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Optimization of Culture Conditions for Carotenoid Production by Rhodotorula mucilaginosa Isolated from Mangrove Sediments

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Abstract

Rhodotorula mucilaginosa is a carotenoid-producing yeast with high potential for biotechnological and industrial applications, particularly in the fields of pharmaceuticals, cosmetics, food additives, and animal feed, due to the antioxidant, immunomodulatory, and pigment properties of carotenoids. This yeast species is commonly found in diverse ecosystems, including both aquatic and terrestrial habitats. It is recognized for its ability to assimilate a wide range of carbon sources, making it an attractive candidate for cost-effective bioprocesses. Cultivation conditions are known to significantly influence carotenoid biosynthesis and overall productivity. This study aimed to optimize the cultivation parameters for carotenoid production by *R. mucilaginosa* SMY40, a strain isolated from mangrove sediments in Chonburi province, Thailand. Several factors were systematically varied, including glucose concentration, nitrogen source, initial pH, and incubation time. The optimal conditions identified were 15 g/L glucose as the carbon source, ammonium sulfate as the nitrogen source, an initial pH of 5.0, and incubation under shaking conditions at 150 rpm at room temperature for 120 hours. Under these optimized conditions, the highest biomass (dry cell weight) and carotenoid production were 4.26 ± 0.03 g/L and 41.98 ± 0.93 µg/g wet weight, respectively. Additionally, glucose consumption reached 13.36 ± 0.11 g/L. These results suggest that *R. mucilaginosa* SMY40 exhibits strong potential for industrial-scale carotenoid production under economically viable conditions. The high efficiency of conversion and stability of pigment synthesis under moderate culture conditions highlight its applicability for future development in microbial pigment biotechnology.

Keywords: *Rhodotorula mucilaginosa*, Carotenoid, Optimization, Mangrove sediment

Introduction

Carotenoids are pigments found in animals, plants, and microorganisms, exhibiting a color range from yellow to red. They are classified into two major groups: the carotene group, which includes β-carotene, α-carotene, and lycopene, and the xanthophyll group, which comprises astaxanthin, β-cryptoxanthin, canthaxanthin, capsanthin, fucoxanthin, lutein, and zeaxanthin[1]. Carotenoids possess antioxidant, anticancer, and therapeutic properties. Additionally, they serve as precursors for vitamin A (provitamin A)[2]. Due to these properties, carotenoids are widely utilized as food colorants and food additives in various industries, including food production, medicine, pharmaceuticals, cosmetics, and animal feed. Consequently, the industrial demand for carotenoids has significantly increased.

Microorganisms have emerged as an important alternative source for carotenoid production, offering advantages such as lower space requirements for cultivation, scalable production capacity, rapid biosynthesis within a limited time frame, and ease of optimizing culture conditions to maximize yield compared to plant-derived carotenoids[3]. Carotenoid-producing microorganisms include filamentous fungi, yeasts, certain bacterial species, algae, and lichens. Some microbial species have already been commercialized for carotenoid production, such as the alga *Dunaliella* sp. and the yeasts *Phaffia rhodozyma* and *Rhodotorula glutinis*[2].

Yeasts are promising microorganisms for carotenoid production. Carotenogenic yeasts, capable of synthesizing carotenoids, belong to various genera, including *Rhodotorula*, *Sporobolomyces*, *Rhodosporium*, and *Cryptococcus*. Among them, *Rhodotorula* sp. has been reported to produce β-carotene at levels reaching up to 70% of the total carotenoid content[4]. Gupta et al.[5] highlighted that carotenoids such as β-carotene, lycopene, and astaxanthin derived from yeasts have been commercialized as dietary supplements.

Rhodotorula mucilaginosa is classified as a highly carotenoid-producing yeast species, commonly found in both aquatic and terrestrial environments. The major carotenoids produced by *R. mucilaginosa* include β-carotene, torulene, and torularhodin[6]. The composition and yield of carotenoids synthesized by *R. mucilaginosa* are influenced by environmental and cultivation conditions[7]. Rodrigues et al.[8] reported that *R. mucilaginosa* can utilize diverse carbon sources, including agricultural and industrial waste such as rice-steaming wastewater, crude glycerol, sugarcane molasses, and corn molasses. Understanding the factors influencing carotenoid biosynthesis

