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PREPARATION OF RED YEAST RICE USING VARIOUS THAI GLUTINOUS RICE AND *Monascus purpureus* CMU001 ISOLATED FROM COMMERCIAL CHINESE RED YEAST RICE SAMPLE

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ABSTRACT

A strain of *Monascus purpureus* CMU001 was isolated from locally available commercial Chinese red yeast rice (Ankak or Ang Khak) by surface sterile technique. The identity of the strain was then confirmed by conventional methods. Preparation of red yeast rice using isolated strain was carried out on various kinds of rice, and especially the most well known varieties of Thai glutinous rice such as Korkor 6 white glutinous rice (RD6), Kam black glutinous rice (Kam) and Sanpatong 1 white glutinous rice (SPT1). The red rice products obtained were studied for the intensity of red pigment by measuring the absorbance of red extracts at 500 nm. The results indicate the most intense red pigment when using RD6 with 3 week cultivation. In order to know the effect of nutrient variation, soybean milk was added during cultivation as amino acids source. The highest intensity was also expressed when RD6 was used with 2 week cultivation. The supplement of 1 ml of 0.25 g/ml soybean milk gave the product with more intense red color.

KEYWORDS: *Monascus purpureus*, red pigment, red yeast rice

1. INTRODUCTION

Red yeast rice is a product obtained by fermentation using *M. purpureus* [1]. Solid culture of the fungus on rice grain produces intense red pigment as well as characteristic odor after drying by heat. The product is called differently depending on local language. Chinese calls “Ankak” or “Anka” or “Ang Khak” or “Hong Qu” while Japanese calls “Beni-Koji” or “Anka-Koji” [1-4]. Red yeast rice is widely used in food industry, and especially the red pigment is used as colorant in Sake, red soil bean curd, red pork, ham, sausage, Nham (fermented pork), sour fish (fermented fish) and candy [5]. Pinthong *et al.* [5] studied the application of red yeast rice in pork sausage as red color additive. The use of red pigment of *Monascus* instead of nitrite for the production of chicken ham was done successfully by Andrea *et al.* [6]

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Chinese red yeast rice is used in Thailand as food additive to make pleasant colored food such as red soil bean curd, sausage, Nham, and sour fish. Sausage and Nham made from pork give stable product without dissociation of red color comparing to conventional product treated with E-249 (nitrite salt) and E-252 (potassium nitrate). The nitrogen compounds mentioned is one of the carcinogenic compounds causing cancer [7]. Nham is one of the most popular food that used to be added with potassium nitrate to give good appearance. The use of red yeast rice to substitute potassium nitrate is a safety way. The glutinous rice abundantly grown in the northern part of Thailand is also interesting to be used as potential raw materials for making red yeast rice.

In this work, *M. purpureus* CMU001 was isolated from commercial Chinese red yeast rice. The *Monascus* was used to prepare glutinous red yeast rice using the known varieties of the rice grown in the northern region. Optimized red glutinous red yeast rice products were obtained and a comparison of their red color production was carried out.

2. MATERIALS AND METHODS

2.1 Isolation of *M. purpureus*

Locally available commercial Chinese red yeast rice was used for *M. purpureus* isolation. Surface sterilization [8] is the preferred method to eliminate the contaminants. The experiment was carried out by preliminary soaking the rice in 95 % ethanol, washing by 0.1% - 0.5% sodium hypochlorite solution for 1- 4 min and finally washing by 95% ethanol for 30 seconds. Twelve washed red rice grains were placed on Rose Bengal agar plates. One week culture was used for isolation of uncontaminated grains by cultivation on potato dextrose agar (PDA) . The pure culture were kept in a refrigerator at 5 ^{o}C as a stock culture.

2.2 Identification of *M. purpureus*

The strain named CMU001 isolated from commercially available red yeast rice was identified by conventional methods and compared with compendium of soil fungi [9]. The strain was inoculated on PDA and incubated at room temperature to observe morphological characteristics such as colonies, ascospores and conidial stage. Pigment production was also observed as well as 6% sodium hypochlorite and 30% ethanol tolerance. The starch hydrolyzing enzyme activity was studied by cultivation of the strain on medium following by clear zone detection using iodine solution [10].

