German J Pharm Biomaterials 2025; 4(3): 13-22
https://doi.org/10.5530/gjpb.2025.3.8

GJPB

Research Article

Studies on the production and optimization of yellow pigment extracted from the SB_2 strain and evaluation of its antioxidant properties

Sandanakirouchenane Aroumougame[1,2]*, Kottakota Chandrasekhar[3], Douglas J.H. Shyu[4]

[1]Department of Microbiology, School of Life Sciences, Pondicherry University, Puducherry, India.
[2]Department of Agricultural Microbiology, Sri Krishnadevaraya College of Agricultural Sciences (SKCAS), Andhra Pradesh, India
[3]Department of Plant Biochemistry and Biotechnology, Sri Krishnadevaraya College of Agricultural Sciences (SKCAS), Andhra Pradesh, India.
[4]Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung 912, Taiwan.
*Correspondence: sandana03@yahoo.co.in

Received: 01 April 2025; Revised: 05 June 2025; Accepted: 20 July 2025

Abstract

Actinobacteria are filamentous bacteria that exist independently in soil, oceans, and the atmosphere, producing secondary metabolites with antioxidant and anti-cancer properties. Among these, the SB_2 strain is a yellow-pigmented, gram-positive, coccus-shaped bacterium. Raman spectroscopy of SB_2 cultures revealed a wave number (cm^{-1}) of 1382.47, which indicates pigment production and was further supported by UV-Vis spectral analysis at 450 nm that confirmed the presence of carotenoid pigment in methanolic extracts of SB_2. For optimal pigment synthesis, SB_2 required a pH of 7, a temperature of 30 °C, and a nutritional medium with 1% molasses, 1.2% olive oil, and 1% peptone. The yellow pigment extracted from SB_2 (500 μg ml^{-1}) demonstrated DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity.

Keywords: Actinobacteria; SB_2 strain; antioxidant

Introduction

In the twentieth century, the term "pigment" referred to a compound composed of small particles with unique properties, such as colorant properties, protective agents, antioxidants, and other features, that are insoluble in water and are widely used across various industries [1]. Natural pigments have been used for body ornamentations, cosmetics, and textile dyeing. Microorganisms were to determine life and death on this planet, the most powerful creature. For centuries, natural sources were used as substrates for producing organic pigments. Still, nowadays, most people are interested in using synthetic organic or inorganic materials, which can cause carcinogenic reactions, induce allergic reactions, induce hyperactivity, and are unsafe for the environment and human health, and are manufactured from petroleum compounds and heavy metals [1]. Naturally occurring pigments range from light yellow to orange and deep red, known as carotenoids (approximately 1158), sourced from 691 different organisms [2] and synthesized as hydrocarbons and their oxygenated derivatives [3]. The global market for carotenoids was valued at $1577 million in 2017, driven by increased consumption of carotenoid-containing products, and is anticipated to grow to $2 billion by 2026. The market for dyes and pigments is projected to be worth between $33.2 and $49.1 billion by 2027 [4]. The yellow pigment isolated from the *Pseudomonas balearica* strain U7, which controls phytopathogenic strains, was identified as melanin [5]. The ability of microorganisms to synthesize pigments from inexpensive culture media is an advantage for pigment production [6]. To meet market demand, statistical and mathematical procedures must become more efficient, reliable, and economically viable through the optimization of media for large-scale manufacturing [7]. The microbial fermentation of carotenoid production may be used as an inexpensive medium, which is a byproduct of the beet sugar industry [8]. Bacterial pigments can act as antimicrobials, anticancer agents, antioxidants, anti-inflammatories,

and antiallergics, with significant economic potential. Astaxanthin binds to free radicals to form inert compounds, quenches electrons from membranes, and prevents them from becoming pro-oxidant molecules. It is also associated with immunity, inflammation, the antioxidant defense system, and the mitigation of the negative effects of oxidative stress [9]. As harmful substances accumulated within the bacteria, it was determined that terpenoids and carotenoids affected ATP hydrolysis and disrupted the activation of the efflux pump [10,11]. Astaxanthin from *H. pluvialis* provides the greatest defense against free radicals, followed by lutein and β-carotene [12]. This study evaluated the pigments extracted from the SB_2 isolates and optimized them with various nutrients for antioxidant activities.

Materials and Methods

Isolation of pigment producing SB_2 isolate and SEM analysis

In our earlier work [13], we isolated the yellow-pigmented bacterial strain SB_2 from marine water collected at Marakanam (Tamil Nadu), India, to assess the optimization and effectiveness of carotenoid pigments in antibacterial activity. The SB_2 isolate was grown in TS (Tryptic Soy) broth, and the resulting bacterial cells were used for scanning electron microscopy (SEM) analysis. The bacterial culture was centrifuged, and the sample was fixed with 2% glutaraldehyde solution and left to alcoholic dehydration for 6 to 12 h. The bacterial cells were then washed three times with saline phosphate buffer (pH 7.0) to remove salts. The material was dehydrated and analyzed by SEM [14].

Raman Spectroscopy analysis for pigment production

To verify the pigment produced by the isolate's pure culture, the SB_2 strain was grown on sterilised TS (Tryptic Soy) agar for 72 h to obtain pigmented pure cultures. This allowed the identification of the strains by a strong Raman signal in Raman spectroscopy, measured in terms of wave number (cm^{-1}) [15].

