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Optimization of Biopigments Production from *Monascus purpureus* Using Different Substrates

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Monascus purpureus **was cultivated under solid state fermentation and submerged state fermentation in the laboratory. The substrates corn, sweet potato and double beans were used and these substrates were assayed at different sizes and concentrations in solid state and submerged state fermentation respectively for highest pigment production. Under solid state fermentation, reference strain (*Monascus purpureus* MTCC410) produced highest quantity of yellow pigment (92. 7 CVU / g) in the substrate sweet potato of 2 - 4 mm size and more red pigment yield (97.33 CVU / g) was observed in the substrate maize of 3-6 mm size. Isolate MP (PMPO) produced highest yellow pigment (90.93 CVU / g) in sweet potato of 2-4 mm size and where as red pigment yield (83.0 CVU / g) was more in sweet potato of 2-4 mm size. Sweet potato proved to be the best substrate for pigment production. Under submerged state fermentation conditions, reference strain produced higher quantity of yellow pigment in all the tested substrates at 1: 1 dilution of substrate filtrate. Red pigment production by reference strain was highest in maize followed by sweet potato at concentration of 1:1 dilution of where as 1:2 dilutions gave good pigment values in case of double beans.**

Key words: Substrate, Fermentation, Pigment, Monascus, CVU (colour value units).

Pigments are formed in nature by variety of plants, animals and microorganisms. The production of many currently approved natural pigments has a number of disadvantages including dependence on the supply of raw materials and variance in pigments. In this regard, microorganisms are particularly of interest because they proved to be a readily available alternative source of naturally derived food colorants that could easily be produced in large quantities and there is an increasing pressure to minimize the damage to the environment. The industries are continuously looking for cheaper, more eco-friendly pigments. As a result, there has been considerable interest world wide in the development of pigments from natural sources. Colourants from microbial species offer advantages since they are not subjected to the vagaries of nature and they are good alternatives and safer.

The pigments produced by the *Monascus* fungi are well known in orient. *Monascus* sp. is well known for its production of water soluble pigments These pigments are rubropunctatine, monascorubrine, rubropunctamine, monascorubramine, monacine and ankaflavine

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(Sweeny *et al*., 1981). *Monascus* pigments have a great potential in food processing industry. It is not only used as food additive or colouring agent in industries but also used as medicine in the treatment of various diseases. In recent years, secondary metabolic products of *Monascus* sp. become the key research topic. This includes *Monascus* pigments (red, orange and yellow pigments) (Blanc *et al.,* 1994), antibacterial activity substances (Wong *et al.*, 1981), cholesterol synthetic inhibitor monacolin-K (Endo, 1979), antihypertensive substance-γ-aminobutyric acid GABA) (Kono and Himeno 2000), antioxidant action (Anyia *et al.,* 1999), antitumor promotion action (Yasukawa *et al.,* 1994), and immunosuppressive action (Martinkova *et al.,* 1995).

In India not much work has been accomplished in the field of bio-pigment production from microorganisms. This appears to have a wide scope in the industrial production of bio-pigments from *Monascus* sp. With this review, the present study was undertaken to standardize the use of different substrates for highest pigment production.

MATERIAL AND METHODS

The monascus isolate MP (POMO) was isolated from the phyllosphere of pomegranate. Isolate MP (POMO) isolated from phyllosphere of pomegranate was used along with the reference strain *Monascus purpureus* MTCC 410 obtained from the Microbial Type Culture Collection. IMTECH, Chandigarh, India and maintained on malt extract agar media. Mycelial discs of 0.6 cm diameter were cut from the margin of the mycelial mat with the help of sterile incinerated cork borer and used as inoculum. Reference strain *Monascus purpureus* MTCC 410 and *Monascus* isolate MP (POMO) were grown under solid-state fermentation and submerged liquid culture fermentation conditions using three different substrates.

For solid state fermentation, three substrates were used *viz.,* corn, double bean, and sweet potato of different sizes. Sweet Potato (6-10, 4-6 and 2-4 mm), Corn (Whole grain, 3-5 and 1-3 mm) and Double bean. (Cotyledons, 4-6 and 2-5 mm)

A quantity of 50 g of substrate was taken in 250 ml conical flasks and soaked for 10 hours in water. After draining the excess water, the flasks were steam sterilized. Two 0.6 cm diameter discs of *Monascus purpureus* fungal mat were inoculated to the flask. The contents of the flask were stirred with sterile scalpel. After incubation of 15 days, the contents were dried at 40 ^{0}C in an oven. After thorough drying, the material was ground in to fine powder in a porcelain pestle and mortar. The fine powder was used to estimate the pigment production.

