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Light and Temperature Effects on the Accumulation of Carotenoids in *Rhodotorula* spp. Yeasts

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Abstract

Carotenoids are widely recognized for their antioxidant and health-beneficial properties, making them attractive for applications in the food, pharmaceutical, medical, and agricultural sectors. *Rhodotorula* yeasts are considered one of the most suitable alternatives for carotenoid synthesis due to their rapid biomass growth and high pigment yield. During this study, based on the sequences of the ITS region between 18S and 28S rRNA genes, the yeast strains were identified as belonging to *Rhodotorula babjevae*, *R. dairenensis*, *R. diobovata*, *R. glutinis*, *R. graminis*, *R. ingeniosa*, *R. kratochvilovae*, and *R. mucilaginosa*. The production of carotenoids by different *Rhodotorula* yeast strains was analyzed under the combined effects of lighting and temperature. Among all tested strains, the isolate identified as *R. ingeniosa* exhibited the lowest carotenoid content, ranging from 0.18 to 0.23 mg/g biomass. The highest levels of pigment were accumulated in dark conditions by *R. babjevae* (0.86 mg/g biomass) and *R. graminis* (0.76 mg/g biomass) cultivated for 14 days at a constant temperature of 26 °C, and by *R. glutinis* (0.89 mg/g biomass) after incubation at 4 °C. The majority of yeasts tested produced more carotenoids at a higher temperature. It was observed that in *R. babjevae*, *R. glutinis*, and *R. graminis*, lighting negatively affected the pigment content regardless of incubation temperature. In these strains, the pigment content decreased by 1.2- to 1.4-fold after one week of cultivation under light conditions at 26 °C, compared to cultures grown in the dark. The results suggest that the isolated *Rhodotorula* strains could be attractive candidates for the efficient synthesis of carotenoids.

Keywords: *Rhodotorula*; carotenoids; β-carotene; temperature; light

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1. Introduction

Carotenoids are natural lipid-soluble pigments from the terpenoid group that give many microorganisms their characteristic red, orange, and yellow colors. These lipophilic molecules, synthesized through multiple enzyme-catalyzed biochemical reactions, accumulate in cell membranes and act as membrane-protective antioxidants [1]. The most important structural features of carotenoids rely on conjugated double bonds, which are responsible for light absorption and color [2]. Carotenoids are generally classified into two groups: xanthophylls (containing oxygen) and carotenes (oxygen-free) [2]. The first group includes astaxanthin, lutein, and zeaxanthin, while the second group comprises of α-carotene, β-carotene, γ-carotene, lycopene, and others [2]. Among these, β-carotene is

the predominant pigment produced by *Rhodotorula* yeasts in most cases [2–4]. This compound is particularly well known for its provitamin A activity, as well as its antioxidant, anticancer, and antimicrobial properties, making it highly valuable for human health [1–8]. Carotenoids, including β-carotene, have high attractiveness in the food industry, medicine, pharmacy, and agriculture [1,2] due to their functional and health-promoting properties. In particular, β-carotene derived from red yeasts such as *Rhodotorula* spp. is gaining recognition as a natural and sustainable alternative to synthetic pigments and supplements. This yeast-derived β-carotene is considered safe by ESFA and FDA and suitable for use as a food colorant, dietary supplement, and feed additive, especially in poultry and aquaculture to enhance pigmentation and health [1,2,4,5]. *Rhodotorula* strains can produce significant amounts of β-carotene when cultivated under optimal environmental conditions [2,9].

Carotenoid production by microorganisms is influenced by many factors, such as light, pH, temperature, metal ions, and salts [9]. Photoinduction may increase the growth of microorganisms and activate enzymes important for the biosynthesis of carotenoids [9]. On the other hand, carotenoids, particularly β-carotene, are light-sensitive molecules whose degradation can depend on pH, temperature, and oxygen concentration. It was demonstrated that light-induced degradation of β-carotene follows zero- or first-order kinetic patterns [10]. Under near-UV or visible light illumination, β-carotene degrades in dichloromethane at a constant rate irrespective of its concentration. At higher molecular concentrations and under intense visible light (about 1000 lux), β-carotene degrades even faster [11]. Light intensity, wavelength, and exposure duration can either enhance or inhibit carotenoid production. For example, *Rhodotorula toruloides* exhibited increased pigmentation and higher carotenoid levels when subjected to light, suggesting that light acts as a stimulant for carotenogenesis in this species [12]. Conversely, excessive light exposure can induce stress responses, adversely affecting both growth and metabolic activities [11,12].

Temperature variations also play a crucial role in carotenoid biosynthesis. Several studies have indicated that low temperatures can lead to an increase in carotenoid production in certain red yeast species. For example, *Rhodotorula gracilis* synthesizes higher amounts of intracellular lipids and carotenoids when cultured at lower temperatures [13]. However, it is important to note that while low temperatures may boost carotenoid production, they can also limit overall microbial growth. Conversely, high temperatures might denature enzymes essential for carotenoid synthesis, thereby reducing production efficiency [14]. Understanding the interplay between light, temperature, and other stress factors is essential for developing effective biotechnological processes to enhance carotenoid production in red yeasts [2].

