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A Marine Derived Carotenoid from *Planococcus Maritimus* KA01 with Promising Bioactivity

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Abstract: Marine ecosystems are recognized as rich reservoirs of bioactive pigment-producing microorganisms with potential industrial applications. In this study, an orange-pigmented bacterium was isolated from marine sediment collected from Colachel, Tamil Nadu, India, and identified as *Planococcus maritimus* KA01 through 16S rRNA sequencing and phylogenetic analysis. Optimization of culture conditions showed that maximum carotenoid production occurred at 25 °C, pH 7, after 96 h of incubation, with 1.0% NaCl, glucose as the carbon source, and under shaking conditions. Methanol showed the highest efficiency solvent for pigment extraction. Spectral characterization *via* UV-Vis, FTIR, and GC-MS suggested the presence of carotenoid-like compounds, with squalene identified as a major metabolite. The pigment extract exhibited antibacterial activity, with lower MIC and MBC values against *Staphylococcus aureus* compared with *Pseudomonas aeruginosa,* indicating greater susceptibility of Gram-positive bacteria under the tested conditions. Antioxidant analysis using the DPPH assay demonstrated dose-dependent radical-scavenging activity. Overall, the study suggests that *P. maritimus* KA01 is a promising source of carotenoid-containing pigments with antimicrobial and antioxidant properties. Further studies involving purification, toxicity assessment, and large-scale production are required to establish potential applications.

Keywords: *Planococcus Maritimus,* Marine Bacterium, Carotenoid Pigment, Antibacterial Activity, Antioxidant Activity.

1. Introduction

Pigments are compounds that impart color by absorbing specific wavelengths of light. Unlike luminescent materials, pigments remain stable and don't dissolve in whatever they're mixed with. This makes them useful across industries, in medical applications, and for environmental purposes [1]. There's growing concern about the harm caused by artificial dyes – they can be toxic and cause birth defects, mutations, and even cancer. As a result, there's a stronger push for natural, eco-friendly colorants. Consequently, demand for environmentally friendly colorants has surged. Consider the textile industry, it consumes around 8×10^5 tons of dyes annually. Yet a significant share of these dyes escapes during fixation and washes into rivers and lakes. This creates colored wastewater that disrupts entire ecosystems [2]. These concerns have heightened the need to find pigments from natural sources that won't harm the planet and living things.

Plant-based, animal-based, and microorganism-based natural pigments offer several benefits, including biodegradability, low toxicity, biological activities such as antimicrobial and antioxidant properties, and protection against UV radiation [3]. Among pigment types, microbial pigments have attracted considerable interest for industrial applications due to their high stability, rapid production rates, and favorable physicochemical characteristics. Microbial pigments remain stable year-round, whereas plant-based pigments are affected by seasonal changes; microbial pigments can be produced year-round without disruption. In addition, microorganisms can use inexpensive substrates, including agricultural waste and by-products, thereby making microbial pigment production economically viable [4].

Improvisation in analytical techniques has led to enhancements in the characterization and use of microbial pigments. Some analytical methods include TLC, UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), High-Performance Liquid Chromatography (HPLC), and Gas Chromatography-Mass Spectrometry (GC-MS). They have enabled the characterization of the microbial pigments and their biological activity [5]. This paved the way for the use of microbial pigments in the food, pharmaceutical, cosmetic, textile, and health care industries. Pigments produced by microbes are classified into different

categories, such as carotenoids, melanins, flavins, quinones, violacein, and monascins [6]. These pigments possess antioxidant, antibacterial, anticancer, and anti-inflammatory properties [7]. Some pigments, such as riboflavin and β-carotene, canthaxanthin, and astaxanthin, have been widely used in industry owing to their nutritional and functional relevance [8]. Among microbial pigments, carotenoids are an important class due to their broad biological significance and commercial applications. Carotenoids are lipophilic pigments composed of a polyene chain that imparts their characteristic colors and antioxidant capacity [9]. Carotenoids serve as a form of protection for microbes against oxidative and photo-oxidative damage by neutralizing free radicals and stabilizing cellular membranes. In addition to their biological significance in microorganisms, carotenoids have attracted much attention in the large-scale industrial sector due to their antioxidant and anti-inflammatory actions [10]. Rising demand for antioxidants from natural sources has spurred further research to identify microbial sources that produce stable carotenoids.

Marine microorganisms are considered rich sources of structurally unique and biologically active substances. The marine ecosystem is characterized by extreme and rapidly changing conditions, including salinity, UV radiation, nutrient availability, pressure, and temperature variations. Marine microorganisms must adapt to these challenging conditions and therefore produce adaptive substances, including pigments that help maintain cell membrane stability and tolerance to stressors [11]. In recent decades, marine microorganisms have yielded numerous bioactive metabolites, many of which remain poorly studied in terms of their potential applications [12]. Bacteria adapted to high salt concentrations, such as halophiles and halotolerants, are notable for their pigment production, as their adaptation strategies often include pigment biosynthesis. Pigments may help cope with adverse factors such as osmotic and oxidative stress, as well as radiation [13]. One of the most common groups of marine bacteria includes representatives of the *Planococcus* genus. They occur in abundance throughout marine and hypersaline environments. Representatives of the genus Planococcus possess a strong ability to adapt to changing environmental conditions and to produce carotenoids, among other compounds.

Despite the enormous studies that have been carried out on pigment-producing marine bacteria, only a few research efforts have concentrated on the carotenoids produced by the *Planococcus* genus. Most research has focused on identifying and initially characterizing the pigments. On the other hand, very few scientific studies have addressed the isolation of the strains, optimization of production, full characterization of the pigments, and their bioactivities within a single study [14]. Hence, the efficiency of production of such marine bacteria, their multiple functions, and applications can be well determined. Hence, the current study focused on the isolation and characterization of a carotenoid-producing marine bacterium, *Planococcus maritimus* strain; the optimization of pigment-producing conditions; the characterization of the purified pigment; and the evaluation of its biological activities [15].

2. Materials and Methods

2.1 Isolation and Characterization of the Carotenoid Pigment-Producing Strain

Marine sediment from Colachel, Tamil Nadu, India, was serially diluted and plated on nutrient agar. Orange-pigmented colonies were selected based on colony morphology and pigment characteristics. Pure isolates were maintained on nutrient agar slants for further characterization and pigment production studies.

2.2 Molecular Identification

Genomic DNA was isolated from the purified bacterial culture obtained in the present study. The quality and integrity of the extracted DNA were examined by electrophoresis on a 1.0% (w/v) agarose gel. This revealed a distinct, high-molecular-weight DNA band, confirming successful DNA extraction. A fragment of the 16S rRNA gene was amplified using universal bacterial primers 16S rRNA-F and 16S rRNA-R. Polymerase chain reaction (PCR) produced a single discrete amplicon of about 1500 bp, as visualized on an agarose gel. The amplified product was then purified to eliminate residual primers, nucleotides, and other impurities. Forward and reverse DNA sequencing reactions of the PCR amplicon were performed using the 16S rRNA-F and 16S rRNA-R primers with the BDT v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer. A consensus sequence of the 16S rRNA gene was generated from forward and reverse sequence data using alignment software. The 16S rRNA gene sequence was used to carry out BLAST with the ‘nr’ database of the NCBI GenBank database. Based on the maximum identity score, the first 10 sequences were selected and aligned using the multiple alignment software program Clustal W. A Distance matrix and phylogenetic tree were constructed using MEGA 10.

