



Effect of media composition on citrinin and bio-pigments production by *Monascus ruber*

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ABSTRACT

Monascus species are used for production of red bio-pigments, which are used as food coloring agents. However, presence of the mycotoxin citrinin, which is a secondary metabolite produced in conjunction with the red pigments, prevents the use of these pigments in large scale. In the present study, *Monascus ruber* Van Tieghem was isolated from the Egyptian soil and identified. Effect of media composition on nine of the pigments produced by this fungus in addition to citrinin was studied. The fungus was grown in four different media containing different carbon sources (glucose, ethanol, yeast malt extract and potato dextrose broth). On the other hand, effect of glucose concentration was also studied. The produced pigments and citrinin were extracted by butanol and analyzed by LC/MS/MS as well as HPLC with fluorescence detection of citrinin. The data revealed that the highest amount of red pigments production (4.3g/l) was obtained at glucose concentration 10g/l. This was combined with complete absence of citrinin. Rubropunctamine (26.1%) and ankuflavine (48.7%) were the most abundant pigments in the glucose and ethanol media, respectively; while Moanascin (61.6%) and N-glutaryl-rubropunctamine (39.4%) were the most abundant in the PDB and YMB media, respectively. This result enables the large scale production of the red pigments by *M. ruber* without production of the mycotoxin citrinin.

1. INTRODUCTION

Food natural colorants are extracted from plants, animals and microorganisms. More than fifty patents concerning the use of pigments of *Monascus* spp. (a fungus first discovered by Van Tieghem) for colorization of food have been issued in Japan, USA, France and Germany [1]. The genus *Monascus* includes three species (*M. pilosus*, *M. purpureus* and *M. ruber*) belonging to the family Monascaceae and to the class *Ascomyceta* [2]. The well-known *Monascus* pigments are the yellow (monascin and ankaflavin), orange (monascorubrin (MBN) and rubropunctatin (RTN) and red-purple (monascorubramine and rubropunctamine, N-Glucosylrubropunctamine, PP-R, 7-(2-hydroxyethyl)-monascorubramine), N-glutarylmonascorubramine, N-glutaryl-rubropunctamine. These pigments are produced mainly in the cell-bound state [3-5]. Fungi from the genus *Monascus* are traditionally used in Asian countries, mainly in China and

Thailand, to prepare fermented rice with strong red color. Also, these fungi were found to be common in several applications ranging from conferring color to products such as wine, cheese and meat, to medicinal uses, as substitute for traditional food additives such as nitrites and cochineal in sausages and/or other meat products [6- 9]. Recently, several companies sell the dry, pulverized fermented rice product as a nutritional supplement with ability to reduce cholesterol levels and others sell the dried product or purified extracts as food colors. In such filamentous fungi, many secondary metabolites with complex chemical structures are synthesized from the polyketide pathway.

These metabolites display a wide range of biological activities, including antimicrobial, immuno-suppressive and anticancer properties such as hypocholesteremic [10- 14]. Other components of *Monascus* pigments have anti-inflammatory activity and have been reported to suppress skin cancer caused by tumor promoters in experimental animals [15, 16]; in addition to their clinical benefits in treating hypertension in humans [17]. The *M. ruber* was used for lovastatin production, which reduces the serum total cholesterol and triglycerides and acts as an inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A reductase.

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A maximum lovastatin production of 2.83 mg/g fungal dry weight was obtained [18]. Effect of different substrates (sweet potato, potato, cassava and dioscorea) on monacolin-K production was investigated. Monacolin-K is the secondary metabolite of *Monascus* species and an inhibitor for cholesterol synthesis [19].

M. ruber excretes a mycotoxin namely citrinin [20]. Citrinin is a fungal metabolite known since 1931, when it was isolated from *Penicillium citrinum* and later from the Australian plant *Crotolaria crispate*. After ten years, it was characterized as an antibacterial antibiotic and later it was tested for its activity against bacteriophages, sarcomas, protozoa and animal cells. It exhibits antibiotic activity against gram-positive bacteria, but its nephrotoxic properties in addition to other serious health problems prevent its use as a therapeutic drug [21]. Production of citrinin together with red pigments (Anka) prevents use of *M. ruber* as a producer of natural colorants for food technology [22]. However, not all *Monascus* strains do produce citrinin. Furthermore, the used nitrogen source and medium composition affect the citrinin production by the citrinin-producing strains as well as the type of pigments produced.

