Glutamic acid promotes monacolin K production and monacolin K biosynthetic gene cluster expression in *Monascus*

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**Abstract**

This study investigated the effects of glutamic acid on production of monacolin K and expression of the monacolin K biosynthetic gene cluster. When *Monascus* M1 was grown in glutamic medium instead of in the original medium, monacolin K production increased from 48.4 to 215.4 mg L⁻¹, monacolin K production increased by 3.5 times. Glutamic acid enhanced monacolin K production by upregulating the expression of *mokB*-*mokI*; on day 8, the expression level of *mokA* tended to decrease by Reverse Transcription-polymerase Chain Reaction. Our findings demonstrated that *mokA* was not a key gene responsible for the quantity of monacolin K production in the presence of glutamic acid. Observation of *Monascus* mycelium morphology using Scanning Electron Microscope showed glutamic acid significantly increased the content of *Monascus* mycelium, altered the permeability of *Monascus* mycelium, enhanced secretion of monacolin K from the cell, and reduced the monacolin K content in *Monascus* mycelium, thereby enhancing monacolin K production.

**Keywords:** Gene expression, Glutamic acid, Monacolin K, *Monascus* RT-qPCR

**Introduction**

*Monascus* species, which are characteristically found in East Asian countries, have been influential in local life and culture, and have received attention worldwide because of their diverse products (Cheng et al. 2016) and abundant beneficial metabolites (Ming-Jen et al. 2010). *Monascus* species are known to produce various secondary metabolites with polyketide structures, including monacolins (Ming-Tao et al. 2013), pigments (Dajung et al. 2014), γ-aminobutyric acid (Su et al. 2003), and citrinins (Radu et al. 2012a; Zhang et al. 2016).

Monacolin K, an inhibitor of cholesterol biosynthesis, was the first monacolin isolated from the cultures of *Monascus ruber*. This compound has also been independently found in *Aspergillus terreus*, in which it is designated lovastatin (Nezami et al. 2012). Monacolin K is able to act on cholesterol biosynthesis (Radu et al. 2012b) and can block the activity of HMG-CoA reductase as a competitive inhibitor (Suzuki and Imai 2010). Moreover, monacolin K is thought to have wide uses in the clinical setting. For example, monacolin K is one of the most effective drugs available for the treatment of hyperlipidemia (Feuerstein and Bjerke 2012). Monacolin K can reduce the expression of pro-inflammatory transcription factors, lower the extent of atherosclerosis, and promote apoptosis in malignant thyroid cells (Chen et al. 2014). In addition, monacolin K has the ability to inhibit breast cancer cell proliferation (Patel 2016).

Monacolin K can be produced by *Monascus* during liquid or solid fermentation (Yu et al. 2013). The general production of monacolin K in liquid fermentation is lower (5-130 mg/L) than that in solid fermentation. However, the liquid fermentation process is relatively simpler than the solid fermentation process (Vendruscolo et al. 2016). Moreover, Previous studies have shown that the secondary metabolites of *Monascus* fermentation are strongly influenced by the environmental factors
such as particularly nitrogen sources (Vendruscolo et al. 2016), can influence the production of secondary metabolites in Monascus. However, the effects of amino acids on the secondary metabolites of Monascus are multifaceted, the impact mechanisms are till unclear.

The monacolin K biosynthetic gene cluster (Sakai et al. 2009) has been identified in previous studies (Fig. 1). According to the similarities with lovastatin synthetic genes (LNKS) in Aspergillus, researchers have identified nine genes (mokA-mokI) associated with the monacolin K synthesis and have proposed the functions of these genes (Chen et al. 2008). Each gene has different functions, and they are all important to the synthesis of monacolin K, i.e., mokA (polyketide synthase), mokB (polyketide synthase), mokC (P450 monooxygenase), mokD (oxidoreductase), mokE (dehydrogenase), mokF (transesterase), mokG (HMG-CoA reductase), mokH (transcription factor) and mokl (efflux pump).

In the study, we analyzed the effects of glutamic acid as a nitrogen source on the complex regulation of gene expression for monacolin K synthesis. We further assessed the influence of glutamic acid on mycelium content, pH, mycelium morphology, and monacolin K production in liquid culture medium, which are main factors to monacolin K production in Monascus.

