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NOTE

Induction of a Mutant, *Monascus anka* 732Y3 from *Monascus anka* KFCC 11832 and its Morphological Observations

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***Monascus anka* 732Y3 was induced from *Monascus anka* KFCC 11832 (IFO 4478, ATCC 16360) by ultra-violet light irradiation. The growth of this new fungus is frequently more dependent on sexual propagation than asexual propagation, compared with that of its parental strain, *M. anka* KFCC 11832. Less conidia than those of *M. anka* KFCC 11832 were observed by a microscope. The optical density of the red pigments (OD_{500}) produced by *M. anka* 732Y3 was 157, which was about 10 times higer than that of *M. anka* KFCC 11832. Such high production of the red pigments by the mutant could be explained by the following observations.**

Nowadays with the increasing doubt about the safety of artificial dyestuff, new dyestuffs which can replace the artificial ones are being searched for from natural sources. One of such sources are the pigments produced by *Monascus* fungi (8). *Monascus* species have been used in Asian countries for the production of fermentative foods such as red rice, rice wine and kaoliang brandy, and soya bean cheese and food-coloring pigments (3, 5, 6, 7, 11, 13). Already, some developed countries like America, Japan and Taiwan have been successful in the mass production of the pigments (8, 12, 14). The components of the pigments are rubropunctatin ($C_{21}H_{22}O_5$-red color), monascorubrin ($C_{23}H_{26}O_5$-red color), monascin ($C_{21}H_{26}O_5$-yellow color), ankaflavin ($C_{23}H_{30}O_5$-yellow color), rubropunctamine ($C_{21}H_{23}NO_4$-purple color), and monascorubramine ($C_{23}H_{27}NO_4$-purple color) (12).

Su and Hiroi developed *M. anka* V-204 (12) and *M. anka* UN5504-4 (14), respectively. The optical densities of the produced red pigments by those mutants cultured in their own optimal conditions were 156 and 167, respectively. They were applied to plant-scaled process. According to Su, such hyper-production of Monascus pigment by those mutants is due to the their more frequent dependence of growth on sexual propagation than on asexual propagation (12).

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In this report, we deal with a mutant from *Monascus anka* KFCC 11832 (IFO 4478, ATCC 16360), *Monascus anka* 732Y3, which produces far more pigments and is often more dependent on sexual propagation than its parental fungus.

MATERIALS AND METHODS

Media Used for Incubation of *Monascus* Species

The compositions of the media used in this experiment are as following; Medium C: 10% sucrose, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.2% $NaNO_3$, 0.05% KCl, 0.001% $FeSO_4 \cdot 7H_2O$, 0.3% yeast extract, 0.5% casamino acid, and 2.0% agar (4).

PDA (potato dextrose agar): 20% potato infusion, 2.0% glucose, and 2.0% agar.

Pigment production medium: 7.0% rice powder, 0.3% peptone, 0.25% KH_2PO_4, and 0.1% $MgSO_4 \cdot 7H_2O$.

Mutagenesis of *Monascus anka* KFCC 11832

A medium C slant was inoculated with *M. anka* KFCC 11832 (IFO 4478), and incubated at 30℃ for 7 days. And then, 10 ml of sterilized water was added to the slant for the suspension of spores. The suspended spores were filtered with a cotton filter in order to remove the mycelia and the filtrates were diluted 10 times. 10 ml of the diluted spore solution was poured into a petridish

of 9 cm diameter, and an ultra-violet light (100 V, 13 W) was irradiated to it from a 40 cm distance for 2 minutes for 99% lethal rate. 100 μl of this UV light-irradiated spore solution was smeared onto the plates of medium C and potato dextrose agar, and after a 7 day incubation at 30℃, the colonies with relatively deep red color and wide diameter were selected (4).

Production and Analysis of Monascus Pigments

M. anka KFCC 11832 and its mutants were subcultured at 30℃ for 7 days on media C. Each of the subcultured *Monascus* strains was inoculated into a 500 ml flask with 50 ml of pigment production medium. And then, the inoculated *Monascus* strain was incubated with shaking at 120 rpm at 30℃ for 7 days.