in *R. mucilaginosa* is essential for optimizing large-scale production. Culture conditions such as carbon source, nitrogen source, pH, temperature, and oxygen availability significantly affect both the yield and composition of carotenoids[7]. Among these factors, the choice of carbon source plays a critical role, as it provides not only energy but also carbon skeletons for carotenoid synthesis[9]. Similarly, the nitrogen source influences primary metabolism and can act as a regulatory factor for secondary metabolite production. Inorganic nitrogen sources such as ammonium sulfate have been reported to support higher carotenoid yields compared to organic sources, likely due to their influence on intracellular C:N balance and the regulation of biosynthetic gene expression[10]. The initial pH of the culture medium also modulates enzyme activity and membrane transport systems. *R. mucilaginosa* typically exhibits optimal carotenoid production under mildly acidic conditions, particularly at pH 5.0, which supports favorable redox balance and precursor availability[11]. Furthermore, temperature and oxygen availability are essential for maintaining cellular respiration and redox balance. Carotenoid biosynthesis is an oxygen-dependent process that involves several oxidative enzymes; thus, aerobic conditions are critical. Agitation and sufficient aeration enhance oxygen transfer and improve carotenoid yields[12]. Several studies have reported optimal conditions for carotenoid production by *Rhodotorula mucilaginosa*. For instance, Sharma and Ghoshal[13] demonstrated that *R. mucilaginosa* (MTCC-1403) possesses high carotenoid-producing potential when cultivated on agro-industrial waste substrates such as onion peels, potato peels, green gram husks, and pea pods. The optimal cultivation conditions for growth and carotenoid synthesis were determined to be at pH 6.1, a temperature of 25.8 °C, and an agitation speed of 119.6 rpm. Similarly, Rodríguez et al.[14] identified that the highest carotenoid production by *R. mucilaginosa* was achieved when cultured on artichoke agro-industrial waste at pH 5.0, 30 °C, 120 rpm, and an incubation time of 72 hours. In another study, Elasanhoty et al.[15] reported that *R. mucilaginosa* exhibited efficient growth and carotenoid production when cultivated in a synthetic medium containing glucose as the carbon source and peptone as the nitrogen source, under conditions of pH 6.0 and a temperature of 28 °C. Therefore, the findings from this study can be applied to the design of industrial-scale carotenoid production processes and may contribute to the development of environmentally

friendly bioprocesses using low-cost alternative raw materials, such as agricultural waste. This approach could help reduce production costs and enhance the competitiveness of bio-based carotenoids in the global market. Given the potential of *R. mucilaginosa* as a carotenoid producer, this study aimed to determine the optimal cultivation conditions for *R. mucilaginosa* isolated from mangrove sediment in Chonburi province, Thailand. This particular isolate was selected for investigation because it was previously obtained from sediment samples and preserved in the laboratory culture collection. Preliminary studies indicated that this strain exhibits notable potential for lipid accumulation, suggesting its applicability for carotenoid and bio-based compound production under optimized cultivation conditions. The results obtained from this research will serve as a fundamental basis for the development of alternative yeast-derived carotenoid production and the industrial-scale application of *R. mucilaginosa* in carotenoid biosynthesis.

Methods

1. Yeast and starter preparation

The yeast *Rhodotorula mucilaginosa* SMY40 was isolated from mangrove sediment collected in Bang Sai subdistrict, Mueang Chonburi district, Chonburi province, Thailand. The isolate was maintained on slant YPD (Yeast extract–Peptone–Dextrose) agar at 4 °C. A single loopful of the yeast colony was inoculated into 50 mL of liquid YPD medium contained in a 250-mL Erlenmeyer flask and incubated at room temperature (approximately 28–30 °C) for 48 hours in a rotary shaker at 150 rpm[16].

2 . Optimization of growth and carotenoid production

A 5-mL aliquot of the seed culture (10% v/v) was inoculated into 45 mL of production medium in a 250-mL Erlenmeyer flask. The medium contained the following components (per liter): glucose, 15 g; yeast extract, 2 g; malt extract, 1 g; ammonium sulfate [$(NH_4)_2SO_4$], 1 g; potassium phosphate (KH_2PO_4), 1 g; and magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), 0.25 g[2]. Subsequently, the carbon source was varied by replacing glucose with sucrose and xylose, and the concentration of each carbon source was adjusted to five levels: 10, 15, 20, 25, and 30 g/L. The nitrogen source was also modified by replacing ammonium sulfate with alternative nitrogen sources including ammonium chloride (NH_4Cl), urea (CH_4N_2O), and potassium nitrate (KNO_3). The initial pH of the medium was adjusted to 3.0, 5.0, 7.0, and 9.0. Additionally, the cultivation time was varied

at 96, 120, 144, and 168 hours. Cultivation was conducted under agitation at 150 rpm at room temperature. Yeast growth was monitored every 24 hours by measuring the optical density at 600 nm (OD_{600}). The residual sugar concentration was analyzed, and the cells were harvested to determine dry biomass and carotenoid content produced by the yeast. The conversion factors determined as the amount of biomass produced per unit weight of substrate consumed ($Y_{X/S}$), the amount of carotenoids produced per unit of dry weight of cells ($Y_{P/X}$), and the amount of carotenoids produced per unit weight of substrate consumed ($Y_{P/S}$), were calculated using the following equations:

$$Y_{X/S} = \frac{X}{S}; \qquad Y_{P/X} = \frac{P}{X}; \qquad Y_{P/S} = \frac{P}{S}$$

where: Y = conversion factor; X = biomass (g/L); S = substrate (glucose)(g/L); P = product (carotenoid)(µg/g).