It was noticed that in a synthetic medium, citrinin production was completely inhibited using methionine as nitrogen source [20]. The assimilation of exogenous octanoic acid during growth of *M. ruber* resulted in a slight enhancement of the pigment excretion and inhibition of citrinin production [23]. Effect of cyclic adenosine monophosphate on development and secondary metabolites of *Monascus ruber* was found to be in a dose-dependent pattern.

The red pigment might convert to citrinin under changing cAMP concentrations [24]. Glycerol and glutamate as substrate on the red pigments production by *M. ruber* affected the production, where it was 40-70 g L⁻¹ and 7-8 g L⁻¹ for glycerol and glutamate, respectively [25]. Effect of different amino acids on bio-pigments production showed that histidine was the most valuable amino acid resulted in the highest production of red pigments and almost completely eliminated the formation of the mycotoxin [26]. The present work aimed to isolate *Moanscus ruber* fungus from the Egyptian soil and testing effect of media composition and glucose concentration on the bio-pigments and mycotoxin production.

2. MATERIALS AND METHODS

2.1. Isolation and identification of the pigments-producing fungus

Soil samples were obtained from an area irrigated by treated sewage waste-water at the district of Abu Rawash, Giza, Egypt. Ten grams of soil was added to 90 ml of sterilized water and serial dilutions up to 10⁷ fold were made. One ml from each dilution was added into sterile Petri dishes poured with soil extract agar medium.

The plates were incubated at 28 ±2°C for 7 days [25]. The red dye-producing colony was picked and purified on the same medium. Identification of the isolated fungi was carried out to the genus and species levels according to the macroscopic (culture features) and microscopic features using the following

references [27] and [28] at Assuit University Mycology Center (AUMC).

Petri-dishes containing aliquots of soil extract agar medium were inoculated with 1 ml of diluted soil and incubated at 28±2°C for 7 days [29]. The red dye-producing colony was picked, purified and identified at Assuit University Mycology Center (AUMC).

2.2. Fungus propagation and pigments production

Four types of media were used: 1) The fungus isolate was cultivated on the medium containing 3, 5, 10 or 20 g glucose, 5 g monosodium glutamate (MSG); 5g K₂ HPO₄; 5 g KH₂PO₄; 0.1 g CaCl₂; 0.5 g Mg SO₄ - 7H₂O; 0.01g FeSO₄- 7H₂O, 0.01 g Zn SO₄- 7H₂O and 0.03 g Mn SO₄ - H₂O per liter of distilled water [26]; 2) The same medium supplemented with ethanol (20g/l) instead of glucose, in addition to methionine (5g /l) as a nitrogen source, 3) Potato dextrose broth (PDB) (Difco) and 4) Yeast malt extract broth (YMB) that composed of (g/L): Glucose: 20, peptone: 5, malt extract: 3 and yeast extract: 3 [30]. The initial pH of the medium was adjusted to 6.5 with phosphoric acid. The stock culture was maintained on potato dextrose broth medium. Half-liter baffled Erlenmeyer flask containing 100-ml of the liquid medium was inoculated by 2×10⁶ fungus spores suspension. The flasks were incubated in shaking-incubator at a rate of 150 rpm for 7 day at 30°C. The culture pH values at the different glucose concentrations were determined at the end of the incubation period. The developed fungus growth was separated by filter paper No. 42 and dried in an oven at 80°C for determination of the fungal dry weight [31].

2.3. Pigments extraction and purification

Fifty ml of each culture filtrate was extracted three times with water saturated n-butanol (20 ml). The organic phase was dried under anhydrous Na₂SO₄ followed by vacuum concentration [22]. The pigments were purified by TLC technique using silica gel plates (Merck, Germany) with chloroform-methanol-water (65:25:4, v/v/v) as the solvent system. The red band was scraped off from the silica plates and solubilized in ethanol 70%. This solution was evaporated and analyzed by HPLC to confirm the purity. A known concentration of the pure pigment (40mg/100ml solvent) was used as standard solution for spectrophotometer analysis of the red pigment.

