Nep J Environ Sci (2025), 13(1), 71-77
https://doi.org/10.3126/njes.v13i1.75856
ISSN 2350-8647 (Print) 2542-2901 (Online)

Research Article

Application of Melanin Pigment from Actinobacteria in Textile Industry

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(Received: 22 February 2025; Revised: 25 June 2025; Accepted: 28 June 2025)

Abstract
Actinobacteria are known to produce pigments which has many industrial applications. Melanin is one of the pigments produced by many of the actinobacteria. In this paper, the application of melanin in textile industry is studied. The actinobacteria were isolated from the soil samples. The three isolates were shown to produce melanin which was identified to belong to the genus *Streptomyces* and *Nocardia*. Melanin pigment was extracted from actinobacteria using starch casein, tyrosine and oatmeal media. The production of the melanin pigment was checked on both solid agar and liquid media. The application of melanin pigment was checked in colouring the white cotton fabric. When used for colouring the fabric, the extracted pigment from actinobacteria can be used as dyes and these will be eco-friendly. This study has paved the way for important application of melanin pigment in textile industries.

Keywords: Biological, eco-friendly, economical, environment, pigment

Introduction
Melanin is a pigment with wide variety of industrial applications which is recognized for its remarkable ability to absorb a variety of radiations. The versatility of the pigment has led to reports of its use as an antioxidant and radical scavenger, a photo-protector that effectively absorbs and dissipates solar radiation as heat, chelator of metals and a binding agent for organic compounds and organic semiconductors (Tran-Ly et al., 2020). Melanin acts as a functional additive or coating that has recently emerged in materials science and green technology as a way to significantly enhance the performance of conventional materials in a variety of applications. Actinobacteria are known to produce melanin pigment in the culture media (Dastager et al., 2006). Melanin pigments normally have a brown or black colour. *Pseudomonas stutzeri, Gliocephalotrichum simplex*, *Rhizobium* sp., *Brevundimonas* sp., *Aspergillus fumigatus*, *Bacillus safensis*, *Streptomyces lusitanus*, *Streptomyces kathirae* etc. are among the species that have been utilized to produce melanin pigment. These studies suggest that pigment from microorganisms has gained importance due to their wide applications. Microbial melanin is a valuable source of natural melanin due to the benefits of employing microorganisms to manufacture melanin, such as the lack of seasonal growth restrictions, economical and eco-friendly (Tran-Ly et al., 2020).

The microbial pigments for use in textile industries will be eco-friendly and economical in comparison to the natural pigments. Also, the production of these pigments from microorganisms is easy and it will be in large quantity. Melanin pigments are now converted into useful materials in many fields of green technology, materials science, biomedicine, cosmetics and environmental remediation due to the emergence of new knowledge and technologies (Singh et al., 2025). Melanin is a natural "sunscreen" which absorbs a broad range of ultraviolet (UV)-visible light. This pigment also functions as an effective UV filter and antioxidant. The bioavailability, biocompatibility and biodegradability of microbial melanin are additional benefits that make it an attractive choice for biomedical applications, such as implantable devices. Melanin is used in an alternate context to create ecologically friendly silver nanostructures (Kiran et al., 2014). The food and health industries may benefit from the utilization of these melanin-mediated silver nanostructures, which exhibit broad-spectrum antibacterial activity against food pathogens. Melanin has several uses in dermatology and cosmetics, including hair colouring and sunscreen. Melanin has the potential to function as metal chelators, which has use in the environment. The melanin-based composites are found to remove 94% lead [Pb (II)] from water systems by combining fungal melanin with other polymers including polycaprolactone and polyurethane. Microbial melanin research has not yet reached its full potential in the era of the switch to sustainable materials (Ribera et al., 2019). There is limited experimental work on directly applying actinobacterial melanin to cotton textiles under lab conditions, particularly when comparing media-specific pigment yields (Ribera et al., 2019).

Materials and Methods
Sample Collection
The soil samples were collected from the top layer 8.0 to 10.0 cm from Morwadi, Nehru Nagar, and Bhosari, Pune, Maharashtra in clean plastic bags and labelled properly. All the soil samples were kept at 4 °C for further studies.

