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Isolation and Characterization of Melanin Producing Bacteria from Compost Soil

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ABSTRACT
In this study microorganisms that produce melanin were isolated from compost soil on a nutrient agar medium enriched with L- tyrosine. Gram staining and biochemical testing were used to aid with the identification. To characterize microbial strain MALDI-TOF MS and MALDI Biotyper database were used. Melanin production activity was studied in nutrient broth by visual perception. In the present study optimization parameters of melanin production were studied. The optimum conditions for melanin production were pH 7, L- tyrosine 0.05 gm/ml, and temperature 32°C. Nutritional factor peptone, glucose, and yeast extract are widely chosen as carbon and nitrogen sources. The over-righteous addition of L-tyrosine enhances melanin production. After 48 hours of incubation, the second day showed the maximum tyrosine activity. By centrifuging for 15 minutes at 5000 rpm, melanin was extracted. FTIR samples with melanin concentrations used to detect melanin. Staphylococcus aureus, Salmonella Typhimurium, Klebsiella pneumoniae, Proteus vulgaris and Escherichia coli were examined for melanin's antimicrobial properties.
Keywords: *Melanin, L- tyrosine, compost, U.V spectroscopy, MALDI-TOF MS, MALDI Biotyper, FTIR, antimicrobial*

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INTRODUCTION

Melanins are the naturally occurring pigments found in most microbes, plants, and animals [1]. They are the high molecular weight, darkly coloured, negatively charged pigments that result from the polymerization of phenolic and/or indolic chemicals. The amorphous structure of these complex polymers prevents them from being soluble in either aqueous or organic solutions. They demonstrated resilience to concentrated acids and are vulnerable to oxidizing agents' bleaching [2]. They are essential for defense and protection processes that increase the organisms' ability to compete and survive [3]. Melanin is well recognized for its ability to absorb light of all wavelengths, with an optimal absorbance at the UV range [4], which guards against photo-induced harm. As a result, it is utilized to create bioplastics and photo-absorbing optical lenses. In addition to sun protection, it has a variety of biological functions, including radical scavenging, antioxidant, anti-inflammatory, anti-tumor, and immune-stimulating agents [5, 6].

Considering the increasing demand and potential uses for melanin pigment there is a need to conduct studies on the production of melanin from microorganisms Numerous bacteria, including Bacillus species, which are well documented for their capacity to produce pigment in a variety of stressful situations, have been reported to produce melanin [4], [7]. Economic considerations have a role in the substrate choice for melanin synthesis. For instance, costly substrates have been employed in the past to produce melanin in high yields, including NCM media [4], LB (Luria-Bertani) media [7], minimum media supplemented with L-tyrosine [8], amino acid-enriched tryptone broth agar [9], and others [10], [11]. Due to the cost-effectiveness and practicability of the melanin synthesis process, it is essential to select economically viable substrates and optimize the important variables.[12]

In this study, melanin-producing bacteria were isolated from compost soil. The strain was grown on a Nutrient agar supplemented with L-tyrosine medium to produce melanin. The purified and characterized melanin was examined for its antimicrobial ability.

MATERIAL AND METHODS

Sample Collection

Melanin-synthesizing bacteria were isolated primarily from gathered compost samples utilizing a medium consisting of Nutrient agar added with L-tyrosine. For 15 min at 15 psi (121°C) the glassware and media were autoclaved proceeding the experiment; these plates of agar containing media were kept

for incubation for 4 days at 37 °C Enrichment and Isolation of melanin synthesizing bacteria. The collection of Compost soil samples was done from the village of Satara, Maharashtra. On the selective medium, the streaking of the sample was done – tyrosine containing Nutrient agar medium as the melanin pigment is synthesized by the L-tyrosine oxidation. Separation of selected colonies was done for the characterization of culture.

Isolation and Screening

The media utilized for the cloaking of the species of melanin producer is Peptone iron media (gm/liter), sodium thiosulphate-0.8, Agar-32, peptone-20, pH-7, Ferric ammonium citrate-0.5, K2HPO4-1. This medium was Inoculated with a 1 gm sample of soil in the flask (250 ml) including sterile peptone iron media (100 ml). At 37°C in static for 5 days the medium which was inoculated was incubated. Post period of incubation the media was diluted serially and on tyrosine agar plate was plated. The plate of Cysteine casein agar (inoculated) was consisting of 1g of agar (32g), sodium nitrate (10g), casein hydrolysate (25g) L-tyrosine (1g). For 5-6 days at 30 °C incubation of plates was done. Post period of incubation the cysteine casein Agar plates was checked for diffusible pigment showing colonies and pure culture was obtained by streaking on the plate of Tyrosine Agar. Biochemical and morphological characteristics of isolated bacteria were studied. All the colonies isolated on the nutrient agar medium were checked for gram nature and colony characteristics.

