



Isolation And Characterization of Pigment Producing Bacteria from Air and Water and Its Various Applications

Deep Dattatray Munde

Department of Microbiology, Dr. D. Y. Patil Arts, Commerce & Science College, Akurdi, Pune- 411044

Corresponding Author –Deep Dattatray Munde

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Abstract:

Microbial pigments are bioactive secondary metabolites produced by diverse microorganisms including bacteria, fungi, and actinomycetes. In recent years, increasing environmental and health concerns associated with synthetic dyes have led to a growing interest in natural pigments derived from microorganisms. The present study focuses on the isolation and characterization of pigment-producing bacteria from air and water samples and evaluates their potential applications in pharmaceutical and industrial sectors. Air samples were collected using the settle plate method, while water samples were collected from ponds, lakes, and tap water sources under sterile conditions. Samples were cultured on nutrient agar and incubated at 30–37°C for 24–48 hours. Pigmented colonies were selected based on distinct coloration and purified through repeated streaking. Morphological characterization included colony morphology, Gram staining, and motility testing. Biochemical identification involved catalase, oxidase, indole, MR-VP, citrate utilization, urease, and carbohydrate fermentation tests. Pigments were extracted using organic solvents such as methanol and acetone, followed by spectrophotometric analysis to determine absorbance maxima. Antimicrobial activity of extracted pigments was evaluated using the agar well diffusion method against test pathogens. The study successfully isolated multiple pigment-producing bacterial strains exhibiting red, yellow, violet, and blue-green pigments. Spectrophotometric analysis confirmed characteristic absorption peaks corresponding to known microbial pigments. Several isolates demonstrated significant antimicrobial activity. The findings suggest that air and water are rich reservoirs of pigment-producing bacteria with promising pharmaceutical, agricultural, cosmetic, and textile applications. Microbial pigments represent sustainable and eco-friendly alternatives to synthetic dyes.

Introduction:

Microorganisms synthesize a diverse array of bioactive secondary metabolites, among which pigments represent a particularly significant group. Microbial pigments are naturally occurring coloured compounds produced by bacteria, fungi, and algae that contribute not only to cellular coloration but also to physiological protection and ecological fitness. These pigments are often synthesized in response to environmental stress and play essential roles in microbial adaptation and survival (Venil et al., 2013; Dufossé, 2018).

In bacteria, pigment production is frequently associated with multiple functional advantages, including:

- Protection against ultraviolet (UV) radiation
- Defence against oxidative stress through reactive oxygen species (ROS) scavenging
- Antimicrobial and competitive interactions with other microorganisms
- Regulation of energy metabolism and electron transport processes
- Enhancement of environmental stress tolerance (temperature, salinity, desiccation)

Dynamic environments such as air and aquatic ecosystems present fluctuating conditions of temperature, humidity, radiation exposure, nutrient availability, and osmotic pressure. Microorganisms inhabiting these niches must rapidly adapt to survive. Pigment production enhances cellular resilience by stabilizing membranes, reducing oxidative damage, and improving resistance to environmental stressors (Soliev et al., 2011; Dufossé, 2018).

Several bacterial species are well recognized for their pigment production capabilities. For example, *Serratia marcescens* produces the red Tri pyrrole pigment prodigiosin, which exhibits antimicrobial and anticancer properties. *Chromobacterium violaceum* synthesizes violacein, a violet pigment with antioxidant and antimicrobial activities. *Pseudomonas aeruginosa* produces blue-green pigments such as pyocyanin and pyoverdine, which are involved in iron acquisition and virulence. Additionally, *Micrococcus luteus* generates yellow carotenoid pigments that provide protection against photooxidative damage.

The growing global demand for natural colorants in the food, pharmaceutical, cosmetic, and textile industries has intensified interest in microbial pigments as sustainable alternatives to synthetic dyes. Unlike many artificial colorants, microbial pigments are biodegradable, environmentally friendly, and may possess additional bioactivities such as antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Venil et al., 2013).

Materials And Methods:

1. Sample Collection:

Air Samples: Nutrient agar plates were exposed to the ambient air for 20 minutes at various locations using the settle plate method. After exposure, the plates were incubated at 30–37°C for 24–48 hours

to allow microbial growth and pigment production (Atlas, 2010; Madigan et al., 2018).

Water Samples: Water samples were collected from ponds, lakes, and tap water in sterile containers. The samples were transported to the laboratory under cooled conditions and stored at 4°C until further processing. Serial dilution and spread plate techniques were subsequently employed to isolate bacterial colonies capable of producing pigments (Atlas, 2010; Madigan et al., 2018).

2. Isolation of Pigment-Producing Bacteria:

Water samples were serially diluted up to 10^{-6} using sterile saline or distilled water. Aliquots of the diluted samples were spread onto nutrient agar plates and incubated at 37°C for 24 hours. Colonies exhibiting distinct pigmentation were selected based on visible coloration and purified through repeated streaking to obtain pure cultures. The bacterial load was quantified by calculating colony-forming units (CFU/ml) from the dilution plates (Madigan et al., 2018; Atlas, 2010).

