KOREAN J. FOOD SCI. TECHNOL.
Vol. 21, No. 1, pp 31~36(1989)

Production of Red Pigment by Mutants of *Monascus anka*

Beung-Ho Ryu, Byeong-Ho Lee*, Bub-Gyu Park**
Hee-Sock Kim, Hye-Sung Kim Dong-Seuk Kim and Ju-Hwa Lee
Department of Food Science and Technology, Kyungsung University, Pusan
**Department of Food and Nutrition, Dongeui University, Pusan*
***The Institute of Food Science and Microbiology, Kyungsung University, Pusan*

Monascus anka 균의 돌연변이주에 의한 적색색소의 생산

류병호·이병호*·박법규**·김희숙·김혜성·김동석·이주화
경성대학교 식품공학과, *동의대학교 식품영양과, **경성대학교 종합식품연구소

Abstract

Optimal conditions of producing red pigment by *Monascus anka,* Nakazawa, *et* Sato, IFO 4478 and 6540 were found to be at pH 6.0 and 30℃ for 10 days. When 3.0% steamed rice and 1.0% defatted sesame exfracts were used as substrates, the highest production of red pigment was yielded. Furthermore, mutants such as *Monascus anka* 4478-27 and 6540-185 induced by N-methyl -N-nitro-N-nitrosoguanidine (MNNG) treatment were found to produce pigment much more than parent strains.

Key words: red pigment productivity, *Monascus anka,* MNNG treatment

Introduction

Monascus sp. has traditionally been used for making red rice wine and red soy bean cheese in china. The main components of red pigment are monoscoflavin[1], monoscorubin[2], rubropunctatin[3], and monascamin[4,5]. Since *Monascus* sp. has begun to be used for the processing foods as red color additive, the pigment was produced by the solid medium[6] and submerged medium[7,8] using various substrates. Although the mold were performed to screen by suitable cultivative medium, the attempts were not achieved for mass production. However, Lin[7] reported that mutants of *Monascus* sp. for pigment production was effectively by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treatment.

Lin and Suen[9] suggested that red koji processing was improved by mutants of *Monascus* sp. induced from an ultraviolet-ray. Mutants induced by MNNG treatment produced higher pigment than parent strains[10,11]. But there is very limited information regarding the production of *Monascus* pigment. This paper aims at investigation the optimal culture conditions for high pigment production by *Monascus* sp. and their mutants.

Corresponding author: Beung-Ho, Ryu, Department of Food Science and Technology, Kyungsung University. 110-1, Dae Yeon-dong, Nam-gu, Pusan 608-73

Materials and methods

Microorganisms

Monascus anka, nakazawa, *et* Sato IFO 4478 and *Monascus anka,* Nakazawa, *et* Sato IFO 6540 were obtained from Institute of Fermentation, Osaka.

Medium

The strains were maintained 10% glucose, 1.0% peptone 1.0% potato. 2.0% agar, pH 5.0. Pigment producing medium contained 3.0-5.0% steamed rice, 0.15% $NaNO_3$, 0.1% $MgSO_4 \cdot 7H_2O$, 0.25% KH_2PO_4[10,11] and several kinds of substrates such as sweet potato and cassava.

Estimation of pigment

Extraction of red pigment cultured from pigment producting medium was carried out according to Tsukioka *et al.*[11] with some modification. Pigment producing broth with various substrates were incubated with the solution of 2×10^5 conidial/m*l* at 30℃ for 8 or 10 days. After incubation, the culture broth was filtered with filter paper (Toyo paper No. 2) and then washed twice with 80% ethyl alcohol.

Pigment solution was adjusted at pH 4.2 and extracted with 80% ethyl alcohol on the rotary shaker for 1hr at 60℃. Pigment content produced from *Monascus anka* was measured at 500*n*m. The pigment productivity of *Monascus anka* was expressed optical density per gr of mycelial dry weight.

Isolation of highly pigment productive mutants

Isolation of mutants induced by MNNG treatment were conducted to improve the pigment production according to Hiroi *et al.*[9] with some modification. For conidiation of *Monascus* sp. medium used contained 10% sucrose, 0.1% KH_2PO_4, 0.05% $MgSO_4\ 7H_2O$, 0.2% $NaNO_3$, 0.05% KCl, 0.3% yeast extract and 2.0% agar.