2.3 Optimization of Growth Conditions for Enhanced Pigment Production

To improve pigment yield from the isolated strain from a marine sediment sample, key growth parameters were systematically optimized. Carbon sources, including sucrose, glucose, fructose, maltose, lactose, and mannitol, were tested at 1% (w/v) to evaluate their effects on bacterial growth and pigment synthesis. The effects of temperature on biomass accumulation and carotenoid production were assessed across seven

conditions: 4°C, 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C. This helps to identify the optimal environment for metabolite biosynthesis. The pH of the culture medium was adjusted from 1 to 14 to determine the optimal acidity for growth and pigment formation. Salinity tolerance and its effect on carotenoid synthesis were studied by varying the sodium chloride concentration from 0% to 5%. Incubation time was also optimized by harvesting cultures at 24, 48, 72, 96, 120, and 144 hours. This determined the peak period for carotenoid accumulation. Both static and agitated conditions were examined to assess the impact of aeration and oxygen availability on pigment production. After optimal growth conditions were established, carotenoids were extracted using several solvents: acetone, methanol, ethanol, ethyl acetate, chloroform, DMSO, and petroleum ether. The most efficient solvent for pigment recovery was selected based on extraction yield and color intensity. This systematic optimization enabled the identification of conditions that maximize carotenoid production by the isolated strain. It highlights its potential as a sustainable and valuable source of bioactive pigments for various industrial applications [5, 6].

2.4 Statistical Analysis

All the experiments were performed in triplicate and expressed as the mean ± SD. Statistical analyses were conducted, and one-way ANOVA followed by Tukey's post hoc test was used. Statistical significance was considered at $p < 0.05$.

2.5 Preliminary Test for Carotenoids

Bacterial biomass was collected by centrifugation at 8,000 rpm for 10 minutes at 4°C and then washed with distilled water. Pigments were extracted by suspending the pellet in methanol and incubating at 60°C for 15 minutes. The extract was clarified by filtration through Whatman No. 1 paper. Preliminary confirmation of carotenoids was performed by adding concentrated sulfuric acid diluted in water (1:9). The development of a blue-green color indicated the presence of carotenoid pigments. The pigment extract was also scanned at 400-550 nm to support its identification [16].

2.6 Characterization of Bacterial Pigments

The carotenoid-containing pigment extract was characterized using UV-Visible spectrophotometry. This technique measures the amount of light a substance absorbs at different wavelengths. Fourier Transform Infrared (FTIR) spectroscopy was also used to identify chemical bonds through infrared absorption [17]. Gas Chromatography-Mass Spectrometry (GC-MS), which separates and identifies compounds, was employed to determine optical properties, functional groups, and chemical composition.

2.7 Antibacterial Activity

The antibacterial potential of the extracted carotenoid-containing pigment extract was evaluated using the agar well diffusion method against *S. aureus* and *P. aeruginosa*. Overnight cultures of the test bacteria were spread evenly on nutrient agar plates. Wells of 6 mm diameter were aseptically punched into the agar. 100 µL of the carotenoid extract (dissolved in methanol) was added to each well. Plates were incubated at 37°C for 24 h, and zones of inhibition around the wells were measured in millimeters. Wells containing acidified ethanol served as negative controls, while standard antibiotics were used as positive controls. All experiments were performed in triplicate to ensure reproducibility. This method follows the procedures described in previous studies on bacterial pigment bioactivity with slight modification [18, 19].

2.8 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of the pigment extract was determined by broth microdilution in accordance with CLSI guidelines. Serial two-fold dilutions of the extract were prepared in Mueller-Hinton broth in 96-well microtiter plates. Each well was inoculated with a standardized bacterial suspension (~5×10^5 CFU/mL). Plates were incubated at 37 °C for 18-24 h, and the MIC was recorded as the lowest concentration that completely inhibited visible bacterial growth. Sterility, growth, and solvent controls were included, and all assays were performed in triplicate to ensure reproducibility. Aliquots (10 µL) from MIC wells showing no visible growth were aseptically plated onto fresh nutrient agar and incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration of the pigment extract required to prevent colony formation, corresponding to ≥99.9% bacterial killing. All experiments were performed in triplicate and expressed as the mean ± SD [20].

2.9 Antioxidant Activity

The antioxidant capacity of carotenoid-containing pigment extract was determined using the DPPH free-radical scavenging method [21], with slight modifications. A 0.1 mM DPPH solution was prepared using methanol. Different concentrations of pigment extract (3, 6, 9, 12, and 15 µg/mL) were diluted to 1 mL with methanol, then mixed with 2 mL of DPPH solution. The mixtures were incubated for 30 min in the dark at room temperature, after which the absorbance was recorded at 517 nm using methanol as the blank. Ascorbic acid served as the positive control. The percentage of DPPH radical scavenging was calculated using the formula:

$$\%\ inhibition = [(A\ control - A\ sample) / A\ control] \times 100 \quad (1)$$

All measurements were performed in triplicate, and the IC_{50} value was determined from the inhibition curve.

3. Results and Discussion

Thirty-five morphologically distinct bacterial isolates were obtained from marine sediment. A consistent orange-pigmented strain was identified using DNA sequencing. BLASTn search of the consensus sequence against the NCBI GenBank database revealed 100% identity with multiple strains of *Planococcus maritimus* and ≥99.8% identity with other closely related *Planococcus* species. Phylogenetic analysis using the Maximum Likelihood method supported the placement of KA01 within the *P. maritimus* group. The validated 16S rRNA gene sequence of strain KA01 was submitted to the NCBI GenBank database under the accession number: OM349618.1. Based on sequence identity and phylogenetic analysis, the orange-pigmented isolate KA01 was identified as *P. maritimus* strain KA01.

Based on the optimization study, the influence of temperature on carotenoid production was assessed over 5-35°C, and the results showed a clear temperature-dependent pattern (Figure 1). Pigment production was minimal at 5°C (absorbance 0.18), indicating reduced metabolic activity at low temperatures. A gradual increase in carotenoid synthesis was observed at 15°C (0.68) and 20°C (1.14), reflecting improved enzyme performance and cellular metabolism. The highest carotenoid yield occurred at 25°C, with an absorbance of 1.86, suggesting that this temperature provides optimal conditions for the enzymatic steps involved in carotenoid biosynthesis. Beyond this optimum, pigment levels declined, with absorbance decreasing to 1.42 at 30°C and 0.54 at 35°C, likely due to thermal stress affecting enzyme stability and pigment integrity. These observations are consistent with previous reports indicating that microbial pigment production is generally favored at moderate temperatures [22]. Similar temperature optima (20-30°C) have also been reported for several carotenoid-producing bacteria. Quadratic regression analysis explained a moderate proportion of the variation in carotenoid production (R^2 = 0.66). Although the regression model was not statistically significant (F = 3.85, p = 0.117), the highest absorbance value was experimentally observed at 25°C. Therefore, 25°C was selected as the optimum temperature for subsequent experiments. All experiments were performed in triplicate, and results are presented as mean ± SD.

Carotenoid production varied significantly across the tested pH conditions, with a noticeable decrease in pigment yield at acidic (pH < 5) and highly alkaline (pH > 9) levels, highlighting pH as a key factor affecting enzyme activity and pigment stability (Figure 2). The highest carotenoid production occurred at pH 7.0, suggesting that neutral conditions offer the most favorable environment for the biosynthetic enzymes involved in pigment formation. At pH 6.0, pigment synthesis reached 1.33, about 67% of the maximum at pH 7.0. A moderate decline was observed at pH 8.0, with an absorbance of 1.24 (approximately 62% of the maximum), whereas at pH 9.0, absorbance dropped to 1.12, a 44% decrease relative to the optimal pH. A near-neutral pH likely supports enzymatic activity by favoring the ionization of catalytic residues, thereby maintaining their structural stability [23]. Lower pH values cause excessive protonation, disrupting these residues, decreasing enzyme activity, and increasing carotenoids' vulnerability to acid-mediated breakdown [24].