Materials and methods
Fungal strain and culture conditions
Monascus M1 was obtained from The Chinese General Microbiological Culture Collection Center (Strain Number, CGMCC 3.0568), China. Monascus M1, which is a stable producer of monacolin K, was maintained on potato/dextrose/agar (PDA) for 5 days at 30 °C. All Monascus were cultured with 50 ml seed medium containing (per liter): 30 g glucose, 15 g Soybean powder, 1 g MgSO₄·7H₂O, 2 g KH₂PO₄, 70 g glycerol, 2 g NaNO₃, and 10 g peptone at a neutral pH. The cultures were incubated at 30 °C for 48 h with shaking at 200 rpm. For monacolin K production and gene expression testing, 5 ml of the seed medium was inoculated into two types of fermentation medium (50 ml). The original fermentation medium contained (per liter): 20 g rice powder, 1 g MgSO₄·7H₂O, 2 g ZnSO₄·7H₂O, 2.5 g KH₂PO₄, 90 g glycerol, 5 g NaNO₃ and 10 g peptone at a neutral pH. The glutamic acid fermentation medium contained all of the above components of the original fermentation medium plus 10 mM glutamic acid. The cultures were incubated at 30 °C for 48 h with shaking at 150 rpm, followed by incubation at 25 °C for 240 h with shaking at 150 rpm.

Analysis of Monascus content and pH
For analysis of Monascus content, 5 ml liquid fermentation medium was placed on four layers of gauze for filtering, and the gauze was then washed with sterile water until the liquid was colorless. The gauze was dried in an oven at 60 °C overnight and then weighed. The pH value was determined in different media using a pH meter. The experiment was performed in triplicate, and values are presented as average values of three independent measurements.

Analysis of monacolin K production
For analysis of monacolin K production, 5 ml of the fermentation medium was inoculated into 15 ml of 75% methanol (v/v), sonicated for 10 min, and allowed to settle overnight. Monacolin K production was determined by high-performance liquid chromatography (HPLC) on a C18 column at 25 °C (5 μm, 150 × 4.6 mm) after filtration of the supernatant through a 0.45 μm filter. The mobile phase was 0.1% H₃PO₄/methanol (1:3, v/v), running at 1 ml min⁻¹. The eluate was monitored by ultraviolet spectroscopy at a wavelength of 237 nm. Monacolin K from the National Institutes for Food and Drug Control in China was used as the standard.

Quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) analysis of monacolin K biosynthetic gene clusters
Monascus mycelia were harvested from the monacolin K production medium and stored in liquid nitrogen for RNA extraction. Total RNA was extracted from mycelia using an RNAprep Pure Plant Kit (Tiangen-bio, Beijing, China) according to the manufacturer’s protocol. First-strand cDNA was synthesized using a FastQuant RT Kit (with gDNase) (Tiangen-bio, Beijing, China), with the FQ-RT Primer Mix. Gene expression was monitored by RT-qPCR, carried out using SYBR Green PCR master
mix (Tiangen-bio, Beijing, China). Primers for moka, mokB, mokC, mokD, mokE, mokF, mokG, mokH, moki (NCBI accession No. DQ176595.1) and GAPDH genes (NCBI accession No. HQ123044.1) were designed by Beacon Designer8 (Table 1).

RT-qPCR was performed to determine the expression patterns of Monascus M1 in different fermentation culture media using a CFX96 Real-Time PCR Detection System version with 3.0 software (Bio-Rad, Hercules, CA, USA). After reverse transcription of total RNA into cDNA, cDNA was then used for qPCR with unigene-specific primers. The amplification program was as follows: 95 °C for 15 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s and 72 °C for 30 s. Amplification was performed using SuperReal Premix Plus (SYBR Green) for the fluorophore SYBR Green with fluorescein. The relative abundance of transcripts was calculated by the comparative threshold cycle (CT) method. GAPDH was used as the housekeeping reference gene. RT-qPCR was carried out in triplicate for each sample.