2.3 Preparation of Red Yeast Rice

Inoculation and cultivation of isolated *M. purpureus* CMU001 were done using difference varieties of glutinous rice (*Oryza sativa* L.). The glutinous rice used were Korkor 6 white glutinous rice (RD6), Kam black glutinous rice (Kam) and Sanpatong 1 white glutinous rice SPT1. For comparison, non glutinous rice, Khao Hom Mali105 (Mali105) was used to make red yeast rice. The controlled condition with appropriate weight, culture age, inoculation volume, temperature, humidity and pH according to Boonsangsom *et al.* [11] was used. Sample after 2 and 3 week cultivation were finally obtained and used for further study. Stepwise preparation of red yeast rice is shown in Figure 1. Glutinous rice grains were immersed in water for 6 hours following by steaming for 20 minutes. After cooling, 50 grams of steam rice was put in 250 ml flask and sterile at 15 psi and 121 ^{o}C for 15 minutes. One week old precultured *M. purpureus* CMU001 was used as inoculum. The inoculated rice was incubated at 30 ^{o}C for 2 or 3 weeks. Humidity and pH were measured before and after inoculation. The end-product was dried in the oven at 65 ^{o}C for 6 hours to obtain dried red yeast rice. In case of non glutinous rice (Mali105) which was used for comparison, the red yeast rice preparation was done without immersion of rice grains in water. In order to study the effect of adding nitrogen compounds, addition of 1 ml of 0.25 g/ml soybean milk solution was also performed. The yield of red yeast rice was

evaluated as percentage yield. The percentage yields were obtained by using the following equation.

$$\text{Percentage yield} = \frac{\text{Weight of dried end product}}{\text{Weight of steamed rice used}} \times 100$$

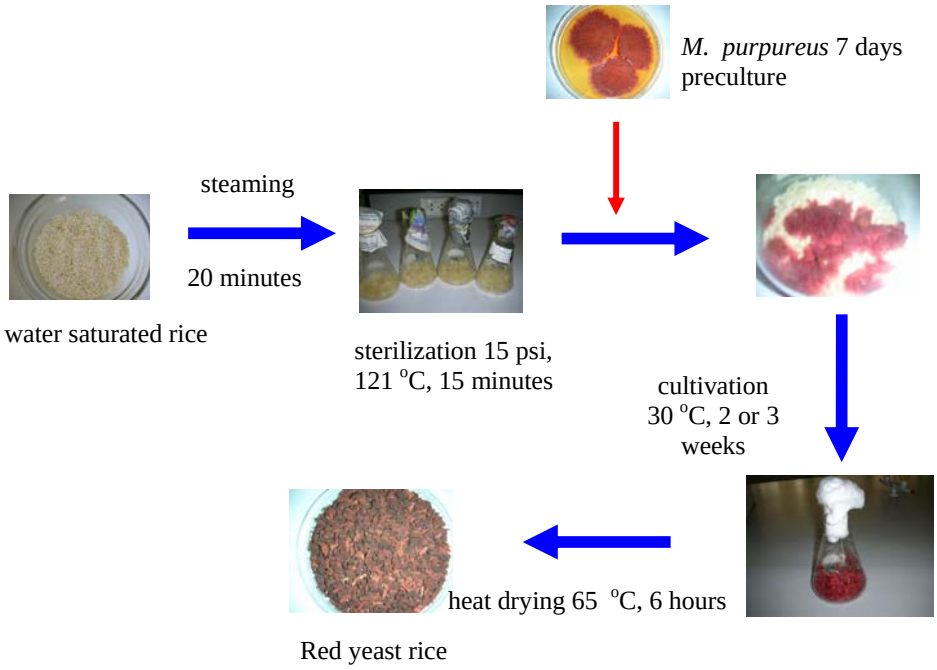

Figure 1 Stepwise preparation of red yeast rice

2.4 Measuring the Content of Red Pigment

The red pigment content was measured by determination of absorbance at 500 nm [11-14]. 0.5 g of red yeast rice powder was used for extraction by 10 ml of 75% ethanol (HPLC grade) [1]. The mixture was vibrated in ultrasonic bath for 60 minutes.