Pigment extraction from SB_2 isolate

The yellow-pigmented SB_2 isolate was inoculated into sterile Tryptic Soy (TS) broth and incubated at 30 °C with shaking at 160 rpm for one week. After centrifugation at 8000 rpm for 15 min, the supernatant was discarded, and the pellet was washed with 5 ml of sterile distilled water and centrifuged again at 4000 rpm. Then, 5 ml of methanol was added to the pellet, and the mixture was sonicated for 2 h to extract the pigments. The supernatant was filtered through Whatman No. 1 filter paper, yielding the crude yellow pigment, which was dried and analyzed by UV-visible spectrophotometry over 200-800 nm [16].

Different concentrations of molasses used for biomass production

Molasses concentrations of 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% were prepared in Tryptic Soy (TS) broth in conical flasks and sterilized at 121 °C for 15 min. The log-phase culture of the SB_2 strain was inoculated into each flask and incubated for 7 days at 30 °C, shaking at 160 rpm. The biomass (g/l) was estimated based on molasses concentration [17].

Effect of pigment production utilized in various concentrations of olive oil

To examine the impact of olive oil on cell pigment production, varying concentrations (0.2% to 1.2%) were added to sterilize Tryptic Soy (TS) broth in conical flasks. A log-phase culture of SB_2 was inoculated and incubated for one week at 30 °C, shaking at 160 rpm. To effectively measure the production of cell pigment (expressed in mg/l) in relation to the different concentrations of olive oil, demonstrating the potential influence of olive oil on pigment synthesis in the isolated culture [18].

Growth of isolated strain on the effect of pH

We investigated the impact of pH levels (4 to 12) on a nutritional broth by inoculating a pigmented SB_2 culture into Tryptic Soy (TS) broth at various pH values (4 to 12). After incubating for 7 days at 30 °C, shaking at 160 rpm, we measured cell pigment production (mg/l), revealing important insights into the relationship between pH and pigment synthesis [19].

Effect on temperature

The TS broth was prepared and sterilized in seven different conical flasks. The pigmented culture was inoculated into TS broth and incubated at 20, 25, 30, 35, 40, 45, and 50 °C, shaking at 160 rpm. After one week, the growth in the culture broth for carotenoid production (mg/l) and biomass (g/l) was determined [20].

The SB_2 isolate grown on various concentrations of peptone in carotenoid production

The concentration of peptone (0.2, 0.4, 0.6, 0.8 and 1.0 %) was added into Tryptic Soy (TS) broth sterilized at 121 °C for 15 min. The pigmented culture was inoculated and incubated at 30 °C shaking at 160 rpm for seven days. The yield of biomass (g/l) and carotenoids (mg/l) was recorded in each broth [21].

Determination of DPPH scavenging assay for extracted carotenoid pigment

After preparing a 2.5 ml 0.5 mM DPPH methanolic solution, add 0.5 ml of the extracted pigment, shake the mixture briskly, and let it sit for 30 minutes at room temperature in the dark. The mixture was measured at 517 nm [22]. Ascorbic acid was used as a control. The formula for calculating the percentage of scavenging was: % of inhibition = (absorbance of control - absorbance of sample)/ absorbance of control × 100. The antioxidant activity of the pigment extracted from the isolate was evaluated by measuring the scavenging (%) at different pigment concentrations (μg/ml).

Statistical analysis

The experiments in this study were conducted in triplicate, and the results were analysed using OriginPro 2022b. The average of the three replicate data was analysed, and a one-way ANOVA was performed.

Results

Colony characteristics of SB_2 isolate

The growth characteristics of the SB_2 strain have gram-positive bacteria with coccus by SEM image, the ability to grow 0-15% salt, yellow-pigmented, and non-spore-forming, result was presented in Figure 1.

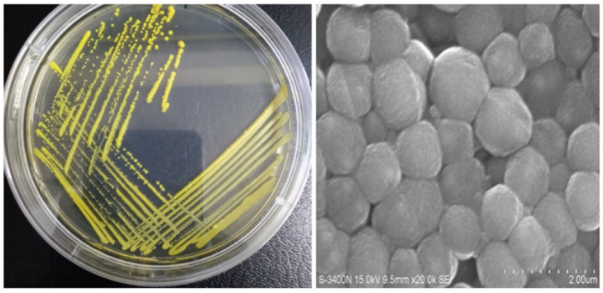

Figure 1. Yellow pigmentation on Zobell's marine agar and SEM image of the isolate SB_2.

Raman spectroscopy analysis for SB_2 isolate

The results for the SB_2 strain are shown in Figure 2, and pigment production was analyzed by Raman spectroscopy. The strain revealed a maximum peak at a wave number (cm^{-1}) in the Raman intensity (1382.47). In this analysis, the pure culture of the isolate was used to authenticate the colour-producing organisms, which is an advantage of the isolate screening technique, as it allows the pigment to be produced and separated from the UV-VIS analysis.

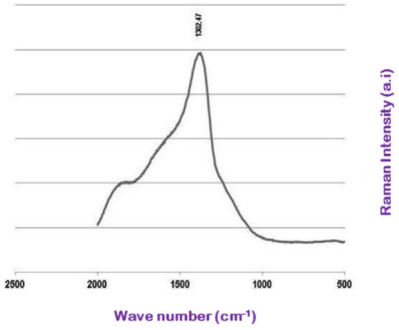

Figure 2. The pigment production of isolate SB_2 in Ramen spectroscopy.

