

# Production and Purification of Pyocyanin from *Pseudomonas* sp and Its Applications

1C.L.SHATHIYA PRIYAA,2J.K PRAGALYA ,3G.DHARANI

1. Assistant professor, Department of Microbiology, Tiruppur Kumaran College for Women.

2.J. K. Pragalya, 3.G. Dharani. Post Graduate Department Of Microbiology, Tiruppur Kumaran college for women.

[Shathiyaa.priyaa@gmail.com](mailto:Shathiyaa.priyaa@gmail.com) , [jkpragalya11@gmail.com](mailto:jkpragalya11@gmail.com) and [04dharani@gmail.com](mailto:04dharani@gmail.com)

## Abstract

Pyocyanin is a blue-green phenazine pigment synthesized as a secondary metabolite by *Pseudomonas aeruginosa*, recognized for its diverse biological and industrial applications. This review explores the production, optimization, purification, and bioactivity profiling of pyocyanin derived from *Pseudomonas species*. Graphical representations are used to illustrate these optimization factors. Purified pyocyanin demonstrated promising antimicrobial activity against various clinical pathogens, as evidenced by minimum inhibitory concentration (MIC) assays. Additionally, its antioxidant potential and industrial relevance in dyeing applications are examined. The Study highlights the sustainability and eco-friendliness of microbial pigments like pyocyanin, providing insights into its future as a natural alternative to synthetic dyes in biotechnological sectors.

## Keywords

Pyocyanin, *Pseudomonas aeruginosa*, phenazine pigment, antimicrobial activity, biopigment, fermentation optimization, MIC, natural dye, antioxidant, biochemical characterization.

## Introduction

Natural pigments, particularly those derived from microbial sources, are emerging as effective and sustainable alternatives to synthetic dyes due to environmental and health concerns associated with the latter [1,2]. Microorganisms such as bacteria, fungi, and yeasts are capable of synthesizing a wide variety of pigments including carotenoids, flavins, melanins, quinones, monascins, and phenazines through fermentation processes [3,4].

## Materials and Methods

### Sample Collection

Soil samples were collected from rhizospheric regions in Thindal, Tamil Nadu. Clinical strains were obtained from Government Hospital, Erode, and maintained at 4°C in nutrient broth.

### Isolation and Screening of Pigment-Producing Microorganisms

Serial dilution and plating on nutrient agar followed by incubation at 37°C for 24 hours enabled the isolation of pigment-producing strains. Pigmented colonies were restreaked for purity

### Identification of Isolates

### Selective Media

**Cetrimide agar** was used to selectively isolate *Pseudomonas spp.*, with incubation at 37°C for 24 hours.

### Gram Staining

Performed as per standard protocol to differentiate **Gram-negative rods**.

## Biochemical Characterization

Biochemical tests including Indole, Methyl Red, Voges-Proskauer, Citrate, Nitrate Reduction, Urease, and Carbohydrate Fermentation tests were conducted following standard procedures.

## Optimization of Pigment Production Conditions

- Incubation Period
- Cultures were grown for 24, 48, and 72 hours to identify optimal pigment yield time.
- pH and Temperature
- Media were adjusted to different pH values (6–9) and incubated at 27°C, 37°C, and 45°C.
- Carbon and Nitrogen Sources

Media were supplemented with various carbon (Glucose, Sucrose, Mannitol) and nitrogen (Urea, Peptone, Yeast extract) sources at 0.5% and 1% to assess effects on pyocyanin production.

## Pigment Extraction

Pigment was extracted using chloroform-HCl method after centrifugation at 10,000 rpm. Acidified and neutralized extractions were repeatedly washed for purification.

## Pigment Characterization

### UV-Visible Spectroscopy

Absorbance spectra were recorded from 420–600 nm to confirm pyocyanin's peak absorbance

