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STUDIES ON MELANIN PIGMENT PRODUCED BY *PHAEOSPHAERIOPSIS MUSAE* ISOLATED FROM SALINE WATER OF LONAR LAKE IN WEST VIDARBHA REGION

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ABSTRACT

The aim of this study was to isolate a fungus producing melanin pigment, its morphological, biochemical and molecular identification, studying effects of parameters like ph and temperature on the isolate and its melanin producing ability, extraction of melanin pigment from the isolate, chemical characterization of the melanin pigment and performing analytical techniques to confirm the pigment as melanin. A fungus producing melanin pigment was isolated from a saline water sample of lonar lake using potato dextrose agar medium enriched with L-tyrosine amino acid. It was identified as *Phaeosphaeriopsis musae* using molecular technique of partial internal transcribed spacer (ITS) region analysis. It was found to survive at pH-3 and produce melanin at pH-5 to 8 but could only grow and produce melanin at 30°C. The extracted crude melanin pigment was water soluble and techniques like Ultraviolet-Visible spectrum analysis and Fourier Transmission Infra-red spectrum analysis proved that the pigment extract is melanin.

KEYWORDS: melanin producing fungus, *Phaeosphaeriopsis musae*, saline water, lonar lake.

INTRODUCTION

Many fungal species produce melanin, a biologically important pigment. Melanin is found throughout nature, often providing a protective role such as from ultraviolet radiation (Helene C. Eisenman and Arturo Casadevall, 2012). Melanin is nearly a ubiquitous pigment synthesized by living organisms in the course of hydroxylation and polymerization of organic compounds. Melanins have immense application potentials in the field of agriculture, cosmetics and pharmaceutical industries (photoprotection and mosquitocidal activity isolated from *Streptomycete*). Several types of melanin have been described in bacteria, fungi and animals; eumelanins, phaeomelanins, allomelanins and pyomelanins.

Eumelanins are formed from Quinines and free radicals. Fungal melanins are complex pigments which are produced by two different synthetic pathways, known as the DHN (1, 8-dihydroxynaphthalene) and L-DOPA (L-3, 4-*dihydroxyphenyl-alanine*) pathways, depending on the species. The DHN pathway of melanin biosynthesis is very common in the fungi kingdom. (M Kalaiselvam et.al, 2013).

Some of the fungi known to produce melanins are *Cryptococcus neoformans*, *Sporothrix schenckii*, *Sepia officinalis*, *Aspergillus niger*, *Penicillium marneffei*, *Paracoccidioides brasiliensis Histoplasma-capsulatum*, *C. neoformans* (Korumilli Tarangini and Susmita Mishra, 2013).

MATERIAL AND METHODS

Chemicals used

Potato dextrose agar media, L-tyrosine and all other media required for this study were purchased from HiMedia chemicals, Mumbai, India. Ethanol, HCL and all other chemicals used were of analytical reagent grade throughout the study. Ultrapure water used for the experiments and aseptic conditions were maintained wherever necessary.

Screening, isolation and identification of the melanin producing fungi

A saline water sample from Lonar lake in west Vidarbha region of Maharashtra, India was collected. Its geographical location is 19.978909N 76.511468W. By pour plate technique, the isolate was screened using potato dextrose agar medium enriched with amino acid L-tyrosine which is the initial precursor of melanin biosynthesis pathway. As it was able to utilize the l-tyrosine in the media, it was selected for further isolation. The same media was used to isolate the pure culture and culture maintenance. The isolate was named

KRDF6 temporarily. The media and the glassware were autoclaved at 15 psi (121°C) for 20 min prior to the experiment. The incubation was done at 28-30°C for 6 days. The fungus was identified by performing morphological, biochemical and molecular technique (partial ITS region analysis).

Optimization studies

The effect of different parameters namely pH and temperature conditions on the fungal isolate and on its melanin production ability were studied. The pH values taken into consideration in this study were 3, 5, 7, 8, 10, 12 and 14. The temperatures studied were 5°C, 15°C, 28°C, 37°C, 45°C and 55°C.