For submerged liquid state fermentation *Monascus purpureus* MTCC 410 and isolate MP (POMO) were grown in different substrate filtrates at different concentrations. Maize and double beans substrates (500 g) were soaked for 8 hours. Sweet potato were washed with water and cut into small pieces. Substrates were ground with double volume of distilled water and made into solution, solutions were filtered through two layers of muslin cloth, and filtrates were diluted to different concentration (1:1, 1:2, 1:3 and 1:4) by using distilled water. The filtrate solution was steam sterilized and then inoculated with reference *Monascus purpureus* strain and *Monascus* isolate. The flasks were placed over a wrist action shaker set at 150 strokes per min. The flasks were incubated at room temperature (28^{0}C) for 15 days.

Powdered solid state fermented product (1g) and liquid state fermented broth (1ml) was taken in a test tube and 10 ml of 70 % ethyl alcohol was added and the pigment was extracted by boiling on a water bath for 10 min. Then the contents were centrifuged at 5000g for 10 min. The clear supernatant was diluted with nine ml of distilled water; absorbance of the solution was read in a spectrophotometer at 300 and 500 nm for yellow and red pigments respectively. The absorbance values were converted into pigment units using formula given by Ratana and Toshioma (1987)

$$\text{Colour value} = \frac{\text{O.D X dilution}}{\text{Amount of samples (g)}} \times \text{Volume of extracts}$$

RESULTS AND DISCUSSION

Under solid state fermentation, reference strain *Monascus purpureus* MTCC 410 produced

highest yellow pigment in sweet potato of 2 - 4 mm size (92. 7 CVU / g) and least was in maize whole grain and red pigment yield was highest in maize of 3-6 mm (97.33 CVU / g), followed by sweet potato of 2-4 mm (94.16 CVU / g).

Monascus isolate MP (POMO) produced highest yellow pigment in sweet potato of 2-4 mm (90.93 CVU / g) and least was in whole maize grain (22.0 CVU / g) where as red pigment yield was more in sweet potato of 2-4mm (83.0 CVU / g) and minimum in whole maize grain (19.86 CVU / g). Red pigment production was higher in case reference strain with all the substrates and sizes. Sweet potato proved to be the best substrate with respect to pigment production. The smallest size of sweet potato *i.e*., 2-4 mm encouraged higher yield of pigment. The results revealed that MP (POMO) isolate and *M. purpureus* MTCC 410 produced more yellow and red pigment in sweet potato followed by maize and double beans. *Monascus* sp. is a starch loving fungi. Sweet potato is rich source of starch, which favoured the growth and pigments biosynthesis by *Monascus* sp. and soft texture of sweet potato helps for easy penetration and efficient utilization of nutrients. In maize, though it is starch rich but waxy skin and tight construction of the corn starch become impediments for efficient establishment and nutrient utilization.

Pigments synthesis and growth are markedly affected. Pigment synthesis was less in double beans, which may be due to its proteineous nature. As for the size of substrates is concerned, more pigments synthesis was observed in maize (1-3 mm), sweet potato (2-4 mm) and double beans (2-3 mm). As the size of the substrates decreases, organisms get more surface area, which helps, for better mycelial growth and efficient utilization of nutrients. Pigments production was more in solid state compared to submerged conditions. It might be the availability of support as an anchor for optimal growth and productivity. In case of solid state fermentation, more aeration also supported more pigment synthesis and it provided natural environment for better establishment. It is interesting to note that the solid state fermentation has advantages over submerged conditions in terms of pigment yield

Table 1. *Monascus* pigments production using different substrates under solid state fermentation.

Treatment	*Monascus purpureus* MTCC 410 Colour value units / g		MP (POMO) Colour value units / g	
	Yellow	Red	Yellow	Red
S_1M	21.66	26.50	22.00	19.86
S_2M	72.46	67.33	40.10	33.23
S_3M	70.46	81.73	73.00	68.48
S. Em±	0.94	1.63	0.51	1.07
C.D. at 5%	3.25	5.67	1.77	3.70
S_1S	82.30	82.90	78.16	86.16
S_2S	83.20	91.50	80.83	88.00
S_3S	92.70	94.16	83.00	90.93
S. Em±	0.47	0.32	0.86	0.49
C.D. at 5%	1.64	1.11	2.98	1.70
S_1B	63.86	63.56	61.23	65.93
S_2B	70.63	74.50	66.00	72.43
S_3B	76.30	75.90	70.43	74.53
S. Em±	0.64	1.47	0.45	0.53
C.D. at 5%	2.22	5.09	1.56	1.85

Note: MP (POMO) - Isolated from phyllosphere of Pomegranate.

