International Journal of Biological Research (IJBR)

SREEKANTH B et.al, 2014

ISSN-2321-0524

Research Paper

# ISOLATION ANDSCREENINGOFFUNGALSTRAINSFOR BIOCOLOR PRODUCTION

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## ABSTRACT

Color is the main feature of any food item as it enhances the appeal and acceptability of food. During processing, a substantial amount of color is lost, and makes any food commodity attractive to the consumers; synthetic or natural colors are added. The main food biocolorants are carotenoids, flavanoids, anthocyanidins, chlorophyll, betalain and crocin, which are extracted from several horticultural plants. In addition to food coloring, biocolorants also act as antimicrobials, antioxygens and thereby prevent several diseases and disorders in human beings. Although, biocolorants have several potential benefits, yet tedious extraction procedures, lower color value, higher cost than synthetic dyes, instability during processing etc., hinder their popularity. Although, it is presumed that with the use of modern techniques of biotechnology, these problems in extraction procedures will be reduced. To meet the growing demand, more detailed studies on the production and stability of biocolorants are necessary while ensuring biosafety and proper legislation. Therefore, the present study is one of the approaches that, to isolate a new strain in order to produce appreciable amounts of biocolor.

**Keywords:**biocolorants, isolation, screening

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## INTRODUCTION

Color becomes the most sensitive part of any commodity not only for its appeal but also it enhances consumer acceptability. In addition, the color of a food substance is important to indicate its freshness and safety that is also indices of good aesthetic and sensorial values. The demand for natural source of such compounds is increasing day by day because of awareness of positive health benefit out of natural compounds. It therefore, necessitates looking into natural sources of food grade colorants and their use potentials. It is found more justified to use the term biocolorant instead of biopigment. Since the pigments are mostly water insoluble with exceptions of certain pigments of biological origin (Pritam Chattopadhyay *et al.*2008).

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Not all fungi produce an appropriate color, and not all fungal colors can be used successfully. To date, rather drab lichens and fruiting bodies of *Macromycetes* have been used as a source of dyes. Most microscopic forms of fungi have not been tested for their staining properties. Because many fungi can become colored in culture, they may be a useful source of dye, especially if the dye is required in any great quantity. Some fungi from obvious colors in their spore carp. However, the colors of many fungi change rapidly or fade during extraction, limiting the value of the commercial product. Other fungi have a color that is unpredictable. The lack of predictability of color also limits the use of the dye. The search for new dyes among the fungi, and determination of their properties require a more scientific approach (Alison and Downham, 1999).

## MATERIALS AND METHODS

### Collection of samples

The survey for isolating some of the untapped wild strains was carried out. In the survey, the areas covered were very diverse, major focused on the areas that were never or very minimally surveyed. So different soil samples in and around Bengaluru district from diversified habitats were collected for the isolation of biocolor producing micro-organisms from following habitats.

**a. Soil samples:** Garden Soil, agricultural soils, soil sample around general sewage and domestic sewage.

**b. Rotten vegetables:** Vegetable market

**c. Rotten Fruits:** Fruit market

**Isolation of fungi for biocolor production**

Samples collected from the said location were brought to the laboratory in sterile polythene bags. These samples were aseptically plated on Potato Dextrose Agar (PDA) media by using 0.1 mL of sample (by serial dilution method) was to avoid the overcrowding of the organisms on the plates. The plates were incubated for 48-72 hrs at 28°C. The plates were exhibited isolated colonies of varied fungi. The isolates obtained were preserved in PDA slants at a temperature of 4°C (Aneja, 1993). Further, the tentatively identified strains were labeled serially as **FS1 to FS20.**

**Rapid plate assay for screening of biocolor producer**

The fungal isolates obtained from the above steps were further subjected to rapid plate assay for screening of biocolor producing isolates on Sabourauds Dextrose Agar (SDA) media. The plates containing SDA medium were inoculated with the spore suspension of the fungi and incubated at 28°C for a period of one week. The control plate was maintained. The isolate which gave maximum production of biocolor was selected for further studies.

**Identification and characteristics features of biocolor producing fungi**

The fungal isolates based on the prominent zone of brownish- red pigment production on SDA plates were studied intensively for colony characteristics, microscopic and growth characteristics.

**Colony characteristics:** The incubated plate was observed for the standard colony characters as per the method of K. H. Domsch and W. Gams (1972). The details of the colony growth and texture were also studied.

**Microscopic characters**: The fungal isolate was picked up and examined for the essential microscopic features by doing a wet mount under a microscope with lacto phenol blue staining. The isolate was then maintained as stock at 4°C on a slant of PDA for further studies.

