PRODUCTION OF FUNGAL PIGMENTS AS POTENTIAL NATURAL COLORANTS FOR VARIOUS INDUSTRIAL APPLICATIONS

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Abstract:Natural colorants are preferred over synthetic colorants owing to their tremendous marketing potential and healthy image [kumar et al.]. Fungal pigment production offers higher yields, varied color range and independence over agro-climatic conditions, and thereby minimizing batch to batch variations. Pigment producing ability of filamentous fungi was tested. Five different lab strains of filamentous fungi were used in present study. Pigment production was assessed on five different solid media. Pigment producing ability was expressed in Sabouraud's dextrose agar and Czapek yeast extract agar, by two different fungal strains. One of them expressed pigment production in Czapek'sdox broth. Extraction of pigments was performed using polar and non-polar solvents from both solid and liquid media. One of the fungal strains produced extracellular pigment in solid media which was extracted in methanol, while intracellular pigments were extracted in hexane. Also, extracellular Polar pigments were produced in liquid media. Crude pigment extracts were characterized spectrophotometrically. The present study brought out two promising pigment producing fungal strains and their water soluble colorants. Further work is in progress in terms of toxicity evaluation of crude pigment extract and process optimization for optimal pigment production[Nielsen S. R.et al. 2002].

Index terms- Natural colorants, filamentous fungi, solid and liquid growth medium,pigment, polar and non-polar solvents.

Introduction:Natural colorants offer better alternatives for synthetic ones and have a broad spectrum of applications that include food, paint, cosmetics and textile industries.Natural colorants are healthier alternatives of synthetic colorants, owing to eventual harmful effects of synthetic colorants.The market for natural colorants is rising due to the healthier lifestyles and growing awareness of consumers[Mortensen A. et al. 2006]. Naturally derived colorants have the potential to overtake synthetic food colorants in market because of the demand for clean label ingredients.Among microbial sources of natural colorants filamentous fungi are preferred because of their ability to be grown easily under laboratory conditions unlike micro-algae, the growth of which is time consuming and have lower yields and/or productivity.Fungal pigment production offers advantage of minimal batch to batch variation, higher yields, extraordinary color range and independence over agro-climatic conditions compared to plant based pigments[Dufosse et al.].*Monascus* is one of the best examples for fungi used in industrial pigment production, however their ability to coproduce a mycotoxincitrinin limits their use. Nevertheless, *Monascus* pigments have been successfully used as food colorants for hundreds of years [Dufosse*et al*. 2005]. Recent studies have brought out the potential of pigment producing genera other than *Monascus*[Jung H. et al.],the color hues of which resemble some of the commercially available plant based food colorants [Mapari et al. 2006].

Pigment producing ability of fungi is dependent on media and/or culture conditions. Submerged Liquid fermentation is preferred in industries due to easy product recovery and standardized culture conditions. Studying the morphology and pigment producing ability of microorganisms and substrate choice is equally important as they can be manipulated to get desired products, higher yields and convenient procedures. Present study was carried out to test the hypothesis that the pigment production by filamentous fungi can be regulated by changing media and/or culture conditions, and pigments of newer color hues with better functionality can be obtained.

Research Methodology:

Fungal strains: Five Fungal strains were procuredas lab contaminants and designated as F1,F2,F3,F4 and F5.

Growth medium used:Assessment of pigment producing ability of fungal strainswas tested on five differentsolid nutrient media*viz.* Nutrient Agar, Sabouraud's Agar, Potato Dextrose Agar, Minimal Agar and Czapek'syeastextract agar. The fungalstrains were also inoculated in liquid nutrientmedia viz. Czapek'sdox broth and Sabouraud's Broth. All five fungal strains were also inoculated on sterile rice with $ZnSO_4$.

Culture Conditions: Fungi inoculated on solid growth media were kept for incubation at room temperature [22^0C-25^0C] for an incubation period of six days. The two selected strains inoculated in liquid media [Semesgaard, J] were incubated at room temperature [22^0C-25^0C] under stationary conditions for an incubation period of 7 days.

Pigment Extraction: For extraction of colorants from both solid and liquid growth media polar and non-polar solvents were used; methanol (polar solvent) and hexane (non-polar solvent). Micro-extraction technique[Mapari et al. 2006] was used to extract colorants from solid nutrient mediumby taking plugs using cork-borer and then extracted in both polar and non-polar solvents. The polar solvent used was methanol and non-polar solvent was hexane.Sonicator was used to cause cell lysis and extract possible intracellular colorants. The two fungal strains grown in liquid medium were extracted using the same solvents.

