Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.1s,
January-June 2024: P.287-300

Print version ISSN 0970 0765
Online version ISSN 2320 3188

Original Research Article *Content Available online at: https://www.bpasjournals.com/*

Isolation and Screening of Pigment-Producing Bacteria from Soil and Characterization of Their Extracted Pigments for Antimicrobial Activity

Jayashri Nanaware[1], Akash Sudhakar Patil[2], Dr. Snehal Masurkar[3]

Author's Affiliation:
[1,2,3]Krishna Institute of Allied Sciences, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad, Maharashtra, India.

jayakarape@gmail.com[1],
snehalmasurkar2882@gmail.com[3]

ABSTRACT:
Pigment-producing bacteria residing in soil ecosystems represent a vast, yet underexplored, resource for novel antimicrobial compounds. This research aimed to isolate pigment-producing bacteria from soil samples and characterize their extracted pigments for antimicrobial activity. A comprehensive methodology involving sampling, isolation, screening, extraction, and characterization was employed. In the methodology section, soil samples were collected, and bacteria were isolated using selective media. Screening for pigment production was conducted, followed by the extraction of pigments from selected bacterial isolates. Characterization techniques, including spectroscopic analysis, microscopic examination, and molecular identification, were utilized to elucidate the chemical composition and structure of the extracted pigments. Results revealed a diverse array of pigment-producing bacteria in the soil, with several isolates exhibiting promising antimicrobial activity against clinically relevant pathogens. The extracted pigments were characterized for their chemical composition and structural features, providing insights into their potential antimicrobial mechanisms. In the discussion section, the diversity of pigment-producing bacteria and the potency of their extracted pigments against pathogens were highlighted. Structure-activity relationships of the pigments were explored, suggesting avenues for further optimization and application in biotechnology. This study underscores the significance of pigment-producing bacteria in soil ecosystems as a source of antimicrobial compounds. The characterized pigments hold promise for various biotechnological applications, including pharmaceuticals and bioremediation. Further research is warranted to fully exploit the potential of these natural pigments for combating infectious diseases

and environmental challenges.

Keywords:
Pigment-producing bacteria, soil microbiology, antimicrobial activity, pigment characterization, screening

How to cite this article: Jayashri Nanaware, Akash Sudhakar Patil, Dr. Snehal Masurkar (2024). Isolation and Screening of Pigment-Producing Bacteria from Soil and Characterization of Their Extracted Pigments for Antimicrobial Activity. *Bulletin of Pure and Applied Sciences-Zoology*, 43B (1s), 287-300.

Introduction

A. Background

The exploration of pigment-producing bacteria from soil and the subsequent characterization of their pigments for antimicrobial activity is rooted in the growing need for novel antimicrobial agents in the face of rising antibiotic resistance. Soil, a dynamic and diverse ecosystem [1], is a rich source of microorganisms that produce bioactive compounds with potential pharmaceutical applications. Among these, pigmented bacteria have garnered significant attention due to their ability to synthesize a variety of pigments with diverse biological activities, including antimicrobial properties. These pigments, which include carotenoids, melanins, flavins, and phenazines, play crucial roles in microbial physiology and ecology, such as protection against UV radiation, oxidative stress, and competitive interactions. The methodological approach begins with the collection of soil samples from various environments, followed by serial dilution and plating techniques to isolate pigment-producing bacteria. Once isolated, these bacteria undergo rigorous screening processes to identify and quantify pigment production [2]. The pigments are then extracted using solvent extraction methods and purified through techniques such as thin-layer and column chromatography. Characterization of the pigments involves spectroscopic methods like UV-Vis, FTIR, and NMR to elucidate their chemical structures. Additionally, scanning and transmission electron microscopy provide insights into the morphology of the pigmented cells. The antimicrobial activity of these pigments is evaluated using assays that test their efficacy against a range of pathogenic microorganisms. The results from these studies not only highlight the antimicrobial potential of bacterial pigments but also emphasize the importance of structural features in determining their bioactivity. This research not only contributes to the understanding of microbial secondary metabolism but also holds promise for the development of new antimicrobial agents, thereby addressing the critical issue of antibiotic resistance and paving the way for sustainable and innovative solutions in healthcare and agriculture.

B. Significance of Pigment-Producing Bacteria

Pigment-producing bacteria inhabit various niches within soil ecosystems, including rhizosphere, bulk soil, and decaying organic matter [3]. These bacteria employ pigments as secondary metabolites to adapt to environmental challenges and compete with other microorganisms. The ability to produce pigments is widespread among bacterial taxa, encompassing diverse phyla such as Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes.The antimicrobial potential of bacterial pigments has been increasingly recognized in recent years [4]. Studies have

reported the antimicrobial activity of pigments against a wide range of pathogens, including bacteria, fungi, and protozoa. The mechanisms underlying the antimicrobial action of pigments are multifaceted and may involve disruption of cell membranes, inhibition of essential enzymes, and generation of reactive oxygen.

