Dynamic Biochemistry, Process Biotechnology and Molecular Biology ©2011 Global Science Books

A Water-Soluble, Non-aromatic, Nitrogenous Compound from a Hyper-red Pigment-Producing Mutant of *Monascus purpureus*

Sandipan Chatterjee[1] • Pritam Chattopadhyay[1] • Sharmistha Maity[1] • Angshuman Sarkar[2] • Subrata Laskar[3] • Sukanta Kumar Sen[1*]

[1] Microbiology Division, School of Life Sciences, Department of Botany, Visva-Bharati University, Santiniketan 731 235, India
[2] Department of Statistics, Visva-Bharati University, Santiniketan 731 235, India
[3] Department of Chemistry, The University of Burdwan, Burdwan 713 104, India

Corresponding author: * **sksenvb@rediffmail.com**

ABSTRACT

Attempts have been made to develop hyper-pigment-producing mutants of *Monascus purpureus* MTCC 1090 by UV irradiation. Harvested spores of *M. purpureus* were irradiated and LD_{50} was determined. Out of several mutants, M^{10c} was selected as the most potent. Total protein profile of M^{10c} was compared with the prototroph of *M. purpureus* to confirm its mutant nature. Pigment extraction was standardized using different test solvents. The extracted pigment was purified by thin layer chromatography and column chromatography. The final yield of red pigment at the laboratory scale was 6.8 g Kg^{-1}. For characterization of the principal compound, spectral analysis using UV-Vis, IR and GC-MS were performed. The purified compound was also checked for antimicrobial efficacy and toxicity. The purified compound was active against Gram-positive test bacteria, *Bacillus* sp. and *Staphylococcus* sp. and found to be non-toxic against test Swiss albino mice.

Keywords: characterization, *Monascus*, mutant, over production, purification

INTRODUCTION

The value of a commodity is generally enhanced by the addition of color. Commonly used colorants are of synthetic nature. Therefore, long-term intake of such compounds is a matter of great concern both in terms of physiological and environmental aspects (Evans and Wang 1984; Carvalho *et al.* 2003; Babitha *et al.* 2007; Chatterjee *et al.* 2009; Li *et al.* 2010). As a result, a series of synthetic food colors were banned or placed on a red list, e.g., tartrazine was banned in Norway and Austria, quinoline yellow in the U.S, brown HT in Denmark, Belgium, France, Germany, USA and Norway, etc. (Kapoor 2006). While using synthetic compounds, many toxic effluents result from the dyeing process. Thus, suitable alternatives that allow for the exploitation of pigment-producing microorganisms are required (Mapari *et al.* 2005; Chairote *et al.* 2008). Several species of microalgae, bacteria as well as fungi produce a range of water-soluble pigments, but low productivity is the main bottleneck for their commercialization. The number of approved colorants for the food industry is also limited (Roy *et al.* 2008).

Monascus species have been traditionally used in Oriental countries for coloring and preserving a number of fermented foods ranging from red rice wine, red soyabean cheese (Patakova *et al.* 1998; Lin *et al.* 2008), *angkak* or *hongqu* (Chinese) or *benikoji* (Japanese) or red rice. In some countries, such colors are used in fish (fish sauce, fish paste), bean (bean curd or tofu), milk (yogurt), and wine products for preserving and to intensify colors. However, red rice is a unique fermented product enriched with amino acids like (+) monascumic acid, (-) monascumic acid, tryptophan, etc., antioxidant and pigments vary in concentration when fermented with different strains of *Monascus* (Klimek *et al*. 2009).

Monascus purpureus, an ascomycetous fungus, is known to produce a complex mixture of pigments (**Table 1**) (Kumasaki *et al.* 1962; Blanc *et al.* 1994; Zhang 2000; Lin *et al.* 2008; Loret and Morel 2010). Therapeutic properties (Hajjaj *et al.* 2000; Erdogrul and Azirak 2004; Hsu *et al.* 2010; Shi and Pan 2010) and relatively high stability with respect to pH (2.0-12.0) and temperature (4-110°C) are some added features that favor their use as natural colorants (Pastrana *et al.* 1995; Carvalho *et al.* 2005; Panda *et al.* 2007). To overcome the bottleneck of pigment overproduction by *M. purpureus*, genetic improvement is supposed to be one of the promising approaches as reported for other fungi (Wong and Koehler 1981).