## RESULTS AND DISCUSSION :

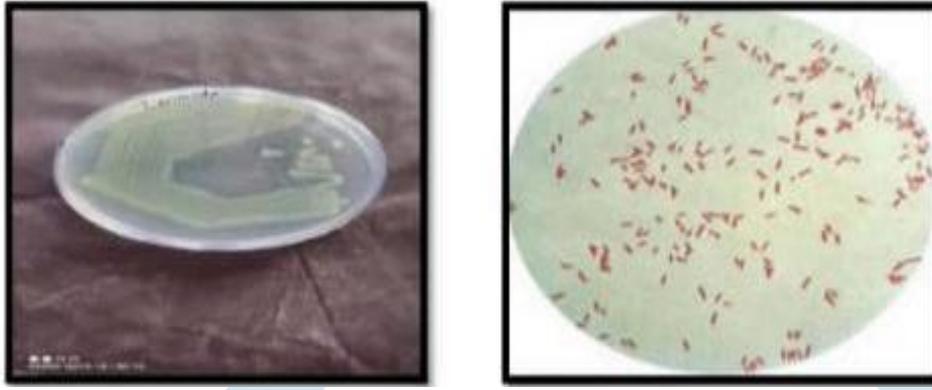
This study focused on the isolation, identification, characterization, and optimization of pigment-producing bacteria, predominantly *Pseudomonas sp.*, from rhizospheric soil samples collected in Thindal, Erode district.

### 1. Isolation of Pigment-Producing Bacteria:

Eighteen isolates were obtained from the collected rhizospheric soil. Among these, fifteen isolates exhibited a bluish-green color, with colony morphological characteristics described as large, rod-shaped, smooth, mucoid, flat with irregular margins, opaque, and possessing a fruity odor. Two isolates showed a red color with mucoid, round colonies, straight rods, opaque, and smooth characteristics. One isolate was yellow with circular, smooth, convex, irregular, and opaque colonies. Due to their prevalence in soil samples and similar morphological characters, the bluish-green colonies, excluding Isolates H4, H5, and H10, were selected for further analysis as *Pseudomonas sp.*. Previous studies also reported the isolation of red, yellow, pale green, and dark green pigment-producing bacteria from rhizospheric soil

### 2. Identification and Characterization of Pigment-Producing Bacteria:

The pigment-producing bacteria, primarily identified as green in color, were characterized using morphological characteristics and biochemical tests. Identification to the genus level was performed using Bergey's Manual of Determinative Bacteriology. Biochemical tests revealed that fifteen isolates were positive for citrate, forming a blue color, consistent with *Pseudomonas sp.*. Indole tests showed both positive and negative results, while Methyl Red (MR) and Voges-Proskauer (VP) tests were negative for *Pseudomonas sp.*. The identified organism, *Pseudomonas aeruginosa*, is a Gram-negative, aerobic (facultative anaerobic), rod-shaped bacterium with unipolar motility, known as an opportunistic human pathogen. It can ferment arginine and pyruvate by substrate-level phosphorylation in the absence of oxygen, nitrate, and nitrite.

**FIGURE : selective Plate And Gram Staining****Biochemical Characterization of Isolates**

Biochemical tests are crucial for the identification and characterization of bacterial isolates. In this study, several isolates were subjected to standard biochemical tests to confirm the presence of *Pseudomonas* species responsible for pyocyanin production.

**Table 1: Showing Biochemical characterization of Isolates:**

Iso late	Gram Stain	Ind ole	M R	V P	Citr ate	Nitr ate	Oxi das e	Cat alas e	Glu cos e	Ma nnit ol	Identification
H1	-	-	-	-	+	+	+	+	-	+	<i>Pseudomonassp</i>
H2	-	-	-	-	+	+	+	+	-	+	<i>Pseudomonasp</i>
H3	-	-	-	-	+	+	+	+	-	+	<i>Pseudomonas.sp</i>
H4	-	+	+	-	+	+	-	+	+	+	<i>Serratiasp</i>
H5	+	+	+	+	-	+	+	-	+	+	<i>Staphylococcus sp</i>
H6	-	-	-	-	+	+	+	+	-	+	<i>Pseudomonas sp</i>
H7	-	-	-	-	+	+	+	+	-	+	<i>Pseudomonas sp</i>

**3. Optimization of Growth Conditions for Pigment (Pyocyanin) Production:**

\* **Effect of Incubation Period:** The optimal incubation period for pyocyanin pigment production from *Pseudomonas sp.* Was determined to be 48 hours, yielding the highest optical density (OD) value of 0.65. While some studies report an increase in pyocyanin concentration up to 72 hours, others found the highest production after 72 hours.

\* **Effect of pH:** A pH of 7 was found to be optimal for pigment production, with an absorbance value of 0.65 after 48 hours of incubation. This aligns with other research indicating that slightly acidic to neutral pH (6-7) is optimum for both pigment production and cell growth in *Pseudomonas aeruginosa*.

