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Isolation and Identification of Pigment-Producing Bacteria from Soil and Water in Oke-Osun, Osogbo, Osun State

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Abstract

Background: Pigments can be natural or synthetic and play an essential role in various industries, such as textiles, food, cosmetics, and pharmaceuticals. **Objective**: This study aims to identify pigment-producing bacteria from soil and water samples, which serve as natural habitats for diverse microbial communities capable of producing pigments. **Methodology**: Soil and water samples were randomly collected from multiple locations in Oke-Osun, Osogbo, and Osun State and cultured on nutrient agar, King B medium, and glycerol-supplemented agar. Microscopy and biochemical tests were used to determine the morphological and biochemical properties of the isolated bacterial strains, and molecular characterisation was further used to identify specific isolates. **Results**: Seven pigment-producing bacteria were isolated. The pigments include green, yellow, brown, orange and red colourations. The identified bacteria include *Microccocus spp.* (yellow), *Serratia spp.* (red), *Bacillus spp.* (brown), *Pseudomonas spp.* (green), with isolate B4 identified specifically as *Pseudomonas aeruginosa*. **Conclusion**: The study demonstrated the presence of diverse pigment-producing bacteria in soil and water environments of Oke-Osun, Osogbo, and established glycerol-supplemented medium as a favourable substrate for enhanced pigment production. These bacterial pigments possess promising potential for application as natural colourants in pharmaceutical, food, cosmetic, and textile industries. **Recommendations**: This research contributes to the growing body of knowledge on microbial pigments and their industrial applications, and also offers an alternative pathway for developing an environmentally friendly pigment industry.

Keywords: Pigment production, bacteria, glycerol-supplemented media

Introduction

Pigments are natural or synthetic substances that impart colour to materials and play essential roles across industries such as textiles, food, cosmetics, and pharmaceuticals. Beyond their aesthetic value, pigments are also used as indicators in biochemical assays (Sharma *et al.,* 2021). In the food industry, colour additives significantly influence consumer acceptance of processed products. However, many synthetic colourants pose health risks at high concentrations, driving a growing demand for safer, natural alternatives.

Pigments are widely distributed in the microbial world, occurring in bacteria, fungi, yeast, algae, and protozoa. These pigments are typically produced as secondary metabolites and serve protective functions, particularly against damage from visible and near-ultraviolet radiation (Noirbent *et al.,* 2021). In addition to photoprotection, microbial pigments contribute to physiological and molecular processes such as adaptation to environmental stress and, in some cases, photosynthesis (Celedón & Díaz, 2021).

Pigment-producing microorganisms can be isolated from diverse environmental sources,

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including soil, water, plants, insects, and animals (Deveikaite & Zvirdauskiene, 2023). These habitats, especially soil and aquatic ecosystems, represent rich reservoirs of microbial diversity and offer strong potential for the discovery of novel pigment-producing strains. Such discoveries are important for expanding the range of natural pigments with unique properties and industrial relevance (Sanuj & Parveen, 2021).
The use of microbial pigments offers a cost-effective, sustainable alternative to synthetic pigments, with the added advantage of scalability via controlled fermentation. This is particularly important given the high cost and environmental concerns associated with conventional industrial pigment production methods (Fakorede *et al.,* 2020). Several microorganisms are known for high pigment yield, including species of *Monascus*, *Serratia*, *Streptomyces*, *Penicillium*, *Rhodotorula*, *Cryptococcus*, *Phaffia rhodozyma*, *Bacillus*, and *Yarrowia* (Seang-On *et al.,* 2023). These organisms produce a wide spectrum of pigments, some of which are water-soluble and suitable for diverse industrial applications.

Materials and Methods

Study Area

The study was conducted in Oke-Osun, Osogbo, Osun State, Nigeria. A stratified random sampling design was employed to ensure spatial representation of sampling locations. Sampling was conducted in February between 8:00 and 11:00 a.m. to minimise temporal variation.

Sample Collection

Fourteen (14) soil and water samples were collected from various locations. Soil samples were collected at a depth of 2–5 cm using sterile spatulas and composited from three sub-points within a 1 m² radius. Water samples were aseptically collected in sterile bottles. All samples were transported under cooled conditions and processed within 24 hours.

Culture Media Preparation

Media used included:

- Nutrient Agar (NA)
- Glycerol-Supplemented Nutrient Agar (GS-NA)
- King’s B medium
- Cetrimide Agar

Isolation of Bacteria

Serial dilution was performed on 1 g of soil and 1 ml of water samples. Aliquots (0.1 ml) of 10^{-2} and 10^{-4} dilutions were inoculated using pour plate and spread plate techniques and incubated at 30°C for 48 hours. Pigmented colonies were subcultured and preserved on nutrient agar slants at 4°C.

Identification of Isolates

Identification involved:

- Colony morphology
- Gram staining
- Biochemical tests (performed in duplicate)

Molecular identification of isolate B4 was performed using 16S rRNA sequencing with appropriate positive and negative PCR controls.

