




Research Article

Production, Characterization, and Antimicrobial Activity of Pigment from *Streptomyces* Species

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Production of potential pigments using the bacterial source can be an important area of research to disclose its possible value in diverse industrial applications. Soil samples from various terrestrial, rhizosphere, and forest habitats were collected from Karnataka, and pigment-producing actinomycetes were recovered. In the present investigation, 25 strains were isolated using starch casein agar medium; further, phenotypic, biochemical, and morphological and the 16S rRNA gene sequence studies have suggested that the strain belonged to the *Streptomyces* species, strain BJZ10, an actinomycetes. With higher production of pigments, starch casein broth medium was used, and the extraction of actinobacterial pigments was done by exclusively using methanol solvent. The pigment was characterized by UV-visible spectroscopy; absorption spectra range from 220 to 250 nm. The FTIR characterization was carried out, and the spectrum obtained for the strain BJZ10 indicated alkane, alkyls, alkynes, alcohols, esters, and sulfate functional groups. Antibacterial activity of the pigments was tested against *Bacillus cereus* (Gram-positive) and *Escherichia coli* (Gram-negative), and significant results were compared. The present study revealed the brown pigment synthesized by *Streptomyces* sp. strain BJZ10, known for its potential antimicrobial activity.

1. Introduction

Colour is as variable and evanescent in the form of pigment as invisible nature. Pigments are the major sources of colours and are also key components in manufacturing

paint; each thread of cloth, the core building of the phone case, is a pigment. Pigments are restorative materials used for various applications. Their main benefit is associated with imparting colour. Amongst microorganisms, multiple groups of bacterial species can produce a wide range of by-

products, and one of the main by-products is pigment. Various researchers have investigated the production and application of bacterial pigments as natural colorants [1]. However, several pigment-producing industries practiced the traditional methods in extracting pigments and thus later translating them into clay. In modern days, pigments can be synthesized under laboratory conditions. Colour plays a crucial role in food. The freshness and safety of food can only be determined by its colour. The colour of the food is visually impactful and can stimulate people appetite. Because of the consumer's health concerns on using the synthetic dyes, there is a huge scope in promoting natural colours. Authorized, artificial colours are more popular due to their lower cost, providing a strong and consistent colour [2]. They can also blend easily to give a mixture of shade. The utility of artificial food colours is slowly coming down upon realizing their destructive effects on human health. However, the shift from synthetic colours will be an extremely slow process due to comparatively higher costs of natural colours [3, 4].

Hence, switching to obtain biological colouring agents from different sources like plants or microorganisms is an hour long. Microbial pigments are of great interest considering the stability of obtained pigments, easy availability, and easy cultivation and extraction methods [5, 6]. Microorganisms produce many bioactive compounds such as carotenoids, melanins, flavins, and quinones. Filamentous fungi such as actinomycetes are a well-known producers of many pigments primarily used in the food and pharmaceutical industry [7–9]. In our present investigation, we have reported that actinomycetes are an economically and biotechnologically potential producers of natural dyes and pigments. Actinomycetes are prokaryotic organisms that are generally rod-shaped and filamentous. They form long branching hyphae and have some common characteristics with fungi.

Actinomycetes are popularly known as the producers of many bioactive molecules [10, 11]. Actinomycetes represent around 7,000 of the metabolites which have been announced in the Dictionary of Natural Products. Bacterial pigment production is now one of the emerging research fields to demonstrate its potential for various industrial applications [3]. Recent studies have shown that microorganisms are a promising source of natural colours. The significant characteristics of actinomycetes were found to be pigment production. Research on pigment production from natural sources has grown due to its nontoxic nature. The FDA (Food and Drug Administration) has permitted several biocolors and presently endures in market; they are β -carotene, riboflavin, ArpinkRed, astaxanthin, lycopene, and *Monascus* pigments [3]. FDA and EFSA (European Food Safety Authority) tend to evaluate the food safety of additives via specified international guidelines and codes of practice. In the pharmaceutical industry, bacterially derived pigments have indicated their prolific agents in treating numerous diseases, as they possess the properties such as immunosuppressive, anticancer, and antimicrobial.