3. Analysis

The supernatant obtained after yeast cell separation was analyzed for residual sugar content using the 3,5-dinitrosalicylic acid (DNS) method[17]. The absorbance was measured at a wavelength of 540 nm (OD_{540}) (U-2900, Hitachi) and the residual reducing sugar concentration was calculated by comparing the absorbance values to a standard glucose calibration curve[10]. A 10-mL aliquot of yeast cell suspension was centrifuged (Centrifuge 5804 R, Eppendorf) at 6,000 rpm for 20 minutes at 4 °C. The resulting cell pellet was washed three times with sterile distilled water. The washed cell pellet was resuspended in sterile distilled water and transferred to a pre-weighed evaporation dish. The dish was dried (UNE – Memmert) at 80 °C until a constant weight was achieved. The dry weight of the yeast biomass was recorded and used to calculate the dry biomass yield[18]. A 30-mL sample of yeast cell suspension was centrifuged at 6,000 rpm for 20 minutes at 4 °C. The supernatant was discarded, and the cell pellet was washed three times with sterile distilled water. The wet weight of the yeast cell pellet was recorded prior to carotenoid extraction. Carotenoids were extracted using a slightly modified method of Passorn et al.[19] The extraction was carried out by mixing

(Vortex Mixer Genie, Scientific Industries) the cell pellet with a solution consisting of petroleum ether, 1 M sodium chloride, and acetone in a 1:1:1 (v/v/v) ratio in an amber bottle. Cell disruption was performed using a sonicator bath (Elmasnic S60/H, Elma) operating at 50/60 Hz for 30 minutes. The mixture was subsequently centrifuged at 4,000 rpm for 5 minutes. The upper orange-colored layer (petroleum ether phase) was collected, and its absorbance was measured at 474 nm. Carotenoid content was quantified using an extinction coefficient (E_1%/1 cm) of 2,100, corresponding to astaxanthin in petroleum ether at a concentration of 1% (w/v). The results were recorded and used to calculate the total carotenoid content as equation;

$$\text{Carotenoid (ug/g)} = \frac{\text{(Volume of Petroleum ether)(A474)(10,000)}}{\text{(2100)(dry biomass)}}$$

All experiments were performed in three replicates, and all data were analyzed statistically using the mean, standard deviation (SD), one-way ANOVA, and Tukey HSD tests by Minitab for Windows, version 18.

Results and Discussion

1. Optimal carbon sources for growth and carotenoid production of *R. mucilaginosa* SMY40

R. mucilaginosa SMY40 exhibited the highest biomass production and carotenoid accumulation when cultivated with glucose as the sole carbon source (Table 1). The biomass yield and carotenoid content obtained were 4.21 ± 0.12 g/L and 40.24 ± 0.81 µg/g wet weight, respectively. These values were significantly higher ($P < 0.05$) than those obtained with maltose (2.87 ± 0.15 g/L; 28.13 ± 0.78 µg/g wet weight) and xylose (1.39 ± 0.13 g/L; 11.48 ± 0.96 µg/g wet weight). Glucose consumption was also the highest at 13.33 ± 0.26 g/L, followed by maltose (9.52 ± 0.56 g/L) and xylose (5.12 ± 0.69 g/L). Regarding conversion efficiencies, the maximum biomass yield per gram of sugar consumed ($Y_{X/S}$) was observed in the glucose-treated group (0.32 g/g sugar), while maltose and xylose yielded 0.30 and 0.27 g/g sugar, respectively. Similarly, glucose supported the highest carotenoid yield per biomass ($Y_{P/X}$) and per sugar consumed ($Y_{P/S}$), with values of 9.56 µg/g biomass and 3.02 µg/g sugar, respectively.

Table 1. Biomass yield, carotenoid production, sugar consumption and effect of medium composition on the conversion factors ($Y_{X/S}$, $Y_{P/X}$, and $Y_{P/S}$), biomass and carotenoids productivities of *R. mucilagonosa* SM40 cultivated with different carbon sources.

Carbon source	Biomass (g/L)	Carotenoid (g/g wet weight)	Sugar consumed (g/L)	$Y_{X/S}$ (g/g sugar)	$Y_{P/X}$ (ug/g biomass)	$Y_{P/S}$ (ug/g sugar)
Glucose	4.21 ± 0.12^{a}	40.24 ± 0.81^{a}	13.33 ± 0.26^{a}	0.32	9.56	3.02
Maltose	2.87 ± 0.15^{b}	28.13 ± 0.78^{b}	9.52 ± 0.56^{b}	0.30	9.80	2.96
Xylose	1.39 ± 0.13^{c}	11.48 ± 0.96^{c}	5.12 ± 0.69^{c}	0.27	8.29	2.26

Data presented as mean value $\pm$ standard deviation (n = 3).

Values followed by the different letters in the same column indicated significant differences ($P < 0.05$, Turkey's HSD).

The results clearly demonstrate that *R. mucilaginosa* SMY40 responds differentially to various carbon sources in terms of both growth performance and carotenoid biosynthesis. Glucose was the most efficient carbon source, promoting the highest biomass and carotenoid production. These findings are in agreement with previous studies indicating that glucose is readily assimilated by yeasts through facilitated diffusion and is directly channeled into glycolysis, enabling rapid energy generation and precursor supply for biomass formation and secondary metabolite synthesis[9] . In contrast, maltose must first be hydrolyzed by maltase, and xylose requires metabolic conversion via the pentose phosphate pathway, both of which are relatively less efficient and energy-intensive processes[16] . This may account for the comparatively lower biomass and pigment production observed with these carbon sources. The highest conversion efficiencies ($Y_{X/S}$, $Y_{P/X}$, and $Y_{P/S}$) recorded in the glucose condition reflect superior metabolic efficiency in converting available substrate into cellular biomass and carotenoids. A high $Y_{P/S}$ value specifically suggests that glucose was effectively utilized for carotenoid biosynthesis, which proceeds via the mevalonate pathway requiring acetyl-CoA and NADPH as key intermediates generated through glycolysis and the pentose phosphate pathway[16]. These results align with Maldonade et al.[10] reported enhanced carotenoid production in *Rhodotorula* species grown on simple sugar as glucose. The findings highlight the potential of *R. mucilaginosa* SMY40 for efficient carotenoid production using glucose-based media, offering promising implications for biotechnological and industrial applications.

