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Screening of culture media for growth and pigment production by *Fusarium sp.*

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Abstract

The present study was aimed to select a suitable medium for enhanced pigment production by *Fusarium* sp. The fungus was isolated from air by plate exposure method using potato dextrose agar media. Different types of solid and liquid media were screened for growth and pigment production using *Fusarium* sp. It was observed that media components influenced growth as well as pigment production. Highest growth rate, after 6 days of incubation, was obtained in CYEA followed by PDA and MEA. Maximum pigment on agar media was seen in CYEA and PDA. In broth medium, crude pigment was estimated by spectral analysis in different wavelengths ranging from 380nm to 720nm. PDB and SDB were found to yield pigment having maximum red and orange components while in Lin's broth media pigment produced by *Fusarium* sp. consisted primarily of yellow component. The results therefore indicate that growth and pigment production by the fungus is dependent on the media components.

Key words : *Fusarium sp.*, pigment, radial growth, culture media, spectral analysis.

INTRODUCTION

In biology the word pigment is much more loosely used; it includes insoluble materials that may be coloured or visible by virtue of being refractile or birefringent. There are several organisms that produce pigments as secondary metabolites and are often referred to as biopigments. These biopigments can be obtained from two major sources, plants and microorganisms [1]. Biopigments from micro-organisms have been preferred over those from plants because of their stability and the availability of their cultivation technology throughout the year [2].

There has been increasing interest in using microorganisms as a colour source due to the cost factor, labour, extensive land requirement and use of expensive solvent for extraction from higher plant material. Therefore investigations are being undertaken on microbial dyes. Microorganisms are known to produce a wide variety of pigments, and therefore are a promising source of food colorants because of their rapid growth and ease of control. Among microorganisms, fungi display higher rates of growth and adaptation which makes production of secondary metabolites by means of biotechnological processes conducive. Also, fungi are reported as potent pigment producing microorganisms [3].

The application of fungal pigments such as anthraquinone, anthraquinone carboxylic acids, pre-anthraquinones extracted from filamentous fungi in dyeing industries has been reported. Many fungi such as *Aspergillus, Fusarium* and *Trichoderma* produce pigments during their growth as intermediate metabolites. The fungi such as *Fusarium oxysporum, Trichoderma viride* and *Alternaria sp*. were studied for colour production and tested on various cellulose fibers [4].

During the recent past, there is wider range of search for pigment producing fungi and their potential use as replacement for synthetic dyes. Therefore, the study aims to explore *Fusarium* sp. for pigment production. *Fusarium* is a genus of filamentous fungi that contains many agronomically important plant pathogens, mycotoxin producers, and opportunistic human pathogens. Comparative analyses have revealed that the *Fusarium* genome is compartmentalized into regions responsible for primary metabolism and reproduction, and pathogen virulence, host specialization, and possibly other functions [5]. In order to utilize the potential of *Fusarium* as a pigment producer, it is essential to choose a fermentation medium which has positive effect on growth and pigment production. The study is therefore aimed to assess the influence of culture media on growth and pigment production by *Fusarium* species.

MATERIALS AND METHODS

Isolation and identification of Fusarium sp.

Fusarium strain was isolated from air for the production of pigment. Potato Dextrose Agar (PDA) plates in duplicate were exposed to air for 5-10 mins and then incubated at 28±1°C for 3-4 days or until the colored sign of mycelial growth. The fungal isolates were identified to genus level by lacto phenol cotton blue staining. The morphological structure of fungi was observed under light microscope at 40 X magnification. *Fusarium* sp. identified thus, was used for further studies.

Radial Growth and Pigment Production of Fusarium sp. on different agar media

Fungi grow on nutrient medium forming colonies of different shape, size and colour. Fungal growth was estimated by measuring the size of colonies with mean diameter per day. The *Fusarium* isolate was cultured on six different nutrient media *viz.*, Malt Extract Agar (MEA), Czapek -Yeast Extract Agar (CYEA), Nutrient Agar (NA) and Rose Bengal Agar (RBA), oat meal agar (OMA) and Potato Dextrose Agar (PDA). After autoclaving the media, 20ml was poured in each petriplate and allowed to solidify. With the help of inoculating loop, 5mm diameter of 7-day old *Fusarium sp.* grown on PDA was taken and inoculated at the centre of each petri plate containing different media and

incubated at 28±2°C. The diameter was calculated for each colony in millimeters in two directions at right angles to each other after every 24 hrs.

