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Full Length Research Paper

Optimization of fermentation conditions for red pigment production from *Penicillium* sp. under submerged cultivation

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An extracellular pigment-producing ascomycetous filamentous fungi belonging to the genera *Penicillium* was obtained from soil and its optimal culture conditions investigated. The optimal culture conditions for pigment production were as follows; soluble starch 2% (670 units), peptone (880 units), pH 9.0 (900 units); temperature 30˚C (950 units), agitation 200 rpm (920 units), and inoculums age 4 days (850 units). The properties of pigments and their residual content after exposing to the various physico-chemical conditions like sunlight, fluorescent light, UV light, high temperature and preservatives (sodium bisulfate, ascorbic acid and citric acid) are also investigated.

Key words: *Penicillium*, pigment, physical factor, stability.

INTRODUCTION

There is worldwide interest in process development for the production of pigments from natural sources due to a serious safety problem with many artificial synthetic colorants, which have widely been used in foodstuff, cosmetic and pharmaceutical manufacturing processes (Kim et al., 1995). The existing authorized natural food colorants are of either plant or animal origin and have numerous drawbacks such as instability against light, heat or adverse pH, low water solubility, and are often non-availability throughout the year. Many fungi have been reported to produce non-carotenoid pigments but only a few of those have been explored as possible food colorants (Sameer et al*.,* 2006). There are number of micro-organisms which have the ability to produce pigments in high yields, including species of *Monascus*, *Paecilomyces, Serratia, Cordyceps, Streptomyces* and yellow-red and blue compounds produced by *Penicillium herquei* and *Penicillium atrovenetum.* Amongst them, many species of fungus have attracted special attention because they have the capability of producing different coloured pigments showing high chemical stability (Hajjaj et al., 2000). For industrial applications of microbial pigments, higher production of pigment yield, chemical and light stability are essential features. Isolation of new strain is still of particular interest because of necessity to obtain microorganisms with suitable characteristics for submerged cultivation (Rasheva et al., 1998). *Penicillium,* a new fungus strain produces a chromophore of the anthraquinone type, a red colorant which can be applied in the food and cosmetic industries. Some strains of same species are effective biological control agent and others have been used for production of milk clotting enzyme (Sardaryan et al*.,* 2004). Our attention was focused to isolate and screen the efficient pigment producers as a potential role in food, cosmetics and pharmaceutical industry. For commercial application, optimization of fermentation condition in order to produce more yield and stability of pigment from *Penicillium* is necessary.

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MATERIALS AND METHODS

Microorganism and inoculum preparation

Penicillium sp. was isolated from western Ghat region and identified according to Alexopolus and Mims (1979). The stock culture was maintained on a potato dextrose agar (PDA) slant. For inoculum preparation, the fungus was initially grown at 25℃ on a PDA plate for 7 days. A 0.7 cm^2 plug from the outer zone of the colony was

Table 1. Effect of carbon source on the mycelial growth and pigment production of *Penicillium* sp.

Carbon source	Pigment at 530 nm	Mycelial dry weight (g/l)	Final pH
Glucose	450	4.52	5.4
Fructose	140	5.50	5.2
Dextrose	280	4.72	6.7
Lactose	78	2.42	5.8
Sucrose	142	4.20	6.2
Maltose	550	3.78	6.5
Mannose	30	2.23	4.2
Galactose	110	3.24	4.2
Soluble starch	670	3.45	6.2
Xylose	70	5.42	6.8
Glycerol	0.0	1.2	4.7

Fermentations were carried out in flasks for 5 days at 30°C.

punched with a sterile cutter and transferred to 25 ml potato dextrose broth medium in a 250 ml flask, and grown at 25°C under basal conditions (static) or on a rotary shaker at 200 rpm for 7 days.

Optimization of culture condition

The culture conditions examined were carbon, nitrogen, pH, temperature, aereation, inoculum age and C: N ratio. Experiments were conducted in shake flasks, fungal growth and pigment production were monitored in 5 - 7 days interval, and all experiments were performed in duplicate (Cho et al*.,* 2002)

Extracellular red pigments

Red pigment production was indirectly evaluated by measuring the absorbance of the culture filtrate at 530 nm in spectrophotometer (Shimadzu).

Dry cell weight

The culture broth was centrifuged at 10 000 *g* for 20 min and the supernatant fluid filtered through a filter paper (WhatmanNo.2). The mycelial biomass yield was estimated by washing with deionized water and drying at 50°C for 48 h (Olsson and Nielsen, 1997).