### Table 1 Primer pairs used for amplification of the monacolin K gene cluster in Monascus

| Genes | Primer pairs | Length (bp) | Tm value |
|-------|--------------|-------------|----------|
| moka-F | 5'-GACCTCGGTCACTCTGGC-3' | 18 | 59.1 |
| moka-R | 5'-TTGGTCAAGCGGTCTTC-3' | 18 | 57.3 |
| mokb-F | 5'-AAACATGTCACAGCTGCT-3' | 18 | 50.7 |
| mokb-R | 5'-CTAAGTCGGGATCTCTAC-3' | 18 | 53.1 |
| mokc-F | 5'-CAAGCTGGAAATACACCAAGCCTC-3' | 25 | 63.6 |
| mokc-R | 5'-AGCGGTGCTTCATTCTTGTC-3' | 25 | 65.3 |
| mokd-F | 5'-TTATCTGTGCTGTGATAT-3' | 18 | 59.8 |
| mokd-R | 5'-AACCTCTACGGCAGGATG-3' | 18 | 58.7 |
| moke-F | 5'-ATGCGGAGTCACGGACATCAA-3' | 25 | 72.3 |
| moke-R | 5'-GAAAGGCCAACC-GCACGCTTCC-3' | 25 | 71.1 |
| mokf-F | 5'-GAGATCATATACTGCGAGAC-3' | 22 | 59.8 |
| mokf-R | 5'-ACGTCCTCATCACCACCAAG-3' | 22 | 56.1 |
| mokg-F | 5'-CCAGTAAAACACGAGAGGAT-3' | 19 | 56.0 |
| mokg-R | 5'-GATCAGACGTCAGGCCAG-3' | 18 | 52.0 |
| mokh-F | 5'-CAGGAATCTGGGACTACCTC-3' | 25 | 65.8 |
| mokh-R | 5'-TGTCGATGATGATGATGATGAT-3' | 25 | 59.2 |
| mokl-F | 5'-AGTGGGATGGGATGGGATGG-3' | 20 | 60.9 |
| mokl-R | 5'-CAATGGGATGGGATGGGATGG-3' | 18 | 61.7 |
| GAPDH-F | 5'-CGTAGTGTCTTCCGTCAAC-3' | 19 | 55.4 |
| GAPDH-R | 5'-CTGGGGTCTGCATAGTTG-3' | 19 | 57.6 |

### Scanning electron microscopy (SEM) analysis of Monascus mycelial

Scanning electron microscopy was used to observe morphological differences in mycelia for in different media. M1 mycelium cells were fixed in 25% glutaraldehyde solution in phosphate-buffered saline (PBS) for 12 h at 25 °C temperature. Mycelium cell suspensions were rinsed with 0.1 M H3PO4 solution in PBS (pH 7.2) and harvested by centrifugation (12,000 rpm, 5 min). The supernatants were removed, and the mycelia were resuspended, dehydrated using a graded ethanol series (30, 50, 70, 80, 90 and 100%), and the mycelia were harvested by centrifugation (12,000 rpm, 5 min). The ethanol was removed with isomyl acetate-ethanol solution (1:1, v:v), incubated for 10 min, and centrifuged at 12,000 rpm for 5 min. The mycelia were resuspended in a suitable amount of hexamethyl disilazane (HMDS), with cotton wool plugging the upper part of the tube during centrifugation, and the sample was dried to a powder at 60 °C. After primary fixation, the mycelia were coated with gold–palladium for 2 min. Photomicrographs were then acquired using a VEGA 3LMU/LMH scanning electron microscope (TESCAN, Brno, Czech republic).

### Results

#### Effects of glutamic acid on monacolin K production and biomass

At the beginning of the study, we constructed growth curves and determined the four key stages of Monascus M1 growth and the most suitable amount of glutamic acid. This analysis showed that the adjustment phase, logarithmic phase, and stabilization stage of M1 occurred on days 2, 5 and 11, respectively. The maximum monacolin K biosynthesis quality was achieved on day 8. A number of pre experimental results showed that the amount of 10 mM glutamic acid and the time of initial fermentation is the most optimal program of promoting the monacolin K production in Monascus. Figure 2 shows accumulation of monacolin K content from Monascus M1 in the original medium and glutamic acid-containing medium. In glutamic acid medium, maximal production of monacolin K (215.4 mg l⁻¹) was observed on day 11 by HPLC (Fig. 3a). In the original medium, maximal monacolin K production (68.6 mg l⁻¹) was observed on day 8 by HPLC (Fig. 3b). Glutamic acid was found to substantially alter the biomass of Monascus M1. For example, in glutamic acid medium, the maximal biomass (36.7 mg l⁻¹) was found on day 5, whereas that in the original medium (31.3 mg l⁻¹) was found on the day 8.