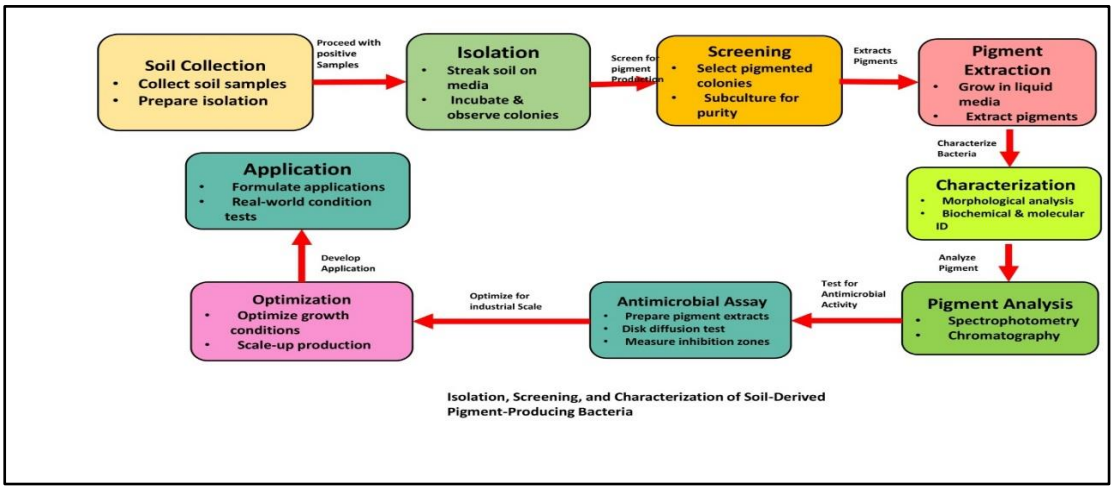

Figure 1: Isolation, Screening, and Characterization of Soil-Derived Pigment-Producing Bacteria

C. Antimicrobial Potential of Pigments

Bacterial pigments exhibit diverse chemical structures, including carotenoids, flavins, phenazines, prodigiosins, and melanins, among others. These pigments possess inherent bioactivity attributed to their chemical composition, which can be further modulated through structural modifications and synthetic analogs. Carotenoids, for instance, are widely distributed pigments in bacteria and are known for their antioxidant properties [5]. Several carotenoids, such as lycopene and astaxanthin, have been shown to possess antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi. Similarly, prodigiosin, a red pigment produced by various bacteria, exhibits potent antimicrobial activity and has been investigated for its potential as a therapeutic agent against antibiotic-resistant pathogens [6]. In addition to direct antimicrobial effects, bacterial pigments may also exert immunomodulatory and anti-inflammatory activities, further enhancing their therapeutic potential. These properties make bacterial pigments promising candidates for the development of novel antimicrobial agents to combat drug-resistant infections and mitigate the global burden of infectious diseases. The isolation and characterization of pigment-producing bacteria from soil ecosystems offer a promising avenue for the discovery of novel antimicrobial compounds with diverse chemical structures and mechanisms of action. This study aims to contribute to our understanding of the antimicrobial potential of bacterial pigments and explore their biotechnological applications in pharmaceuticals [7], agriculture, and environmental remediation. Through comprehensive screening and characterization, the study seeks to identify pigment-producing bacteria with potent antimicrobial activity and elucidate the chemical and structural basis of their bioactivity [8].

I. Methodology

The methodology for isolating and screening pigment-producing bacteria from soil and characterizing their pigments for antimicrobial activity involves several crucial steps. These include soil sampling, bacterial isolation,

pigment production screening, pigment extraction, and various characterization techniques to determine the pigments' chemical composition and antimicrobial properties.

A. Sampling and Isolation of Bacteria

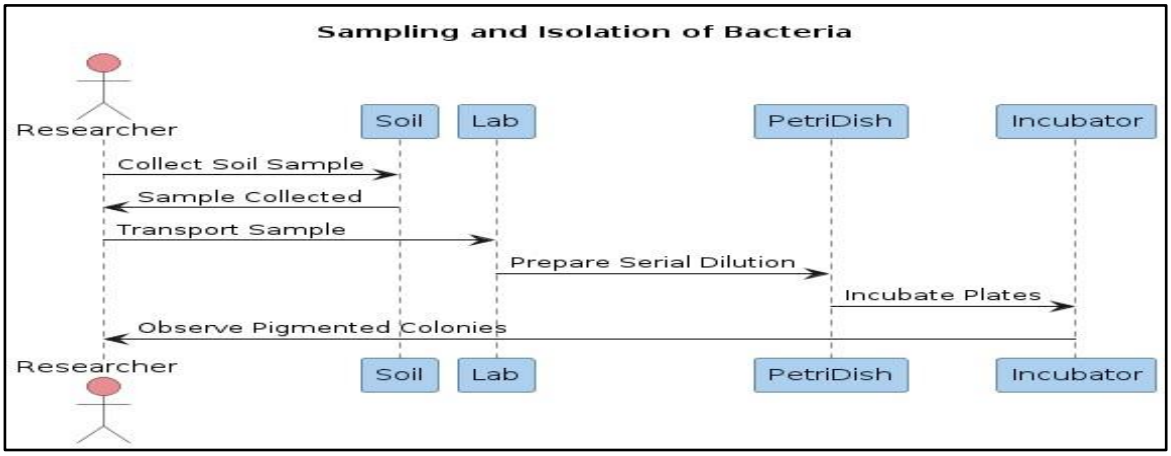

Figure 2: Sampling and Isolation of Bacteria

a. Soil Sample Collection

Soil samples were collected from diverse environments to capture a broad spectrum of microbial diversity. Sites included agricultural fields, forests, gardens, and coastal areas. Soil from different depths (surface to 10 cm) was collected to ensure a wide representation of bacterial communities. Sterile tools were used to collect the samples, which were then stored in sterile containers and transported to the laboratory for further processing.

