Determination and Genetic Variation Analysis of Wild-type and Albino's Mutant of *Monascus spp.* by Random Amplification of Polymorphic DNA (RAPD) Method

Tutus Gusdinar[1)], Marlia Singgih[1)], Tiana Milanda[2)], Haryanto Dhanutirto[1)]

[1)]School of Pharmacy ITB-Indonesia
[2)]Dept. of Pharmacy Padjadjaran University-Indonesia

ABSTRACT

Determination and genetic variation analysis of wild-type and albino's mutan of *Monascus* spp. isolated from Cikapundung River at Bandung area, were carried out using Random Amplification of Polymorphic DNA (RAPD) method. The albino's mutant, was obtained by Ethyl Methane Sulphonate (EMS)-treated spores of the wild-type. RAPD result of *Monascus* spp wild-type was compared with *Monascus purpureus* CECT 2955T as reference standard, and it was found that both RAPD profiles observed was identical. These result showed that the wild-type is proved to be *Monascus purpureus*. However, one of the DNA bands of albino's mutant at 1150 bp was not found on the wild-type and the standard, and it was considered as a genetic variation resulted from the mutation process.

Key words : RAPD, *Monascus purpureus*, wild-type, albino's mutant

INTRODUCTION

Monascus purpureus are filamentous ascomycetes that form asexual uninucleate spores. They well known producers of natural pigments which have been widely used in the Far East as food colorants for rice (anka, angkak), processed meats (sausage or ham), wine or in Chinese medicine (Fabre *et al*. 1993; Jacobson and Wasileski, 1994). Moreover*, M. purpureus* produces statins (such as lovastatin), with HMG-CoA reductase inhibitory properties, used to reduce cholesterol levels in blood (Endo, 1985). They are

also used commercially to produce alcohol, organics acids and enzymes (Chen and Tseng, 1989; Martinkova *et al*., 1995)

M. purpureus was first isolated from Chinese anka in Java, Indonesia (Wong and Bau, 1977). Based on morphological and biochemical reactions, 12 species and two varietas of *Monascus* were identified from isolates from Asian countries (Lizuka and Lin, 1981). Hawksworth and Pitt (1983) identified *Monascus* species using microscopic morphological characteristic on different culture media. But variation due to mutations or genetic recombination in reproductive structure can make identification of *Monascus* difficult at specific level.

Determination of genetic variation in fungi can be assessed by several biology techniques such as protein profile analysis, isoenzyme analysis, RFLP (Cruz *et al*., 1996), PCR (Mullis *et al*., 1986), RAPD (Welsh and and McClelland, 1990) and AFLP (Peleman *et al*., 1995) have provided rapid, accurate indications of genetic relationships. K. Lakrod *et al.* (2000) initiated genetic investigation of *Monascus* spp using RAPD markers and cluster analysis to determine the level of genetic variation within a collection of isolates from food products from Asian countries. S. Campoy *et al.* (2003) characterized a high-level pigment-producing *Monascus* IBCC1 as *M. purpureus* by RAPD method too.

In this work, we determine and analysis of genetic variation of a wild-type and an albino's mutant of *Monascus spp.* by RAPD method. Our objective was to identified species of the wild-type and to asses the genetic variation resulted from the mutation process in albino's mutant. This mutant will be used as cell receptor in a genetic transformation system for *Monascus purpureus*.

MATERIALS AND METHODS

Strains and culture conditions

The wild-type, *Monascus* spp. KM1 was isolated from Cikapundung River at Bandung area. The albino's one, a strain non-producing azaphilone pigments, was obtained by Ethyl Methane Sulphonate (EMS)-treated spores of the wild-type. As a reference strain, *Monascus purpureus* CECT 2955T was used.

All fungal strains were grown in YMP agar (0.3% yeast extract, 0.3% malt extract, 0.6% peptone, 2% glucose, 1% bacto-agar). Spores from one big glasstube of YMP agar were collected using sterile aquadest. The spores were used to inoculate liquid cultures in YMP broth.

