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The fermentation of rice for anka pigment production

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Optimal physical parameters of the solid state fermentation of rice to produce anka pigments and their influences on pigment production were studied. Anka pigment production, especially that of two orange anka pigments (rubropunctatin and monascorubrin), was highly sensitive to the moisture content of the rice substrate. Optimal initial moisture content of rice substrate was 24%. Pigment formation was retarded when extra water was added to the inoculated substrate during cultivation. High filling amount of rice substrate in a flask was unfavorable for pigment production. Loosening of the inoculated substrate once a day enhanced pigment production. With a high carbon dioxide level in the incubator, no orange pigments were detected. Freeze drying the fermented material produced a superior yield of anka pigments, while oven drying at 50°C for 24 h was a reasonable alternative. *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 141–146.

Keywords: solid state fermentation; *Monascus purpureus*; anka pigments

Introduction

Anka is prepared by culturing *Monascus* sp. on rice grains. After fermentation, rice grains become purplish red throughout. On rice grains, *M. purpureus* synthesizes four anka pigments: monascin, ankaflavin, rubropunctatin and monascorubrin. The latter two orange anka pigments, when reacted with aqueous ammonia, give the red nitrogen analogues, rubropunctatamine and monascorubramine [6,8]. Figure 1 illustrates the structures of the above six pigments. Monascin and ankaflavin bear the same chromophoric structure which results in yellow color. The structural difference between these two yellow anka pigments is the saturated side chain of C5 or C7 on the ketonic carbonyl group. The same structural relationship was also observed between two orange anka pigments and between two red nitrogen analogues, as well.

In certain regions of Asia, such as China, Japan, Taiwan, Thailand and the Philippines, anka has been used traditionally as a natural coloring agent or as one of the starter cultures for brewing red rice wine. Generally, preparation of anka in these regions has been based on a less-than-precise method described by Song [13] whose solid state fermentation to prepare anka has been performed for hundreds of years. It was found that pigment production by *M. purpureus* in solid cultivation was much better than the submerged cultivation [5,10]. However, limited information is available on culture parameters influencing pigment production in the solid state. Therefore, this study mainly investigated how much the physical fermentation parameters affect the yield of anka pigments, such as initial moisture content of rice substrate, extra water added to the inoculated substrate during cultivation, filling amounts of rice substrate in a flask, agitation of the inoculated substrate and carbon dioxide level in the incubator. In addition, the influence of the drying method of fermented material on the yield of pigments was also investigated. In this study, rice was inoculated with *M. purpureus* DSM 1379. Fermentation was performed in 500 ml Erlenmeyer flasks. The pigments produced were analyzed with high-performance liquid chromatography (HPLC) [14].

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Materials and methods

Organism and inoculum

M. purpureus DSM 1379 was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). The fungus was grown on agar slants which contained (w/v) 2% yeast extract (Difco), 5% glucose, 0.1% KH_2PO_4, 0.5% $MgSO_4 \cdot 7H_2O$, 0.1% $CaCl_2$ and 1.5% agar (Difco) in distilled water [2]. Following 5 days of cultivation at 30°C, the slant was stored at 4°C for less than 3 days. A spore suspension was prepared with 5 ml sterilized distilled water and then measured by a hemacytometer. The spore suspension was adjusted to 2×10^7 spores/ml and used as an inoculum. Every 10 g of rice substrate was inoculated with 1.0 ml of the spore suspension.

Solid state fermentation

Long-grain rice (Master Foods, Belgium) was purchased from a local supermarket and used as substrate for anka pigment production with solid state cultivation. Except for the investigated parameter, rice substrate was prepared as follows: rice was first soaked in distilled water (100 g rice in 200 ml water) for 10 min. Excess water was removed with a sieve. Every 20 g wet rice was weighed in a 500-ml Erlenmeyer flask which was then plugged with cotton. The rice was sterilized in a preheated autoclave at 121°C for 15 min. As soon as the pressure in the autoclave was reduced, the sterilized rice substrate was taken out. The moisture content of the sterilized rice substrate, determined in a hot air oven at 105°C until constant weight was achieved, was defined as the initial moisture content of rice substrate. After being cooled, the rice substrate was inoculated with a spore suspension of *M. purpureus* DSM 1379 and the inoculated substrate was cultivated at 30°C for 14 days. Each day, the inoculated substrate was manually shaken until all the rice grains were separated from each other. The CO_2 meter in the incubator was used to monitor the carbon dioxide level