3. Morphological Characterization:

Colony characteristics recorded: Size, shape, color, margin, elevation, consistency, opacity, gram character.

4. Biochemical Characterization:

The following biochemical tests were conducted: Catalase, oxidase, indole test, methyl red, vogues Proskauer, citrate utilization. These tests helped in tentative identification of isolate.

5. Pigment Extraction:

Isolated bacterial colonies were inoculated into nutrient broth and incubated at 30–37°C for 48–72 hours under shaking conditions to promote pigment production. After incubation, the cultures were centrifuged at 6000 rpm for 10 minutes to separate the cells from the culture medium. Depending on the pigment location, either the supernatant (extracellular pigments) or the cell pellet (intracellular pigments) was collected.

Organic solvents such as methanol, acetone, or ethanol were added to the collected fraction to extract the pigments. The resulting mixture was filtered to remove cell debris, and the solvent was evaporated under reduced pressure or at room temperature to obtain the crude pigment. The extracted pigment was then stored for further analysis (Venil et al., 2013; Dufossé, 2018).

6. Spectrophotometric Analysis:

The crude pigment solutions were analysed using a UV-Visible spectrophotometer. The absorbance spectra were recorded over a wavelength range of 200–800 nm, and the wavelength corresponding to the maximum absorbance (λ_{max}) was noted for each pigment (Venil et al., 2013; Dufossé, 2018).

7. Antimicrobial Activity:

Antimicrobial testing was performed using agar well diffusion method against:

- Escherichia coli
- Pseudomonas
- Staphylococcus aureus

Zones of inhibition were measured in millimeters.



Figure 1: Antimicrobial Activity

Results:

1. Isolates:

A total of 30 colonies were isolated from air and water samples. Among these, **10 isolates** showed distinct pigmentation.

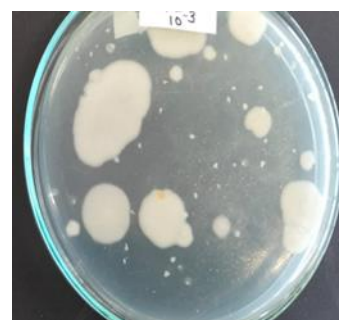
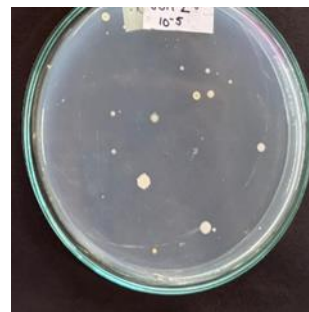


Fig.2: Isolates from water and air sample on Nutrient agar plate

2. Morphological Findings:

Most isolates were circular, smooth, and convex. Gram staining revealed both Gram-positive and Gram-negative bacteria.



Fig. 3: gram negative rods

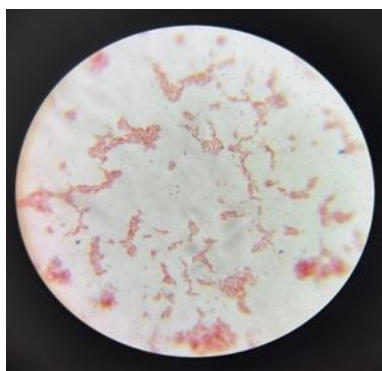


Fig. 4: gram negative rods

Colony No.	Catalase	Oxidase	MR	VP	Citrate	Indole
1	+	-	-	-	-	-
2	+	-	-	-	-	-
3	+	+	-	+	+	-
4	+	+	-	+	+	-
5	+	-	-	+	+	-
6	+	-	-	+	+	-
7	+	-	+	-	+	+
8	+	-	+	-	-	-
9	+	+	-	+	+	-
10	+	-	-	-	+	-

Catalase positive: 80% isolates

Oxidase positive: 60% isolates

Citrate utilization: 70% isolates

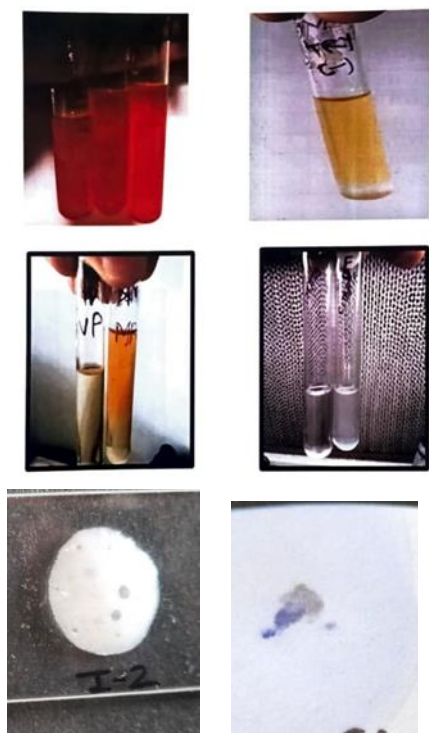


Figure 5: Biochemical Tests

By comparing to Bergey’s Manual of Determinative Bacteriology the isolated organisms from water and air samples- Isolates were found to belong to genus *Micrococcus*, *Serratia* and *Pseudomonas* respectively