S_1M: whole grain (maize) S_1S: 6-10 mm (sweet potato)

S_1B: cotyledons (double beans) S_2M: 3-6 mm S_2S: 4-6 mm

S_2B: 3-5 mm S_3M: 1-3 mm S_3S: 2-4 mm

S_3B: 2-3 mm

but disadvantages in terms of down stream process of separation of pigments from the substrates. In the present investigation, sweet potato of 2-4 mm size was found to be the best for pigment synthesis. Under solid state fermentation, the production of pigments and the other metabolites were more compared to submerged conditions (Lin, 1973; Mark *et al*., 1990)

Under submerged conditions, concentrations like 1:1, 1:2, 1:3 and 1:4 dilutions of substrate filtrate made by using distilled water were used as substrate for inoculation of the mold. Reference strain produced highest quantity of yellow pigment in 1: 1 dilutions in all the substrates and highest was observed in maize (71.0 CVU / ml). The lowest pigment production was observed in 1:1 dilution of double beans (20.26 CVU / ml). Red pigment production by reference strain was highest in maize (72.60 CVU / ml) and lowest recorded in double beans 1:1 dilution (18.16 CVU / ml). Isolate produced highest yellow pigment in 1:1 dilution of maize (78.30 CVU / ml), followed by 1:2 dilution of double beans (71.43 CVU / ml). Similarly, highest red pigment production was recorded in 1:2 dilution of maize (70.00 CVU / ml) and the least was observed in 1:1 dilution of double beans (19.40 CVU / ml). Substrate concentration of 1:1 performed better in case maize and sweet potato, where as 1:2 dilutions performed better in case of double beans.

Among the substrates, maize yielded more quantity of pigment, followed by sweet potato and double beans. Maize and sweet potato are good sources of starch that must have favoured the growth and pigment synthesis by *Monascus.* As for concentration ratios is concerned, 1:1 dilution of maize and sweet potato, 1:2 dilution of double beans yielded high quantity of pigments. Lower concentrations of substrates markedly

Table 2. *Monascus pigments* production using different substrates under submerged conditions

Treatment	*Monascus purpureus* MTCC 410 Colour value units / g		MP (POMO) Colour value units / g	
	Yellow	Red	Yellow	Red
C1M	71.00	72.60	78.30	70.00
C2M	70.60	66.40	71.30	68.00
C3M	64.30	62.40	68.10	64.00
C4M	22.06	21.13	35.50	29.00
S. Em±	0.47	1.47	0.61	0.82
C.D. at 5%	1.53	4.80	2.0	2.68
C1S	67.20	64.40	70.70	64.93
C2S	66.60	63.33	67.70	62.04
C3S	61.50	61.20	56.73	57.20
C4S	46.20	39.03	43.10	37.60
S. Em±	0.31	0.47	0.29	1.59
C.D. at 5%	1.03	1.55	0.94	5.18
C1B	20.26	18.16	24.30	19.40
C2B	66.09	64.89	71.43	68.10
C3B	65.06	62.03	66.50	69.13
C4B	59.70	58.04	63.46	60.03
S. Em±	0.17	0.38	0.51	0.28
C.D. at 5%	0.57	1.26	1.68	0.91

MP (POMO) - Isolated from phyllosphere of Pomegranate.
M= Maize; S= Sweet potato; B= Double beans
C1 = substrate filtrate: Distilled water (1:1) C 2= substrate filtrate: Distilled water (1:2)
C3= substrate filtrate: Distilled water (1:3) C4= substrate filtrate: Distilled water (1:4)

affected the pigment synthesis and growth. It might be due to the depletion of nutrients and growth factors in the media, where as in double beans, 1:2 dilutions yielded better pigmentation compared to 1:1 dilution. This might be due to, high viscosity of the substrate at 1:1, which might have prevented diffusion of oxygen in to media. With respect to pigment production, it was less under submerged condition compared to solid state fermentation. The reason can be attributed to the nutrient depletion by the organisms for its growth and deficiency of oxygen in the environment. These two factors may have affected the pigment synthesis. Enzyme degradation and catabolic repression is more in submerged condition, which results in less pigments production. The results of the present investigation are in conformation with the findings of Lee *et al.* (1994)