Yellow pigment extraction from a bacterial isolate

The production of carotenoid pigment by the SB_2 isolates supports the finding that the yellow pigment in the crude extract had a maximum peak at 450 nm in the UV-VIS spectrum. The outcome is shown in Figure 3.

The growth of SB_2 isolate on molasses as a nutrient

Molasses is a readily available substrate for microorganisms. The strain was evaluated differently in the concentration of nutrient medium, in which 1% concentration was recorded as the maximum biomass production (2.57 gm/l) (F = 13.90361, P = 0.005798), followed by 0.8 and 0.6%. The results are shown in Figure 4.

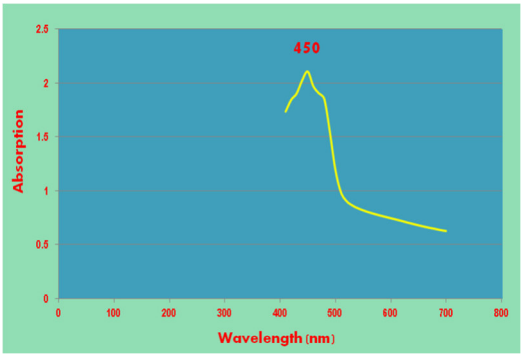

Figure 3. The UV range of the extracted yellow pigment from the strain SB_2.

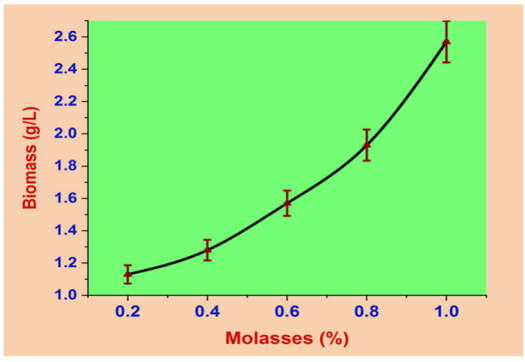

Figure 4. The isolate utilize various concentration of molasses.

Effect of olive oil as a nutrient source

Higher carotenoid production was observed when increased salt concentration enhanced the uptake of nitrogen and organic carbon sources. The results in Figure 5 showed that the strain utilizes readily available olive oil nutrients for growth, and the 1.2% concentration resulted in higher pigment production (0.179 mg/l) (F = 20.37104, P = 0.001967), followed by the 1% concentration.

The SB_2 strain grows in response to pH

The growth of bacteria and the synthesis of pigment depend on a variety of environmental parameters and nutrient sources. The pH range of 4 to 12 was used to test the SB_2 pigmented isolate. The results shown (Figure 6) indicate that the highest pigment synthesis (1.65 mg/l) (F = 62.78536, P = 6.2779) occurred at pH 7.

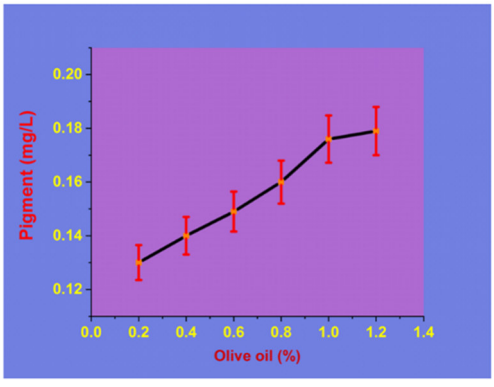

Figure 5. Utilization of olive oil concentration of the isolate.

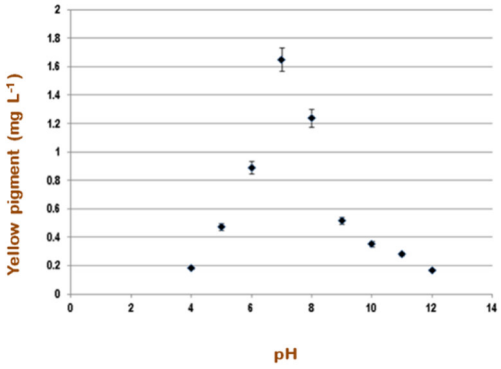

Figure 6. Growth of the isolate at various pH.

Effect on temperature

The isolate SB_2 was grown from 20 to 50 °C on a selective medium, and the higher pigment production (0.97 mg/l) (F = 71.08805, P = 2.18741) and biomass (2.42 g/l) (F = 66.68988, P = 3.04354) were recorded at 30 °C. The result was shown in Figure 7.

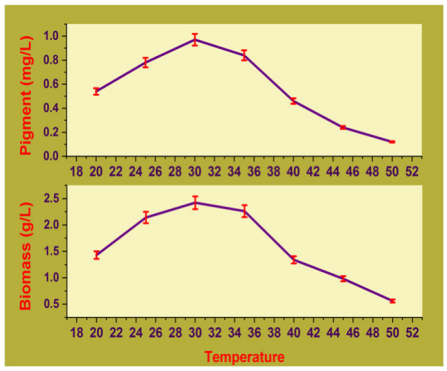

Figure 7. Growth of the isolate occurs at different temperature.