The carotenoid biosynthetic pathway in *Rhodotorula* species has been extensively studied, revealing their ability to produce compounds like β-carotene [1,4,15]. Most of the research has focused on the species *Rhodotorula glutinis*, *Rhodotorula gracilis*, *Rhodotorula mucilaginosa*, *Rhodotorula toruloides*, and *Rhodotorula graminis. R. glutinis* is of particular interest for pigment production capacity and its biocontrol potential against post-harvest diseases [16,17]. *R. mucilaginosa* is notable for its high β-carotene content and has been explored for applications in the food and feed industries. Its widespread occurrence makes it an attractive candidate for biotechnological processes [18]. Due to its adaptation to cold environments, *R. kratochvilovae* serves as a valuable model organism for studying low-temperature tolerance mechanisms and the role of carotenoids in stress adaptation [19,20]. To the best of our knowledge, there is a lack of information on carotenoid production in yeasts such as *R. dairenensis*, *R. diobovata*, and *R. babjevae*.

This study aimed to investigate the combined effects of environmental stressors, including light/dark regimes and temperature shifts (26 °C to 4 °C), on the production and accumulation of carotenoids in different yeast strains from the *Rhodotorula* genus. Given the in-

creasing demand for natural pigments, ongoing research focuses on optimizing environmental conditions and exploring new yeast strains capable of efficient carotenoid synthesis.

2. Materials and Methods

2.1. Yeast Strain Isolation and Media

Red colony-forming yeasts were isolated from various environmental samples, including water and berries. The samples were collected during the summer months from 2019 to 2024 in Lithuania. Samples were placed in sterile bottles or bags and transported to the research laboratory in portable coolers with ice batteries.

Water samples were obtained from Lithuanian water bodies in public bathing areas. Sample aliquots in a quantity of 12 mL were concentrated by centrifuging to 100 µL for 5 min at 5000× *g* and plated onto Yeast Extract-Peptone-Dextrose (YEPD) medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 20 g/L agar) supplemented with chloramphenicol (34 mg/mL).

Berry samples (about 25 g) were immersed in 50 mL of liquid MD medium (20 g/L dextrose, 10 g/L $(NH_4)_2SO_4$, 0.9 g/L KH_2PO_4, 0.5 g/L $MgSO_4$, 0.23 g/L K_2HPO_4, 0.1 g/L NaCl, 0.1 g/L $CaCl_2$) and incubated at room temperature with shaking at 100 rpm for 1 h. The wash solutions were serially diluted and plated onto YEPD medium with chloramphenicol. All culture plates were incubated at room temperature (22 °C) for 3–4 days. Colonies exhibiting red pigmentation were isolated by streaking onto YEPD agar medium and subjected to molecular identification.

2.2. Yeast Species Verification

For the identification of cultivable yeasts, genomic DNA was isolated from overnight-grown yeast cells using a genomic DNA purification kit (Thermo Fisher Scientific Baltics, Vilnius, Lithuania) following the manufacturer's instructions. The region between 18S rRNA and 28S rRNA genes was PCR amplified using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers [21]. The following PCR conditions were utilized: 94 °C for 5 min, followed by 25 cycles of 94 °C for 1 min, 53 °C for 1 min 30 s, and 72 °C for 2 min, and final extension at 72 °C for 10 min. The PCR reaction mixture consisted of 5 µL DreamTaq buffer, 1 µL of 2 mM dNTP mix, 1 µL of each primer (10 µmol/L), 2.5 units of Dream Taq DNA polymerase (all from Thermo Fisher Scientific Baltics, Vilnius, Lithuania), 1 µL of DNA template (5 ng), and sterile distilled water up to 50 µL. PCR products were purified using the GeneJet PCR purification kit (Thermo Fisher Scientific Baltics, Vilnius, Lithuania) and sequenced at Base-Clear (Leiden, The Netherlands). Sequencing results were compared with those offered by the FASTA network service of the EMBL-EBI database (https://www.ebi.ac.uk/jdispatcher/sss/fasta/nucleotide, accessed on 8 May 2025) and deposited in the National Center for Biotechnology Information (NCBI) under accession numbers PV746256–PV746261, MZ185345.1, MZ185346.1. Multiple sequence alignment of related nucleotide sequences was performed using the ClustalW algorithm [22] to ensure accurate homology assessment. A phylogenetic tree was constructed using the Maximum Likelihood (ML) method implemented in MEGA12 [23]. Evolutionary distances were estimated using the Kimura 3-parameter (K3P) model to account for varying substitution rates [24]. The robustness of the inferred tree topology was evaluated through bootstrap analysis with 1000 replicates. Representative nucleotide sequences from the same species and targeting the same genomic region as our sequences were retrieved from the NCBI GenBank database to ensure comprehensive representation of related taxa.