Isolation and extraction of fungal DNA

Mycelial masses of each *Monascus* were grown and increased in 20 mL YMP broth using a spore suspension (10^5-10^6 spores/mL) from 7-10 days culture in YMP agar as starter. Liquid cultures were incubated at room temperature on an orbital shaker (150 rpm) for 20 hours. Mycelial masses were harvested by filtration using Whatman No. 1 paper.

DNA's of each isolate were extracted in CTAB extraction buffer using small potter for several minutes. Mycelial lysate were incubated at 65°C for 45 minutes, then extracted with chloroform. Nucleic acid were precipiteted using one of isopropanol, then were redissolved in Tris-EDTA pH 8 (100 mM Tris pH 8, 1 mM EDTA). RNA's were removed by adding DNAse free RNAse. DNA's were further extracted using phenol-chloroform-isoamylalcohol (25 :24 :1) at least twice, followed by chloroform extraction at least once. DNA's were precipitated using 0.1 vol 3 M sodium acetate pH 5.2 and 1 vol. isopropanol. DNA pellets were washed with 70% ethanol and redissolved with Tris-EDTA pH 8.

RAPD analysis

Total genomic DNA's from each *Monascus* were spectrophotometrically quantified at 260-280 m and were diluted to 50 mg/mL before using as a DNA template in a RAPD analysis. RAPD rections were performed using oligonucleotides CRL9 (5'-CAGCCGCCCC-3') and CRL12 (5'-CGCCGCCCG-3'), according to Kubelik And Szabo (1995). The PCR reaction were performed in DNA thermocycler (Perkin-Elmer) using 25 µL volume containing 0.5 units of *Taq* DNA polymerase, *Taq* polymerase buffer (Sigma Chemical Co.), 90 nmol $MgCl_2$, 8 fmol DNA primer, 5 nmol of each dNTP and 280 ng of DNA template. The PCR program was as follows : an initial 4 min

at 94°C, followed by 44 cycles of 40 s at 94°C, 60 s at 34°C and 120 s at 72°C, with a final 10 min at 72°C.

RAPD products were electrophoretically separeted in 1.6% agarose in 0.5 TBE. RAPD fragments were examined by ethidium bromide staining and transmitted uv lights. Gels were photographed with a Polaroid camera using Polaroid film.

RESULTS

DNAs from *Monascus* spp. KM1, the albino's mutant and reference strains (*Monascus purpureus* CECT 2955T) were compared by RAPD (Fig 1 and Fig.2). Identical bands were observed both of the wild type and the reference. However, one of the DNA bands of albino's mutant at 1150 bp was not found on the wild-type and the reference.

