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Efficient carotenoid biosynthesis by *Rhodococcus corynebacterioides* utilizing nutrient-rich oilseed cakes as sustainable substrates

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ABSTRACT

Carotenoid pigments are in growing demand across the food, feed, and pharmaceutical industries, yet their commercial production remains expensive and reliant on unsustainable feedstocks. While various agricultural wastes have been explored to lower costs, achieving commercially viable yields remains a challenge. This study presents a low-cost, environmentally sound strategy for scaling up carotenoid production using the bacterium *Rhodococcus corynebacterioides*. Notably, this represents the first successful report of utilizing this specific strain grown on four de-oiled agro-waste cakes: peanut, sesame, sunflower, and coconut, which are otherwise treated as low-value byproducts of the oil industry. Among the four substrates tested, sesame cake proved the most productive. Optimization of the fermentation conditions established that the following conditions led to the highest crude pigment production: a temperature of 30°C, pH 7.0, 10% (w/v) sesame cake, 3% (w/v) NaCl, and constant light exposure. The optimized medium and process parameters resulted in a high crude pigment extract yield of 5.4 g/L, which strongly competes with or exceeds previously reported agro-waste-based carotenoid studies. Spectroscopic and chromatographic analysis tentatively identified the crude extract as containing carotenoid/xanthophyll-class pigments. Given the high lipid and protein content of these agro-waste cakes, it is plausible that their metabolic breakdown supplies key biosynthetic precursors that feed into the carotenoid methylerythritol phosphate pathway of *Rhodococcus*. Taken together, these findings position de-oiled agro-waste cakes, particularly sesame cake, as a promising, low-cost feedstock for sustainable carotenoid production at scale.

1. INTRODUCTION

Carotenoids are a class of extremely useful isoprenoid pigments with a large conjugated polyene chromophore, with characteristic absorbance in the green part of the visible spectrum (400–550 nm), imparting yellow, orange, or red color [1]. Carotenoids, such as β-carotene, lycopene, astaxanthin, lutein, and zeaxanthin, have immense market demand for their applications as antioxidants, nutraceuticals, and natural colorants [2,3]. They find wide application in industries, including food and beverages, animal feed, dietary supplements, pharmaceuticals, and cosmetics. In order to meet the industrial demand, many types of natural carotenogenic microorganisms have been widely applied. For instance, commercial production of β-carotene and lycopene mainly relies on the filamentous fungus *Blakeslea trispora* and the halophilic microalga *Dunaliella salina*. The major sources of astaxanthin are *Haematococcus pluvialis*, a microalga; *Xanthophyllomyces dendrorhous* (formerly known as *Phaffia rhodozyma*), a yeast; and *Paracoccus carotinifaciens*, a bacterium. Lutein and zeaxanthin are known to be produced by microalgae such as *Scenedesmus* spp. and *Chlorella* spp. and by bacteria in the genus *Sphingomonas* [4,5]. Moreover, microbial natural carotenoids are being increasingly preferred as sustainable alternatives to synthetic colors in the health and food sectors [6,7].

The global market of carotenoids is projected to reach USD 3.4 billion by 2029 with a CAGR of 6.3% [8]. Although carotenoids have traditionally been sourced from plants, these are also produced by microorganisms. Plant carotenoid extraction is often hindered by seasonal availability and inconsistent yields. In contrast, the microbial fermentation of de-oiled sesame cakes enables the continuous, climate-independent production of carotenoids. Microbial fermentation is an attractive alternative to plant carotenoids due to its scalability, better control in laboratory conditions, economy, and significantly higher yields [5].

Cold-pressed oil cake is the nutrient-rich solid residue remaining after oil extraction from seeds such as groundnut, coconut, sesame, and sunflower. These de-oiled cakes are protein-rich, energy-dense by-products commonly used as livestock feed and organic fertilizer

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and were evaluated here as a microbial growth substrate. Residual saturated and unsaturated fatty acids, along with substantial protein, vitamins, and minerals, can provide essential nutrients that support bacterial growth and secondary metabolite production [9]. Oleic acid, a monounsaturated fatty acid, makes up the majority of peanut oil; linoleic acid, a polyunsaturated fatty acid, is abundant in sesame oil; linoleic acid and other polyunsaturated fatty acids are abundant in sunflower oil; and saturated fats make up the majority of coconut oil.

It is hypothesized that lipids in oilseed cakes support carotenoid production by intracellular carbon channeling. Specifically, the β-oxidation of abundant lipids yields excess acetyl-CoA, which is assimilated through the glyoxylate shunt. This pathway routes carbon directly into gluconeogenesis, ensuring a steady supply of both pyruvate and glyceraldehyde-3-phosphate (G3P), the two essential precursors for the methylerythritol phosphate (MEP) pathway [10,11]. The current study investigated the possibility of employing de-oiled seed cake as a base medium for the synthesis of carotenoids using an isolate of *Rhodococcus corynebacterioides*. It produces a very attractive orange pigment (Supplementary Fig. 1), is non-pathogenic, is easy to culture on agro-waste, and has a short production period, which makes it an ideal candidate organism for industrial carotenoid pigment production.

2. MATERIALS AND METHODS

2.1. Screening and Isolation of Pigment-producing Bacteria

Soil samples were collected from the MIT-WPU garden in Pune in sterile containers. Samples were then serially diluted in sterile saline solution and spread on nutrient agar plates. These were incubated at 30°C for 24–48 hours. The pure culture was obtained and maintained in nutrient broth. The genomic DNA was isolated using the HiPureA Bacterial DNA Purification Spin Column Kit (MB505-250PR, HiMedia, India). The 16S rRNA gene was amplified by PCR using universal primers F27 (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' GGTTACCTTGTTACGACTT 3') [12]. The PCR product was purified and submitted to the National Collection of Industrial Microorganisms (NCIM), CSIR-NCL, Pune, Maharashtra (India), for Sanger sequencing.

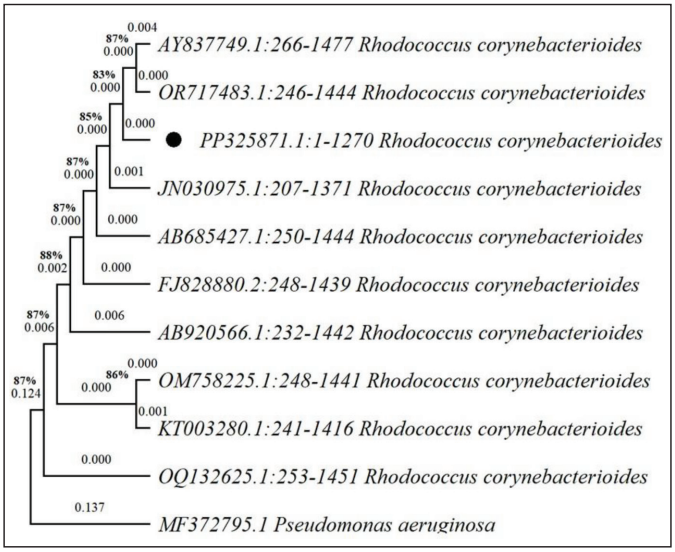

Figure 1. Molecular phylogenetic analysis of *R. corynebacterioides* strain PPSC 1604 based on 16S rRNA gene sequences. Maximum Likelihood tree (MEGA 11, Tamura–Nei model) constructed from a MUSCLE alignment (1,270 bp), with node values showing bootstrap support from 1,000 replicates. Taxon labels include GenBank accession numbers for all comparator sequences. Isolate PP325871 (●) shares 100% sequence identity with references OR717483.1, AB685427.1, and FJ828880.2 and 99.2-99.9% identity with all other *R. corynebacterioides* comparators shown (pairwise identity matrix, ClustalW). *Pseudomonas aeruginosa* (MF372795.1) is the outgroup (76.4% identity to the isolate).