This manuscript deals with the development of a hyper pigment-producing mutant of *M. purpureus* under UV-irradiation, following purification, characterization, and toxicity test of the pigment compound.

Table 1 Pigments obtained from *Monascus*. Sources (see text)

Pigment name	Color	Molecular weight (Da)
Monascorubramine	Red	381
Rubropunctamine	Red	353
Monascorubrine	Orange	382
Rubropunctatine	Orange	354
Ankaflavin	Yellow	386
Monascine	Yellow	358

MATERIALS AND METHODS

Organism used

Monascus purpureus MTCC 1090 was obtained from the Microbial Type Culture Collection, Chandigarh, India and used as parent strain for mutagenesis. The strain was maintained on YPSS medium at 4°C.

Seed medium

The strain was grown in modified malt-yeast extract medium (Mak *et al.* 1990) comprised of (g L^{-1}) malt extract 2.5, yeast ex-

Received: 7 October, 2010. Accepted: 20 January, 2011.

Original Research Paper

tract 1.5, and glucose 5.0. The pH was adjusted to 8.0. Fermentation was carried out at 32°C in total darkness.

Fermentation

Low grade broken rice was used as the substrate (Wang *et al.* 2004). Fermentation was carried out at 32°C in complete darkness for 13 days.

Mutagenesis

UV radiation was used as the mutagenic agent. The seed medium containing the spores (1×10^8 CFU ml^{-1}) of the parental strain was irradiated for varied time periods from 5 to 30 min separately 10 cm from the UV source (Lim *et al.* 2000), to determine the LD_{50} value. UV exposure was followed by 5 hrs of incubation in the dark to avoid photo reactivation. A suitable untreated control was maintained. After UV exposure at a determined dose, the plated spore suspension (0.1 ml) was incubated at 32°C for 4 days. The stable mutants were selected visually based on consistent over-pigment production up to six generations and maintained.

Mutant establishment

For establishment of the isolate M^{10c} as mutant, total protein profile was done on SDS-PAGE, which was prepared using the buffer system of Laemmli (1970), 10% acrylamide separating gel, and 4% acrylamide stacking gel, containing 0.1% SDS. The calibration curve was prepared using BSA as stock solution. Protein sample (10-15 µg) dissolved in 1.25% SDS-sample buffer and denatured by keeping the sample at 60°C for 15 min prior to loading. The sample was compared with standard protein marker (P7702S, New England Biolabs) after silver staining.

Extraction and purification

Pigment was extracted using a Soxhlet apparatus with water and ethanol separately as eluting solvent. Fermented rice (1 Kg) was placed in the Soxhlet apparatus and pigment was extracted with 1 l of eluting solvent (five cycles for each loading) and concentrated accordingly.

Pigment characterization

1. UV-analysis

UV-analysis was done with a Shimadzu UV-Vis scanning spectrophotometer (Model No. UV- 2101 PC).

2. Infrared analysis

The sample was kept in a vacuum desiccator over solid KOH for 48 hrs and then IR-spectral analysis was done by FTIR (Jasco, Model No. FT/IR-420).

3. GC-MS

GC-MS analysis was done in a gas chromatograph (Shimadzu, Model No. GC-17A) and molecular weight of the compound was determined in GC-MS (Shimadzu, Model No. GCMS-QP5050A). Column used: DB-225. Gas used-helium at isothermal condition (at 210°C) where injection and detector port temperatures were the same (250°C).

Antibacterial efficacy

The antibacterial activity was tested both by pour plate and by spread plate methods, placing paper discs, containing sample compound (300 µg), in the center of the test plates, incubated for 24 hrs at 37°C (Roy *et al.* 2006).

Toxicological assessment

A toxicity test was performed with healthy adult mice (Swiss albino strain, average body weight 12.0-14.0 g, obtained from local animal suppliers). Mice were acclimatized to laboratory conditions for 5 days at room temperature with 30-70% relative humidity under artificial lighting with a light: dark ratio of 12: 12. For each experiment male and female mice were divided randomly into six groups with eight individuals in each group (Naidu *et al.* 1999). The mice were fed a pigment-mixed basal diet with a graded dose (0.1, 0.25, 0.5 and 1.0 g Kg^{-1} day^{-1}) and observed for the appearance of any symptoms of toxicity and mortality up to 10 weeks. The control group was maintained on a basal diet under the same conditions.