After cultivation, in order to extract the pigments, 200ml of 95% Ethanol was added to the culture and shaked reciprocally at 120 rpm for one hour at 30℃. And the extracts were filtered through Toyo No. 2 filter paper, and the optical densities of the red and yellow pigments which were respectively detected at wavelengths of 500 nm and 400 nm were accepted to represent the amounts of them as the other researchers did (8, 9, 12, 14).

RESULTS AND DISCUSSION

Pigment Productions of *M. anka* KFCC 11832 and Its Mutants

Seven mutants of *M. anka* KFCC 11832 (IFO 4478) were selected and their pigment productions were tested (Table 1). *M. anka* 7 is a mutant of *M. anka* KFCC 11832; *M. anka* 7-3 and 7-4 mutants of *M. anka* 7, *M. anka* 732Y and 741C mutants of *M. anka* 7-3 and 7-4 respectively, *M. anka* 732Y3 a mutant of *M. anka* 732Y, and *M. anka* 704L-55 a mutant of *M. anka* 732Y3. Until the fourth mutagenesis, the pigment productions of the mutants of *M. anka* KFCC 11832 increased. The pigment production by the fifth mutant, *M. anka* 704L-55, on the contrary, decreased. Therefore, *M. anka* 732Y3 was concluded to be the most hyperpigment-productive mutant of those from *M. anka* KFCC 11832. Along with the increases of the pigment production, the dry cell weights also increased through mutagenesis.

Table 1. Pigment production of *M. anka* KFCC 11832 and its mutants.

Strain	Optical density of the Pigments produced		Dry cell weight (mg/50 ml)
	OD_{500} (red)	OD_{400} (yellow)	
M. anka KFCC 11832	15.9	21.1	604
M. anka 7	29.6	17.2	760
M. anka 7-3	20.6	14.3	812
M. anka 7-4	27.3	25.6	880
M. anka 732Y	87.2	76.7	960
M. anka 741C	78.8	67.6	943
M. anka 732Y3	157	148	955
M. anka 704L-55	124	109	912

Su developed *M. anka* V-204 from *M. anka* IFO 4478 (12), and Hiroi induced *M. anka* UN5504-4 from *M. anka* IFO 4478 (14). The optical densities at 500 nm of the red pigments produced by *M. anka* V-204 and *M. anka* UN5504-4 were 156 and 167, respectively, when they were cultured in their own optimal conditions. In the case of *M. anka* V-204, it was introduced to the plant-scaled process. With consideration of that case, *M. anka* 732Y3 is also believed to be appicable enough to a plant-scaled process if the optimal condition for the production of Monascus pigments by it is set up.

Fig. 1 represents the colonies of *M. anka* 732Y3 and *M. anka* KFCC 11832. In media A, both *M. anka* KFCC 11832 and *M. anka* 732Y3 had wrinkles, the colors of the two colonies were almost the same, and *M. anka* 732Y3 had very short mycelia. In media B, however, the colony of *M. anka* 732Y3 had very strong red color and very short mycelia.

Microscopic Observations of *M. anka* KFCC 11832 and *M. anka* 732Y3

Monascus fungus is homothallic, and its life cycle is composed of asexual propagation accompanied by one-celled conidia and sexual propagation by ascospores in cleistothecia(10). Monascus fungi are known to have to be classified, independently, into a Family of Monascaceae. But because of the formation of conidia and cleistothecia, they are generally classified into the Family of Aspergillaceae (2). According to Su's report, the inhibiting the formation of conidia stimulates the production of Monascus pigments (12). Therefore, a *Monascus* sp. which propagates sexually more often than asexually will probably be effective in the production of Monascus pigments. But the factors which control sexual and asexual propagations are not yet discovered, and also, the roles of the pigments have not been discovered yet.

Fig. 2 shows the conidia of *M. anka* KFCC 11832 and *M. anka* 732Y3 observed after 8 days of incubation. At that moment, *M. anka* 732Y3 was forming fewer conidia than its parental strain.