Isolation of Actinobacteria
Each of the three soil samples (1.0 g) was suspended in 9.0 ml of sterile distilled water (DW) and diluted to 10^{-5}. The dilutions (0.1 ml) of 10^{-3}, 10^{-4}, and 10^{-5} was spread

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on nutrient agar (NA) [NA (g/lit): beef extract - 3.0, peptone - 5.0, sodium chloride (NaCl) - 8.0, agar - 15.0, distilled water (DW) - 1000 ml] plates. The plates were kept for incubation in the incubator at 37 °C for four days and checked for actinobacteria colonies based on the morphology. The isolates were further purified and maintained on NA slants.

Characterization and Identification of the Isolates

The isolates were characterized and identified based on morphological characters, production of oxidase and catalase enzymes (York et al., 2004) and biochemical tests such as sugar fermentation (Verma *et al.*, 2022). The isolates were compared using Bergey's Manual of Determinative Bacteriology (Holt, 1993).

Optimization Studies of Pigment Production

Media optimization

The three isolates (M1, M3 and M4) were streaked on different media viz., starch casein [starch casein (g/lit): soluble starch - 10.0, casein - 0.3, NaCl - 2.0, potassium hydrogen phosphate (K_2HPO_4) - 2.0, calcium carbonate ($CaCO_3$) - 0.02, ferrous sulphate ($FeSO_4.7H_2O$) - 0.01, potassium nitrate (KNO_3) - 2.0, pH - 7.4, agar - 15.0, DW - 1000 ml], tyrosine [tyrosine agar (g/lit): L-tyrosine - 5.0, peptone - 5.0, beef extract - 3.0, pH - 7.4, agar - 15.0, DW - 1000 ml] and oatmeal [oatmeal agar (g/lit): rolled oats - 30.0, pH - 7.4, agar - 15.0, DW - 1000 ml] agar medium. The plates were incubated in the incubator at 37 °C for seven days to observe which medium gave the best optimum pigmentation.

Temperature Optimization

The three isolates (M1, M3 and M4) were inoculated in starch casein, tyrosine and oatmeal broth in 100 ml conical flask and incubated for seven days at 37 and 50 °C respectively to observe which broth gave the best optimum pigmentation.

Extraction of the Pigment

The extraction of pigment from isolates M1, M3 and M4 was done by using chloroform as the solvent. For control, water was used as the solvent. The pigment was scrapped off from the plates in a clean crucible and solvent was added to it. It was then kept for 24 hr to evaporate the solvent.

Quantitative Estimation of the Pigment

For quantitative estimation of the pigment, colorimetric method was used. The three isolates M1, M3 and M4 were inoculated respectively in starch casein, tyrosine and oatmeal broth in 250 ml conical flask. All the flasks were kept in the incubator at 37 °C for seven days. After seven days, the broths were centrifuged at 5000 rpm for 20 min and supernatant was collected in clean test tubes. The absorbance was noted at 540 nm using the colorimeter (Benito-Martinez et al., 2020).

Application of the Pigment Extracted from Actinobacteria

The isolates M1, M3 and M4 were used to extract the melanin pigment, which was then collected in crucibles. It was utilized to find a novel application, which was to use it as a dye to color a white cotton fabric (4.0 × 4.0 cm) using various pigment extracts which were collected from starch casein, tyrosine and oatmeal agar medium. The white cotton fabric was washed with water properly initially, then dipped in chloroform to solubilize the pigment, covered with aluminum foil with holes made to allow the solvent to evaporate and left for 24 hr in the crucibles which contained pigment extracts (Kim & Jang, 2010). For control, instead of chloroform, the white cotton fabric was dipped in water and the same procedure was followed.

Results and Discussion

Three isolates, M1, M3 and M4 which were chosen based on their morphological characteristics and biochemical tests were identified as actinobacteria. The isolates M1, M3 and M4 were Gram positive rods and non-motile. The isolates were able to ferment glucose, fructose, lactose, sucrose and maltose sugars. The isolates M1 and M3 were identified as the Genus *Streptomyces* and M4 as *Nocardia* (Table 1).

