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Effect of Carbon and Nitrogen Sources on Stimulation of Pigment Production by *Monascus purpureus* on Jackfruit Seeds

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Abstract: Food colorants from natural sources are preferred over the synthetic variants which elicits various adverse effects including teratogenicity and carcinogenicity. Pigments produced from *Monascus spp.* are of microbial origin and can be used as food grade biocolorants. The aim of the present work was to investigate the feasibility of Jackfruit seed as a substrate supplemented with carbon sources like mannitol, lactose, starch and fructose and nitrogen sources like yeast extract, peptone, ammonium sulphate and ammonium nitrate for the production of pigments by *Monascus purpureus* in solid-state fermentation (SSF). The absorbance maxima of pigment extract was measured by spectral analysis. The maximum pigment production was found when *Monascus purpureus* culture is supplemented with fructose as carbon source and yeast extract as the nitrogen source.

Key words: Solid state fermentation · *Monascus purpureus* · Pigment · Carbon and Nitrogen sources

INTRODUCTION

The most commonly used food grade pigments are chemical compounds containing nitrite and nitrate salts. These synthetic compounds have been reported to have carcinogenic and teratogenic effects. This has been one of the major reasons for the increased interest in producing pigments from biological origin like plants and microorganisms [1]. The best known of the microbial colorants are produced by *Monascus* group, specifically *M. ruber, M. anka* and *M. purpureus*, because of their chemical stability. During growth, *Monascus* spp. breaks down starch substrate into several metabolites, of which pigments are produced as secondary metabolites. The structure of pigments depends on type of substrate and other specific factors during culture such as pH, temperature and moisture content [2].

Monascus fungi produce at least six major related pigments which can be categorized into 3 groups based on color as follows: yellow pigments: monascin ($C_{21}H_{26}O_5$) and ankaflavin ($C_{23}H_{30}O_5$); orange pigments: monascorubrin ($C_{23}H_{26}O_5$) and rubropunctatin ($C_{21}H_{22}O_5$); and red pigments: monascorubramine ($C_{23}H_{27}NO_4$) and rubropuntamine ($C_{21}H_{23}NO_4$) [3-5]. The color of the pigment is influenced by the culture conditions, pH value and the carbon and nitrogen sources in the substrate [6, 7]. *Monascus* pigments can be obtained from both solid-state and submerged culture. In Solid state fermentation process, the substrates not only supply the nutrients to the microbial culture growing on it, but also serve as an anchorage for the cells resulting in high pigment productivity. Moreover, it is a low-cost process using agro-industrial residues as substrates [8, 9]. Cheap agricultural products and residues were used as substrates for pigment production such as rice broken, wheat bran, jackfruit seeds, palm kernel cake, cassava starch etc. [10-13]. Since the jackfruit seeds are rich in carbohydrate, protein and trace elements, it can be used as a potential substrate for the production of food grade pigments. In the present study, the effects of carbon and nitrogen sources on red and yellow pigment production were evaluated.

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MATERIALS AND METHODS

Materials: Jackfruit seeds were procured from the local market in Thanjavur and air dried. *Monascus purpureus* was cultured at Indian Institute of Crop Processing and Technology and used for pigment production. *Monascus purpureus* was maintained in potato dextrose agar slant and sub cultured in yeast phosphate soluble starch medium (yeast extract-4.0 g, soluble starch-15.0 g, K_2HPO_4-1.0 g, KH_2PO_4-1.0 g, agar-20.0 g, distilled water-1000 ml, pH 6.5).

Production of Pigments by Solid State Fermentation: Jackfruit seeds were grated and about 20 g in weight was used as substrate. Different carbon sources like mannitol, lactose, starch and fructose at the concentration of 3% w/w of the substrate were used. The jackfruit seed substrate was supplemented with nitrogen source like yeast extract, peptone, ammonium sulphate or ammonium nitrate at a concentration of 0.5% w/w of substrate. This medium was then moistened to 60% as analysed by moisture analyser [14]. The samples were labelled as C1-Jackfruit seed + mannitol, C2-Jackfruit seed + fructose, C3-Jackfruit seed + lactose, C4-Jackfruit seed + starch, control-Jackfruit seed alone, N1-Jackfruit seed + yeast extract, N2-Jackfruit seed + peptone, N3-Jackfruit seed + ammonium sulphate, N4-Jackfruit seed+ ammonium nitrate and autoclaved. All the samples were inoculated with 1 ml of 14-days-old culture of *M. purpureus* and incubated at 30°C for 7-14 days. After incubation, the flasks were sterilized and *Monascus* fermented products were dried at 50°C for 24 hours. Dried Monascus Fermented Product (MFP) was pulverized in a Udy cyclone mill and used for further analysis.

Extraction and Estimation of Pigments: The soluble extra-cellular pigments were extracted with ethanol by adding 50 mL of 70% Ethanol to 0.5 g of MFP and incubated in water bath at 60°C for 2 hours and ?ltered through Whatmann No.1 ?lter paper. Pigment estimation was done as described by Tseng *et al.* [15] in which the optical density of the ethanol extract was expressed as a function of the pigment concentration. The absorbance maxima of pigment extract was measured by spectral analysis at 510 and 410 nm as an estimate of red and yellow pigments, respectively using a double beam spectrophotometer (Shimadzu UV 1601). Pigment yield was expressed as OD at its λ_{max} per gram dry fermented matter [16].