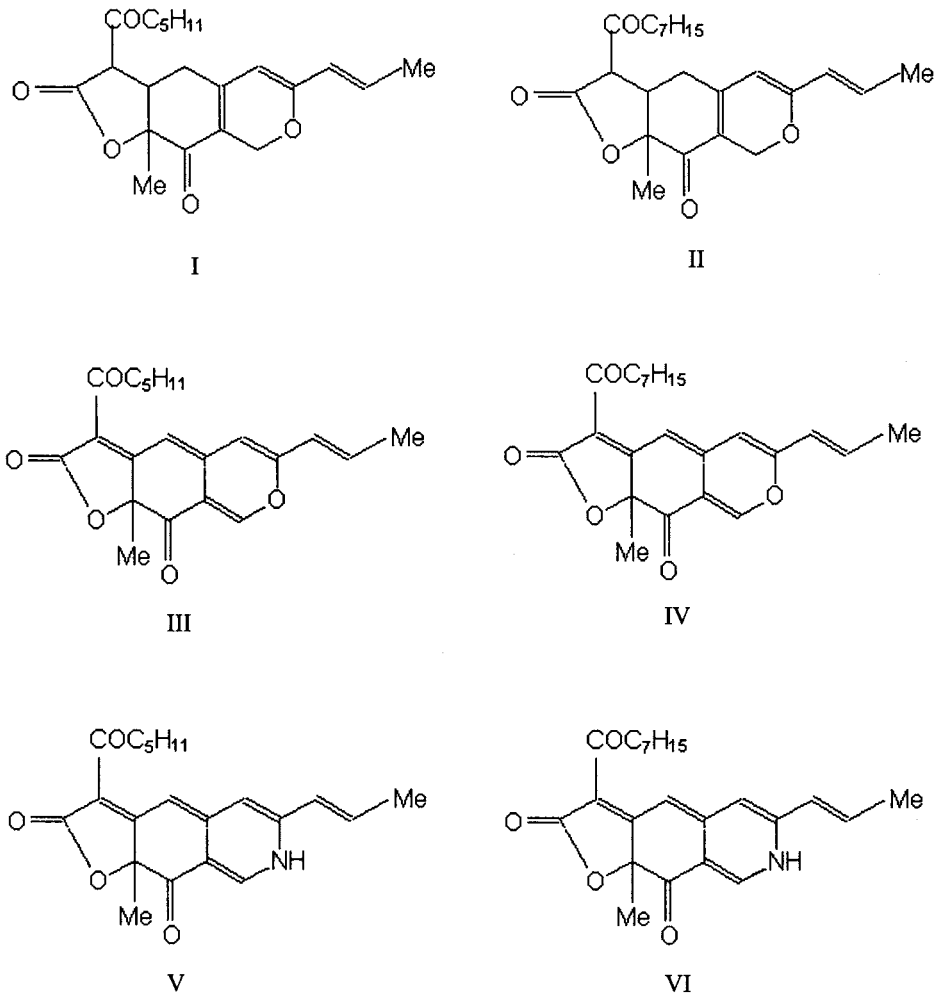

Figure 1 Structure of anka pigments and two red nitrogen analogs: I = monascin, II = ankaflavin, III = rubropunctatin, IV = monascorubrin, V = rubropunctatamine, VI = monascorubramine.

under 4%. After 14 days, the fermentation was completed and the fermented material was freeze-dried at $-60^{\circ}C$ for 24 h. The resultant red dried rice was anka, which was stored at $-26^{\circ}C$ until analysis was conducted. Yield of pigments was analyzed with HPLC [14].