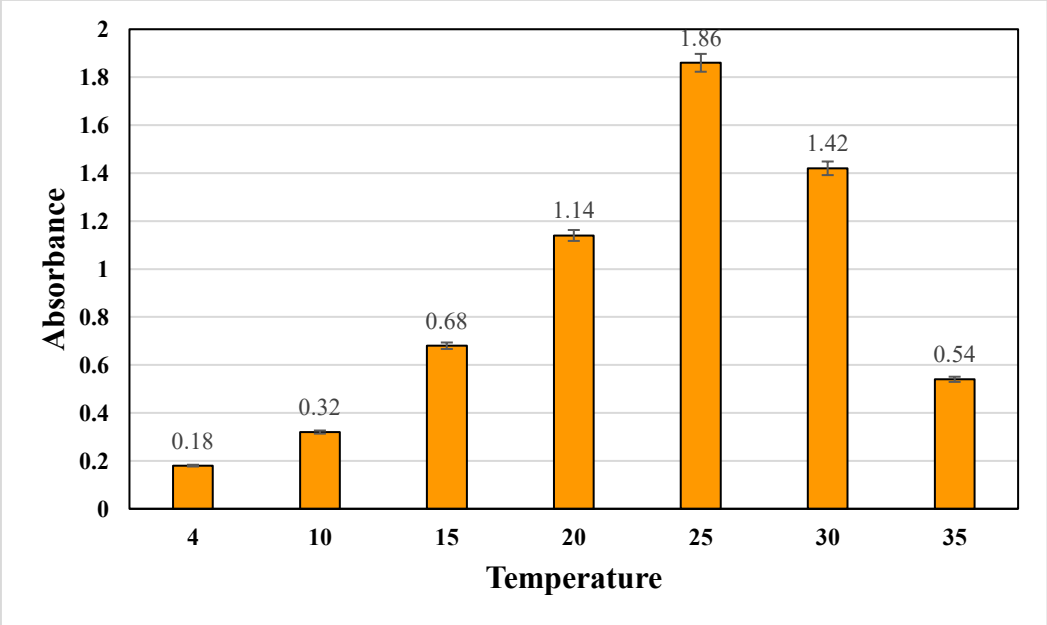


Figure 1. Effect of temperature on carotenoid pigment production.

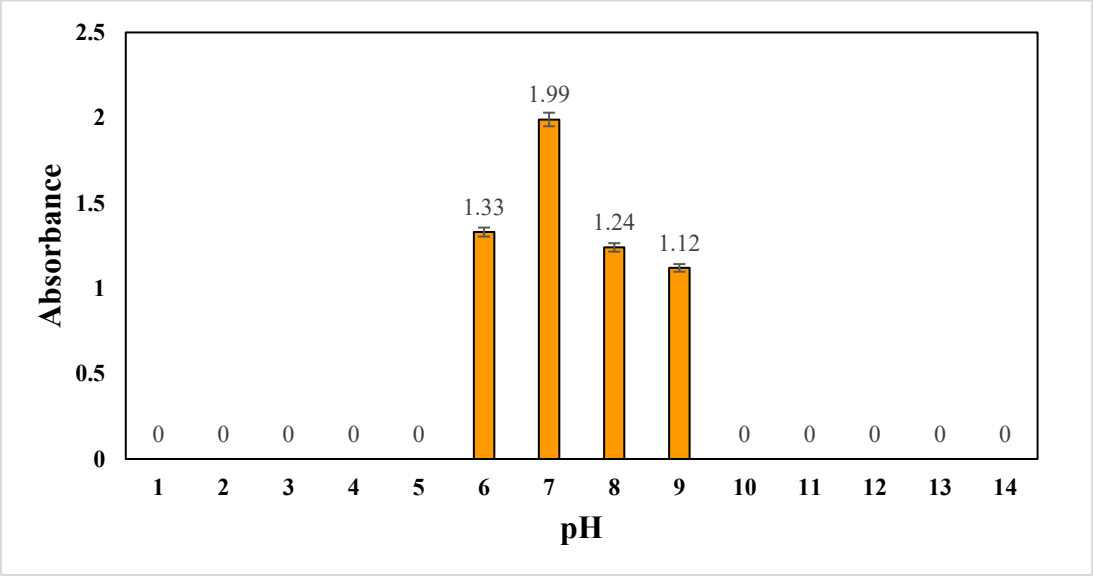


Figure 2. Effect of pH on carotenoid pigment production.

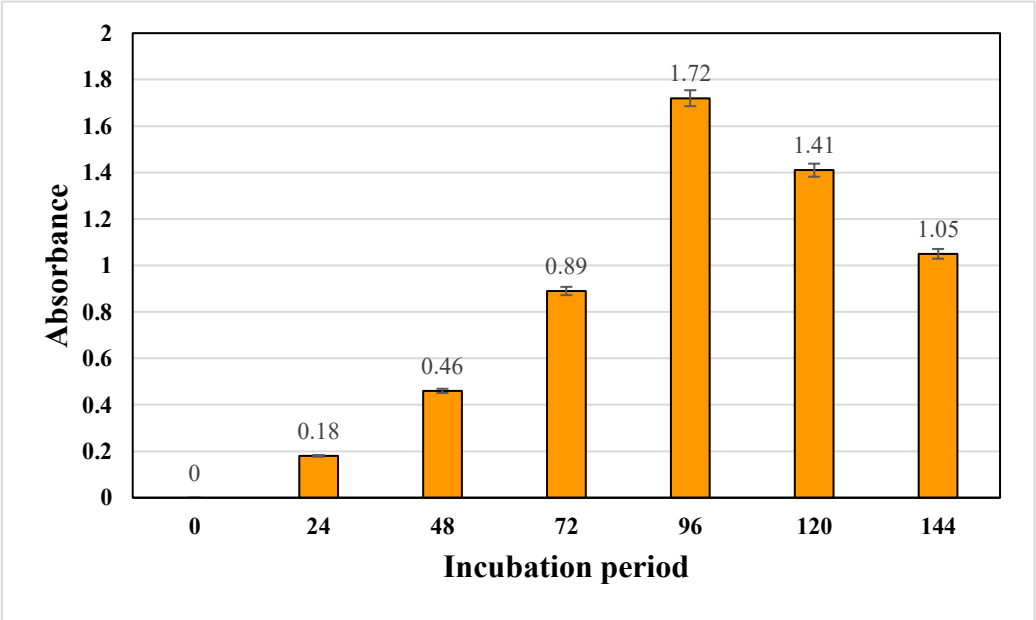


Figure 3. Effect of incubation period on carotenoid pigment production.

In alkaline conditions, deprotonation of amino acids such as cysteine and tyrosine may alter the active-site structure, reducing catalytic efficiency. Elevated pH levels also encourage isomerization and base-catalyzed degradation of carotenoids, contributing to the lower pigment levels seen at pH 9.0. Quadratic regression analysis demonstrated a relationship between pH and carotenoid production. Although the overall model approached significance ($F = 3.80$, $p = 0.056$), maximum pigment production was observed experimentally at pH 7.0, which was considered the optimum pH.