Statistical Analysis

Descriptive statistics (mean ± standard deviation) were used to summarize bacterial load across media.

Results

Sample collection and bacterial isolation

Fourteen (14) soil and water samples were collected from different locations within Oke-Osun, Osogbo, Osun State. Enumeration on Nutrient Agar (NA), Glycerol-Supplemented Nutrient Agar (GS-NA), and King B medium yielded diverse bacterial populations. A total of seven (7) distinct pigment-producing bacterial isolates were obtained, designated as B1–B7. The isolates exhibited yellow, red, green, brown, and orange pigmentation, indicating the production of metabolically diverse secondary metabolites. The bacterial load of each sample was measured in colony-forming units per millilitre (CFU/ml) using the three different media. The samples showed varying bacterial loads, as shown in

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Table 1a, and bacterial loads across media were summarised using mean ± standard deviation (SD) as shown in Table 1b. The occurrence of pigment-producing bacteria in the culture media used **is** shown in Table 2**.**

Morphological and Gram-Staining Characteristics

Gram staining was carried out to determine the Gram Characteristics of the isolates. This procedure helps identify whether the bacteria are Gram-positive or Gram-negative based on the structure of their cell walls (Table 3)

Biochemical Characterization

These isolated pigments were subjected to biochemical tests (Table 4), which helped further identify the isolates. The suspected species of pigment-producing bacteria are shown in Table 5

Molecular Characterization Of Isolate B4

Further molecular characterisation of isolate B4 confirmed it to be *Pseudomonas aureginosa.* This is shown in Table 6.

Discussion

The pigment-producing bacteria isolated from soil and water samples exhibited a range of colours, including yellow, red, green, brown, and orange. These pigments are secondary metabolites with well-documented ecological and biotechnological roles, including protection against ultraviolet (UV) radiation, mitigation of oxidative stress, antimicrobial activity, and use as natural colourants in the food and pharmaceutical industries (Maldonado-Ruiz & Zurek, 2024). Seven distinct bacterial isolates (B1–B7) were identified based on morphological and biochemical characteristics, and their pigmentation can be associated with specific classes of microbial pigments. Isolates B1 and B2, identified as *Micrococcus* spp. (Gram-positive, yellow-pigmented, catalase-positive), They are known to produce carotenoids, a group of isoprenoid pigments responsible for yellow to orange colouration and recognised for their antioxidant and photoprotective properties.

Isolate B3, identified as *Serratia* spp. (Gram-negative, red-pigmented, catalase-positive, Voges–Proskauer positive), likely produces prodigiosin, a characteristic red tripyrrole pigment with documented antimicrobial, anticancer, and immunosuppressive activities. Isolates B4 and B7, identified as *Pseudomonas* spp. (Gram-negative, green-pigmented, catalase-positive), are associated with the production of pyocyanin (a blue-green phenazine pigment) and pyoverdine (a fluorescent yellow-green siderophore).

These pigments play roles in iron acquisition, oxidative stress mediation, and virulence. Molecular characterisation confirmed isolate B4 as *Pseudomonas aeruginosa*, which is widely reported to produce both pigments, consistent with previous studies (Fakorede et al., 2020; Anzum et al., 2022; Kiki, 2023).

Table 1a: Bacterial load of collected soil samples from soil and water across various locations in Oke-Osun, Osogbo, Osun State.

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ID	Sample site	NA		Glycerol-NA		King B	
		Cfu/ml ($x10^2$)	Cfu/ml ($x10^4$)	Cfu/ml ($x10^2$)	Cfu/ml ($x10^4$)	Cfu/ml ($x10^2$)	Cfu/ml ($x10^4$)
W1	A	1.5	0.15	3.0	0.12	0.5	0.07
W2	B	1.1	0.15	2.5	0.11	0.3	0.04
W3	C	1.0	0.12	2.0	0.15	0.3	0.02
W4	D	1.2	0.18	3.5	0.13	0.4	0.10
W5	E	1.3	0.12	2.2	0.20	1.0	0.04
W6	F	1.4	0.19	2.3	0.34	1.0	0.07
W7	G	1.2	0.14	2.4	0.48	0.6	0.05
S1	H	1.8	0.15	2.0	0.16	0.3	0.08
S2	I	1.5	0.17	2.0	0.13	0.1	0.07
S3	J	1.0	0.22	3.4	0.18	0.2	0.10
S4	K	1.6	0.13	2.0	0.11	0.3	0.02
S5	L	1.4	0.12	2.5	0.15	0.1	0.05
S6	M	1.7	0.16	2.2	0.20	0.8	0.09
S7	N	2.0	0.18	2.8	0.13	0.6	0.02