Plate 1. *Monascus purpureus* MTCC 410 on malt extract media

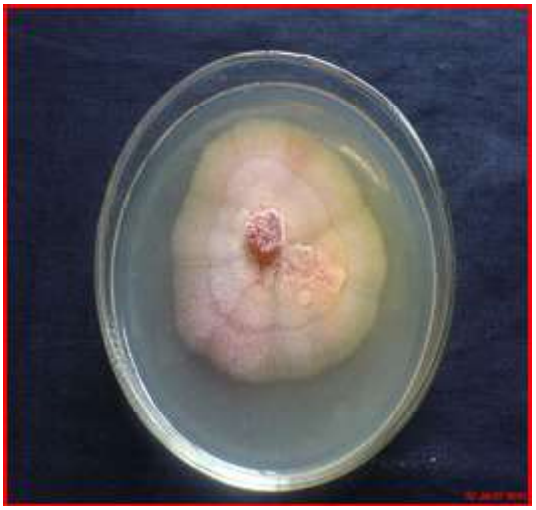
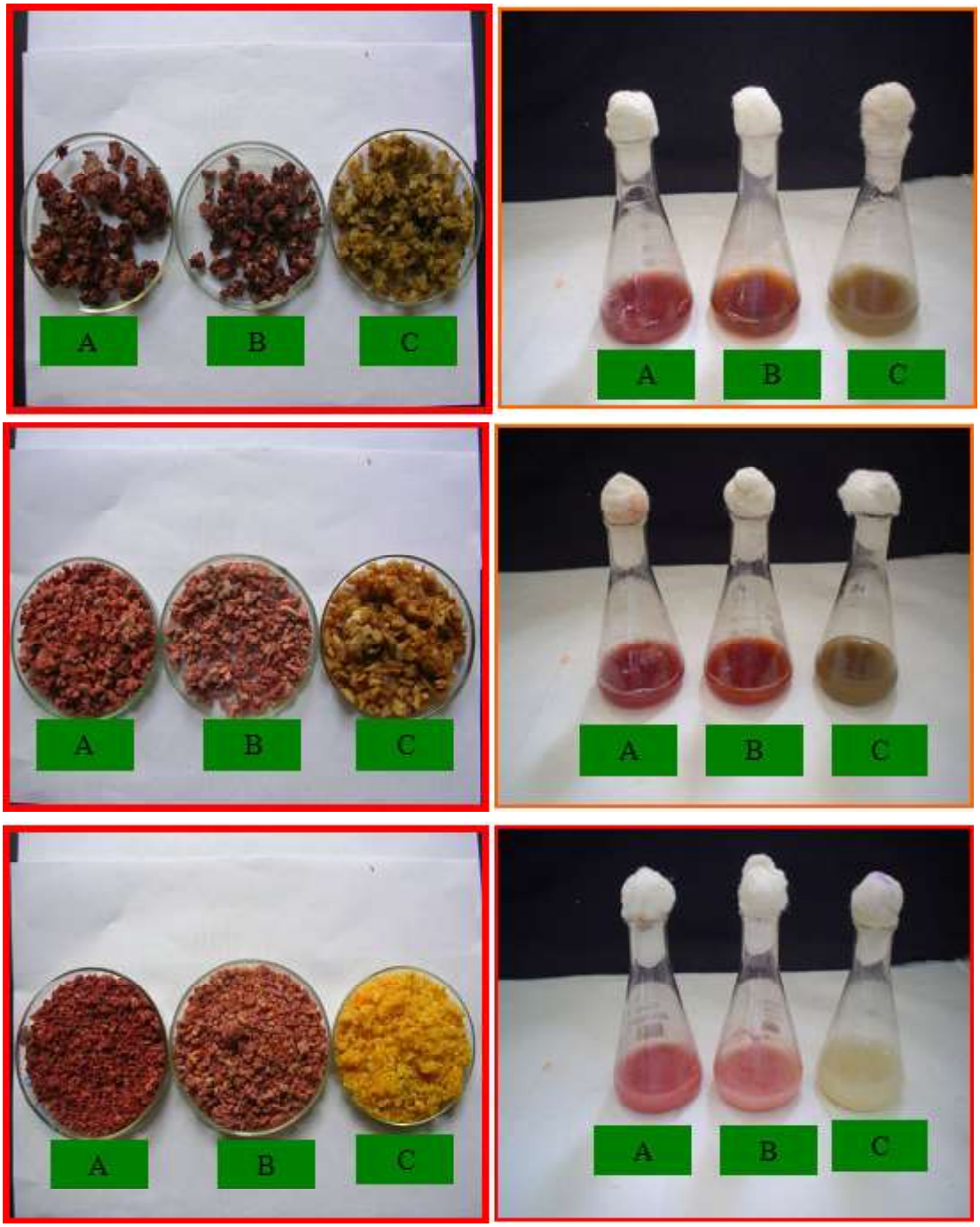


Plate 2. *Monascus* isolate on PDA media

Plate 3. *Monascus purpureus* MTCC 410 on Malt extract broth

Plate 4. *Monascus* isolate on PD broth



A: *Monascus purpureus* MTCC 410.
B : *Monascus* isolate MP (POMO)
C : Control (Un inoculated)

Solid sate fermentation:
Maize : 1-3 mm
Sweet potato : 2-4 mm
Double beans : 2-3 mm

Submerged conditions :
Maize : 1:1 dilution
Sweet potato : 1:1 dilution
Double beans : 1:2 dilution

solid state fermentation

submerged condition

Plate 5-7. Pigment production by *Monascus purpureus* using different substrates under solid state and submerged conditions

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