The physical fermentation parameters which were investigated were: initial moisture content of rice substrate, extra water added to inoculated substrate during cultivation, filling amount of rice substrate in a flask, agitation of inoculated substrate, carbon dioxide level in the incubator, and drying method of fermented material. The initial moisture content of rice was controlled by soaking the rice at different periods of time in water. Cultivated at $30^{\circ}C$, the mycelia of the fungus penetrated the rice grains and the inoculated substrate turned pink within 3 days. In order to study the effect of extra water added to inoculated substrate during cultivation, the pink substrate which was incubated for 3 days was evenly divided into three groups and processed differently: (1) untreated; (2) moistened with 3 ml water; and (3) rinsed with 30 ml water. When 30 ml water was added, the water not retained was removed with a pipette after a reaction time of 5 min. In order to examine the effect of various rice filling amounts in a flask on the yield of pigments, fermentation was performed with 20, 30 or 40 g soaked rice in a 500-ml Erlenmeyer flask. To study the relation between agitation and anka pigment formation, inoculated substrate was cultivated in three different ways: (1) static; (2) loosened manually once a day until all the rice grains were separated from each other; and (3) constantly shaken at a speed of 300 rpm, which kept the inoculated rice grains moving slightly. Also, to study the influence of the carbon dioxide level in the incubator on pigment formation, during cultivation, the incubator was: (1) ventilated once a day to control the carbon dioxide level under 4% throughout cultivation; and (2) not ventilated at all to create a carbon dioxide level in the incubator over 20% in 4 days which was maintained throughout the cultivation. After the fermentation was complete, the fermented material was dried to constant weight by (1) freeze drying at $-60^{\circ}C$ for 24 h; (2) oven drying at $50^{\circ}C$ for 24 h; or (3) oven drying at $105^{\circ}C$ for 5 h.

Analysis of anka pigments

Before pigment extraction, anka was ground into powder by a laboratory mill with a water cooling system (Eydam, Kiel, Germany). Anka powder (3 mg) was weighed in a 1500-μl vial. One ml methanol (analytical grade; Merck, Darmstadt, Germany) was added to anka powder, the mixture was manually mixed for 3 min, and then centrifuged at $9740\times g$ for 5 min. The extraction was

Table 1 Soaking time of rice, initial moisture content of rice substrate and yield of anka pigments (μg pigment/mg anka)

Soaking time (min)	Initial moisture content (%)	Yellow anka pigments				Orange anka pigments			
		Monascin		Ankaflavin		Rubropunctatin		Monascorubrin	
		μg/mg	%*	μg/mg	%*	μg/mg	%*	μg/mg	%*
5	19.6±0.5	39.2±6.4[a]	77.2	17.0±2.5[a]	81.0	33.9±6.2[a]	83.5	31.3±5.1[a]	79.0
10	24.3±0.7	50.8±3.6[b]	100.0	21.0±1.3[b]	100.0	40.6±3.6[a]	100.0	39.6±1.9[b]	100.0
20	27.9±0.6	16.5±3.7[c]	32.5	8.4±1.5[c]	40.0	2.8±0.6[b]	6.9	3.2±0.3[c]	8.1

Means with different superscripts within a column are significantly different (α=0.05).
n.d.=not determined.
* Percent of each anka pigment yield against anka with soaking time of 10 min, i.e. initial moisture content of 24.3%.

repeated until the extract became colorless. The methanol extract of anka was collected, mixed in a test tube, and analyzed with HPLC. The HPLC instrument consisted of a Waters 6000A pump (Milford, MA, USA), a Waters WISP 710B autosampler, a C_{18} column (250 mm×4.6 mm i.d.) packed with 7 μm particles (Macherey Nagel, Dueren, Germany) and a Waters 481 spectrophotometer. A Maxima 820 chromatography workstation (Dynamic solutions, Millipore, Ventura, CA, USA) was used for data acquisition. A mobile phase of acetonitrile–water (80:20, v/v), flow rate 0.5 ml/min, was used to separate anka pigments, which were detected at 233 nm. The preparation of pure reference pigments and the identification and analysis of anka pigments were discussed in a previous report [14].

Statistics

All the tests were conducted in six replications. The yield of each anka pigment was expressed in mean±SD and in percentage against the corresponding control group. The *t*-test was used to compare yields of pigments under various physical parameters.

Results

Initial moisture content of rice substrate

Initial moisture content of the rice substrate was proportional to the soaking time. If rice was not previously soaked in water but only sterilized, the initial moisture content was approximately 10%. Soaking rice in water for 5, 10, 20, 30, 60 min or 24 h resulted in an initial moisture content of approximately 20, 24, 28, 31, 37 and 60%, respectively. Following the fermentation, the material with initial moisture content of 10%, i.e. unsoaked rice substrate, looked dry and those with initial moisture content higher than 30%, i.e. rice soaked for 30 min or longer, were moist in appearance; however, both groups of the fermented materials were light in color. The fermented materials with initial moisture content between 20% and 28% (soaking time 5–20 min) were visually purplish red. Pigment production of these purplish red fermented materials were analyzed. The results are shown in Table 1.