Carotenoid production increased steadily over the 144-hour incubation period (Figure 3). During the first 24 hours, pigment levels were low (absorbance 0.18), indicating that the culture was still in the lag phase, adapting to the growth medium and showing minimal secondary metabolite formation. By 48 hours, absorbance rose to 0.46, marking the onset of carotenoid synthesis. Production continued to increase, reaching 0.89 at 72 hours, consistent with active growth and enhanced biosynthetic capacity. The maximum pigment level was observed at 96 hours, with an absorbance of 1.72, suggesting that this incubation period provides the most favorable conditions for carotenoid formation, supported by optimal cellular activity and an adequate precursor supply [25]. After 96 hours, pigment levels declined. Absorbance decreased to 1.41 at 120 hours and further to 1.05 at 144 hours, indicating reduced enzyme activity [26]. The results demonstrate that 96 hours is the optimal incubation period for maximum carotenoid production, whereas extended incubation yields lower pigment levels due to diminished metabolic activity and instability of

accumulated carotenoids. All experiments were performed in triplicate, and results are presented as mean ± SD. Maximum pigment production was experimentally observed at 96 h and was therefore selected as the optimum incubation period.

Carotenoid production showed a distinct salt-dependent pattern across the tested NaCl concentrations (Figure 4). At 0% salt, pigment formation was low (absorbance 0.45). Production increased progressively to 0.5% (1.20) and peaked at 1.0% NaCl, with an absorbance of 1.98, indicating that a moderate salt level supports optimal carotenoid synthesis. Beyond this optimum, pigment levels declined steadily. Absorbance decreased to 1.36 at 1.5%, 0.92 at 2.0%, and 0.46 at 2.5%, with only minimal pigment detected at higher concentrations (3–5%). These findings indicate that salinity influences carotenoid production in *P. maritimus* KA01, with moderate salt concentrations supporting higher pigment yield. Moderate salinity conditions favored metabolite production in pigment-producing marine microorganisms [27, 28]. A gradual reduction in pigment production at higher NaCl concentrations suggests that elevated salinity may negatively affect carotenoid synthesis under the tested conditions. The highest carotenoid production was observed at 1.0% NaCl, which was selected as the optimum salt concentration for subsequent experiments. All experiments were performed in triplicate, and results are presented as mean ± SD.

The influence of six different carbon sources, such as sucrose, glucose, fructose, maltose, lactose, and mannitol, was evaluated by measuring pigment absorbance at 426 nm. A clear variation in carotenoid production was observed among the tested carbon sources, indicating that substrate type influences pigment production (Figure 5).

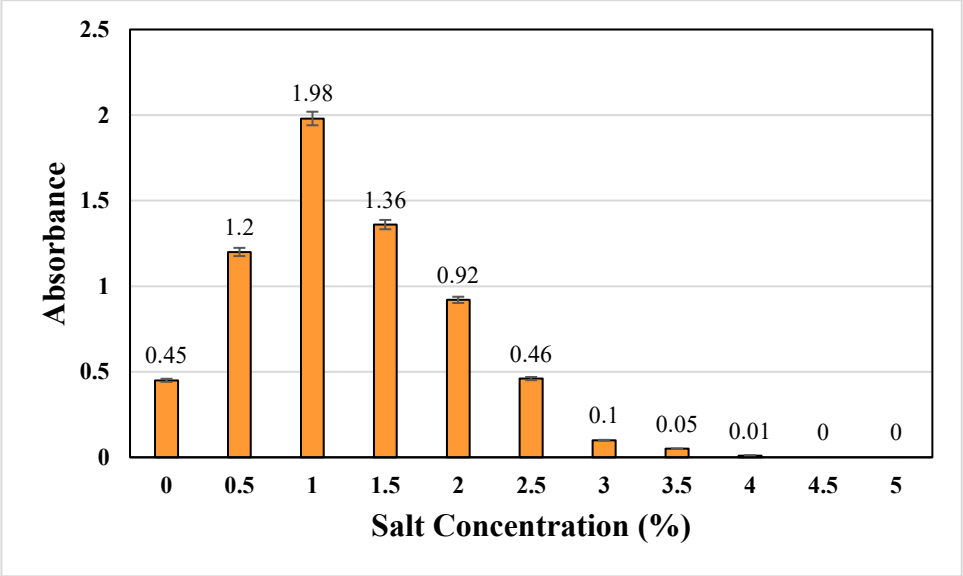


Figure 4. Effect of salt concentration on carotenoid pigment production.

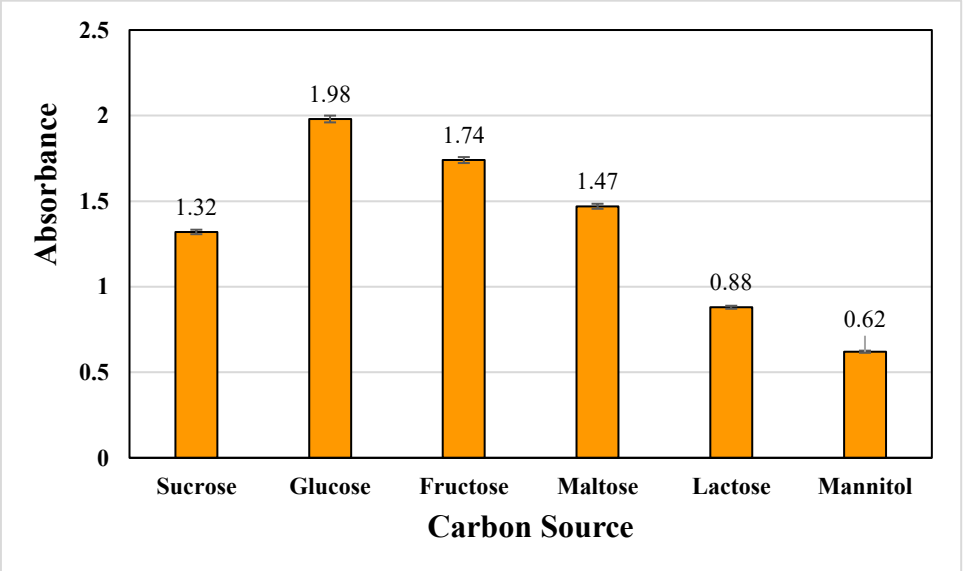


Figure 5. Effect of the Carbon source on carotenoid pigment production.

Glucose supported the highest carotenoid production, with an absorbance of 1.98, followed by fructose (1.74) and maltose (1.47). The pigment-producing marine and halophilic bacteria, in which glucose is the most favorable substrate for carotenoid biosynthesis due to its rapid uptake and catabolic efficiency [26, 27]. Sucrose resulted in moderate pigment production (1.32). In contrast, lactose (0.88) and mannitol (0.62) produced comparatively lower absorbance values. These differences suggest that carbon source utilization may influence pigment production efficiency under the tested conditions, and slowly metabolized carbon sources result in lower pigment production [29, 30]. The one-way ANOVA indicated significant carotenoid pigment production among the six tested carbon sources. Glucose showed the highest pigment production, whereas mannitol showed the lowest. Statistical comparisons among the tested conditions were further evaluated using Tukey HSD analysis. All experiments were performed in triplicate, and results are presented as mean ± SD.

Carotenoid production varied noticeably between shaking and static conditions (Figure 6). Under shaking conditions, pigment production reached an absorbance of 1.71, indicating that agitation provides a more favorable environment for carotenoid synthesis. Continuous mixing enhances aeration and ensures uniform distribution of dissolved oxygen, supporting oxygen-dependent steps in the biosynthetic pathway. Under static conditions, pigment production was considerably lower, with an absorbance of 0.82. In the absence of agitation, oxygen transfer depends solely on passive diffusion at the liquid surface, resulting in limited availability of dissolved oxygen [31]. The results indicate that shaking conditions were more favorable for carotenoid production and were therefore selected for subsequent experiments. All experiments were performed in triplicate, and results are presented as mean ± SD. The effect of different solvent systems on carotenoid extraction was evaluated, with methanol yielding the highest pigment recovery (1.53 absorbance at 426 nm, Figure 7).

