

Original Research Article

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Extraction and Purification of Bacterial Yellow Pigment from Soil Isolate *Micrococcus luteus*

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ABSTRACT

Microbial pigments are important secondary metabolites with ecological, biological and industrial significance. The present study investigated the isolation, identification, extraction and preliminary purification of a yellow pigment-producing bacterium from sediment collected from Puzhal Lake. The sample was processed by serial dilution and spread-plate techniques, followed by purification and phenotypic and biochemical characterization. The selected isolate showed Gram-positive characteristics and positive catalase and oxidase reactions, while indole, methyl red and Voges–Proskauer tests were negative. Genotypic identification was performed using 16S rRNA PCR, and an approximately 671 bp product was obtained for sequencing. After confirmation of the organism as *Micrococcus luteus*, mass culture was prepared and the yellow pigment was extracted using methanol. UV-visible spectrophotometric analysis showed a prominent absorbance peak around 400 nm, consistent with a carotenoid-like pigment. Thin-layer chromatography using a methanol: chloroform solvent system produced separated pigment fractions, with an R_f value of 0.66 reported for the methanol extract. Antioxidant activity was evaluated by the DPPH assay, with radical-scavenging activity increasing from 6.1% at 10 mg/ml to 72.2% at 50 mg/ml. The findings indicate that the soil isolate *M. luteus* is a promising source of a natural yellow pigment with potential biological and biotechnological relevance; further advanced chemical characterization is required.

Keywords

Micrococcus luteus, bacterial pigment, yellow pigment, carotenoid, soil isolate, extraction, thin-layer chromatography, DPPH assay

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Introduction

Microorganisms are remarkable producers of a wide range of bioactive compounds, among which pigments occupy a unique position as secondary metabolites with biological, ecological and industrial relevance. Bacterial pigments are synthesized by diverse microbial species and may contribute to protection against ultraviolet

radiation, reactive oxygen species and other environmental stresses.

Soil is a complex and biodiverse ecosystem that provides a rich habitat for bacteria with diverse metabolic capabilities. Pigment-producing soil bacteria have been reported in several genera and may produce carotenoids, melanins, prodigiosins, violaceins and other colored

metabolites. The diversity of pigments reflects the metabolic versatility and environmental adaptation of microorganisms.

Microbial pigments have attracted attention as potential alternatives to synthetic dyes because they are naturally derived and may be biodegradable. In addition to coloration, several microbial pigments have been investigated for antimicrobial, antioxidant and other biological activities, increasing their potential applications in food, pharmaceutical, cosmetic and textile industries.

The investigation of pigment-producing bacteria generally involves isolation of colored colonies, identification of the producing organism, optimization of culture conditions, extraction of pigment using appropriate solvents, purification and characterization. UV-visible spectroscopy and thin-layer chromatography are useful preliminary techniques for studying pigment properties, while advanced methods can provide more definitive chemical identification.

The present study focused on the extraction and purification of yellow pigment produced by a soil-isolated *Micrococcus luteus* and on the preliminary evaluation of its pigment characteristics and antioxidant activity.

Materials and Methods

Collection of Sample

Sediment/soil samples were collected from Puzhal Lake using sterile spatulas and transferred into sterile containers.

The project record specifies collection at approximately 5–10 cm depth, with the sample labelled and transported to the laboratory under aseptic conditions for microbiological analysis.

Serial Dilution and Spread Plate

One gram of the soil sample was transferred into 9 ml sterile diluent to prepare the initial 10^{-1} dilution. Serial ten-fold dilutions were prepared up to $10^{-6}/10^{-7}$. Aliquots were spread on nutrient agar and incubated at approximately 25–30°C for 24–72 h. Colony-forming units were estimated from the plates.

The project records a microbial load of 5×10^6 CFU/ml for the 10^{-6} spread plate and 4×10^7 CFU/ml for the 10^{-7} plate. These values are reproduced as recorded in the project.

Identification of the Isolate

Distinct colonies were purified by subculturing. (Fig 1) Identification included Gram staining, hanging-drop motility, oxidase, catalase, indole, methyl red and Voges–Proskauer tests. The selected isolate was recorded as Gram-positive and catalase- and oxidase-positive; indole, methyl red and Voges–Proskauer reactions were negative. (Table 1)

Genotypic Identification

Primers were designed for 16S rRNA sequencing. PCR was performed using forward and reverse primers, followed by agarose gel electrophoresis. The project records an approximately 671 bp PCR product that was subjected to sequencing (Figure 2) and subsequent identification. (Table 2) & (Figure 3)

Mass Culture and Pigment Extraction

After confirmation of the organism, mass culture was produced in nutrient broth under the project conditions. Following incubation for 48–72 h, intense pigmentation was observed. The bacterial biomass was processed and the pigment was extracted using methanol. The extracted material was retained for spectrophotometric and chromatographic analysis.