2.3. Yeast Culturing Conditions

Cultures were grown under different conditions to evaluate the carotenoid accumulation under varying temperature and illumination conditions. There were 4 testing settings (Table 1).

Table 1. Design of experiment.

Conditions	Settings	Temperature (°C)	Days	Temperature (°C)	Days
Dark	I	26	2, 3, 5, 7, 14		
	II	26	2	4	1, 3, 5, 12
Light	III	26	2, 3, 5, 7, 14		
	IV	26	2	4	1, 3, 5, 12

Rhodotorula yeasts were grown on YEPD agar medium under all experimental conditions. In settings I and III, cultures were incubated at 26 °C in the dark (I) or under light (III) for 2, 3, 5, 7, and 14 days. In settings II and IV, yeasts were initially grown at 26 °C for 2 days and then transferred to 4 °C for 1, 3, 5, and 12 days, in the dark (II) or under light (IV), respectively. Lighting was generated by a self-contained lighting system consisting of LED holder modules and a current source (LED lamps L5-N604-NOEF, SLOAN, intensity 8–10 μmol m^{-2} s^{-1}).

2.4. Carotenoids Extraction from Yeasts

At different times, *Rhodotorula* spp. biomass (1 to 1.17 g) was collected from agar plates using a spatula and transferred to glass dishes containing 1 mL of N,N-dimethylformamide (DMF) and 10 glass beads (5 mm in size, Zymo Research, Irvine, CA, USA). The disruption of yeast cells was performed by vortexing (Vortex Gene 2, model SI-0256, Scientific Industries, Bohemia, New York, NY, USA) biomass with glass beads for 20 min at 600 rpm, and then 1 mL of DMF was added, and the mixture was centrifuged at 10,000× *g* for 10 min (Eppendorf Centrifuge 5420, Hamburg, Germany). The supernatant was collected, and absorbance was measured in the spectral range from 390 nm to 600 nm wavelengths (Spectrophotometer "Thermo Scientific GENESYS 20", Fisher Scientific, Waltham, MA, USA).

2.5. Quantification of β-Carotene Equivalents

UV-VIS absorption spectrum of β-carotene in DMF was recorded from 390 nm to 600 nm wavelengths. The standard β-carotene solution in DMF solvent exhibited the maximum light absorption at 440 nm. Repeatability was assessed by measuring absorbance across biological replicates. Based on the obtained data, the extinction coefficient of β-carotene in DMF solvent was calculated according to the Beer–Lambert law:

$\varepsilon = A/(c \times l)$;
ε—extinction coefficient ($M^{-1} \cdot cm^{-1}$);
A—light absorption (O.D.);
l—cuvette width (cm);
c—substance concentration (M).

The carotenoid content in the obtained extract is calculated according to the following formula [25–27]:

$c = A \times V \times 104 \times (m \times \varepsilon)$;
c—β-carotene (mg) / dry biomass (g);
A—light absorption (O.D.);
V—solvent volume (mL);

m—yeast biomass (g);
ε—extinction coefficient DMF ($M^{-1} \cdot cm^{-1}$).

All experiments were performed with at least three biological replicates, and total carotenoid content, expressed as β-carotene equivalents, was presented as the mean ± standard deviation. One-way analysis of variance (ANOVA; $p < 0.05$) was used to identify statistically significant differences in carotenoid level accumulated by a specific yeast strain compared to the 2-day timepoint. Pairwise comparisons between conditions (Dark 26 °C vs. Light 26 °C, Dark 4 °C vs. Light 4 °C, Dark 26 °C vs. Dark 4 °C, and Light 26 °C vs. Light 4 °C) were conducted separately for each isolate. To examine the overall impact of lighting conditions regardless of yeast strain, the same pairwise comparisons were performed across the combined dataset, aggregating replicates from all isolates.

3. Results and Discussion

3.1. Isolation, Identification, and Phylogenetic Characterization of Red Yeast

Yeast strains were isolated from water samples and berries located near aquatic environments. Yeast colonies exhibiting red or reddish pigmentation were selected and subsequently identified to the species level through DNA sequencing. In total, eight distinct yeast species were isolated, displaying colony colors ranging from yellowish to bright red (Figure 1). Microscopic examination was performed to assess cell morphology, revealing that most cells measured between 5 and 8 µm in diameter and exhibited intracellular granularity indicative of pigment accumulation. The selected yeast isolates were identified by analyzing the sequences of the ITS region between 18S and 28S rRNA genes and comparing to GenBank references. The isolates were assigned to the following species: *Rhodotorula babjevae*, *R. dairenensis*, *R. diobovata*, *R. glutinis*, *R. graminis*, *R. ingeniosa*, *R. kratochvilovae*, and *R. mucilaginosa* (Figure 1).