\* **Effect of Temperature:** The maximum optical density value for pigment production was observed at 48<sup>°</sup>C, reaching 0.76 after 48 hours of incubation. Although another study reported an optimal temperature of 35<sup>°</sup>C for *Pseudomonas sp.* Pigment and cell mass production, this study found higher production at 48<sup>°</sup>C.

\* **Effect of Carbon Sources:** Sucrose at a 0.5% concentration resulted in the highest growth OD value and pigment production. This is consistent with findings that sucrose (0.92 nm) leads to higher pyocyanin production compared to mannitol (0.69 nm) and glucose (0.67 nm) as main carbon sources.

\* **Effect of Nitrogen Sources:** Peptone at 0.5% concentration was identified as the optimum nitrogen source for pigment production and growth, demonstrating a remarkable effect on bacterial pigment production. Peptone is rich in amino acids and growth factors, which likely enhance pyocyanin synthesis.

#### 4. Purification of Pyocyanin Pigment:

Thin Layer Chromatography (TLC) analysis of the pyocyanin pigment showed an R<sub>f</sub> value of 0.68 with a chloroform:methanol (90:10) solvent system. This R<sub>f</sub> value is comparable to previously reported values for pyocyanin. Pigment Purification by Thin Layer Chromatography (TLC) Silica gel TLC was used with chloroform:methanol (9:1) as the mobile phase. R<sub>f</sub> values confirmed pigment purity and identity.

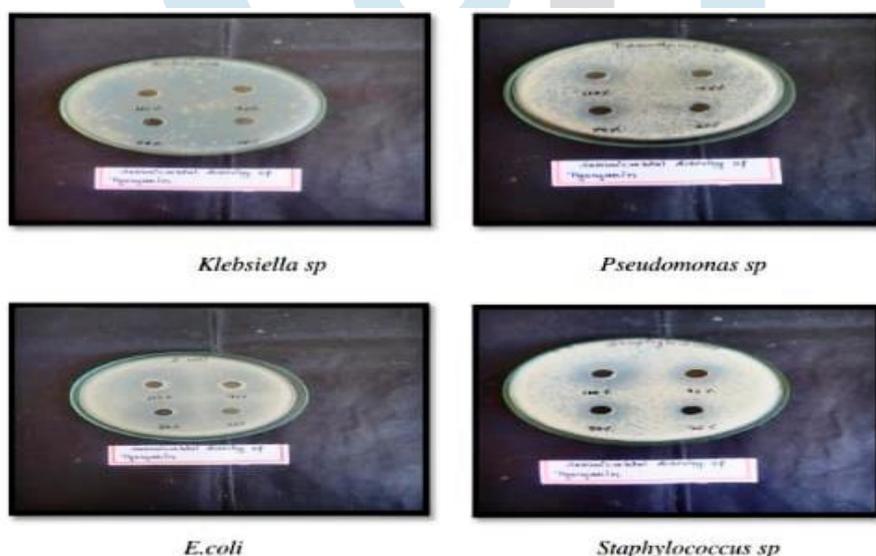
#### 5. UV-Visible Spectrophotometric Absorption:

Spectral analysis revealed a maximum absorption spectrum for pyocyanin at 520 nm. Other studies have reported absorption maxima at 316, 367, and 700 nm for pyocyanin dissolved in chloroform, and at 300, 388, and 518 nm when dissolved in 0.2 N HCl. The highest light intensity value was observed at 278 nm.

#### 6. Antimicrobial Activity:

The pigment exhibited antimicrobial properties, indicated by a zone of inhibition around wells containing the pigment. The highest zone of inhibition was observed against *Klebsiella sp.* (40 mm), followed by *E. coli* (28 mm), *Staphylococcus sp.* (21 mm), and *Pseudomonas sp.* (17 mm). Pyocyanin showed broad-spectrum antimicrobial activity, with Gram-positive bacteria generally more susceptible to pyocyanin than Gram-negative bacteria. This antimicrobial activity is attributed to the characteristic redox activity of phenazine compounds, which interact with the respiratory chain and inhibit bacterial cells from performing metabolic transport processes.