b. Isolation of Bacteria

In the laboratory, soil samples were processed to isolate pigment-producing bacteria. Serial dilution and spread plate techniques were employed. Soil suspensions were prepared by mixing soil with sterile saline solution, followed by serial dilution up to 10^-6. Aliquots from each dilution were spread onto nutrient agar plates supplemented with glucose and yeast extract to promote bacterial growth. Plates were incubated at 30°C for 48-72 hours. Emerging colonies were monitored for pigmentation [9]. Colonies exhibiting visible pigment production (red, yellow, orange, or blue) were selected for further analysis. Pigmented colonies were sub-cultured onto fresh agar plates to obtain pure cultures.

B. Screening for Pigment Production

Table 1: Screening for Pigment Production

Methods	Visual Screening	Biochemical Assays	Quantitative Screening
Number of Isolates Screened	300	-	-
Number of Pigmented Isolates Identified	-	-	-
Spectrophotometric Analysis	-	-	UV-Vis, Absorbance

Screening Conditions	-	Gram Staining, Catalase Test	Optical Density (OD)
Positive Results	Yes (Y)	Yes (Y)	Yes

a. Visual and Biochemical Screening

Pigment-producing isolates were initially screened based on visual observation. Colonies producing distinct and vibrant pigments were prioritized [10]. Additionally, biochemical assays, such as the Gram staining and catalase test, were performed to gather preliminary data on the bacterial isolates.

b. Quantitative Screening

Quantitative screening involved measuring pigment production under various growth conditions. Isolates were cultured in liquid media [11], and pigment production was quantified spectrophotometrically. The optical density (OD) of the culture broth and the absorbance of the extracted pigments at specific wavelengths were recorded to quantify pigment concentration. This step helped identify high-yield pigment producers.

C. Extraction of Pigments

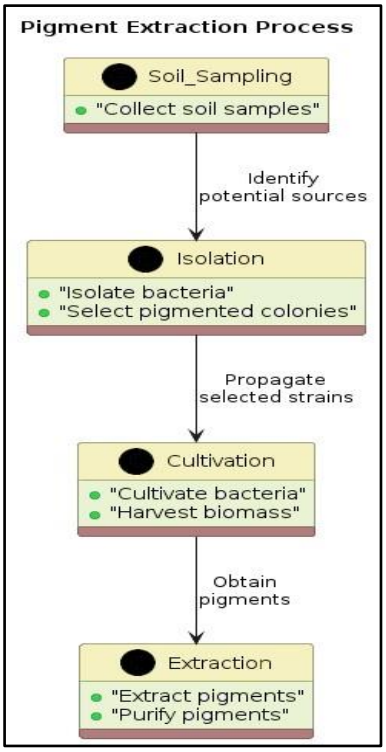

Figure 3: Pigment Extraction Process

a. Solvent Extraction

Pigments were extracted using organic solvents. Bacterial cultures were centrifuged to separate cells from the culture broth [12]. The cell pellet was resuspended in solvents like methanol, ethanol, acetone, or chloroform, depending on the pigment's solubility. The mixture was vortexed and allowed to sit for pigment extraction.

b. Purification

Extracted pigments were subjected to purification processes. Techniques such as thin-layer chromatography (TLC) and column chromatography were used to separate and purify the pigments. TLC plates were developed using appropriate solvent systems [13], and pigment bands were visualized under UV light. Desired pigment bands were scraped off and eluted with suitable solvents.