The bacterial-originated pigments can be helpful in clinical practices in diagnosing various kinds of cancers, diabetes

mellitus, leukemia [12], etc. The naturally derived colorant is scanty in the market and can intensify its production in the food industry. The present requirement of natural pigments can foster researchers in discovering novel natural colouring agents [13]. The bioactive compounds such as violacein (violet), prodigiosin (red), pyocyanin (blue green), flexirubin (yellowish orange), and carotenoids (yellow-orange) from the wide variety of bacterial strains can serve as potential sources for antiviral, anticancer, antitumor, antioxidant, and antimicrobial activities and other biological activities. Bacterial pigment synthesis is currently among an emerging research field and can contribute massively to various industrial applications [14]. The present investigation reveals the production of potential pigment production and characterization of the pigments using UV-visible spectroscopy and FTIR analysis data. Thus, by using the produced pigments, antimicrobial activities were represented and are promising in biomedical applications.

2. Materials and Methods

2.1. Collection of Samples. Overall, three samples (Raynal village in Dharwad district, one each from forest, lake soil, and rhizosphere of cornfield) were collected. Further, the collected samples from different sampling plots were added to the zip lock covers and stored under the refrigerator until they were used and later processed for isolating potential pigment-producing actinomycetes.

2.2. Isolation of Actinomycetes. All three soil samples were subjected to the preheat treatment process. This was done by air drying the samples at about 55°C temperature for up to 60 minutes. SCA (starch casein agar) media (g/l) consists of 10 g of soluble starch, 0.3 g vitamin free casein, 2 g KNO₃, 2 g of NaCl, 2 g of K₂HPO₄, 0.05 g of MgSO₄·7H₂O, 0.02 g of CaCO₃, and 0.01 g of FeSO₄·7H₂O, and the pH was adjusted to 7.0 ± 0.2 and 15 g of agar used for isolation of actinobacteria [15]. Further, all these samples were serially diluted, and the dilutions 10⁻², 10⁻⁴, 10⁻⁶, 10⁻⁸, and 10⁻¹⁰ were spread on the petri plates containing solid SCA medium. After the inoculation, petri plates were kept at 28 ± 2°C, RT (room temperature) for about 15 days. The petri plates were observed for the growth and pigment production daily and a peculiar range of colonies such as chalky to leathery appearance. Different isolated colonies were randomly picked from the petri plates, separately inoculated into the casein broth, and kept at RT for 24 to 48 h afterward; the individual colonies were subcultured and maintained on the SCA slants.

2.3. Morphological Studies. Bergey's systematic bacteriology manual shows that each isolate's colony morphology and culture characteristics have been systematically studied. A total of twenty-five strains were isolated; among them, six were recovered from the forest soil, eight were isolated from lakeshore soil, and six were from rhizosphere soil.

2.4. Identification of Pigment-Producing Actinomycetes. One isolate was identified as *Streptomyces* sp. by 16S rRNA gene