Furthermore, among the conidia of *M. anka* 732Y3, red one could be clearly seen, which could not be seen in *M. anka* KFCC 11832 probably because it would

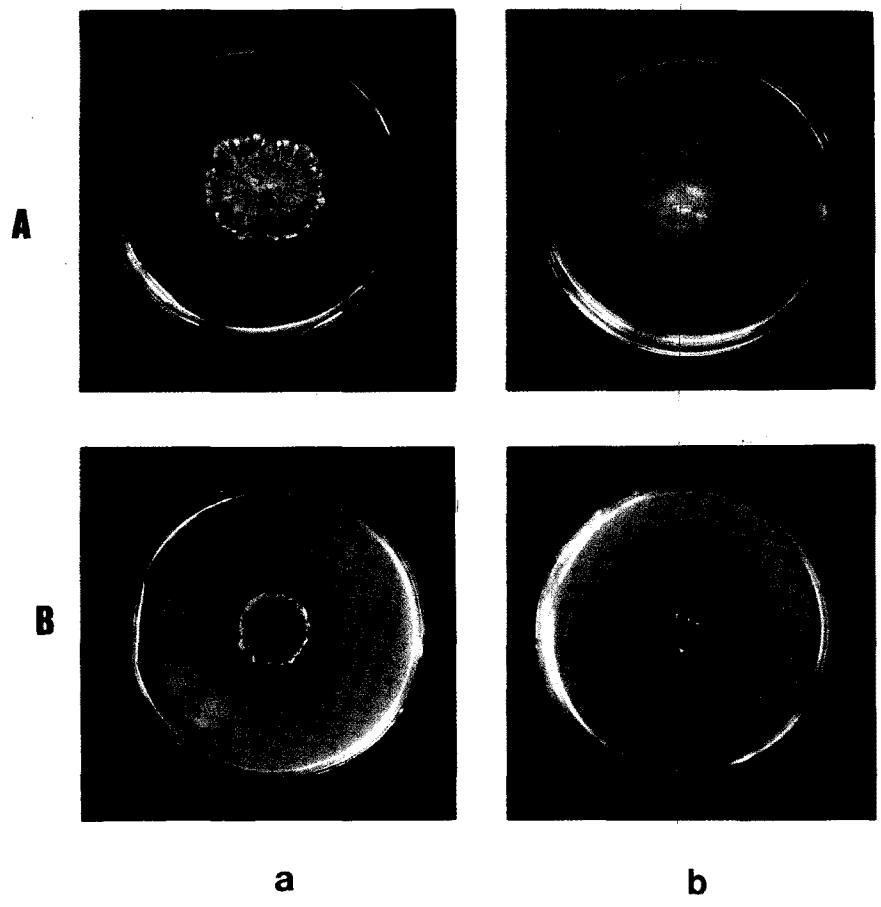


Fig. 1. Colonies of *M. anka* KFCC 11832 and *M. anka* 732Y3 cultured in different media for 7 days.
M. anka KFCC 11832 (A) and *M. anka* 732Y3 (B) were cultured in two different media prepared in plates. The media used for (a) and (b) were potato dextrose agar and medium C.