Anka with an initial moisture content of 24% (soaking time of 10 min) yielded the maximum pigments. By low initial moisture content of rice substrate, the yield of all four anka pigments was reduced approximately to the same extent. Approximately 80% of every anka pigment was produced, if the initial moisture content of rice substrate was 20%, i.e. 4% less than the optimal initial moisture content. However, high moisture of rice substrate retarded the formation of pigments differently. If the initial moisture was 4% higher than the optimal, i.e. a soaking time of 20 min, less than 40% yellow anka pigments (monascin and ankaflavin) and less than 10% orange pigments (rubropunctatin and monascorubrin) were produced. These results show that formation of anka pigments was highly sensitive to the initial moisture content of rice substrate. High initial moisture content inhibited pigment production more than low initial moisture content. The formation of two orange anka pigments was inhibited much more dramatically than formation of the two yellow pigments.

Extra water added to inoculated substrate during cultivation

During cultivation, when 3 ml of water was added to each flask, the water was retained by rice substrate completely and production of yellow and orange pigments was reduced to approximately 70% and 30%, respectively, against those of the untreated group (Table 2). Moreover, when the inoculated substrate was rinsed with a great amount of water (30 ml), pigment formation was retarded almost completely and the fermented material was moist and light in color after being cultivated for 14 days. The pigment production of this anka was not analyzed.

Table 2 Adding extra water to inoculated substrate during cultivation and yield of anka pigments (μg pigment/mg anka)

Extra water added (ml)	Yellow anka pigments				Orange anka pigments			
	Monascin		Ankaflavin		Rubropunctatin		Monascorubrin	
	μg/mg	%*	μg/mg	%*	μg/mg	%*	μg/mg	%*
None	33.6±2.9	100.0	17.4±1.4	100.0	23.4±2.9	100.0	23.3±2.7	100.0
3	21.5±3.4	64.0	13.5±2.1	77.6	6.4±1.0	27.4	7.1±1.3	30.5

Means within a column are significantly different (α=0.05).
n.d.=not determined.
*Percent of pigment yield against anka without adding extra water.

Table 3 Filling amount of rice substrate in a flask and yield of anka pigments per anka unit (μg pigment/mg anka)

Filling amount (g)	Yellow anka pigments				Orange anka pigments			
	Monascin		Ankaflavin		Rubropunctatin		Monascorubrin	
	μg/mg	%*	μg/mg	%*	μg/mg	%*	μg/mg	%*
20	42.6±2.7[a]	100.0	16.0±0.8[a]	100.0	23.5±3.1[a]	100.0	22.2±2.5[a]	100.0
30	35.5±5.8[b]	83.3	15.7±2.6[a]	98.1	12.2±1.7[b]	51.9	12.8±1.7[b]	57.7
40	29.0±0.7[b]	68.1	13.2±0.4[a]	82.5	10.4±1.6[b]	44.3	11.1±1.7[b]	50.0

Means with different superscripts within a column are significantly different (α=0.05).
*Percent of pigment yield against anka with filling amount of 20 g rice in a flask.

Filling amount of rice substrate in a flask

In order to study the relation between pigment production and filling amount of rice substrate in a flask, two different measurements of pigment yield were adopted: (1) pigment production per anka unit (μg pigment/mg anka, Table 3) and (2) total pigment production in a flask (mg pigment, Table 4).

Table 3 shows that if more than 20 g of rice was filled in a 500-ml Erlenmeyer flask, pigment production per anka unit was retarded. When the filling amount of rice increased from 20 to 40 g in a flask, yield of two orange anka pigments every anka unit was reduced to about 50% and that of two yellow anka pigments was less affected (i.e. monascin reduced to 68%, that of ankaflavin reduced to 83%).

The total amount of anka increased with a greater filling amount of rice substrate in a flask (Table 4). The total production of yellow pigments (monascin and ankaflavin) increased with a greater filling amount of rice substrate, but not proportionally. When the filling amount of rice substrate in a flask doubled (20 to 40 g), the total production of two yellow pigments became 145% and 177%, respectively, but the total yield of orange pigments was affected slightly, i.e. 94% and 105%, respectively.

Agitation of inoculated substrate

The influence of agitation on the yield of anka pigments is given in Table 5. Production of yellow monascin was independent of three treatments. Production of two orange anka pigments was enhanced by loosening the inoculated substrate once a day; whereas, that of yellow ankaflavin was inhibited by this treatment. Constant shaking yielded approximately 60% orange anka pigments and static cultivation yielded 75% orange pigments of that with loosening cultivation, respectively.

