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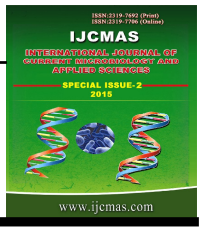

Original Research Article

Pigment Production from Fungi

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A B S T R A C T

There is an ever growing demand of eco-friendly/ non toxic dyes especially for food colours and child textiles. Natural dye producing microbes offer a viable alternative to natural vegetable and harmful synthetic dyes. Fungi are reported as potential biological source of natural pigments like anthraquinones. The present study was under taken to screen fungi for production of different pigments isolated from domestic fridges. Their growth and pigment production were optimized at room temperature. Four fungal strains IDP1, IDP2, IDP3 and IDP4 were isolated which produced four different natural pigments - IDR, IDY, IDG, and IDBr respectively. The maximum production of pigment was seen in mineral salts glucose medium. The microscopic studies and the colony characteristics revealed that all four isolates of the fungi belongs to the genus *Penicillium*. Lambda max of the pigments IDR, IDY, and IDG was 340nm and for IDBr it was 371nm.The yield of the pigments IDR, IDY, IDG, and IDBr were 0.073mM, 0.1056mM, 0.133mM and 0.159mM respectively. FTIR results revealed that the pigments are multicomponent in nature.

Keywords

Fungal pigment, Mineral salts glucose medium, *Penicillium* spp, FTIR

Introduction

Coloured substances known as dyes are used to impart colour to variety of material. The use of such synthetic dyes in dying industry results in dye containing waste water which increases the environmental pollution. Some of these dyes have potential potent carcinogen posing serious health hazard.The use of food colorants as additives in the food industry is a significant factor for both food manufacturers and consumers in determining the acceptability of processed food (Ana Abad *et al.*, 2010).

Many consumers are likely to be unaware of the exotic sources of some of the currently authorized so-called natural colorants. Fungi are reported as potent pigment producing microorganisms (Babitha *et al.*, 2007). The importance of pigments such as anthraquinone, anthraquinone carboxylic acids, pre-anthraquinones extracted from filamentous fungi are already known. The application of these fungal pigments in dyeing of cotton, silk and wool has been reported in several studies (De Santis *et al.*,

2005). Hence, the main objective of the present study is to extract pigment from filamentous fungi and study their properties.

Material and Methods

Isolation and identification of fungi

Sample was collected from domestic fridge. The isolates were purified and stored on PDA as spore stock at -20^{o}C for further studies.

Identification was done microscopically by slide culture method and wet mount.

Cultivation, extraction and purification of fungal pigments

The isolated fungi was cultivated individually on defined mineral salts-glucose medium contains (per liter of de-ionized water): glucose 30 g; 1.0 g $(NH_4)_2SO_4$; 0.5 g $MgSO_4.7H_2O$; 1.4 g K_2HPO_4; 0.6 g KH_2PO_4; 0.8 mg $ZnSO_4.H_2O$; 0.8 mg $FeCl_3.6H_2O$; 0.8 mg $NaMoO_4.2H_2O$; 0.4 mg $MnSO_4$ $2H_2O$; 0.08 mg $CuSO_4.5H_2O$; and pH 5.6 in flask. All flasks were inoculated by a mycelial disk by using cork borer (12 mm diameter) from PDA culture with 4 isolates respectively. They were grown at 37^{o} C in the dark as stationary cultures for 4–6 week (Nagia and EL-Mohamedy, 2007). After an incubation period of 6 week, the mycelium was harvested, and the supernatant was filtered in a sterilized Whatt man's filter paper. Later, two volumes of 95% (v/v) ethanol was added to culture broth according to the following procedure:

(i) After dilution with about 60% of the solvent volume needed, the resulting mixture was kept on the rotary shaker at 180 rpm at 30 C for 30 min

(ii) The ethanolic mixture was centrifuged at 3780 rpm for 15 min

(iii) Once the supernatant had been recovered, the residue was dispersed in the remaining volume of ethanol and centrifuged again at 3780 rpm for 5 min; and

(iv) The supernatants were then collected and filtered through a pre weighed Whattman's filter paper (47 mm) and further diluted with 95% (v/v) ethanol to a final volumetric dilution factor of 20.

1. The absorption spectrum was observed between 300–600 nm using spectrophotometer.

2. Yield is calculated by: E= A/CLr

3. Fourier Transform Infrared spectroscopy analysis for pigment.

Results and Discussion

Microscopic view of fungal strains: By slide culture method (Fig. 1–4). Cultivation, Extraction and Purification of Fungal Pigments: Four fungal strains IDP1, IDP2, IDP3 and IDP4 were isolated which produced four different natural water soluble pigments - IDR, IDY, IDG, and IDBr respectively; maximum absorbance and pigment yield are tabulated in table 1. Figure 5 shows the diffusion of fungal pigments on PDA plates; Figure 6 shows the same in liquid media.