Pigment Production and Mycelial Growth of *Fusarium sp.* on liquid broth media

Three different broth media *viz.* Potato Dextrose Broth (PDB), Sabouraud Dextrose Broth and Lin's Media (carbon source 50 g/litre; mono sodium glutamate (MSG), 12.6 g/litre; K_2HPO_4, 2.4 g/litre; KH_2PO_4, 2.4 g/litre; $MgSO_4.7H_2O$, 1.0 g/litre; KCl, 0.5 g/litre; $ZnSO_4.7H_2O$, 0.01 g/litre; $FeSO_4.7H_2O$, 0.01 g/litre; $MnSO_4.H_2O$, 0.03 g/litre) were used for estimation of pigment production by *Fusarium* sp. The culture was inoculated into flasks containing 50ml of each media. The flasks were kept for incubation at 28°C for 15 days. Samples were collected after incubation period and centrifuged at 5000 rpm for 10 min. Pigment was estimated by measuring optical density (O.D.) of supernatant from 380nm to 720nm by UV-Vis spectrophotometer.

RESULTS

A total of four different fungi were isolated from air and these were studied for cultural and microscopic characteristics. Among the four isolates, one of fungal strain which produced pink colour pigment was selected for microscopic examination (Fig 1).

The selected fungal colony was studied under light microscope at 10X magnification for size, texture, colour, elevation, reverse side colour, and margin. According to the morphological characteristics seen with lacto phenol cotton blue staining at 40X magnification, it was observed that the specimen showed the presence of boat-shaped, hyaline, macro conidia, marked foot cell at the attachment area of spore and hyphal wall confirming that it belongs to genera *Fusarium* (Fig 2).

The radial growth of *Fusarium* sp. was calculated by taking the average value of two readings and dividing the value by total number of days. The findings of this study showed that, different culture media influenced the growth and pigment production of *Fusarium sp.* Out of six nutrient media (MEA, CYEA, NA, RBA, OMA and PDA) used, maximum radial growth and pigment production were observed on CYEA and PDA with a mean of 79±0.5mm, 77±0.5mm respectively followed by MEA with 68±0.5mm (Table 1). Radial growth observed on OMA, RBA and NA were less owing to unsuitable nutrient requirements. The best pigment production was observed on PDA and CYEA after 48 hours. Mycelium was observed to have pink tinge in PDA, CYEA and MEA media while a greenish tinge was seen in RBA media (Figure 3).

After incubation with *Fusarium* for 15 days in broth media, PDB, SDB and Lin's media, growth was observed to be turbid due to biomass production. Chromophoric organic matter released by fungal strains into aqueous medium show absorption in visible and UV spectra. The production of pigment during growth on different media was monitored and measured using spectral measurements

In PDB and SDB media highest absorbance was recorded at 500nm and 540nm while in Lin's media it was 400nm (Fig 4, 5, 6). The absorbance of pigment from 540 to 640nm produced on PDB and SDB were markedly similar as compared to that of pigment produced on Lin's medium. From the results obtained it can be inferred that crude pigment produced on PDB and SDB consisted of more red and orange components whereas that of Lin's medium consisted primarily of yellow component.

DISCUSSION

Sorenson, *et al*. [6] reported that colonies of *Fusarium* species appear red due to production of pigments, such as aurofusarin or bikaverin. Changes in nitrogen source available during growth also affect the hue of pigment. Media containing organic nitrogen source are reported to give enhanced pigment production as compared to inorganic nitrogen source [7].

PDA contains soluble starch which supports fungal growth and pigment production. CYEA contains sucrose which is also reported to influence pigment production [8]. MEA contains peptone as nitrogen source and it is inferred to enhance pigment