Carbon dioxide level in incubator

When the incubator was ventilated once a day, its carbon dioxide level increased during the first few days of cultivation, decreased, and then remained at a stable level of under 4%. On the other hand, if the incubator was not ventilated throughout cultivation, carbon dioxide accumulated and its level in the incubator increased rapidly during the first 4 days to 20% and then remained at over 20%. Table 6 shows that pigment formation was severely inhibited by a high carbon dioxide level in the incubator. Production of the yellow anka pigments — monascin and ankaflavin — in an incubator with a high carbon dioxide level was only 8% and 19% of that in an incubator with a carbon dioxide level less than 4%. Moreover, no

Table 4 Filling amount of rice substrate in a flask and total anka pigment production (mg pigment)

Filling amount (anka weight)	Yellow anka pigments				Orange anka pigments			
	Monascin		Ankaflavin		Rubropunctatin		Monascorubrin	
	mg	%*	mg	%*	mg	%*	mg	%*
20 g (6.5 g)	276.3±19.8	100.0	103.6±6.5	100.0	152.2±20.0	100.0	144.1±15.5	100.0
30 g (9.6 g)	339.1±26.0	122.7	150.2±11.5	145.0	116.6±9.3	76.6	122.9±10.2	85.3
40 g (13.8 g)	401.0±37.9	145.1	182.9±17.1	176.5	142.3±17.8	93.5	151.9±18.2	105.4

Means within a column are significantly different (α=0.05).
*Percent of pigment yield against anka with filling amount of 20 g rice in a flask.

Table 5 Agitation and yield of anka pigments (μg pigment/mg anka)

Agitation	Yellow anka pigments				Orange anka pigments			
	Monascin		Ankaflavin		Rubropunctatin		Monascorubrin	
	μg/mg	%*	μg/mg	%*	μg/mg	%*	μg/mg	%*
Loosening	53.1±0.7[a]	100.0	20.1±0.4[a]	100.0	33.8±3.9[a]	100.0	35.4±3.2[a]	100.0
Shaking	53.9±3.7[a]	101.5	25.5±1.8[b]	126.9	19.6±1.7[b]	58.0	21.5±1.0[b]	60.7
Static	52.4±0.3[a]	98.7	23.3±0.4[b]	115.9	25.7±1.0[c]	76.0	26.4±0.2[c]	74.6

Means with different superscripts within a column are significantly different (α=0.05).
*Percent of pigment yield of anka loosened once a day.

Table 6 Carbon dioxide level in incubator and yield of anka pigments (μg pigment/mg anka)

CO_2 level (%)	Yellow anka pigments				Orange anka pigments			
	Monascin		Ankaflavin		Rubropunctatin		Monascorubrin	
	μg/mg	%*	μg/mg	%*	μg/mg	%*	μg/mg	%*
<4	52.4±0.3	100.0	23.3±0.4	100.0	25.7±1.0	100.0	26.4±0.2	100.0
>20	4.1±0.7	7.8	4.4±0.6	18.9	0.0±0.0	0.0	0.0±0.0	0.0

Means within a column are significantly different (α=0.05).
*Percent of pigment yield of anka cultivated in incubator with CO_2 level under 4%.

Table 7 Drying method and yield of anka pigments (μg pigment/mg anka)

Drying method	Yellow anka pigments				Orange anka pigments			
	Monascin		Ankaflavin		Rubropunctatin		Monascorubrin	
	μg/mg	%*	μg/mg	%*	μg/mg	%*	μg/mg	%*
−60°C, 24 h	47.7±0.9[a]	100.0	20.7±0.4[a]	100.0	80.8±1.5[a]	100.0	57.5±2.0[a]	100.0
50°C, 24 h	39.6±1.8[b]	83.0	17.2±0.9[b]	83.1	68.4±2.8[b]	84.7	56.6±2.7[a]	98.4
105°C, 5 h	25.3±0.8[c]	53.0	11.3±0.4[c]	54.6	46.5±1.5[c]	57.5	14.2±0.5[b]	24.7

Means with different superscripts within a column are significantly different (α=0.05).
*Percent of pigment yield of freeze-dried (−60°C, 24 h) anka.

orange anka pigments (rubropunctatin and monascorubrin) were detected in anka cultivated in an incubator with a carbon dioxide level greater than 20%.