Table.1 Maximum absorbance and pigment yield of all isolates

Sr. No	Name Of Pigment	Maximum Absorbance	Pigment Yield (mM)
1.	IDP1	340	0.073
2.	IDP2	340	0.1056
3.	IDP3	371	0.159
4.	IDP4	340	0.133

Fig.1 Fungal strain IDP1

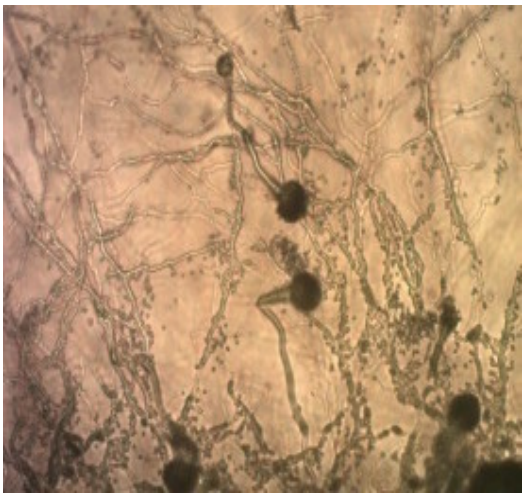


Fig.2 Fungal strain IDP2

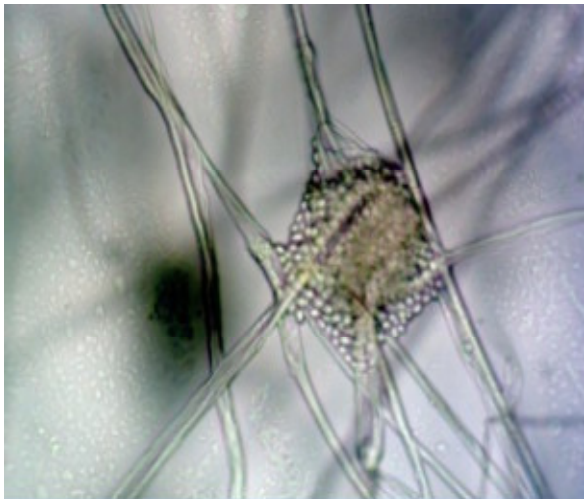


Fig.3 Fungal strain IDP3

Fig.4 Fungal strain IDP4

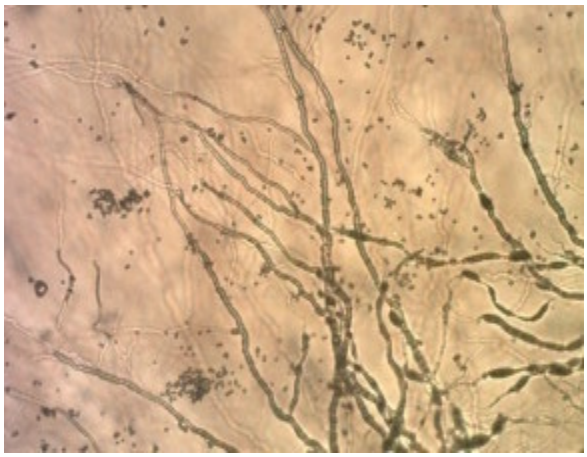


Fig.5 Diffusion of fungal pigment on PDA plates

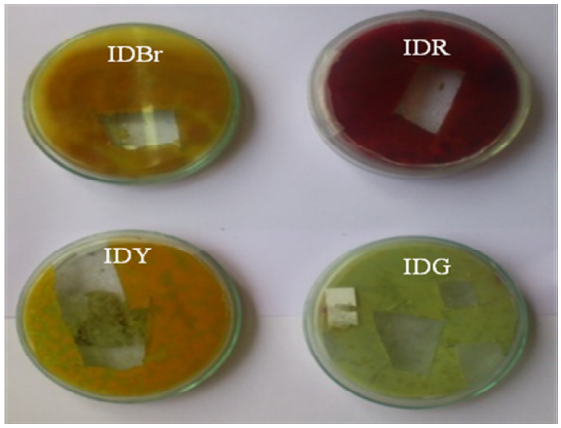


Fig.6 Fungal pigment in liquid media

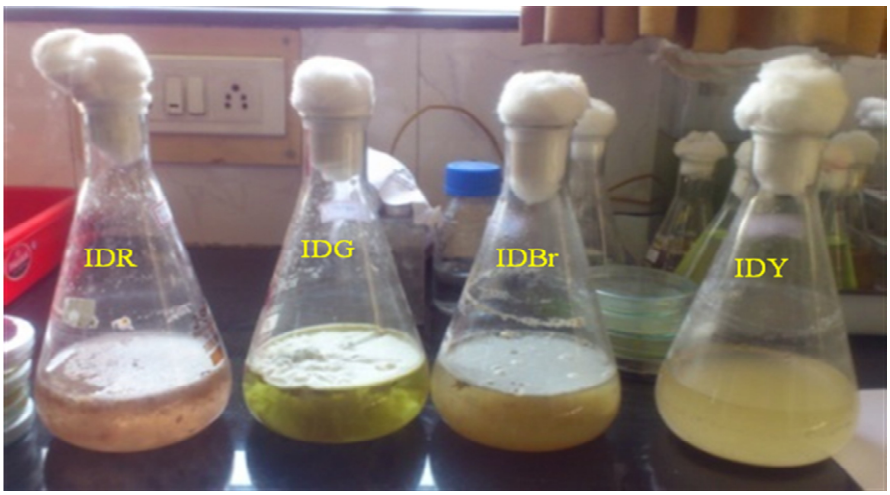


Fig.7 FTIR results for IDRY

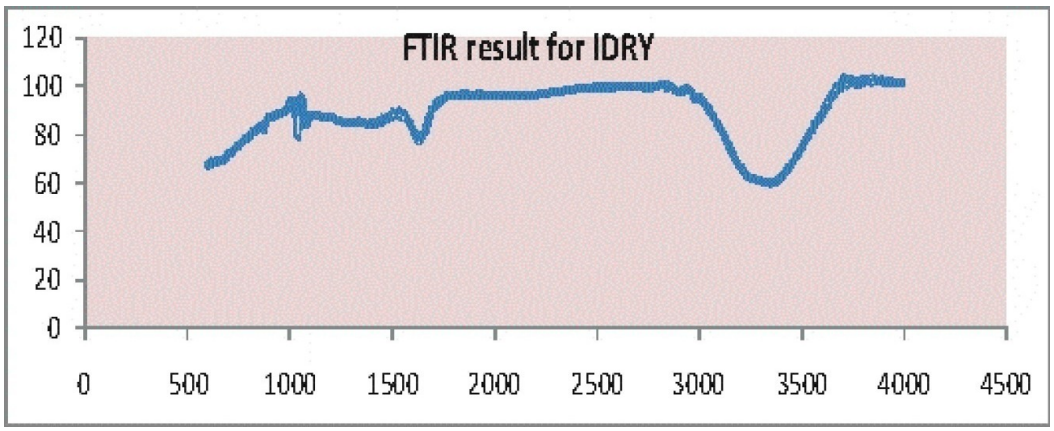


Fig.8 FRIT results for IDG

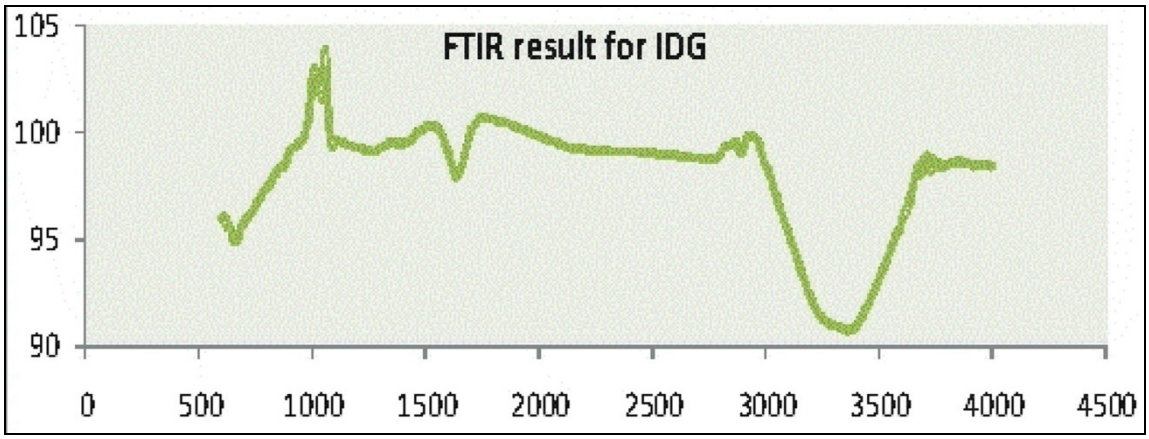


Fig.9 FTIR result for IDR

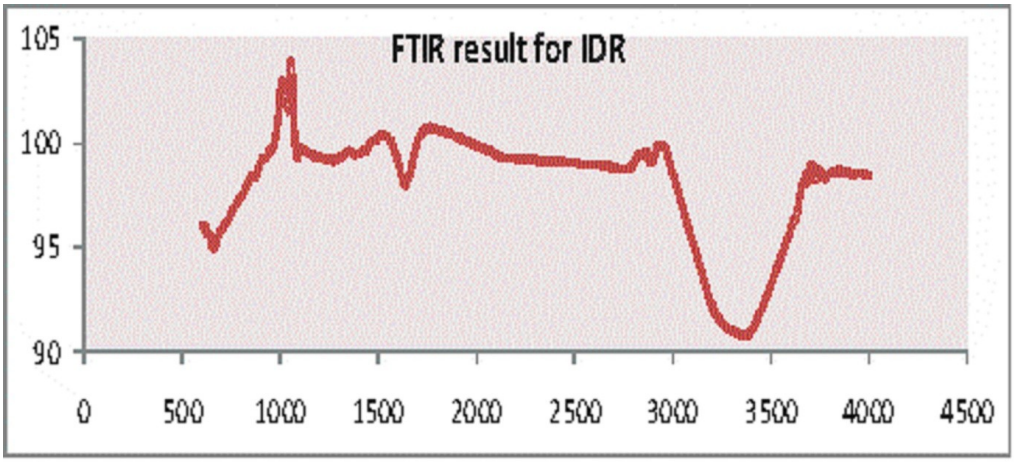
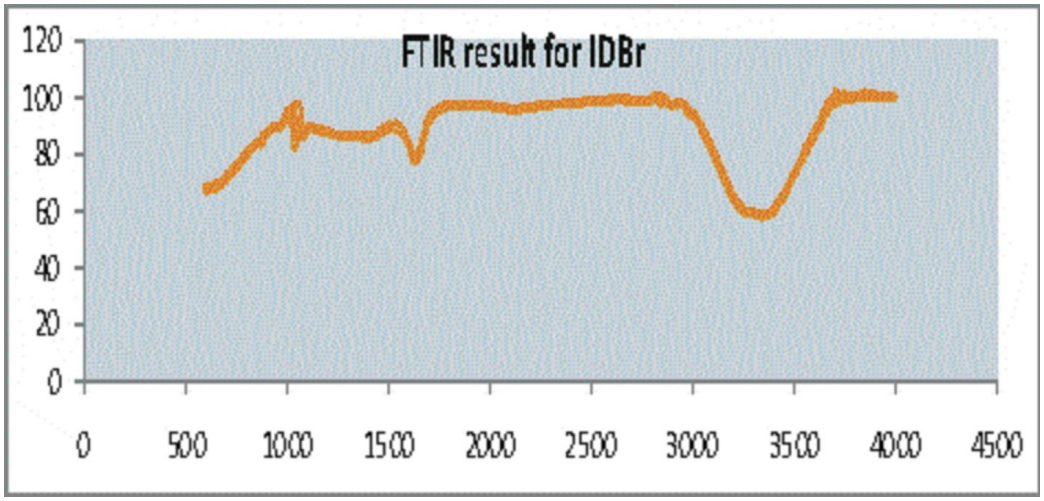