In addition, we tracked changes in pH values in different culture media. Table 2 shows changes in pH values in different culture media. In the adjustment and logarithmic phases (on days 2 and 5, respectively) of Monascus
M1, the pH value of the glutamic acid fermentation medium was generally low. During the stabilization stage (on day 8) and the monacolin K biosynthesis quality maximum stage, minor changes in the pH values of the different culture media were observed.

Effects of glutamic acid on the mycelial morphology of Monascus

As shown in the SEM images in Fig. 4, compared with the control group (mycelia in original culture medium), the mycelia morphology in glutamic acid-containing culture medium exhibited more folds and larger bulges, forming a variety of oddly shaped mycelia. In contrast, the radius of M1 mycelia in the original culture medium was uniform, and the top branches of the mycelia were formed of a single or multiple conidia, with spherical or ellipsoidal spores. Compared with the mycelia in original culture medium, those in glutamic acid-containing medium exhibited more prominent bulges, suggesting that the permeability of the mycelia may be enhanced.

Therefore, we speculated that glutamate could improve the permeability of mycelia. Furthermore, secretion of monacolin K outside of the cell was enhanced, monacolin K content in the cell was reduced, and monacolin K production was improved in glutamic acid-containing medium.

Effects of glutamic acid on the expression of monacolin K biosynthetic genes

Figure 5 shows the expression profiles of monacolin K biosynthetic genes in original medium and glutamic acid-containing medium. The expression levels of mokA, mokB, mokC, mokD, mokE, mokF, mokG, mokH, and mokI were positively correlate with monacolin K accumulation when cultured in original medium. On day 8, gene expression and monacolin K production reached a maximum in original medium. A similar trend was observed in glutamic acid-containing medium; however, the expression levels of mokA, mokB, and mokH were always higher in glutamic acid-containing medium than in original medium, and monacolin K production in glutamic acid medium was higher on day 11 than on day 8.

In glutamic acid-containing medium, mokB, mokC, mokD, mokE, mokF, mokG, mokH, and mokI levels were significantly upregulated. In contrast, mokA expression was decreased by 63.5% on day 8 of culture in the presence of glutamic acid compared with its expression in the original medium. Moreover, the expression levels of mokB, mokC, mokD, mokE, mokF, mokG, mokH, and mokI, which are thought to participate in monacolin K biosynthesis as structural genes (Wanping et al. 2015), were highest on day 8. This trend was similar to that observed for monacolin K production in glutamic acid-containing medium, indicating that glutamic acid stimulated monacolin K production via the upregulation of the expression levels of these eight genes. The expression levels of mokA were highest on day 11 in glutamic acid-containing medium, which differed from the trends observed for monacolin K production in glutamic acid medium. Although mokA is a key gene that encodes monacolin K polyketide synthase, our data showed that mokA was not essential for controlling the quantity of monacolin K production in glutamic acid.
Monacolin K, also known as lovastatin, is able to act on cholesterol biosynthesis, which can reduce the function of HMG-CoA reductase as a competitive inhibitor. The monacolin K biosynthetic gene clusters in Monascus have received much attention because of the various biological activities of this compound. The monacolin K biosynthetic gene cluster has been identified according to the similarities with lovastatin synthetic genes (LNKS) in Aspergillus. Nine genes (mokA-mokI) have proposed the functions of these genes which were associated with the monacolin K synthesis. The mokA-deficient mutant in M. pilosus BCRC38072 cannot produce monacolin K, indicating that mokA encodes the polyketide synthase responsible for monacolin K biosynthesis in M. pilosus BCRC38072. Additionally, the mokB-deficient mutant of M. pilosus NBRC4480 cannot produce monacolin K, but exhibits accumulation of monacolin J, indicating that mokB is responsible for the synthesis of the diketide side chain of monacolin K (Sakai et al. 2009; Chen et al. 2015). Overexpression of the mokH gene in M. pilosus results in significantly higher monacolin K production than that in wild-type strains, indicating that mokH positively regulates monacolin K production (Chen et al. 2010). Based on previous reports, many factors, particularly nitrogen sources, such as amino acids, can influence the production of secondary metabolites in Monascus. For example, monacolin K production is increased in glutamic acid or leucine culture conditions; an ideal nitrogen source can be selected to control the low final pH and then produce citrinin-free Monascus pigments (Kang et al. 2013b).