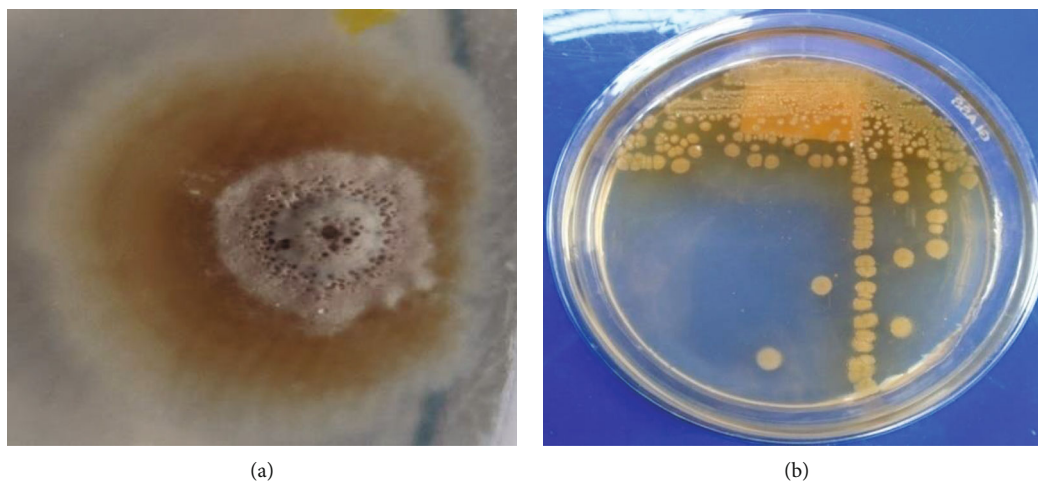


FIGURE 1: (a) Isolated colony and (b) pigment production using BJZ10 strain.

sequence analysis. The identified isolates BJZ10 were subcultured in starch casein agar slants and preserved.

2.5. Production of Pigments. Starch casein broth has been prepared and preincubated overnight; 2 ml of 24 h old suspension cultures of strain have been inoculated into 100 ml into starch casein broth and incubated at room temperature in a rotary shaker at 120 rpm for one month; the produced pigments were extracted by following steps.

2.6. Extraction of Pigments by Using Different Solvents. Equal volume of different solvents ethanol, methanol, acetone, propanol, hexane, water, and supernatant sample was taken and mixed well. The mixture was centrifuged at 8,000 rpm for 10 minutes; the supernatant was monitored at 540 nm to check the optical density. The extract was lyophilized and used for further characterization studies [7].

2.7. Partial Characterization of Pigments

2.7.1. UV/Vis Spectrophotometer. UV-visible spectrophotometer assay was carried out to determine the range of λ_{\max} ; during the process, methanol was used to dissolve the extracted pigment from the microbe. The sample was measured in the range of 200-800 nm, and the λ_{\max} of the pigment was observed and determined [7].

2.7.2. FTIR Spectroscopy (Fourier Transform Infrared Spectroscopy). Powdered form of the extract was used for FTIR analysis to determine the number of functional groups in the extract. The asymmetric, symmetric, and stretching frequencies were analyzed for the important IR bands (OH, N-H, C=C, C-H, C-N, C-H, and C-O) [16].

2.8. Biological Assay

2.8.1. Antimicrobial Activity. The antimicrobial activity of dried methanol-extracted pigment against pathogenic bacteria was performed using an agar well diffusion assay. Various concentrations 50 mg/ml, 25 mg/ml, and 12.5 mg/ml of pigments were used. The Gram-negative bacteria, including *Escherichia coli*, and Gram-positive bacteria *Bacillus cereus*

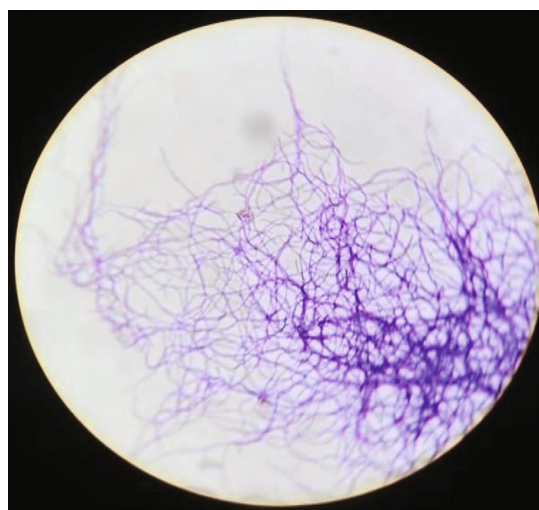


FIGURE 2: Microscopic observation of the BJZ cells.

were used for antimicrobial activity assay. The selected bacteria were allowed to grow on nutrient broth (NB) medium at a temperature of 30°C for about 24 h, and about 100 μ l of the bacterial culture was added to the prepared petri plates. Four wells (measuring diameters of 6 mm) pierced on NB agar plates, with different concentrations, 12.5 mg/ml, 25 mg/ml, and 50 mg/ml of dried methanol extract, were used. 50 μ l of each sample was loaded into the wells [17]. Further, the inoculated plates were allowed to grow for 24 h, and after that, the clear zone of inhibition was determined around the inoculated well.