Fig.10 FTIR result for IDBr



Fourier Transform Infrared Spectroscopy Analysis: Figures 7–10 display the results obtained for each of the pigments.

Bond bound in the range of 3300 to 3550 cm^{-1} in the all 4 pigments shows the presence of –OH group. A peak between 1600 to 1700 cm^{-1} the presence of –C=O group.

UV analysis of the compound at 340nm except for one (IDG) which is at 371nm the presence of highly conjugated compound.

All four isolates of fungi can tolerate lower temperature but the production of pigment can be observed at 30-32ºC (Dikshit Rashmi and Tallapragada Padmavathi 2013). The four potent isolates were identified as Penicillium spp. based on the morphological characteristics. The shades of the pigment extracted from fungus exhibited a marked difference in the color component and pigment concentration. Maximum production of pigment was observed in the IDP3 *Penicillium* spp. However, production in IDP1, IDP2, and IDP4 was similar. Most

probably the pigments belong to the Poly phenolic group (flavenoids).

Future prospect

The extracted pigments can be tested for their use in industries such as cloth or leather dying, cosmetics, food colorant, and pharmaceuticals industries etc. To enhance the binding ability of the pigments appropriate binders can be used. Further affinity of extracted pigments with different types of binders will be investigated to check the effectiveness of the colouring capacity of the pigment to be used as a potent colouring agent.

Acknowledgement

I would like to thank Dr. E.M. Khan, Principal, Abeda Inamdar Senior College for providing us with the necessary infrastructure conducive for research.

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