Pigment production and extraction

The fungal culture was grown on potato dextrose agar plates enriched with L-tyrosine for about two weeks at 28-30°C. The extraction process is explained in the flowchart in detail.

Chemical characterization of crude melanin extract

The solubilities of the crude melanin pigment extract in distilled deionized water, 1M NaOH, ethanol, acetone, chloroform, were checked. Reactions with oxidizing agents such as 6% sodium hypochlorite (NaOCl) and 30% hydrogen peroxide (H_2O_2) were determined and its precipitation in 3N HCl was observed.

UV-visible spectroscopy and FTIR spectrum analysis

UV-visible spectrum of the melanin extract obtained from the fungal isolate was analyzed from 200nm to 900 nm using UV-Visible spectrophotometer (Systronics-118 model). The FTIR analysis of pigment was carried out after mixing with IR grade KBr (1:10) using FTIR spectrophotometer (Shimadzu IR affinity1).

RESULTS

The morphological observations and biochemical test results are shown in table no.1. and Fig.1 shows the fungal isolate KRDF6.

Figure 1: Fungal isolate KRDF6.

Table 1: Morphological and Biochemical characteristics of KRDF6 strain.

Characteristics	**Results**
Colony form	Filamentous
Colony elevation	umbonate
Colony margin	filiform
Colony surface	smooth
Colony opacity	Opaque
Color (pigmentation)	Blackish
***Enzyme Utilization*:**	
Oxidase	-
Catalase	+
Amylase	+
Gelatinase	+
Urease	-
Caseinase	-

The molecular identification of the fungal isolate was done using partial ITS region sequencing technique and was identified as closest neighbour of *Phaeosphaeriopsis musae.* Its phylogenetic tree is shown in fig. 2.

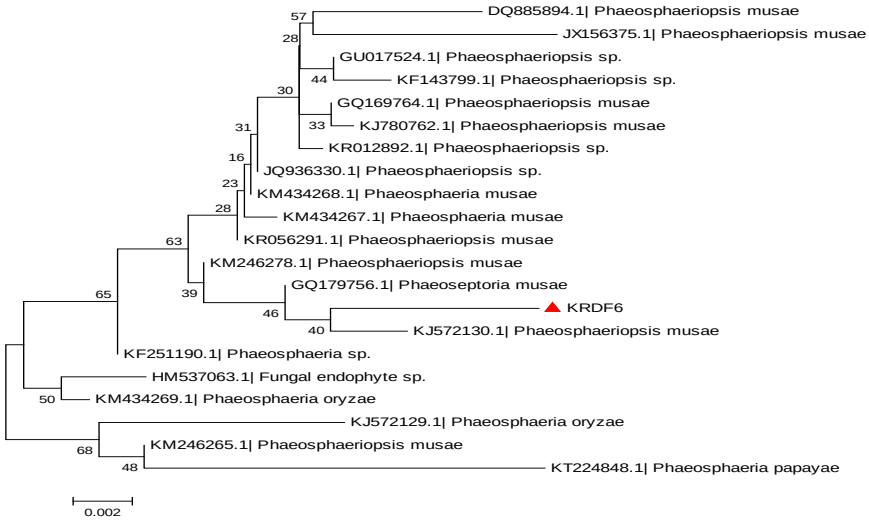


Figure 2: Phylogenetic tree for KRDF6 on basis of partial ITS region analysis.

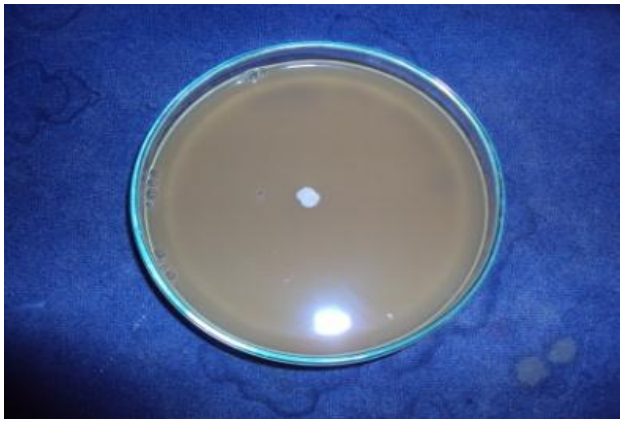


Figure 3: Effect of pH-3 on growth of *Phaeosphaeriopsis musae* and its melanin production.