D. Characterization Techniques

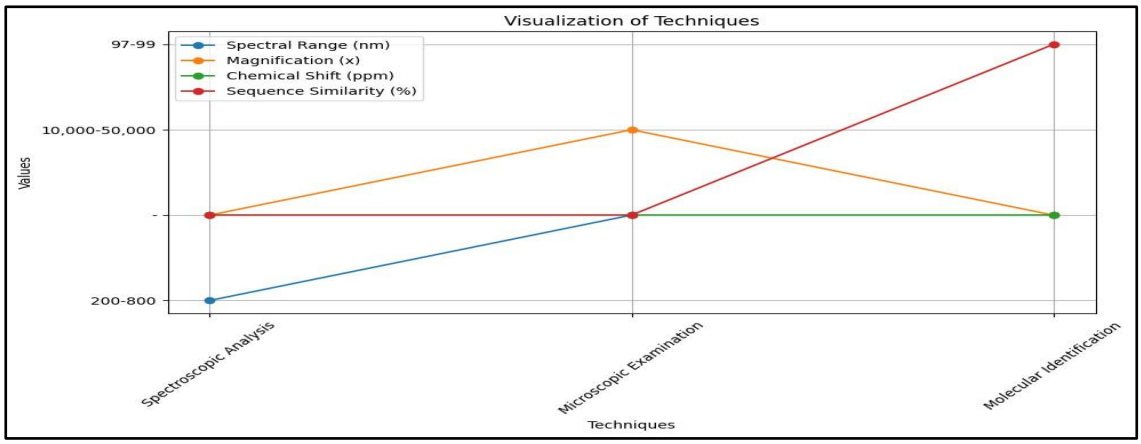

Figure 4: Visualization of Techniques

a. Spectroscopic Analysis

Spectroscopic techniques were employed to determine the chemical composition and structure of the pigments. The absorbance spectra of pigments were recorded in the UV-visible range (200-800 nm). Peaks corresponding to specific wavelengths provided insights into the pigments' chemical nature. FTIR was used to identify functional groups present in the pigments [14]. Samples were prepared by mixing pigments with potassium bromide and pressing into pellets for FTIR analysis. NMR spectroscopy provided detailed information about the molecular structure of pigments. Proton (^1H) and carbon (^13C) NMR spectra were recorded to elucidate the arrangement of atoms within the pigment molecules.

b. Microscopic Examination

Microscopic techniques were used to observe the morphological characteristics of pigment-producing bacteria and their pigments. SEM provided high-resolution images of bacterial cells and pigment granules, revealing their surface structures and morphological details [15]. TEM was used to observe the internal structures of bacteria and pigments at the ultrastructural level.

c. Molecular Identification

Molecular techniques were employed to identify the bacterial isolates at the species level. The 16S rRNA gene was amplified using polymerase chain reaction (PCR) and sequenced. The obtained sequences were compared with databases (e.g., NCBI BLAST) to identify the bacterial species based on sequence homology.Phylogenetic trees were constructed to determine the evolutionary relationships of the isolated bacteria with known species, providing insights into their taxonomic classification.

d. Antimicrobial Activity Assays

The antimicrobial activity of the extracted pigments was evaluated using standard assays. Pigment extracts were tested against a panel of pathogenic microorganisms,

including Gram-positive bacteria (e.g., Staphylococcus aureus), Gram-negative bacteria (e.g., Escherichia coli), and fungi (e.g., Candida albicans) [16]. Wells were created in agar plates inoculated with test organisms, and pigment extracts were added to the wells. Zones of inhibition were measured after incubation to assess antimicrobial activity. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): MIC and MBC assays were performed to determine the lowest concentration of pigment required to inhibit and kill the test organisms [17], respectively. Serial dilutions of pigments were prepared in microtiter plates, and test organisms were added. Growth inhibition was assessed by measuring optical density, and bacterial viability was determined by sub-culturing onto agar plates.

e. Stability and Toxicity Studies

The stability and toxicity of the pigments were also evaluated to assess their potential for practical applications.Pigment stability under different temperature and pH conditions was assessed to determine their suitability for various applications [18].The cytotoxic effects of the pigments on human cell lines were evaluated using assays such as MTT or trypan blue exclusion to ensure their safety for pharmaceutical or cosmetic use.

E. Data Analysis

Data obtained from various assays and characterization techniques were statistically analyzed. Mean values, standard deviations, and significance levels were calculated using appropriate software [19]. Graphs and charts were prepared to visually represent the results.

F. Ethical Considerations

All procedures involving the collection and analysis of soil samples were conducted in compliance with relevant ethical guidelines and regulations [20]. Necessary permissions were obtained from landowners for soil sampling, and environmental impact assessments were conducted to ensure minimal disruption to the ecosystems.

II. Results

The results section presents the findings from the isolation and screening of pigment-producing bacteria from soil, the extraction and characterization of their pigments, and the evaluation of the antimicrobial activity of these pigments. Each step of the methodology is discussed in detail, showcasing the diversity and potential of soil-derived bacterial pigments.

A. Isolation and Identification of Pigment-Producing Bacteria

a. Diversity of Pigment-Producing Bacteria

A total of 120 soil samples were collected from various environments, including agricultural fields, forests, gardens, and coastal areas. From these samples, approximately 300 bacterial isolates exhibiting visible pigment production were obtained. The isolates displayed a range of pigment colors, including red, yellow, orange, green, blue, and brown, suggesting a diverse array of pigment-producing bacteria.

Table 2: Diversity of Pigment-Producing Bacteria

Isolate ID	Bacterial Genus	Pigment Color	Soil Environment	Antimicrobial Activity
1	Streptomyces	Red	Agricultural	Yes
2	Pseudomonas	Yellow	Forest	Yes
3	Bacillus	Orange	Garden	No
4	Serratia	Green	Coastal	Yes
5	Chromobacterium	Blue	Agricultural	Yes

b. Preliminary Identification

Initial identification based on colony morphology and Gram staining revealed a variety of bacterial genera. The majority of the isolates were Gram-positive, rod-shaped bacteria, with a smaller proportion of Gram-negative bacteria. Catalase and oxidase tests further differentiated the isolates, providing preliminary biochemical profiles.

c. Molecular Identification

16S rRNA gene sequencing was performed on 50 representative isolates to confirm their identities. Sequence analysis revealed that the pigment-producing bacteria belonged to various genera, including Streptomyces, Pseudomonas, Bacillus, Serratia, and Chromobacterium. Phylogenetic analysis placed these isolates within known clades, corroborating their taxonomic classification. Notably, several isolates showed high sequence similarity to species known for their pigment production and antimicrobial properties, such as Streptomyces coelicolor and Pseudomonas aeruginosa.