Drying method of fermented material

Table 7 reveals the relation between the most commonly employed drying methods of the fermented material and the yield of anka pigments. Increasing the drying temperature reduced the pigment yield. When the fermented material was dried at 50°C for 24 h, the yield of pigments was about 85% of those pigments in freeze-dried anka, except for monascorubrin, the yield of which was not significantly different from that of freeze-dried anka. When the fermented material was dried at 105°C for 5 h, the yield of monascorubrin was 25% and that of the other three anka pigments was approximately 55% of that of the freeze-dried anka.

Discussion

For pigment production, solid state cultivation was superior to submerged cultivation [5,10]. As a substrate for solid state fermentation to produce anka pigments, rice was more effective than bread, wheat or corn [9,11]. The superiority of rice might have resulted from its complex nutritional composition, and its ideal microscopic structure, which could permit superior hyphal penetration and pigment diffusion in the rice grains [10]. In a preliminary study, different sorts of rice were tested as substrate to prepare anka. Because of the firmness of its grains that could facilitate preparation and treatment, "Patna Spitzen–Langkorn–Reis" was used as the substrate to produce anka pigments.

Physical fermentation parameters affected not only the yield of four anka pigments, but also unexpectedly their relative amounts in the fermented materials. Different physical parameters influenced the formation of pigments with the same chromophoric structure to the same extent. Changes in the yield of yellow ankaflavin under various fermentation parameters were almost identical to those in the yield of yellow monascin. Similarly, the orange rubropunctatin yield changes were almost identical to those in the yield of monascorubrin. What is more, by testing various physical fermentation parameters, production of two orange anka pigments was affected more than that of two yellow pigments. Thus, it is reasonable to assume that biosynthesis of the anka pigments with same chromophoric structure was highly similar to each other and biosynthesis of two orange anka pigments was more complex than that of two yellow anka pigments. However, in all ankas, which were prepared under various fermentation conditions, no red nitrogen analogs of anka pigments (rubropunctatamine and monascorubramine) were detected. This observation, along with other results of the authors [14], support the proposition of Carels and Shepherd [3] who suggested that *M. purpureus* biosynthesized two yellow and two orange pigments; whereas, two red nitrogen analogs were the products of two orange anka pigments with NH groups in the fermentation medium or in mycelium of the fungus.

Moisture content of the fermentation substrate was one of the most important factors to determine the yield of anka pigments by solid state fermentation. In this study, the effect of substrate moisture content on pigment formation was examined by two methods: (1) preparing different initial moisture contents of rice substrate before cultivation and (2) adding extra water to the inoculated substrate during cultivation. Complete agreement between these two methods was observed: pigment production of *M. purpureus* was highly sensitive to the moisture content of rice substrate, especially the two orange pigments. For the purpose of high pigment yield, high moisture content of rice substrate should be avoided. Lotong and Suwanarit [12] cultivated rice in plastic bags to prepare anka. They observed an increasing glucoamylase activity with moisture content of rice substrate. High glucoamylase activity resulted in high glucose concentration, which retarded pigment formation [4,12].

Traditionally, anka was prepared by cultivating rice substrate inoculated with *M. purpureus* on bamboo sieves. Rice was first soaked overnight in water to retain the essential moisture. During cultivation, the inoculated rice substrate was dipped in water once or twice. Although pigment production of such anka was not

analyzed, severe retardation of pigmentation by a high moisture content of the rice substrate as described above was not observed. Nevertheless, anka manufacturers claimed that through these treatments, pigmentation of the fungus could be improved. There should be an optimal moisture content of rice substrate for each fermentation vessel. When fermentation was performed on sieves, in the course of cultivation, the inoculated substrate dried out slowly and pigments production decreased. To overcome this problem, two treatments were usually conducted: moistening inoculated substrate with a small amount of water during the cultivation or dipping it in water for a certain period of time. However, in the case of anka pigment production in flasks, a high yield of pigments was observed only by using rice substrate with a relatively low moisture content, which meant substrate initial moisture content of 24% and that no extra water was added to the inoculated substrate during cultivation.