Conidia of *Monascus anka* were allowed on agar slant with medium above for 6 days at 30℃, diluted to about 500 conidia/m*l* with sterilized water and then irradiated with U.V. (100V, 13W) from distance of 40cm for 3 min. On the basis of diameter and pigmentation of the giant colony, the mutants were selected among mutants induced by U.V. irradiation. For selecting pigment productive mutants, medium contained 10% sucrose, 1.0% peptone, 0.1% KH_2PO_4, 0.05% $MgSO_4\ 7H_2O$, 0.05% KCl and 0.3% asparagine, pH 4.5.

MNNG(Aldrich Chemical Co.) was dissolved in 0.2M citrate buffer, pH 5.0 which adjusted to a concentration of 18mg/m*l*. 10m*l* MNNG solution was added to 40m*l* of 3.6mg spore/m*l* and the mixtures were allowed to stable for 90 min without shaking. For selection of mutants, the mutants were allowed to grow with medium above on the rotary shaker at 30℃ for 10 days. These mutants were selected in comparision with parent strains.

Mycelium dry weight(MDW)

Mycelia were collected on the filter paper and washed twice with distilled water and then dried in a vaccum desicator for a couple of days.

Results and discussion

Improvement of culture conditions for high pigment production

Effect of pH :

The initial pH of the medium for pigment production was adjusted ranging from 5.0 to 8.0 with 0.1N-hydrochloric acid or 0.1N-sodium hydroxide. *M. anka*, 4478 and 6540 were incubated at 30℃ for 8 days on the rotary shaker.

Table 1 showed that pigment production was greatly influenced by initial pH, while growth of mycelium was not effected. The highest productivity of pigment was observed at pH 6.0. But the pigment production noticeably decreased at lower than pH 6.0 and higher than pH 7.0.

The mycelium of *Monascus anka*, 4478 yielded 4, 34g dry cell/*l*, that of *Monascus anka*, 6540, 2.63g dry cell/*l*. These results was in accordance with those of Lin[7].

Table 1. Effect of the initial pH of the medium on the high pigment production by M. anka, 4478 and 6540

	4478			6540		
	Final pH	M.D.W (%)	Pigment (OD,500nm)	Final pH	M.D.W (%)	Pigment (OD,500nm)
5	5.81	0.2828	1.55	6.00	0.3126	1.37
6	5.90	0.3039	2.34	5.96	0.3284	2.63
7	6.54	0.2743	1.27	5.94	0.3276	0.71
8	6.94	0.2723	0.50	7.49	0.3324	0.50

Both strains were incubated on a rotary shaker (160rpm) at 30℃, for 7 days, with the medium containing 3% steamed rice powder, 0.1% $MgSO_4\cdot7H_2O$, 0.2% KH_2PO_4, 0.15% $NaNO_3$, M.D.W.: Mycelium by weight

Effect of temperature

Pigment production of *Monascus anka*, 4478 and 6540 was investigated at the temperature of 26℃, 30℃ or 34℃ on the rotary shaker. As shown in Fig. 1, we found that pigment production was influenced by the temperatures, and optimal temperature for pigment production was at 30℃ for 10 days. While both strains were being incubated at 26℃, the amount of pigment production was observed to be decreasing. In the case of *Monascus anka*, 6540, pigment production decreased markedly at higher temperature than 30℃.

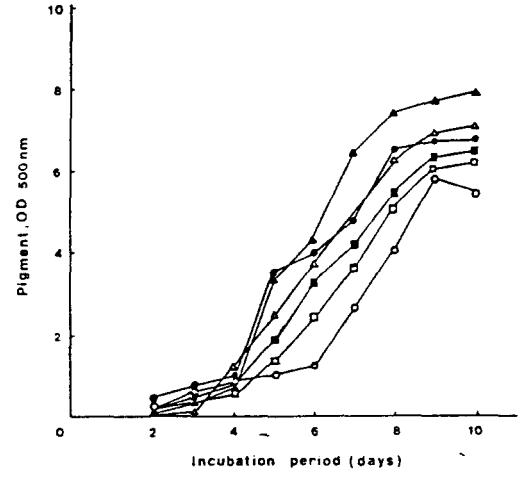

Fig. 1. Effects of incubation temperature on the pigment production by M. anka 4478 and 6540.
Both strains wer incubation on a rotary shaker at 26°C, 30° C and 34°C for 10 days in the medium containing same as Table 1.
□—□: *M. anka*, 4478(26°C) ■—■: *M. anka*, 6540(26°C)
△—△: *M. anka*, 4478(30°C) ▲—▲: *M. anka*, 6540(30°C)
○—○: *M. anka*, 4478(34°C) ●—●: *M. anka*, 6540(34°C)