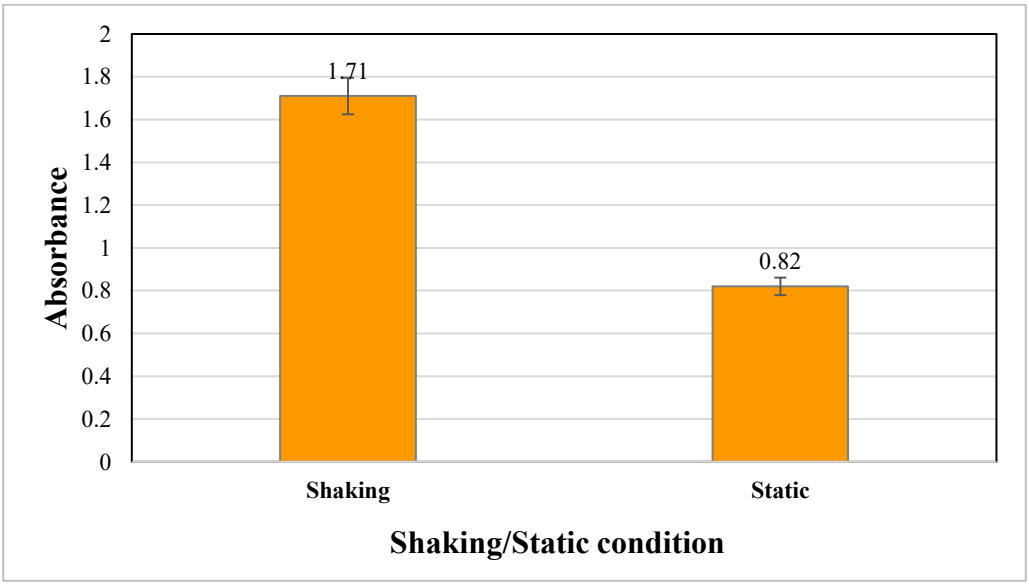


Figure 6. Effect of shaking/static condition on carotenoid pigment production.

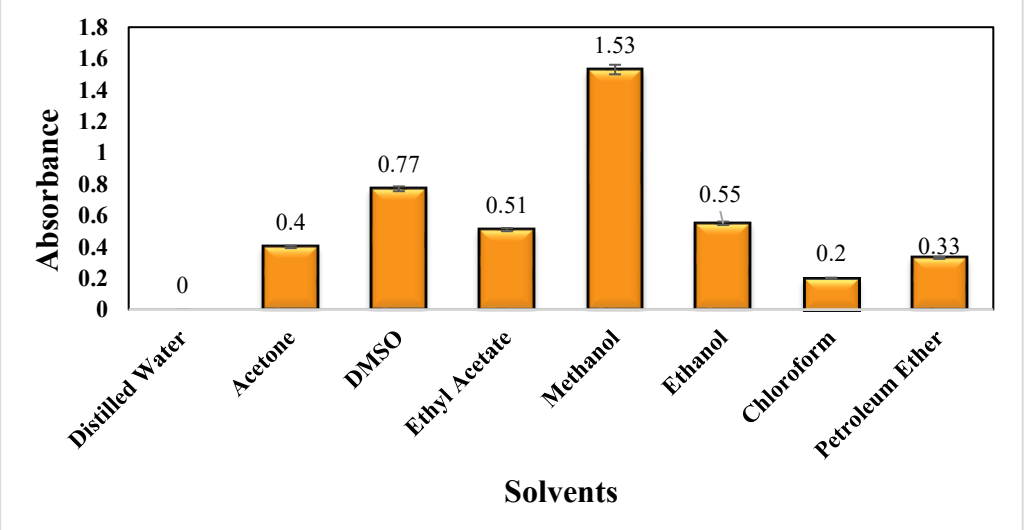


Figure 7. Effect of different solvent systems on carotenoid pigment production.

Higher extraction efficiency was observed with methanol, suggesting that solvent polarity influences pigment recovery [32]. In comparison, solvents such as DMSO and ethanol showed moderate extraction efficiency, while non-polar solvents like chloroform and petroleum ether were less effective. The one-way ANOVA indicated that carotenoid production varied significantly among the eight solvents, with the solvent factor accounting for most of the variation (Sum of Squares = 1.505). This suggests that the extraction depends on the solvent type. Among the solvents evaluated, methanol produced the highest pigment production, and nonpolar solvents were comparatively less effective. Statistical comparisons among the tested conditions were further evaluated using Tukey HSD analysis. All experiments were performed in triplicate, and results are presented as mean ± SD.

3.1 UV–Vis Spectrophotometer

The UV–Vis absorption spectrum of the pigment extracted from *P. maritimus* is shown in Figure 8. The spectrum exhibited two notable absorption peaks: a minor peak near 365 nm in the near-UV and a major peak at approximately 426 nm in the visible region. The absorbance pattern indicates that the pigment strongly absorbs light in the violet-blue region of the spectrum [33]. The major absorption peak at 426 nm is characteristic of carotenoid pigments, which exhibit maxima in the 400–500 nm range due to their conjugated double-bond systems [34]. This observation aligns with previous studies reporting carotenoid pigments in *Planococcus sp.,* with absorption maxima ranging from 447 to 466 nm [22]. The minor absorption peak at 365 nm may indicate the presence of additional conjugated structures or oxygenated derivatives, reflecting pigment complexity.

Carotenoid pigments are known to contribute to protection against photo-oxidative stress by absorbing light and scavenging reactive oxygen species [35 - 37]. Therefore, the observed absorption may indicate a potential photoprotective role of the pigment in marine environments. However, UV–Vis analysis alone cannot confirm pigment identity or functional properties, and additional structural characterization is required to establish compound composition and biological significance. The observed spectral characteristics nevertheless provide preliminary evidence supporting the carotenoid nature of the extracted pigment.

3.2 FTIR Spectral Analysis

The FTIR spectrum of the pigment extracted from *P. maritimus*, as shown in Figure 9, exhibited several characteristic absorption bands associated with carotenoid-like compounds. Strong aliphatic C–H stretching bands appeared at ~2920 cm^{-1} and ~2852 cm^{-1}, corresponding to asymmetric and symmetric stretching of $–CH_2–$ groups, confirming the presence of a long isoprenoid hydrocarbon chain typical of carotenoids [15, 38]. Prominent absorption peaks in the 1600-1700 cm^{-1} region, particularly near 1650 and 1665-1680 cm^{-1}, represent C=C stretching vibrations of an extended conjugated polyene system, the defining chromophoric feature of carotenoids. In addition, absorption bands detected within the 1700-1750 cm^{-1} region indicate oxygenated functional groups [39], such as carboxyl or ester linkages. Additional structured peaks in the 1000-1300 cm^{-1} range may be attributed to C-O stretching vibrations. The observed functional-group profile is generally consistent on oxygenated carotenoid compounds produced by halophilic bacteria, including members of the genus *Planococcus* [40].