Growth on Different Media and Broth

Starch Casein Agar Media and in Broth

Pigmentation production was seen on starch casein agar plates by the three isolates M1, M3 and M4 (Fig. 1). The colonies' centers were observed to contain a black color pigment. All the three isolates M1, M3 and M4 showed yellow-orange pigmentation in the starch casein broth at 37 °C (Fig. 2). The isolates M1, M3 and M4 did not show pigmentation at 50 °C in starch casein broth.

Figure 1 Growth of the isolates M1, M3 and M4 on starch casein agar media

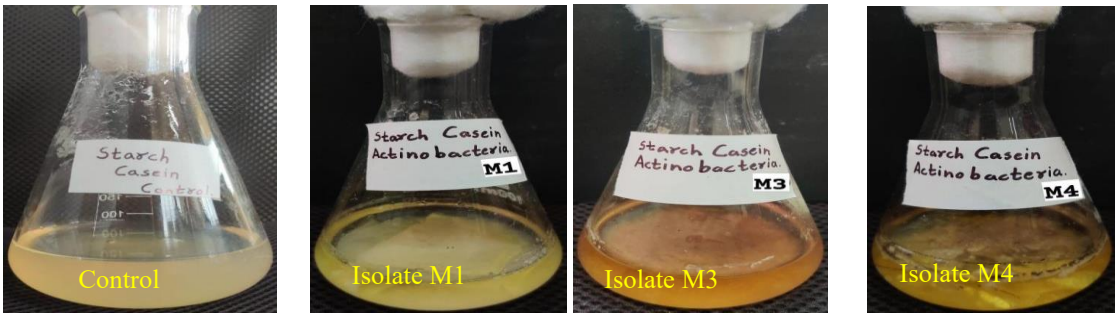


Figure 2 Growth of the isolates M1, M3 and M4 in starch casein broth compared with control

Table 1. Morphological characteristics of the isolates

Morphological characteristics	M1	M3	M4
Size (mm)	2.0	2.0	2.0
Shape	circular	circular	circular
Color	white	white	white
Elevation	raised	raised	raised
Margin	regular	regular	regular
Transparency	opaque	opaque	opaque
Gram character	Gram positive rod	Gram positive rod	Gram positive rod
Motility	non-motile	non-motile	non-motile
Enzymes			
Oxidase	-	-	-
Catalase	+	+	+
Sugar fermentation			
Glucose	A + G	A + G	A + G
Fructose	A + G	A + G	A + G
Lactose	A + G	A + G	A + G
Maltose	A + G	A + G	A + G
Sucrose	A + G	A + G	A + G

A: Acid; G: Gas; -: Negative; +: Positive

Growth on Tyrosine Agar Media and in Broth

The three isolates M1, M3, and M4 were observed to show pigmentation on tyrosine agar plates. Brown-black colour pigmentation was seen diffused in the agar by the colonies (Fig. 3). The isolates M1, M3 and M4 showed brown-black pigmentation in tyrosine broth at 37 °C (Fig. 4). The isolates M1, M3 and M4 did not show pigmentation at 50 °C in tyrosine broth.