2.2. Nutritional Profile of Deoiled Cake Media

2.2.1. De-oiled cake collection and preparation

The 10% oil seed cake medium was formulated by homogenization in a mechanical grinder. Homogenized de-oiled seed cake was centrifuged and sieved using muslin cloth to remove separable solids. The dry matter content was determined by oven-drying the samples at 105°C until a constant weight was achieved. The nutritional profile was characterized using established analytical protocols. Total crude protein was quantified by Bradford's assay, using bovine serum albumin as the standard [13]. Lipid determination was performed using a chloroform-methanol extraction system as per the Bligh and Dyer method [14]. Total carbohydrate content was determined by the phenol-sulfuric acid method [15]. Total ash content was quantified by gravimetric analysis after complete mineralization of the samples in a muffle furnace at 550°C [16]. All analyses were performed in triplicate.

2.3. Oil Cake Media Preparation and Optimization for Pigment Production

The de-oiled oilseed cakes, such as groundnut, sesame, sunflower, and coconut, were procured from local oil mills, screened for impurities, and ground into fine powder. The media were made by grinding oil cakes to a concentration of 6% w/v in distilled water. After grinding, the mixture was sieved and centrifuged at 4,000 rpm at 25°C for 10 minutes. The particle-free supernatant was added to agar-agar powder for solid media preparation, and liquid media were autoclaved at 121°C for 20 minutes, and an actively growing culture was inoculated and incubated at 30°C for 72 hours at 180 rpm of shaking.

2.3.1. Effect of initial pH on pigment production

A series of growth media was prepared with varying pH levels using formulated sesame as a base medium. The pH of the media was adjusted to varying pH values of 5, 6, 7, 8, and 9 using sterile 0.1N HCl or 0.1N NaOH. These were inoculated with an actively growing bacterial culture ($OD_{600\ nm}$ = 0.5) and incubated at 30°C for 72 hours with shaking at 180 rpm. After incubation, the culture was harvested, and the crude pigment was extracted with ethanol. The yield of the crude pigment extract was determined gravimetrically after solvent evaporation [17].

2.3.2. Effect of temperature on pigment production

A series of 6% (w/v) sesame-based growth media were prepared as per section 2.3 and inoculated with actively growing bacterial culture (OD600 nm = 0.5). The cultures were incubated at different temperatures (20°C, 30°C, 40°C, and 50°C) for 72 hours at 180 rpm. After incubation, the cells were harvested by centrifugation, and the crude pigment was extracted with ethanol; the crude pigment extract yield was determined after solvent evaporation using the established protocol [18].

2.3.3. Effect of substrate concentration on pigment biosynthesis

The sesame oil seed cake medium was formulated using the methodology given in section 2.3 but by varying the concentration of sesame cake in distilled water (10% w/v, 8% w/v, 6% w/v, and 4% w/v) and inoculated with an actively growing bacterial culture (OD600 nm = 0.5). The inoculated flasks were incubated at 30°C for 72 hours at 180 rpm. The pigment was extracted with ethanol using the established extraction protocol, and the yield of the crude pigment extract was determined gravimetrically after solvent evaporation.

2.3.4. Effect of salinity on pigment synthesis

Sesame-based liquid media (6% w/v) were amended with varying salinity using sodium chloride (1% w/v, 2% w/v, 3% w/v, 4% w/v, and 5% w/v NaCl) and inoculated with a bacterial culture at OD600 nm = 0.5. Cultures were incubated at 30°C for 72 hours with shaking at 180 rpm. Biomass was harvested and the pigment extracted using the optimized ethanol protocol (including sonication). The solvent was evaporated, and the final crude pigment extract yield was gravimetrically quantified [19].

2.3.5. Effect of photoperiod on pigment synthesis

Three different light regimes were created to assess the effect of photoperiodic conditions on *R. corynebacterioides* pigment biosynthesis. A 6% sesame medium was prepared, sterilized, and inoculated with a 0.5 OD standardized bacterial culture. The cultures were then split into three groups: photopic (continuous illumination), photoperiod (standard laboratory light exposure), and scotopic (achieved by tightly wrapping the flasks in aluminum foil). For 72 hours, all cultures were incubated at 30°C and 180 rpm under standard ideal conditions. Following incubation, the microbial biomass from each photoperiod condition was harvested (via centrifugation and drying), and the pigment was extracted using ethanol following the previously optimized protocol (including sonication). The solvent was subsequently removed via evaporation, and crude pigment extract yield was gravimetrically quantified to determine the effect of light exposure on pigment synthesis [20].

2.3.6. Validation of optimized bioprocess parameters

A validation experiment was carried out to determine the performance of the entire bioprocess by implementing all the optimal conditions that have been identified before. The fermentation mixture was formulated using a constant concentration of the base medium of 10% (w/v) sesame cake mixed with 3% (w/v) NaCl, with a working volume of 50 ml in a 250 ml Erlenmeyer flask. The initial pH of the solution was set to 7.0 and inoculated using a standardized 2% (v/v) bacterial inoculum at an optical density of 0.5. The fermentation was allowed to continue for 72 hours at 30°C in the presence of continuous illumination at 180 rpm. The fold increase in pigment production was calculated by dividing the absolute crude pigment extract yield (g/L) achieved under the optimized integrated parameters by the baseline yield obtained in the unoptimized nutrient broth.

2.4. Pigment Extraction from Biomass

The cells were separated by centrifugation at 9,000 for 5 minutes. After decanting the supernatant, the cell pellet was dried at 50°C for 10 minutes to remove residual moisture. The pigment was extracted into ethanol (or other solvents). The cells were subsequently allowed to incubate in the respective solvents for a period of 24 hours at 30°C to facilitate cell wall breakdown and pigment solubilization. Subsequently, an ultrasonication cycle was also carried out for 10 minutes to facilitate complete pigment extraction. The cell debris was separated by centrifugation at 9,000 for 20 minutes after sonication. The supernatant containing solubilized pigment in solvent was evaporated to obtain dry crude pigment extract. Solvents with varying polarity indices (P'), specifically methanol (5.1-6.6), ethanol (4.3-5.2), acetone (5.1), isoamyl alcohol (3.9), n-butanol (4.0), chloroform (4.1), diethyl ether (2.8), and hexane (0.1) were selected for extraction. For each trial, dried biomass from 2 ml of broth was accurately measured and resuspended in a 2 ml volume of the respective solvent [18,21,22]. For every gravimetric determination, a matched uninoculated-medium blank, solvent-only blank, and pigment-free biomass control (model *Bacillus velezensis*) were carried through the identical extraction, evaporation, and weighing procedure in parallel, and the corresponding blank mass was subtracted from the crude extract mass to correct for non-microbial, solvent-extractable material (residual lipids, phenolics, and other cake-derived compounds) native to the oilseed cake medium.

2.5. Time Course of Bacterial Growth and Pigment Production

A pure culture was used to inoculate 500 ml of 6% sesame broth to determine the growth kinetics and concurrent pigment production profile of *R. corynebacterioides*. The culture was incubated at the optimized temperature of 30°C with continuous orbital agitation maintained at 180 rpm in a shaker incubator until pigment production reached saturation. Regular samples were obtained aseptically at predetermined intervals of 6 hours. Serial 1:10 dilutions were made to calculate the total viable count (CFU/ml). Appropriate dilutions were surface-plated onto nutrient agar plates and incubated at 30°C for 24–48 hours. A cell pellet was obtained by centrifuging a predetermined volume of culture at each time point. To track the crude pigment extract yield and correlate its biosynthesis with the various stages of the culture's growth cycle, pigment was extracted from this pelleted biomass using the standard solvent extraction protocol (using ethanol and sonication). This was then quantified gravimetrically.

2.6. Structural Analysis of Carotenoid Pigment

2.6.1. UV-visible spectroscopy

The dried crude pigment was re-dissolved in ethanol, and a spectral scan (200 to 800 nm) was captured using a BMG Labtech Vantastar® multimode plate reader.

2.6.2. Fourier-transform infrared spectroscopy (FTIR)

The orange crude pigment extracted from *R. corynebacterioides* was subjected to attenuated total reflectance Fourier-transform infrared spectroscopy using a Bruker Alpha II. The resulting infrared spectrum (4,000–400 cm^{-1}) was recorded and examined for distinctive absorption bands.