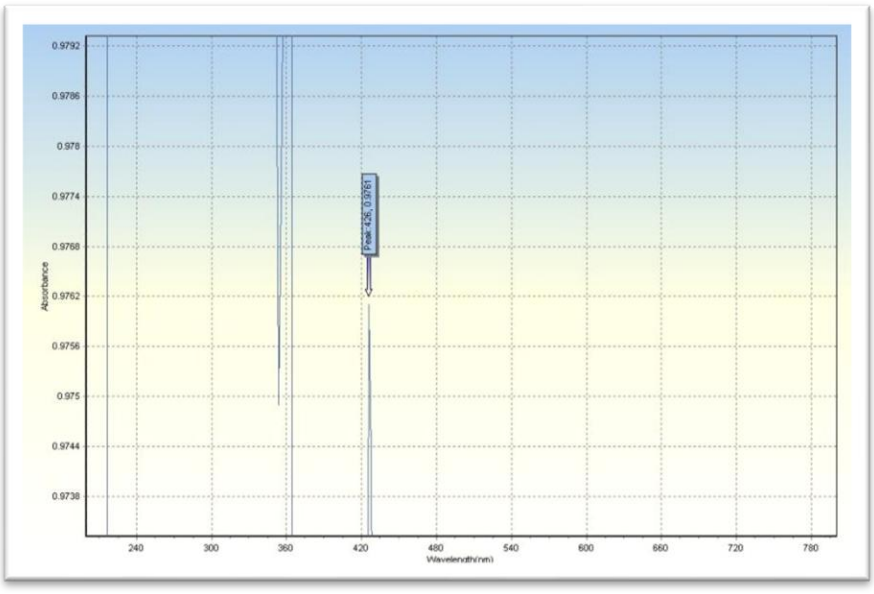


Figure 8. UV-Visible analysis of the carotenoid pigment isolated from *P. maritimus* KA01.

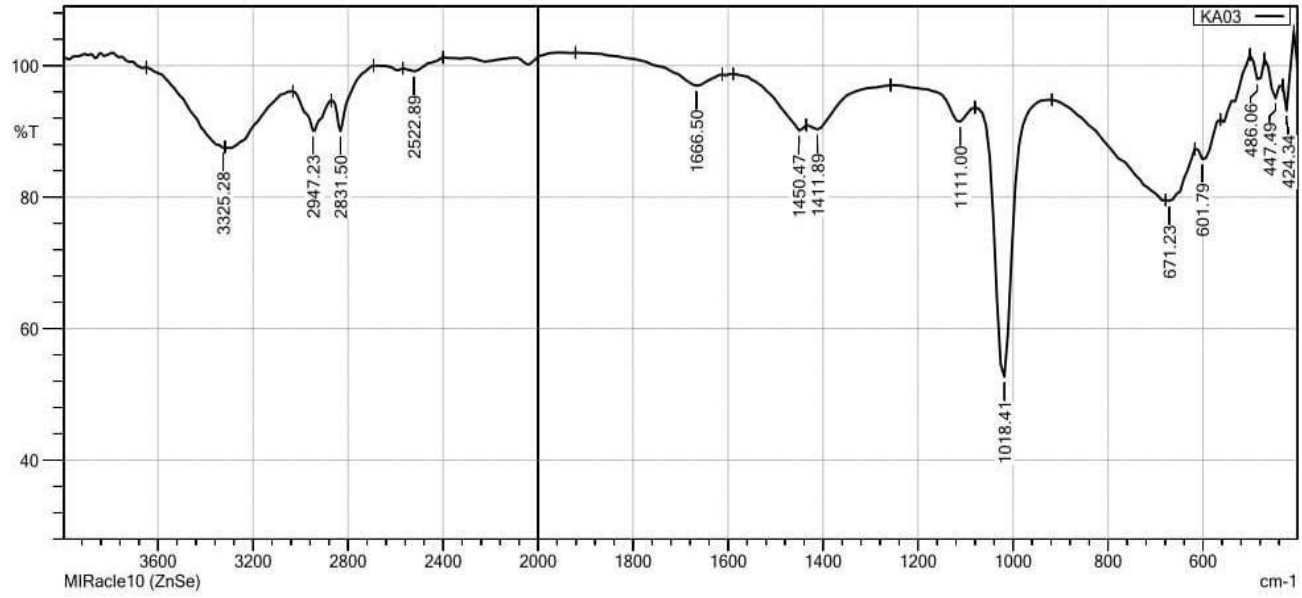


Figure 9. FTIR analysis of the carotenoid pigment isolated from *P. maritimus* KA01.

Furthermore, when interpreted together with the UV-Vis spectral profile, the FTIR results provide supporting evidence for the carotenoid-like nature of the extracted pigment. However, FTIR analysis alone is insufficient to confirm the precise molecular structure or the presence of glycosylated carotenoid derivatives; further structural analyses are required for definitive identification.

3.3 GC-MS Analysis

GC-MS analysis of the pigment extract revealed a complex mixture of hydrocarbons, fatty acid methyl esters, heterocyclic molecules, long-chain esters, and terpenoid metabolites (Figure 10). A total of 35 compounds were identified based on mass spectral similarity to NIST libraries. Among the detected compounds, squalene showed high abundance with a retention time of 17.885 min and represented 30.74% of the total peak area. The second major peak corresponded to oxalic acid monoamide n-propyl dodecyl ester (21.26%), a long-chain fatty acid derivative.

Squalene is a well-known triterpenoid and a key intermediate in carotenoid and sterol biosynthesis, commonly produced by marine bacteria [37]. It's in the extract, which may indicate involvement of isoprenoid-associated metabolic pathways commonly linked to carotenoid biosynthesis in *Planococcus* species. However, further targeted metabolic studies are required to confirm pathway-level associations [41]. Long-chain esters and lipid-based compounds are commonly found in halophilic bacteria, where they play essential roles in membrane stabilization under osmotic stress [42]. A diverse set of fatty acid methyl esters (FAMEs) was also detected, including methyl nonadecanoate (1.32%), methyl docosanoate (1.82% and 5.35%), methyl tridecanoate (3.17%), tetracosanoic acid methyl ester (1.65%), and methyl 18-fluorooctadecanoate (0.99%). FAMEs are typical products of bacterial lipid turnover and are frequently identified in GC–MS profiling of marine/halophilic organisms [43]. Their presence indicates active synthesis of long-chain fatty acids, which are required to maintain membrane fluidity and salt tolerance. Many minor compounds (<5%), including nitrogen-containing heterocycles (pyridazine, oxazine derivatives), cyclic ketones, benzoate esters, and long-chain hydrocarbons such as octatriacontane derivatives. Such compounds are reported as secondary metabolites or intermediates in the biosynthesis of fatty acids, polyketides, and aromatic compounds in bacteria [44].

However, compound identification in the present study was based primarily on spectral library matching, and GC–MS analysis alone cannot confirm definitive structural identity or direct involvement in carotenoid biosynthetic pathways. Therefore, additional purification and targeted analytical approaches are required for compound-level confirmation and pathway validation.

3.4 Antibacterial Activity

The antibacterial activity of the pigment extract was evaluated against *S. aureus* and *P. aeruginosa* using the agar well diffusion method (Figure 11). Distinct zones of inhibition were observed around wells containing the pigment extract, whereas control wells showed negligible or no inhibition, indicating that the antibacterial activity was associated with the applied extract. For *S. aureus*, the pigment extract produced a markedly larger inhibition zone than the control, indicating strong activity against Gram-positive bacteria.