### Table 2 Change of pH values in different culture medium

| pH value of fermentation broth | Time (d) | 2  | 5  | 8  | 11 |
|-------------------------------|----------|----|----|----|----|
| Original medium               |          | 5.15 | 6.02 | 5.89 | 5.55 |
| Glutamic acid medium          |          | 4.68 | 4.88 | 6.06 | 5.74 |

### Discussion

Monacolin K, also known as lovastatin, is able to act on cholesterol biosynthesis, which can reduce the function of HMG-CoA reductase as a competitive inhibitor. The monacolin K biosynthetic gene clusters in Monascus have received much attention because of the various biological activities of this compound. The monacolin K biosynthetic gene cluster has been identified according to the similarities with lovastatin synthetic genes (LNKS) in Aspergillus. Nine genes (mokA-mokI) have proposed the functions of these genes which were associated with...
Fig. 4  Morphology (A) of Monascus M1 in different culture medium with different magnifications factor, 2000 x, 3000 x and 5000 x, respectively. M1 was cultured in original medium (A–C), glutamic medium (D–F) for 11 days at 25 °C with shaking at 150 rpm. Spore (arrows #1), Bulges (arrows #2) and Folds (arrows #3) were visible.
is the first report on the inhibition of citrinin biosynthesis by controlling an extremely low pH. Previous also showed that lowering the pH value to 2.5 would result in high monacolin K and citrinin concentrations as well as high biomass in fixed dioscora amount, implying that pH value may stimulate the formation of monacolin K and citrinin through increasing Monascus cell amount (Lee et al. 2007).

Previous studies have shown that the most suitable pH value for Monascus growth is about 4 (Lee et al. 2007). Interestingly, in this study, we found that the pH varied during different stages of growth; during the adjustment and logarithmic phases of Monascus growth, the pH was lower in glutamic acid-containing medium (4.68 and 4.88, respectively) than that of the original medium (5.15 and 6.02, respectively). Thus the amount of Monascus mycelia was greater in glutamic acid-containing medium than that in original medium. We hypothesized that glutamate may increase monacolin K production by increasing the density of Monascus.

In this study, we demonstrated, for the first time, the correlation between the expression levels of monacolin K biosynthetic genes and monacolin K production in Monascus. At any stage of cell growth, we found that glutamic acid enhanced monacolin K production in Monascus M1, compared with cultivation in original medium. When Monascus M1 was grown in glutamic acid-containing medium rather than original medium, monacolin K production increased from 48.4 to 215.4 mg l⁻¹. Thus, these data showed that glutamic acid promoted the production of monocolinK.

Monascus expresses nine genes related to monacolin K synthesis, and monacolin K accumulation was found to be positively correlated with the expression of the monacolin K biosynthetic gene cluster. Indeed, RT-qPCR analysis showed that the maximal monacolin K biosynthesis quality was reached on day 8, at which point most genes related to monacolin K synthesis showed higher transcription in glutamic acid-containing medium, with the exception of moka. These data indicated that mokB-mokl were eight key genes mediating monacolin K production in the presence of glutamic acid. According to the speculation on the function of monacolin K synthesis genes, we speculated that mokH acts as a transcription factor, it will promote the expression of key genes in monacolin K biosynthesis.

**Fig. 5** Expression of monacolin K biosynthesis-related genes during fermentation. Gene expression for various monacolin K biosynthesis-related genes was analyzed by RT-qPCR. Test samples corresponded one-to-one with samples used for monacolin K testing. Transcript levels were normalized to that of the GAPDH gene. The mRNA expression levels on day 2 in original medium were used as reference values (value: 1.0). Data are expressed as the relative mRNA level for each gene and represent the average values from three separate experiments. Error bars represent the standard deviation (n = 3). **P** < 0.001; *P* < 0.05 compared with mRNA ratio in glutamic acid-containing medium.