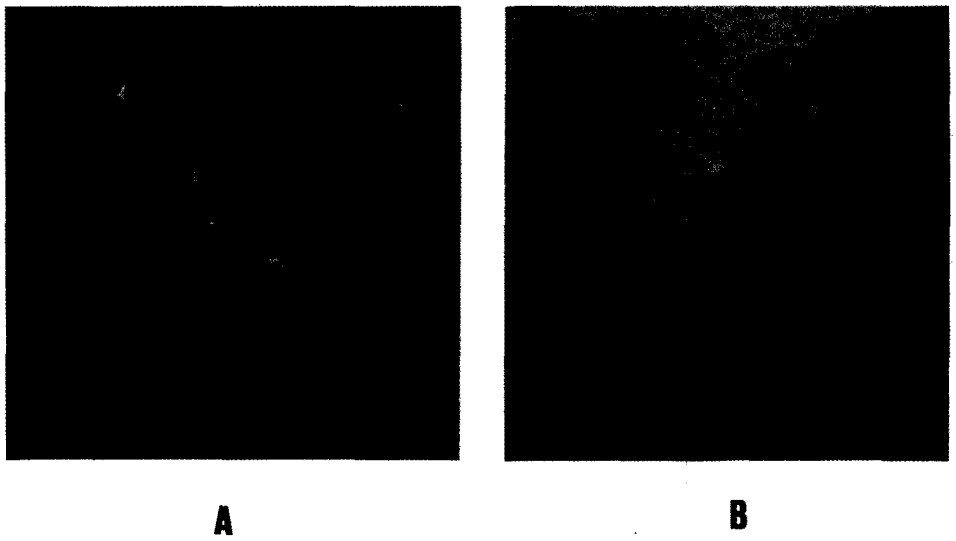
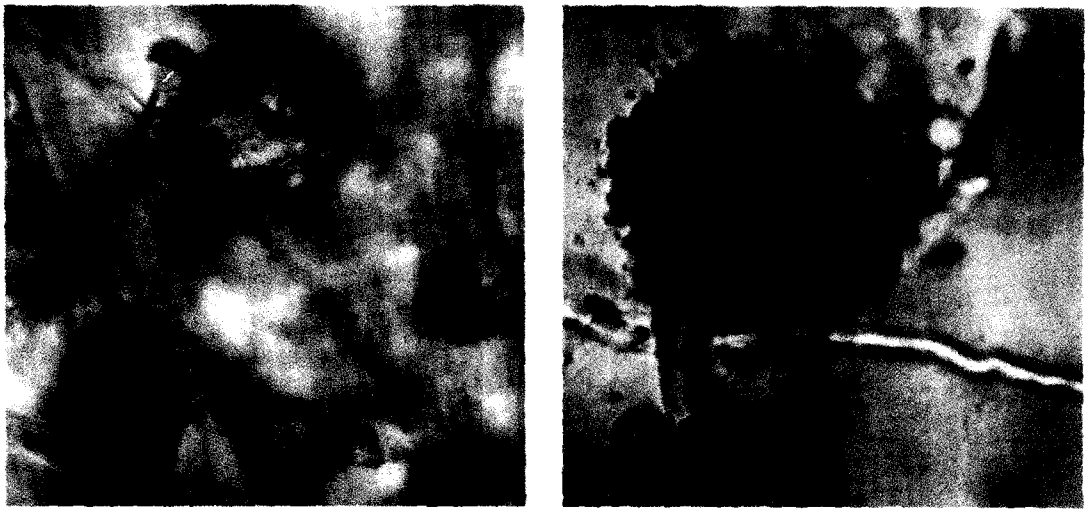


Fig. 2. Microscopic observation of conidia of *M. anka* KFCC 11832 (A) and *M. anka* 732Y3 (B) after 7 days' cultivation in potato dextrose agar at 30℃ (×400).



A B

Fig. 3. Microscopic observation of cleistothecia of *M. anka* KFCC 11832 (A) and *M. anka* 732Y3 (B) after 7 days' cultivation in potato dextrose agar at 30℃ (×400).

be too brightly red.

The cleistothecia of *M. anka* 732Y3 and *M. anka* KFCC 11832 are shown in Fig. 3. The formation of the red pigments could be seen around the cleistothecia, but the depth of the color was stronger in *M. anka* 732Y3 than in *M. anka* KFCC 11832. And also, the cleistothecium of *M. anka* 732Y3 was bigger than that of *M. anka* KFCC 11832.

Many of the cleistothecia of the two strains were red even though their depth differed. But, in the case of conidia, only one of *M. anka* 732Y3 was red. These phenomena are in accord with Su's comment that the production of red pigments such as rubropunctatin and monascorubrin is accompanied by an inhibition of conidiation (12). Carel and Shepherd (1) also said that pigment production is better when conidiation is reduced. Therefore, it was made clear that the production of Monascus pigments would increase if a *Monascus* species developed cleistothecia more often than conidia. But as seen in Fig. 3(A), the formation of cleistothecia is not always accompanied by the formation of pigments. And also, one red conidium in Fig. 2 suggests that *Monascus* pigments do not always have relation with the types of propagation, sexual and asexual propagations. The reason why *M. anka* 732Y3 produces more pigments than *M. anka* KFCC 11832 is that the former develops bigger cleistothecia and fewer conidia than the latter. Therefore, developing or screening a *Monascus* sp. with bigger cleistothecia and fewer conidia solves one of the problems in the hyper-production of Monascus pigments.

Up to now, the most effective method to produce high amount of Monascus pigments has been mutagenesis by NTG or ultrviolet light-treatment (12, 14). By the way, the synthetic pathway and the role of the pigments in *Monascus* strains are not discovered yet out. Therefore, finding out the role of Monascus pigments, accordingly, seems to be an important problem in increasing their production.

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