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Isolation of melanin producing fungi from air source

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Abstract
Today, fungal melanins isolated from environmental sources are thought to have a tremendous scope. In this study, we have isolated melanin producing fungi from air source. Isolate able to utilize L-tyrosine which was supplemented with potato dextrose medium producing brown to black color pigment was chosen. Its morphological, biochemical and molecular studies were done. The melanin producer was found to be *Aspergillus niger*.

Keywords: melanin pigment, air, L-tyrosine

1. Introduction
Biodiversity boosts ecosystem productivity where each species, no matter how small and tiny, all have an important role to play. Melanin producers include various bacteria, fungi, algae, plants, animals and human beings. Such a vast diversity gives rise to variations in the chemical structure and properties exhibited by different types of melanin pigments. Melanin is multifunctional, providing defense against environmental stresses such as ultraviolet (UV) light, oxidizing agents and ionizing radiation. It contributes to the ability of fungi to survive in harsh environments. In addition, it plays a role in fungal pathogenesis. Fungi may synthesize melanin from endogenous substrate via a 1,8-dihydroxynaphthalene (DHN) intermediate. Alternatively, some fungi produce melanin from L-3,4-dihydroxyphenylalanine (L-dopa). The detailed chemical structure of melanin is not known. However, microscopic studies show that it has an overall granular structure. In fungi, melanin granules are localized to the cell wall where they are likely cross-linked to polysaccharides. Some of the fungi known to produce melanins are *Cryptococcus neoformans*, *Sporothrix schenckii*, *Sepia officinalis*, *Penicillium marneffei*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*. (Helene C. *et al*. 2012) [1].

2. Material & Method
2.1 Chemicals and Sample collection
L-tyrosine and media used for isolation were purchased from Himedia chemicals, Mumbai, India and all other chemicals used were of analytical reagent grade.
A sterile potato dextrose agar medium supplemented with L-tyrosine plate was exposed to air in the working laboratory, Department of Microbiology, Shri Shivaji College, Akola, Maharashtra (India) for 15 minutes.

2.2 Fungal isolation
The air exposed potato dextrose agar plate was incubated at 28°C for 7 days. From the mixed culture plate, fungus able to utilize L-tyrosine in the medium was picked and freshly grown on the same media with same temperature and incubation conditions mentioned above. The pure culture was maintained on the above mentioned agar medium plates and slants.

2.3 Morphological and Biochemical Studies
The morphological characteristics of the fungal colony such as colony form, size, margin, elevation, surface, opacity and color/pigmentation were studied.

Then, the biochemical studies of the fungus were also performed including enzyme tests (oxidase, catalase, urease, caseinase, amylase and gelatinase) and carbohydrate utilization tests (glucose, lactose, fructose, sucrose, mannitol, starch and maltose).

2.4 Molecular identification of melanin producing bacteria

DNA Extraction was carried out using Uniflex DNA Isolation Kit (GeNei, 612117000051730). The DNA isolated from fugal samples was subjected to polymerase chain reaction (PCR) amplification using Biometra thermal cycler (T-Personal 48). Gel electrophoresis was performed using 1.0% agarose (Seakem, 50004L) to analyze the size of amplified PCR product. The size obtained was approx. 600bp for ITS region. The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, AP-PCR-50). It was further sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. The DNA sequences were analyzed using online BLASTn (nucleotide Basic Local Alignment Search Tool) facility of National Center for Biotechnology Information (NCBI).

2.5 Phylogenetic characterization of the Fungus

The BLAST results were used to find out evolutionary relationship of fungus. Altogether twenty sequences, including sample were used to generate phylogenetic tree. The tree was constructed by using MEGA 5 software (Saitou N. and Nei M., 1987; Felsenstein J. 1985 and Tamura K. *et al* 2011) [6-8].

3. Results & Discussion

Table 1: Morphological characteristics of KRDF1.