Effect of different concentrations of peptone in carotenoid production

The isolates were grown at different peptone concentrations, and the results are shown in Figure 8, which shows that pigment production and cell biomass gradually increased from 0.2 to 1.0%. The highest pigment production was observed at 1% (2.12 mg/l) (F = 14.20176, P = 0.005477) and biomass (3.49 g/l) (F = 56.68156, P = 6.7422), and the lowest was at a 0.2% peptone concentration.

Determination of DPPH scavenging assay for extracted carotenoid pigment

The antioxidant properties of the pigment extracted from the SB_2 strain were assessed at a concentration of 500 µg/ml, which showed 65.74 % scavenging activity (F = 2.439963, P = 0.156904) with an IC_{50} value of 3.265 ± 15. In contrast, ascorbic acid, used as the control, exhibited a scavenging activity of 75.64% (F = 2.642329, P = 0.155176) with an IC_{50} value of 2.658 ± 19. These results are shown in Figure 9.

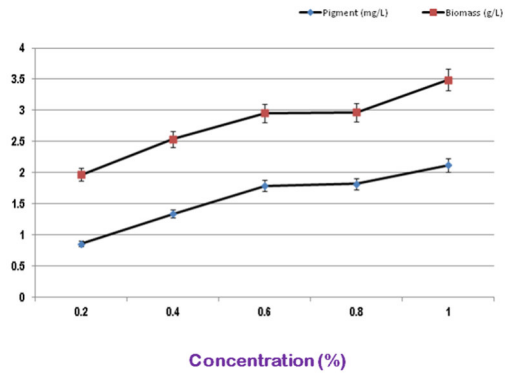

Figure 8. Effect of growth of isolate on peptone.

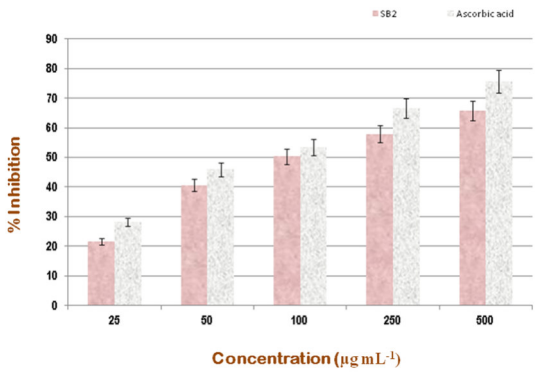

Figure 9. Antioxidant activity of the pigmented SB_2 isolates.

Discussion

Characteristics of SB_2 colonies

The isolated strain SB_2 was observed as yellow-coloured colonies with smooth margins, in gram reaction exhibits gram-positive, may be thick and rigid cell wall which is aerobic in nature and inability to produce endospore, round or coccus shapes, and non-motile were observed in a microscope. Similar characteristics were studied by colonies grown on Tryptic Soy Agar (TSA) medium for 48 h at 28 °C [23], whereas some colonies grown on TSA, preferably 48 h at 30 °C, exhibit the same morphological characteristics [24].

Raman spectroscopy study of extracted yellow pigment characterization

One of the most advanced tools used in microbiological research recently to characterise microbial pigments is Raman spectroscopy. In this analysis, the pure culture of the isolate was used to authenticate the colour-producing organisms, which is an advantage of the isolate screening technique, as it allows the pigment to be produced and separated from the UV-VIS analysis. Using Raman spectroscopy, the pure culture SB_2 strain was examined for pigment production. The isolate revealed a peak at 1382.47 wave number cm^{-1} in Raman intensity, which was also confirmed by distinctive changes in the carotenoid bands at 1527 and 1158 cm^{-1}, indicating the presence of a complex mixture of carotenoids [25]. The movement of atoms in the chromophore during vibrations is reflected in the activation of electronic absorption within the spectrum. This leads to a specific increase in certain Raman intensities (bands), corresponding to the part of the molecule where the electronic transition takes place [26]. This approach uses qualitative and quantitative analysis to investigate microorganisms, focusing on their pigments and biomolecules. Strong Raman signals facilitate an in-depth exploration of microbial pigments, including pure cultures and extracted samples, enhancing our understanding of their significance [27,28]. It has been used to monitor pure cultures of microorganisms and environmental samples to authenticate various natural pigments [15].

Identification of carotenoid pigment from a bacterial isolate

The spectral absorption of β-carotene is around 450 nm, according to Kaiser and group similar results were obtained by the extraction of yellow pigment from the SB_2 isolate [29]. Carotenoid pigments are produced by bacteria, specifically *Exiguobacterium* sp. and *Staphylococcus aureus*, and range in colour from yellow to orange [30].

Cultivation of the SB_2 isolates using molasses as the substrate

The production medium recorded the highest pigment production using molasses [18]. The SB_2 strain also exhibited a 1% concentration in the medium for maximum biomass production. The sugarcane molasses had the highest carotenoid content among *Rhodotorula glutinis* NRRL Y-842 [31]. Higher biomass and beta-carotene can be obtained by optimizing the fermentation process with *Exiguobacterium acetylicum* S01 [32].