2.6.3. High-performance liquid chromatography

Reversed-phase high-performance liquid chromatography analysis was performed using a method modified from Gupta et al. [23] with WATERS HPLC system equipped with a photodiode array (PDA) detector, manual injector, C30 column (3 µm, 250 × 4.6 mm) with a matched guard column, mobile phase A: methanol/MTBE (tert-methyl butyl ether) (98:2 v/v), and B: MTBE, gradient elution at 1 ml/minute. 0 minutes: 100% A (0% B), 15 minutes: 70% A (30% B), 30 minutes: 50% A (50% B), 45 minutes: 20% A (80% B), 46 minutes: 100% A (0% B), 55 minutes: 100% A, 20 µl injection volume, 20°C column temperature, and PDA acquisition (250–700 nm) [23].

2.7. Statistical Analysis

All experiments were performed in triplicate (n = 3 biological replicates). Data are expressed as the mean ± SD. Statistical analyses were performed using GraphPad Prism software. Homogeneity of variance was confirmed using the Brown–Forsythe test ($p > 0.05$). Statistical significance among multiple independent groups for each optimization parameter was determined using a one-way analysis of variance (ANOVA), followed by a Tukey's post-hoc multiple-comparison test. A p-value of <0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Carotenoid-Producing Isolate Identified as *R. corynebacterioides*

Soil samples were collected from a garden located in Dr. Vishwanath Karad MIT World Peace University. The initial screening and isolation process yielded several bacterial colonies on nutrient agar plates. A single colony exhibiting a notably bright orange phenotype was selected for further study. The bright orange pigment producer colonies were 2–3 mm in size, round, opaque, and slightly elevated (Supplementary Fig. 1). The strain was found to be Gram-positive. The identity of the isolated strain was determined using 16S rRNA gene sequencing. The 16S rRNA gene sequence was submitted to the National Center for Biotechnology Information (accession number PP325871). Sequences were aligned using the MUSCLE algorithm, and a maximum likelihood phylogenetic tree was reconstructed in MEGA 11 using the Tamura–Nei substitution model, with node support assessed from 1000 bootstrap replicates; *Pseudomonas aeruginosa* (MF372795.1) served as the outgroup (Fig. 1). Pairwise sequence identity analysis (ClustalW) showed that isolate PP325871 shares 100% nucleotide identity with three *R. corynebacterioides* reference sequences (OR717483.1, AB685427.1, and FJ828880.2) and 99.21%–99.91% identity with the remaining *R. corynebacterioides* comparators included in the analysis (JN030975.1: 99.91%; OM758225.1: 99.82%; AY837749.1: 99.82%; KT003280.1: 99.74%; AB920566.1: 99.65%; OQ132625.1: 99.21%) (Supplementary data Clustal2.1), compared with only 76.35% identity to the *P. aeruginosa* outgroup (Fig. 1). Several shallow intra-clade nodes show comparatively low bootstrap support, consistent with the high sequence conservation of the 16S rRNA gene among closely related *Rhodococcus* strains; this does not affect the species-level assignment, which is independently supported by the pairwise identity values above.

3.2. Nutritional Analysis of De-oiled Cakes

The nutritional analysis of the prepared oil cake media revealed high nutrient density across all samples. The protein, carbohydrate, lipid, and ash contents of the dry mass are tabulated in Table 1. Peanut and sesame cake contained higher concentrations of protein than the oilseed cakes tested. Coconut oilseed cake contained the highest concentration of lipids, whereas peanut oilseed cake had the lowest. The total carbohydrates were quantified, with peanut oil cake showing the highest concentration. Mineral content, indicated by total ash, was most prominent in sesame (19.53%) (Table 1). For context, literature values of other elemental parameters such as ash, calcium, and phosphorus content for these cakes are well-documented in previous studies [24,25]. Collectively, these findings confirm that the tested oilseed cakes provide essential macronutrients and minerals. Consequently, they represent a highly suitable and nutrient-dense substrate fully capable of supporting robust bacterial growth. The oilseed cakes evaluated in this study were procured from a local cold-pressed oil mill in Jalgaon, Maharashtra; however, as with all agricultural byproducts, they are subject to inherent compositional variability. Batch-to-batch fluctuations in the residual protein, lipid, carbohydrate, and ash fractions are common and can be largely attributed to pre-harvest factors, including geographical origin, soil fertility, climatic conditions, and the specific genetic cultivars of the seeds.

3.2.1. De-oiled cakes as microbial nutritional media

The investigation demonstrated that agro-industrial de-oiled seed cakes can serve as substrates for *R. corynebacterioides* growth in solid medium (supplementary Fig. 2) and support pigment biosynthesis in liquid medium (supplementary Fig. 2) as well as support high microbial pigment biosynthesis in liquid medium (supplementary Fig. 3). The highest crude pigment yield was achieved in the sesame oil cake medium, yielding 4.4 g/L (Fig. 2a). This yield was substantially higher than the control medium nutrient broth. Oil cakes are more than just carbon sources; they are "nutrient-dense" reservoirs that include leftover lipids, proteins, and minerals such as magnesium, calcium, and selenium, which can function as cofactors for enzymes in the carotenoid biosynthesis pathway [26,27]. Higher yield in sesame oil cake can be attributed to its unique composition of higher content of soluble macronutrients as well as high micronutrient content, such as bioactive phenolic compounds and sulfur-containing amino acids. We hypothesize that these specific compounds shown to support the synthesis of antioxidant pigments in related actinobacteria [28]. The improved nutritional profile of oil cakes can explain the much greater carotenoid pigment output found in the sesame oil cake medium 4.4 g/L compared to prior results employing molasses as a substrate. Unlike molasses, which primarily supplies carbon sources, sesame oil cake contains a varied range of nutrients such as proteins, lipids, carbohydrates, minerals, and bioactive substances, all of which may contribute to increased microbial growth and metabolic activity. This dietary richness most likely promotes the activation of carotenoid biosynthesis pathways, resulting in significantly increased pigment accumulation. Thus, changes in substrate complexity and nutritional content fundamentally explain the diversity in crude pigment extract yields.

Table 1. Proximate nutritional composition of the 10% four de-oiled oilseed cake media (crude protein, total carbohydrate, lipid, and ash content), expressed as a percentage of total dry matter, with the unaccounted fraction representing crude fiber and other non-assayed components.

Oil cake media	Total dry matter (% w/v)	Protein (% of DM)	Carbohydrate (% of DM)	Lipid/Fat (% of DM)	Total Ash (% of DM)	Unaccounted (Fiber/ Other)
Peanut	5.10 ± 0.05	47.06 ± 0.02	23.53 ± 0.03	10.00 ± 0.02	9.22 ± 0.02	10.19 ± 0.04
Sesame	6.40 ± 0.04	43.75 ± 0.04	15.63 ± 0.02	15.47 ± 0.03	19.53 ± 0.01	5.62 ± 0.03
Sunflower	5.90 ± 0.03	33.90 ± 0.04	16.95 ± 0.06	14.41 ± 0.05	10.17 ± 0.02	24.57 ± 0.03
Coconut	4.80 ± 0.03	22.92 ± 0.05	20.83 ± 0.04	16.88 ± 0.04	8.75 ± 0.03	30.62 ± 0.02

*Note: The unaccounted percentage represents unassayed organic matter, primarily comprising crude fiber and non-starch polysaccharides inherent to the oil cake biomass. DM = Dry Matter.