## RESULTS AND DISCUSSION

### Isolation of fungi for biocolor production

The isolation pattern of fungi is presented in table1. In the present study, 20 isolates were isolated and named serially from FS1 to FS20. Amongst the samples used for the isolation of fungi, soil from rotten vegetables yielded maximum number of isolates (12), whereas rotten fruits, garden soil and agricultural soil yielded 5, 2, 1 isolates respectively.

| SL.NO | SOURCES | NO. OF FUNGAL ISOLATES |
|---|---|---|
| 01 | Garden Soil | 02 |
| 02 | Agricultural Soil | 01 |
| 03 | Rotten Vegetables | 12 |
| 04 | Rotten Fruits | 05 |

**Table 1:** Isolation pattern of fungal isolates for biocolor production

### Rapid plate assay for screening of biocolor producer

The results from plate assay are presented in Figure1. The results revealed that all isolates showed different range of zone of diameter. Therefore, for the convenience, the grouping of strains of fungi has been done on the basis of zone of diameter they exhibited. It is proposed that the strain exhibiting zone of diameter above 0.9 mm are referred as good or high bicolor producers, those strains with zone of diameter 0.6 to 0.9 mm and those having below 0.6mm zone of diameter may be referred to as moderate and poor biocolor producers respectively. As per this grouping the isolate FS10 exhibited higher zone of diameter and considered as potential strain for biocolor production among isolates obtained from the different samples. As such, strain FS2, FS9, FS15 can be treated as moderate biocolor producers and remaining isolates treated as poor biocolor producers.

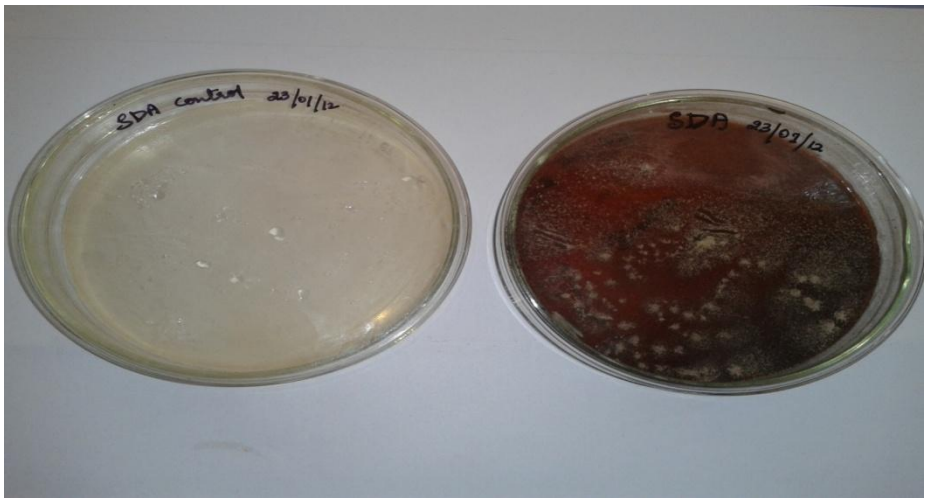


**Figure 1:** Rapid Plate Assay for Biocolor Production

**Identification of potential biocolour producing isolate FS10**

On the basis of its morphology (Figure 2) and microscopic features the strain was identified as *Penicillium sp.*

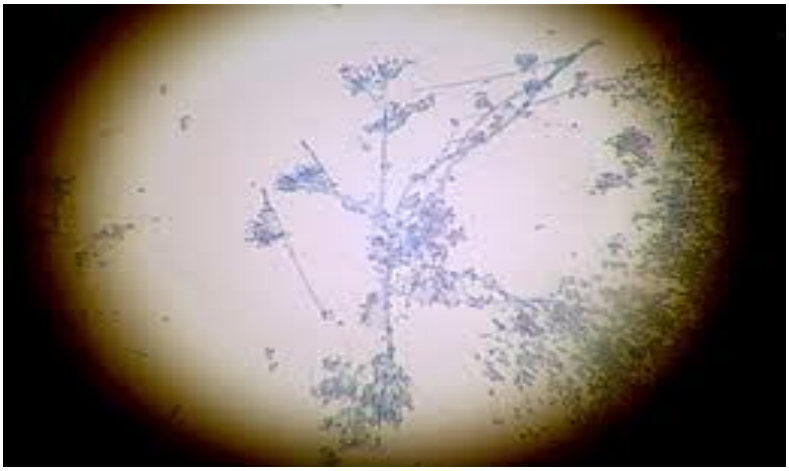

**Figure 4.2** Microscopic appearance of *Penicillium FS10*

**CONCLUSION**

Fungi from the genus *Penicillium sp.* are a promising source for natural color additive and reducing blood cholesterol. However, before effectively applying *Penicillium*to foods or dietary supplement, it is important to select and control the fermentation condition to obtain large amounts of required substances such as pigment but with little or no citrinin. Despite this toxicity problem, *Penicillium* pigments may be quickly produced in large scale throughout the year in industrial facilities, so that it might become an industrially important pigment. The key is to find strain which produce pigment with as little citrinin as possible. In the present study, fungi as source of biocolor production are studied. Mainly due to the enhanced yield and exceptional stability of biocolor, it has a broader

perceptive in varied commercial applications. Here, it aimed to isolate and select biocolor producing potent fungi, using standard screening techniques. *Penicillium FS10* is isolated and screened for the production of biocolor.