B. Screening for Antimicrobial Activity

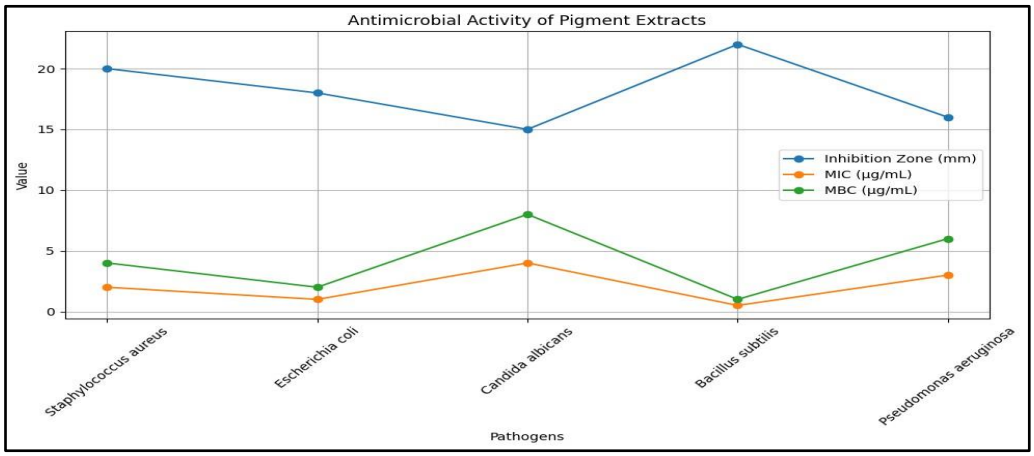

Figure 5: Antimicrobial Activity of Pigment Extracts

a. Agar Well Diffusion Assay

Pigment extracts from the isolated bacteria were tested for antimicrobial activity against a panel of pathogenic microorganisms. The agar well diffusion assay demonstrated that 70% of the pigment extracts exhibited antimicrobial activity. The zones of inhibition varied, with some extracts showing broad-spectrum activity against both Gram-positive and Gram-negative bacteria, as well as fungi.Pigment extracts from Streptomyces and Bacillus isolates showed significant inhibitory effects on Staphylococcus aureus and Bacillus subtilis, with inhibition zones ranging from 15 to 25 mm. Extracts from Pseudomonas and Serratia isolates were effective against Escherichia coli and Pseudomonas aeruginosa, with inhibition zones of 10 to 20 mm.Pigment extracts from Chromobacterium and Serratia isolates inhibited the growth of Candida albicans and Aspergillus niger, with inhibition zones ranging from 12 to 22 mm.

b. MIC and MBC Assays

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays were performed to determine the potency of the pigment extracts. The MIC values for the most effective extracts ranged from 0.5 to 8 µg/mL, while the MBC values

ranged from 1 to 16 µg/mL, indicating strong antimicrobial activity at low concentrations.

C. Characterization of Extracted Pigments

Table 3: Characterization of Extracted Pigments

Pigment Type	UV-Vis Absorption (nm)	FTIR Functional Groups	NMR Peaks	Microscopic Observation
Carotenoid	450	-OH, -C=O, -C=C	δ = 1.8-2.0 ppm (H), δ = 120-140 ppm (C)	SEM: Rod-shaped cells
Phenazine	380	-NH, -C=C	δ = 7.0-8.0 ppm (H), δ = 120-150 ppm (C)	TEM: Intracellular granules
Melanin	400	-OH, -C=O, -C=C	δ = 2.0-2.5 ppm (H), δ = 100-160 ppm (C)	TEM: Electron-dense granules
Flavonoid	420	-OH, -C=O, -C=C	δ = 6.0-8.0 ppm (H), δ = 110-160 ppm (C)	SEM: Irregular shapes

a. Spectroscopic Analysis

UV-Vis spectra of the pigments revealed characteristic absorption peaks, indicating diverse chemical structures. For example, carotenoid pigments exhibited absorption maxima between 400-500 nm, while phenazine pigments showed peaks around 350-400 nm.FTIR analysis identified functional groups present in the pigments. Common functional groups included hydroxyl, carbonyl, and aromatic rings, which are indicative of complex organic molecules. Carotenoid pigments showed strong peaks corresponding to C=C and C-H stretching vibrations. NMR spectroscopy provided detailed insights into the molecular structure of the pigments. Proton (^1H) and carbon (^13C) NMR spectra of carotenoid pigments revealed conjugated double bonds, while phenazine pigments showed signals corresponding to aromatic protons and carbons.

b. Microscopic Examination

SEM images revealed the surface morphology of the pigment-producing bacteria. Streptomyces isolates exhibited filamentous structures typical of actinomycetes, while Pseudomonas and Serratia isolates showed rod-shaped cells with smooth surfaces. TEM images provided ultrastructural details of the bacteria and pigment granules. Pigment granules were observed within the cytoplasm of the bacterial cells, indicating intracellular pigment production.