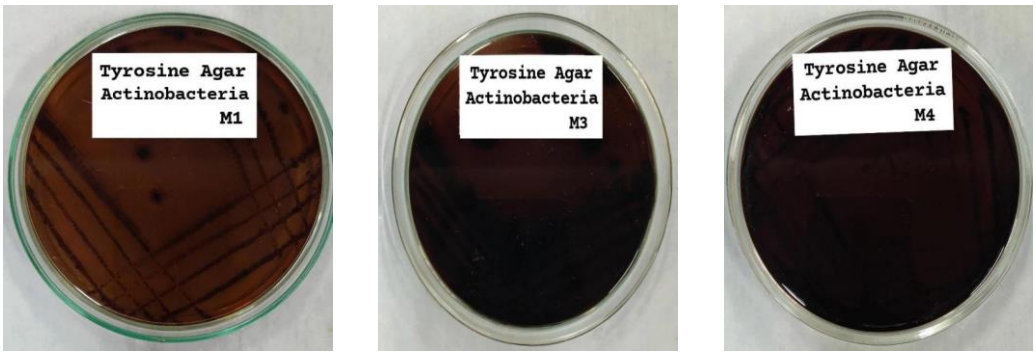


Figure 3 Growth of the isolates M1, M3 and M4 on tyrosine agar media

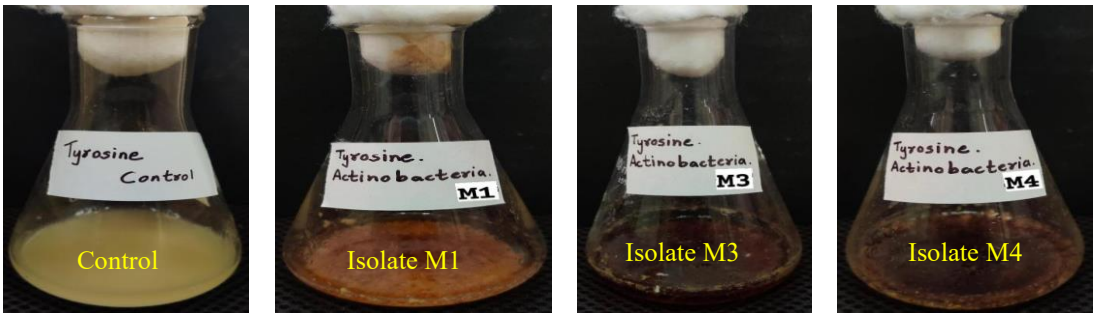


Figure 4 Growth of the isolates M1, M3 and M4 in tyrosine broth compared with control

Growth on Oatmeal Agar Media and in Broth

Only isolates M3 and M4 showed yellow colour pigmentation on oatmeal agar plates after incubation at 37 °C for three days (Fig. 5). After the incubation period was completed, it was observed that the isolates M3 and M4 showed yellow pigmentation in oatmeal broth at 37 °C (Fig. 6). The isolates M1, M3 and M4 did not show pigmentation at 50 °C in oatmeal broth. The dry weight of pigment from isolates M1, M3 and M4 was 0.35, 0.38 and 0.25 g, respectively.

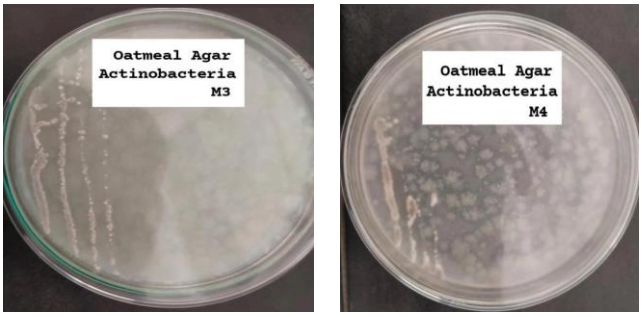


Figure 5 Growth of the isolates M3 and M4 on oatmeal agar media

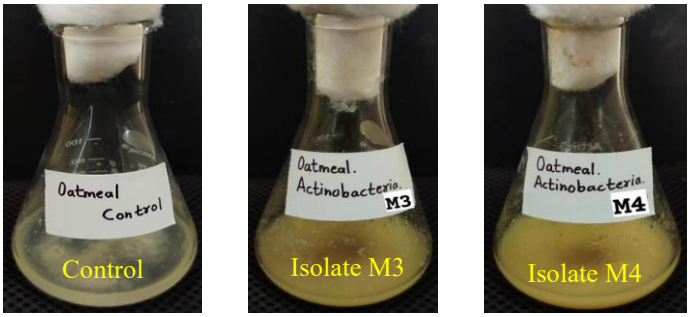


Figure 6 Growth of the isolates M3 and M4 in oatmeal broth compared with control

Figure 7 Pigment extracted from the isolates M1, M3 and M4 using starch casein agar medium

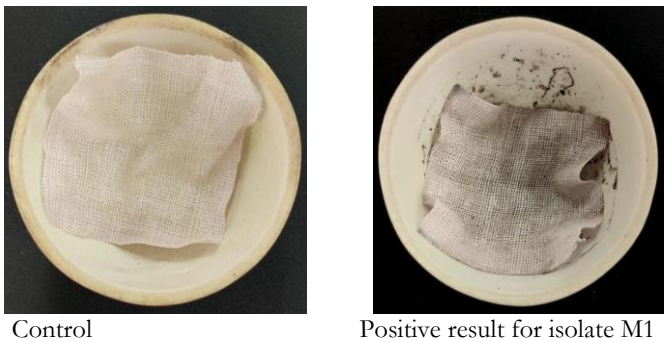
Figure 8 Pigment extracted from the isolates M1, M3 and M4 using tyrosine agar medium

