

Original Research Article

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Isolation and Characterization of Pigment Producing Bacteria from Rhizosphere Soil to Evaluate its Potential Application in Textile Industry

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ABSTRACT

Microbial pigments have gained increasing attention as potential alternatives to synthetic dyes because of their natural origin and potential environmental advantages. The present study was undertaken to isolate and characterize pigment-producing bacteria from rhizosphere soil and to evaluate the potential application of the produced pigment in textile coloration. Rhizosphere soil samples were collected aseptically and subjected to serial dilution and plating on nutrient agar. Pigmented colonies were selected, purified by repeated subculturing and characterized using morphological and biochemical methods. The selected isolate exhibited green pigmentation and was identified as *Pseudomonas aeruginosa* based on its Gram-negative rod morphology, motility, oxidase and catalase positivity, non-fermentative characteristics and biochemical profile. Pigment production was evaluated in nutrient broth and starch casein broth. Green pigmentation was observed in nutrient broth after 24 h and increased after 48 h, whereas no obvious pigment production was observed in starch casein broth. The pigment was extracted using acetone and subjected to Fourier Transform Infrared Spectroscopy (FTIR) for partial characterization. FTIR analysis showed functional group regions corresponding to hydroxyl/amine, aliphatic C–H, carbonyl, aromatic/amine, C–N and C–O groups. The extracted pigment was applied to cotton fabric, which showed visible and relatively uniform colour uptake without obvious leaching during shade drying. The findings indicate that rhizosphere soil can serve as a source of pigment-producing bacteria and that the recovered microbial pigment has potential for textile coloration.

Keywords

Microbial pigments; Pigment-producing bacteria; Rhizosphere soil; *Pseudomonas aeruginosa*; Textile coloration.

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Introduction

Microbial pigments are naturally occurring coloured compounds produced by microorganisms such as

bacteria, fungi and algae. They have attracted increasing interest as potential alternatives to synthetic dyes because of their natural origin and possible environmental advantages. Microbial pigments have applications in

textile, pharmaceutical, cosmetic and food industries, and their production can be carried out under controlled laboratory conditions. The rhizosphere is a biologically active region surrounding plant roots that contains diverse microorganisms capable of producing various secondary metabolites, including pigments.

Therefore, rhizosphere soil represents a potential source for the isolation of pigment-producing bacteria. Previous studies have reported pigment-producing microorganisms from soil and demonstrated their potential for textile applications. Darwesh *et al.*, (2016) and Muneefa *et al.*, (2021) reported pigment-producing microorganisms from environmental samples with potential applications in textile coloration. Nanaware *et al.*, (2024) also reported diverse pigment-producing bacteria from soil and highlighted the use of FTIR for partial characterization of microbial pigments. Microbial pigments are being investigated for textile applications as alternatives to synthetic colourants. Nair *et al.*, (2024) demonstrated the application of microbial pigments on different textile materials, while Basto *et al.*, (2025); Patil *et al.*, (2025); Priya *et al.*, (2025) highlighted the importance of microbial isolation, pigment production, extraction, characterization and textile application. Although several pigment-producing microorganisms have been reported, further investigation of bacteria from different ecological niches and their suitability for textile coloration is required.

Therefore, the present study was undertaken to isolate and characterize pigment-producing bacteria from rhizosphere soil, extract and partially characterize the pigment using FTIR, and evaluate its potential application in cotton textile coloration.

Materials and Methods

Materials

Materials Standard microbiological glassware, culture media, reagents and laboratory equipment were used throughout the study. Nutrient agar and nutrient broth were used for isolation, cultivation and pigment production. Indole, citrate, urease and triple sugar iron agar media were used for biochemical characterization. Acetone was used for pigment extraction and cotton fabric was used for textile application. The media and reagents used were prepared according to the laboratory requirements described in the original project.

Sample Collection

Rhizosphere soil samples were collected from garden regions around plant roots using sterile containers. The samples were transported to the laboratory under suitable conditions and processed for isolation of pigment-producing bacteria. The sampling approach was based on the procedure reported by Sreedhar *et al.*, (2025), with modifications according to the requirements of the present study.

Isolation of Pigment-Producing Bacteria

1 gm of the sample was added to 9 ml of sterile distilled water to obtain a 10^{-1} dilution and mixed well. From this tube, 1 ml of the diluted sample was transferred into another tube containing 9 ml of sterile diluent to get a 10^{-2} dilution. The same procedure was repeated by transferring 1 ml each time into fresh tubes with 9 ml diluent to obtain further dilutions such as 10^{-3} , 10^{-4} , and till 10^{-8} . Careful pipetting and proper mixing were carried out to ensure accurate dilution. Appropriate dilutions were plated on sterile nutrient agar using the spread plate technique.

The inoculated plates were incubated at 37°C for 24–48 h. This general isolation approach is consistent with the methodology reported for soil-derived pigment-producing bacteria by Sreedhar *et al.*, (2025). Following incubation, colonies exhibiting distinct pigmentation were selected and purified by repeated streaking on nutrient agar. A green-pigmented colony was selected for further characterization.