A phylogenetic tree was constructed based on ITS sequences and revealed distinct evolutionary lineages among *Rhodotorula* species tested (Figure S1). The highest bootstrap values of the phylogenetic analysis determined clades significantly corresponding to respective yeast species. Among the isolates, *R. ingeniosa* was the most phylogenetically distant species. *R. mucilaginosa* and *R. dairenensis* strains, similar to *R. kratochvilovae* and *R. diobovata*, form separate clusters. Yeast strains identified as *R. glutinis*, *R. graminis*, and *R. babjevae* represent closely related taxa. This suggests potential for physiological and ecological diversity among the members of this group. The genus *Rhodotorula* has undergone several taxonomical revisions in recent years, with *R. ingeniosa* now reclassified under the genus *Sampaiozyma* [28]. *R. ingeniosa* yeasts are distinguished from other yeast species by their yellowish colony morphology (Figure 1). The intensity of this coloration could be related to the amount of β-carotene present in the cells. Strains exhibiting yellow to orange coloration typically have β-carotene as their major pigment, whereas red-hued strains are richer in torulene and torularhodin [29,30].

Understanding the phylogenetic relationships within *Rhodotorula* can provide insights into the evolutionary patterns underlying carotenoid biosynthesis. The ability to synthesize carotenoids varies among *Rhodotorula* species and appears to correlate to some extent with their phylogenetic proximity. Species such as *R. glutinis*, *R. graminis*, and *R. babjevae* are closely related and are known for high β-carotene production. *R. glutinis* has been reported to produce up to 118 mg/L of β-carotene under optimized conditions [31]. *R. mucilaginosa* demonstrates moderate carotenoid production, influenced significantly by the growth substrate and environmental parameters [32]. *R. kratochvilovae* produces 2.03 mg/L of β-carotene depending on the carbon source and culture conditions [20]. *R. diobovata* and *R. dairenensis* have limited information on β-carotene yields but are recognized as

carotenoid-producing species. *R. ingeniosa*, being phylogenetically distant, lacks substantial documentation on its carotenoid biosynthesis capabilities.

The observed patterns suggest that phylogenetically close *Rhodotorula* species often share similar biosynthesis capabilities, including conserved gene clusters responsible for carotenoid metabolism. However, carotenoid biosynthesis in yeast cells can be enhanced by changing the conditions of the culture environment [33].

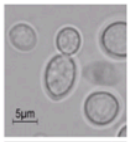
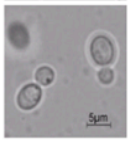
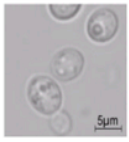
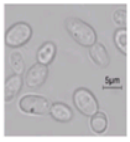
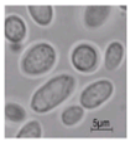
Morphological analysis			Species/ Acc. No.	Reference/ Acc. No.	Identity %
Colony		Microscopy			
			*S. ingeniosa* W-16_1 PV746259	*S. ingeniosa* PP905642.1	100
			*R. dairenensis* W-32_3 PV746258	*R. dairenensis* AF444501.1	100
			*R. mucilaginosa* S-9 PV746257	*R. mucilaginosa* PP067095.1	100
			*R. diobovata* SW-3_1 PV746256	*R. diobovata* PQ351472.1	100
			*R. kratochvilovae* W-8_2 PV746260	*R. kratochvilovae* NR_073282.1	100
			*R. glutinis* PC-L-8.1 MZ185346.1	*R. glutinis* MN913569.1	99.64
			*R. babjevae* W-32_2 PV746261	*R. babjevae* MZ098654.1	99.82
			*R. graminis* PC-6-10.1 MZ185345.1	*R. graminis* MN006688.1	100

Figure 1. Identification of *Rhodotorula* strains.

3.2. Carotenoid Accumulation in Rhodotorula spp. *Yeasts Cultured at a 26 °C Temperature and Differing Lighting Conditions*

Temperature is one of the main factors influencing yeast metabolism, thus affecting biomass growth and accumulation of the products. For *Rhodotorula* species, the optimal growth temperature ranges from 20 °C to 30 °C, as higher temperatures have been shown to reduce growth rates [34,35]. Previously, it was found that yeast obtained high biomass and total carotenoid content when incubating at 30 °C for 24 h [36]. Various cultural conditions, such as correct temperature, pH, light, nutrition availability, etc., can influence carotenoid

synthesis in fungi [12,37,38]. Some fungi may produce more carotenoids under higher temperatures, while others may require the absence of light [14].