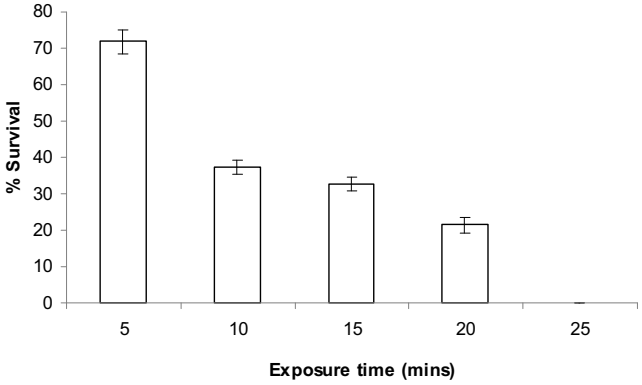


Fig. 1 Determination of LD_{50} dose of spores of *Monascus purpureus*. Values presented as the mean ± SD. A correlation coefficient (r = - 0.956) indicating the variance were 91% inversely correlated.

Statistical analyses

Experiments were performed in triplicate and repeated three times. The results presented are mean values of each triplicate. The variations among data were calculated in terms of standard deviation of mean values. For the mutagenesis study, the correlation between time of exposure to UV and percentage of survival was calculated. Data of toxicological assessment were subjected to analysis of variance (ANOVA) using S-PLUS 4.0, MathSoft Inc. $P < 0.05$ was considered to be statistically significant.

RESULTS

Mutagenesis

The percentage survival decreased with an increase in UV exposure time and reached a maximum within 25 min (**Fig. 1**). The increased period of exposure at a distance of 10 cm from the UV source and the parent to be mutated was directly correlated with the mortality rate. After treating at the LD_{50} dose, 60 over-producing mutants were isolated and screened on the basis of their pigment production (**Scheme 1**). On the basis of pigment production at the 10^{th} day of fermentation, a short list was prepared and two isolates (M^{5e} and M^{10c}) were found to be potent. However, on the basis of growth characteristics and pigment concentration, isolate M^{10c} was selected for further experiments.

Protein profile study

The fungal mat was crushed with silicon dioxide and suspended in PBS buffer to extract the total protein that was quantified against BSA (Lowry 1951). SDS-PAGE was cast; silver staining revealed differences in banding pattern and also in protein intensity that reflected the varied level of gene expression (**Fig. 2**).

Extraction and purification

The concentrated crude was passed through a silica gel 60-120 mesh column. Hexane, ethylacetate and ethanol separately and in different ratios (with increasing polarity index) were used as eluting solvents. Finally the target compound was eluted with ethanol. Each fraction was collected and

Organism grown on solid medium (MY agar) in Petri dishes
↓ Incubated for 4 days
Conidia harvested and suspended in a medium containing NaCl (0.9%) and Tween-80 (0.2%)
↓ Conidia (10^8 CFU ml^{-1}) exposed to UV
Plated on malt extract agar plates
↓ Incubated for 4 days at 30°C
Selected colonies transferred to slant
↓
Screened on yield basis

Scheme 1 Screening for mutants.

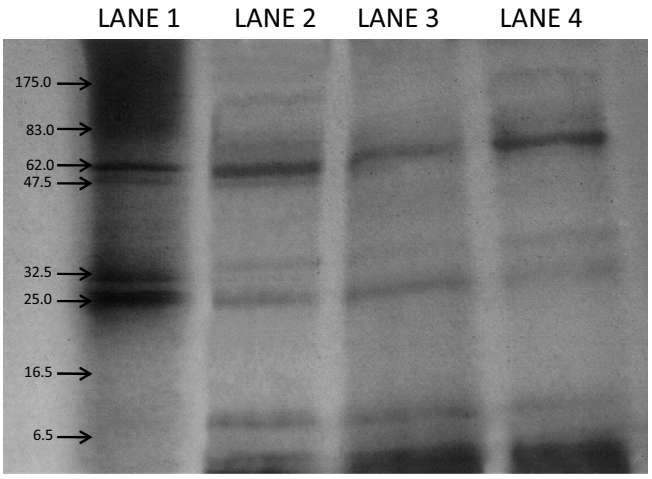


Fig. 2 Total protein profile of mutants M^{5c} and M^{10c} along with prototroph. Lane 1: Protein marker, molecular weight in kDa (BioLabs, New England), Lane 2: MTCC 1090, Lane 3: M^{5c}, Lane 4: M^{10c}.