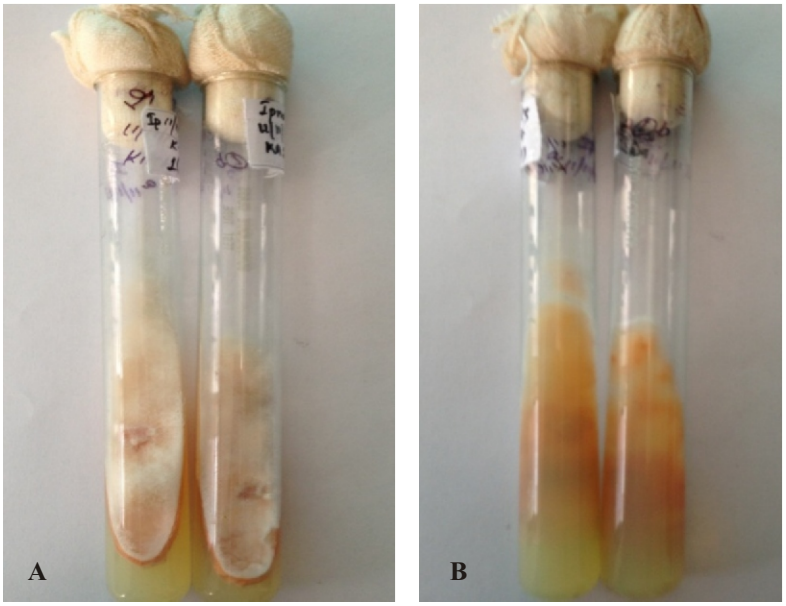

Fig 1: (A) Growth of *Fusarium sp.* on PDA slant; (B) Reverse side showing pinkish hue.

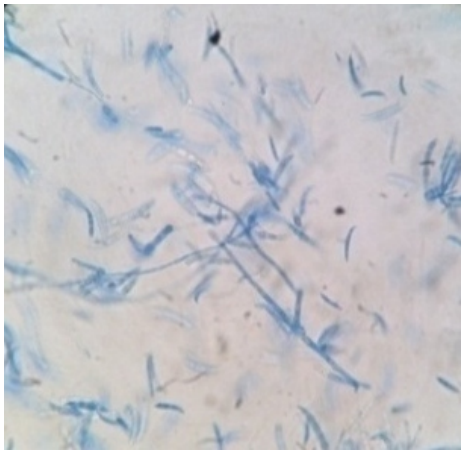

Fig 2: *Fusarium sp.* (40X)

Table 1: Radial growth, cultural characteristics and pigment production by *Fusarium sp.* on different agar media.

S.No	Medium	Colony Diameter (mm)		Growth and pigment production
		Day-4	Day-6	
1	MEA	43.5±0.5	68±0.5	White cottony growth, pinkish tinge, raised colony with irregular margin.
2	CYEA	65.5±0.5	79±0.5	White cottony growth, pinkish tinge, raised colony with irregular margin, pink tinge on reverse side of Petri plate.
3	NA	8±0.5	8±0.5	No proper growth.
4	RBA	33±0.5	37±0.5	White cottony growth, pinkish tinge, raised colony with irregular margin.
5	OMA	42.5±0.5	58.5±0.5	Green cottony growth, raised colony with irregular margin.
6	PDA	69.5±0.5	77±0.5	White cottony growth, pinkish tinge, raised colony with irregular margin, pink tinge on reverse side of Petri plate.

Results are mean of independent experiments ± SD and are expressed as colony diameter (mm).

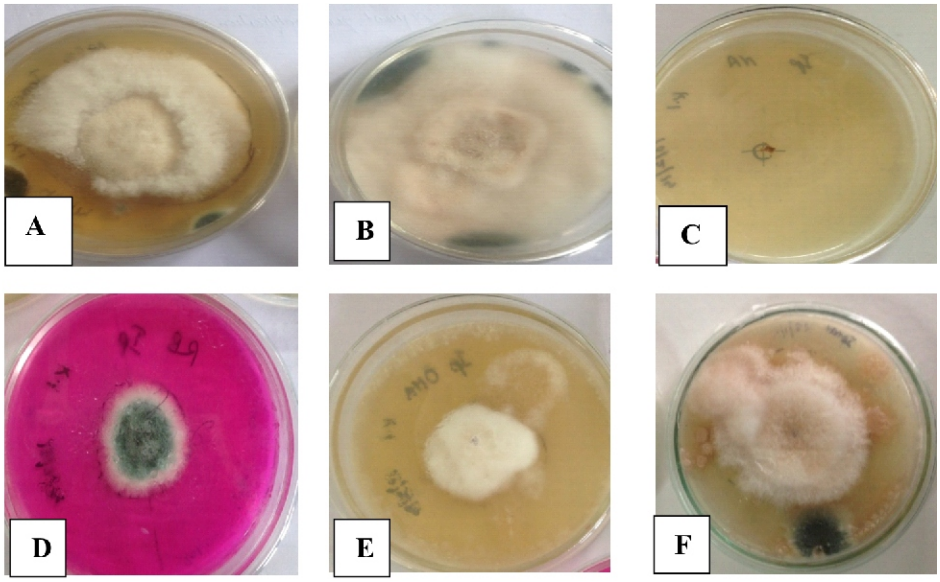

Fig 3: Growth of *Fusarium sp.* on (A)Malt extract agar (B)Czapex-Yeast agar (C)Nutrient agar (D) Rose Bengal Agar (E)Oat meal agar (F) Potato dextrose agar.

production as compared to inorganic nitrogen source. In OMA, oat meal is a source of nitrogen, carbon, protein and nutrients and does not contain nitrogen source separately. Papaic digest of soyabean meal in RBA does not support growth and pigment production as compared to peptone. Finally, in NA, even though peptone and yeast extract are present, there is no growth in it due to lack of carbohydrate.