Carotenoid accumulation in *Rhodotorula* yeasts was quantified as β-carotene equivalents under varying temperature and light conditions. It was demonstrated that the carotenoid content increased over the entire test period (14 days) in all *Rhodotorula* strains cultivated at 26 °C in the dark (Figure 2). Starting from the second day, based on pigment level, the yeast could be divided into 2 groups. The first group of yeasts (*R. ingeniosa*, *R. dairenensis*, *R. mucilaginosa*, and *R. diobovata*) produced lower levels of carotenoids, ranging from 0.18 to 0.23 mg/g biomass. The second group of yeasts (*R. babjevae*, *R. glutinis*, *R. graminis*, and *R. kratochvilovae*) accumulated a higher amount of carotenoids, ranging from 0.3 to 0.36 mg/g biomass. For all tested *Rhodotorula* strains, β-carotene equivalent level was significantly higher on the seventh and fourteenth days compared to those cultivated for 2 days (Figure 2A). After 14 days, the carotenoid amount was doubled in the first group of yeasts and reached 0.41–0.53 mg/g biomass. Among the second group, after 5–7 days of incubation, *R. graminis* isolate dominated in terms of β-carotene equivalent level (0.58–0.59 mg/g biomass), compared to the other tested yeast strains. After 14 days, the pigment content in this strain increased by approximately 30%, reaching 0.76 mg/g biomass. The highest accumulation of carotenoids at 26 °C in the dark conditions was observed for *R. babjevae* yeasts—0.86 mg/g biomass (based on β-carotene equivalent level). The pigment amount increased about 2.5 times after 14 days of yeast cultivation.

When yeasts were cultivated under light conditions at 26 °C, the amount of carotenoids in all *Rhodotorula* spp. tested remained almost unchanged over 3 days (Figure 2B). Starting from the fifth day, the accumulation of pigment significantly increased in all strains tested, except *R. mucilaginosa*, compared to those grown for 2 days. After one week, the highest level of carotenoid was observed in the second group of yeasts. *R. kratochvilovae* reached 0.54 mg of pigment per g of yeast biomass, *R. glutinis*—0.60 mg/g, *R. graminis*—0.47 mg/g, and *R. babjevae*—0.69 mg/g biomass, respectively. Carotenoid production increased by approximately 1.5-fold in *R. graminis*, 1.7-fold in *R. kratochvilovae*, 2-fold in *R. babjevae*, and 2.6-fold in *R. glutinis* compared to yeasts cultured for two days (Figure 2B). It is most likely that the 14-day-lasting lighting conditions cause inhibition of carotenoid synthesis or even degradation in *R. glutinis*, *R. kratochvilovae*, and *R. babjevae* yeast strains. The amount of pigment decreased 1.2- to 1.4-fold in the yeasts mentioned above compared to one week of culturing under light conditions at 26 °C.

Previous experiments with *R. toruloides* showed that cells grown in light increased carotenoid production five-fold compared to those grown in the dark [9]. Exposure to white light has been shown to increase β-carotene production in *R. glutinis* during its logarithmic growth phase [14]. Additionally, some studies have reported that blue light can stimulate carotenoid production in other fungi [12,14]. Conversely, light exposure has been associated with reduced astaxanthin production in certain fungal species [14].