was then read on a spectrophotometer (300-700 nm) to check its purity. The fraction with the target compound was further purified to near homogeneity through TLC (Silica gel G). The target band was scraped off, dissolved in ethanol and centrifuged at 8000 × *g* for 30 min, decanted and concentrated to obtain the final yield, which was 5.62 g and 6.80 g Kg^{-1} for water and ethanol extraction, respectively (**Scheme 2**).

Fermented substrate (1 Kg)
↓
Soxhlet extraction for 5 cycles with 1 L water or ethanol
↓ Loaded to column
Fractions collected and concentrated
↓ Applied for TLC
Scrapped sample
↓ Eluted in water or ethanol
Centrifuged
↓ Evaporated to dryness
Purified product

Scheme 2 Extraction and purification of the target compound.

Chemical characterization

1. UV analysis

The purified compound showed λ_{max} in ethanol at 595.3 nm.

2. IR analysis

The IR spectrum (**Fig. 3**) of the compound showed peaks at 3436 cm^{-1} (strong band for N-H stretching in secondary amide), 2954 cm^{-1} (C-H stretching in the CH_3 group), 2926 cm^{-1} and 2859 cm^{-1} (C-H stretching in $–CH_2$), 1730 cm^{-1} (C=O stretching, 5-membered cyclic ketone: γ-lactone system), 1637 cm^{-1} (C=C stretching: conjugated double bond system) and 1458 cm^{-1} for unsaturation.

3. GC-MS analysis

GC-MS of the principal compound showed a single band, having a retention time of 13.843 min (**Fig. 4**). MS analyses of the compound showed M^+ = m/z 375 (**Fig. 5**). Therefore, the molecular weight of the compound is 375 and is a non-aromatic nitrogenous compound having -CONH, $-CH_3$ and -CHO groups (**Fig. 6**).

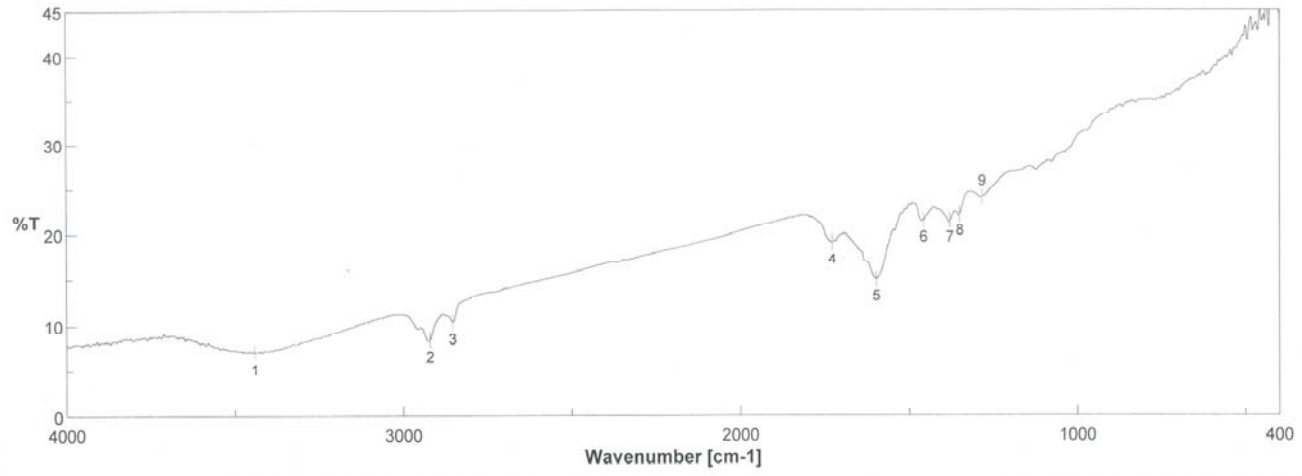


Fig. 3 IR spectral analysis of the purified red compound from M^{10c}.