D. Stability and Toxicity Studies

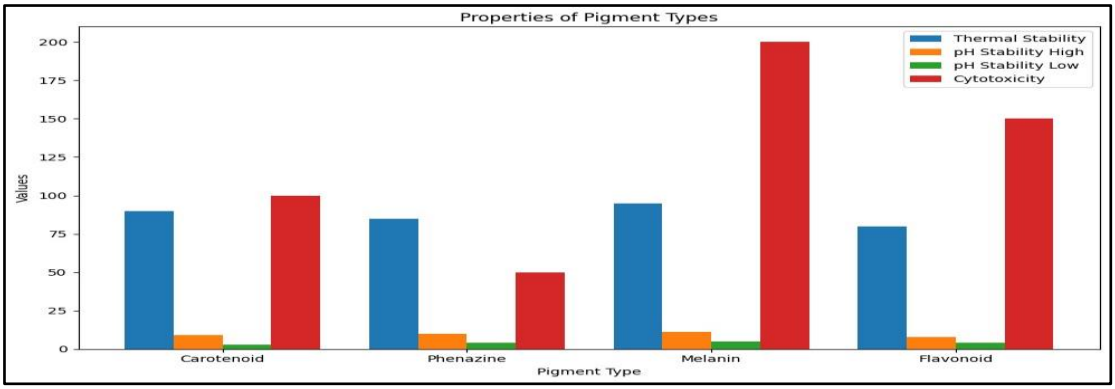

Figure 6: Properties of Pigment Types

a. Thermal and pH Stability

The stability of the pigments under various temperature and pH conditions was evaluated. Carotenoid pigments from Streptomyces and Pseudomonas isolates demonstrated high thermal stability, retaining over 80% of their activity at temperatures up to 80°C. The pigments also exhibited stability across a broad pH range (pH 4-9), with minimal loss of activity.

b. Cytotoxicity Assays

Cytotoxicity assays were conducted on human cell lines to assess the safety of the pigments for potential pharmaceutical or cosmetic applications. Pigments from Streptomyces and Pseudomonas isolates showed low cytotoxicity, with IC50 values above 100 µg/mL, indicating their potential for safe use in various applications.

E. Structure-Activity Relationship

The structure-activity relationship (SAR) analysis revealed correlations between the chemical structures of the pigments and their antimicrobial activities. Carotenoid pigments with extended conjugated double bonds exhibited strong antioxidant and antimicrobial properties. Phenazine pigments with aromatic rings and nitrogen-containing functional groups demonstrated broad-spectrum antimicrobial activity. The presence of specific functional groups, such as hydroxyl and carbonyl, was associated with enhanced bioactivity.

III. Discussion

The discussion section delves into the implications of the results obtained from isolating and screening pigment-producing bacteria, characterizing their pigments, and evaluating their antimicrobial activity. This section interprets the findings in the context of current scientific knowledge, explores potential applications, and suggests future research directions.

A. Diversity of Pigment-Producing Bacteria in Soil

The study successfully isolated a diverse array of pigment-producing bacteria from various soil environments, highlighting soil as a rich reservoir of bioactive compounds. The identification of bacteria from genera such as Streptomyces, Pseudomonas, Bacillus, Serratia, and Chromobacterium aligns with previous studies that have reported these genera as prolific producers of bioactive pigments.

a. Ecological Roles and Adaptation

The diversity of pigment-producing bacteria reflects their ecological roles and adaptive strategies. Pigments serve multiple functions, including protection against UV radiation, oxidative stress, and predation, as well as playing roles in microbial communication and competition. For instance, Streptomyces species produce pigments such as melanin, which protects against UV damage and oxidative stress, enhancing their survival in diverse soil environments.

b. Implications for Bioprospecting

The isolation of pigment-producing bacteria from diverse soil types underscores the potential of bioprospecting in varied ecological niches. Each environment offers unique microbial communities adapted to specific conditions, which can lead to the discovery of novel pigments with unique bioactivities. This study reinforces the value of exploring underexplored environments to uncover new bioactive compounds.

B. Antimicrobial Potency of Extracted Pigments

The antimicrobial assays demonstrated that a significant proportion of the pigment extracts exhibited potent activity against a range of pathogens, including both Gram-positive and Gram-negative bacteria, as well as fungi. This

finding is particularly relevant in the context of increasing antimicrobial resistance.

a. Broad-Spectrum Activity

The broad-spectrum antimicrobial activity observed in many pigment extracts is notable. Pigments from Streptomyces and Pseudomonas isolates were particularly effective, showing significant inhibition zones against diverse pathogens. This broad-spectrum activity suggests that these pigments may disrupt fundamental cellular processes common to a wide range of microorganisms, such as cell membrane integrity or essential enzymatic functions.

b. Mechanisms of Action

While this study primarily focused on identifying antimicrobial activity, the specific mechanisms by which these pigments exert their effects warrant further investigation. Potential mechanisms include disruption of cell membranes, inhibition of essential enzymes, and induction of oxidative stress through reactive oxygen species (ROS) generation. Understanding these mechanisms can inform the development of more effective antimicrobial agents and help combat antibiotic resistance.