b

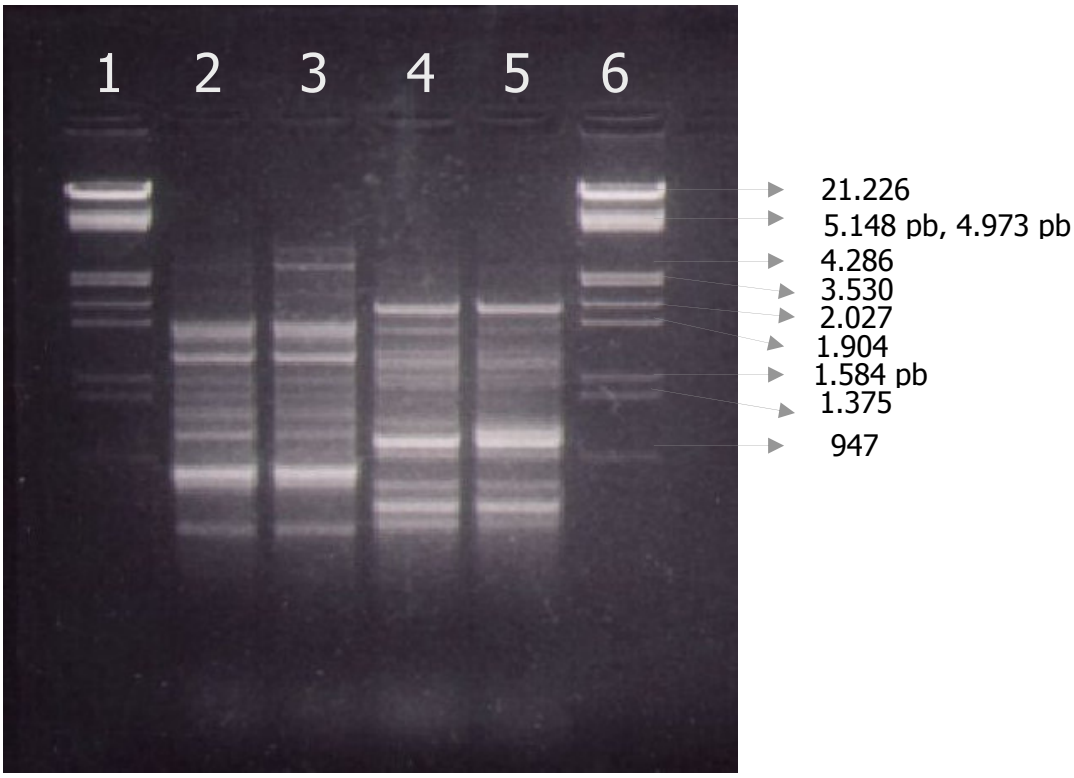

Fig. 1a RAPD amplification of *Monascus* spp. DNA using oligonucleotides CRL9 and CRL12.
Lane 1 and 6 DNA markers
Lane 2 *Monascus purpureus* CECT 2955T/CRL9
Lane 3. *Monascus* spp KM1/CRL9
Lane 4 *Monascus purpureus* CECT 2955T/CRL12

Lane 5. *Monascus* spp KM1/CRL12

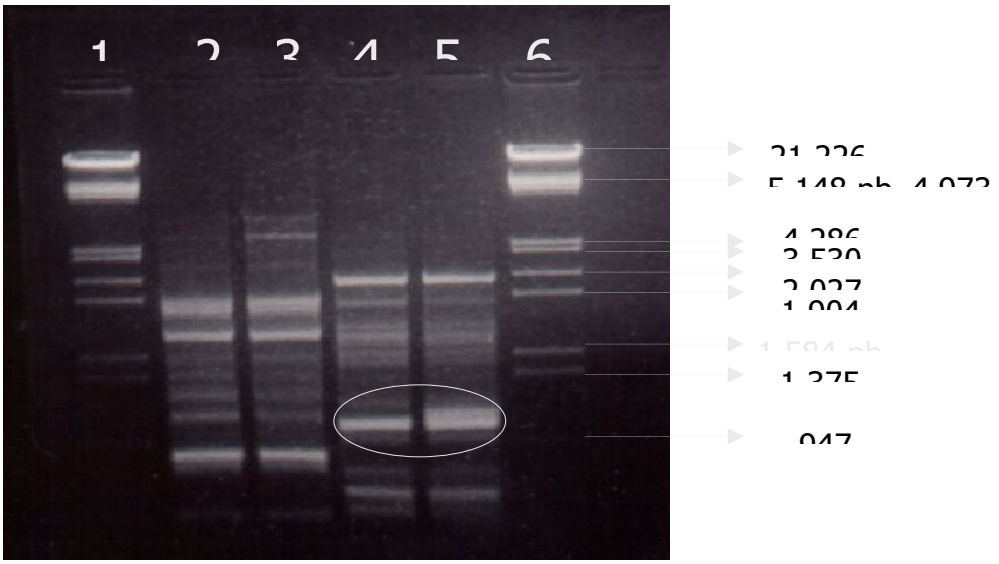

Fig. 2 RAPD amplification of *Monascus* spp. DNA using oligonucleotides CRL9 and CRL12.
Lane 1 and 6 DNA markers
Lane 2 *Monascus purpureus* CECT 2955T/CRL9
Lane 3. *Monascus* albino's mutant/CRL9
Lane 4 *Monascus purpureus* CECT 2955T/CRL12
Lane 5. *Monascus* albino's mutant/CRL12

DISCUSSION

The identical of RAPD profiles of *Monascus* spp wild-type and *Monascus purpureus* CECT 2955T showed that the wild-type is proved to be *Monascus purpureus*. One of the DNA bands of albino's mutant at 1150 bp was not found on the wild-type and the standard, and it was considered as a genetic variation resulted from the mutation process.

