenoid biosynthesis in yeast cells begins in the late log phase and continues into the stationary phase. Therefore, the fed-batch mode can reliably provide nutritional supplements during the stationary phase [50]. For example, studies carried out on *R. mucilaginosa* demonstrated that fed-batch cultivation feeding with crude glycerol achieved a 2-fold increase in carotenoid production compared to batch culture [87]. Rodrigues et al. [35] reported that carotenoid production from *R. mucilaginosa* grown in an agro-industrial environment also had a 12-fold increase in carotenoid production in a fed-batch fermentation setup compared to batch fermentation. Fallahi et al. [50] studied the effect of various fermentation systems on carotenoid production in *R. toruloides* KP324973. They compared the systems in batch culture mode and fed-batch culture in a bubble column reactor. The authors reported an increase in carotenoid production of 42% in fed-batch culture. Sriphuttha et al. [88] demonstrated a 12-fold increase in carotene production with the fed-batch strategy compared to batch.

6. Fermentation by Adhesion to Magnetic Nanoparticles

The use of magnetic nanoparticles in yeast fermentations holds promise for enhancing the production of metabolites of interest. The binding of nanoparticles to the surface of cells is attributed to various forces, including electrostatic forces, hydrogen bonds, hydrophobic interactions, and van der Waals forces [89]. Alonso-Estrada et al. [90] investigated these interactions by fermenting the yeast *R. toruloides* with manganese ferrite nanoparticles coated with chitosan. They demonstrated that the nanoparticles adhered to the cell surface without adversely affecting the growth and viability of *R. toruloides*. Taghizadeh et al. [91] immobilized recombinant *Pichia pastoris* cells on magnetic magnetite nanoparticles coated with aminopropyltriethoxy silane, and also found that magnetic immobilization did not affect cell growth or viability during three cycles of reuse of the immobilized cells. A study was carried out in a magnetically assisted bioreactor, with the addition of Fe_3O_4 nanoparticles, to produce ethanol using the yeast *Saccharomyces cerevisiae*, demonstrating that the maximum specific growth rate showed a positive effect in this magnetically assisted bioprocess [92]. Núñez Caraballo et al. [93] demonstrated that ethanol production by *S. cerevisiae* immobilized on chitosan-coated manganese ferrite increased biomass in the presence of nanoparticles. On the other hand, Firoozi et al. [94] produced ethanol from molasses as a carbon source using *S. cerevisiae* immobilized on L-lysine-coated Fe_3O_4 nanoparticles. They demonstrated that the magnetically immobilized yeast was quickly separated from the culture media with an appropriate magnetic field. Decoration of *S. cerevisiae* cells with L-lysine-coated nanoparticles showed no adverse effect on yeast cell metabolism. The use of polymer-coated magnetic nanoparticles in fermentation processes by surface adhesion to yeast cells is promising for producing metabolites such as enzymes, lipids, and ethanol. This approach facilitates the transport of metabolites through membrane permeability and allows for cell immobilization using an external magnetic field, which can be reused in multiple fermentation cycles, thus reducing costs in biotechnological processes. This analysis highlights the potential for further research on the impact of magnetic nanoparticles on yeast fermentation, particularly in increasing carotene production. It is important to determine optimal nanoparticle concentrations that stimulate carotene production without compromising cell growth and viability in defined fermentation processes.

7. Conclusions

Carotenoids are playing an increasingly important role in human health. The nutritional value of carotenoids has been known for many years, as well as their antioxidant properties and their effectiveness in preventing certain human diseases. Considering the carotenoid biosynthesis capacity of *Rhodotorula*, there is great potential for its application

in medical and industrial production. It is essential to control fermentation methods and conditions to efficiently produce microbial carotenes. Since carotenoids are secondary metabolites, it is crucial to ensure optimal growth in the first stage of cultivation. It may be that certain pH and C/N ratios are not optimal for growth, but are optimal for carotenoid biosynthesis. For this reason, fermentation strategies that involve gradual increases may be ideal for controlling pH and C/N ratios in stages. Fed-batch fermentation has the advantage of reducing substrate inhibition, in addition to providing a constant supply in the stationary phase, which can be guaranteed by a constant flow for carotene formation. Currently, neither torulene nor torularhodin are produced industrially. However, due to its valuable properties, new strains of *Rhodotorula* are sought and yeast mutation methods can be applied that provide high performance and reduce production costs for future applications.

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