**REFERENCES**


AkramNeshati (2010). "Extraction and Characterization of Purple Pigment from *Chromobacterium Violaceum* Grown in Agricultural Wastes". Faculty of Science, *Universiti Teknologi Malaysia.* 34-36.

Aneja K. R. (1993). "Experimental in Microbiology Plant Pathology and Tissue Culture". *VishwaPrakashan*, IBBN: 81-7328-0118, 117-120.

Rymbai, H, R.R. Sharma, Manish Srivastav (2011), "Biocolorants and its implications in Health and Food Industry - A Review", *International Journal of Pharmaceutical Technology and Research* Vol.3, No.4, pp 2228-2244.

Julova P., Martinkona L. and Kren V. (1996) "Secondary Metabolites of the Fungus *Monascus:* a review" *Journal of Industrial Microbiology* 16: 163-170.

K. H. Domsch and W. Gams (1972). "A Book of Soil Fungi of Agricultural Soils".
Lingappa K. and C. S. VivekBabu (2005). "Production of Lovastanin by Solid State Fermentation of Carab Pods Using A. Terrus KLVB-28". *Indian Journal of Microbiology*, 45(4), 283-286.

Masahiro, K.O., Mine, K., Taya, M., Tone, S. and Ichi, T. (1994) "Production and release of anthraquinone pigments by hairy roots of madder (Rubia tinctorum L.) under improved culture conditions". *Journal of Fermentation and Bioengineering* 77, 103–106.

Mohan A. Dhale, Vijay-Raj A. S. 2009. "Pigment and amylase production in *Penicillium sp* NIOM-02 and its radical scavenging activity". *International Journal of Food Science Technology*, vol. 44(12); 2424-2430.

P.S. Hamano and B.V. Kilikian (Dec 2006). "Production of Red Pigments by *M. ruber* in Culture Media Containing Corn Steep Liquor". *Brazilian Journal of Chemical Engineering*, 23 (4): 443-449.

Park, J.P., Kim, S.W., Hwang, H.J. and Yun, J.W. (2001) "Optimization of submerged culture conditions for the mycelial growth and Exo-biopolymer production by Cordyceps militaris". *Letters in Applied Microbiology* 32, 1–6.

Patcharee Pattanagul, RenuPinthong, Aphirak Phianmonghol, Noppol Leksawasdi (2007). "Review of Ang-kak Production *(Monascus. Purpurens)*". *Chiang Mai Journal of Science*, 34 (3): 319-328.

Patrick J. Evans and Henry Y. Wang. (June 1984). "Pigment Production from Immobilized *Monascus sp.* Utilizing Polymeric Resin Adsorption". *Applied and Environmental Microbiology*, 47 (6): 1323-1326.

Pritam Chattopadhyay, SandipanChatterjee and Sukanta K. Sen (2008), "Biotechnological potential of natural food grade biocolorants", *African Journal of Biotechnology* Vol.7 (17), pp. 2972-2985.

Pattanagul P. (2002). "Use of vegetable oil, angkak soy protein isolate and tropica starch to improve the quality of sausages", Chiang Mai University, Thailand.

Sameer A M, Anne SM, Ulf T (2006). "Colorimetric Characterization for Comparative Analysis of Fungal Pigments and Natural Food Colorants". *Journal of Agriculture and Food Chemistry* 54: 7027-7035.

Sanae Chiba, Naoko Tsyoshi, Ryosuke Fudou, Makoto Ojika, Yoshimasa Murakami, Yoshiro Ogoma, Masakatu Oguchi, Yamanaka, 2003. "Magenta pigment produced by fungus". *Journal of General Applied Microbiology*, 52, 201-207.

Su, Y.C., Chen, W.L. and Lee, Y.H. (1973), "Studies on the Anka-Pigment Produced by a Mutant of *M.anka*. Memorial Collection of Agriculture". National Taiwan University, 14: 41-56.

Walford J. (1980). "Historical Development of Food Colouration In: Developments in Food Colours". London. UK: *Applied Science Publishers*, 1: 1-25.

Y.J. Cho, J.P. Park, H.J. Hwang, S.W. Kim, J.W. Choi and J.W. Yun (2002), "Production of red pigment by submerged culture of *Paecilomyces sinclairii*", *Letters in Applied Microbiology*, 35, 195–202.

Y. R. Tseng, M. T. Chen and C. F. Lin (2000). "Growth pigment production and protease activity of Monascus purpures as affected by salt, sodium nitrite, polyphosphate and various sugars". *Journal of Applied Microbiology*, 88: 31-37.

Zhang J. and Gresaham R. (1999). "Chemically defined media of commercial fermentations". *Applied Microbiology, Biotechnology*, 51: 407-421.