2.4. Spectrophotometer analysis

The maximum absorbance was scanned using spectrophotometer (U.V-160 Shimadzu) in a range 200-1100 nm. The obtained λ-max (500 nm) was used for HPLC detection of the red pigment as well as for quantitative analysis of the red pigment in all treatments.

2.5. HPLC analysis of the red pigment

The HPLC (HP-1100) was used with a separation column of HP-C₁₈ 25cm X 4.6cm, the mobile phase was a gradient mixture of water-methanol (80:20 to 0:100 (v/v) over 30 min with a flow

rate of 1 ml min⁻¹. The detector was HP-1100 spectrophotometer set at 500 nm.

2.6. vHPLC analysis of citrinin

Presence of citrinin was analyzed by HP-1100 HPLC system with fluorescence detection. The separation column was HP-C₁₈, 5µm 200 mm × 4.6 mm.; The mobile phase consisted of methanol / acetonitrile / water (3: 3: 4 v: v: v). The pH value of the mixture was 2.5 and the flow rate was 1 ml/min.; injection volume: 20µl. Fluorescence detector: HP (1100) set at 331 nm excitation and 500 nm emission wave lengths [32].

2.7. LC/MS/MS analysis

The pigments types as well as the mycotoxin (citrinin) produced in all of the tested media were analyzed by LC-MS/MS (Waters) at the Chemical Analysis laboratory of National Gene Bank of Egypt. The system utilized a Surveyor MS pump, a Surveyor auto-sampler, and a XEVO-TQD triple quadrupole mass spectrometer equipped with an ESI source. Data acquisition was performed with Xcalibur 1.3 software. The used column was XDB-C18 column (150 mm × 2.1 mm, 3 µm). The mobile phase was A: water (0.1% formic) and B: Acetonitrile (0.1 % formic), gradient from 15% acetonitrile to 100% acetonitrile in 10 min.

The column temperature was maintained at 30 °C. The mass spectrometer was operated in the positive mode. The analysis was performed using Single Ion Recording (SIR) of the ions are listed in table (1). The optimized parameters were as follows: Cone volt 20V, capillary voltage 3.6 KV. Nitrogen (N₂) was used as sheath gas and auxiliary gas at a flow of flow 500L/hr. The de-solvation temperature was 220°C, and the collision energy was 20 eV. The column temperature was maintained at 30 °C. The mass spectrometer was operated in the positive mode. The analysis was performed using Single Ion Recording (SIR) of the ions are listed in table (1). The optimized parameters were as follows: Cone volt 20V, capillary voltage 3.6 KV. Nitrogen (N₂) was used as sheath gas and auxiliary gas at a flow of flow 500L/hr. The de-solvation temperature was 220°C, and the collision energy was 20 eV.

Table 1: The compounds and M+H ions detected by SIR-LC-MSMS analysis:

Compound	M+H
N-glutarylmonascrubramine	512
N-glutarylrubropunctamine	484
PPR-7-OHME-monascrubramine	427
ankuflavine	387
monascrubtin	383
Monascrubramine	382
Moanascin	359
Rubropunctatin	355
Rubropunctamine	354
Citrinin	251

3. RESULTS AND DISCUSSION

3.1. Morphological characters of *Monascus ruber* Van Tieghem

Figure (1) shows the morphological features of the fungus *Monascus* which has an isolated taxonomic position (Fam.: Monascaceae Shröt) and is characterized by stalked, non-ostiolate

ascostoma (cleistothecia), (Fig. 1a), developing from a terminal vesicle, (Fig. 1b), which become surrounded by hyphae grown out from the base and forming a 4 µm thick wall. The ascostoma contain a limited number of globose, soon evanescent asci, which are no longer recognizable at maturity when the ascospores fill the cavity. Hyaline to brownish ascostoma, 20-70 µm in diameter profusely produced. Ascospores are ellipsoidal, hyaline or slightly orange, thick and smooth-walled 4-7 × 3-5 µm. Conidia (Fig. 1c) are mostly in short or rather long chains, subglobose to pyriform with a little broadly truncate base, 4.5-16 × 4.5 – 10 µm, thin-walled, hyaline or pale brownish, sometimes thick-walled and larger, in short chains or solitary and functioning as clamydospore.