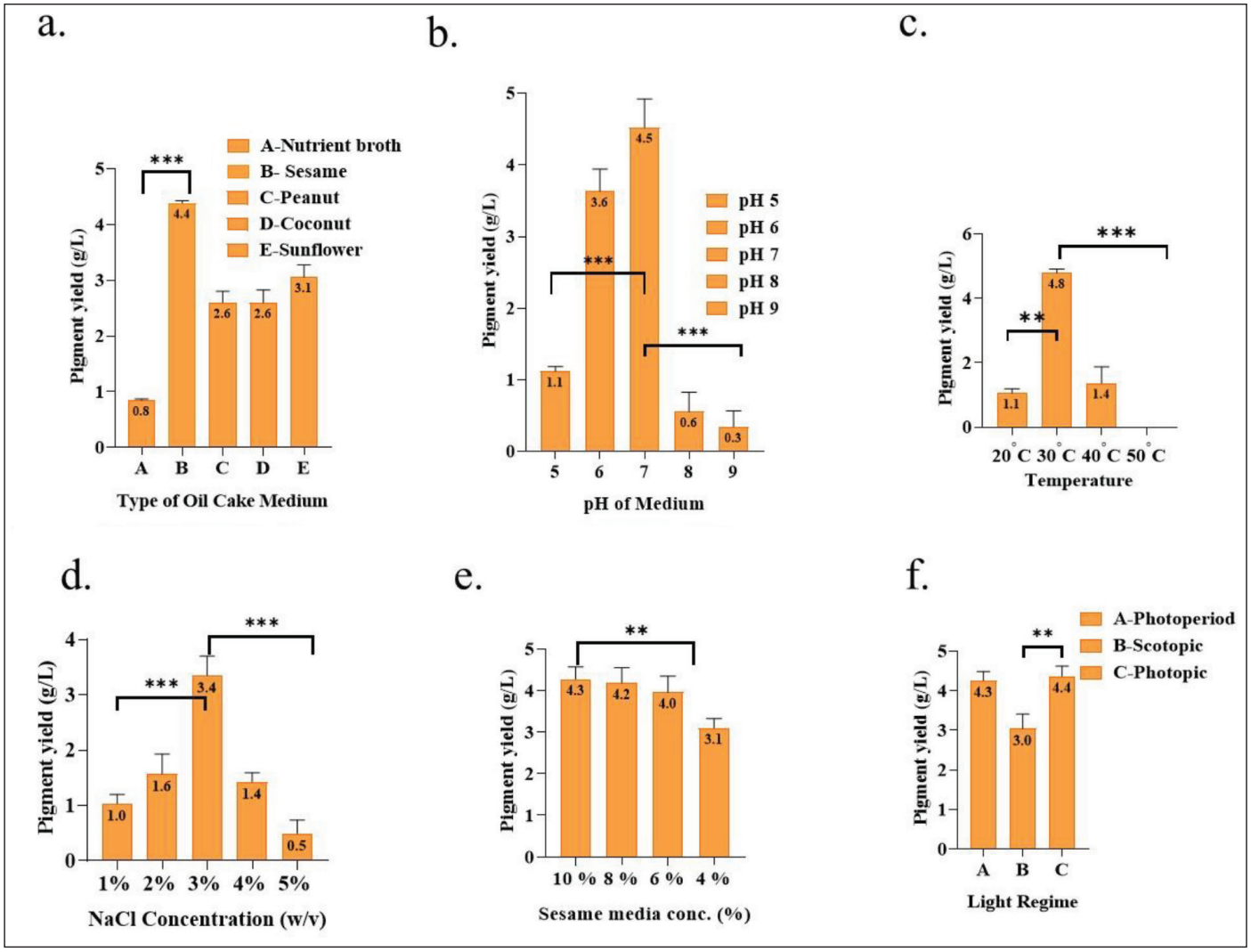

Figure 2. Optimizing carotenoid production by *R. corynebacterioides*. **a.** Oil cakes and crude pigment extract yield: Sesame oil cake shows the highest yield (4.4 g/L) (***, $p < 0.001$), indicating a *significant difference* between the sesame media and the control (A-nutrient broth). **b.** Effect of initial pH on pigment production: the maximal crude pigment extract yield (4.5 g/L) was at the neutral optimum of pH 7.0 (***, $p < 0.001$). **c.** Temperature optimization of pigment biosynthesis: the maximal yield (4.8 g/L) confirms 30°C as the optimal temperature (**, $p < 0.01$; ***, $p < 0.001$). **d.** Effect of sesame oil cake concentration (%): The maximal yield (4.3 g/L) was achieved at the 10% (w/v) concentration, representing a significant increase compared to the 4% treatment group (**$p < 0.01$). **e.** Effect of NaCl % in media (Salinity): The optimal salinity was found to be 3% NaCl, yielding 3.4 g/L of crude pigment. This optimal salinity outperformed both lower (1%) and higher (5%) NaCl concentrations (***, $p < 0.001$). **f.** Effect of exposure to light (Photoperiod): Continuous illumination (photopic) achieved the highest crude pigment extract yield (4.4 g/L). (**, $p < 0.01$). Data are presented as mean ± SD ($n = 3$ independent biological replicates). Statistical significance was determined by one-way ANOVA with Tukey's post-hoc test. Brackets indicate specific pairwise comparisons.

3.2.2. Effect of media pH

The initial growth medium pH of 7.0 was found to be the most favorable for pigment production (4.5 g/L), while alkaline environments were the most detrimental, with pH 9.0 yielding only 0.3 g/L (Fig. 2b) (Supplementary Fig. 4). This sensitivity suggests that the enzymatic pathways responsible for pigment synthesis are highly pH-dependent, likely facing denaturation or inhibited substrate transport under strongly alkaline conditions. Consequently, maintaining a neutral environment is essential for maximizing secondary metabolite accumulation in this bioprocess [17].

3.2.3. Effect of culture temperature

The ideal growth temperature for pigment production was found to be 30°C, with high crude pigment production of 4.8 g/L. Specifically, at lower temperatures, 20°C, production was limited to 1.1 g/L, while thermal stress at 50°C proved nearly inhibitory, with yields dropping to 0 g/L (Fig. 2c). The significant variance ($p < 0.001$) between 30°C and 50°C highlights the narrow thermal window required for efficient bioprocessing in this system. Pigment accumulation decreased significantly at both lower and higher temperatures, indicating the sensitivity of the biosynthesis pathway to thermal deviations [29].

3.2.4. Effect of oilseed cake concentration

The maximal crude pigment extract yield was consistently achieved with 10% w/v sesame, which resulted in 4.3 g/L of pigment (Fig. 2d). Pigment accumulation showed a clear concentration-dependent decrease as the substrate was diluted: the 8% medium produced a yield of 4.2 g/L, and the 6% medium yielded 4 g/L. The lowest crude pigment extract yield, 3.1 g/L, was recorded at the most dilute concentration, 4% w/v (Fig. 2d). The concentration-dependent rise in carotenoid