Olive oil effect as a source of nutrients

Using corn cob, sugarcane bagasse, and corn steep liquor as economically viable substrates for the manufacture of carotenoid pigments determines the bioprocess's cost [33,34]. The substrate olive oil induces increased pigment production in the culture medium using a 1.2% concentration from the isolate SB_2. Olive oil utilized by the *Micrococcus* sp. for pigment production was observed at 0.8 gm/l [18].

Influence of pH on an SB_2 strain growth

However, this organism grows over a pH range of 6.5 to 7.5. Certain environmental conditions affect pigment stability [6,35]. After incubating at pH 7 for 24 and 36 h, the pH optimization revealed the highest growth and pigment production [36]. The SB_2 pigmented isolate also exhibited the highest pigment production at pH 7; a similar result was found at neutral or alkaline pH [37]. Optimum production for most increased at pH 7, where *Micrococcus flavus* ranged from 6.5 to 7.5. The pH favours the formation of lycopene and β-carotene from neutral to slightly alkaline, and from neutral to acidic [38]. *Micrococcus roseus* was reported at pH 8.0 and 37 °C. Constant growth was observed for *Micrococcus luteus* at 35 °C and pH 7.0 [39]. Growth occurs at constant pH ranges of 6-8 and at a constant pH of 7.0-8.0 [23]. The pigmented strain grows at pH 5-12 [27]. The maximum growth and pigment formation were observed at pH 8 and pH 7 in *Micrococcus luteus* (B1) and *Micrococcus varians* (B2), respectively [19]. Higher levels of beta-carotene production were observed as the salt concentration increased. Simultaneously, lutein and xanthin production were shown to be declining [40].

Effect of temperature on pigment production

Microbial growth occurs at temperatures of 4-45 °C [24]. The temperature range for growth tolerance is 4-45 °C, and optimal growth occurs at 28 °C [23]. The *Micrococcus* grows at various temperatures, from 29 to 37 °C. Ibrahim (2008) reported that carotenoid production and the growth of marine *Micrococcus* sp. depend on the optimal temperature of the growth medium [41]. The optimal temperature range for our isolate was 29 to 35 °C, with peak pigment production of the SB_2 culture observed at 30 °C. Both *Micrococcus luteus* (B1) and *Micrococcus varians* (B2) thrived at this temperature, indicating it is the optimal condition for maximizing their growth and pigment formation [19]. The pigment-producing bacteria *Serratia* sp. and *Micrococcus* sp. were grown at various temperatures, and higher pigment production was noticed at 30 °C [42]. The high-temperature denaturation of the enzyme may occur in microorganisms because cell biomass increases at 36 °C and decreases at 45 °C. In contrast, the growth and pigment production of *Pseudomonas* sp. require 35-36 °C [43].

E*ffect of various peptone concentrations on the synthesis of carotenoid*

Using 2% peptone from *Serratia marcescens* increases beta-carotene production [21]. The SB_2 isolate was observed at a 1% peptone concentration to produce carotenoid pigment. A similar result was recorded by Abdelaziz et al. (2023) [44]. Zobell and Upham reported in 1944 that seawater enriched with neopeptone possibly increases pigment production by bacteria [45]. Several recent optimization studies also identify peptone as one of the best nitrogen sources for enhancing carotenoid production [46].

Assessment of extracted carotenoid pigment's DPPH scavenging assay

Human diseases can result from changes in DNA, proteins, and lipids caused by unpaired electrons in the body. Consequently, *Planococcus* derived carotenoids have attracted growing interest for their potential applications in the food, cosmetic, and pharmaceutical industries as natural antioxidants [47]. Stress-mediated cytotoxicity, by blocking apoptosis and activating the intracellular antioxidant defense system, showed that carotenoids, such as neoxanthin [48]. DPPH is a stable radical, and similar properties of natural colorant products were checked for the antioxidant activity of pigment extracted from the SB_2 strain concentration (500 μg/ml), which showed the highest scavenging activity with an (65.74%) ($F = 2.439963$, $P = 0.156904$) with an IC_{50} value of 3.265±15. Carotenoids donate electrons to free radicals and neutralise them; they are called antioxidants. When the carotenoid compound was concentrated to 200 μg/ml and 40 μg/ml, its maximal scavenging capability, which is caused by conjugated double bonds in its chemical structure, was 54.3% and 44.1%, respectively revealing that lutein has potent antioxidant activity [49] which is also confirmed by Zhang et al. (2022) [50] using lutein extracted from *G. rubripertincta* GH-1. Arthroxanthin with IC_{50} values of 69.8 and 21.5 gm/ml exhibited excellent radical scavenging properties of DPPH and ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid), respectively [51]. Strain QL17 produces pigment compounds that interact with free radicals and singlet oxygen, conferring antioxidant activity [52]. *M. roseus* and *M. luteus* demonstrated significant UV protection and antioxidant IC_{50} values of carotenoid pigments of 3.5-4.5 mg/ml [39]. The inhibitory concentration values may vary due to acyclic carotenoids compared to other pigments [53]. The cell death of a carotenoid extracted from *Halobacterium halobium* was caused by oxidative stress [54]. Carotenoids exhibit total antioxidant activity in the hydrogen peroxide scavenging assay [55]. The presence of the polyene chain that possesses several conjugated double bonds confirmed that the carotenoid pigment is an ideal antioxidant substance [56]. The percentage of free radical scavenging activity in the crude extract of astaxanthin, as evaluated by the DPPH assay, yielded an IC_{50} value of (63.20 ± 2.64 μg/ml) [57]. The antioxidant potential of the extracted carotenoid for DPPH and ABTS inhibition was 65 ± 0.06% and 42 ± 07%, respectively, at 20 μg/ml concentration [58]. Pigments such as lycopene, carotenoids, flavonoids, anthocyanins, and chlorophylls are used to scavenge free radicals in DPPH and ABTS assays [59].