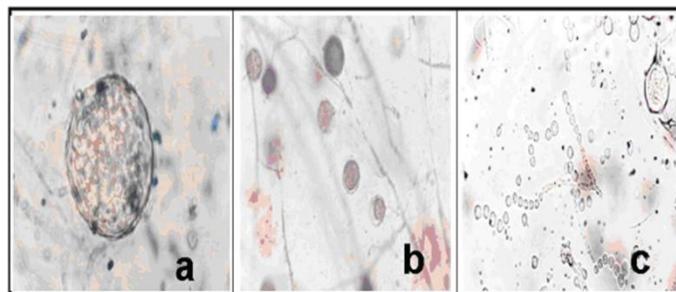


Fig. 1: Morphological features of *Monascus ruber*. a) Stalked, non-ostiolate cleistothecia, b) Hyphae grown out from the base and c) Conidia in short chains.

3.2. Purification of the red pigment

Thin layer chromatography (TLC) showed that the RF of the red pigment at all glucose concentrations was 0.27 (Fig. 2). The UV spectrum of the purified red pigment showed the λ max at 500 nm. The calculated percent extinction coefficient ($\epsilon_{\text{percent}}$) was 2.777 (g/100 ml)⁻¹ cm⁻¹.

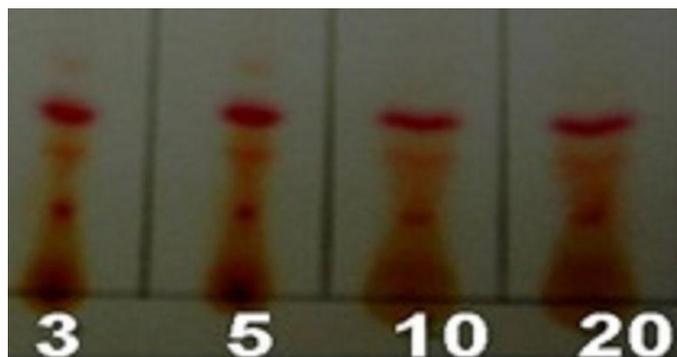


Fig. 2: TLC analysis of the pigments produced by *M. ruber* at different glucose concentrations (3, 5, 10 and 20 g l⁻¹).

3.3. Determination of red pigments production

The HPLC analysis of the red pigment band obtained from TLC plate showed that this band is a pure compound and has a retention time (Rt) of 1.6 min. (Fig. 3). Data presented in table

(2) reveal that the changes of glucose concentration in the medium composition resulted in a favorable effect on excretion of the red pigment; since the increase in glucose concentration from 3 to 10 g l⁻¹ resulted in increase in the pigment production from 2.8 to 4.3 g l⁻¹. The higher glucose concentration (20 g l⁻¹) decreased the production to 2.8 g l⁻¹. On the other hand, the production was lower (2.3g l⁻¹) when either ethanol or PDB medium was used, followed by 2.7g l⁻¹ in the YMB medium.

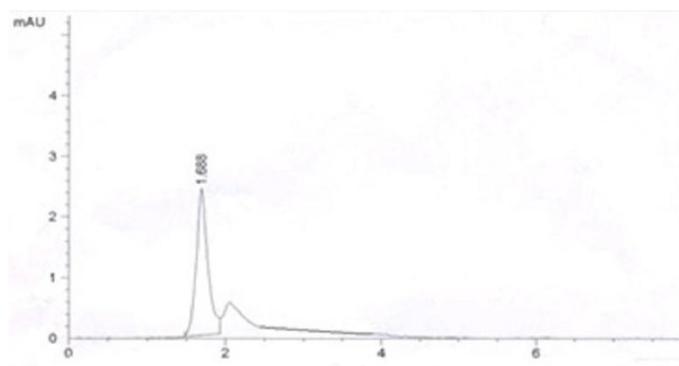


Fig. 3: HPLC chromatogram of the red pigment produced by *M. ruber*.