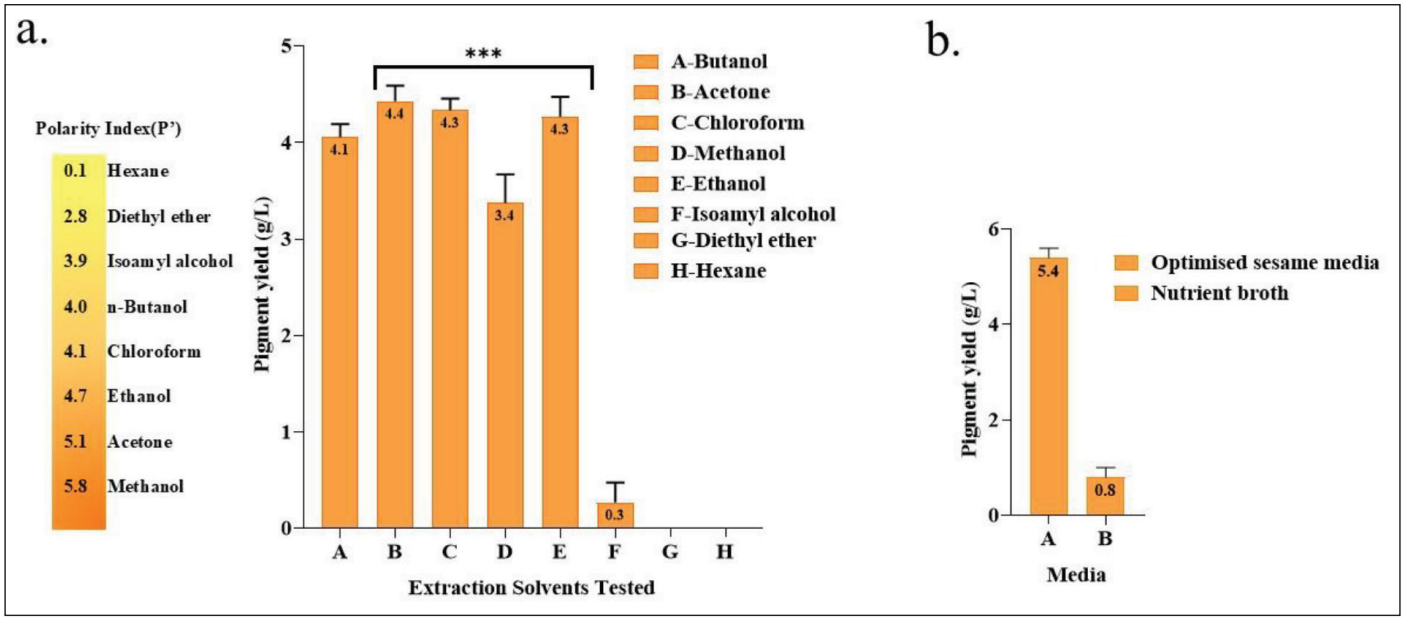

Figure 3a. Selection of Extraction Solvent: Acetone (4.4 g/L) and Chloroform (4.3 g/L) demonstrated maximal extraction efficiency, which was highly significant (***, $p < 0.001$) compared to the negligible recovery observed with nonpolar solvents such as hexane (H). Data are presented as mean ± SD ($n = 3$ biological replicates). Statistical significance was determined by one-way ANOVA with Tukey's post-hoc test. Brackets indicate specific pairwise comparisons. **3b**. Optimized Bioprocess Parameters Integrated Validation: Absolute yield of crude pigment extract on the optimum sesame cake medium compared to nutrient broth as the baseline.

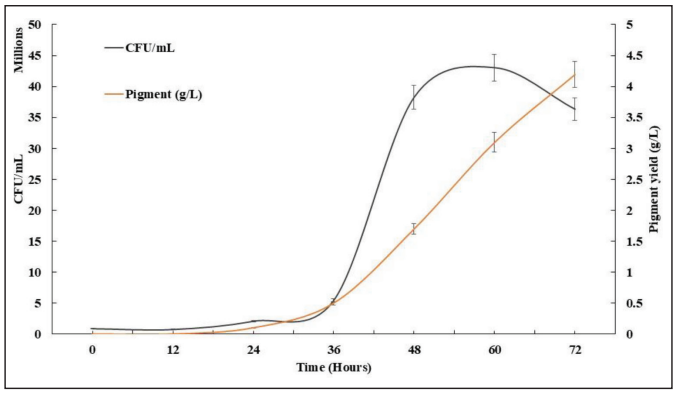

Figure 4. Growth kinetics and pigment production profile of *R. corynebacterioides*: bacterial growth (CFU/ml, left axis, grey line) and crude pigment extract yield (g/L, right axis, orange line) over a 72-hour incubation in sesame cake medium at 30°C, 180 rpm. Data are mean ± SD ($n = 3$ independent biological replicates). Viable cell count peaked at 4.3×10^7 CFU/ml at 60 hours before plateauing; pigment accumulation continued through the transition to stationary phase, reaching a maximum of 4.3 g/L at 72 hours, indicating partially growth-associated biosynthesis.

output, culminating at 10% (w/v) sesame substrate, highlights the need for high carbon and nitrogen availability associated with the energy-intensive isoprenoid pathway. The high crude pigment extract yields achieved on oilseed cakes suggest that their nutritional profile may support MEP pathway activation. Based on established *Rhodococcus* metabolism, we hypothesize that, while protein catabolism contributes basic carbon skeletons, the predominant lipid fraction likely undergoes β-oxidation, generating Adenosine triphosphate, reducing equivalents, and excess acetyl-CoA. Theoretically, rather than entering the standard Tricarboxylic Acid Cycle, this acetyl-CoA could be assimilated via the glyoxylate shunt. This would bypass decarboxylation and funnel carbon into gluconeogenesis, thereby potentially sustaining the intracellular pools of pyruvate and G3P necessary for robust MEP pathway biosynthesis. Increasing the concentration of solid oilseed cakes beyond 10% visibly increased the viscosity of the medium; as noted in previous literature, such high viscosity can hinder the oxygen transfer rate [30].

3.2.5. Media salinity enhances carotenoid biosynthesis

The maximal crude pigment extract yield recorded was 3.4 g/L at a 3% (w/v) NaCl concentration (Fig. 2e). Crude pigment extract yield was lower at the extremes of the tested range. This response pattern suggests that *R. corynebacterioides* is moderately halotolerant; the 3% NaCl level appears to enhance the metabolic activity specifically linked to pigment synthesis, while excessive salinity may inhibit overall bacterial growth and subsequent pigment production [31]. Research indicates that carotenoid compound levels can rise by 2–4 times at 1–3 M NaCl concentrations compared to low salinities. Environmental conditions such as salinity can lead bacteria to accumulate carotenoids as a defensive response to oxidative stress. Strategic use of abiotic stressors such as salt can significantly increase carotenoid accumulation in bacteria [29].

3.2.6. Photopic conditions critically enhances carotenoid yield in R. corynebacterioides

The investigation into the influence of different photoperiodic conditions on pigment biosynthesis demonstrated that light exposure positively correlates with yield. The maximal crude pigment extract yield was recorded under photopic (continuous illumination), resulting in 4.4 g/L (Fig. 2f), with the day-night cycle following closely at 4.3 g/L. Conversely, the lowest crude pigment extract yield, 3.0 g/L, was observed in scotopic. This significant reduction suggests that the biosynthetic pathway for this orange pigment is likely photo-inducible or that light energy plays a direct or indirect role in enhancing the metabolic rate necessary for maximal pigment accumulation in *R. corynebacterioides*. Carotenoids predominantly function as

photoprotectants in non-phototrophic bacteria, serving as antioxidants to quench reactive oxygen species and singlet oxygen produced by light exposure. The considerable increase in production reported under continuous lighting is consistent with processes described in other bacteria [32,33].

3.2.7. Integrated parameters showed enhanced pigment production

The combined optimum parameters—10% (w/v) sesame cake, 3% (w/v) NaCl, starting pH 7.0, temperature 30°C, and continuous light exposure—resulted in a total crude pigment extract yield of 5.4 g/L (Fig. 3b). Cultivation under the integrated optimal conditions resulted in a remarkable 6.75-fold enhancement in crude pigment extract yield (5.4 g/L) compared to the unoptimized baseline in standard nutrient broth (0.8 g/L). Importantly, replacing the expensive commercial NB with a 10% sesame cake medium not only maximized biosynthetic output but also established a highly cost-effective, agro-industrial byproduct-based approach for economically viable scale-up. The main goal was to identify the primary driving variables and establish proof-of-concept for using sesame cake, and evaluating the complex multi-variable interactions is the necessary next step for bioreactor scale-up. Alongside optimization and physical scale-up, the safety aspects are equally critical for the commercialization of bacterial pigments. While natural microbial colorants are generally seen as eco-friendly alternatives to synthetic dyes, their move from the lab to the factory depends on thorough safety checks. Before approval from agencies like the FDA, these pigments must go through detailed toxicological testing, including tests for cell damage and genetic changes[34]. Additionally, safety checks must show that the bacteria used to make the pigments are not harmful and are considered safe (GRAS). The final pigment must also be free from any substances from the bacteria. When using things like sesame cake from farming as a medium for growing the bacteria, the process of cleaning up the pigment afterward must be very strong. This process must remove not only parts of the cells but also any possible problems from the farming materials. This ensures the pigment is clean enough for use in the market [35,36].