Conclusion

The SB_2 strain is a notable yellow-pigmented, gram-positive coccus. We confirmed pigment synthesis by Raman spectroscopy, which showed a strong peak at 1382.47 cm^{-1}. We extracted a vibrant yellow carotenoid pigment using methanol, evidenced by a UV-Vis peak at 450 nm. To optimize the SB_2 culture, we assessed biomass production across various concentrations of olive oil and molasses, achieving the highest yields at 1.2% olive oil and 1.0% molasses. The ideal pH for pigment production was 7, with optimal results at 30 °C. We also noted increased pigment and biomass when supplementing with 1% peptone nutrients. Importantly, the isolated carotenoid pigment exhibited significant antioxidant qualities, highlighting its potential applications in health and nutrition. Our findings showcase the remarkable capabilities of the SB_2 strain and its promise for future exploration.

Abbreviations

SB_2= Saline Bacteria 2, TSA= Tryptic Soy Agar, *DPPH*= 2, 2-diphenyl-1-picrylhydrazyl, IC_{50} = Inhibitory Concentration, ABTS= 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid.

Acknowledgements

This research was made possible by funding a PhD program from the National Fellowship for Persons with Disabilities (NFPWD), 2017–18 (Grant number: F No. 01-01/2019-sch, dated October 21, 2019). The author sincerely thanks the University Grant Commission (UGC), New Delhi, India. The supervisor, Dr. Joseph Selvin, Department of Microbiology, Pondicherry University, enabled the research effort, for which the author was grateful.

Declaration of competing interest

The authors state that none of their known financial conflicts or interpersonal connections could have influenced the work reported in this paper.

References

1. Usman AI, Aziz AA, Noqta OA. Bio-synthesis of triangular and hexagonal gold nanoparticles using palm oil fronds extracts at room temperature. Materials Research Express. 2018;5(1):[8p].
2. Yabuzaki J. Carotenoids Database: structures, chemical fingerprints and distribution among organisms. Database (oxford). 2017;2017(1):bax004.
3. R Priya, Ramamoorthy S. Analysis of phylogenetic and functional diverge in plant nine-cis epoxycarotenoid dioxygenase gene family. J Plant Res. 2015;128(4):519-34.
4. Rebelo BA, Farrona S, Ventura MR, Abranches R. Canthaxanthin, a red-hot carotenoid: applications, synthesis, and biosynthetic evolution. Plants (Basel). 2020;9(8):1039.
5. Zerrad A, Anissi J, Ghanam J, Sendide K, Mohammed El H. Antioxidant and antimicrobial activities of melanin produced by a *Pseudomonas balearica* strain. J Biotechnol Lett. 2014;5(1):87-94.
6. Dufossé L. Microbial pigments from bacteria, yeasts, fungi, and microalgae for the food and feed industries. Natural and Artificial Flavoring Agents and Food Dyes. 2018;113-32.
7. Singh V, Haque S, Niwas R, Srivastava A, Pasupuleti M, Tripathi C. Strategies for Fermentation Medium Optimization: An In-Depth Review. Front Microbiol. 2017;7:2087.
8. Asadi M. Beet-sugar handbook. John Wiley & Sons; 2006.
9. Singh S, Verma DK, Thakur M, Tripathy S, Patel AR, Shah N, et al. Supercritical fluid extraction (SCFE) as green extraction technology for high-value metabolites of algae, its potential trends in food and human health. Food Res Int. 2021;150(Pt A):110746.
10. Mahizan NA, Yang SK, Moo CL, Song AA, Chong CM, Chong CW, et al. Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. Molecules. 2019;24(14):2631.
11. Jackson Seukep A, Zhang YL, Xu YB, Guo MQ. In vitro antibacterial and antiproliferative potential of *Echinops lanceolatus* Mattf. (Asteraceae) and identification of potential bioactive compounds. Pharmaceuticals (Basel). 2020;13(4):59.
12. Rao AR, Baskaran V, Sarada R, Ravishankar GA. In vivo bioavailability and antioxidant activity of carotenoids from microalgal biomass—A repeated dose study. Food Res Int. 2013;54(1):711-7.