Table 2: The red-pigments production as affected by glucose concentration and media composition.

Glucose con. and media types	Glucose Con. (g.l ⁻¹)				Ethanol	PDB	YMB
	3	5	10	20			
Total red pigments (g.l ⁻¹)	2.8	4.1	4.3	2.8	2.3	2.3	2.7

3.4. Effect of carbon source concentration and type on the citrinin production

The relative concentration of citrinin was determined by HPLC in the fungal cultures that showed the highest red pigment production (glucose medium 3, 5 and 10 g l⁻¹). The obtained results were compared with the citrinin production in the medium containing ethanol as the sole carbon source. This comparison was carried out according to Blanc *et al.* (1995), who noticed that the citrinin was not produced by *M. ruber* when ethanol was used as the sole carbon source [20].

The HPLC analysis of citrinin (fig. 4) showed that the Rt. of citrinin is 2.6 min. and the citrinin amount decreases with glucose concentration increase. The relative citrinin amounts to the red pigment were 33.8, 23 and 0 % for glucose concentrations 3, 5 and 10 g l⁻¹, respectively. The corresponding citrinin percentage in the ethanol medium was 43.1 %.

In contrast, Blanc *et al.* (1995) found that when the initial glucose concentration in the medium increased from 5 to 20g.l⁻¹, the production of citrinin increased two folds [32]. But Hajjaj *et al.* (1999) reported that the production of citrinin started after about 45 hr of cultivation and the production continue after the medium glucose has been consumed [33]. Hassan *et al.*, (2015) studied effect of different C/N ratios on the red pigments and mycotoxin

production. They reported that the specific production of the red pigments was optimal at a glucose/glutamate ratio of about 10; while the production of the mycotoxin increased with increased C/N with an optimum in the range of 30–45 [34]. On the other hand, effect of several other parameters such as solid-state fermentation [35] and oxygen supplementation [36] on the red pigments and citrinin production has been investigated.

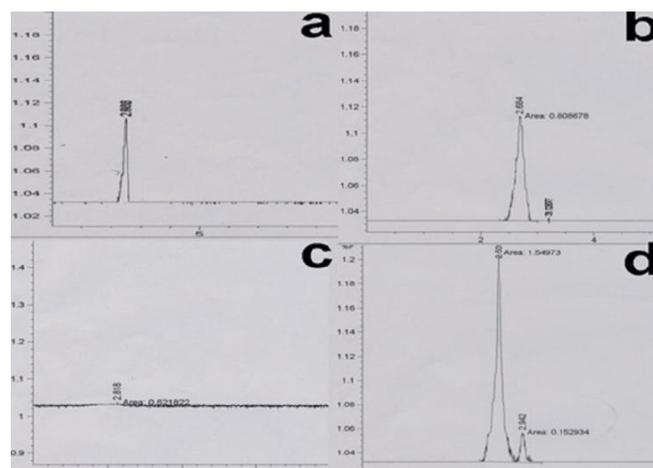


Fig. 4: HPLC analysis of citrinin at different glucose concentrations (g l⁻¹): (a: 3, b: 5 and c: 10) and (d): ethanol medium.

3.5. LC/MS/MS analysis of the bio-pigments and citrinin:

The bio-pigments types as well as citrinin production as affected by the media type was illustrated in table (3).

Table 3: The percentages of bio-pigments and citrinin produced by *M. ruber* in different media.