3.3. Intermediate Polarity Solvents Maximize Carotenoid Extraction

The efficacy of various organic solvents for pigment recovery demonstrated a strong correlation between solvent polarity and extraction yield, consistent with the "like dissolves like" principle for carotenoids. Quantitative analysis revealed that solvents of intermediate polarity achieved the highest crude pigment extract yield, while highly nonpolar or highly polar solvents were less effective. Specifically, acetone yielded the highest crude pigment yield at 4.4 g/L (Fig. 3a). This was closely followed by chloroform (4.3 g/L); ethanol also demonstrated high efficiency with a yield of 4.3 g/L. Butanol also showed high efficiency, yielding 4.1 g/L. In contrast, the highly nonpolar solvents, diethyl ether and hexane, yielded minimal or no detectable pigment, confirming the semi-polar nature of the extracted carotenoids. Isoamyl alcohol and methanol produced yields of 0.3 g/L and 3.4 g/L, respectively (Supplementary Fig. 5). This primarily highlights the necessity of membrane-disrupting solvents for efficient intracellular pigment recovery. Because ethanol is highly effective, generally recognized as safer ("greener"), and food-compatible compared to acetone and chloroform, it was selected for all subsequent optimization experiments [37]. Conversely, the poor extraction yields observed with hexane and diethyl ether may reflect their inability to adequately penetrate the bacterial cell wall. The extraction profile favors polar and moderately polar solvents [18,22,38].

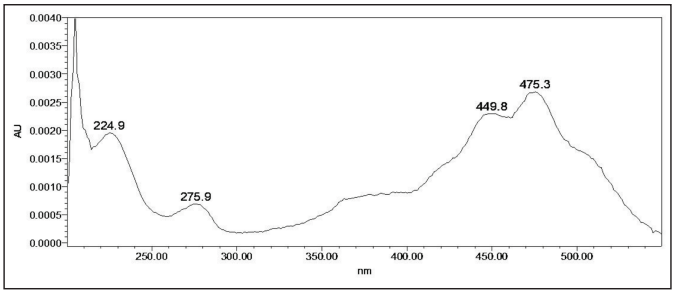

Figure 5. UV-visible absorption spectrum of the crude pigment extract. The spectral profile exhibits characteristic absorption maxima (*λmax*) at 475.3 nm (primary) and 449.8 nm (secondary), along with minor peaks at 224.9 nm and 275.9 nm in the UV region.

3.4. Maximal Carotenoid Accumulation at Late Exponential and Early Stationary Phases

The kinetics associated with growth and production illustrated in (Fig. 4) provide the profile of microbial growth and production. Maximum viable cell count was achieved at about 4.3×10^7 CFU/ml at hour 60. Production of pigments started to become apparent around 42 hours at the late exponential phase. Accumulation of the metabolite proceeded even in the stationary phase, with the final concentration of around 4.3 g/L. This indicates that the most effective harvest time for maximum crude pigment extract yield is during the late stage of active growth, which is around 72 hours. It can be seen that the pigment production follows mixed growth-associated production kinetics, where the production kinetics have growth-associated as well as non-growth-associated components [39].

3.4.1. UV-visible analysis indicates carotenoid nature of the orange pigment

The resulting absorption spectrum exhibited distinct peaks in the visible region, which is typical for conjugated double-bond systems found in carotenoids. The pigment showed its maximum absorbance wavelength (λ_{max}) at 475.3 nm (Fig. 5). Furthermore, the spectrum displayed secondary absorption maxima at 449.8 nm and 275.9 nm, as well as a peak at 224.9 nm. The characteristic triple-peak absorption pattern, with the primary maximum centered around 475 nm and a major peak at 449.8 nm, is indicative of a polyene chain typical of carotenoid-like chromophores [40]. These spectral signatures classify the extracted orange pigment as belonging to the broad carotenoid family. The λ_{max} value of 475.3 nm falls within the typical absorption range for carotenoids routinely generated by members of the *Rhodococcus* genus [18,41]. The primary absorption bands for the majority of carotenoids are in the 400–500 nm wavelength area; however, the structure of the spectrum varies greatly amongst carotenoids, typically displaying three separate peaks rather than a single absorption band [40,42].

3.4.2. FTIR spectroscopy reveals presence of hydroxyl and ester moieties in pigment

Fourier-transform infrared spectroscopy was conducted to identify the key functional groups present in the crude pigment extract. The spectrum displayed in (Fig. 6) has a broad and intense band at 3,343.54 cm^{-1}, which is diagnostic of O-H stretching vibrations originating from hydroxyl groups (OH). Signals in the aliphatic region, specifically at 2,924.03 cm^{-1} and 2,853.96 cm^{-1}, showed the presence of asymmetric and symmetric (C-H) stretching vibrations from the alkyl backbone. The presence of a key peak at 1,625.94 cm^{-1} was assigned to the (C=C) stretching vibrations within the conjugated polyene chain. A band

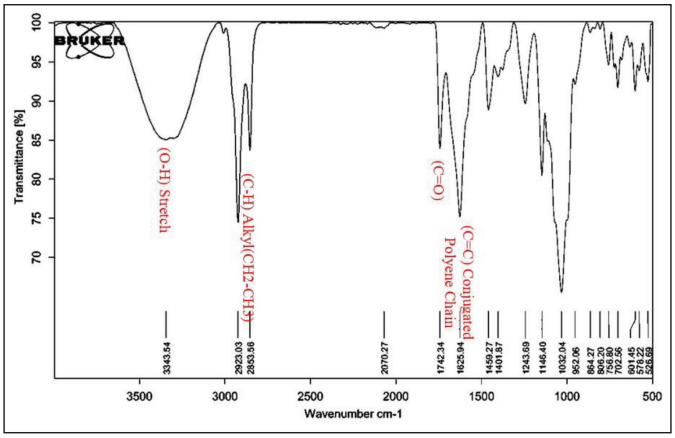

Figure 6. Fourier-transform infrared spectroscopy (FTIR) spectrum: shows characteristic functional groups of carotenoids, including the broad band at 3,343.54 cm^{-1} (O-H) and the peak at 1,625.94 cm^{-1} (C = C polyene), reinforcing the carotenoid core and hydroxyl groups. The peak at 1,742.34 cm^{-1} suggests a carbonyl (C = O) group, proposed to be an esterified structure.

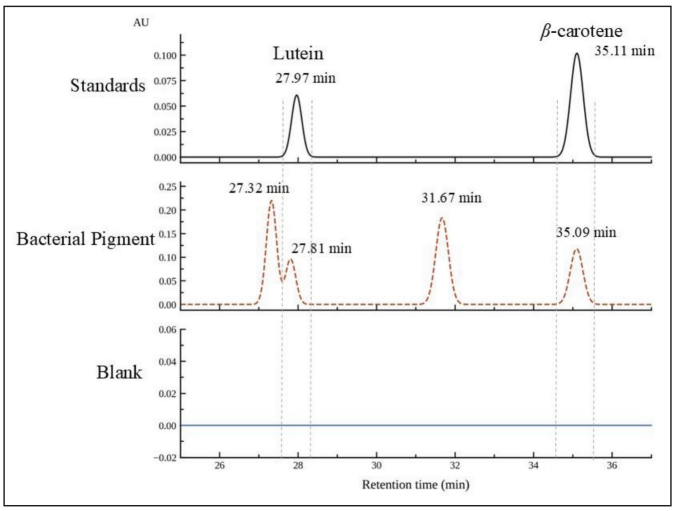

Figure 7. High-Performance liquid chromatography-photodiode array (HPLC-PDA) chromatogram: Illustrates the absorbance (AU) plotted against retention time (minutes). The top panel displays the reference standards, identifying peaks for lutein (27.97 minutes) and β-carotene (35.11 minutes). The middle panel depicts the bacterial crude pigment extract, which exhibits peaks at 27.81 minutes and 35.09 minutes, alongside additional unidentified peaks at 27.32 minutes and 31.67 minutes. The bottom panel confirms a stable baseline using a media and solvent blank control. Although the observed peaks in the sample closely match the retention times of the standard references, the bacterial extract is possibly a mixture of carotenoids.

observed at 1,742.34 cm^{-1} suggests the presence of a carbonyl group (C=O stretching). In the context of a crude extract from *Rhodococcus*, this signal, along with (C-O) stretching in the fingerprint region at 1,243.69 cm^{-1} and 1,032.04 cm^{-1}, likely reflects a combination of the pigment's functional groups and the ester linkages of co-extracted cellular lipids. Further (C-H) bending vibrations from methyl groups were noted at 1,458.27 cm^{-1} and 1,401.87 cm^{-1}.