The supernatant was obtained by centrifugation at 3000 rpm at 4 °C for 10 minutes. The extraction procedure was repeated 3 times to obtain 30 ml solution which was then made up to 50 ml in a volumetric flask with 75% ethanol. After standing for 30 min, the solution was filtered through 0.2 µm membrane and its absorbance was measured at the wavelength range of 400-700 nm.

3. RESULTS AND DISCUSSION

3.1 The Number of Rice Grains Showing the Growth of Fungus after Treatment by Surface Sterilization

After washing with 95% ethanol and 0.1-5.0% sodium hypochlorite solution, the number of rice grains with the growth of fungus was obtained. The results showed different number of fungal growth on rice grains when washing with different concentration of sodium hypochlorite and with different time of washing as shown in Table 1.

The number of rice grains with fungal growth was highest when washed with 0.1% solution of sodium hypochlorite for 1 minute. The fungal colonies are shown in Figure 2 (a) and (b).

Table 1 Number of rice grains with fungal growth

	Sodium hypochlorite solution															
	5.0 %v/v				2.5 %v/v				1.0 %v/v				0.1 %v/v			
	Time(minutes)															
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
No. of grains	6	5	4	4	5	4	4	3	8	6	6	5	12	10	9	9

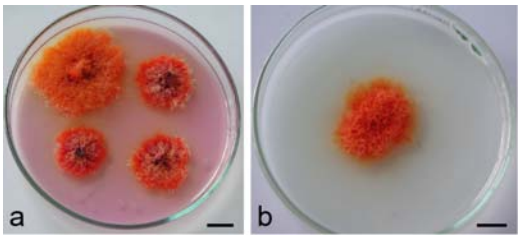

Figure 2 Isolation of *Monascus purpureus* from commercial Chinese red yeast rice available in local market. a) Cultivation of surface sterile red yeast rice on Rose Bengal agar, b) Isolated *M. purpureus* growing on PDA showing pure strain (Bars = 1 cm.)

The best condition for isolation by surface sterile technique was to wash with 95% ethanol followed by 1 minute washing with 0.1% sodium hypochlorite solution. The highest numbers of growth obtained are 12 rice grains.

The Rose Bengal agar used in this study contains chloramphenicol which is antibacterial agent, therefore, the growth of fungal colonies are confirmed [15-17].

3.2 Identification of Fungal Strain

The colonies of *M. purpureus* strain CMU001 on PDA (Figure 3a) were initially white and later became orange to red. Ascomata (36–72 μm), non-ostiolate (cleistothecia), hyaline to reddish brown, superficial, globose, stalked, soon evanescent asci are shown in Figures 3b,c. Ascospores (5–6.2 × 5 μm), hyaline or slightly orange, smooth-walled (Figures 3d,f). Anamorph is present. Conidia 7.5–8.5 × 5–6.2 μm, hyaline, thick- and smooth-walled, subglobose with a very broadly truncate base are shown in Figures 3e,f. The morphological characteristics of stalked cleistothecia and soon evanescent asci, which is no longer recognizable at maturity, those of *Monascus*, as described by Tieghem [18]. The characteristics of ascomata, ascospores and conidia of the strain CMU001 show similarity to *M. purpureus* [19].

In growth observation of the strain CMU001 on PDA, colonies also show the lava shaped, helix mycelium, red pigment production and starch hydrolysis activity (Figure 4). The growth is impossible in 6% NaOCl and 30% ethanol. These confirmed characteristics of *M. purpureus* [20].