Characteristic \ Isolate	KRDF1
Colony form	filamentous
Colony size (in mm)	40
Colony margin	filiform
Colony elevation	raised
Opacity	opaque
Surface	rough
Color/Pigmentation	black, non-diffusible

Table 2: Biochemical characteristics of KRDF1.

Tests \ Isolate	KRDF1
Enzyme Utilization:	
Oxidase	-ve
Catalase	+ve
Amylase	-ve
Gelatinase	-ve
Urease	+ve
Caseinase	-ve
Carbohydrate Utilization:	
Lactose	-ve
Glucose	+ve
Sucrose	-ve
Mannitol	-ve
Fructose	+ve
Maltose	-ve
Starch	+ve

A melanin producing fungus was isolated from an air source when grown on potato dextrose agar medium supplemented with L-tyrosine for 4-5 days. The colony capable of producing brown to black colored pigment was picked (labelled temporarily as KRDF1) and was identified as Aspergillus *niger* based on its morphological (Table 1) and biochemical characteristics (Table 2). Later, it was confirmed as according to the phylogenetic identification using 18S rDNA sequencing technique, the closest neighbor of KRDF1 was found to be *Aspergillus niger*. The phylogenetic relationship of this strain is shown in Fig.1.

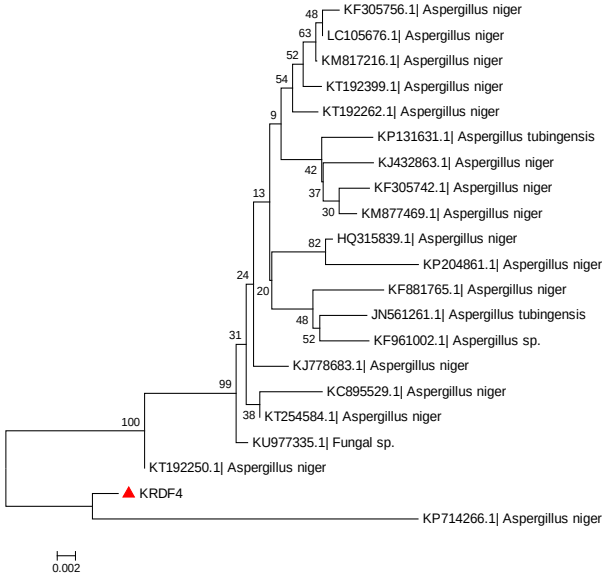

Fig 1: Phylogenetic tree showing the position of isolate KRDF1 with reference to related strains.

4. References

1. Helene C Eisenman, Arturo Casadevall. Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol 2012; 93(3):931-940.
2. Eric Jacobson S. Pathogenic Roles for Fungal Melanins. Clinical microbiology reviews, 2000, 08-717.
3. Rajagopal K, Kathiravan G, Karthikeyan S. Extraction and characterization of melanin from Phomopsis: A phellophytic fungi Isolated from Azadirachta indica A. Juss. African Journal of Microbiology Research. 2011; 5(7):762-766.
4. Shosuke Ito. A Chemist's View of Melanogenesis. Pigment cell research 2003; 16:230-236.
5. Przemyslaw Plonka M, Maja Grabacka. Melanin synthesis in microorganisms — biotechnological and medical aspects. Acta Biochimica Polonica 2006; 53(3):429-443.
6. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 1987; 4:406-425.
7. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 1985; 39:783-791.
8. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution (In Press), 2011.
9. Butler MJ, Day AW. Fungal melanins: a review. Can J Microbiol. 1998a; 44:1115-1136.
10. Caesar-Tonthat T, Van Ommen KF, Geesey GG, Henson JM. Melanin production by a filamentous soil

fungus in response to copper and localization of copper sulfide by sulfide-silver staining. Appl Environ Microbiol. 1995; 61:1968-1975. [PubMed: 16535031]
11. Wakamatsu K, Ito S. Advanced chemical methods in melanin determination. Pigment Cell Res 2002; 15:174-183. [PubMed: 12028581]
12. Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA. Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol 2003; 38(2):143-58.