3. Results and Discussion

Overall, twenty-five isolates were isolated through serial dilution technique by spread plate method on the SCA (starch casein agar) medium. The colony characteristics of each isolate have been studied and photographed (Figures 1 and 2). Out of 25 isolates, only six isolates were pigment-producing. Gram's staining has been performed

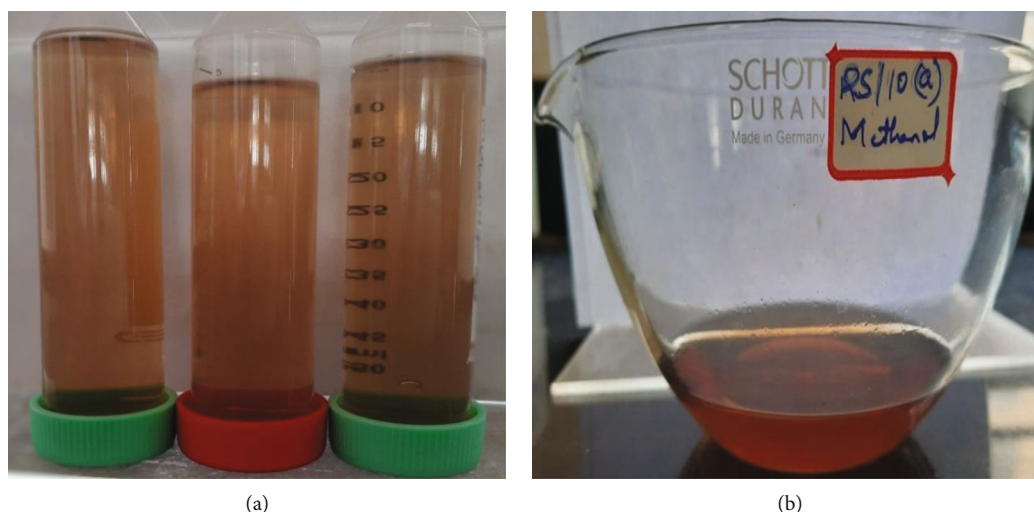


FIGURE 3: (a) Crude pigment extract and (b) pigment extracted using methanol solvent.

on all selected isolates (out of 25 isolates, 22 were Gram-positive, and three were Gram-negative). BJZ10 strain was selected for further studies; the colony was hard, round-shaped, 3–4 mm in size; the pigment was diffused into the media. By Gram's staining, the cells of the organism was blue-coloured, rod-shaped, long-chain formation and in cluster form. Among all isolates, a brown pigment-produced organism was sent for 16S rRNA sequence analysis, and the results indicated that the potential isolate was almost similar to *Streptomyces* sp. strain RF-2. The synthesis of diffusible dark brown pigments itself has long been considered a vital factor in description of the *Streptomyces* species [18]. The *Streptomyces* species was cultured on a yeast extract-malt extract medium at a temperature of 28°C for about 7–10 days of incubation. Afterward, spores of freshly grown culture in slant were picked and inoculated into seed medium which consisted of yeast extract 4 g/l, malt extract 10 g/l, dextrose 4 g/l, and tyrosine 1 g per liter at 220 rpm for about 48 h. During extraction of the pigment from the isolate, the strain was allowed to grow on the solid-state fermentation, and wheat bran was added to the production medium, showing promising pigment production, where constituents for the production of pigment consist of 50 g of sterilized wheat bran along with starch (1%) as a carbon source and also peptone (1%) as a nitrogen source. About 10% (*w/v*) of seed culture was supplemented to the production medium and incubated at a temperature of 28°C for about 10–14 days. As shown in Figure 3, a matured spore of aerial mycelium that produced brown-coloured pigment extracted using the ethyl acetate solvent (100 ml) via solid-liquid extraction methods was found to be different from the reports from Jayanthi and Ritika [19]; they found red-coloured pigment. Thus, the recovered extract was vacuum dried at a lower pressure, and the sample was defatted with the cyclohexane. The isolate can be classified and described under the genus *Streptomyces* based on these morphological and sequence results. In addition, microorganisms obtained from various harsh regions have biotechnological applications [20–27].