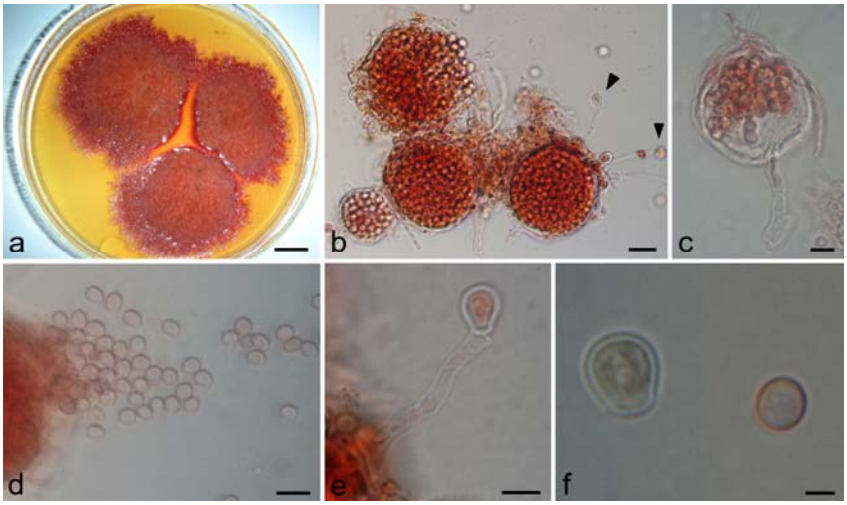

Figure 3 *Monascus purpureus* strain CMU001 a) Colonies in PDA, b) Various stages of ascomata development and conidial formation (arrowed), c) Several asci developed in stalked cleistothecium, d) Subglobose or ellisoidal ascospores, e) Conidiophore bearing conidium, f) Comparison of broadly truncate base conidium and globose ascospore (Bars: a = 1 cm, b-d = 10 μm, e = 5 μm, f = 2 μm.)

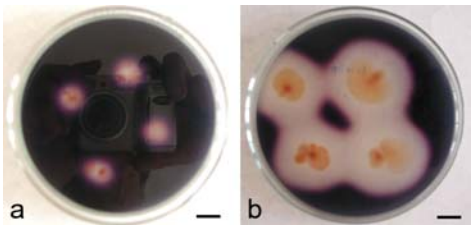

Figure 4 Starch hydrolysis activities of *M. purpureus* strain CMU001 after one (a) and two (b) weeks (Bars = 1 cm.)

3.3 Yield of Red Yeast Rice

The percentage yields of prepared red yeast rice after 2 and 3 week cultivation using Mali105, Kam, RD6 and SPT1 rice are shown in Table 2.

All varieties gave red yeast rice production varying from pale red to darker red depending on the kind of rice used as shown in Figure 5.

Table 2 Percentage yield of red yeast rice

Rice varieties	Without soybean milk		With soybean milk	
	2 weeks	3 weeks	2 weeks	3 weeks
	% yield	% yield	% yield	% yield
Mali105	16.39	12.37	25.22	13.69
Kam	28.50	22.56	22.92	19.81
RD6	18.41	16.02	15.24	14.10
SPT1	17.03	15.28	15.93	13.40