Spectrophotometric Analysis: All the extracted colorants were spectrophotometrically analyzed using UV-VIS Spectrophotometer [Jasco V-630].Intensity of the colorant and the wavelength at which it absorbs the most was analyzed, different absorption spectra were obtained[Mapari et al.].

Results and discussions:The metabolic potential of pigment production was explored using various complex media and minimal media.Pigment production ability of five filamentous fungi was tested on solid and liquid growth media.Colorant production was observed in fungiF3[Sabouraud's agar] and F5[Czapek's yeast extract agar]. In F3,yellowish green color was observed and in F5 faint pink color was observed on the plates[Table 1]. The strains labeled F1, F2 and F4 did not produce significant colorants. Since pigment producing ability in liquid media is desirable for the large scale pigment production and easy downstream processing, the two potential pigment producing fungal strains were also grown in liquid nutrient media, Czapek'sdox broth, [Table 2].Chemical nature of colorants was determined by extraction using polar and non- polar solvents [methanol and hexane respectively]. UV-VIS Spectrophotometry was done to study the nature of chemicals, concentration of colorants, and the colorhues. Pigments were extracted in methanol and hexane, then analysedspectrophotometrically. Characteristic absorption spectra were obtained,of polar and non-polar pigment extracts.Orange colorant production[extracellular] was observed in strain F5 [Czapek'sdox broth] after incubation for 7 days. F5 in liquid media was extracted and characteristic absorption spectra were obtained.

Table 1.Growth and pigment production of fungal strains on different solid media:

	Fungal strains	Nutrient Agar	Minimal Agar	Sabouraud's Agar	Potato Dextrose Agar	Czapek's Yeast Extract Agar
Growth	F1	+	+	+	+	+
Pigment		-	-	-	-	-
Growth	F2	+	+	+	+	+

	Fungal strains	Nutrient Agar	Minimal Agar	Sabouraud's Agar	Potato Dextrose Agar	Czapek's Yeast Extract Agar
Pigment		-	-	-	-	-
Growth	F3	+	+	+	+	+
Pigment		-	-	+	-	-
Growth	F4	+	+	+	+	+
Pigment		-	-	-	-	-
Growth	F5	+	+	+	+	+
Pigment		-	-	-	-	+

As it can be seen from table 1, growth and pigment producing ability of fungal strain was not positively correlated and vice-versa. Distinctly F3 and F5 produced pigments on Sabouraud's agar and Czapek Yeast extract agar respectively.

Table 2.Growth and pigment production of fungal strains in liquid Media:

	Fungal strain	Sabouraud's Broth	Czapek'sDox Broth
Growth	F3	+	+
Pigment		-	-
Growth	F5	+	+
Pigment		-	+

As it can be seen from table 2, F5 produced orange pigment in Czapek'sDox broth

Table 2.Growth and pigment production of fungal strains on rice as solid growth medium

Fungal Strain	F1	F2	F3	F4	F5
Growth	-	-	+	-	-
Pigment Production	-	-	-	-	-

As we can see from table 3, F3 showed growthin rice as growth medium.

Figure 1
F3 in Sabouraud Agar Day 4,(Obverse side)

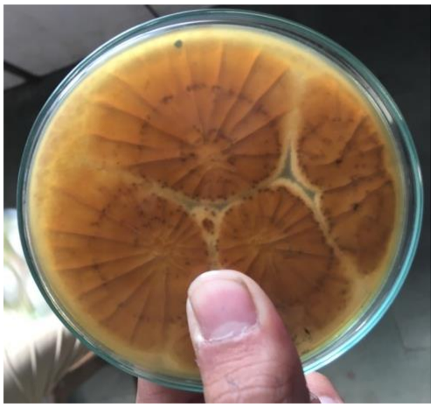

Figure 2
F3 in Sabouraud Agar Day 4, (Reverse side)

Figure 3
F5 Czapek'syeast Agar, day 4 (obverse side)

Figure 4
F5 Czapek'syeast Agar, day 4 (obverse side)