Compound	Rt (min.)	Glucose (10 g.l ⁻¹)	Ethanol medium	PDB	YMB
N-glutarylmonascorubramine	0.82	1.3	5.3	1.2	3.7
N-glutarylrubropunctamine	8.31	3.4	0.0	5.2	39.4
PPR-7-OHME-monascorubramine	0.94	5.6	12.2	3.0	3.0
ankuflavine	0.88	20.7	48.7	12.0	5.7
monascorubtin	0.93	11.0	26.6	1.7	3.5
Monascorubramine	1.4	18.9	3.5	5.4	1.5
Moanascin	7.3	7.2	0.4	61.6	5.2
Rubropunctatin	7.3	5.7	0.1	2.2	0.0
Rubropunctamine	7.3	26.1	1.2	4.4	4.2
Citrinin	0.58	0.0	2.1	3.4	33.7

Rubropunctamine (26.1%) and ankuflavine (48.7%) were the most abundant pigments in the glucose and ethanol media, respectively. Moanascin was the most abundant in the PDB medium (61.6%), while N-glutarylrubropunctamine (39.4%) was the most abundant in the YMB medium. Absence of citrinin in the 10% glucose medium was also confirmed from the LC-MS analysis, where there is no peak at the SIR chromatogram at MW 251 detection channel (fig. 5).

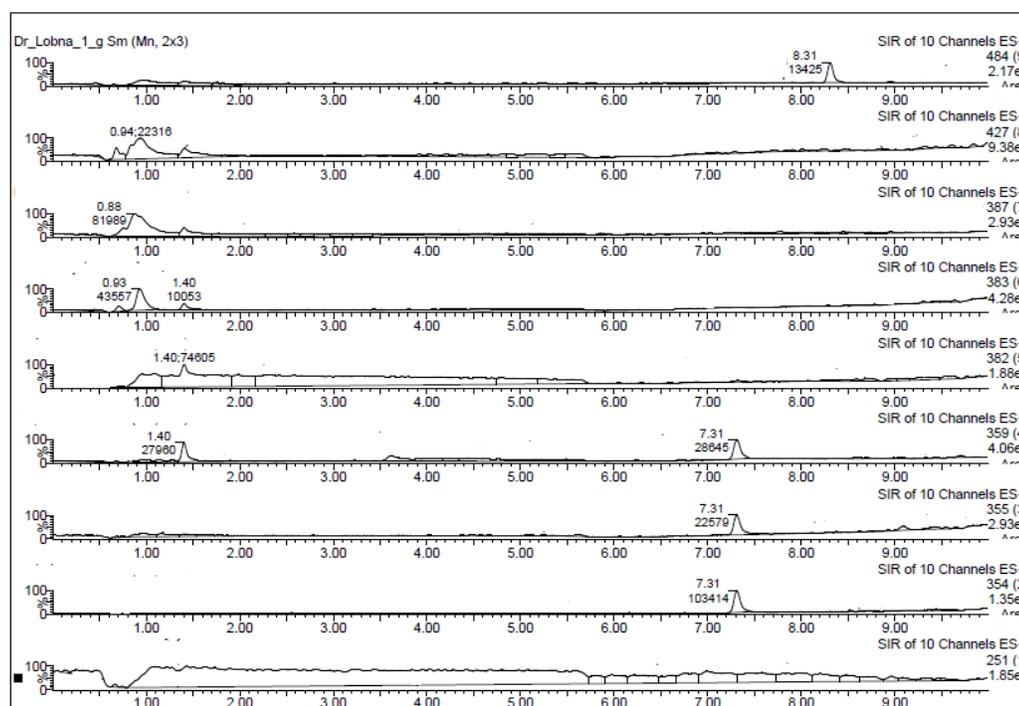


Fig. 5: The SIR chromatograms of the LS/MS/MS analysis for the bio-pigments and citrinin produced by *M. ruber* in 10% glucose medium. The result showed absence of any signals at the SIR chromatogram of M+H =251 for citrinin.

4. CONCLUSION

A high yield of red pigments production (4.3g l^{-1}) by *M. ruber* was obtained in the medium containing glucose 10g l^{-1} and 5g l^{-1} monosodium glutamate (MSG), without production of citrinin. Rubropunctamine (26.1%) was the most abundant pigment produced under these conditions.