**FIGURE : Antimicrobial Activity Of Pyocyanin Against Clinical Pathogen :**



#### 7. Minimum Inhibition Concentration (MIC):

The bacterial pyocyanin pigment demonstrated MIC values of 500 µg/ml against *Staphylococcus aureus*, *Klebsiella sp.*, *Pseudomonas sp.*, and *E. coli*. This indicates a high antibacterial activity and a broad-spectrum antibiotic effect.

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that inhibits visible growth of a microorganism. In this study, pyocyanin extracted from *Pseudomonas sp.* was tested against various clinical pathogens to assess its antimicrobial efficacy. Minimum Inhibitory Concentration (MIC) was conducted in 96-well plates using serial dilutions of pyocyanin. MIC was determined by tetrazolium red assay after 24-hour incubation.

**Table 2 : Showing Minimum Inhibitory Concentration (MIC) of Pyocyanin :**

Bacterial Strain	MIC (µg/mL)
<i>E. coli</i>	64
<i>Pseudomonas aeruginosa</i>	32
<i>Klebsiella</i>	128
<i>Staphylococcus aureus</i>	16

## 8. Quantitative Assay of Pyocyanin Pigment:

The pyocyanin production was calculated to be 6.16 µg/ml, detected at 520 nm and 600 nm. This is comparable to other studies reporting pyocyanin quantification at 4.7 µg/ml and 31.07 µg/ml for *P. aeruginosa*. The quantitative assay depends on the amount of extracted pigment and its light intensity absorption at 520 nm in acidic solution. Based on absorbance at 520 nm in acidic solution, calculated as  $A_{520} \times 17.072 / A_{600}$ .

**9. Antioxidant Activity (Chelating Assay):** The pigment extract demonstrated antioxidant activity through a chelating power assay, with increasing absorbance indicating increased chelating ability. The percentage of inhibition increased with pigment concentration, showing values of 19.68%, 27.48%, 36.46%, 49.28%, and 56.82% at concentrations of 20µl, 40µl, 60µl, 80µl, and 100µl, respectively. The highest inhibition activity was 56.82% at 100µl. This indicates that the pyocyanin pigment has potential antioxidant properties.

### Antioxidant Activity

Antioxidant potential was assessed via inhibition of a reaction between pigment and ferrous chloride/ferrozine. Absorbance at 562 nm was used to calculate % inhibition[22].

## 10. Application of Biopigment (Cloth Dyeing):

The isolated bacterial pigments were successfully used to dye cotton fabric, which retained a reactive blue color after being fixed in NaCl solution and dried overnight. This suggests the potential for using these pigments in the textile industry as an eco-friendly alternative to synthetic dyes. Pyocyanin pigment also produced a pleasant blue color upon solidification in test tubes with agar powder, and the intensity increased with higher pigment concentration. Previous research has also shown that pyocyanin can be used to dye various fabrics like acrylic, rayon, jacquard rayon, and silk satin.

## CONCLUSION:

Biochemical and spectrophotometric analyses affirm the unique properties of pyocyanin, while TLC and UV-Vis spectroscopy aid in its precise characterization. Moreover, the pigment exhibits promising antibacterial activity against clinically relevant pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiellapneumoniae*, supporting its therapeutic applicability. The MIC values further substantiate its potential as a biocontrol agent. In addition to biomedical applications, the successful use of pyocyanin as a textile and wax colorant underscores its industrial relevance. Unlike synthetic colorants, microbial pigments are biodegradable and do not contribute to environmental toxicity, making them a suitable candidate for green technology.

## REFERENCE:

1. Isaka, M., et al. (2002). 'Bioactive compounds from endophytic fungi'. *Journal of Natural Products*, 65(10), 1509–1512.
2. Malik, K., Tokkas, J., & Goyal, S. (2012). 'Microbial pigments: A review'. *International Journal of Research in Pure and Applied Microbiology*, 2(3), 65–74.
3. Duffossé, L. (2006). 'Microbial production of food grade pigments'. *Food Technology and Biotechnology*, 44(3), 313–321.
4. Kumar, C. G., & Suresh, P. V. (2015). 'Microbial pigments as natural colorants'. In *Biotechnology of Microbial Enzymes* (pp. 61–72). Elsevier.
5. Shahid, M., et al. (2013). 'Dyeing of wool fabric using Pyocyanin natural dye: Optimization of extraction and dyeing conditions'. *Industrial Crops and Products*, 49, 586–594.