Figure 5
F3 and F5 in Czapek's Broth,Orange Dye produced by F5

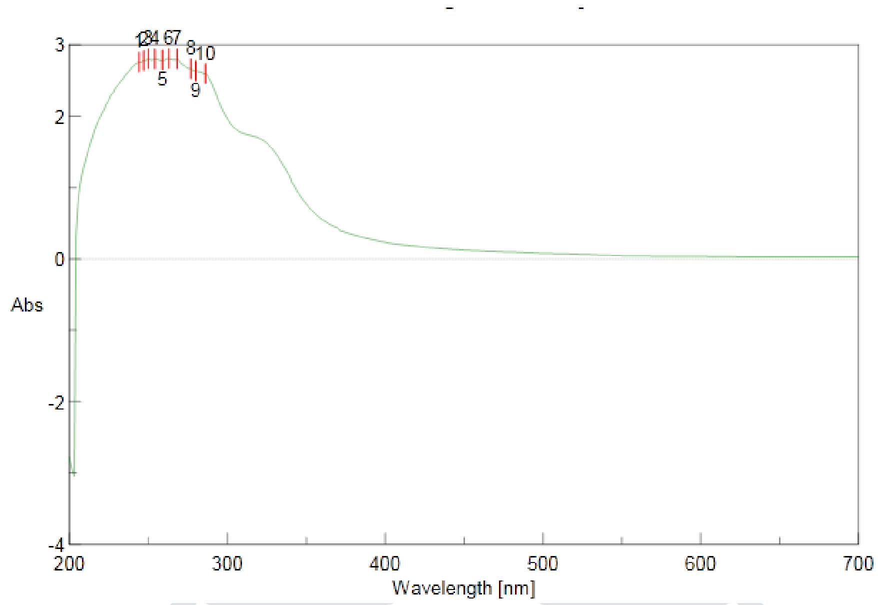

Figure 6: Absorption Spectra of F5 [Czapek's] in Hexane extract

Figure 6 shows characteristic absorption spectra due to presence of non-polar pigment,

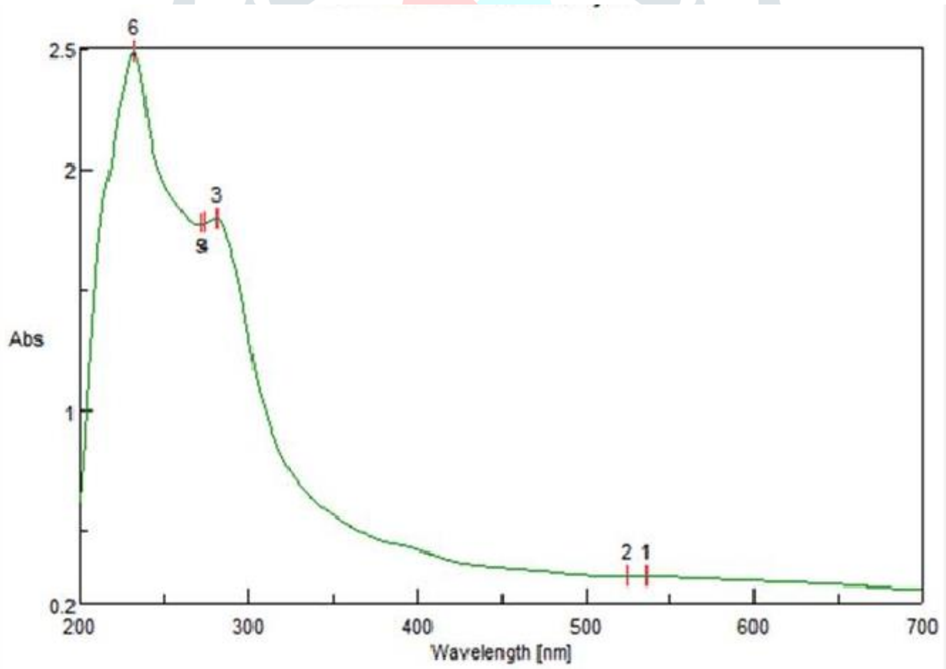

Figure 7:Absorption Spectra of F3inSabouraud's Agar -Methanol Extract

It is seen from figure 7 that greenish-yellow pigment was produced by fungi F5 in Sabouraud's Agar. Chemical nature of pigment was polar