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6. REFERENCES

- Lin T F, Yakushijin K, Buchi G H and Ldemain A. Formation of water-soluble *Monascus* red pigments by biological and semi-synthetic processes. *J. Ind. Microbiol.* 1992; 9: 173 - 179.
- Pitt J I and Hocking A D. *Fungi and Food Spoilage*. 2nd ed., London, Springer; 1997
- Wong H and Koehler P E. Production of red water-soluble *Monascus* pigments. *J. Food Sci.* 1983; 48: 1200 - 1203.
- Hassan H, Alain K B, Marie O. L, Theo T D, Gerard G and Philippe J B. Production and Identification of N-Glucosylrubropunctamine and N-Glucosylmonascorubramine from *Monascus ruber* and Occurrence of Electron Donor-Acceptor Complexes in These Red Pigments. *Applied and Env. Microb.* 1997; 36 (7): 2671–2678.
- Sameer AS, Anne S M, Ulf T and Jens C F. Identification of potentially safe promising fungal cell factories for the production of polyketide natural food colorants using chemotaxonomic rationale. *Microbial Cell Factories.* 2009; 8:24, doi:10.1186/1475-2859-8-24.
- Wong H C and Koehler P. Production and isolation of an antibiotic from *Monascus purpureus* and its relationship to pigment production. *J. Food Sci.* 1981; 46: 589 - 592.
- Ariel R, Eduardo A, Javier D and Ricardo P C. Solid Substrate Fermentation of *Monascus purpureus*: Growth, Carbon Balance, and Consistency Analysis. *Biotechnol. Prog.* 2000; 16, 152-162.
- Vidyalakshmi R, Singaravadivel K, Muruges S. Optimization of fermentation conditions and effect of nutritional supplementation in production of food grade pigments by *Monascus ruber*. *Journal of Pure and Applied Microbiology.* 2010; 4(2): 779- 784.
- Fabre C E, Goma G and Blanc P J. Production and food applications of the red pigments of *Monascus ruber*. *J. Food Sci.* 1993; 58: 1099 - 1102.
- Turner W B. *Fungal Metabolites*. London, United Kingdom: Academic Press. 1971; p. 446.
- Simpson T J. Studies of polypeptide chain-assembly processes, Mycotoxins. In. "Mycotoxins and Phycotoxins" (Eds. Steyn, P. S. and Vleggaar, R.), Amsterdam, Netherlands: Elsevier Science Publishers. 1986; p. 85 - 96.
- Martinkova L, Jzlova P and Vesely D. Biological- activity of polyketida pigments produced by the fungus *Monascus*. *Journal of Applied Bacteriology.* 1995; 79: 609 - 616.
- Vendruscolo F, Tosin I, Giachini A J, Schmidell W, Ninow J L. Antimicrobial Activity of *Monascus* Pigments Produced in Submerged Fermentation. *Journal of Food Processing and Preservation.* 2014; 38 (4): 1860-1865.
- Eman M M and Abbadly M S. Secondary Metabolites and Bioactivity of the *Monascus* Pigments Review Article. *Global Journal of Biotechnology and Biochemistry.* 2014; 9 (1): 01-13.
- Yongsmith B, Krairak S and Bavavoda R. Production of yellow pigments in submerged culture of a mutant of *Monascus* spp. *J. Ferment. Bioeng.* 1994; 78: 223 - 228.