13. Aroumougame S. Studies on optimization of pigment production of SB2 isolate from the Saltern region of Marakanam (TN). World Journal of Biology Pharmacy and Health Sciences. 2022;12(1):86-102.
14. McDougall LA, Holzapfel WH, Schillinger U, Feely DE, Rupnow JH. Scanning electron microscopy of target cells and molecular weight determination of a bacteriocin produced by *Lactococcus lactis* D53. Int J Food Microbiol. 1994;24(1-2):295-308.
15. Jehlička J, Edwards HG, Oren A. Raman spectroscopy of microbial pigments. Appl Environ Microbiol. 2014;80(11):3286-95.
16. Arulselvi I, Sasidharan P, Raja R, Karthik C, Gurumayum RS. Isolation and characterization of yellow pigment producing *Exiguobacterium* sps. J Biochemical Technol. 2013;4(4):632-5.
17. Galal GF, Rania FA. "Using of some agro-industrial wastes for improving carotenoids production from yeast *Rhodotorula glutinis* 32 and bacteria *Erwinia uredovora* DSMZ 30080." Microbiol Res J Int. 2020;30(1):15-25.
18. Karbalaei-Heidari HR, Partovifar M, Memarpoor-Yazdi M. Evaluation of the bioactive potential of secondary metabolites produced by a new marine *Micrococcus* species isolated from the Persian Gulf. Avicenna J Med Biotechnol. 2020;12(1):61.
19. Garg N, Ram RA, Priti C. Microbial properties of pigmented bacteria isolated from bioenhancer 'Amrit pani'. Ind J Trad Knowl. 2017;16:669-73.
20. Cojoc LR, Enache MI, Neagu SE, Lungulescu M, Setnescu R, Ruginescu R, et al. Carotenoids produced by halophilic bacterial strains on mural paintings and laboratory conditions. FEMS Microbiol Lett. 2019;366(21):243.
21. Haisheng W, Wang F, Zhu X, Yan Y, Yu X, Jiang P, et al. Biosynthesis and characterization of violacein, deoxyviolacein and oxyviolacein in heterologous host, and their antimicrobial activities. Bioche Engg J. 2012;67:148-55.
22. Mukherjee T, Bose S, Mukhopadhyay SK. Antioxidant properties of the carotenoid extracts of three *Deinococcus*–Thermus phylum bacteria, *Meiothermus* sp. strains RP and TP and *Thermus* sp. strain YY from Paniphala hot spring, India. Nutrire. 2017;42(7).
23. Zhao GZ, Li J, Qin S, Zhang YQ, Zhu WY, Jiang CL, et al. *Micrococcus yunnanensis* sp. nov., a novel actinobacterium isolated from surface-sterilized Polyspora axillaris roots. Int J Syst Evolu Microbiol. 2009;59(10):2383-7.
24. Huang CH, Wang CL, Liou JS, Lee AY, Blom J, Huang L. Watanabe K. Reclassification of *Micrococcus aloeverae* and *Micrococcus yunnanensis* as later heterotypic synonyms of *Micrococcus luteus*. Int J Syst Evolu Microbiol. 2019;69(11):3512-8.
25. Novikova NI, Matthews H, Williams I, Sewell MA, Nieuwoudt MK, Simpson MC, et al. Detecting phytoplankton cell viability using NIR Raman spectroscopy and PCA. ACS Omega. 2022;7(7):5962-71.
26. Vítek P, Jehlička J, Edwards HG, Osterrothová K. Identification of β-carotene in an evaporitic matrix—evaluation of Raman spectroscopic analysis for astrobiological research on Mars. Analytical and Bioanalytical Chem. 2009;393:1967-75.
27. Huang YY, Beal CM, Cai WW, Ruoff RS, Terentjev EM. Micro-Raman spectroscopy of algae: composition analysis and fluorescence background behavior. Biotechnol Bioengg. 2010;105(5):889-98.
28. Lu X, Al-Qadiri HM, Lin M, Rasco BA. Application of mid-infrared and Raman spectroscopy to the study of bacteria. Food Bioproc Technol. 2011;4:919-35.
29. Kaiser P, Surmann P, Vallentin G, Fuhrmann H. A small-scale method for quantitation of carotenoids in bacteria and yeasts. J Microbiol Meth. 2007;70(1):142-9.
30. Mani M, Keerthana G, Preethi K. Evaluation of antioxidant potential of bioactive colored metabolite isolated from *Exiguobacterium profundum* BC2-11 and its bioactivities. Int J Recent Sci Res. 2015;6:3612-7.
31. Selim AEI, Kassem MM, Bayoumy SM, El-Sawah MM, Ali DF. Effect of some sugars and agro-industrial by-products on carotenoids production by some yeast strains of *Rhodotorula* SPP. J Pl Prod. 2013;4(7):1097-107.
32. Jinendiran S, Kumar BD, Dahms HU, Arulanandam CD, Sivakumar N. Optimization of submerged fermentation process for improved production of β-carotene by *Exiguobacterium acetylicum* S01. Heliyon. 2019;5(5):e01730.
33. Velmurugan P, Hur H, Balachandar V, Kamala-Kannan S, Lee KJ, Lee SM, et al. *Monascus* pigment production by solid-state fermentation with corn cob substrate. J Biosci Bioengg. 2011;112(6):590-4.
34. Silvana TS, Daroit DJ, Sant'AV, Brandelli A. Stability modeling of red pigments produced by *Monascus purpureus* in submerged cultivations with sugarcane bagasse. Food Bioproc Technol. 2013;6:1007-14.
35. 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.
36. Benjamin S, Surekha P, Dhanya P. *Micrococcus luteus* strain BAA2, a novel isolate produces carotenoid pigment. Electro J Biol. 2016;12(1):83-9.