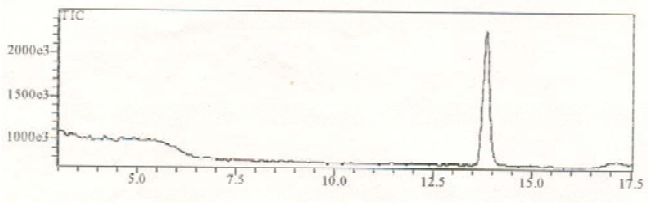
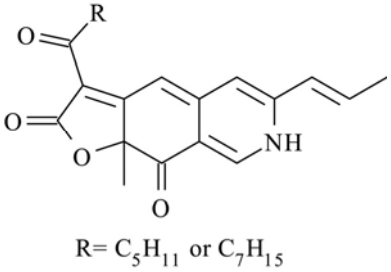


Fig. 4 GLC of the purified red compound from M[10c].



$R = C_5H_{11}$ or C_7H_{15}

Fig. 6 Chemical structure of the purified red compound from M[10c].

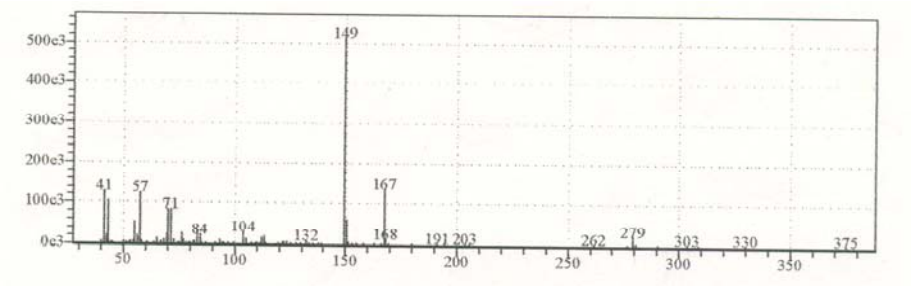


Fig. 5 Mass spectrograph of the purified red compound from M[10c].

Antibacterial activity

To judge its therapeutic property, the antibacterial activity of the pigment compound was determined against selected test bacteria. It was observed that the purified red fraction was active only against Gram-positive bacteria (**Table 2**).

Table 2 Antibacterial spectra of the purified red fraction from M[10c].

Test bcteria	Inhibition zone diameter (mm)[a]
Bacillus subtilis MTCC 121	24.9 ± 0.07
Staphylococcus aureus MTCC 737	18.1 ± 0.08
Escherichia coli MTCC 443	1.4 ± 0.05
Salmonella typhi MTCC 531	0.8 ± 0.06

[a] values presented as mean ± SD

Toxicological assessment

Feeding trials did not produce any significant symptoms of toxicity or mortality either immediately or in the post-feeding period (**Table 3**).

DISCUSSION

Industrial production of microbial pigments for use as biocolorants is considered still at its infant stage (Roy *et al.* 2008). Technological limitations are the major bottleneck for the commercial exploitation of the source material (Chattopadhyay *et al.* 2008).

Pigments from *Monascus* has immense potential for its ever-increasing use in food, feed, pharmaceutical and cosmetic industries (John and Stuart 1991; Babitha *et al.* 2007; Jaivel and Marimuthu 2010). Significant improvement in *Monascus* pigment production, both in submerged and in solid state culture has been achieved in the last few decades (Su and Huang 1976; Lin and Iizuka 1982; Babitha *et al.* 2006).

Genetic improvement by UV irradiation is one of the most promising approaches for increased production of secondary metabolites by industrially important microorganisms, especially in fungi like *Monascus* (Wong and Koehler 1981; Jaivel and Marimuthu 2010). Selection of mutants is one of the consolidated ways to get over-producers.