ACKNOWLEDGEMENT

This research was financially supported by Hibah Penelitian Tim Pasca Sarjana (HPTP), ITB, Indonesia, 2003-2004

REFERENCES

Campoy, S., F. Perez, J.F. Martin, S. Guiterrez and P. Liras (2003) Stable transformants of the azaphilone pigment-producing *Monascus purpureus* obtained by protoplast transformation and *Agrobacterium*-mediated DNA transfer. *Curr Genet* **43**, 447-452.

Chen, M. and Y.Y. Tseng (1989) Efficacy of antimicrobial substance from *Monascus* metabolites on preservation of meat. *Science and Technology* **2**, 483-485.

Endo, A. (1985) Compactin (ML-236B) and related compound s as potential cholesterol-lowering agents that inhibit HMG-CoA reductase. *J Med Chem* **28**, 401-405.

Fabre, C.E., A.L. Santerre, M.O. Loret, R. Barberian, G. Pareilleux, G. Goma and P.J. Blanc (1993) Production and food application of the red pigments of *Monascus ruber. J Food. Sci* **58**, 1099- 1110.

Hawksworth, D.L. and J.I. Fitt (1983) A new taxonomy for *Monascus* species by on cultural and microscopical characters. *Australian J Bot.* **31**, 51-61.

Jacobson, G. and J. Wasileski (1994) Production of food colorants by fermentation In : A. Gabelman (ed) **Bioprocess production of flavour, fragrance and color ingredients**. Wiley Interscience. New York, 205-237.

Kubelik, A.R., L.J.Szabo (1995) High-GC primers are useful in RAPD analysis of fungi. *Curr Genet* **28**, 384-389.

Lakrod, K., C. Chaisrisook, B. Yongsmith and D.Z. Skinner (2000) RAPD analysis of genetic variation within a collection of *Monascus* spp. isolated from red rice (ang-kak) and sofu. *Res* **104**, 403-408

Lin, C.F. and H. Lizuka (1982) Production of extracellular pigment by a mutant of *Monascus kaoliang* sp. nov. *Applied and Enviromental Microbiology* **43**, 671-676.

Martinkova, L., P. Juzlova and D. Vesely (1995) Biological activity of polyketide pigments produced by the fungus *Monascus. Journal of Applied Bacteriology* **79**, 609-616.

Mullis, K.B., F.A. Faloona, S. Scharf, R.G. Saiki, G. Horn and H. Erlich (1986). Spesific enzymatic amplification of DNA in vitro : the polymerase chain reactions. *Cold Spring Habour Symposium on Qualitative Biology* **51**, 263-273.

Peleman, J., M. Kuiper and M. .Zaeban (1995) AFLP : a new concept for DNA fingerprinting. *Nucleic Acids Research* **23**, 4407-4414.

Vera Cruz, C.M., E.Y. Ardales, D.Z. Skinner, J. Talag, R.J. Nelson, F.J. Louws, H. Leung, T.W. Mew and J.E. Leach (1996) Measurement of halotopic variation in *Xanthomonas oryzae* pv. o*ryzae* within a single field by repPCR and RFLP analysis. *Phytopathology* **86**.1352-1359.

Welsh, J. and M. McClelland (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18**, 7213-7218.

Wong, H.C. and Y.S. Bau (1977) Pigementation and antibacterial activity of fast neutron and X-ray induced strains of *Monascus purpureus* Went. *Plant Physiology* **60**, 578-581.