3.4.3. HPLC characterization

To qualitatively confirm the biosynthetic capability of the strain to produce carotenoids, cells were cultivated in standard nutrient broth and analyzed via HPLC-PDA. Triplicate injections of the crude pigment extract (n = 3) produced two early peaks at 27.32 ± 0.01 minutes and 27.81 ± 0.01 minutes, respectively, in all three runs. There was also a later peak at 35.09 ± 0.01 minutes and an additional peak at 31.67 ± 0.01 minutes. Comparing the retention times to the independently run standards of lutein and β-carotene, the early doublet of the extract (27.32/27.81 minutes) was within 0.16–0.65 minutes of the lutein standard peak (27.97 minutes). The extract's later peak (35.09 minutes) was within 0.02 minutes of the β-carotene standard peak (35.11 minutes) (Fig. 7) (Supplementary Tables 1 and 2). From these comparisons, the crude extract can be described as a blend of carotenoid-class pigments. This matches earlier reports that carotenoid production occurs in *R. corynebacterioides* strains [43]. This analysis is only a qualitative comparison of retention times and spectral data. It should not be taken as a precise measurement of compound identity. Further studies such as LC-MS and NMR will be required to fully establish the identity. Bacteria often produce many different carotenoids, including those with additional oxygen and sugar due to the specialized metabolism typical of the organism [42,44]. Recent work by Yürümez Canpolat and Artan Onat [43] has explicitly demonstrated and characterized carotenoid production in *R. corynebacterioides*.

4. CONCLUSION

This study establishes a robust and sustainable platform for carotenoid biosynthesis using *R. corynebacterioides* cultivated on nutrient-rich de-oiled oilseed cakes. Sesame oil cake was found to be the most effective substrate for high crude pigment extract yield. Optimized bioprocess conditions, including a temperature of 30°C, a pH of 7.0, an oilseed cake concentration of 10% w/v, NaCl of 3% w/v, and continuous exposure to light, significantly enhanced carotenoid production, underscoring the decisive role of physicochemical parameters in microbial metabolite synthesis. UV-visible spectroscopy, FTIR, and HPLC-PDA data show similarities with a carotenoid/xanthophyll-type pigment profile. Although the sample demonstrated similarities to β-carotene and lutein reference standards, the current characterizations confirm its general classification as a carotenoid, with exact molecular elucidation requiring further high-resolution mass spectrometry. Based on the high lipid content of the favored substrate, we hypothesize that oilseed cake upcycling may be driven by lipid β-oxidation coupled with the glyoxylate shunt. This proposed routing of excess acetyl-CoA through gluconeogenesis provides a plausible mechanism for how the system channels carbon into the pyruvate and G3P pools required to sustain MEP pathway biosynthesis. By integrating waste valorization with microbial bioprocessing, this study offers a cost-effective, scalable route for carotenoid production from agro-industrial residues. Future studies involving high-resolution mass spectrometry with standards are required for the confirmation of pigment identity. Statistical optimization using response surface methodology or other methods may be based on the identified parameters in the present study. These findings advance the circular bioeconomy paradigm and open new avenues for sustainable pigment production with broad applications across the food, pharmaceutical, and cosmetic industries.

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6. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

7. ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

8. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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All the data are available with the authors and shall be provided upon request.

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI) tools for writing and editing of the manuscript, and no images were manipulated using AI.

13. SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the journal's website: Link here [https://jabonline.in/admin/php/uploadss/1542_pdf.pdf]

REFERENCES

1. Britton G. Structure and properties of carotenoids in relation to function. FASEB J 1995;9:1551–8; doi: https://doi.org/10.1096/fasebj.9.15.8529834
2. Joel J. Carotenoids market by type (astaxanthin, beta-carotene, lutein, lycopene, canthaxanthin, zeaxanthin, and others) for feed, food, supplements, cosmetics, and pharmaceuticals: global industry perspective, comprehensive analysis, size, share, growth, segment, trends and forecast, 2015–2021. Zion Market Research; 2016. Available from: https://www.marketresearchstore.com/report/carotenoids-market-z76031
3. Joshi A, Purandare D, Bhope A, Chaudhari S, Patil A, Polshettiwar S. Challenges and future prospects in the field of microbial pigments. In: Madan A, Mitra D, Andjelkovic S, editors. Biopigments of microbial origin: innovations and applications across industries. Wallingford: CABI; 2025. p. 320–35; doi: https://doi.org/10.1079/9781836990505.0018
4. Saini RK, Keum YS. Microbial platforms to produce commercially vital carotenoids at industrial scale: an updated review of critical issues. J Ind Microbiol Biotechnol 2019;46:657–74; doi: https://doi.org/10.1007/s10295-018-2104-7
5. Mata-Gómez LC, Montañez JC, Méndez-Zavala A, Aguilar CN. Biotechnological production of carotenoids by yeasts: an overview. Microb Cell Fact 2014;13:12; doi: https://doi.org/10.1186/1475-2859-13-12
6. Mendes-Silva T de CD, da Silva Andrade RF, Ootani MA, Mendes PVD, Da Silva MRF, Souza KS, *et al.* Biotechnological potential of carotenoids produced by extremophilic microorganisms and application prospects for the cosmetics industry. Adv Microbiol 2020;10:397–410; doi: https://doi.org/ 10.4236/aim.2020.108029
7. Al-Monofy KB, Farghali MH, Al-Madboly LA, Kamer AMA, Abdelaziz AA. Advances in biomanufacturing and recent biological applications of the fundamental bacterial carotenoids: a comprehensive review. Microb Cell Fact 2025;24:225; doi: https://doi.org/10.1186/s12934-025-02847-1
8. MarketsandMarkets. Carotenoids market worth $3.52 billion by 2031. 2026. Available from: https://www.marketsandmarkets.com/PressReleases/carotenoid.asp
9. Talwar B, Chopra R, Chaudhary S. Sustainable valorization of oilseed cake using solid-state fermentation for enhanced nutritional profile. Discov Food 2026;6:187; https://doi.org/10.1007/s44187-026-00912-1
10. Ahn S, Jung J, Jang IA, Madsen EL, Park W. Role of glyoxylate shunt in oxidative stress response. J Biol Chem 2016;291:11928–38; doi: https://doi.org/10.1074/jbc.M115.708149
11. Yano T, Yoshida N, Yu F, Wakamatsu M, Takagi H. The glyoxylate shunt is essential for CO2-requiring oligotrophic growth of Rhodococcus erythropolis N9T-4. Appl Microbiol Biotechnol 2015;99(13):5627–37; doi: https://doi.org/10.1007/s00253-015-6500-x
12. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl Environ Microbiol 2008;74:2461–70; doi: https://doi.org/10.1128/AEM.02272-07
13. Kruger NJ. The Bradford method for protein quantitation. In: Walker JM, editor. The protein protocols handbook. 3rd ed. Totowa (NJ): Humana Press; 2009. p. 17–24; doi: https://doi.org/10.1007/978-1-59745-198-7_4.
14. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7; doi: https://doi.org/10.1139/o59-099
15. Masuko T, Minami A, Iwasaki N, Majima T, Nishimura SI, Lee YC. Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. Anal Biochem 2005;339:69–72; doi: https://doi.org/ 10.1016/j.ab.2004.12.001
16. Ismail BP. Ash content determination. In: Ismail BP, Nielsen SS, editors. Nielsen's food analysis laboratory manual. 4th ed. Cham: Springer; 2024. p. 129–31. doi: http://doi.org/10.1007/978-3-031-44970-3_14
17. Loh WLC, Huang KC, Ng HS, Lan JCW. Exploring the fermentation characteristics of a newly isolated marine bacteria strain, Gordonia terrae TWRH01 for carotenoids production. J Biosci Bioeng 2020;130:187–94; doi: https://doi.org/ 10.1016/j.jbiosc.2020.03.007
18. López GD, Álvarez-Rivera G, Carazzone C, Ibáñez E, Leidy C, Cifuentes A. Bacterial carotenoids: extraction, characterization, and applications. Crit Rev Anal Chem 2023;53:1239–62; doi: https://doi.org/10.1080/10408347.2021.2016366
19. Wang H, Yang A, Zhang G, Ma B, Meng F, Peng M, *et al.* Enhancement of carotenoid and bacteriochlorophyll by high salinity stress in photosynthetic bacteria. Int Biodeterior Biodegradation 2017;121:91–6; doi: https://doi.org/ 10.1016/j.ibiod.2017.03.028
20. Zhou Q, Zhang P, Zhang G, Peng M. Biomass and pigments production in photosynthetic bacteria wastewater treatment: effects of photoperiod. Bioresour Technol 2015;190:196–200; doi: https://doi.org/10.1016/j.biortech.2015.04.092
21. Meckel RA, Kester AS. Extractability of carotenoid pigments from non-photosynthetic bacteria with solvents and detergents: implications for the location and binding of the pigments. Microbiol (N Y) 1980;120:111–6; doi: https://doi.org/10.1099/00221287-120-1-111
22. Jiang Y, Ye J, Hu Y, Zhang J, Li W, Zhou X, *et al.* Extraction and synthesis of typical carotenoids: lycopene, β-carotene, and astaxanthin. Molecules 2024;29:4549; doi: https://doi.org/10.3390/molecules29194549

