EFFECTS OF METHOD AND CULTIVATION CONDITIONS ON PIGMENTS AND MONACOLIN PRODUCTION BY STRAINS OF *MONASCUS PURPUREUS* 5057 AND 5085

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1. INTRODUCTION

The red fungus of *Monascus* sp. have been used for preparation of oriental fermented foods such as red soyabean cheese, preservaties, color and flavor in processing of meat, fish, red rice wine and beverages in many Asean countries such as China, Japan, Taiwan, Korea, Philippines, etc for centuries [1, 3]. After fermentation by soilid state or submerged methods *Monascus* sp. produced at least six moleculas of natural pigments of three group as yellow (ankaflavin and monascin), orange (rubropunctatin and monascorubrin) and red (rubropunctamine and monascorubramine). *Monascus* fungus also able to produce other secondary metabolites as ethanol, coenzyme, and compounds with anticholesterollemic activity (Monacolin), antihypertension as γ - aminobutyric acid (GABA) [4, 5, 7].

The aim of the work is to screen a suitable fermentation method and conditions for pigment and Monacolin production by *Monascus purpureus* 5057 and 5085.

2. MATERIALS AND METHODS

2.1. Strains and culture conditions

Two strains of *Monascus purpureus* 5057 and 5085 from Food Industries Research Institute's Microorganism Culture Collection were employed in this study. *Monascus* strains were maintained on potato dextrose agar (PDA) slopes at 4^{o}C.

The strains first cultured on potato dextrose agar (PDA) to induce spore formation. After cultivation at 30^{o}C for 7 days, spores were suspended in distilled water and used as innoculum for solid state fermentation or as seed culture for submerged fermentation.

Solid state fermentation was carried out as follow: 100 gram rice was steamed with 70 ml tap water. After cooling, 50 gram of steamed rice was put in 500ml Erlenmyer flask and sterilized at 121^{o}C for 15 min. Then 5 ml of distilled water suspension from a 7 days old PDA slope of *M. purpureus* strains grown at 30^{o}C was used for inoculation. After cultivation at 30^{o}C for 4-7 days, the fermented steamed rice having color from bright red to dark red was dried at 60^{o}C, then pigment and Monacolin were extracted and measured.

Submerged fermentation was carried out on a rotory shaker at 250 rpm, 30^{o}C for 4 days. Fermentaion medium contain (g/l) glucose 10, $MgSO_4.7H_2O$ 4.8; KH_2PO_4 1.5; $K_2HPO_4$1.5; NaCl 0.4; $FeSO_4.7H_2O$ 0.01; $ZnSO_4.7H_2O$ 0.01; Yeast extract 1.0; MSG (Monosodium

glutamate) 7.6. Medium for inoculum was prepared in (g/l): glucose 10, meat extract 3.0, peptone 5.0. All media were prepared with tap water and pH was adjusted to 5.5 prior to sterilization at 121^{o}C for 20 minutes.

2.2. Analytical methods

Pigment value of *Monascus's* products was determined by spectrophotometric analysis on a spectrophotometer at 370, 400, and 500 nm, corresponding to yellow, orange and red pigment concentrations, respectively [7]. Monacolin and pigments were extracted by ethanol, methanol, acetone and butanol. The results are expressed as optical density units per ml of fermented broth or per gram of wet or dried steamed rice multiplied by the dilution factor.

Analysis of Monacolin was performed by Thin Layer Chromatograms - TLC with mobile phase of chloroform, methanol and benzene. Monacolin was detected by iodine vapour of dried TLC. Commercial pure Mevinolin (Monacolin or Lovastatin) obtained from Sigma Chemical Co. was used as reference [6].

3. RESULTS AND DISCUSSION

3.1. Screening the ability of pigment and Monacolin producing by two strains of *Monascus purpureus* 5057 and 5085

The growth and pigmentation in two *M. purpureus* 5057 and 5085 in the PDA, soilid state and submerged cultures are shown in Figure 1 and Table 1.