The working strain could produce a range of pigments that was dominated by a red fraction under suitable conditions. The quality of pigments varied notably with initial pH of the seed culture. Low initial pH (2.0 – 4.0) supported yellow pigmentation, while at higher pH (6.0 – 6.2), the red fraction was found to be more pronounced (Chatterjee *et al.* 2009). In this study, *M. purpureus* MTCC 1090 yielded an over-producing mutant (M^{10c}) when exposed to UV. To avoid photoreactivation, UV-exposed spores were incubated in the dark for at least 5 hrs immediately after UV exposure. An over-producing mutant of *Monascus* by UV mutagenesis was also reported by Lim *et al.* (2000). Changes in macro- and micromorphology upon mutagenesis was observed (Lin and Suen 1973; Su and Huang 1976; Campoy *et al.* 2006; Jaivel and Marimuthu 2010). Primarily, pigment estimation was done spectrophotometrically (λ_{max} = 500 nm), then extracted through a Soxhlet apparatus. Purification was done by column chromatography and thin layer chromatography, finally the purified product was quantified (g Kg^{-1} substrate). The final yield of the selected mutant was 6.80 g Kg^{-1} and improved 5.8-fold more than the prototroph. Pigment production by *Monascus* was also enhanced when co-cultured with *Saccharomyces cerevisiae* (Ju *et al.* 1999).

Upon mutagenesis, significant improvements in *Monascus* pigment production might also be linked to increased leakage of nucleotide related substances from cells (Lin and Izuka 1982; Xu *et al.* 2009). This presumes that the pigments are excreted as fast as they are synthesized.

To confirm isolate M^{10c} as a mutant, the total protein

Table 3 Effect of feeding of freeze dried red pigment of *Monascus* M^{10c} on absolute body weight (g) of female/male albino mice.*

Pigment dose	Duration of feeding (in weeks)				
	2	4	6	8	10
Control	15.0 ± 0.5a	17.2 ± 0.4 a	18.7 ± 0.4 a	20.5 ± 0.4b	21.2 ± 0.5 b
0.1%	15.2 ± 0.5 a	17.4 ± 0.3 a	18.9 ± 0.3 a	20.2 ± 0.3 b	20.7 ± 0.5 b
0.25%	15.1 ± 0.5 a	17.1 ± 0.3 a	18.5 ± 0.6 a	19.7 ± 0.4 b	20.9 ± 0.3 b
0.5%	15.2 ± 0.4 a	17.3 ± 0.3 a	18.8 ± 0.3 a	20.6 ± 0.4 b	22.5 ± 0.3 b
1.0%	14.8 ± 0.2 a	16.9 ± 0.3 a	18.6 ± 0.5 a	20.7 ± 0.4 b	21.3 ± 0.3 b

* Values presented as the mean ± SD. Mean values followed by the same letter are not significantly different at 0.05 level.

profile was compared against the prototroph by SDS-PAGE (Yongsmith *et al.* 2000; Wang *et al.* 2004). After silver staining, differential nature of the expression of the similar protein sub-units was observed; confirming the difference in gene product, thereby substantiate the genetic difference (Yu *et al.* 2008).

Spectral and GC-MS analysis of the principal compound indicate the non-aromatic nature of the purified red fraction, having a relative molecular weight of 375.

While understanding the therapeutic property, the red pigment was tested against bacteria and resulted as low spectra showing effects only against Gram-positive test bacteria. The antibacterial and antimalarial (Martinkova *et al.* 1995; Erdogrul and Azirak 2004), antitubercular (Hsu *et al.* 2010), anti-diabetic (Shi and Pan 2010), anticholesteromic (Hajjaj *et al.* 2000) and antifungal activities (Jongrungruangchok *et al.* 2004; Panda *et al.* 2007) of *Monascus* pigments have been determined. To check its toxicological effects, the mice were maintained under a pigment-mixed basal diet for 10 weeks in laboratory conditions: no clinical signs of toxicity or mortality were found, even after the post-feeding period.

Due to increased human concern and current legislation, biotechnological applications of natural pigments are increasing day by day. Thus, the present study is of immense importance to this increasing dimension and adds knowledge for biotechnological process development for industrial production of pigments.

ACKNOWLEDGEMENTS

Authors are thankful to DBT, Government of India for financial assistance. Also to the DST-FIST and UGC-DRS, New Delhi for financial support to the Department. Authors are also indebted to the Editor-in-Chief for thorough revision of the English language.