23. Gupta P, Sreelakshmi Y, Sharma R. A rapid and sensitive method for determination of carotenoids in plant tissues by high-performance liquid chromatography. Plant Methods 2015;11:5; doi: https://doi.org/10.1186/s13007-015-0051-0
24. Gohl B. Tropical feeds. Feed information summaries and nutritive values. Food and Agriculture Organization of the United Nations, Rome, Italy, 1981.
25. Ramachandran S, Singh SK, Larroche C, Soccol CR, Pandey A. Oil cakes and their biotechnological applications–a review. Bioresour Technol 2007;98:2000–9; doi: https://doi.org/ 10.1016/j.biortech.2006.08.002
26. Usman I, Saif H, Imran A, Afzaal M, Saeed F, Azam I, *et al.* Innovative applications and therapeutic potential of oilseeds and their by-products: an eco-friendly and sustainable approach. Food Sci Nutr 2023;11:2599–609; doi: https://doi.org/10.1002/fsn3.3322
27. Vichare SA, Morya S. Exploring waste utilization potential: nutritional, functional and medicinal properties of oilseed cakes. Front Food Sci Technol 2024;4:1441029; doi: https://doi.org/10.3389/frfst.2024.1441029
28. Kuddus M, Singh P, Raveendran S, Singh R. Microbial pigments: applications in Food and Beverage Industry. CRC Press, Boca Raton, FL, 2024.
29. Styczynski M, Rogowska A, Gieczewska K, Garstka M, Szakiel A, Dziewit L. Genome-based insights into the production of carotenoids by Antarctic bacteria, *Planococcus sp*. ANT_H30 and *Rhodococcus sp*. ANT_H53B. Molecules 2020;25:4357; doi: https://doi.org/10.3390/molecules25194357
30. Grewal J, Wołacewicz M, Pyter W, Joshi N, Drewniak L, Pranaw K. Colorful treasure from agro-industrial wastes: a sustainable chassis for microbial pigment production. Front Microbiol 2022;13:832918; doi: https://doi.org/10.3389/fmicb.2022.832918
31. Flores N, Hoyos S, Venegas M, Galetović A, Zúñiga LM, Fábrega F, *et al.* Haloterrigena sp. strain SGH1, a bacterioruberin-rich, perchlorate-tolerant halophilic archaeon isolated from halite microbial communities, Atacama Desert, Chile. Front Microbiol 2020;11:324; doi: https://doi.org/10.3389/fmicb.2020.00324
32. Sumi S, Suzuki Y, Matsuki T, Yamamoto T, Tsuruta Y, Mise K, *et al.* Light-inducible carotenoid production controlled by a MarR-type regulator in *Corynebacterium glutamicum*. Sci Rep 2019;9:13136; doi: https://doi.org/10.1038/s41598-019-49384-7
33. Padmanabhan S, Monera-Girona AJ, Pérez-Castaño R, Bastida-Martínez E, Pajares-Martínez E, Bernal-Bernal D, *et al.* Light-triggered carotenogenesis in Myxococcus xanthus: new paradigms in photosensory signaling, transduction and gene regulation. Microorganisms 2021;9:1067; doi: https://doi.org/10.3390/microorganisms9051067
34. Tuli HS, Chaudhary P, Beniwal V, Sharma AK. Microbial pigments as natural color sources: current trends and future perspectives. J Food Sci Technol 2015;52:4669–78; doi: https://doi.org/10.1007/s13197-014-1601-6
35. Afroz Toma M, Rahman MH, Rahman MS, Arif M, Nazir KHMNH, Dufossé L. Fungal pigments: carotenoids, riboflavin, and polyketides with diverse applications. J Fungi 2023;9:454; doi: https://doi.org/10.3390/jof9040454
36. Barreto JVDO, Casanova LM, Junior AN, Reis-Mansur MCPP, Vermelho AB. Microbial pigments: major groups and industrial applications. Microorganisms 2023;11:2920; doi: https://doi.org/10.3390/microorganisms11122920
37. Viñas-Ospino A, López-Malo D, Esteve MJ, Frígola A, Blesa J. Green solvents: emerging alternatives for carotenoid extraction from fruit and vegetable by-products. Foods 2023;12:863; doi: https://doi.org/10.3390/foods12040863
38. Morón-Ortiz A, Mapelli-Brahm P, Meléndez-Martínez AJ. Sustainable green extraction of carotenoid pigments: innovative technologies and bio-based solvents. Antioxidants 2024;13:239; doi: https://doi.org/10.3390/antiox13020239
39. Gharibzahedi SMT, Razavi SH, Mousavi M. Kinetic analysis and mathematical modeling of cell growth and canthaxanthin biosynthesis by Dietzia natronolimnaea HS-1 on waste molasses hydrolysate. RSC Adv 2013;3:23495–502; doi: https://doi.org/10.1039/c3ra44663h
40. Britton G, Liaaen-Jensen S, Pfander H. Carotenoids: handbook. Birkhäuser, Basel, Switzerland, 2012.
41. Elsayed Y, Refaat J, Abdelmohsen UR, Fouad MA. The Genus *Rhodococcus* as a source of novel bioactive substances: a review. J Pharmacogn Phytochem 2017;6:83–92.
42. Klassen JL, Foght JM. Differences in carotenoid composition among Hymenobacter and related strains support a tree-like model of carotenoid evolution. Appl Environ Microbiol 2008;74:2016–22; doi: https://doi.org/10.1128/AEM.02306-07
43. Yürümez Canpolat E, Artan Onat T. The effect of carbon sources on carotenoid synthesis by the novel *Rhodococcus* corynebacterioides TAO1. Fermentation 2025;11:599; doi: https://doi.org/10.3390/fermentation11100599
44. Silva TR, Tavares RSN, Canela-Garayoa R, Eras J, Rodrigues MVN, Neri-Numa IA, *et al.* Chemical characterization and biotechnological applicability of pigments isolated from Antarctic bacteria. Mar Biotechnol 2019;21:416–29; doi: https://doi.org/10.1007/s10126-019-09892-z

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