2. Optimal carbon source concentrations for growth and carotenoid production of *R. mucilaginosa* SMY40

The influence of varying carbon source concentrations on biomass production, carotenoid production, and conversion efficiencies by *R. mucilaginosa* SMY40 is summarized in Table 2. The results indicate that increasing carbon concentrations from 10 to 25 g/L led to a corresponding increase in biomass yield, peaking at 25 g/L with a value of 4.41 ± 0.14 g/L. However, no statistically significant difference ($P > 0.05$) was observed between biomass levels at 15, 20, 25, and 30 g/L concentrations. The highest carotenoid content was obtained at 15, 20 and 25 g/L, with values of 42.51 + 0.46 42.50 ± 1.11 and 42.91 ± 1.03 µg/g wet weight, respectively, which were not significantly different ($P > 0.05$). Sugar consumption increased with higher initial carbon concentrations, reaching a maximum of 14.46 ± 0.46 g/L at 20 g/L substrate. However, the biomass yield per sugar consumed ($Y_{X/S}$) decreased from 0.39 g/g at 10 g/L to 0.30 g/g at 30 g/L, indicating reduced conversion efficiency at higher substrate concentrations. Similarly, the carotenoid yield per sugar consumed ($Y_{P/S}$) decreased from 3.88 to 3.01 µg/g sugar, although the carotenoid yield per biomass ($Y_{P/X}$) remained relatively stable, with a slightly increased at 15 g/L glucose as 10.03.

Table 2. Biomass yield, carotenoid production, sugar consumption and effect of medium composition on the conversion factors ($Y_{X/S}$, $Y_{P/X}$ and $Y_{P/S}$), biomass and carotenoids productivities of *R. mucilagonosa* SM40 cultivated with different carbon source concentrations.

Carbon source concentration	Biomass (g/L)	Carotenoid (g/g wet weight)	Sugar consumed (g/L)	$Y_{X/S}$ (g/g sugar)	$Y_{P/X}$ (ug/g biomass)	$Y_{P/S}$ (ug/g sugar)
10	3.65 ± 0.11^{c}	36.24 ± 0.82^{c}	9.35 ± 0.26^{b}	0.39	9.93	3.88
15	4.24 ± 0.10^{a}	42.51 ± 0.46^{a}	13.51 ± 0.16^{a}	0.31	10.03	3.15
20	4.29 ± 0.14^{a}	42.50 ± 1.11^{a}	13.90 ± 0.15^{a}	0.31	9.91	3.06
25	4.30 ± 0.14^{a}	42.30 ± 1.03^{a}	13.48 ± 0.25^{a}	0.32	9.83	3.14
30	4.10 ± 0.06^{b}	40.80 ± 0.98^{b}	13.55 ± 0.38^{a}	0.30	9.95	3.02

Data presented as mean value ± standard deviation (n = 3).

Values followed by the different letters in the same column indicated significant differences ($P < 0.05$, Turkey's HSD).

The data suggest that moderate carbon concentrations enhance both biomass production and carotenoid biosynthesis in *R. mucilaginosa* SMY40, with optimal values observed at 15–25 g/L. At lower concentrations (10 g/L), limited substrate availability may have constrained growth and metabolite synthesis, while excessive sugar levels (30 g/L) appeared to inhibit growth and reduce conversion efficiency. While Machado et al. (2019)[20] reported the optimum glucose concentration for carotenoid production by *R. mucilaginosa* URM 7409 was 30 g/L. This phenomenon is commonly attributed to substrate inhibition, osmotic stress, or repression of metabolic pathways at high sugar concentrations[9]. The decline in $Y_{X/S}$ and $Y_{P/S}$ values at elevated carbon concentrations indicates that not all available sugar was effectively converted into biomass or pigments. These findings are consistent with previous studies that reported reduced metabolic efficiency in yeast cultures under high substrate conditions[21,22]. It is also plausible that carbon overflow metabolism or the accumulation of inhibitory by-products may have played a role, as observed in other oleaginous and pigment-producing yeasts[2,3]. Interestingly, the carotenoid content per unit biomass ($Y_{P/X}$) remained stable across most concentrations, suggesting that once a threshold level of biomass is achieved, carotenoid biosynthesis proceeds at a relatively consistent rate, possibly due to constitutive or carbon-responsive regulation of carotenogenic genes[2,2]. This supports the suitability of glucose concentrations as 15 g/L for maximizing both pigment yield and process efficiency.