Three broth media (PDB, SDB and Lin's media) were used for quantification of pigment production by *Fusarium sp.* Several studies involving different strains of fungi on these media were done earlier [8]. The extraction of pigment is easier in broth as compared to solid media. Extracellular pigments are preferred as they are soluble in liquid culture media which makes downstream processing simpler and cheaper. Moreover, production of extracellular pigment is reported to be higher than intracellular pigment.

Wavelength below 380nm corresponds to yellow component, 400nm to 520nm corresponds to orange component, 540 nm to 720nm corresponds to red components. *Fusaium* sp. are reported to produce pigment components which include aurofusarin (red), fuscofusarin (yellow), rubrofusarin (red), 2,7-dimehoxy-6-(acetoxyethyl) juglone (yellow); bikaverin (red), bostrycoidin (red), nectriafurone (yellow), norjavanicin (red), O-methyl-6-hydroxynorjavanicin (yellow, o-methylanhydrofusarubin

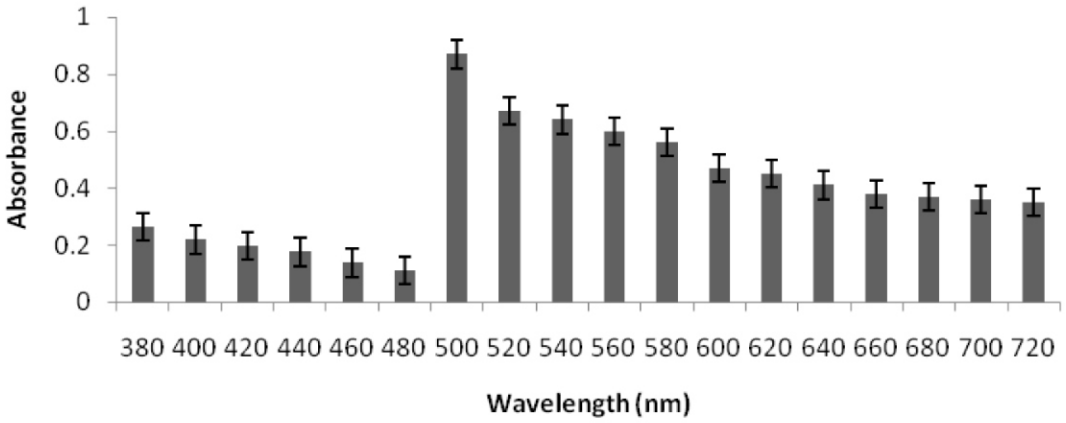

Fig 4: Absorption spectra of pigment produced by *Fusarium sp* on PDB.

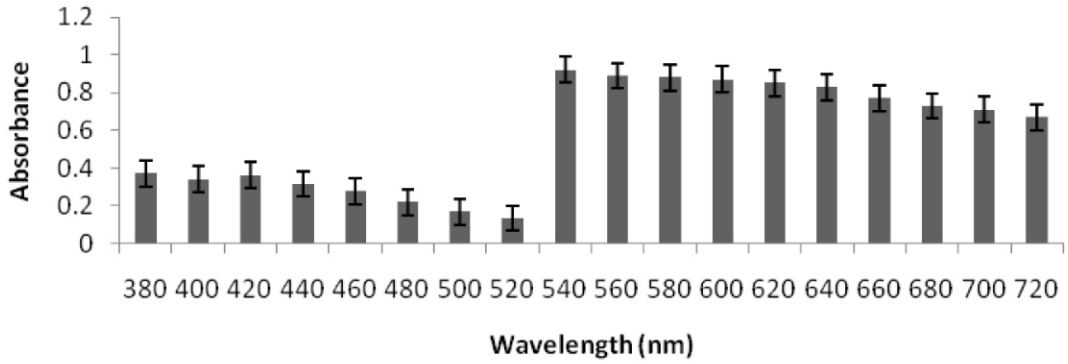

Fig 5: Absorption spectra of pigment produced by *Fusarium sp* on SDB.