REFERENCES

Babitha S, Soccol CR, Pandey A (2006) Jackfruit seed - a novel substrate for the production of *Monascus* pigment solid-state fermentation. *Food Technology and Biotechnology* **44**, 465-471

Babitha S, Soccol CR, Pandey A (2007) Solid state fermentation for the production of *Monascus* pigments from jackfruit seed. *Bioresource Technology* **98**, 1554-1560

Blank PJ, Loret MO, Santerre AL, Pareilleux A, Prome D, Prome JC, Laussac JP, Goma G (1994) Pigments of *Monascus. Journal of Food Science* **59**, 862-865

Campoy S, Rumbero A, Martin J, Liras P (2006) Characterization of a hyperpigmenting mutant of *Monascus purpureus* IB1: Identification of two novel pigment chemical structures. *Applied Microbiology and Biotechnology* **70**, 488-496

Carvalho JC, Pandey A Babitha S, Soccol CR (2003) Production of *Monascus* biopigments: an overview. *AgroFOOD Industry Hi–tech* **14**, 37-42

Carvalho JC, Oishi BO, Pandey A, Soccol CR (2005) Biopigments from *Monascus*: Strain selection, citrinin production and color stability. *Brazilian Archives of Biology and technology* **48**, 885-894

Chairote E, Chairote G, Niamsup H, Lumyong S (2008) The presence and the content of monacolins in red yeast rice prepared from Thai glutinous rice. *World Journal of Microbiology and Biotechnology* **24**, 3039-3047

Chatterjee S, Maity S, Chattopadhyay P, Sarkar A, Laskar S, Sen SK (2009) Characterization of red pigment from *Monascus* in submerged culture. *Journal of Applied Sciences Research* **5**, 2102-2108

Chattopadhyay P, Chatterjee S, Sen SK (2008) Biotechnological potential of natural food grade biocolorants. *African Journal of Biotechnology* **7**, 2972-2985

Erdogrul O, Azirak S (2004) Review of the studies on the red yeast rice (*Monascus purpureus*). *Turkish Electronic Journal of Biotechnology* **2**, 37-49

Evans PJ, Wang HY (1984) Pigment production from immobilized *Monascus* sp. utilizing polymeric resin adsorption. *Applied and Environmental Microbiology* **47**, 1323-1326

Hajjaj H, Klaebe A, Goma G, Blanc PJ, Barbier E, Francois J (2000) Medium-chain fatty acids affect citrinin production in the filamentous fungus *Monascus ruber*. *Applied Environmental Microbiology* **66**, 1120-1125

Hsu W-H, Lee B-H, Pan T-M (2010) Protection of *Monascus* fermented *Dioscorea* against DMBA-induced oral injury in hamster by anti-inflammatory and antioxidative potentials. *Journal of Agricultural and Food Chemistry* **58**, 6715-6720

Jaivel N, Marimuthu P (2010) Strain improvement of *Aspergillus terreus* for increased lovastatin production. *International Journal of Engineering Science and Technology* **2**, 2612-2615

John MR, Stuart DM (1991) Production of pigments by *Monascus purpureus* in solid culture. *Journal of Industrial Microbiology* **8**, 23-28

Jongrungruangchok S, Kittakoop P, Yongsmith B, Bavovada R, Tanasupawat S, Lartpornmatulee N, Thebtaranonth Y (2004) Azaphilone pigments from a yellow mutant of the fungus *Monascus kaoliang*. *Phytochemistry* **65**, 2569-2575

Ju J-Y, Kim D-Y, Suh J-H, Shin C-S (1999) Optimization of *Monascus* red pigment fermentation by regulating chitinase activity level in fermentor. *Bioprocess Engineering* **21**, 25-29

Kapoor VP (2006) Natural food colors: Present status and scopes. In: Daniel M, Bhattacharya SD, Arya A, Raole VM (Eds) *Natural Dyes: Scope and Challenges*, Scientific Publishers, Jodhpur, India, pp 34-43

Klimek M, Wang S, Ogunkanmi A (2009) Safety and efficacy of red yeast rice (*Monascus purpureus*) as an alternative therapy for hyperlipidemia. *Pharmacy and Therapeutics* **34**, 313-327

Kumasaki S, Nakanishi K, Nishikawa E, Ohashi M (1962) Structure of monacorubrin. *Tetrahedron* **18**, 1171-1184

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685

Li J-J, Shang X-Y, Li L-L, Liu M-T, Zheng J-Q, Jin Z-L (2010) New cytotoxic azaphilones from *Monascus purpureus* fermented rice (red yeast rice). *Molecules* **15**, 1958-1966