UV-Visible Spectrophotometry

The pigment extract was analyzed using a UV-visible spectrophotometer. A prominent absorbance peak around 400 nm was recorded in the project and interpreted as characteristic of a carotenoid-like pigment. (Table 3)

Thin-Layer Chromatography

TLC was used for preliminary purification and separation of pigment components. A methanol: chloroform solvent system was used. The developed plate was visualized under UV illumination (Figure 4) and the R_f value was calculated as the distance travelled by the substance divided by the distance travelled by the solvent front. (Table 4)

DPPH Antioxidant Assay

Antioxidant activity was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay. Different concentrations of the pigment extract were reacted with 0.1 mM DPPH and incubated in the dark for 20 min. Absorbance was measured at 517 nm. Radical-scavenging activity was calculated using the project formula: $[(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100$. (Table 5)

Results and Discussion

Isolation and Identification

The Puzhal Lake sediment sample yielded distinct bacterial colonies after serial dilution and spread plating. Colonies differing in morphology, size and colour were selected and purified. The selected pigmented isolate was characterized by Gram staining and biochemical tests. The isolate showed Gram-positive staining, positive catalase and oxidase reactions, and negative indole, methyl red and Voges–Proskauer reactions. The project subsequently identified the isolate as *Micrococcus luteus* by 16S rRNA-based genotypic analysis.

PCR Amplification

PCR amplification of the 16S rRNA target produced an approximately 671 bp product as recorded in the project. The amplicon was used for sequencing and subsequent identification of the selected isolate.

Pigment Extraction and UV-Visible Analysis

Following confirmation of *Micrococcus luteus*, mass culture was prepared and the yellow pigment was extracted using methanol. The extracted pigment exhibited strong absorbance in the UV-visible region, with a reported peak around 400 nm. This observation was interpreted in the project as supporting the presence of a carotenoid-like pigment.

TLC Analysis

The methanol extract produced separated bands on TLC using a methanol: chloroform solvent system. An R_f value of 0.66 was reported for the analyzed pigment fraction.

Antioxidant Activity

The DPPH assay showed a concentration-dependent increase in radical-scavenging activity. The recorded values are presented below.

The present study investigated the extraction and preliminary purification of yellow pigment from the soil isolate *Micrococcus luteus*. The isolation of a pigmented bacterium from Puzhal Lake sediment supports the view that soil and sediment environments contain microorganisms with diverse secondary metabolic capabilities.

The selected isolate was characterized by conventional microbiological and biochemical tests and subsequently identified by 16S rRNA-based analysis. The project recorded a 671 bp PCR product for sequencing. The identification of *M. luteus* is consistent with the known occurrence of yellow-pigmented carotenoid-producing bacteria.

Extraction with methanol yielded a yellow pigment, and UV-visible analysis showed a prominent absorbance around 400 nm. The project interpreted this spectral behaviour as characteristic of a carotenoid-like pigment. TLC further provided preliminary separation of pigment components, with an R_f value of 0.66 reported for the methanol extract using a methanol: chloroform solvent system.

The DPPH assay demonstrated increasing radical-scavenging activity with increasing extract concentration, reaching 72.2% at 50 mg/ml. This concentration-dependent response suggests antioxidant potential of the crude pigment extract under the assay conditions used. However, the activity cannot by itself establish the identity of the active compound.

The project also recognizes limitations in the level of pigment characterization. Advanced analytical techniques such as high-performance liquid chromatography and mass spectrometry would be useful for more precise identification of individual pigment molecules. Further optimization of pH, temperature and carbon and nitrogen sources may also improve pigment yield.