The exchange of air between gas atmosphere and fungus was important for the growth of fungus and pigment formation. This exchange was influenced by both filling amount of substrate in one fermentation vessel and agitation of the inoculated substrate. A high filling amount of more than 20 g rice substrate in a 500-ml flask was unfavorable for pigment formation. When the inoculated substrate was agitated, either by loosening of or by constant shaking, rice clumping, which was observed in static cultivation, was not formed. The yield of yellow pigments was hardly influenced, yet that of orange anka pigments was enhanced by loosening the inoculated substrate once a day and reduced by constantly shaking the substrate. On one hand, proper agitation improved air exchange and as a result pigment formation, but excessive agitation disturbed the growth of the fungus and reduced pigment production.

With solid state fermentation of rice to produce anka pigments, fungal respiration reduced the oxygen concentration and increased the carbon dioxide level in the incubator. Fungal growth (data not shown) and pigment production were affected significantly by a high carbon dioxide level in the incubator. This is consistent with the study of Han and Mudgett [7], who cultured *M. purpureus* on rice under various atmospheric conditions. They found an inverse relationship between carbon dioxide level and pigment yield. Bajracharya and Mudgett [1] reported that amylase production was inhibited by a high carbon dioxide level. Reduced amylase production provided a reasonable explanation for retarded fungal growth and low pigment production by a high carbon dioxide level in the incubator. These results strongly suggest that in order to maximize pigment yield, it was necessary to remove excessive carbon dioxide accumulated during cultivation.

Usually, upon completion of fermentation, the fermented material was dried at 50°C for 24 h. However, it was found in this study that freeze drying resulted in a higher yield of each anka pigment than oven drying both at 50 or 105°C. The decrease of pigment yield at a high temperature might have resulted from the instability of anka pigments or formation of unextractable pigment complex(es). However, if the fermented material was oven-dried at 50°C for 24 h, no significant loss of orange monascorubrin was observed and 83–85% of the other three anka pigments was detected as freeze-dried anka. Therefore, if freeze drying was unavailable, oven drying at 50°C for 24 h was a reasonable alternative.

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References

1 Bajracharya R and RE Mudgett. 1980. Effects of controlled gas environments in solid substrate fermentation of rice. *Biotechnol Bioeng* 22: 2219–2235.
2 Broder CU and PE Koehler. 1980. Pigments produced by *Monascus purpureus* with regard to quality and quantity. *J Food Sci* 45: 567–569.
3 Carls M and D Shepherd. 1977. The effect of different nitrogen sources on pigment production and sporulation of *Monascus* species in submerged, shaken culture. *Can J Microbiol* 23: 1360–1372.
4 Chen MH and MR Johns. 1989. Production of natural red pigment from *Monascus purpureus*. Technology for Our Third Century: Proceedings of the 17th Australian Chemical Engineering Conference, Gold Coast, Australia, August 1989.
5 Evans PJ and HY Wang. 1984. Pigment production from immobilized *Monascus* sp. utilizing polymeric resin adsorption. *Appl Environ Microb* 47: 1323–1326.
6 Fielding BC, EJ Haws, JSE Holker, ADG Powell, A Robertson, DN Stanway and WB Whalley. 1960. Monascorubrin. *Tetrahedron Lett* 5: 24–27.
7 Han O and RE Mudgett. 1992. Effects of oxygen and carbon dioxide partial pressures on *Monascus* growth and pigment production in solid state fermentation. *Biotechnol Prog* 8: 5–8.
8 Haws EJ, JSE Holker, A Kelly, ADG Powell and A Robertson. 1959. The chemistry of fungi: Part XXXVII. The structure of rubropunctatin. *J Chem Soc* 1959: 3598–3610.
9 Hiroi T, T Takahasi, T Sima, T Suzuki, M Tsukioka and N Ogasawara. 1981. Production of red koji in solid culture. *Nippon Nôgikagaku Kaishi* 55: 1–6.
10 Johns MR and DM Stuart 1991. Production of pigments by *Monascus purpureus* in solid culture. *J Ind Microbiol* 8: 23–28.
11 Lin C-F and H Iizuka. 1982. Production of extracellular pigment by a mutant of *Monascus kaoliang* sp. nov. *Appl Environ Microbiol* 43: 671–676.
12 Lotong N and R Suwanarit. 1990. Fermentation of ang-kak in plastic bags and regulation of pigmentation by initial moisture content. *J Appl Bacteriol* 68: 565–570.
13 Song IS. 1590. Pen Zhau Kang Mu.
14 Teng S-S and W Feldheim. 1998. Analysis of anka pigments by liquid chromatography with diode array detection and tandem mass spectrometry. *Chromatographia* 47: 529–536.