37. Deb PA, Madhugiri MJ. Optimization of apple-pomace based medium for pigment production by *Micrococcus flavus*. Bioscan. 2012;7(1):57-60.
38. Joshi VK, Attri D, Bala A, Bhushan S. Microbial pigments. Ind J Biotechnol. 2003;2:362-9.
39. Mohana DC, Thippeswamy S, Abhishek RU. Antioxidant, antibacterial, and ultraviolet-protective properties of carotenoids isolated from *Micrococcus* spp. Radiation Protect Environ. 2013;36(4):168-74.
40. Gómez PI, Barriga A, Cifuentes AS, González MA. Effect of salinity on the quantity and quality of carotenoids accumulated by *Dunaliella salina* (strain CONC-007) and *Dunaliella bardawil* (strain ATCC 30861) Chlorophyta. Biol Res. 2003;36(2):185-92.
41. Ibrahim ASS. Production of carotenoids by a newly isolated marine *Micrococcus* sp. Biotechnology. 2008;7(3): 469-74.
42. Asker D, Awad TS, Beppu T, Ueda K. Novel zeaxanthin-producing bacteria isolated from a radioactive hot spring water. Methods Mol Biol. 2012;892:99-131.
43. Kumar A, Vishwakarma HS, Singh J, Dwivedi S, Kumar M. Microbial pigments: production and their applications in various industries. Int J Pharm Chem Biol Sci. 2015;5(1):203-12.
44. Abdelaziz AA, Kamer AMA, Al-Monofy KB. *Pseudomonas aeruginosa* greenish-blue pigment pyocyanin: its production and biological activities. Microbes Cell Fact. 2023;22:110.
45. Zobell CE, Upham HC. A list of marine bacteria including descriptions of sixty new species. Bulletin of the Scripps Institution of Oceanography, University of California. 1944;5:239-92.
46. Sajjad W, Din G, Rafiq M, Iqbal A, Khan S, Zada S, et al. Pigment production by cold-adapted bacteria and fungi: colorful tale of cryosphere with wide range applications. Extremophiles. 2020;24(4):447-73.
47. Mohan K, Ravichandran S, Muralisankar T, Uthayakumar V, Chandirasekar R, Seedevi P. Carotenoids as a source of natural antioxidants: a review on their characterization and applications. Marine Drugs. 2021;19(11):619.
48. Udayawara RD, Maradagi T, Stephen NM, Niraikulam A, Nambi RK, Ponesakki G. Neoxanthin prevents H2O2-induced cytotoxicity in HepG2 cells by activating endogenous antioxidant signals and suppressing apoptosis signals. Molecular Biol Rep. 2021;48:6923-34.
49. Foong LC, Loh CW, Ng HS, Lan JC. Recent development in the production strategies of microbial carotenoids. World J Microbiol Biotechnol. 2021;37(1):12.
50. Zhang Q, Wang J, Li C, Zheng M, He Z, Zou Y, et al. Characterization and Bioactive Potential of Carotenoid Lutein from Gordonia rubripertncta GH-1 Isolated from Traditional Pixian Douban. Foods. 2022;11(22):3649.
51. Yu X, Jiang K, Zhang W, Dong S, Wu Y, Zhang G, et al. Purification, identification, and properties of a novel carotenoid produced by *Arthrobacter* sp. QL17 isolated from Mount Qomolangma. Antioxidants. 2022;11(8):1493.
52. Young AJ, Lowe GL. Carotenoids—antioxidant properties. Antioxidants. 2018;7(2):28.
53. Mandelli F, Miranda VS, Rodrigues E, Mercadante AZ. Identification of carotenoids with high antioxidant capacity produced by extremophile microorganisms. World J Microbiol Biotechnol. 2012;28(4):1781-90.
54. Abbes M, Baati H, Guermazi S, Messina C, Santulli A, Gharsallah N, et al. Biological properties of carotenoids extracted from *Halobacterium halobium* isolated from a Tunisian solar saltern. BMC Complement Altern Med. 2013;13:255.
55. Sudhakar T, Karpagam S, Premkumar J. Biosynthesis, antibacterial activity of pyocyanin pigment produced by *Pseudomonas aeruginosa* SU1. J Chem Pharmaceu Res. 2015;7(3):921-4
56. Zare M, Norouzi Roshan Z, Assadpour E, Jafari SM. Improving the cancer prevention/treatment role of carotenoids through various nano-delivery systems. Crit Rev Food Sci Nutri. 2021;61(3):522-34.
57. Entesar A, Mohamed HE, Abd El-Salam HS. In vitro antimicrobial activity of astaxanthin crude extract from *Haematococcus pluvialis*. Egypt J Aquat Biol Fisheries. 2022;26(2):95-106.
58. Naik R, Gupte S. Characterization of pigment produced by high carotenoid yielding bacteria *Paracoccus marcusii* RSPO1 and evaluation of its anti-diabetic, anti-microbial and antioxidant properties. Natural Product Research. 2024;38(6):968-77.
59. Li J, Xian J, Zhang S, An Y, Li N, Zhou D, Sun S, Wang J. Influence of pigment composition on antioxidant capacity of different tomato (*Solanum lycopersicum* L.). LWT. 2025:117871.

How to cite this article:
Aroumougame S, Chandrasekhar K, Shyu DJH. Studies on the production and optimization of yellow pigment extracted from the SB_2 strain and evaluation of its antioxidant properties. German J Pharm Biomaterials. 2025;4(3):13-22.