Table.1 Preliminary and biochemical characteristics of the selected isolate

Test	Observation
Gram staining	Gram-positive (G+)
Catalase	+ positive
Oxidase	+ positive
Indole	Negative
Methyl Red	Negative
Voges-Proskauer	Negative

Table.2 PCR conditions recorded in the project

PCR step	Temperature	Time	Cycles
Pre-denaturation	95°C	10 min	1
Denaturation	95°C	45 s	40
Annealing	58°C	45 s	40
Elongation	72°C	45 s	40
Final elongation	72°C	10 min	1

Table.3 Optical density of the pigment extract at different concentrations

Concentration	Optical density (OD)
10 mg/ml	0.971
20 mg/ml	0.743
30 mg/ml	0.695
40 mg/ml	0.482
50 mg/ml	0.287

Table.4 Thin-layer chromatographic separation of the pigment extract

Extract	Solvent system	Reported Rf value
Methanol extract	Methanol : Chloroform	0.66

Table.5 Antioxidant activity of the pigment extract by DPPH assay

Concentration	DPPH radical-scavenging activity
10 mg/ml	6.1%
20 mg/ml	28.2%
30 mg/ml	32.8%
40 mg/ml	53.4%
50 mg/ml	72.2%

Figure.1 Isolation of *Micrococcus luteus*



Figure.2 Agarose Gel Electrophoresis

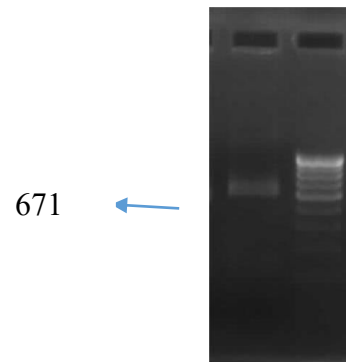


Figure.3 Phylogenetic tree construction using Mega 11 software

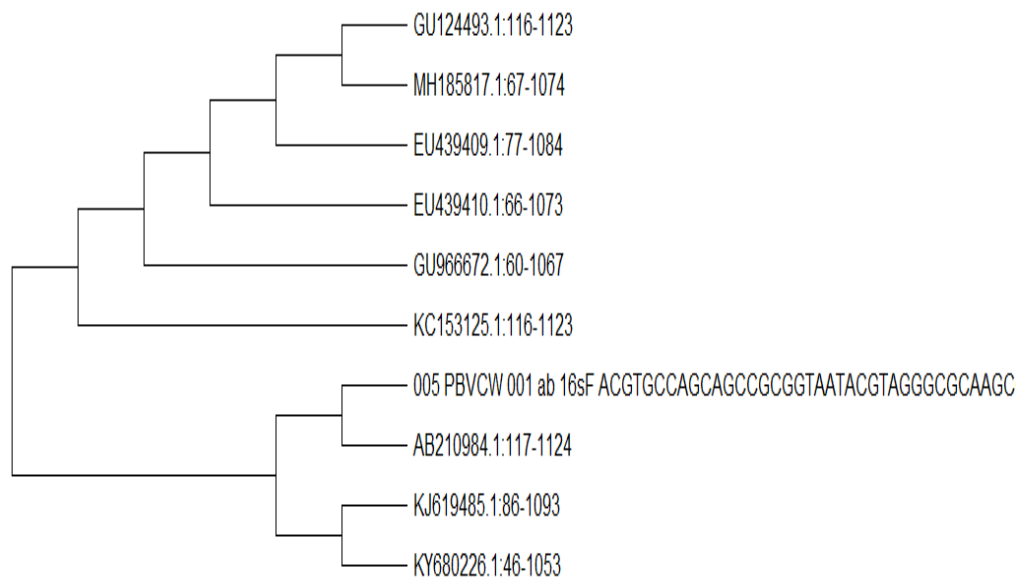


Figure.4 Thin layer chromatography



In conclusion, the study successfully isolated and identified *Micrococcus luteus* as a producer of yellow pigment from Puzhal Lake soil/sediment. The pigment was extracted using methanol, showed a prominent UV-visible absorbance around 400 nm and was partially separated by TLC.

The DPPH assay showed increasing antioxidant activity with increasing extract concentration, reaching 72.2% at 50 mg/ml. These findings indicate the potential of *M. luteus*-derived yellow pigment as a natural microbial pigment with possible biotechnological applications. More advanced chemical characterization, optimization and scale-up studies are required before practical or commercial applications can be established

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Ethics statement

Not applicable.

Conflict of interest

The authors declare no competing interests.

Author Contributions

Suresh Kumar K: sample processing, molecular assays, sequence analysis and manuscript preparation. Mrs. Priyadharsini CV: supervision, scientific guidance and manuscript review.

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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