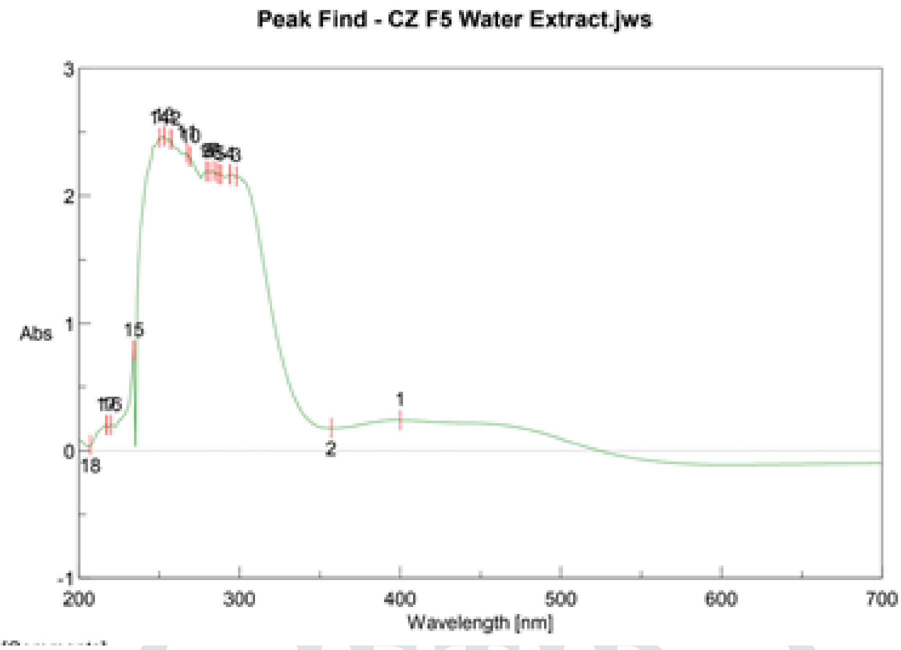

Figure 8:Absorption Spectra of F5 in Czapek'sDox broth-Water extract.Absorbance Unit at max.wavelength (398) per 100ml=2.25

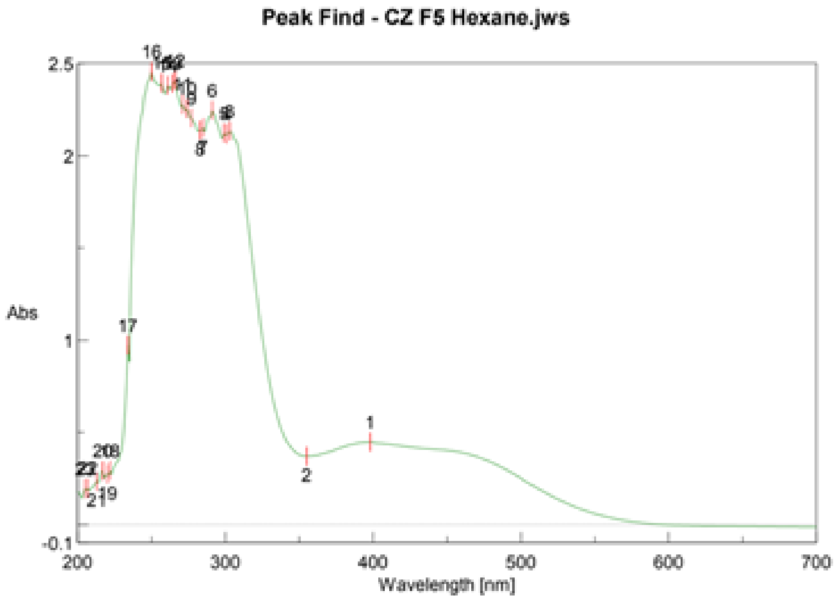

Figure 9: Absorption Spectra of F5 in Czapek'sDox broth-Hexane extract.Absorbance Unit at max.wavelength (400nm) per 100ml=1.2

Significant findings of our work was production of water soluble orange pigment in liquid media that can be easily recovered and may find water based applications .In liquid media both polar and non-polar pigments were produced. Non-polar pigments were extracted in hexane and extracellular pigments were extracted in water.

Rice was used as growth medium as *Monascus* grows readily on rice, the strains F1, F2, F3, F4 and F5 were innoculated on rice as solid growth medium. Zinc Chloride was added as strains may produce pigments in its presence. Butno growth was observed except strain F3[Table 3] in rice as medium. Strain F3 showed growth and may also produce pigments by further manipulation of media components.The present study brought out two promising pigment producing fungal strains.The chemical nature of pigments varied from polar to non-polar, which can be advantageously used in industrial applications. Color range can be enhanced by manipulating media and/or culture conditions.Orange dye was produced by strain F5 in modified Czapek's Broth. Novel and/or well known color leads could be obtained further by purifying pigment extracts. Toxicity evaluation, stability studies, process optimization, assessment of pigment composition and stability studies are the future perspectives.

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