Note

Hyperpigment-productive Mutant of *Monascus anka* for Solid Culture

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Recently, monascus-pigment is being used as a food coloring additives. It is extracted from solid cultures of the mold with ethanol or propylene glycol.

Lin¹⁾ reported that *Monascus* sp. F-2, an effective pigment-productive strain in submerged culture, was improved by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) treatment, and Su *et al.*²⁾ reported that red *koji* processing was improved by using an ultraviolet ray-induced mutant of *Monascus anka*.

However, isolation of a suitable hyperpigment-productive strain in aerated solid culture with rice or fine flakes of bread has not yet been reported. We have investigated strains belonging to genus *Monascus* to obtain hyperproductive strains for pigment-production using mechanical *koji* processing.

Experiments with ultraviolet ray (UV) irradiation followed by NTG treatment were conducted to improve the yield of pigment-production by *Monascus anka* Nakazawa *et Sato* after screening tests of strains on pigment-production and growth. For conidiation of fungus, the following medium (medium C) was used: sucrose 10 g, KH₂PO₄ 0.1 g, MgSO₄·7H₂O 0.05 g, NaNO₃ 0.2 g, KCl 0.05 g, FeSO₄·7H₂O 0.001 g, yeast extract powder 0.3 g, casamino acids 0.5 g, agar 2 g, in 100 ml of distilled water. Conidia of *Monascus anka* were allowed to grow on agar slant with the above medium for 4 days at 30°C, diluted to about 500 conidia/ml with sterile water and irradiated with UV (100 V, 13 W) from a distance of 40 cm for 2.5 min. On the basis of diameter and pigmentation of the giant colony, three mutants were selected among 98 mutants induced by UV-irradiation. For selecting pigment-productive mutants, the following medium (medium P) was used: sucrose 10 g, peptone 1 g, KH₂PO₄ 0.1 g, MgSO₄·7H₂O 0.05 g, tartaric acid 0.1 g, L-asparagine 0.3 g, in 100 ml of tap water, and pH of medium was 4.4. With the selected mutant strain U2, U26 and U55, NTG-treatment was carried out. To 10 ml of conidial suspension of UV mutant, 0.2, 0.4 or 0.6 ml of NTG

TABLE I. GROWTH AND PIGMENT-PRODUCTION OF ISOLATED MUTANTS ON SHAKING CULTURE

Each organism was cultured by rotary shaker (150 rpm) at 30°C for 8 days.

Organism	Dry weight of mycelia (mg/200 ml)	Pigment produced (OD 500 nm)	Pigment-production ratio of mutant to parent organism
<i>M.anka</i> Nakazawa <i>et Sato</i>	228.2	0.088	1.00
U2	180.3	0.353	4.01
UN202-6	240.4	0.201	2.28
UN202-12	224.9	0.463	5.26
UN202-13	262.8	0.629	7.15
UN202-14	186.5	0.379	4.32
UN202-15	220.3	0.256	2.91
UN204-6	179.0	0.402	4.57
UN204-8	269.7	0.382	4.34
UN204-9	210.0	0.403	4.58
UN204-15	234.0	0.470	5.34
U55	316.5	0.503	5.72
UN5502-2	284.2	0.502	5.70
UN5502-3	281.2	0.414	4.70
UN5502-8	274.8	0.532	6.05
U26	249.3	0.612	6.95
UN2602-8	375.9	0.324	3.68
UN2602-27	248.4	0.350	3.98
UN2604-4	296.4	0.644	7.32

solution which has a concentration of 1 mg/ml was added. By these procedures, 18 mutants were selected, and the mutants were allowed to grow in medium P on a rotary shaker for 8 days at 30°C, and mycelium yield and pigment-production were measured. As shown in Table I, pigment-productivity of each of these mutants, which was measured by absorbancy at 500 nm, was improved in comparison with parent organisms as follows: 7.32, 7.15 and 6.05 times for UN2604-4, UN202-13 and UN5502-8, respectively.

To examine the pigment-production of mutants on rice *koji*, seven mutants and the original strain were incubated on steamed rice in Erlenmeyer flasks for 8 days at 34°C. The preparation of rice medium for solid culture was as follows: hulled rice of 72% grade was soaked in aqueous solution, which was adjusted to pH 2.0 with lactic acid, for 15 hr at 10°C. After the solution was removed, the soaked hulled rice was autoclaved for 15 min at 120°C and cooled to room temperature. As shown in Table II, two mutants, strain UN2604-4 and UN202-13, showed hyperpigment-production, when the absorbancy of their ethanol-extracts were measured at 500 nm. The former was 17.7 times and the latter 21.4 times greater, respectively, in comparison with the parent organism.

Some differences in thermostability of conidia and

TABLE II. PIGMENT-PRODUCTION OF ISOLATED MUTANTS ON SOLID CULTURE WITH STEAMED HULLED RICE

Each organism was cultured in 500 ml Erlenmeyer flask containing 20 g of steamed hulled rice at 34°C for 8 days.

Organism	Pigment in rice <i>koji</i> (OD 500 nm)	Pigment-production ratio of mutant to parent organism
<i>M.anka</i> Nakazawa <i>et</i> Sato	31.1	1.0
U2	398.0	12.8
UN202-13	666.0	21.4
UN202-14	450.0	14.5
U55	544.0	17.5
UN5502-8	464.0	14.7
U26	492.0	15.8
UN2604-4	552.0	17.7

ascospores between mutant UN202-13 and the parent organism were observed. Conidia and ascospores of the parent organism died at 65°C and 70°C, respectively, whereas those of mutant UN202-13 died at 55°C and 60°C, respectively. The results indicated that mutant UN202-13 has slightly lower thermostability in comparison with that of the parent organism.

For comparisons of conidia and ascospores in pigment-productivity, mutant UN202-13 was cultured on agar slants with two media, that is, medium C and medium P for obtaining conidia and ascospores, respec-

tively. To obtain conidia, conidial suspension prepared from organisms grown on medium C, was filtered through absorbent cotton to separate mycelia. Isolation of ascospore was performed by heating the suspension, which was prepared from organisms grown on medium P, for 20 min at 55°C to kill conidia. The serial transfers with each medium were made 10 times by using the conidia and ascospores obtained, then the giant colonies were cultured for 10 days at 30°C with each strain of conidial and ascospore origin. Both giant colonies macroscopically showed the same growth rate and pigmentation characteristics. The properties of mutant UN202-13, which were derived from conidium with UV irradiation followed by NTG treatment, appeared to be inherited by the ascospore.

From these results, we decided to use mutant UN202-13 for large scale production of monascus-pigment by solid culture with aeration using rice or fine flakes of bread.

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