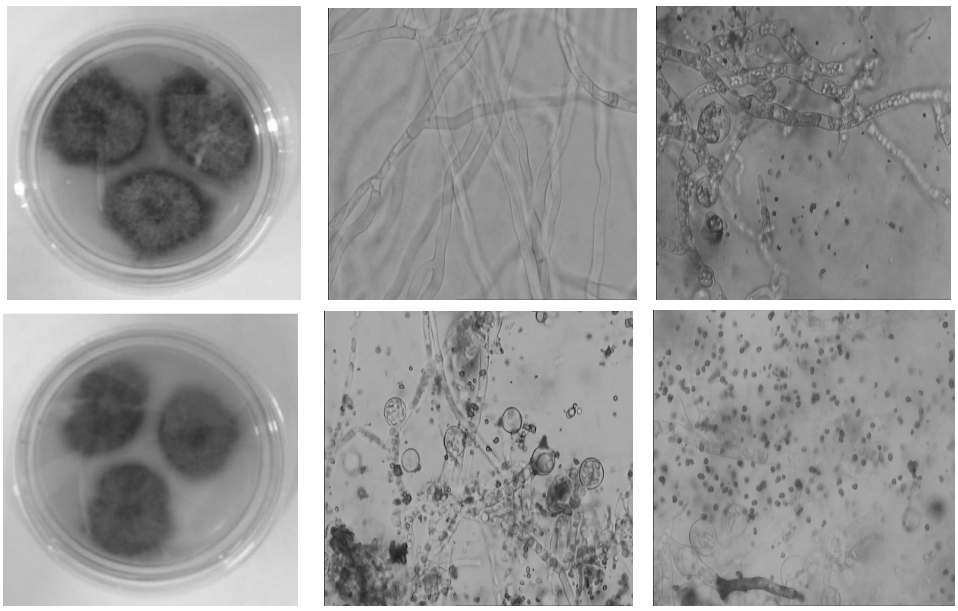


Figure 1. Showing colonies, hyphae, conidia, ascocarp and pigment crystal of *M. purpureus* 5057 and *M. purpureus* 5085 on PDA at 30^{o}C for 7 days of cultivation

- The pigmentation in two strains differed. In the PDA medium, strain 5057 was slightly orange/yellow on the surface of the colony, but orange underneath. Strain 5085 was initial orange, but became deep red underneath

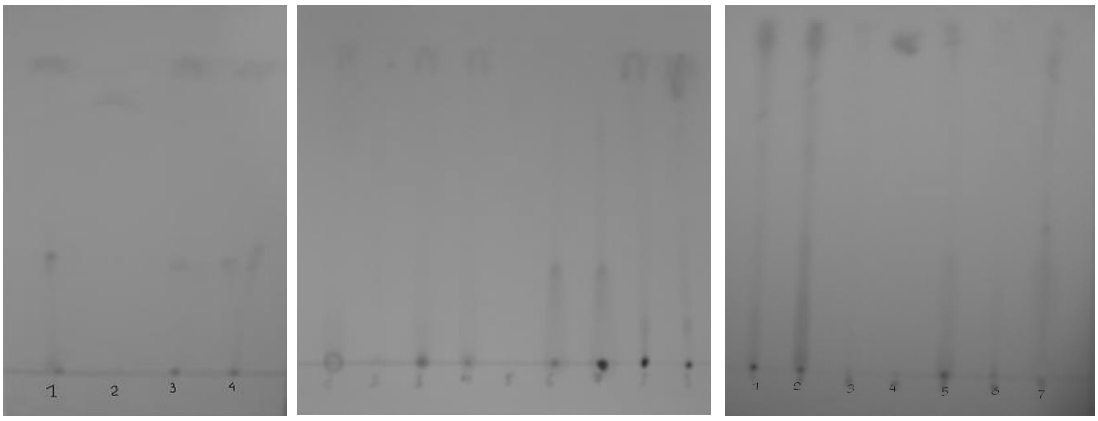
- *M. purpureus* 5085 grown and pigmentation faster more than 3 days *M. purpureus* 5057 when cultured by soilid state fermentation in the steamed rice medium. Both two strains production of yellow, orange and red pigments, but productivity of yellow and orange absorption at A_{370} and A_{420} higher from 1.5 - 1.8 times in comparison with red pigments at A_{500}.

- In submerged fermentation on medium containing glucose, yeast extract, monosodium glutamate and mineral salts, produced pigments by *Monascus* strains were not only bound to the cells but also excreted in to medium**.** *M. purpureus* 5057 produce yellow, orange and red pigments higher than *M. purpureus* 5085 of 2.5, 1.7 and 4.4 fold, respectively.

Fermentation broth, extracts of fermented stemed rice and mycelium biomass of *M. purpureus* 5057 and 5085 was analysis for Monacolin productivity by TLC method. The results are obtained in Figure 2 shown that Monacolin was produced by *M. purpureus* 5085 in both of solid state and submerged fermentation, whereas *M. purpureus* 5057 produce Monacolin only by submerged fermentation.