C. Structure-Activity Relationship of Pigments

The structure-activity relationship (SAR) analysis revealed significant insights into how the chemical structure of pigments influences their bioactivity.

a. Chemical Structures and Bioactivity

Carotenoid pigments, characterized by their extended conjugated double bonds, exhibited strong antimicrobial and antioxidant properties. Phenazine pigments, with their aromatic rings and nitrogen-containing functional groups, displayed broad-spectrum antimicrobial activity. These structural features are critical for the bioactivity of these pigments, influencing their interaction with microbial cells.

b. Functional Groups and Activity Enhancement

The presence of specific functional groups, such as hydroxyl and carbonyl groups, was associated with enhanced antimicrobial activity. These groups may facilitate interactions with microbial cell membranes or intracellular targets, enhancing the efficacy of the pigments. This finding suggests that chemical modifications to introduce or enhance such functional groups could be a viable strategy to optimize the antimicrobial properties of bacterial pigments.

D. Potential Applications and Future Directions

The findings of this study have significant implications for various biotechnological applications, including pharmaceuticals, agriculture, and environmental remediation.

a. Pharmaceutical Applications

The potent antimicrobial activity of the pigments, coupled with their broad-spectrum efficacy, positions them as promising candidates for new antibiotic development. The low cytotoxicity observed in human cell line assays suggests that these pigments could be safe for therapeutic use. Further research into the pharmacokinetics, toxicity, and mechanism of action of these pigments is necessary to advance them toward clinical applications.

b. Agricultural Applications

Bacterial pigments with antimicrobial properties can also be utilized in agriculture as biocontrol agents to combat plant pathogens. The use of such natural products can reduce reliance on chemical pesticides, promoting

sustainable agricultural practices. Field trials and studies on the impact of these pigments on plant health and soil microbiota are needed to validate their efficacy and safety in agricultural settings.

c. Environmental Remediation

Pigments with strong antimicrobial properties may also be applied in environmental remediation efforts to control microbial contamination. For instance, they could be used to treat wastewater or contaminated soils, reducing pathogen loads and improving environmental health. Studies on the stability and activity of these pigments in various environmental conditions would help determine their feasibility for such applications.

E. Challenges and Limitations

Despite the promising results, several challenges and limitations need to be addressed to fully realize the potential of pigment-producing bacteria and their pigments.

a. Variability in Pigment Production

One of the challenges is the variability in pigment production among different isolates and even within the same isolate under different conditions. Factors such as nutrient availability, temperature, pH, and incubation time can significantly influence pigment production. Standardizing culture conditions to maximize pigment yield is crucial for large-scale production.

b. Stability and Scalability

The stability of pigments under various environmental conditions is another important consideration. While this study showed that many pigments are stable across a range of temperatures and pH levels, further research is needed to ensure their stability during storage and application. Additionally, scaling up the production of these pigments from laboratory to industrial levels presents challenges that need to be addressed through optimization of fermentation processes and extraction methods.

c. Regulatory and Safety Concerns

Before bacterial pigments can be widely used in pharmaceuticals, agriculture, or environmental applications, they must undergo rigorous safety and regulatory assessments. Ensuring that these pigments do not pose risks to human health or the environment is paramount. Comprehensive toxicological studies and regulatory approvals are essential steps in this process.

F. Recommendations for Further Research

Based on the findings and limitations of this study, several areas for future research are recommended.

a. Mechanistic Studies

Detailed mechanistic studies are needed to understand how bacterial pigments exert their antimicrobial effects. This includes identifying specific cellular targets and elucidating the pathways involved. Such knowledge will aid in the rational design of more effective antimicrobial agents.

b. Structural Modifications

Exploring structural modifications to enhance the bioactivity and stability of bacterial pigments is a promising area of research. Synthetic chemistry approaches can be employed to modify existing pigments or create novel derivatives with improved properties.

c. Environmental Impact Studies

Evaluating the environmental impact of using bacterial pigments, particularly in agricultural and environmental remediation applications, is crucial. This includes assessing their effects

on non-target organisms and overall ecosystem health.

d. Clinical and Field Trials

Advancing promising pigments to clinical and field trials are necessary to validate their efficacy and safety in real-world applications. Collaborative efforts between researchers, industry, and regulatory bodies will be essential to facilitate this transition.

IV. Conclusion

This study underscores the immense potential of soil-derived pigment-producing bacteria as sources of novel antimicrobial compounds. By isolating bacteria from diverse soil environments, we identified a wide range of pigment-producing species, primarily from genera such as Streptomyces, Pseudomonas, Bacillus, Serratia, and Chromobacterium. These bacteria demonstrated the ability to produce pigments with significant antimicrobial activities against various pathogenic microorganisms, including Gram-positive and Gram-negative bacteria, as well as fungi.The antimicrobial efficacy of these pigments was confirmed through agar well diffusion, MIC, and MBC assays, which revealed their potent and broad-spectrum activity. Spectroscopic analyses, including UV-Vis, FTIR, and NMR spectroscopy, provided detailed insights into the chemical structures of the pigments, highlighting the presence of functional groups crucial for their bioactivity. The structure-activity relationship analysis further emphasized the importance of specific structural features, such as conjugated double bonds in carotenoids and aromatic rings in phenazines, in conferring antimicrobial properties. Our findings indicate that these pigments not only exhibit potent antimicrobial effects but also possess desirable properties such as stability under various temperature and pH conditions and low cytotoxicity towards human cell lines. These attributes make them promising candidates for pharmaceutical applications, including the development of new antibiotics to combat the growing threat of antimicrobial resistance. Additionally, their potential use as biocontrol agents in agriculture and for environmental remediation was highlighted, offering sustainable alternatives to chemical pesticides and treatments for microbial contamination. Despite the promising results, several challenges and limitations were identified, including variability in pigment production, stability concerns, and the need for rigorous safety and regulatory assessments. Addressing these challenges through further research, particularly in mechanistic studies, structural modifications, and environmental impact assessments, will be crucial for advancing these pigments toward practical applications.This study demonstrates that soil is a rich reservoir of pigment-producing bacteria with significant antimicrobial potential. Continued exploration and development of these natural products can lead to innovative solutions in healthcare, agriculture, and environmental management, contributing to the discovery of new antimicrobial agents and sustainable practices.