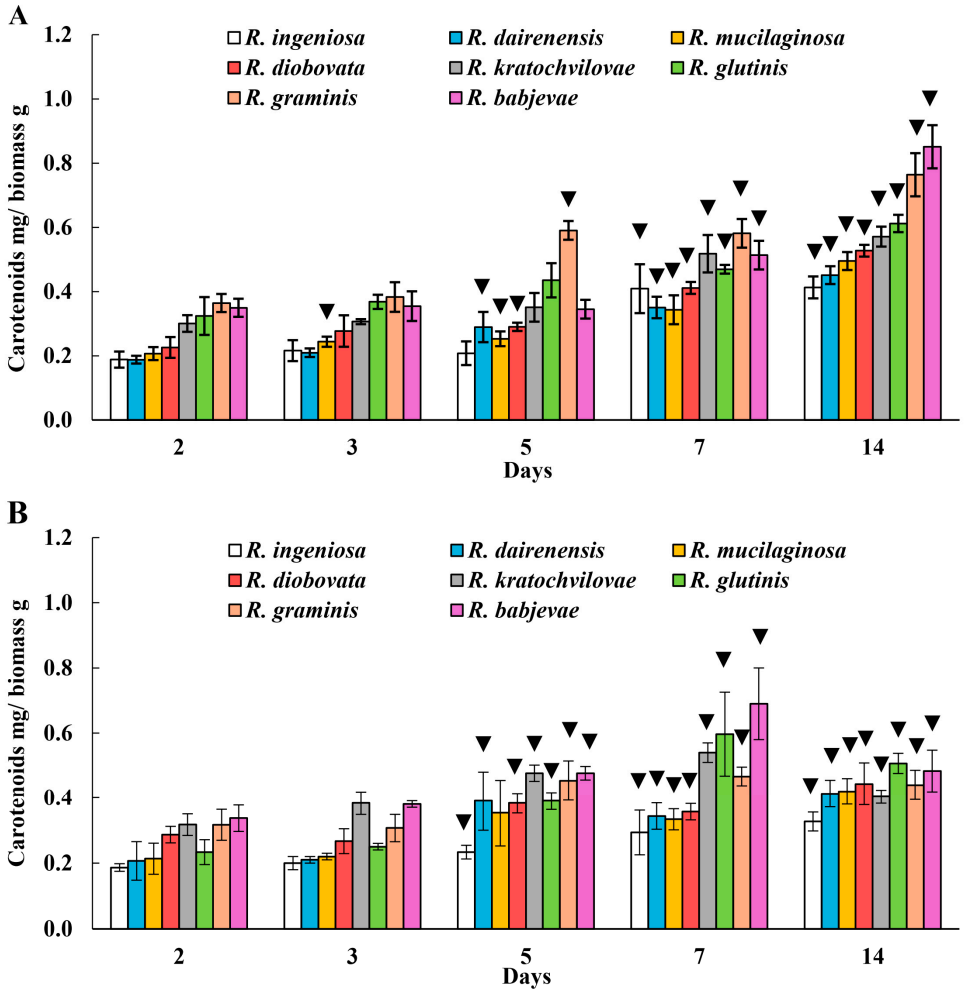


Figure 2. Carotenoid accumulation in *Rhodotorula* strains cultured for different periods at a constant 26 °C temperature under dark (**A**) and light (**B**) conditions. Data were quantified as β-carotene equivalent levels, and results were reported as mean ± SD. Statistically significant differences ($p < 0.05$) in carotenoid level accumulated by a specific yeast strain compared to the 2-h timepoint were marked by triangles.

3.3. Effect of Low Temperature on Carotenoid Content in Rhodotorula spp.

The content and composition of carotenoids are closely associated with ambient temperature. To analyze the effect of low temperature on the accumulation of carotenoids, the *Rhodotorula* yeast strains were grown for 2 days at 26 °C and then transferred to 4 °C for 12 days. Plates were incubated in parallel under dark and light conditions, and carotenoid content was measured as β-carotene equivalents at different time points (Figure 3). The pigment level in *R. ingeniosa*, *R. dairenensis*, *R. diobovata*, *R. mucilaginosa*, and *R. kratochvilovae* yeasts remained similar over one week of incubation at low temperature in the dark. The maximum concentration of carotenoids was observed after 2 weeks of incubation of *Rhodotorula* yeasts. In *R. ingeniosa*, *R. dairenensis*, and *R. kratochvilovae*, the pigment content increased by approximately 1.3- to 1.4-fold (Figure 3A). In *R. diobovata* and *R. mucilaginosa*, carotenoid level increased by 1.9- and 1.7-fold, respectively, over the entire incubation period. However, it was still lower (20 to 28%) than that accumulated at 26 °C temperature in dark conditions (Figure 2A). Low temperature slows down carotenoid synthesis in *R. graminis* cells—only a 20% increase in pigment level was observed during the entire culture incubation (Figure 3A). A similar inhibitory effect of low temperature was observed in nearly all tested *Rhodotorula* species. *R. glutinis* was the only strain exhibiting significantly increased pigment accumulation at 4 °C in the dark, reaching 0.89 mg/g

biomass. In contrast to the other strains, the carotenoid content in the *R. glutinis* was approximately 32% higher at 4 °C than at 26 °C.