Production and purification of melanin

For the preparation of inoculum Nutrient broth NaCl (5 g/L), beef extract (3 g/L) and peptone (5 g/L) was used. Around 108 CFU/mL (10 µL) suspension of culture was add up to the nutrient broth supplemented with tyrosine. Nutrient broth added with L- tyrosine was utilized for the preparation of inoculums and the production of pigment. The addition of bacterial cultures was done in 200 ml nutrient broth. The incubation was done at 40°c in the incubator. After incubation for 10-15 days until the dark pigmentation of the liquid medium and nearly opaque appearance, the centrifugation of the medium was done for 15 min at 5000 rpm to separate the cells and supernatant (broth). The separation of the solid pellet was done which was suspended in distilled water. Again centrifugation was done to separate the supernatant.

Antibacterial activity of melanin pigment

Staphylococcus aureus, *Salmonella Typhimurium, Klebsiella pneumoniae, Proteus vulgaris*, and *Escherichia coli* were swabbed on a Muller Hinton agar, and 3 µl of pigment extraction was poured in the well and incubated at 37 °C for 24 hours to assess the antibacterial activity.

Parameters optimization on melanin production

By altering the pH of the production medium in the range of 3 to 9 using 1 N NaOH and 1 N HCl after sterilization, the impact of the initial pH of the medium on melanin formation was investigated. By incubating the production medium with 5% of the inoculum at various temperatures, including 27°C, 37°C, and 60°C for 5–6 days under static circumstances, the effect of temperature on melanin formation was examined. The study examined how several simple and complicated carbon sources, such as lactose, sucrose, and glucose, affected the melanin-producing medium. The flasks were incubated at 37°C for 5–6 days under static circumstances after being infected with 5% of the inoculum. After 5 days, the samples were taken, and the culture solution was centrifuged for 20 minutes at 5000 rpm. The melanin was obtained from the cell-free supernatant. After 5 days, the samples were retrieved, and they served as a source for the production of melanin.

Characterization and analytical methods

The microbial strain was characterized by using MALDI-TOF MS and MALDI Biotyper database. Structure binding capacity, affinity and sites of metal ions in melanin were identified by FTIR.

RESULTS

Antibacterial activity of melanin pigment

By using the agar well diffusion method, the antibacterial activity of melanin was tested against 24-hour-old cultures of Escherichia coli, Staphylococcus aureus, Salmonella Typhimurium, Klebsiella pneumoniae, and Proteus vulgaris. By measuring the growth inhibition zone surrounding the wells on Muller-Hinton agar plates were 1 cm-diameter well, the antibacterial activity of the melanin pigment was ascertained. On the agar's surface, bacterial cultures were swabbed, and melanin pigment was applied to the wells.

Parameters optimization on melanin production

In a lab environment, the effects of pH, temperature, and sugar supply were investigated.

The amount of melanin created in a 100 ml flask during an experiment at experimental circumstances (pH 7, 37°C, and sugar glucose) was at its highest. Each of these variables, including pH, temperature, and sugar, had a considerable impact on the formation of melanin and were each shown as having a major effect. Nutrient agar supplemented with l-tyrosine seemed to be the best medium for melanin formation

under normal circumstances. At a pH of 7, a temperature of 37°C, and glucose as the sugar, an intensive colouring of the medium to straw colour to brownish black was seen within 24 hours.

FTIR analysis

The interpretation of the metal ion binding capacity, affinity, and sites in melanin depend on IR spectroscopy demonstrates high absorptions for both acquired bacterial melanin and conventional melanin at 3500 cm-1, 1700 cm-1, and 1300 cm-1. The signals between 3600 and 2800 cm-1 are attributed to the carboxylic, phenolic, and aromatic amino functional groups of indolic and pyrrolic systems stretching vibrations of (O—H and N—H).[13]