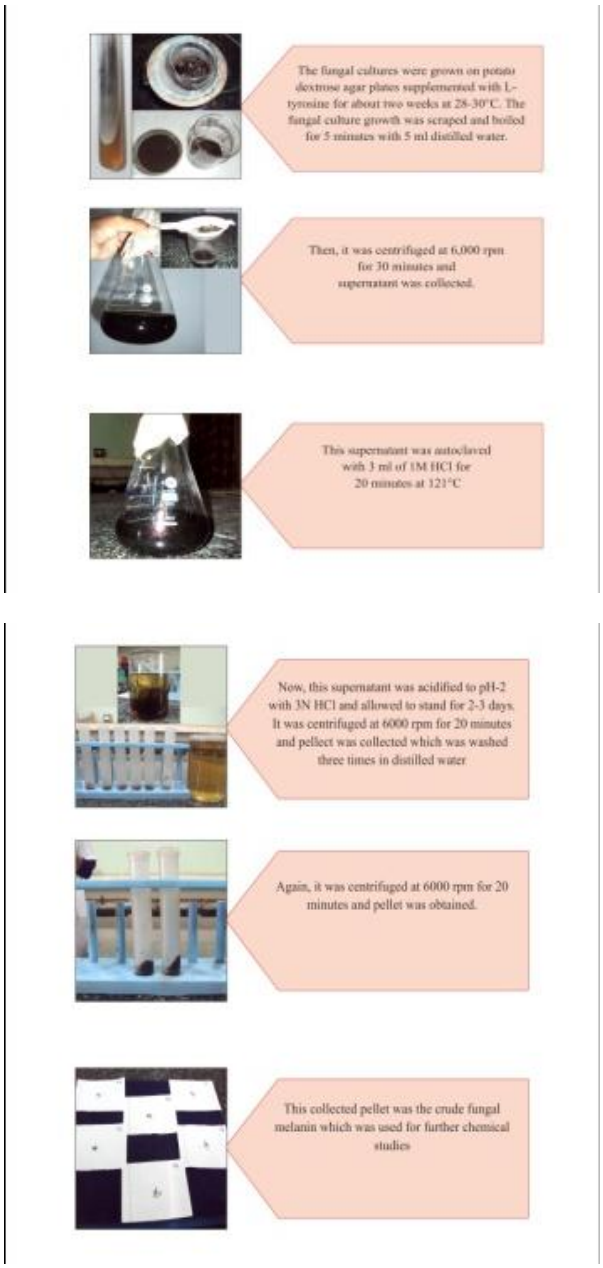
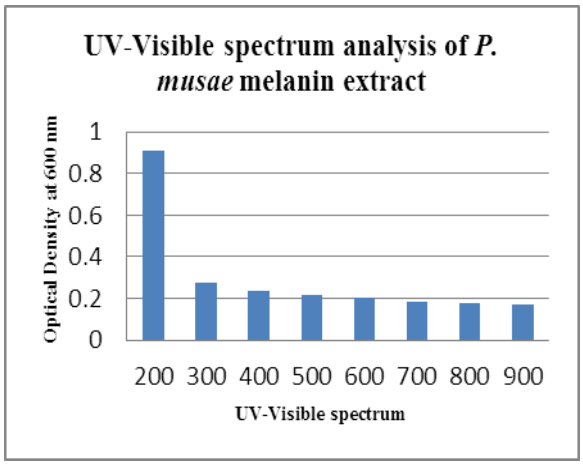


Figure 4: flowchart for extraction of melanin extract from *Phaeosphaeriopsis musae*

Table 2 - Chemical characterization of crude melanin extract from *Phaeosphaeriopsis musae*

Characteristics	*P. musae* crude melanin extract
Solubility	
Water	Soluble
Ethanol	soluble
Chloroform	Insoluble
Acetone	Insoluble
1M NaoH	Soluble
Color	Blackish brown
Precipitation in 3N HCl	Precipitated readily
Reaction with oxidizing agent H2O2	Decolorized
Reaction with NaOCl	Decolorized



Graph 1: UV-Visible spectrum of P. musae melanin extract.