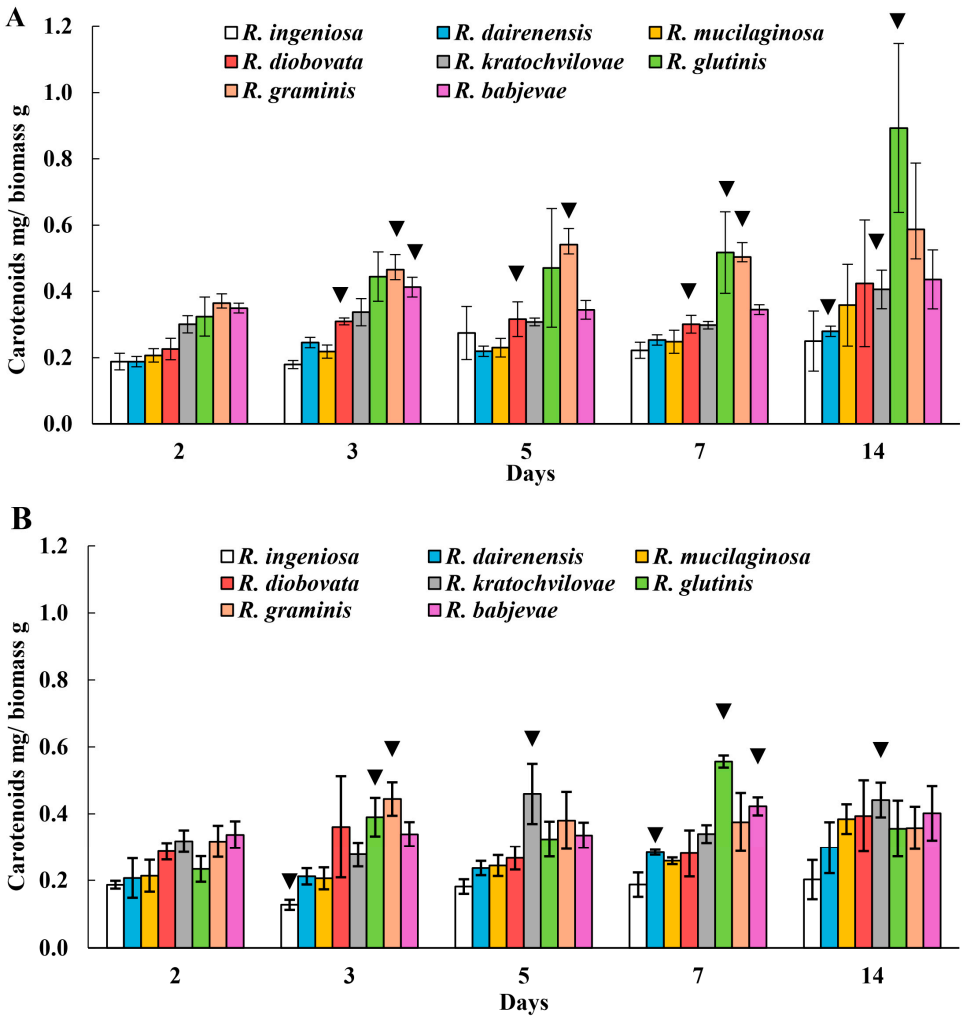


Figure 3. Carotenoid accumulation in *Rhodotorula* strains cultured at 4 °C under dark (**A**) and light (**B**) conditions. Carotenoid content, expressed as β-carotene equivalent, was reported as mean ± SD. Statistically significant differences ($p < 0.05$) in carotenoid level accumulated by a specific yeast strain compared to the 2-h timepoint were marked by triangles.

To investigate the effect of lighting on carotenoid accumulation in various *Rhodotorula* strains grown at low temperature, yeasts were first cultivated for 2 days under light conditions at 26 °C, then transferred to 4 °C and continuously exposed to LED illumination. In most strains, the pigment levels remained unchanged during the entire incubation period at a 4 °C temperature (Figure 3B). Only *R. graminis* and *R. kratochvilovae* showed an increase in carotenoid content by about 30% on the third and fifth days, respectively. In *R. glutinis*, carotenoid content increased by approximately 60% after one week of incubation, followed by a gradual decline.

It has been observed that a lower temperature favors carotenoid production in *R. acheniorum*, whereas *R. diobovata* produces higher levels of carotenoids at 30 °C [12]. The low temperature caused increased carotenoid biosynthesis in *R. toruloides* yeasts [39]. It was demonstrated that carotenoid content and composition in *R. glutinis*, *R. mucilaginosa*, and *R. gracilis* yeasts changed after they were cultured at a low temperature [19]. In some *R. glutinis* strains, carotenoid synthesis increases at temperatures above 30 °C, while for *R. mucilaginosa*, carotenoid production decreases at temperatures over 30 °C [14].

3.4. Comparative Analysis of Temperature and Lighting Impact on Carotenoid Content in Rhodotorula spp.

To highlight the influence of temperature and light exposure on carotenoid production and stability, we conducted one-way ANOVA on the pigment content in *Rhodotorula* spp. isolates after two weeks of incubation under different conditions (Figure 4). A comparative analysis of carotenoid levels, expressed as β-carotene equivalents, in *Rhodotorula* strains cultivated at 26 °C under light or dark conditions demonstrated that dark conditions promote carotenoid accumulation and stability across all strains tested. A statistically significant difference in carotenoid content was observed across *Rhodotorula* isolates between light and dark treatments at 26 °C ($F = 20.56$, $p = 0.000041$). A comparison of pigment levels, in yeasts cultivated at 4 °C and at 26 °C under dark conditions, revealed that overall carotenoid content per gram of biomass was significantly higher at the elevated temperature ($F = 5.29$, $p = 0.026$).