Mokl acts as an efflux pump, it will promoted the process of transferring monacolin K out of the Monascus cells, reduced the content of monacolin K in cells and promoted the final mknacolin K content. MokB-mokG participated in the monacolin K biosynthesis process and promoted the production of monacolin K in glutamic acid medium directly. Thus, based on the expression of monacolin K synthesis-related transcripts, these data supported that glutamic acid promoted the production of monocolin K has an internal power and the promoting effect is stable.

In summary glutamic acid increased the content of Monascus mycelia, altered the pH value in fermentation broth, changed the permeability of Monascus mycelia, enhanced the secretion of monacolin K to the outside of the cell, and reduced monacolin K content in the Monascus mycelia, thereby enhancing monacolin K production. In addition, glutamic acid may not only be used to provide energy for Monascus growth and metabolize, but also generate the production of acetyl coenzyme A, which is a substrate for monacolin K, and ultimately increase the content of metabolites. Our findings also demonstrated that glutamic acid could enhance monacolin K production by upregulating the expression of mokB, mokC, mokD, mokE, mokF, mokG, mokH, and mokl. So further studies are needed to elucidate the molecular pathways through which glutamic acid regulates monacolin K production.

**Abbreviations**

LNKS: lovastatin synthetic genes; PDA: potato/dextrose/agar; HPLC: high-performance liquid chromatography; RT-qPCR: quantitative real-time reverse transcription polymerase chain reaction; SEM: scanning electron microscopy; PBS: phosphate-buffered sline; HMDS: hexamethyl disilazane.

**Authors’ contributions**

JL, SC, CZ, and LY performed experiments, and analyzed data. CZ and JL conceived of the study. CZ, BS and CW designed experiments and wrote the manuscript. All authors read and approved the final manuscript.

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Acknowledgements
This study was supported by the National Natural Science Foundation of China (Grant No. 31301411), Beijing Municipal Education Commission (Grant No. KM201410011009), Beijing Municipal Science and Technology Commission (Grant No. Z15110001215008), National Natural Science Foundation of China (Grant No. 31301410), and the National Key Research and Development Program of China (Grant No. 2016YFD0400802).

Competing interests
Informed consent was obtained from all individual participants included in the study. The authors declare that they have no competing interests.

Availability of data and materials
The datasets supporting the conclusions of this article are included within the article additional files named “Monascus RT-qPCR original data”. These data were collected using a CFX96 Real-Time PCR Detection System version with 3.0 software (Bio-Rad).

Consent for publication
Not applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

Ethics approval and consent to participate
Not applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding
This study was funded by the National Natural Science Foundation of China (Grant No. 31301411), Beijing Municipal Education Commission (Grant No. KM201410011009), Beijing Municipal Science and Technology Commission (Grant No. Z15110001215008), National Natural Science Foundation of China (Grant No. 31301410), and the National Key Research and Development Program of China (Grant No. 2016YFD0400802).

Received: 11 December 2016  Accepted: 19 December 2016
Published online: 10 January 2017