The pigments were extracted by using methanol solvents, and further, the sample was dried under a freeze dryer. BJZ10 isolate has produced the pigment of 300 mg/100 ml of production broth.

Considering UV-visible (Figure 4) absorbance result, peak ranging between 220 and 250 nm describes the presence of highly polygene properties of the pigment extract. In addition, similarities were observed in the UV spectrum and absorbance maxima peaks. This promising result supports the potential ability of strain *Streptomyces* in the production of antibiotics. Moreover, the highlighted spectral data are most similar to the reports from Saadoun et al. [28]. In other investigation, Slavica et al. [29] proposed that the absorbance maxima of UV spectral data 215 and 270 nm from the *Streptomyces* strains recovered from the soil samples of South-Eastern Serbia, which is more or less similar to the data presented in the current investigation. Therefore, the present work would provide new avenues to produce novel cost-effective antibacterial compounds that can be produced on a large scale.

FTIR analysis: the FTIR characterization was carried out, and the spectrum obtained for the strain BJZ10 has indicated the presence of alkane, alkene, alkynes, alcohols, esters, and sulfate functional groups (Figure 5). Especially, the broadband at 3362 indicated the presence of O-H stretching of alcohol; this indicates high-energy region that could be likely the presence of the intermolecular bonding [30]; peak observed at 2902 could be belonged to the C-H, and this medium stretching indicated the presence of alkane group and band 3000–3100 indicating the presence of alkene. Peaks at 3267–3333 indicate the presence of alkynes, 1310–1250 (1292) implies the presence of a strong bond of aromatic ester, and band observed at 1388 indicated the presence of S=O stretching that is sulfate group; the band has shown that near 2902 indicated vibration of the CH₂ groups due to the fact of melanin in the extracted pigment.

As the microbes are expected to perform in diverse physiological conditions equally, these bacteria can also be

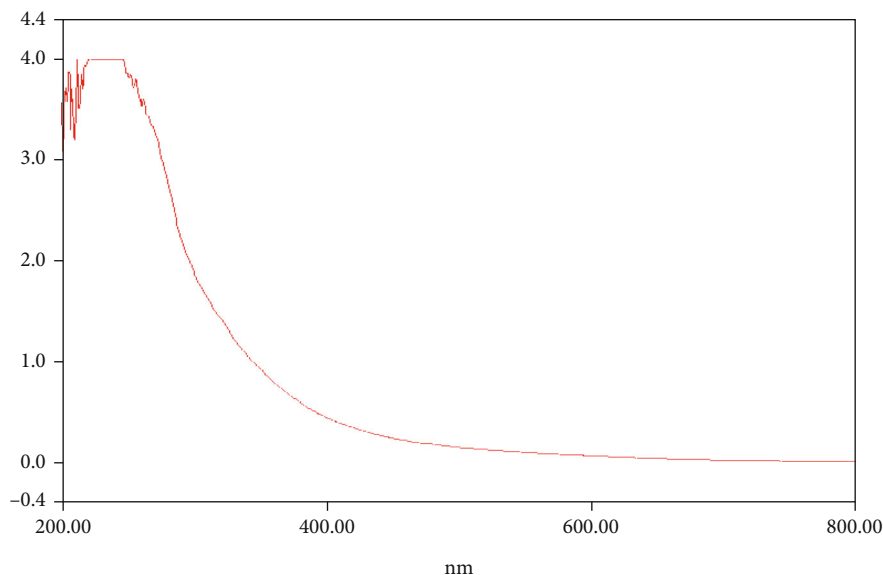


FIGURE 4: UV/vis spectroscopy: the UV absorption spectrum result of BJZ10 strain showed that the maximum peak range from 220 nm to 250 nm, with λ_{\max} at 247 nm.