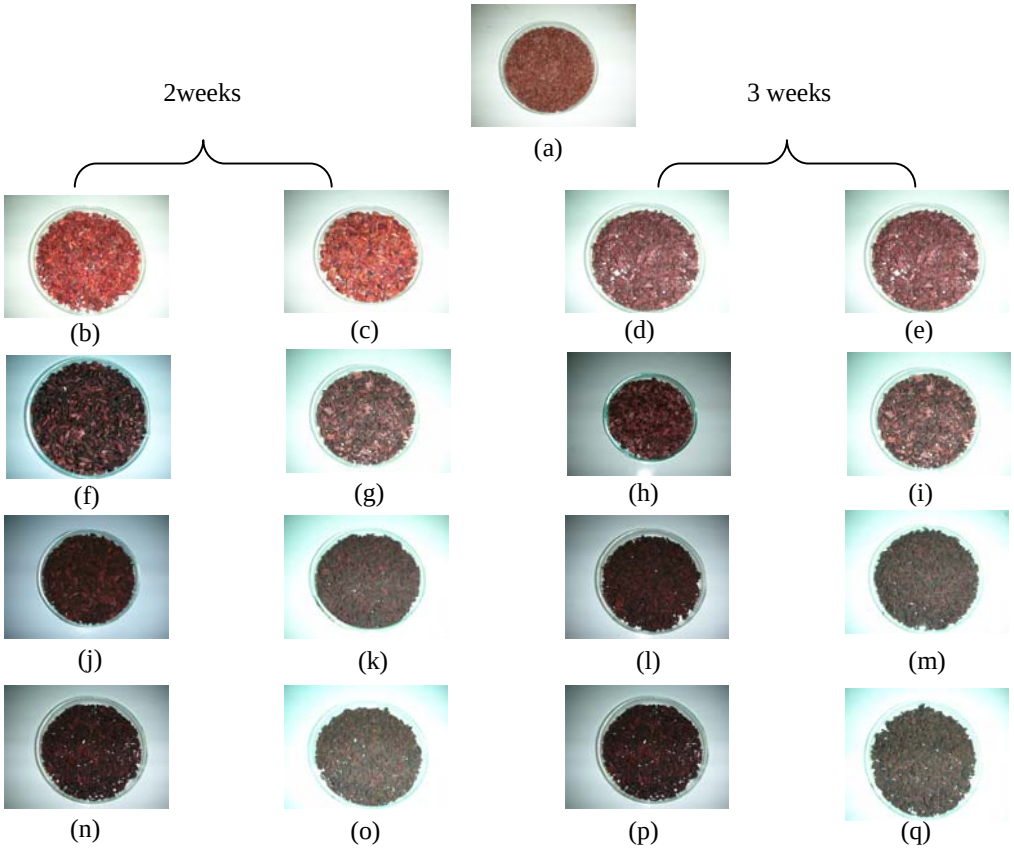

Figure 5 Products of red yeast rice
(a) Commercial Chinese red yeast rice
(b), (f), (j), (n) Mali105, Kam, RD6 and SPT1, respectively, without soybean milk, 2 weeks
(c), (g), (k), (o) Mali105, Kam, RD6 and SPT1, respectively, with soybean milk, 2weeks
(d), (h), (l), (p) Mali105, Kam, RD6 and SPT1, respectively, without soybean milk, 3 weeks
(e), (i), (m), (q) Mali105, Kam, RD6 and SPT1, respectively, with soybean milk, 3weeks

3.4 Red Pigment Content

The contents of red pigment in the 75% ethanol extract are expressed as absorbance unit per gram (AU/g) of red yeast rice powder (Table 3). The absorbance was measured at 500 nm which is the maximum wavelength for red color.

Table 3 Red pigment contents from fermented 1 cultivar of normal rice and 3 cultivars of glutinous rice with *M. purpureus*

Time	AU (AU/g)							
	Mali105		Kam		RD6		SPT 1	
	Without soybean milk	With soybean milk	Without soybean milk	With soybean milk	Without soybean milk	With soybean milk	Without soybean milk	With soybean milk
2 weeks	4.51	3.60	3.63	6.99	30.11	45.04	29.63	36.09
3 weeks	1.42	0.91	6.06	5.06	39.90	42.16	34.13	42.70

Many reports indicate the absorbance of red pigment from *M. purpureus* at 400-500 nm [12-14]. The results show maximum absorbance around 500 nm that was used for measuring of red pigment. The red color is due to the presence of rubrapunctamine and monascorubramine which derives from orange colored compounds rubropunctatin and monascorubrin respectively [21]. Amine compounds seem to play an important role for derivatization of orange colored compounds by ring opening and shift rearrangement reaction. Consequently, from Table 3, most of rice variety except Mali105 expressed more intense red color when adding soybean milk solution during red yeast rice preparation.

The red pigment produced from glutinous rice seems to be higher than normal rice, Mali105. The result is controversial to the work of Pinthong *et al.* [4]. Control of humidity and low efficiency of starch degradation may be reasonable explanation.

4. CONCLUSIONS

In this report, it was found that under controlled condition, glutinous rice gave more intense red color products. The result shows the most intense red color was observed from using RD6 with addition of soybean milk harvested with in 2 weeks. Among the glutinous rice used, it should be noted that the highest yield from RD6 was much higher than Kam rice (natural black rice the lowest quality product).

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