3. Optimal nitrogen sources on the growth and carotenoid production of *R. mucilaginosa* SMY40

The effect of different nitrogen sources on biomass yield, carotenoid production, sugar consumption, and conversion efficiencies of *R. mucilaginosa* SMY40 is presented in Table 3. Among the nitrogen sources tested, $(NH_4)_2SO_4$ resulted in the highest biomass concentration (4.28 ± 0.13 g/L), followed by KNO_3, urea, and NH_4Cl. However, statistical analysis revealed no significant difference ($P > 0.05$) between the biomass yields from $(NH_4)_2SO_4$, KNO_3, and urea. Carotenoid production was also highest with $(NH_4)_2SO_4$ (42.36 ± 0.89 µg/g wet weight), whereas urea yielded the lowest value (39.75 ± 0.90 µg/g wet weight). The highest carotenoid yield per biomass ($Y_{P/X}$) was observed in the $(NH_4)_2SO_4$ treatment (9.90 µg/g), while $Y_{P/S}$ was highest for NH_4Cl as well (3.34 µg/g sugar consumed), despite its lower sugar consumption (11.68 ± 0.63 g/L) compared to $(NH_4)_2SO_4$. Interestingly, the sugar-to-biomass conversion efficiency ($Y_{X/S}$) was comparable among all nitrogen sources, ranging from 0.32 to 0.34 g/g sugar, with NH_4Cl yielding the highest efficiency.

Table 3. Biomass yield, carotenoid production, sugar consumption and effect of medium composition on the conversion factors ($Y_{X/S}$, $Y_{P/X}$ and $Y_{P/S}$), biomass and carotenoids productivities of *R. mucilagonosa* SM40 cultivated with different nitrogen sources.

Nitrogen source	Biomass (g/L)	Carotenoid (g/g wet weight)	Sugar consumed (g/L)	$Y_{X/S}$ (g/g sugar)	$Y_{P/X}$ (ug/g biomass)	$Y_{P/S}$ (ug/g sugar)
$(NH_4)_2SO_4$	4.28 ± 0.13^{a}	42.36 ± 0.89^{a}	13.35 ± 0.32^{a}	0.32	9.90	3.18
NH_4Cl	3.96 ± 0.10^{b}	38.95 ± 0.76^{ab}	11.68 ± 0.63^{b}	0.34	9.84	3.34
KNO_3	4.15 ± 0.08^{ab}	41.04 ± 0.64^{ab}	13.08 ± 0.29^{a}	0.32	9.89	3.14
Urea	4.04 ± 0.05^{ab}	39.75 ± 0.90^{b}	12.25 ± 0.30^{ab}	0.33	9.83	3.25

Data presented as mean value $\pm$ standard deviation (n = 3).

Values followed by the different letters in the same column indicated significant differences ($P < 0.05$, Turkey's HSD).

The findings indicate that nitrogen source type significantly influences both biomass production and carotenoid production in *R. mucilaginosa* SMY40. $(NH_4)_2SO_4$ promoted the highest biomass and carotenoid content, consistent with previous studies reporting enhanced growth of oleaginous yeasts when utilizing inorganic ammonium salts[22]. This is likely due to the rapid assimilation of ammonium ions via the glutamine synthetase–glutamate synthase pathway, supporting both primary metabolism and secondary metabolite synthesis. Although NH_4Cl produced lower biomass compared to $(NH_4)_2SO_4$, it exhibited the highest carotenoid yield per biomass and per sugar consumed, suggesting an elevated flux towards carotenoid biosynthesis under this nitrogen condition. Similar nitrogen-dependent metabolic shifts have been observed in *Rhodotorula* species, where nitrogen limitation or specific nitrogen types can trigger oxidative stress or alter carbon flux distribution, thereby enhancing pigment production[9]. The use of KNO_3 and urea also supported moderate biomass and carotenoid production. Nitrate assimilation requires energy and reductants, which may reduce the resources available for pigment synthesis. Meanwhile, urea, despite being a cost-effective nitrogen source, resulted in the lowest carotenoid content, possibly due to delayed urease activity or suboptimal nitrogen release kinetics[24]. Overall, the results suggest that ammonium-based nitrogen sources, particularly $(NH_4)_2SO_4$ and NH_4Cl, are more favorable for both biomass growth and carotenoid production. However, the choice

between them should consider the intended application, $(NH_4)_2SO_4$ for maximizing biomass or NH_4Cl for maximizing pigment yield efficiency. Based on the findings of this study, $(NH_4)_2SO_4$ was identified as the optimal nitrogen source and was therefore employed in subsequent experimental phases to further enhance carotenoid biosynthesis by *R. mucilaginosa* SMY40.

4. Optimal initial pH of the culture medium on the growth and carotenoid production of *R. mucilaginosa* SMY40

The effect of initial pH on the growth, carotenoid production, sugar utilization, and conversion efficiencies of *R. mucilaginosa* SMY40 is presented in Table 4. Among the tested pH values, an initial pH of 5.0 supported the highest biomass concentration (4.28 ± 0.85 g/L) and carotenoid content (42.20 ± 0.19 µg/g wet weight), which were significantly higher than those obtained at more acidic (pH 3.0) or more alkaline (pH 7.0 and 9.0) conditions ($P < 0.05$). The yeast cultivated at pH 5.0 also exhibited the highest sugar consumption (13.40 ± 0.42 g/L), while the lowest sugar consumption and biomass were observed at pH 9.0 (7.93 ± 0.64 g/L and 2.23 ± 0.24 g/L, respectively). Although the carotenoid content (µg/g wet weight) was highest at pH 5.0, the highest carotenoid yield per biomass ($Y_{P/X}$=9.86 µg/g) was recorded at pH 5.0, followed closely by pH 3.0 (9.86 µg/g). The carotenoid yield per sugar consumed (YP/S) was highest at both pH 3.0 and 7.0 (3.38 µg/g sugar), despite their lower absolute carotenoid content.