Stability test for pigments

The pigment/powder was subjected to heat, light and preservative stability by Lee and Chen (2000).

RESULTS AND DISCUSSION

Effect of carbon and nitrogen source on red pigment production

For the determination of a suitable carbon source for the red pigment production, *Penicillium* was cultivated in the basal medium containing various carbon sources (2%). Of the 10 carbon sources examined, glucose, fructose, mannose and sucrose were relatively favourable to the mycelial growth of *Penicillium* sp., although the pigment production was low. The maximum mycelial growth (5.50 g l^{-1}) was achieved in fructose medium, while the maximum pigment production (670 mg l^{-1}) was obtained in soluble starch medium (Table 1). In this study, peptone, peptone–yeast extract mixture, tryptone and monosodium glutamate had a positive effect on pigment production, whereas soy peptone, beef extract and potassium nitrate strongly inhibited red pigment synthesis. Of all the nitrogen sources tested, peptone gave the highest yield for red pigment production (880 mg l^{-1}) (Table 2). It has been reported that various types of peptone supported greater pigment production in many kinds of pigment-producing fungi (Cho et al*.,* 2002).

Effect of pH and temperature on red pigment production

The pH of the culture medium has been reported to play a key role in pigment synthesis. *Penicillium* was cultivated at different initial pH values (3·0 – 9·0) in shake flask cultures. Our results (Tables 3 and 4) indicated that biomass and pigment production was slightly affected by initial pH of the medium. The highest biomass and pigment production was observed when initial pH of culture medium set at pH 9.0. These correspond with pigment production in other microorganism, in that pH 9.0 was found best for growth and pigment production (Unagul et al*.,* 2005). *Penicillium* was cultivated under various temperatures for mycelial growth and pigment production (15 – 35 °C). Consequently, the optimal temperature for both mycelial growth and pigment production was found to be 30°C. The fungi usually require long periods for submerged culture, exposing them to contamination risk; this optimal temperature is regarded as a favourable for *Penicillium* sp. This is similar to anthroquinone production by *P. oxalicum* (Sardaryan et al*.,* 2004).

Table 2. Effect of nitrogen source on the mycelial growth and pigment production of *Penicillium* sp.

Nitrogen source	Pigment at 530 nm	Mycelial dry weight (g/l)	Final pH
Peptone	880	4.25	4.8
Beef extract	25	1.7	5.2
Yeast extract	14	2.74	4.1
Yeast extract + peptone	140	2.74	4.8
Monosodium glutamate	380	3.65	6.1
Soya peptone	16	3.47	4.8
Ammonium nitrate	0.0	0.75	4.5
Tryptone	200	1.75	4.2
Sodium nitrate	90	0.54	5.3
Potassium nitrate	50	1.04	4.7

Fermentations were carried out in flasks for 5 days at 30°C.

Table 3. Effect of pH on the mycelial growth and pigment production of *Penicillium* sp.

pH	Pigment at 530 nm	Mycelial dry weight (g/l)	Final pH
3.0	120	3.42	4.5
4.0	70	2.78	4.7
5.0	550	3.45	4.8
6.0	600	1.27	4.7
7.0	620	2.87	4.2
8.0	240	5.42	4.5
9.0	900	4.75	4.9
10.0	650	3.47	4.8

Fermentations were carried out in flasks for 5 days at 30°C.

Table 4. Effect of temperature on the mycelial growth and pigment production of *Penicillium* sp.

Temperature	Pigment at 530 nm	Mycelial dry weight (g/l)	Final pH
10˚c	0.0	0.0	4.5
15˚c	0.0	0.9	4.5
25˚c	800	3.89	5.4
30˚c	950	2.65	4.8
35˚c	700	2.45	4.7

Fermentations were carried out in flasks for 5 days at 30°C.

Effect of C/N ratio on red pigment production

The effect of the C/N ratio on pigment production was investigated using soluble starch–peptone medium. As shown in Table 5, mycelial growth (4·2 g l^{-1}) and pigment production (1550 mg l^{-1}) were maximal at a C/N ratio of 1 : 1. It is noteworthy that further change of C/N ratios higher or lower than 1: 1 resulted in a decrease in red pigment production. This is comparable with the findings of Nam and Rhee (1991) and Cho et al, (2002) in that the carotenoid content of pink pigment decreased as the C/N ratio increased.