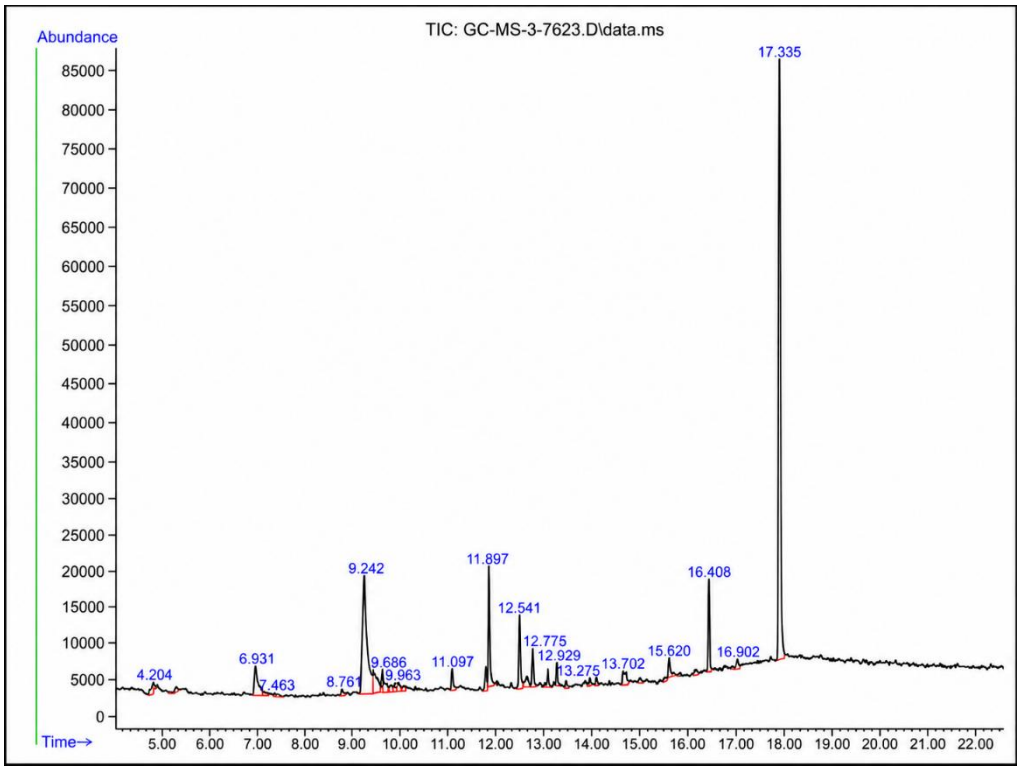


Figure 10. GC-MS analysis of the carotenoid pigment isolated from *P. maritimus* KA01.

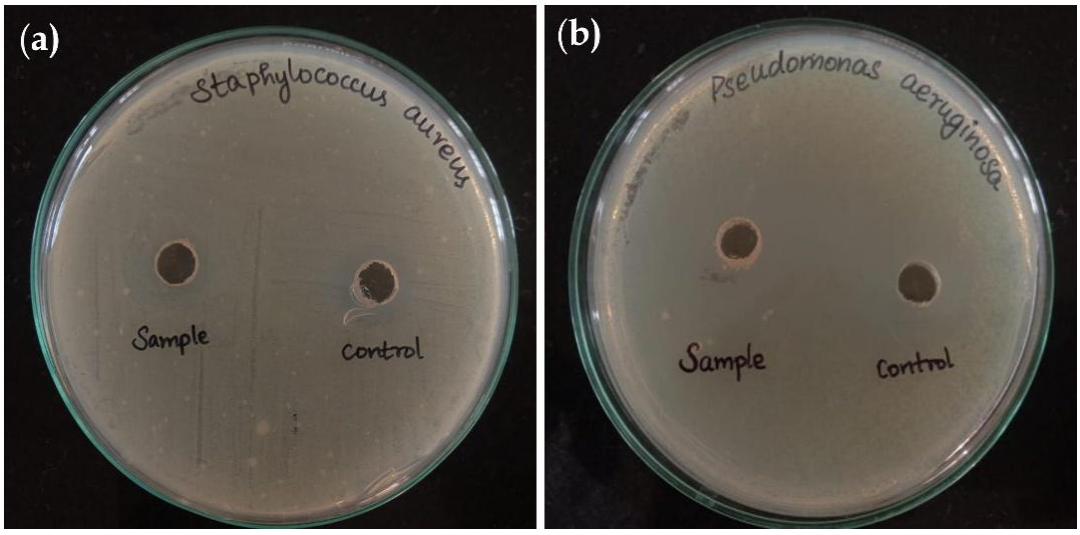
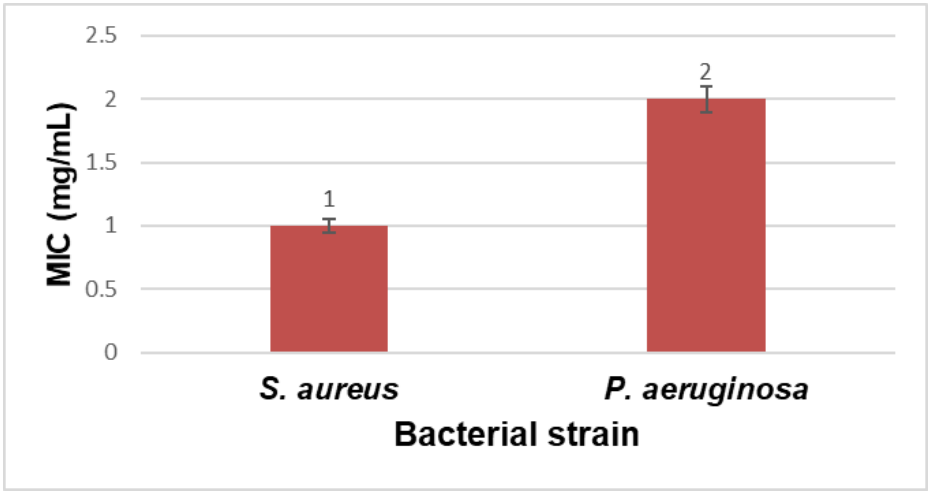


Figure 11. Antibacterial activity of pigment **(a)** *S. aureus*, and **(b)** *P. aeruginosa*.

For *P. aeruginosa*, a moderate inhibition zone was observed, indicating that the extract also exhibits activity against Gram-negative bacteria, albeit to a lesser extent. This pattern aligns with known structural differences between the bacterial groups; the outer membrane in Gram-negative organisms limits the penetration of antimicrobial compounds [45]. Carotenoid pigments from halophilic and marine bacteria, including those reported from *Planococcus* sp., have previously been associated with antimicrobial activity. The observed antibacterial effects in the present study may be related to membrane interactions or other bioactive mechanisms; however, mechanistic studies were not performed, and therefore specific modes of action cannot be confirmed [46, 47]. The present findings are consistent with earlier reports showing that carotenoid extracts strongly inhibit *S. aureus* and exhibit moderate activity against *Pseudomonas* species [48, 49]. The greater susceptibility of *S. aureus* may be linked to its thick but more permeable peptidoglycan layer. In contrast, the reduced sensitivity of *P. aeruginosa* is attributed to intrinsic resistance mechanisms and efflux systems [48]. However, the specific compounds responsible for antibacterial activity and their mechanisms of action were not investigated in this study. Therefore, further purification and mechanistic studies are required to identify the active components and confirm their antibacterial mode of action.