Maintenance and Subculturing of the Isolate

The selected pigmented colony was transferred aseptically to fresh nutrient broth and incubated at 37°C for 24 h. Repeated subculturing was performed to obtain a pure and stable culture. The original project reports that repeated subculturing resulted in more uniform colony morphology and increased pigment intensity.

Morphological Characterization

The selected isolate was examined for colony and cellular characteristics. Gram staining was performed to determine the Gram reaction and cell morphology. The stained preparation was observed under a light microscope using the 100× oil-immersion objective.

Biochemical Characterization

The selected isolate was characterized using biochemical tests including catalase, oxidase, indole, citrate utilization, urease, methyl red, Voges–Proskauer and triple sugar iron agar tests. These tests were selected to obtain a biochemical profile useful for preliminary identification of the isolate.

Pigment Production

The purified pigmented isolate was inoculated into nutrient broth and incubated at 37°C. Pigment production was monitored after 24 and 48 h. Starch casein broth was also evaluated for comparison. The change in colour of the culture medium was recorded visually.

Pigment Extraction

The method of pigment extraction was adopted and followed as per the protocol described by Nair *et al.*, (2017). The solvent extraction process for separating bacterial pigments involved the following steps: Culture Growth: The bacterial culture was grown in a nutrient broth until visible pigment colour appeared. Centrifugation: The culture was centrifuged to separate the bacterial cells from the medium.

Solvent Extraction: Different organic solvents were used to extract the pigments from the cell pellets. 100ml of the bacterial broth (that had produced the green colour pigment) was taken and 50ml of acetone was added into that bacterial broth to extract then pigment. The extracted pigment fraction was separated and concentrated by evaporation of the solvent. The resulting pigment residue was retained for further characterization and textile application.

FTIR characterization

The extracted pigment was subjected to Fourier Transform Infrared Spectroscopy (FTIR) to obtain information about the major functional groups present in the pigment extract. The FTIR spectrum was interpreted based on the major absorption regions observed in the sample. The present study used FTIR as a partial characterization technique to support the presence of different functional groups in the pigment extract rather than as a standalone method for establishing the exact chemical identity of the pigment. FTIR has similarly

been used for partial characterization of microbial pigments in previous studies.

Application of Pigment on Cotton Fabric

Cotton fabric was used to evaluate the dyeing potential of the extracted microbial pigment. The fabric was immersed in the pigment solution and allowed to remain in contact with the pigment for the required period. Following treatment, the fabric was removed and dried under shade.

The colour uptake, uniformity of coloration, visible leaching and changes in fabric texture were observed. The application of microbial pigments on textile materials, including cotton, has been reported in previous studies by Nair *et al.*, (2024) and Basto *et al.*, (2025).

Results and Discussion

Isolation and Characterization of the Pigment-Producing Bacterium

Rhizosphere soil samples yielded bacterial colonies with different morphological characteristics on nutrient agar. Among the isolates, a distinct green-pigmented colony was selected and purified by repeated subculturing, as shown in Figure 1 Gram staining showed that the isolate was a Gram-negative rod and was motile, non-capsulated and non-sporeforming.

The isolate was positive for catalase, oxidase, citrate and urease tests, while indole, methyl red and Voges–Proskauer tests were negative. The isolate showed an oxidative reaction in the O/F test and an alkaline/alkaline reaction in Triple Sugar Iron agar. Based on the morphological and biochemical characteristics, the isolate was presumptively identified as *Pseudomonas aeruginosa*.

The morphological and biochemical characteristics are presented in Table 1 and 2. The results are consistent with established taxonomic descriptions of *Pseudomonas* species. Similar findings were reported by Sreedhar *et al.*, (2025), who isolated pigment-producing bacteria from diverse soil environments and confirmed their identity through biochemical assays, reinforcing the reliability of soil as a source for pigment producing strains.

Table.1 Morphological Characteristics of the Selected Bacterial Isolate

Characteristics	<i>Pseudomonas aeruginosa</i>
Gram staining	Negative
Cell shape	Rods
Motility	Motile
Capsule	Non – capsulated
Spore formation	Non – sporing

Table.2 Biochemical Characteristics of the Selected Bacterial Isolate

Biochemical test	<i>Pseudomonas aeruginosa</i>
Catalase	Positive
Oxidase	Positive
Methyl red (MR)	Negative
Voges proskauer (VP)	Negative
Oxidative/ Fermentative (OF)	Oxidative
Indole	Negative
Citrate	Positive
Urease	Positive
Triple Sugar Iron (TSI)	Slant /Butt:Alkaline /Alkaline
H ₂ S Production	Negative
Gas Production From Nitrate	Positive

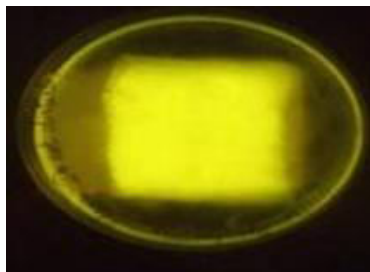
Figure.1 Green-pigmented bacterial colony on nutrient agar



Figure.2 Solvent extraction of pigment on nutrient broth



Figure.3 Dye uptake of microbe



Pigment Production

Pigment production was evaluated in nutrient broth and starch casein broth. Green pigmentation was observed in nutrient broth after 24 h and increased after 48 h, producing a deeper green colour. In contrast, no obvious pigment production was observed in starch casein broth. The difference in pigmentation indicates that the culture medium influenced pigment production.