KEY

A – Male Hostel Borehole
B – Girls Hostel Borehole
C – PF Borehole
D - Osun Groove Stream
E - Oke-Osun under bridge
F – Stagnant water
G – Mosque Borehole
H – Love Garden 1
I – Love Garden 2
J - Waterway soil
K – Dump site
L – Osun Groove soil
M – Mosque farm
N - Sport arena

Table 1b: Bacterial loads across media were summarised using mean ± standard deviation

Medium	Mean CFU/ml (×10²) ± SD
Nutrient Agar (NA)	1.41 ± 0.30
Glycerol-Supplemented NA	2.49 ± 0.49
King B Medium	0.47 ± 0.30

Table 2: Occurrence of pigment-producing bacteria in culture media used

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Pigment	NA	King B	GS- Media
Yellow	3	0	3
Red	1	0	2
Green	1	0	3
Brown	1	0	1
Orange	1	0	2

Key: GS-Media –Glycerol-Supplemented Media

Table 3: Characteristics of the colonies of isolated pigment-producing bacteria.

	Size	**Shape**	**Color**	**Margin**	**Opacity**	**Elevation**	**Consistency**	**Grams Nature**
B1	2mm	Circular	Yellow	Entire	Opaque	Convex	Smooth	Gram-positive
B2	1mm	Circular	Yellow	Entire	Opaque	Convex	Smooth	Gram-positive
B3	2mm	Circular	Red	Entire	Opaque	Convex	Smooth	Gram-negative
B4	2mm	Circular	Green	Entire	Opaque	Flat	Smooth	Gram-negative
B5	1mm	Circular	brown	Entire	Opaque	Flat	Smooth	Gram-positive
B6	1mm	Circular	Orange	Entire	Opaque	Convex	Sticky	Gram-negative
B7	2mm	Circular	Green	Entire	Opaque	flat	Smooth	Gram-negative

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Table 4: Biochemical characterisation of isolated pigment-producing bacteria

	Catalase	Indole	Citrate	Voges Proskauer	Oxidase	Coagulase	Methy red
B1	+	-	+	-	-	-	-
B2	+	-	+	-	-	-	-
B3	+	-	+	+	-	-	-
B4	+	-	+	-	+	-	-
B5	+	-	+	-	+	-	+
B6	+	-	+	+	-	-	+
B7	+	-	+	-	-	-	+

Table 5: Suspected species of pigment-producing bacteria

Isolate	Colour	Pigment	Microorganism
B1	Yellow	Carotenoids	*Micrococcus spp.*
B2	Yellow	Carotenoids	*Micrococcus spp.*
B3	Red	Prodigiosin	*Serratia spp.*
B4	Green	Pyocyanin	*Pseudomonas spp.*
B5	Brown	Melanin	*Bacillus Species*
B6	Orange	Carotenoids	*Erythrobacter spp.*
B7	Green	Pyocyanin	*Pseudomonas spp.*

Table 6: Sequence BLAST prediction Report

Sample ID	Organism	Sequence length	% identity	Accession no of Blast hit	E-value	Alignment score	Highest query coverage (%)
B4	***Pseudomonas aeruginosa***	860	99.19	OQ144895.1	0.0	≥200	100%

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Isolate B5, described as a brown-pigmented organism and tentatively identified as *Bacillus* spp., may produce melanin-like pigments, which are known to confer protection against environmental stressors such as UV radiation and enzymatic degradation. Isolate B6 (identified as *Erythrobacter spp.*, orange-pigmented, Gram-negative) is also associated with carotenoid pigments, particularly bacterioruberin-like compounds, which contribute to membrane stability and protection against photooxidative damage. The diversity of pigmentation observed among the isolates reflects the presence of varied biosynthetic pathways, including carotenoid, phenazine, prodiginine, and melanin pathways. These pigments enhance bacterial survival under environmental stress conditions such as high radiation and oxidative stress, while also offering significant industrial potential as natural dyes, antioxidants, and bioactive compounds for pharmaceutical and cosmetic applications (Kiki, 2023).

Conclusion

This study demonstrated that glycerol-supplemented media can enhance pigment production in selected bacterial isolates, highlighting its potential as a cost-effective strategy for optimising microbial pigment synthesis. The findings also underscore the diversity of pigment-producing bacteria present in soil and water environments, reinforcing their value as reservoirs of biotechnologically relevant strains. The pigments produced by these isolates show promising potential as natural colourants for applications in pharmaceuticals, cosmetics, textile dyeing, and food systems. However, further studies involving molecular identification, pigment characterisation, and toxicity assessment are necessary to validate their suitability for industrial-scale applications.

Professional implications of the study

- Supports development of eco-friendly pigment production systems
- Provides baseline data for industrial-scale microbial pigment production
- Contributes to pharmaceutical and food-grade pigment innovation

Limitations of the study

- Limited molecular characterisation (only one isolate sequenced)
- Absence of pigment extraction and chemical characterisation
- No toxicity or safety evaluation of pigments
- Lack of inferential statistical analysis

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Ethical approval

Not applicable.

Informed consent

Not applicable.

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