References
Chen YP, Tseng CP, Liaw LL, Wang CL, Chen IC, Wu WL, Wu MD, Yuan GF (2008) Cloning and characterization of monacolin K biosynthetic gene cluster from Monascus pilosus. J Agric Food Chem. 56:5639–5646
Chen YP, Yuan GF, Hsieh SY, Lin YS, Wang WY, Liaw LL, Tseng CP (2010) Identification of the mokL gene encoding transcription factor for the upregulation of monacolin K biosynthesis in Monascus pilosus. J Agric Food Chem. 58:267–293
Chen HH, Chen YY, Yeh JZ, Jiang CM, Wu MC (2014) Immune-stimulated anti-tumor effect of different molecular weight polysaccharides from Monascus purpureus on human leukemic U937 cells. Cyta-J Food. 12:134–140
Chen WP, He YY, Zhou YX, Yao HC, Feng YL, Li M, Chen FS (2015) Edible filamentous fungi from the species Monascus: early traditional fermentations, modern molecular biology, and future genomics. Compr Rev Food Sci Food Saf. 14:555–567
Cheng MJ, Wu MD, Chan HY, Chen JJ, Cheng YC, Chen IS, Yuan GF (2016) A new azaphilone metabolite from the fungus Monascus ruber. Chem Nat Compd. 52:231–233
Dajung J, Deokyeong C, Kyoungwha N, Chul SS (2014) Biological evaluation of novel derivatives of the orange pigments from Monascus purpureus sp. as inhibitors of melanogenesis. Biotechnol Lett. 36:1605–1613
Feuerstein J, Bjerke W (2012) P02.88. Powdered red yeast rice and plant stanols and sterols to lower cholesterol. BMC Complement Altern. 12:144
Kang B, Zhang X, Wu Z, Qi H, Wang Z (2013a) Effect of pH and nonionic surfactant on component profile of intracellular and extracellular Monascus pigments. Process Biochem. 48:759–767
Kang Biyu, Zhang Xuehong, Zhenqiang Wu, Wang Zhilong, Park Sunghoon (2013b) Production of citrinin-free Monascus pigments by submerged culture at low pH. Enzyme Microb Technol. 55:50–57
Lee CL, Hung HK, Wang Ji, Pan TM (2007) Improving the ratio of monacolin K to citrinin production of Monascus purpureus NTU 588 under dioscoreamedium through the mediation of pH value and ethanol addition. J Agric Food Chem. 55(16):6493–6502
Ming-Jen Ch, Ming-Der W, Ih-Sheng C, Chung-YCh Wen-Li Li (2010) Secondary metabolites from the red mould rice of Monascus purpureus BCRC 38113. Nat Prod Res. 24:1719–1725
Ming-Tao L, A-Li W, Zhen S, Jin-Jie S, Xiu-Li W (2013) Cytotoxic monacolin analogs from Monascus purpureus-fermented rice. J Asian Nat Prod Res. 15:600–609
Nezami Nariman, Safa Javid, Salari Behzad, Ghorsashi Sona, Khosraviani Khashayar, Davari-Farid Sina, Hashemi-Aghdam Yashar, Nargabad Ourmaan Nezami, Tabrizi Jafar Sadegh (2012) Effect of lovastatin therapy and withdrawal on serum uric acid level in people with type 2 diabetic nephropathy. Nucleus Nucleot Nud 31:353–363
Patel S (2016) Functional food red yeast rice (RYYR) for metabolic syndrome amelioration: a review on pros and cons. World J Microbiol Biotechnol. 32:87
Radu N, Salageanu A, Ferdes M, Rau I (2012a) Cytotoxicity study regarding some products derived from Monascus sp. Mol Cryst Liq Cryst. 37:189–194
Radu N, Doncea SM, Ferdes M, Salageanu A, Rau I (2012b) Biostimulatory properties of Monascus sp Bioproducts. Mol Cryst Liq Cryst. 555:95–201
Rashmi D, Padmavathi T (2014) Statistical optimization of pigment production by Monascus sanguineus under stress condition. Prep Biochem Biotechnol. 44:68–79
Sakai K, Kinoshita K, Hiroshi N (2009) Identification of mokL involved in monacolin K biosynthesis in Monascus pilosus. Biotechnol Lett. 31:1911–1916
Su YC, Wang JJ, Lin TT, Pan TM (2003) Production of the secondary metabolites γ-aminobutyric acid and monacolin K by Monascus. J Ind Microbiol Biotechnol. 30:41–46
Suzuki N, Imai A (2010) HMG-CoA reductase inhibitor lovastatin causes reversible cytoskeleton perturbation by RhoA signalling suppression in peritoneal cell line Met5A. J Obstet Gynaecol. 30:404–407
Vendruscolo F, Buhler RMM, de Carvalho JC, de Oliveira D, Moritz DE, Schmidhell W, Ninow JL (2016) Monascus: a reality on the production and application of microbial pigments. Appl Biochem Biotechnol. 178:211–223
Wanqing C, Yi H, Youxiang Zh, Yanchun Sh, Yanli F, Mu L, Fusheng Ch (2015) Edible filamentous fungi from the species Monascus: early traditional fermentations, modern molecular biology, and future genomics. Compr Rev Food Sci. 14:555–567
Yu Li, Zhang XH, Xie YH, Ma SM, Liu H, Luo YB (2013) Optimization of Fermentation conditions for higher Monacolin K production by Monascus purpureus. Adv Mater Res. 781:1397–1402
Zhang Zh, Ali Z, Khan JI, Khan IA (2016) Cytotoxic monacolins from red yeast rice, a Chinese medicine and food. Food Chem. 202:262–268

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