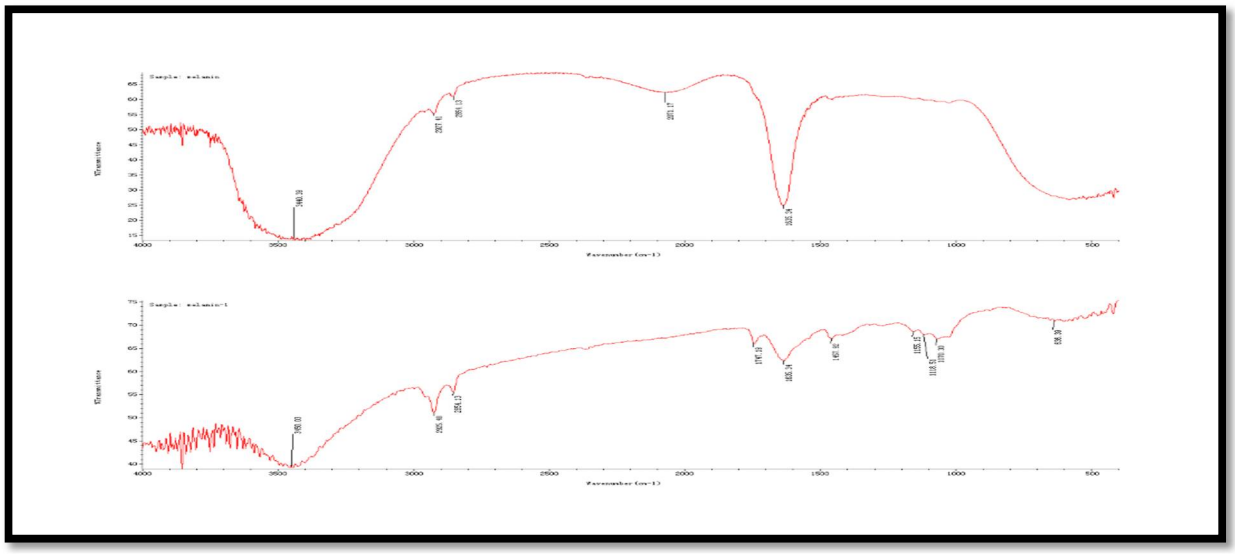

Fig.1. FTIR analysis

MALDI-TOF MS and MALDI Biotype Database analysis

MALDI-TOF MS and MALDI Biotype Database method identifies that organism is *pseudomonas aeruginosa*

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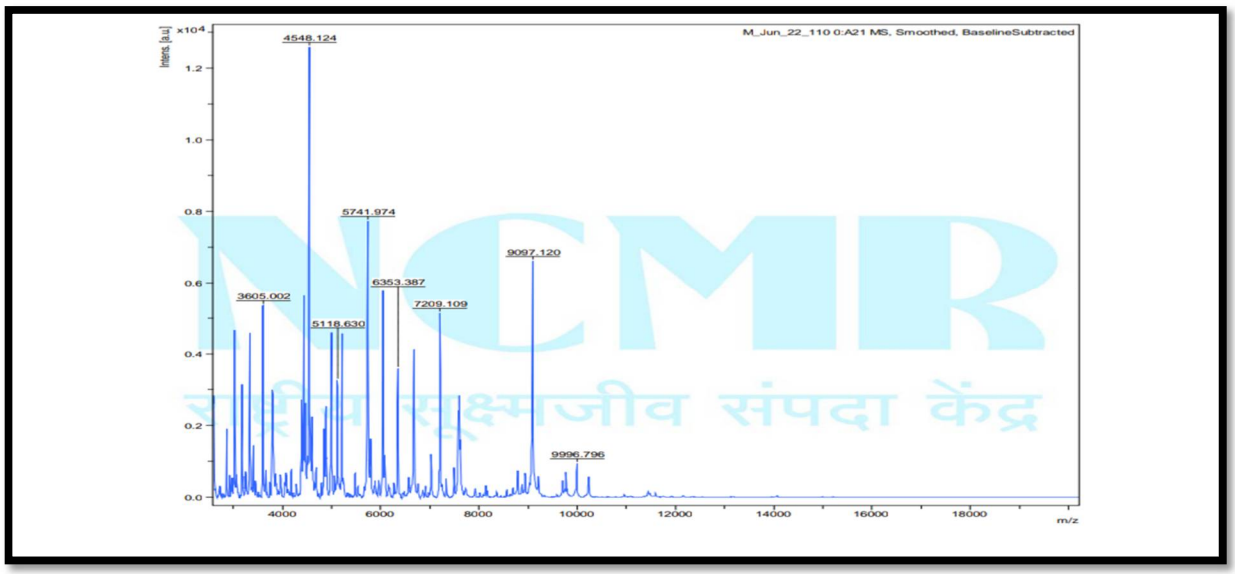

Fig.2. MALDI-TOF MS spectra of Microbial strains indicating the protein profile (2-20KDa)

Table 1 Comparison with the Bruker taxonomy database using Biotyper 3.1 software the test strain

Analyte (PRN)	Sample	Organism (best match)	Score	Organism (second match)	Score
M_Jun_22_110	TB1	*Pseudomonas aeruginosa*	2.503	*Pseudomonas aeruginosa*	2.418

Table 1 Comparison with the Bruker taxonomy database using Biotyper 3.1 software the test strain.

CONCLUSION

From the results of this study, it is concluded that the optimization of vital nutritional parameters significantly enhanced the yield of melanin. The melanin obtained in this study has photo protective, antibacterial activity and metal binding capacity that are significant from an economic standpoint. The melanin obtained in this study exhibits photo protective, antimicrobial, and metal-binding properties that are significant from a business standpoint. Therefore, the isolated pseudomonas aeruginosa from compost can serve as prospective sources for the synthesis of melanin

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