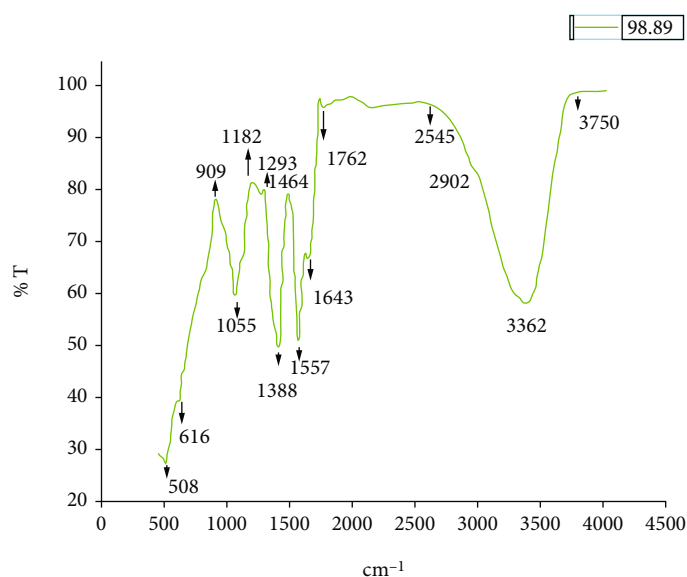


FIGURE 5: Fourier transform infrared (FTIR) peaks of the extracted pigment.

explored for the bionanotechnological applications, including medical and environmental technology [31–38].

The intended antimicrobial analysis was aimed at testing the growth inhibition potentials of the extracted pigment. The selection of the Gram-positive *B. cereus* from intestinal or nonintestinal is closely related to the synthesis of tissue-destructive exoenzymes, and the strain is also known to produce toxins such as three distinct phospholipases, an emesis-inducing toxin, four hemolysins, and proteases. On the other hand, the basis for the selection of Gram-negative *Escherichia coli* was because they can cause septic shock, serious food poisoning, meningitis, or urinary tract infections in humans (see Figure 6). From Table 1, one can conclude that the concentration of the pigment extracted

was the major factor that affects the inhibition activity against various types of microorganisms, especially a concentration of 50 mg/ml yielded the most promising 14 mm zone of inhibition against the Gram-positive, spore-forming, and facultative anaerobic bacterium, this *Bacillus cereus* species. The present investigation showed good results when it was compared with Elbendary et al. [39], where their report revealed a maximum of 10.2 mm zone of inhibition against the *Bacillus cereus* species. However, the pigment recovered from the *Streptomyces* sp. has shown little or nil impact on *Escherichia coli*, a Gram-negative bacteria, when the extract was used at lower concentrations (12.5–25 mg/ml), and is effective if used in higher concentrations (50 mg/ml and above), and also, their strains have not showed any growth