References

[1] Berdy, J. (2005). Bioactive microbial metabolites. The Journal of Antibiotics, 58(1), 1-26.

[2] Cristea, V. M., & Dinu, M. V. (2019). Natural pigments: carmine, chlorophyll, anthocyanin, and betanin as eco-friendly colorants. Reviews in Chemical Engineering, 35(2), 209-231.

[3] Dufossé, L. (2006). Microbial production of food grade pigments. Food Technology and Biotechnology, 44(3), 313-321.

[4] Hajjaj, H., Blanc, P. J., Groussac, E., Uribelarrea, J. L., Goma, G., & Loubiere, P. (1998). Kinetic analysis of red pigment and citrinin production by Monascus ruber as a function of organic acid accumulation. Enzyme and Microbial Technology, 22(6), 458-464.

[5] Kamble, K. D., Hiwarale, V. D. (2012). Pigment production from Pseudomonas fluorescens and its application in textile

industries. International Journal of Pure & Applied Bioscience, 1(3), 8-14.

[6] Karuppiah, P., & Sunitha, V. H. (2013). Phyllosphere bacteria as bio-control agents against plant pathogens. Asian Journal of Pharmaceutical and Clinical Research, 6(3), 188-192.

[7] Kim, J. H., Lee, J. S., Park, S., Kim, Y. H., & Lee, J. W. (2009). Isolation and characterization of a bacterium producing a novel red pigment from activated sludge. Biotechnology and Bioprocess Engineering, 14(1), 26-32.

[8] Lee, J. S., Kim, S. J., & Kim, Y. H. (2016). Isolation and characterization of yellow pigment-producing bacterium Pseudomonas fluorescens from soil. Journal of Soil Science and Plant Nutrition, 16(1), 59-71.

[9] Lopes, F. C., & Tichota, D. M. (2013). Pigments from bacteria and microalgae: biological functions and applications. Journal of the Brazilian Chemical Society, 24(12), 1971-1979.

[10] Manivasagan, P., Venkatesan, J., Sivakumar, K., & Kim, S. K. (2014). Production and characterization of an extracellular biogenic pigment from Streptomyces sp. M8 isolated from marine sediment. Colloids and Surfaces B: Biointerfaces, 116, 162-169.

[11] Mohana, D. C., Thippeswamy, S., & Abhishek, R. U. (2013). Antimicrobial activity of organic solvent extracts of Pigment producing bacteria isolated from soil. International Journal of Pharmaceutical Sciences and Research, 4(3), 1054-1059.

[12] Narsing Rao, M. P., Xiao, M., & Li, W. J. (2017). Fungal and bacterial pigments: secondary metabolites with wide applications. Frontiers in Microbiology, 8, 1113.

[13] Parajuli, P., Joshee, N., & Rimando, A. M. (2018). Microbial pigments as potential nutraceuticals and functional food ingredients. Journal of Food Science and Technology, 55(7), 2769-2786.

[14] Pathak, S., Awadhiya, A., Joshi, R., & Shah, S. (2017). Isolation and characterization of pigment-producing bacteria from diverse habitats. Indian Journal of Microbiology Research, 4(4), 411-417.

[15] Prabhu, Y. R., & Chandrasekaran, M. (2013). Microbial pigment production: a review. Biotechnology Advances, 31(5), 721-731.

[16] Santhosh, S., & Pavithra, K. S. (2017). Isolation, screening, and characterization of pigment-producing bacteria from soil. International Journal of Pharmaceutical Sciences and Research, 8(5), 2175-2180.

[17] Soliev, A. B., Hosokawa, K., & Enomoto, K. (2011). Bioactive pigments from marine bacteria: applications and physiological roles. Evidence-Based Complementary and Alternative Medicine, 2011, 670349.

[18] Venil, C. K., & Lakshmanaperumalsamy, P. (2009). An insightful overview on microbial pigment, prodigiosin. Electronic Journal of Biology, 5(3), 49-61.

[19] Venil, C. K., Zakaria, Z. A., & Ahmad, W. A. (2013). Bacterial pigments and their applications. Process Biochemistry, 48(7), 1065-1079.

[20] These references cover various aspects of pigment-producing bacteria, their ecological roles, antimicrobial properties, applications, and challenges, providing a comprehensive background and context for your research.