Table 4. Biomass yield, carotenoid production, sugar consumption and effect of medium composition on the conversion factors ($Y_{X/S}$, $Y_{P/X}$ and $Y_{P/S}$), biomass and carotenoids productivities of *R. mucilagonosa* SM40 cultivated with different initial pH of the culture medium.

Initial pH	Biomass (g/L)	Carotenoid (g/g wet weight)	Sugar consumed (g/L)	$Y_{X/S}$ (g/g sugar)	$Y_{P/X}$ (ug/g biomass)	$Y_{P/S}$ (ug/g sugar)
pH 3.0	3.92 + 0.05^{ab}	38.36 + 1.01^{b}	11.33 + 0.65^{b}	0.37	9.80	3.38
pH 5.0	4.28 + 0.85^{a}	42.20 + 0.19^{a}	13.40 + 0.42^{a}	0.32	9.86	3.15
pH 7.0	3.52 + 0.10^{b}	35.37 + 1.35^{b}	10.50 + 0.41^{b}	0.35	9.76	3.38
pH 9.0	2.23 + 0.24^{c}	20.37 + 1.18^{c}	7.93 + 0.64^{c}	0.28	9.21	2.81

Data presented as mean value ± standard deviation (n = 3).

Values followed by the different letters in the same column indicated significant differences ($P < 0.05$, Turkey's HSD).

The initial pH of the culture medium exhibited a significant influence on both biomass yield and carotenoid production by *R. mucilaginosa* SMY40. Among the tested pH values (3.0, 5.0, 7.0, and 9.0), pH 5.0 was found to be optimal for cell growth and pigment biosynthesis, yielding the highest biomass (4.28 ± 0.85 g/L) and carotenoid content (42.20 ± 0.19 µg/g wet weight). This observation is in agreement with previous studies that demonstrated the favorable impact of slightly acidic conditions on carotenoid biosynthesis in *Rhodotorula* species[22]. The efficient sugar utilization at pH 5.0 (13.40 ± 0.42 g/L) also suggests that this condition supports enhanced metabolic activity and carbon flux toward biomass and carotenoid biosynthesis pathways. At lower and neutral pH values (3.0 and 7.0), although moderate biomass (3.92 ± 0.05 and 3.52 ± 0.10 g/L, respectively) and carotenoid levels (38.36 ± 1.01 and 35.37 ± 1.35 µg/g, respectively) were observed, these were significantly lower than those achieved at pH 5.0. Interestingly, pH 3.0 and 7.0 both resulted in a similar carotenoid yield per gram of sugar consumed ($Y_{P/S}$ = 3.38 µg/g sugar), indicating relatively stable pigment productivity despite varying growth levels. These results may reflect cellular mechanisms aimed at maintaining carotenoid levels under moderate stress conditions induced by suboptimal pH. At alkaline pH (9.0), a marked decrease in both biomass (2.23 ± 0.24 g/L) and carotenoid production (22.07 ± 1.67 µg/g) was evident, indicating that such basic conditions likely imposed stress on the cells, impairing metabolic activity and pigment biosynthesis. This finding is consistent with previous reports showing that extreme pH levels negatively affect membrane integrity and enzyme function in yeasts, thereby reducing growth and metabolite production[25]. Overall, the findings from this study reinforce the importance of pH optimization in enhancing microbial pigment production. The results confirm that an initial pH of 5.0 provides the most favorable environment for *R. mucilaginosa* SMY40, supporting both efficient biomass accumulation and carotenoid biosynthesis. This finding is consistent with the study by Rodríguez et al.[14], who reported that pH 5.0 was optimal for carotenoid production by *R. mucilaginosa* when cultivated on artichoke agro-industrial waste, serving as a cost-effective carbon source.

5. Optimal cultivation time on the growth and carotenoid production of *R. mucilaginosa* SMY40

The influence of cultivation time on biomass yield, carotenoid production, sugar consumption, and associated conversion efficiencies of *R. mucilaginosa* SMY40 is summarized in Table 5. The biomass concentration reached its peak at 120 hours of cultivation (4.29 ± 0.02 g/L), which was statistically higher than that observed at 96 hours (3.90 ± 0.14 g/L) and comparable to values obtained at 144 hours (4.22 ± 0.12 g/L) and 168 hours (4.10 ± 0.06 g/L). Carotenoid content also increased with time, reaching a maximum at 144 hours (42.62 ± 0.41 µg/g wet weight), although this value was not significantly different from those obtained at 120 hours (42.58 ± 0.74 µg/g) and 168 hours (42.42 ± 0.95 µg/g). The shortest cultivation period (96 hours) resulted in significantly lower carotenoid content (40.02 ± 0.51 µg/g; $P < 0.05$). Sugar consumption showed a gradual increase with cultivation time, from 11.98 ± 0.80 g/L at 96 hours to 13.57 ± 0.19 g/L at 168 hours. Conversion efficiency values revealed that the biomass yield per gram of sugar consumed ($Y_{X/S}$) was highest and identical at 96 and 120 hours (0.33 g/g), then slightly decreased at 144 hours (0.32 g/g) and drastically dropped at 168 hours (0.20 g/g). Although the carotenoid yield per biomass ($Y_{P/X}$) remained relatively stable across all time points, peaking slightly at 96 hours (10.38 µg/g), the carotenoid yield per sugar consumed ($Y_{P/S}$) was also highest at 96 hours (3.39 µg/g), with a gradual decrease observed in later time points.