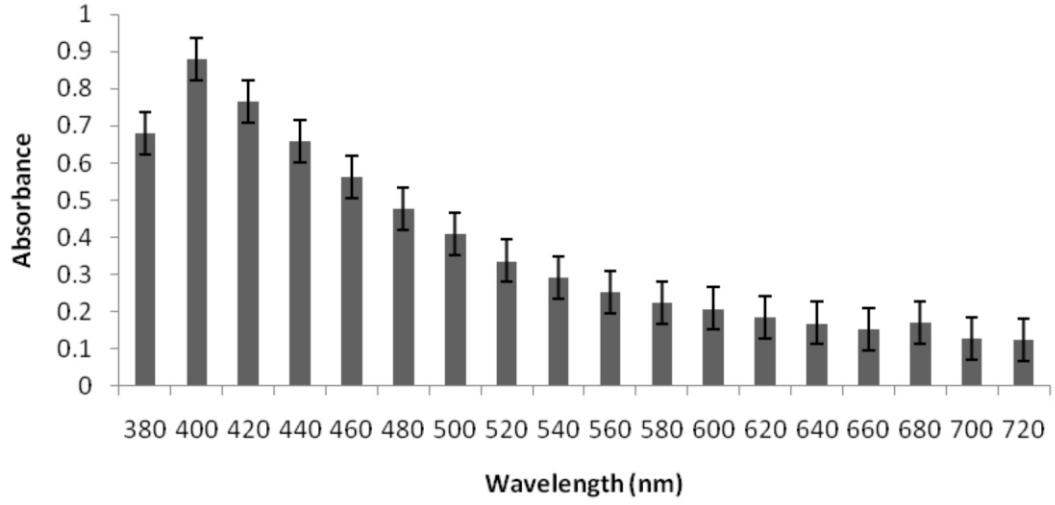

Fig 6: Absorption spectra of pigment produced by *Fusarium sp.* on Lin's

(orange-red), O-methylfusarubin (red), O-methyljavanicin Isomarticins (red), O-ethylfusarubin (red), O-methylsolaniol (orange-red), lycopene, nectriafurone (yellow) [9,10,11]. Cho, *et al.* [1], also stated that generally, the absorbance of pigments are measured at 400nm, 470nm and 500 nm, these wavelengths representing absorption maxima for yellow, orange and red pigments, respectively.

SDB contains peptone and PDB contain potato starch, which support growth and pigment production. Therefore production of pigment was seen to be higher in PDB and SDB media as compared to Lin's media. These findings suggest that PDB and SDB might contain components such as metal ions and micronutrients essential for pigment production mechanisms. These results also corroborate with findings of Boonyapranai, *et*

al. [12], which concluded that naphthoquinone production by *Fusarium* sp. was supported by addition of peptone and yeast extract.

When complex nitrogen source such as peptone and yeast extract are used, multiple extracellular pigments are produced by fungi. Stimulation of pigment formation by nitrogen source has been reported by Hamdi, *et al*. [13]. Meat peptone, casein peptone, peptoneyeast extract mixture and corn steep powder were found to have a positive effect on pigment production, whereas soy peptone and malt extract were found to strongly inhibit pigment synthesis. In their study, among the nitrogen sources tested, meat peptone gave highest yield of red pigment.

In Lin's medium, the yellow and orange components were found to be less as compared to PDB and SBD due to presence of inorganic nitrogen source monosodium glutamate. These results are supported with the findings of Lin and Demain [14]. They suggested that transformation from orange to red happens due to a strong tendency of the orange pigments to react with the primary amino group of amino acids such as MSG. They also inferred that MSG was a far superior nitrogen source compared with other inorganic salts.

CONCLUSION

Growth and pigment production by fungi has been attributed to be influenced by several factors. Some of these factors include the media components like carbon source, nitrogen source, presence of metal ions, micronutrients, pH, temperature, presence or absence of light during growth, fermentation condition like solid state fermentation or submerged fermentation and the fungal strain used. The synthesis of secondary metabolites by fungi is regulated by above factors. *Fusarium* sp. has been reported to produce napthaquinone pigments which possess broad range of biological activities like antibacterial, fungicidal, antiparasitic and insecticidal properties also [15]. Thus *Fusarium* sp. can be explored for pigment production for use in food industries as additives, colour intensifiers and antioxidants, etc. Further characterization of the crude pigment and optimization of production process may be done.

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