Effect of substrates

Various substrate such as 3.0% or 5.0% steamed rice, 3.0% sweet potato, and 3.0% cassava sources for pigment production for 10 days. Fig. 2 showed the results of pigment production obtained by various substrates under individual conditions. When 3.0% and 5.0% steamed rice were used as substrates of 4478 and 6540 strains, the most production of pigment was yielded. *Monascus* sp. pigment was produced from other substrate such as potato and cassava starch[7,12], but the pigment production level was slightly low. These results indicated that substrates of steamed rice were

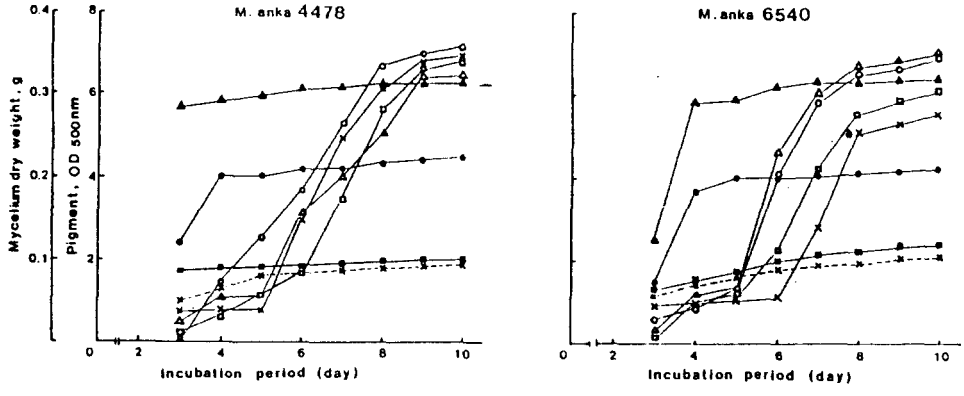

Fig. 2. Effects of carbon sources for the pigment productivity by M. anka, 4478 and 6540.

Each symbol indicated the pigment productivity and mycelium dry weight when strains were incubated at 30°C for 10 days in the medium containing carbon sources plus 0.1% $MgSO_4 \cdot 7H_2O$, 0.15% $NaNO_3$ and 0.25% KH_2PO_4, pH6.0. ○—○, pigment productivity (3.0% steamed rice addition); ●—●, mycelium dry weight; △—△, pigment productivity (5.0% steame rice adddition); ▲—▲, mycelium dry weight; □—□, 3.0% sweet potato; □—□, mycelium dry weight; ×—×, pigment productivity (3.0% cassava starch); X·····X, mycelium dry weight.

served as susperior carbon sources. We examined substrate such as barley, wheat and corn, too. They showed low level of pigment production (data not shown).

Effect of defatted sesame seed extract

In the preliminary experiments, it was found that natural nitrogen sources were essential for pigment productions.

Various concentration (0.2, 0.5, 1.0 or 1.5%) of defatted sesame seed extract were used as the substrate of pigment production(Fig. 3). Defatted sesame seed extract with final concentration of 0.5% and 1.0% were added to the basal medium containing 3.0% or 5.0% steamed rice, 0.25% KH_2PO_4, 0.1% $MgSO_4$ $7H_2O$. Defatted sesame seed extracts having 1.0% final concentration which contained 5.0% steamed rice markedly produced pigment. Defatted sesame seed extract were proved to be a suitable substrate for pigment production in both strains(Fig. 4). Highly pigment producing mutants; *M. anka,* 4478-27 and 6540-185 induced by UV irradiation MNNG treatment. Four mutants such as 4478-18, 4478-27, 4478-36 and 4478-52 induced from *M. anka,* 4478. and 6 mutants 6540-27, 6540-49, 6540-73, 6540-103, 6540-185 and 6540-230 induced from *M. anka,* 6540. They were selected from colonies among about 400 isolates on the basis of pigment productivity. The mutants were allowed

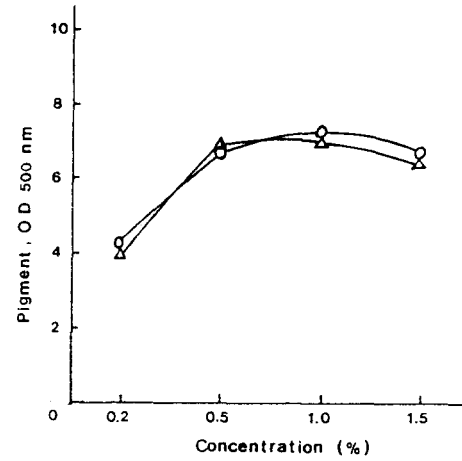

Fig. 3. Effects of various concentration of defatted sesame seed extract on the pigment prouctivity by M. anka, 4478 and 6540.