The higher pigment production in nutrient broth may be associated with its suitability for bacterial growth and pigment biosynthesis. The extracellular secretion of pigment simplifies downstream recovery, making *P. aeruginosa* a practical candidate for pigment production. Comparable results were reported by [Priya et al., \(2025\)](#), who demonstrated that pigment yield and stability varied significantly depending on the growth medium and environmental conditions, emphasizing the importance of optimized culture parameters for microbial pigment production. Extraction using acetone proved efficient, yielding a stable pigment residue that retained its coloration during drying and storage. The chemical stability observed suggests potential for industrial applications, particularly in textiles where pigment retention is essential.

Pigment Extraction and FTIR Analysis

The pigment produced in nutrient broth was extracted using acetone. The extracted pigment retained its green colour after concentration and drying as shown in Figure 2. The spectral analysis of the sample was identified with significant functional groups as followed, O-H and N-H stretching in the wavelength of 3200-3400 cm^{-1} relevance for the presence of phenols, alcohols and amine group. The range between 2920-2850 cm^{-1} denoted the presence of C-H stretching of aliphatic chains of lipid or carotenoid structure. The C=O

stretching in the range of 1650-1700 cm^{-1} indicated the presence of ketones, amides and quinones. The stretch in the range of 1540-1580 cm^{-1} confirmed the presence of N-H bending and C=C of proteins and aromatic rings. Further 1400-1450 cm^{-1} indicated the C-H bending of Alkanes and 1230/1300 cm^{-1} indicated the C-N stretching of amine group. The range of 1000/1150 cm^{-1} correlated with C-O stretching of alcohols and esters. FTIR analysis showed absorption regions corresponding to hydroxyl/amine, aliphatic C-H, carbonyl, aromatic/amine, C-N and C-O functional groups. These findings indicate the presence of different organic functional groups in the extracted pigment. These results are consistent with [Patil et al., \(2025\)](#), who characterized pigments from rhizosphere isolates and identified similar functional groups. FTIR therefore provided preliminary information about the chemical characteristics of the pigment; however, further analysis would be required for definitive identification.

Application of the Pigment on Cotton Fabric

The extracted pigment was applied to cotton fabric to evaluate its potential as a textile colourant. Cotton fabric immersed in pigment solution showed gradual colour uptake within 24 hours. Dye penetration appeared uniform across the fabric surface. The fabric showed visible and relatively uniform green colour uptake, as shown in Figure 3. No obvious leaching was observed during shade drying. The ability of the pigment to impart colour to cotton demonstrates its potential for textile coloration. The observed colour uptake is consistent with previous reports on the application of microbial pigments to textile materials. Further studies on washing, light and colour fastness would be required to evaluate its suitability for practical textile applications. Compared to synthetic dyes, the microbial pigment offered eco-friendly advantages, requiring no mordants and posing minimal environmental risks. This observation resonates

with Nair *et al.*, (2024), who demonstrated that microbial pigments could be applied to multiple textile fibers, including silk, jute, and synthetic materials, with strong adsorption and stability under washing and light exposure. Their work highlighted the versatility of microbial pigments across different substrates, reinforcing their potential as sustainable alternatives to synthetic dyes. Overall, the study demonstrated that rhizosphere soil can serve as a source of pigment producing bacteria. The selected *Pseudomonas aeruginosa* isolate produced green pigment in nutrient broth, which was successfully extracted, partially characterized by FTIR and applied to cotton fabric with visible colour uptake. The successful application of the pigment in textile dyeing underscores its industrial potential, while its eco-friendly nature supports adoption as a sustainable alternative to synthetic dyes.

Overall, the study highlighted the importance of rhizosphere microorganisms as valuable resources for pigment production. The isolation and characterization of pigment producing bacteria provide a foundation for further research aimed at optimizing pigment yield, improving extraction methods, and evaluating large scale production.

Future work can focus on molecular identification of the bacterial isolates, optimization of culture conditions for enhanced pigment production, and detailed evaluation of dyeing efficiency on different textile materials. In conclusion, pigment producing bacteria isolated from rhizosphere soil possess promising potential for application in the textile industry. Their use as natural dyes can contribute to environmentally friendly and sustainable industrial practices while also opening new avenues in microbial biotechnology.

Author Contributions

G. J. Aswini: Original, Draft preparation, Data curation. A. Dharshini Uma: Conceptualization, Project Administration. N. Bharakath Shifana: Methodology, Editing. D. Deepika: Validation, visualization. A. Deiva Siromani: Resources, Investigation. C. Soundhari: Designing Protocols, Evaluation Manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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