Results indicated possible identification as *Serratia*, *Micrococcus*, and *Pseudomonas* species

4. Pigment Distribution:

Isolates were inoculated in nutrient broth for extraction of pigment

Red pigment – 2 isolates, yellow pigment – 3 isolates, Blue-green pigment – 1 isolate, Orange pigment – 2 isolates

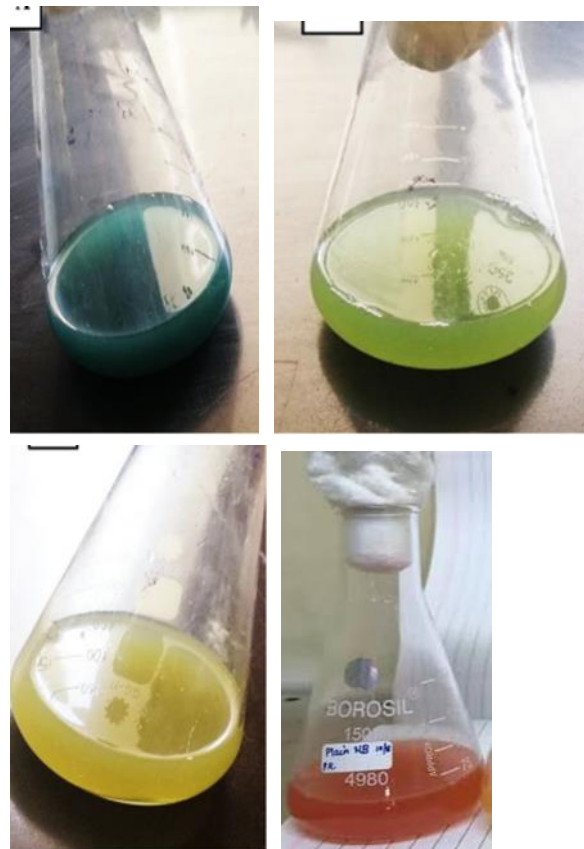
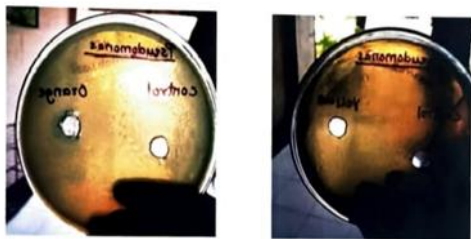


Fig.6: Isolates inoculated in Nutrient broth

5. Antimicrobial Activity:

Several pigment extracts showed inhibition zones ranging from 8–18 mm against test pathogens, indicating antimicrobial potential.

**Discussion:**

This study confirms that environmental air and water harbour diverse pigment-producing bacteria, consistent with previous reports (Barreto et al., 2023). Pigmentation likely provides survival advantages such as UV protection and resistance to oxidative stress (Patkar et al., 2021).

Spectrophotometric analysis showed distinct absorbance peaks, corresponding to known pigment classes like carotenoids and prodigiosin, similar to findings by Daramola & Awojobi (2025). Antimicrobial activity observed in the pigments aligns with earlier studies suggesting that microbial pigments can disrupt pathogen cell membranes or cell wall synthesis (Barreto et al., 2023).

Environmental factors, including pH, temperature, and nutrient availability, influenced pigment production, supporting previous observations that culture conditions affect yield and stability (Patkar et al., 2021). The presence of carotenoids and phenazine derivatives highlights the potential for pharmaceutical and industrial applications, offering eco-friendly alternatives to synthetic dyes (Huang et al., 2024).

Overall, these results corroborate prior studies and reinforce the biotechnological relevance of microbial pigments as natural, multifunctional colorants.

Conclusion: India as a Paradigm for 21st Century Statecraft:

India's foreign policy in the globalized era is a dynamic work in progress, demonstrating both the promise and perils of interdependence. It has successfully transitioned from a post-colonial, defensive posture to that of an aspirational power seeking to shape the international order. Its strategy of multi-alignment, economic pragmatism, and issue-based coalition-building offers a viable model for other middle and emerging powers in an age of uncertainty.

The future will test India's ability to move from strategic balancing to genuine agenda-setting. Its success hinges on its internal economic and technological transformation—the bedrock of comprehensive national power. As globalization enters a phase of reconfiguration, possibly fracturing into competing techno-spheres, India's foreign policy will be judged on its capacity to secure its interests, provide global public goods, and bridge the divides between the developed and developing world. In doing so, India is not just adapting to globalization but actively attempting to sculpt its next phase, asserting that in a world of inevitable interdependence, pluralism, strategic autonomy, and inclusive growth must be its defining principles. The story of India's foreign policy is, therefore, a central chapter in the ongoing story of how nations navigate an interconnected but contested world.

Conclusion:

The present study successfully isolated and characterized pigment-producing bacteria from air and water sources. Extracted pigments demonstrated characteristic absorption spectra and antimicrobial activity. These findings support the potential use of microbial pigments in pharmaceutical, industrial, and environmental applications.

Microbial pigments represent sustainable, biodegradable, and eco-friendly alternatives to synthetic dyes, making them highly valuable for future biotechnological innovations.

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