Both strains were incubated at 30°C for 10 days in the medium containing 3.0% steamed rice powder, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.2% KH_2PO_4
○—○, *M. anka* 4478; △—△, *M. anka* 6540

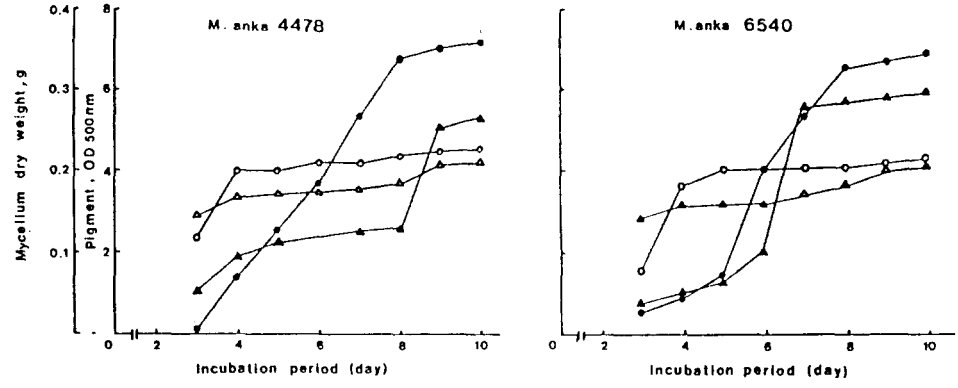

Fig. 4. Effects of defatted sesame extract on the pigment productivity by M. anka, 4478 and 6540.

Each symbol indicated the pigment productivity and mycelium dry weight when strains were incubated in the medium containing 3.0% or 5.0% steamed rice and 1.0% sesame extracts plus 0.1% $MgSO_4 \cdot 7H_2O$, 0.25% KH_2PO_4, pH 6.0.
▲—▲, pigment productivity (3.0% steamed rice plus 1.0% sesame extract); △—△, mycelium dry weight; ●—●, pigment productivity (5.0% steamed rice plus 1.0% sesame extract); ○—○, mycelium dry weight.

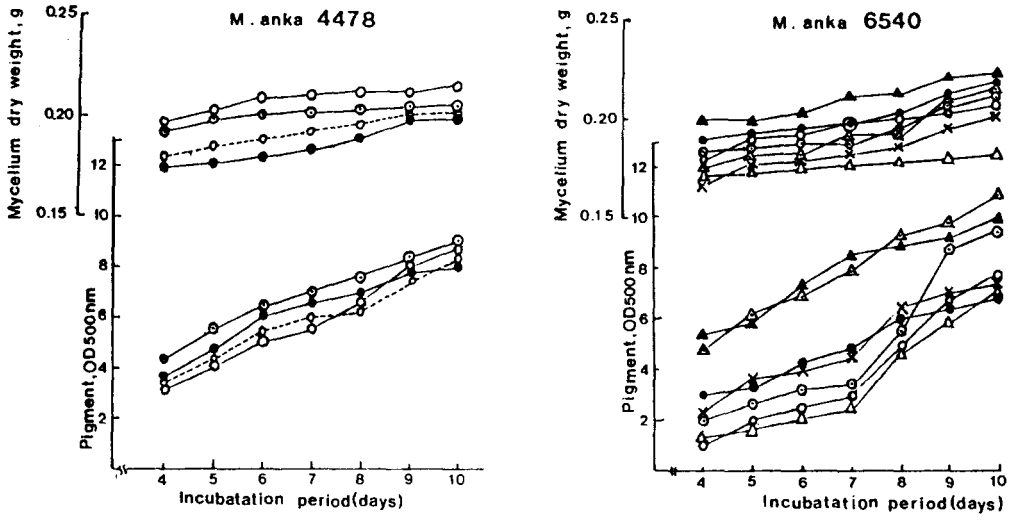

Fig. 5. Pigment productivities by mutants of *M. anka*, 4478 and 6540.

These mutants were incubated pigment production containing 3.0% steamed rice, 1.0% sesame extract, 0.1% $MgSO_4 \cdot 7H_2O$, 0.25% KH_2PO_4 and thiamine on the rotary shaker at 30°C for 10 days.