Table 5. Biomass yield, carotenoid production, sugar consumption and effect of medium composition on the conversion factors ($Y_{X/S}$, $Y_{P/X}$ and $Y_{P/S}$), biomass and carotenoids productivities of *R. mucilagonosa* SM40 cultivated with different times.

Time	Biomass (g/L)	Carotenoid (g/g wet weight)	Sugar consumed (g/L)	$Y_{X/S}$ (g/g sugar)	$Y_{P/X}$ (ug/g biomass)	$Y_{P/S}$ (ug/g sugar)
96	3.90 + 0.14^{b}	40.02 + 0.51^{b}	11.98 + 0.80^{b}	0.33	10.38	3.39
120	4.29 + 0.02^{a}	42.58 + 0.74^{a}	13.20 + 0.22^{a}	0.33	9.93	3.23
144	4.22 + 0.12^{a}	42.62 + 0.41^{a}	13.34 + 0.71^{a}	0.32	10.11	3.20
168	4.10 + 0.06^{ab}	42.42 + 0.95^{a}	13.57 + 0.19^{a}	0.20	10.35	3.13

Data presented as mean value ± standard deviation (n = 3).

Values followed by the different letters in the same column indicated significant differences ($P < 0.05$, Turkey's HSD).

The duration of fermentation plays a crucial role in determining the balance between microbial growth, substrate consumption, and secondary metabolite accumulation, including carotenoids. The present findings indicate that *R. mucilaginosa* SMY40 achieved maximum biomass and carotenoid content at approximately 120–144 hours of cultivation, which is consistent with the typical stationary phase onset reported in other studies of carotenoid-producing yeasts[22]. The significant increase in carotenoid content from 96 to 120 hours suggests active pigment biosynthesis during the late exponential phase, which then plateaued through 144 and 168 hours. This stabilization phase is commonly observed in yeast fermentation systems where secondary metabolite production continues even after biomass accumulation slows[9]. Interestingly, the highest carotenoid yield per sugar consumed ($Y_{P/S}$ = 3.39 µg/g) and per biomass ($Y_{P/X}$ = 10.38 µg/g) were obtained at 96 hours. This suggests that shorter fermentation periods may favor efficient conversion of carbon sources into carotenoids, possibly due to metabolic prioritization toward secondary metabolism under limited biomass expansion. However, the total carotenoid productivity was lower at 96 hours due to reduced biomass formation, making longer cultivation durations more favorable for maximizing volumetric yield. The drastic decline in $Y_{X/S}$ to 0.20 g/g at 168 hours indicates a decoupling between sugar consumption and biomass formation, potentially due to increased maintenance energy requirements, nutrient depletion, or cellular aging, as noted in prolonged yeast cultivations. Therefore, although extended fermentation may sustain carotenoid levels, it is not economically favorable due to reduced conversion efficiencies[24]. A cultivation period between 120 and 144 hours appears optimal for achieving a balance between biomass yield, carotenoid concentration, and conversion efficiency of *R. mucilaginosa*[13,14]. Beyond this period, the benefits in pigment accumulation diminish, and process efficiency declines.

Conclusions

This study identified the optimal cultivation conditions for maximizing the biomass and total carotenoid yield of *R. mucilaginosa* SMY40, a promising carotenoid-producing yeast. Among the tested conditions, the highest productivity was observed when the yeast was cultured in a synthetic medium containing 15 g/L glucose as the carbon source and ammonium sulfate as the nitrogen source, at an initial pH of 5.0. The cultivation was conducted at room temperature under shaking conditions

(150 rpm) for 120 hours. Under these optimized parameters, the maximum dry cell weight reached 4.26 ± 0.03 g/L, with a total carotenoid content of 41.98 ± 0.93 µg/g wet weight and glucose consumption of 13.36 ± 0.11 g/L. These findings suggest that a slightly acidic pH environment facilitates optimal metabolic activity and carotenoid biosynthesis in *R. mucilaginosa* SMY40, likely by enhancing the enzymatic efficiency within the mevalonate pathway. The high yield achieved under relatively simple and cost-effective culture conditions highlights the potential of this strain for biotechnological applications, particularly in the production of natural pigments for use in the food, cosmetic, and pharmaceutical industries.