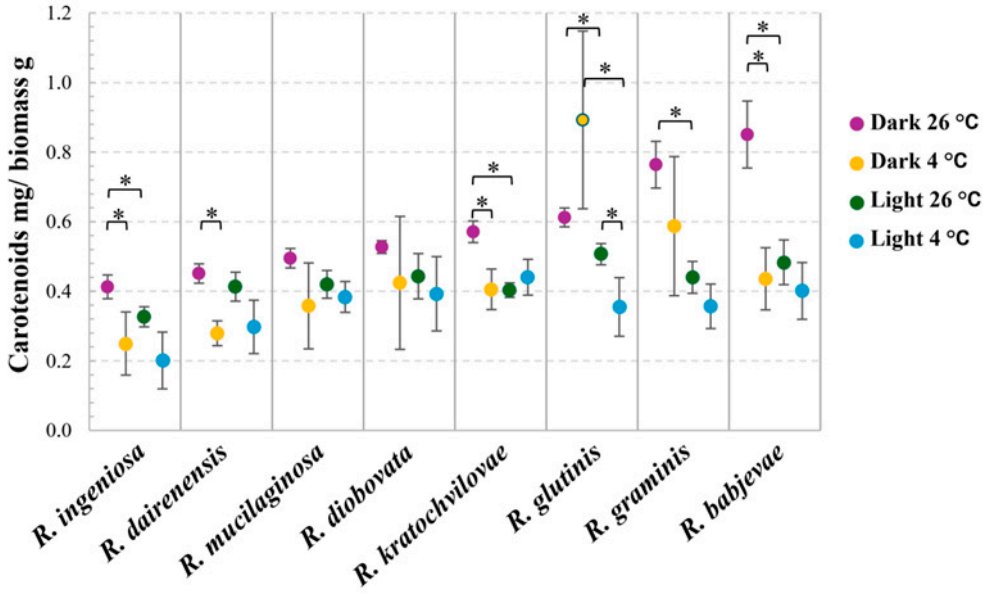


Figure 4. Carotenoid accumulation in *Rhodotorula* strains after 14-day cultivation under varying temperature and lighting conditions. Dark 26 °C (purple), dark 4 °C (orange), light 26 °C (green), and light 4 °C (blue) conditions. Carotenoid content, expressed as β-carotene equivalent, is reported as mean ± SD ($n = 3$). Significant differences ($p < 0.05$) are indicated with asterisks.

R. ingeniosa, *R. kratochvilovae*, and *R. babjevae* strains accumulated significantly higher carotenoid content when cultivated in the dark at 26 °C, compared to both light conditions at the same temperature and dark conditions at 4 °C. These results indicate that both temperature and light significantly influence carotenoid accumulation in these strains. Notably, light exposure had a consistently negative effect on pigment synthesis, as evidenced by the reduced carotenoid levels under illuminated conditions. In *R. dairenensis*, temperature appears to have a significantly greater effect on carotenoid accumulation than light exposure, as the carotenoid content in the dark at 26 °C was approximately 38% higher than under dark conditions at 4 °C. *R. graminis* exhibited significantly higher carotenoid content when cultivated in the dark at 26 °C compared to light conditions at the same temperature, indicating suppressive effect of light, while temperature had no significant impact. Among all tested strains, *R. glutinis* was unique in displaying differences across multiple conditions at 26 °C and 4 °C. Notably, it was the only strain that demonstrated a significant increase in pigment level when incubated in the dark at 4 °C, reaching a carotenoid level of 0.89 mg/g biomass, suggesting enhanced pigment stability at lower

temperatures. *R. mucilaginosa* and *R. diobovata* did not exhibit any statistically significant differences in carotenoid accumulation under the tested temperature and light conditions.

Exogenous factors such as temperature and light are important for carotenogenesis in yeast [34,36,37,39]. Light intensity and duration can either stimulate or inhibit biosynthesis of carotenoids in fungi [12]. High temperatures are generally beneficial for both carotenoid production and cell growth; however, they may also lead to the denaturation of enzymes involved in carotenoid biosynthesis [12,14]. In contrast, low temperatures reduce the metabolic activity and energy production of microbial cells, which may negatively impact carotenoid synthesis [19]. Exposure of cells to visible light or temperature shifts may induce an ROS stress response, increasing the production of free radicals in yeast cells, and thus affecting carotenoid biosynthesis [19,40,41]. The mechanism by which yeasts can adapt to low temperature stress and intensify the biosynthesis of carotenoids needs to be clarified [42]. Nevertheless, not only exogenous conditions but also features of yeast producers may affect the synthesis and accumulation of different types of carotenoids [39].

4. Conclusions

Eight yeast strains of the genus *Rhodotorula* species were isolated from environmental samples and identified via ITS rDNA sequencing. It was demonstrated that carotenoid production by different *Rhodotorula* strains was influenced by both light conditions and temperature. The carotenoid content of the *Rhodotorula* yeasts tested was lower under light than under dark conditions. In most yeast strains, cultivation at 26 °C resulted in higher pigment accumulation than incubation at 4 °C. The highest level of pigment was accumulated by *R. babjevae*, *R. glutinis*, and *R. graminis* cells, while the lowest was by *R. ingeniosa*. The isolated *Rhodotorula* strains, when applied under optimized lighting and temperature conditions, could be promising for the efficient synthesis of carotenoids.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation11070412/s1, Figure S1: Phylogenetic tree of *Rhodotorula* species inferred from partial sequences of the ITS region (ITS1-5.8S-ITS2).

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