Figure 9 Pigment extracted from the isolates M3 and M4 using oatmeal agar medium

Application of Melanin Pigment Extracted from Actinobacteria

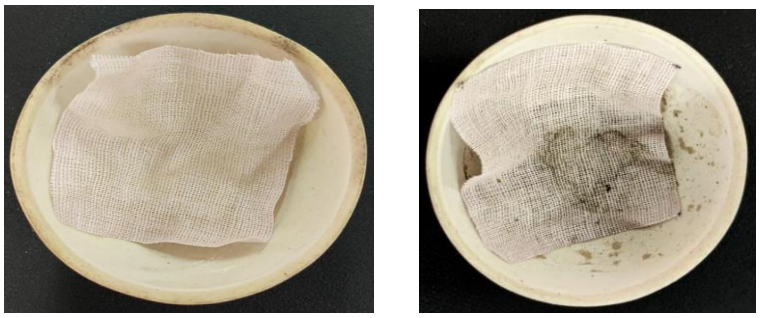
Cotton fabrics (4.0 x 4.0 cm) kept in various crucibles containing pigment extracted from starch casein (Fig. 7), tyrosine (Fig. 8) and oatmeal agar plates (Fig. 9) were observed to see if they had successfully dyed after 24 hr. The pigment extracted from the starch casein agar plate of isolate M1 exhibited positive results (Fig. 10). The pigment extracted from the isolates M3 and M4 on starch casein agar plates did not dye the white cotton fabrics. The results were positive for the pigment extracted from the isolates M1, M3 and M4 on tyrosine agar media plates (Fig. 11) and positive for the pigment extracted from the isolates M3 and M4 on oatmeal agar media plates (Fig. 12).

The yield of melanin pigment extracted from bacteria is more compared to melanin from fungi (Mohana et al., 2020). There is a report on use of melanin pigment in dyeing of wool fabrics (Amal et al., 2011). Also, there is a report on industrial application of melanin pigment (Ghattavi et al., 2022). There is a report on melanin-coated cotton with UV protection and good stability (Pakdel et al., 2022). Also, there is a study on pigments from bacteria for use in textile industry (Thomas et al., 2021). There is a review on use of natural pigments as colorants in cosmetics (Mohana et al., 2020).



Control Positive result for isolate M1

Figure 10 Application of pigment on cotton fabrics extracted from the isolate M1 using starch casein agar medium



Control Positive result for isolate M1

Positive result for isolate M3 Positive result for isolate M4

Figure 11 Application of pigment on cotton fabrics extracted from the isolates M1, M3 and M4 using tyrosine agar medium

Control Positive result for isolate M3 Positive result for isolate M4

Figure 12 Application of pigment on cotton fabrics extracted from the isolates M3 and M4 using oatmeal agar medium

Conclusions

Actinobacteria-produced pigment exhibits great potential for diverse industrial applications in cosmetics, food, and pharmaceuticals. Extensive research has explored pigment production and optimization from species like *Streptomyces* and *Nocardia*, with demonstrated success in dyeing white cotton fabric—highlighting its industrial relevance. The isolation of actinobacteria and extraction of melanin pigment confirm the feasibility of microbial melanin production. However, further optimization is needed to enhance yield, expand applications, and conduct FTIR characterization for deeper analysis. This natural pigment offers a sustainable, cost-effective, and eco-friendly alternative for industries, particularly textiles, where its stability and performance on blended fabrics require further investigation. Melanin from actinobacteria represents a groundbreaking biological solution with significant promise for revolutionizing dyeing processes, paving the way for innovation in sustainable industrial practices.

Author Contributions: RS and CM have performed the experiments. AG has supervised the work, prepared and edited the manuscript.

Conflicts of Interest: The author declare there is no conflict of interest.

Data availability: The data of the current study is accessible upon reasonable request from the corresponding author.

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