References

1. Martins N, Ferreira ICFR. Wastes and by-products: Upcoming sources of carotenoids for biotechnological purposes and health-related applications. Trends Food Sci Technol 2017;62:33-48.
2. Aksu Z, Eren AT. Production of carotenoids by the isolated yeast of Rhodotorula glutinis. Biochem Eng J 2007;35:107-13.
3. Maldonade IR, Rodriguez-Amaya DB, Scamparini ARP. Carotenoids of yeasts isolated from the Brazilian ecosystem. Food Chem 2008;107:145–50.
4. Marova I, Certik M, Breierova E. Production of enriched biomass by carotenogenic yeasts- Application of whole cell yeast biomass to production of pigments and other lipid compound. In D. Matovic. (Ed.), Biomass - Detection, Production and Usage. Shanghai: In Tech China; 2011.
5. Gupta I, Adin SN, Panda BP, Mujeeb M. β-Carotene-production methods, biosynthesis from Phaffia rhodozyma, factors affecting its production during fermentation, pharmacological properties: a review. Biotechnol Appl Biochem 2022;69:2517–29.
6. de Mattos MVC, Michelon M, de Burkert Medeiros JF. Production and stability of food-grade of food-grade liposomes containing microbial carotenoids from Rhodotorula mucilaginosa. Food Structure 2022;33:100282.
7. Moliné M, Flores MR, Libkind D, del Carmen Diéguez M, Farias ME, van Broock M. Photoprotection by carotenoid pigments in the yeast Rhodotorula mucilaginosa: the role of torularhodin. Photochem Photobiol Sci 2010;9:1145-51.
8. Rodrigues TVD, Amore TD, Teixeira EC, de Medeiros Burkert JF. Carotenoid production by Rhodotorula mucilaginosa in batch and fed-batch fermentation using agroindustrial byproducts. Food Technology and Biotechnology 2019;57:388-98.

9. Buzzini P, Martini A. Extracellular enzymatic activity profiles in yeast and yeast-like strains like strains isolated from tropical environments. J Appl Microbiol 2002;93:1020-5.
10. Maldonade IR, Rodriguez-Amaya DB, Scamparini ARP. Statistical optimization of cell growth and carotenoid production by Rhodotorula mucilaginosa. Braz J Microbiol 2012;109-15.
11. Gedela R, Sharma S, Prabhu AA, Pakshirajan K, Veeranki VD. Elevated production of lipids and carotenoids from Rhodotorula mucilaginosa using raw dairy wastewater as crude renewable substrate: a waste to wealth transformation approach. Chemical Papers 2025;79:4403-18.
12. El-Banna AA, El-Razek AMA, El-Mahdy AR. Some factors affecting the production of carotenoids by Rhodotorula glutinis. Food and Nutrition Sciences 2012;3:64–71.
13. Sharma R, Ghoshal G. Optimization of carotenoids production by Rhodotorula mucilaginosa (MTCC-1403) using agro-industrial waste in bioreactor: A statistical approach. Biotechnol Rep (Amst) 2019;25:e00407.
14. Rodríguez NAT, Quiñones-Cerna CE, Castillo HMR, Cruz Monzon JF, Butrón FJH, Soto JCR. Optimization of Total Carotenoid Production by Rhodotorula mucilaginosa from Artichoke Agroindustrial Waste Using Response Surface Methodology. Environ Res Eng and Manag 2023;79:111-21.
15. Elsanhoty RM, Al-Turki AI, Abdel-Razik MM. Production of carotenoids from Rhodotorula mucilaginosa and their applications as colorant agent in sweet candy. J Food Agric Environ 2017;15:21-6.
16. Sriwongchai S, Aksornsamai C, Kitleartpompairoat R. Screening of xylose-utilizing oleaginous yeasts from fermented bio-extracts for microbial oil production. BUU Sci J 2019;24:311-25.
17. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426-8.
18. Sriwongchai S, Pokethitiyook P, Kruatrachue M, Bajwa PK, Lee H. Screening of selected oleaginous yeasts for lipid production from glycerol and some factors which affect lipid production by Yarrowia lipolytica strains. JMBFS 2013;2:2344-8.
19. Passorn S, Sookwanna N, Auputinan P. Screening of Carotenogenic Yeasts from Natural Soil at the University of Phayao. BUU Sci J 2016;21:15-22.
20. Machado WRC, da Silva LG, Vanzela ESL, Bianchi VLD. Evaluation of the process conditions for the production of microbial carotenoids by the recently isolated *Rhodotorula mucilaginosa* URM 7409. Braz J Food Technol 2019;22:e2018267.

21. Buzzini P, Innocenti M, Turchetti B, Libkind D, van Broock M, Mulinacci N. Carotenoid profiles of yeasts belonging to the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces*, and *Cryptococcus*. Can J Microbiol 2007;53:1024–31.

22. Saenge C, Cheirsilp B, Suksaroge TT, Bourtoom T. Potential use of oleaginous red yeast *Rhodotorula glutinis* for the bioconversion of crude glycerol from biodiesel plant to lipids and carotenoids. Process Biochem 2011;46:210–8.

23. Du J, Shao Z, Zhao H. Engineering microbial cell factories for biosynthesis of value-added products. J Ind Microbiol Biotechnol 2011;38:873–90.

24. Ye Z, Sun T, Hao H, He Y, Liu X, Guo M, et al. Optimising nutrients in the culture medium of *Rhodosporidium toruloides* enhances lipids production. AMB Express 2021;14:149.

25. Kot AM, Błażejak S, Kurcz A, Bryś J, Gientka I. *Rhodotorula glutinis*–potential source of lipids, carotenoids, and enzymes for use in industries. Appl Microbiol Biotechnol 2019;103:739–51.