RESULTS

Growth of *M. purpureus* in Solid State Fermentation: The growth of the fungus was monitored at intervals of 2, 7 and 14 days of incubation. Fungal mycelia could be observed from second day in the samples supplemented whereas unsupplemented samples (control) show growth only after five days. After 14 days of incubation, dense growth and pigment production were noted in Nitrogen and Carbon supplemented samples when compared with the control. The results were tabulated in Table 1.

Effect of Carbon Source on Pigment Production: The effect of various carbon sources supplemented to jackfruit seed substrate on the red pigment production was studied by measuring the absorbance at 510 and 410 nm as an estimate of red and yellow pigments, respectively and the results are given in figure 1. Fructose as the carbon source was found to give the maximum yield of 1.304 U/g and 0.497 U/g for red and yellow pigments respectively. Next to fructose, starch yielded 1.079 U/g and 0.401U/g. Lactose supplemented sample produced 0.711 U/g and 0.313 U/g and mannitol with the least pigment yield of 0.579 U/g and 0.282 U/g when compared with other carbon cupplemented samples. Fructose supplementation resulted in about 4.5% increase in yield.

Effect of Nitrogen Source on Pigment Production: From the spectral analysis observed for changes in different nitrogen sources, yeast extract was found to yield maximum pigment of 1.29 U/g and 0.725 U/g followed by peptone 0.921 U/g and 0.551 U/g, ammonium sulphate 0.616 U/g and 0.392 U/g and ammonium nitrate 0.482 U/g and 0.382 U/g. As compared to the control, addition of nitrogen sources increased the yield of pigment upto 8.5% (Fig. 2).

Table 1: Growth of *M. purpureus* in the control and supplemented samples

Incubation Period	Control Jackfruit seed	Jackfruit seed + Mannitol	Jackfruit seed + Fructose	Jackfruit seed + Starch	Jackfruit seed + Lactose	Jackfruit seed + Yeast extract	Jackfruit seed + Peptone	Jackfruit seed + Ammonium nitrate	Jackfruit seed +Ammoniumsulphate
2 days	-	+	+	+	+	+	+	-	-
7 days	+	++	++	++	++	++	++	+	+
14 days	++	++	+++	+++	++	+++	++	++	++

-No Growth; + Moderate Growth; ++ Dense Growth; +++ Dark pigmentation with dense growth

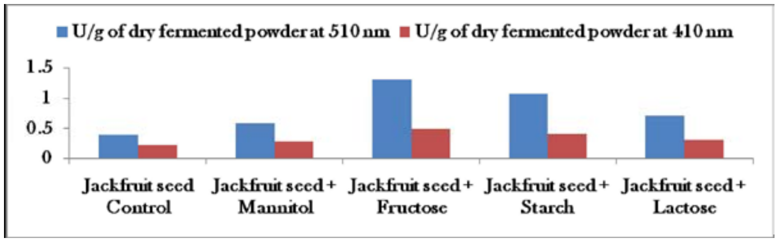

Fig. 1: Effect of carbon source supplementation on pigment yield

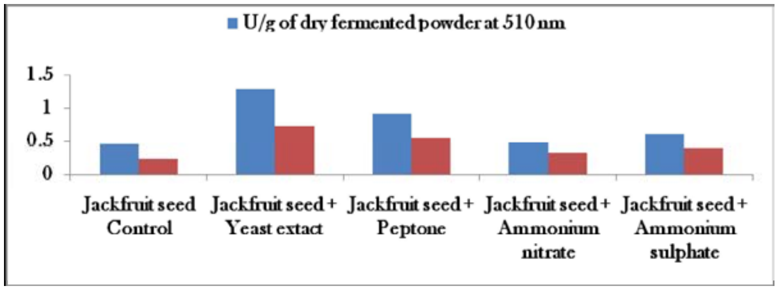

Fig. 2: Effect of nitrogen source supplementation on pigment yield

DISCUSSION

The interest in red pigments produced by *Monascus spp.* for use in the food industry has been mounting given the flexibility in production and easy down streaming process. *Monascus* is reported to produce non-toxic pigments, which can be used as food colorant. Besides to adding color, it enhances the flavor of the food and acts a food preservative. The use of jackfruit seeds for substrate is cost-effective as well as environment friendly. Studies conducted by Sumathy Babitha *et al.,* [12] proved addition of nitrogen sources like monosodium glutamate, peptone, soybean meal and chitin powder enhanced pigment production in Jack fruit seed substrate. Chang Chai Ng *et al.,* [17] proved medium that comprised 5% glucose and 1.5% ammonium phosphate produced the most pigment. Previous studies on effect of nitrogen sources on broken rice proved yeast extract has the maximum yield compared to monosodium glutamate and added that organic nitrogen sources gave a better yield than inorganic sources. The authors could report that supplementation of the substrate with carbon and nitrogen sources for yield enhancement. Furthermore, this study reveals that the addition of nitrogen source significantly increases the pigment production when compared to carbon sources.

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