Table 1. Comparison of pigments production by *M. purpureus* 5057 and 5085 in solid state culture and submerged cultures

Strain	**Medium and cultivation conditions**	**Parameter**	**Value of pigment (OD λnm)**		
			370 nm	***400 nm***	***500 nm***
M. purpureus 5057	*Solid state culture*: Steamed rice medium; static culture at 30°C for 7 days	OD λ nm / g of fermented steamed rice	251.7	**282.5**	166.5
	Submerged culture: Medium contain of glucose, yeast extract, monosodium glutamate and mineral salts; culture on rotary shaker of 250 rpm at 30°C for 4 days	OD λ nm / ml of fermentation broth	6.2	5.8	4.7
		OD λ nm / g of mycelium biomass	**155.8**	144.3	118.5
M. purpureus 5085	*Solid state culture*: Steamed rice medium; static culture at 30°C for 4 days	OD λ nm / g of fermented steamed rice	290.4	**306.4**	172.8
	Submerged culture: Medium contain of glucose, yeast extract, monosodium glutamate and mineral salts; culture on rotary shaker of 250 rpm at 30°C for 4 days	OD λ nm / ml of fermentation broth	4.5	4.9	3.3
		OD λ nm / g of mycelium biomass	62.3	**88.9**	26.9



(A) *M. purpureus* 5057 (B) *M. purpureus* 5085

1,2,3: Fermented rice extracted by ethanol **1,3,4:** Mycelium biomass extracted by ethanol, acetone, butanol **1,2,3:** Fermented rice extracted by ethanol, methanol, butanol

2: Mevinolin standard **2,5,6,7**: Fermentation broth cultured at 25, 30 and 35°C **4:** Mevinolin standard

3: Fermented rice extracted by acetone **8:** Fermentation broth cultured at 30°C was concentrated **5,6,7:** Fermentation broth extracted by ethanol, butanol, acetone

4: Fermented rice extracted by butanol **9**: Mevinolin standard

Figure 2. Thin layer chromatograms of fermentation products of *M. purpureus* 5057 (A) and *M. purpureus* 5085 (B)

3.2. Effect of cultivation medium and conditions of solid state fermentation on pigments production by *Monascus purpureus* 5057

Table 2. Effect of culture temperature to pigments producing by *M. purpureus* 5057 in solid state fermentation

Temperature (°C)	Value of pigment (ODλ nm/g of fermented steamed rice)		
	370 nm	***400 nm***	***500 nm***
25°C	357.6	336.4	113.6
30°C	**437.2**	**428.4**	**252.4**
35°C	301.2	281.6	151.2

In selected medium of 50 steamed rice per 500 ml Erlenmeyer flask with huminity of 35% after inoculum of 5 ml spore suspension, cultivation temperature is important factor for pigment production by *M. purpureus* 5057. The results obtained in Table 2 shown that: Production of yellow and orange pigments increase when cultivation temperature of *M. purpureus* 5057 increase from 25 to 30°C, but pigment productivity significantly decrease when temperatute

increase to 35°C; and at cultivation temperature of 25 and 30°C, *M. purpureus* 5057 produce yellow and orange pigments more than 2.9 - 3.1 fold higher red pigment.

3.3. Effect of cultivation medium and conditions of submerged fermentation on pigments production by *Monascus purpureus* 5057

Table 3. Effect of culture temperature to pigments producing by *M. purpureus* 5057 in submerged fermentation

Cultivation temperature *(°C)*	Wet mycelium biomass *(g/L)*	Value of pigment (ODλ nm/ml of fermentation broth			Value of pigment (ODλ nm/g of wet mycelium biomass		
		370 nm	*400 nm*	*500 nm*	*370 nm*	*400 nm*	*500 nm*
25°C	35.2	8.4	9.1	9.7	77.3	36.3	98.5
30°C	**46.5**	**8.6**	**9.8**	**9.8**	**165.7**	**140.5**	**147.0**
35°C	34.3	0	0	2.9	76.8	3.8	128.7

Table 4. Effect of medium pH to pigments producing by *M. purpureus* 5057 in submerged fermentation

pH of cultivation medium	Wet mycelium biomass (g/L)	Value of pigment (ODλ nm/ml of fermentation broth			Value of pigment (ODλ nm/g of wet mycelium biomass		
		370 nm	400 nm	500 nm	370 nm	400 nm	500 nm
4.0	40.0	5.4	4.8	5.3	116.4	108.2	124.5
4.5	44.0	6.0	5.3	7.0	122.8	120.0	128.7
5.0	45.0	7.1	8.4	8.8	133.0	144.3	151.4
5.5	**46.5**	**8.6**	**9.8**	**9.8**	**165.7**	**140.5**	**147.0**
6.0	46.0	7.5	7.3	7.6	156.4	137.0	146.7
6.5	38.5	7.2	7.8	7.9	144.7	134.8	141.8
7.0	33.5	7.2	7.5	7.3	143.2	134.5	139.5