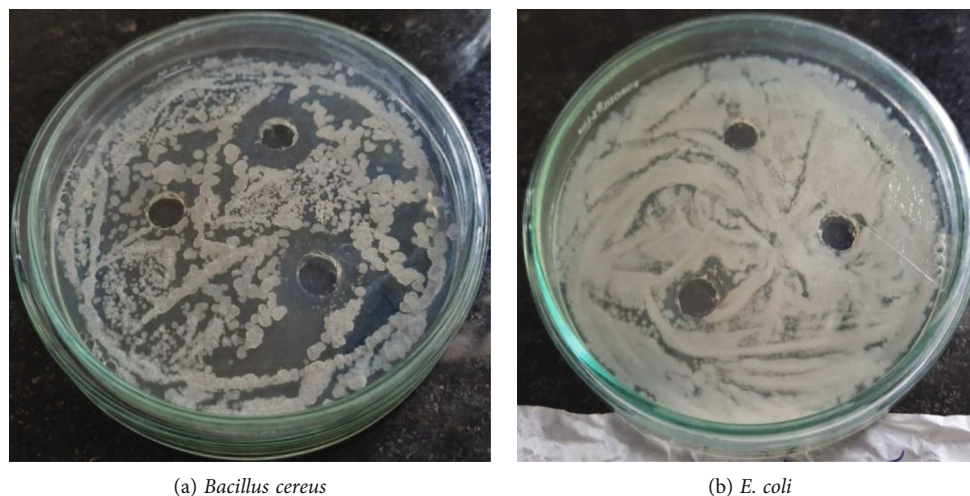


FIGURE 6: The antimicrobial effects of the extracted pigments on Gram-positive (a) and Gram-negative (b) bacteria.

TABLE 1: Antagonistic effects of the extracted pigment against the *Bacillus cereus* and *E. coli*.

Sl. no	Name of pathogen	Concentration of sample	Inhibition size of diameter in mm
1	<i>Bacillus cereus</i>	12.5 mg/ml	0.00 ± 0.02
		25 mg/ml	8.0 ± 0.3
		50 mg/ml	14±
2	<i>Escherichia coli</i>	12.5 mg/ml	0.00 ± 0.01
		25 mg/ml	0.00 ± 0.31
		50 mg/ml	4 ± 0.16

inhibition against the *E. coli* species. It can be concluded that the brown pigment of strain BJZ10 has been assessed against Gram-positive and Gram-negative pathogenic bacteria. The results obtained indicated that *Escherichia coli* has lower susceptibility than *Bacillus cereus* against the tested pigments.

4. Conclusions and Future Scope

The actinobacteria that are recovered from harsh environments have an advantage over their terrestrial counterparts. As they can tolerate and grow well, most adverse conditions are more suitable for the enormous applications in bioremediation, wastewater treatment, and production of the industrial enzyme, antimicrobials, antioxidant, anti-inflammatory, and anticancer metabolites [40–45]. The present investigation emphasized that the bioactive pigments recovered from the strain BJZ10 can be employed as potential antimicrobial agents against Gram-positive bacteria. Strain BJZ10 showed higher antimicrobial activity against *Bacillus cereus*. Microbial pigments have received a huge application in the food and pharmaceutical industry. Further study involves identifying pigment, statistical optimization, and anticancer studies of the brown pigment. The previous report claimed that the melanin pigment produced by the strain of *Streptomyces glaucescens* NEAE-H was found to be promising against the lung fibroblast (WI-38) and human amnion (WISH). Their results also revealed after treatment, IC50 on all cells ranged

from 37.05 ± 2.40 to 48.07 ± 2.76 $\mu\text{g/ml}$. The antiproliferative activity of the purified melanin pigment of *Streptomyces glaucescens* NEAE-H indicated potential cytotoxic activity against the HFB4 skin cancer cell line [18]. Hence, there is a huge scope for utilizing these actinobacterial species to potentially treat cancer and other dreadful diseases. Actinomycetes, especially ant species belonging to the genera *Streptomyces*, are versatile organisms; they carry important metabolites that can be further explored for various biotechnological, agriculture and forestry, environmental, and other industrial applications. There is huge scope for the exploration of pigments produced by the *Streptomyces* sp. Since we believe that the present results are even better significant, when the extracted pigments are capped with the nanoparticles, this process of capping nanoparticles may enhance the efficacy of the antibiotics. Thus, these pigments capped with nanoparticles can be used in the preparation of antimicrobials agent against the antibiotic-resistant pathogenic strains.

Data Availability

Data is available upon request.

Conflicts of Interest

The authors declare no conflict of interest.

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