●—●: *M. anka*, 4478 ○···○: *M. anka*, 4478-36 ⊙—⊙: *M. anka*, 6540-49 △—△: *M. anka*, 6540-185
○—○: *M. anka*, 4478-18 ●—●: *M. anka*, 6540 ▲—▲: *M. anka*, 6540-73 ×—×: *M. anka*, 6540-230
⊙—⊙: *M. anka*, 4478-27 ○—○: *M. anka*, 6540-27 △—△: *M. anka*, 6540-103

to grow in pigment production medium containing 3.0-5.0% steamed rice, 0.5-1.0% defatted sesame seed extract, 0.1% $MgSO_4$ $7H_2O$, 0.25% KH_2PO_4, and thiamine(ug/m*l*) on the rotary shaker at 30℃ for 10 days, and then the amount of pigment production were measured at 500*n*m. As shown in Fig. 4, 4478-27 mutant was yielded the highest pigment production in comparison with the other 3 mutants. 6540-185 mutant yielded the highest pigment production among the other mutants. These experiments were an attempt to check correlations of the highly pigment productivity of strains with their morphological features. There was no significant features which was associated with pigment-producting mutants. From these data, in the practical view point, mutants of *M. anka*, 4478-27 and 6540-185 has been proved to be a potent pigment producer in 5.0% steamed rice and 1.0% sesame extracts cultures in which there seemed to be much possibility of manufacturing pigment.

Acknowledgement

This paper was supported by the grant from the Korean Traders Scholarship Foundation.

요 약

M. anka, Nakazawa et Sato IFO 4478과 6540 균주를 사용하여 적색색소의 최적 생산 조건을 검토한 바 pH 6.0, 온도 30℃에서 10일간이 가장 좋았다. 그리고 기질로서 탄소원으로는, 3.0% 쌀과 질소원으로는 1.0% 깨 추출물이 가장 높은 생산률을 보였다. 한편 MNNG의 처리에 의한 *M. anka* 4478-27과 *M. anka* 6540-185의 변이주는 가장 높은 색소생산능을 보였다.

References

1. Inoue, Y., Nakanishi, K., Nishikawam, H., Ohashi, M., Terahara, A. and Yamamura, S. : Structure monoscoflavin. *Tetrahedron.*, 18, 1195(1962)
2. Kumasaki, S., Nakanishi, K., Nishikawa, E. and Ohashi, M. : Structure of monascorubin. *ibid.*, 18,

1171(1962)

3. Haws, E.J., Holker, J.S.E., Kelly, A., Powell, A.D.G. and Rodbertson, A. : *Structure of rubropunctatin. J. Amer. Chem. Soc.*, **70**, 3598(1956)
4. Fielding, B.C., Haws, E.J., Holker, J.S.E., Powell, A. D.G., Robertson, A. an Whalley, W. : Monascorubin. *Tetrahedron Lett.*, No. **5**, 24(1960)
5. Kobayshi, S., Nakanishi, K. and Ohashi, M. : Unusual spectral behavior of monascamine, isodihydromonascamine and monascaminone. *Tetrahedron.*, **18**, 1185(1962)
6. Suich, I. : Preparation of soluble *Monascus* pigment. *Patent*, **48**-44880, p.245(1973)
7. Lin, C.F. : Isolation and cultural conditions of *Monascus* sp. for the prouction of pigment in a submerge culture. *J. Ferment. Technol.*, **51**(6), 407(1973)
8. Yoshimura, M., Yamanaka, S., Mitsugi, K., Hirose, Y. : Production of *Monascus*-pigment in a submerged culture. *Agric. Biol. Chem.*, **39**(9), 1789(1975)
9. Lin C.F., and Suen, S.T. : Isolation of hyperpigment productive mutants of *Monascus* sp. F-2, *J. Ferment Technol.*, **51**, 757(1973)
10. Hiroi, T., Shima, T., Suzuki, T., Tsukioka, M., Ogsaswara, N. : Hyperpigment-productive mutant of *Monascus anka*-for solid culture. *Agric. Biol. Chem.*, **43**(9), 1975(1979)
11. Tsukioka M., Hiroi, T., Suzuki, T., Konno, T. : Pigment production by mutants of *M. anka. Nippon Nogeikagaku Kaishi.*, **6**, 451(1986)
12. Hiroi, T., Takahsahi, T., Sima, T., Suzuki, T. : Production of red koji in solid culture. *Nippon Nogeikagoku Kaishi.*, **55**, 1(1981)

(1988년 5월 29일 접수)