The UV-visible spectrum of P.musae melanin extract was compared with earlier reports and found the same pattern i.e. higher absorption in the UV region of 200 nm and decreasing towards the visible region.

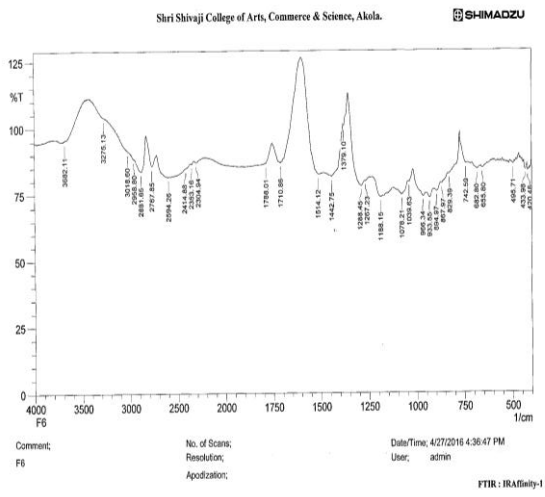


Figure 5: FTIR spectrum analysis of P. musae melanin extract.

CONCLUSION

The fungal isolate, *Phaeosphaeriopsis musae*, was isolated from an environmental sample and basic media was used to isolate and culture maintenance. *P. musae* could only survive at pH-3 but could also produce melanin from 5 to 8. *P. musae* was found to be very specific to grow and produce melanin only at 30°C. *P. musae* was found to be a slow growing fungus. It was soluble in water, ethanol and 1M NaOH but insoluble in chloroform and acetone; readily precipitated in 3N HCl and decolorized while reacting with H_2O_2 and NaOCl. UV-Visible spectrophotometry and FTIR spectrum analysis techniques were used to confirm the extracted pigment as melanin.

REFERENCES

1. Helene C. Eisenman and Arturo Casadevall. 2012. Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol., 2012 February; 93(3): 931–940.
2. M. Helan Soundra Rani, T. Ramesh, J. Subramanian, M. Kalaiselvam. 2013. Production and Characterization of Melanin Pigment from Halophilic Black Yeast *Hortaea werneckii*. International Journal of Pharma Research & Review, August 2013; 2(8): 9-17.
3. Korumilli Tarangini and Susmita Mishra. Production, Characterization and Analysis of Melanin from Isolated Marine *Pseudomonas* sp. using Vegetable waste. *Research Journal of Engineering Sciences*, May 2013; 2(5): 40-46.
4. K. Rajagopal, G. Kathiravan and S. Karthikeyan. Extraction and characterization of melanin from *Phomopsis:* A phellophytic fungi Isolated from *Azadirachta indica* A. Juss. African Journal of Microbiology Research, 4 April, 2011; 5(7): 762-766.
5. Sajjan, Shrishailnath, Guruprasad Kulkarni, Veeranagouda Yaligara, Lee Kyoung, and T. B. Karegoudar. Purification and Physiochemical Characterization of Melanin Pigment from Klebsiella sp. GSK. J. Microbiol. Biotechnol., 2010; 20(11): 1513–1520.
6. V. Vasanthabharathi, R. Lakshminarayanan and S. Jayalakshmi. 2011. Melanin production from marine *Streptomyces.* African Journal of Biotechnology, 19 September, 2011; 10(54): 11224-11234.
7. T.L. Doering, J. D. Nosanchuk, W. K. Roberts & A. Casadevall. Melanin as a potential cryptococcal defence against microbicidal proteins. Medical Mycology., 1999; 37: 175–181.
8. Helene C. Eisenman and Arturo Casadevall. Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol., 2012 February; 93(3): 931–940.