16. Yongsmith B, Tabloka W, Yongmanitchai W and Bavavoda R. Culture conditions for yellow pigment formation by *Monascus* sp. KB10 grown on cassava medium. *World J. Microbiol. Biotechnol.* 1993; 9: 85 - 90.
17. Kushiro T, Hashida J, Kawamura H, Mitsubayashi H and Saito T. Clinical effects of beni-Koji in mild essential hypertension. A multi-center double – blind comparison with placebo. *Jpn. J. Nephrol.* 1996; 38: 625 - 633.
18. Panda B P, Javed S, Ali M. Optimization of fermentation parameters for higher lovastatin production in red mold rice through co-culture of *Monascus purpureus* and *Monascus ruber*. *Food and Bioprocess Technology.* 2010; 3 (3): 373- 378.
19. Chun-Lin L, Jyh-Jye W, Shing-Lin K and Tzu-Ming Pan. *Monascus* fermentation of dioscorea for increasing the production of cholesterol-lowering agent—monacolin K and antiinflammation agent—monascin. *Appl. Microbiol. Biotechnol.* 2006; 72: 1254–1262.
20. Blanc P J, Loret M O and Goma G. Production of citrinin by various species of *Monascus*. *Biotech. Lett.* 1995; 17: 291 - 294.
21. Sankwa U, Ebizuka Y, Noguchi H, Isikawa Y, Kitaghawa S, Yamamoto Y, Kobuyashi T and Litak Y. Biosynthesis of citrinin in *Aspergillus terreus*. *Tetrahedron.* 1983; 39: 3583 - 3591.
22. Hajjaj H, Klaebe A, Loret M O, Tzedakis T, Goma G and Blanc P J. Production and Identification of N-Glucosylrubropunctamine and N-Glucosylmonascorubramine from *Monascus ruber* and Occurrence of Electron Donor-Acceptor Complexes in These Red Pigments *Appl. Environ. Microbiol.* 1997; 63(7): 2671 - 2678.
23. Hajjaj H, Blanc P, Groussac E, Uribelarrea J L, Goma G and Loubiere P. Kinetic analysis of red pigment and citrinin production by *Monascus ruber* as a function of organic acid accumulation. *Enz. Micro. Technol.* 2002; 27: 619 - 625.
24. Lai Y, Wang L, Qing L, Chen F. Effects of cyclic AMP on development and secondary metabolites of *Monascus ruber* M-7. *Letters in Applied Microbiology.* 2011; 25 (4): 420-426.
25. Meinicke RM, Vendruscolo F, Esteves M D, de Oliveira D, Schmidell W, Samohyl RW, Ninow J L. Potential use of glycerol as substrate for the production of red pigments by *Monascus ruber* in submerged fermentation. *Biocatalysis and Agricultural Biotechnology.* 2012; 1 (3): 238-242.
26. Hajjaj H, François J M, Goma G and Blanc P J. Effect of Amino Acids on Red Pigments and Citrinin Production in *Monascus ruber*. *Journal of Food Science.* 2012; 77 (3): 156- 159.
27. Pitt J I and Hocking A D. *Fungi and food spoilage.* 3rd ed. London New York: Springer Dordrecht Heidelberg. 2009; p. 519.
28. Samson R A, Hoekstra E S and Frisvad J C. *Introduction to food and airborne fungi.* 7th ed. The Netherlands. Centraalbureau voor schimmelcultures-utrecht: 2004; p. 389.
29. Holm E and Jenseon V. Aerobic chemoorganotrophic bacteria of a Danish beoon forest. *OIDOS.* 1972; 23: 248 - 260.
30. Juzlova P, Martinkova L and Kren V. Secondary metabolites of the fungus *Monascus*: a review. *J. Ind. Microbiol.* 1996; 16(3): 103 - 167.
31. Al-Sarrani A Q. Amylases, proteases and lipases production by *Monascus ruber* Tieghem. *Int. J. Exp. Bot.* 1998; 63: 1 - 9.
32. Lixue M, Shen X H, Duan Z W and Guo S R. A Validated RP-HPLC Method for the Determination of Citrinin in Xuezhikang Capsule and other *Monascus*-Fermented Products. *E-Journal of Chemistry.* 2012; 9(1), 260-266
33. Hajjaj H, Klaebe A, Loret M O, Goma G, Blanc P and Francois J. Biosynthetic Pathway of Citrinin in the Filamentous Fungus *Monascus ruber* as Revealed by ¹³C Nuclear Magnetic Resonance. *Applied and Environmental Microbiology.* 1999; 65(1): 311 - 314.
34. Hassan H. Gerard Goma and Jean M. François. Effect of the cultivation mode on red pigments production from *Monascus ruber*. *International J. of food science technology.* 2015; 50 (8): 1731–1736.
35. Liang Z, Zhiqiang L, Bing D, Wenxue Z and Yongjun Y. Effect of submerged and solid-state fermentation on pigment and citrinin production by *Monascus purpureus*. *Acta Biol. Hung.* 2013; 64(3):385-94.
36. Jian Y, Qi Chen, Weiping W, Jiajun H, Chuan H. Effect of oxygen supply on *Monascus* pigments and citrinin production in submerged fermentation. *J. Biosci. Bioeng.* 2014; 5;119(5):564-9.

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