Lim H-S, Yoo S-K, Shin C-S, Hyun Y-M (2000) *Monascus* red pigment overproduction by coculture with recombinant *Saccharomyces cerevisiae* secreting glucoamylase. *The Journal of Microbiology* **38**, 48-51

Lin CF, Suen SJT (1973) Isolation of hyperpigment productive mutants of *Monascus* sp. F-2. *Journal of Fermentation Technology* **51**, 757-759

Lin TF, Iizuka H (1982) Production of extracellular pigment by a mutant of *Monascus kaoliang sp*. nov. *Applied Environmental Microbiology* **43**, 671-676

Lin YL, Wang TH, Lee MH, Su NW (2008) Biologically active components and nutraceuticals in the *Monascus*-fermented rice: A review. *Applied Microbiology and Biotechnology* **77**, 965-973

Loret M-O, Morel S (2010) Isolation and structural characterization of two new metabolites from *Monascus*. *Journal of Agricultural and Food Chemistry* **58**, 1800-1803

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275

Mak NK, Fong WF, Wong-Leung YL (1990) Improved fermentative production of *Monascus* pigments in roller bottle culture. *Enzyme and Microbial Technology* **12**, 965-968

Mapari SAS, Neilson KF, Larsen TO, Frisvad JC, Meyer AS, Thane U (2005) Exploring fungal biodiversity for the production of water soluble pigments as potential natural food colorants. *Current Opinion in Biotechnology* **16**, 231-238

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

Naidu KA, Venkateswaran G, Vijayalakshmi G, Manjula K, Viswanatha S, Murthy KN, Srinivas L, Joseph R (1999) Toxicological assessment of the yeast *Rhodotorula gracilis* in experimental animals. *Zeitschrift fur Lebensmitteluntersuchung und Forschung A* **208**, 444-448

Panda BP, Javed S, Ali M (2007) Fermentation process optimization. *Research Journal of Microbiology* **2**, 201-208

Pastrana L, Blanc PJ, Santerre, AL, Loret MO, Goma G (1995) Production of red pigments by *Monascus ruber* in synthetic media with a strictly controlled nitrogen source. *Process Biochemistry* **30**, 333-341

Patakova JP, Rezanka T, Viden I (1998) Identification of volatile metabolites from rice fermented by the fungus *Monascus purpureus* (an-kak). *Folia Microbiologica* **43**, 407-410

Roy RN, Laskar S, Sen SK (2006) Dibutyl phthalate, the bioactive compound produced by *Streptomyces albidoflavus* 321.2. *Microbiological Research* **161**, 121-126

Roy S, Chatterjee S, Sen SK (2008) Biotechnological potential of *Phaffia rhodozyma*. *Journal of Applied Biosciences* **5**, 115-122

Shi Y-C, Pan T-M (2010) Anti-diabetic effects of *Monascus purpureus* NTU 568 fermented products on streptozotocin induced diabetic rats. *Journal of Agricultural and Food Chemistry* **58**, 7634-7640

Su YC, Huang JH (1976) Studies on the production of Anka-pigment. *Journal of Chinese Agricultural Chemical Society* **14**, 45-58

Wang J-J, Lee C-L, Pan T-M (2004) Modified mutation method for screening low citrinin-producing strains of *Monascus purpureus* on rice culture. *Journal of Agricultural and Food Chemistry* **52**, 6977-6982

Wong HC, Koehler PE (1981) Mutant for *Monascus* pigment production.

Journal of Food Science **46**, 956-957

Xu M-J, Yang Z-L, Liang Z-Z, Zhou S-N (2009) Construction of a *Monascus purpureus* mutant showing lower citrinin and higher pigment production by replacement of *ctnA* with *pks1* without using vector and resistance gene. *Journal of Agricultural and Food Chemistry* **57**, 9764-9768

Yongsmith B, Kitprechavanich V, Chitradon L, Chaisrisook C, Budda N (2000) Color mutants of *Monascus* sp. KB9 and their comparative glucoamylases on rice solid culture. *Journal of Molecular Catalysis B: Enzymatic* **10**, 263-272

Yu C-C, Wang J-J, Lee C-L, Lee S-H, Pan T-M (2008) Safety and mutagenicity evaluation of nanoparticulate red mold rice. *Journal of Agricultural and Food Chemistry* **56**, 11038-11048

Zhang ML, Peng CX, Zhou YF (2000) US Patent 6,046,022