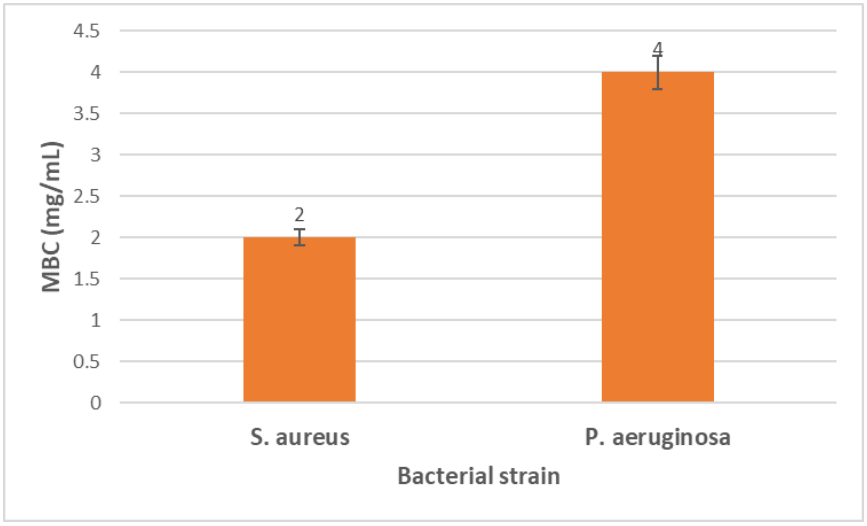
3.5 MIC & MBC

The MIC assay showed that the carotenoid extract inhibited both *S. aureus* and *P. aeruginosa*, with MIC values of 1.0 ± 0.0 mg/mL and 2.0 ± 0.0 mg/mL, respectively (n = 3) (Figure 12). The lower MIC for *S. aureus* suggests a stronger inhibitory effect against Gram-positive bacteria, likely due to differences in cell wall structure. Gram-positive bacteria have a thick peptidoglycan layer without an outer membrane, which facilitates easier penetration by hydrophobic carotenoid molecules, as reported for carotenoid-rich extracts from halophilic *Planococcus* sp. [35, 48]. In contrast, the higher MIC for *P. aeruginosa*, a Gram-negative bacterium, can be attributed to its outer membrane, which is enriched in lipopolysaccharides, limiting permeability and conferring resistance to hydrophobic compounds [49]. These results indicate that carotenoids possess bacteriostatic activity, particularly against Gram-positive pathogens. The MBC values of the carotenoid extract were 2.0 ± 0.0 mg/mL for *S. aureus* and 4.0 ± 0.0 mg/mL for *P. aeruginosa* (n = 3) (Figure 13), demonstrating that the bactericidal activity is concentration-dependent. The requirement for approximately twice the MIC concentration to achieve bacterial killing suggests that carotenoids may exert antibacterial effects through membrane-associated interactions or other mechanisms. However, the present study did not directly investigate the mode of action. This observation aligns with previous reports indicating that C_{30} and C_{50} carotenoids from *Planococcus*, *Deinococcus*, and related extremophiles act by perturbing membrane integrity and inducing oxidative stress [48, 50]. The higher MBC against *P. aeruginosa* reflects its intrinsic resistance mechanisms, including efflux pumps and low outer-membrane permeability [47]. Overall, these findings confirm that carotenoid extracts are effective bactericidal agents, though Gram-negative bacteria require substantially higher concentrations to be killed.



Values are expressed as mean ± SD (n = 3).

Figure 12. MIC analysis of the carotenoid pigment isolated from *P. maritimus* KA01.



Values are expressed as mean ± SD (n = 3).

Figure 13. MBC analysis of the carotenoid pigment isolated from *P. maritimus* KA01.

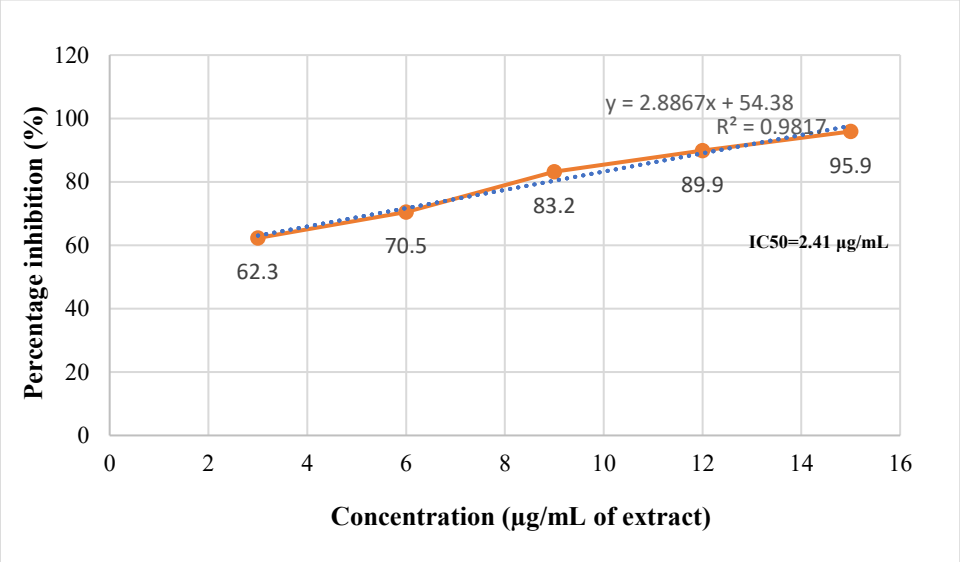


Figure 14. DPPH radical scavenging activity of the carotenoid extract.

3.6 Antioxidant Activity

The antioxidant potential of the carotenoid extract was evaluated using the DPPH radical-scavenging assay, with ascorbic acid as the reference standard. The carotenoid extract exhibited concentration-dependent radical-scavenging activity, with inhibition increasing from 62.30% at 3 µg/mL to 95.90% at 15 µg/mL (Figure 14). The IC_{50} value of the extract was estimated to be approximately 2.4 µg/mL, indicating strong antioxidant activity. The concentration–response curve showed good linearity (R^2 = 0.9817), indicating reliable assay performance. Similar antioxidant activities have been reported for carotenoids derived from marine microorganisms, where pigment molecules contribute significantly to protection against oxidative stress [17, 51]. Further purification and characterization of the active carotenoid compounds are required to identify the specific molecules responsible for the observed antioxidant activity and to evaluate their potential applications in pharmaceutical, nutraceutical, and food industries. Overall, the significance of the present study lies not only in the isolation of a pigment-producing marine *Planococcus* strain but also in the integration of production optimization, pigment characterization, and functional validation within a single workflow. Previous studies on *Planococcus sp*. have primarily focused on pigment occurrence or preliminary characterization. In contrast, the present findings highlight the potential of this marine isolate as a source of bioactive carotenoids with antimicrobial activity and antioxidant properties. Furthermore, the optimized production conditions and observed bioactivities indicate that this strain could serve as a promising candidate for future biotechnological applications.

4. Conclusion

The current investigation identified *P. maritimus* KA01 as a carotenoid-producing marine bacterium with the potential to produce bioactive compounds. Optimization studies indicated that pigment production was highest at 25°C, pH 7, after 96 h of incubation, and in the presence of 1% NaCl, with glucose as the preferred carbon source under shaking conditions. Analytical characterization using UV-Vis, FTIR, and GC-MS suggested the presence of carotenoid-like compounds with oxygenated functional groups, along with a high proportion of squalene, indicating possible involvement of isoprenoid-associated metabolites. The pigment extract exhibited antibacterial properties, with greater sensitivity to *S. aureus* than to *P. aeruginosa*, possibly due to differences in cell wall structure and permeability. The antioxidant screening further demonstrated dose-dependent radical-scavenging activity, supporting the extract's preliminary bioactive potential. However, additional purification, compound-level identification, toxicity evaluation, stability assessment, yield optimization, and large-scale production are required before industrial application can be established. Furthermore, pigment production in the present study was evaluated primarily using absorbance-based measurements, and future studies focusing on biomass-normalized productivity and mass-based pigment quantification would enable better comparison with existing studies and a more robust assessment of industrial applicability. Overall, *P. maritimus* KA01 may serve as a promising source of carotenoid-rich pigments with preliminary bioactive potential, supporting further investigations for future applications.

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Authors Contribution Statement

Aswini Anguraj: Methodology, Investigation, Writing - Original Draft. Subashkumar Rathinasamy: Conceptualization, Supervision, Validation, Writing - Review & Editing. Both the authors have read and agreed to the published version of the manuscript.

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Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Data Availability

The data supporting the findings of this study can be obtained from the corresponding author upon reasonable request.

Has this article screened for similarity?

Yes