Effect of inoculum age on red pigment production

To study the effect of inoculum age on pigment production, *Penicillium* sp. was cultivated in the optimal medium with different inoculum ages from 2 to 6 day old culture at 30°C in shake flask cultures. The optimal inoculum age for pigment production was 4 d and an increase in inoculum age resulted in a decrease in mycelial growth (Table 6). Amongst several fungal physiological properties, the inoculum age usually plays an important role in fungal development (Glazebrook et al., 1992; Bae et al., 2000).

Table 5. Effect of C/N ratio on pigment production of *Penicillum* sp*

Peptone (%) (w/v)	Soluble starch (%) (w/v)				
	0.5	**1.0**	**1.5**	**2.0**	**2.5**
PY	650	690	540	580	600
DW	(3.13)	(3.4)	(3.4)	(3.35)	(3.16)
PY	1100	1550	1250	956	879
DW	(3.48)	(4.2)	(3.56)	(3.54)	(3.16)
PY	1005	1318	1350	1145	1236
DW	(3.45)	(4.1)	(3.32)	(3.47)	(4.12)
PY	396	396	810	196	250
DW	(4.12)	(4.0)	(4.15)	(4.27)	(4.51)
PY	215	250	196	260	213
DW	(4.33)	(4.12)	(4.15)	(3.17)	(4.17)

Fermentations were carried out in flasks for 5 d at 30^{0}C. PY – Pigment yield; DW - Dry weight.

Table 6. Effect of inoculum age on the mycelial growth and pigment production of *Penicillium* sp.

Inoculum age (days)	Pigment at 530 nm	Mycelial dry weight (g/l)	Final pH
2	330	1.2	4.6
3	600	1.9	4.2
4	850	3.2	4.8
5	750	2.8	4.9
6	557	2.1	5.2

Fermentations were carried out in flasks for 5 days at 30^{o}C.

Table 7. Effect of agitation on the mycelial growth and pigment production of *Penicillium* sp.

Agitation (rpm)	Pigment at 530 nm	Mycelial dry weight (g/l)	Final pH
50	320	1.90	4.2
100	550	2.60	4.9
150	600	2.65	4.6
200	920	3.20	4.2
250	350	3.10	4.5

Fermentations were carried out in flasks for 5 days at 30^{o}C.

Effect of agitation on pigment production

The growth of fungi and pigment production was studied in various agitation speeds. The level of pigment production varied at different agitation speeds (Table 7). The pigment production was increased up to 200 rpm (920 mg l^{-1}) and reduced thereafter.

Residual content of pigments after exposing to various physical and chemical conditions

The pigments of fungus were subjected to various physiccal and chemical conditions and the results are presented in Table 8. It was inferred from the results that the pigments were more stable in UV light (99.2%) compared to fluorescent light and sunlight. Furthermore, the pigments were stable at 105ºC for 20 min to an extent of 95.5% whereas the stability reduced to 94.0% when autoclaved at 121ºC for 20 min. When the pigments were treated with preservatives, it was found that citric acid was a good preservative followed by ascorbic acid and sodium bisulfite. Citric acid did not have any effect on the colour intensity of the pigments (Lee and Chen, 2000).

Conclusion

By optimization of culture conditions for *Penicillium* sp. an almost seven fold improvement in red pigment production was achieved under optimal culture conditions by using submerged fermentation. The high concentration of pig-

Table 8. Properties of *Penicillium* sp. red pigment and their residual content after different treatments.

Parameter	Properties
Colour content (OD units)	1550
Water solubility	Soluble
Hue (at 0.1%)	Dark red
Hygroscopy	Little
Residual content	**Amount (%)**
Sunlight for 4 h (11 am to 3 pm)	79.3
Fluorescent light	98.0
UV light	99.2
121˚C for 20 min	94.0
105˚C for 20 min	95.5
Sodium bisulfite (0.1%, w/v), pH 7.0, 80˚C for 1 h)	88.1
Ascorbic acid (0.1%, w/v), pH 7.0, 80˚C for 1 h)	78.2
Citric acid (0.1%, w/v), pH 7.0, 80˚C for 1 h)	100

ment produced in *Penicillium* sp. demonstrates the possibility of commercial production of pigment by this strain, considering its relatively high production yield and light stability. The chemical and biological properties of the pigment will be investigated in detail.

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