Pigments production in submerged fermentation is affected by numberous environmental factors, particularly the temperature and medium pH. In suitable cultivation medium and conditions (glucose 10 g/L; $MgSO_4$ 4.8 g/L; KH_2PO_4 1.5 g/L; K_2HPO_4 1.5 g/L; NaCl 0.4 g/L; $FeSO_4$ 0.01 g/L; $ZnSO_4$ 0.01; yeast extract 1 g/L; sodium glutamate 7.6 g/L; shaking of 250 rpm; 200 ml medium/ 500 ml Erlenmeyer flask; inoculum ratio of 2.5%), the results of pigments producing by *M. purpureus* 5057 at various temperatures and pH medium are presented in Table 3 and 4 shown that:

- At cultivation temperature of 25°C, mycelium biomass of *M. purpureus* 5057 reach 35.2 g/L and contain red and yellow pigments higher than orange. The water – soluble red

pigment higher than orange and yellow pigments; Cultivation temperature of 30°C was favoured for *M. purpureus* 5057 producing both of fungal mycelia biomass (obtained 46.5 g of wet mycelium biomass/L) and intracellular, extracellular pigments of yellow, orange, red pigments; At the higher temperature of 35°C, most of the red and yellow pigments was accumulated within the mycelium biomass of *M. purpureus* 5057 and did not produce water-soluble pigments.

- The produce mycelium biomass of *M. purpureus* 5057 was favoured in pH range of 4.0 - 5.5, obtained 40.0 - 46.5 g/L and significantly decrease when pH medium increase to 6.5 - 7.0; The intracellular and extracellular pigments of yellow, orange, red pigments increase when pH increase from 4.0 - 5.5 and decrease when pH increase to 7.0.

4. CONCLUSIONS

The production of pigments and Monacolin by strains of *Monascus purpureus* 5057 *and* 5085 were investigated utilizing solid state and submerged fermentation.

In both solid state fermentation using rice steam at 30°C for 4-7 days and submerged fermentation using medium contained glucose, yeast extract and monosodium glutamate (MSG) for 4 days, *M. purpureus* 5057 and 5085 produce yellow, orange higher red pigments. Monacolin is detected by Thin Layer Chromatography method in both solid and submerged fermentation products of *M. purpureus* 5085 whereas M. *purpureus* 5057 produce Monacolin only by submerged fermentation.

When culture by solid state method, *M. purpureus* 5057 synthesis yellow and orange pigment higher than red at 25 and 30°C.

By submerged fermentation method, cultivation temperature of 30°C and pH of 5.5 were favoured for *M. purpureus* 5057 producing both of fungal mycelia biomass and intracellular, extracellular yellow, orange, red pigments.

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TÓM TẮT

ẢNH HƯỞNG CỦA PHƯƠNG PHÁP VÀ ĐIỀU KIỆN NUÔI CẤY ĐẾN SỰ TỔNG HỢP CHẤT MÀU VÀ HOẠT CHẤT MONACOLIN CỦA CÁC CHỦNG NẤM MỐC ĐỎ *MONASCUS PURPUREUS* 5057 VÀ 5085

Hai chủng nấm mốc đỏ *Monascus purpureus* 5057 và 5085 trong Sưu tập giống vi sinh vật công nghiệp Viện Công nghiệp thực phẩm đã được nghiên cứu khảo sát khả năng tổng hợp chất màu và hoạt chất Monacolin theo phương pháp nuôi cấy bề mặt và nuôi cấy chìm. Khi nuôi cấy bề mặt trên môi trường lên men gạo hấp, cả hai chủng đều tổng hợp các chất màu vàng, da cam hấp thụ cực đại ở các bước sóng tương ứng là 370 và 400 nm cao hơn so với màu đỏ hấp thụ ở bước sóng 500 nm từ 1,5 đến 1,8 lần. Chủng *M. purpureus* 5085 tổng hợp Monacolin cả khi nuôi cấy bề mặt và nuôi cấy chìm, còn *M. purpureus* 5057 chỉ tổng hợp Monacolin khi nuôi cấy theo phương pháp lên men chìm. Nhiệt độ nuôi cấy là yếu tố ảnh hưởng lớn đến khả năng tổng hợp chất màu khi nuôi cấy *M. purpureus* 5057 theo phương pháp lên men bề mặt: ở 25 và 30^{o}C, *M. purpureus* 5057 tổng hợp màu vàng và màu da cam cao hơn từ 2,9 đến 3,1 lần so với màu đỏ, nhưng khi nhiệt độ nuôi cấy tăng lên đến 35^{o}C, sự tổng hợp các chất màu đều giảm. Khi nuôi cấy *M. purpureus* 5057 theo phương pháp lên men chìm trên môi trường chứa glucoza, cao nấm men, muối khoáng và glutamat natri, pH = 5,5, ở nhiệt độ 30^{o}C, hiệu suất tổng hợp sinh khối hệ sợi cũng như các chất màu vàng, da cam, đỏ trong sinh khối hệ sợi và tiết xuất ra môi trường nuôi cấy đạt giá trị cao nhất.

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