https://doi.org/10.17221/4/2022-CJFS

Effect of low expression level of acetyl coenzyme A synthetase gene on secondary metabolite in *Monascus*

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Citation: Lin L., Zhang C.D., Shao X.M., Liu P.P., Wu Z.F., Lu Y.X., Guo H.Z. (2022): Effect of low expression level of acetyl coenzyme A synthetase gene on secondary metabolite in *Monascus*. Czech J. Food Sci., 40: 414–421.

Abstract: Acetyl-coenzyme A (CoA) is a key metabolite produced by the acetyl-CoA synthetase (ACS) gene in energy metabolism and biosynthetic pathways. ACS is speculated to be the branching site of monacolin K (MK) and citrinin production and related to the metabolite production of *Monascus*. In this study, the ACS expression was inhibited by ribonucleic acid interference (RNAi). T7 was selected for a follow-up analysis of the lowest ACS expression, which was 0.401 times higher than that of the wild-type strain. The effects on the colony morphology of *Monascus* were determined. The morphological characteristics of mycelia and spores were observed under a scanning electron microscope. The contents of secondary metabolites, namely, MK and citrinin, were determined through high performance liquid chromatography (HPLC). Colour values were measured with a spectrophotometer. Results showed that the low ACS expression could inhibit the growth of *Monascus* colonies and the hypha and affect the formation and morphology of *Monascus* M1 spores. It could also inhibit the production of the main secondary metabolites, namely, MK, citrinin, and pigment.

Keywords: citrinin; colonial morphology; monacolin K; pigment; scanning electron microscope

As a food colourant and functional food, red yeast rice has a long history in China. The main metabolites of *Monascus* are monacolin, citrinin, and pigment. Monacolin K (MK), also called lovastatin, is a strong 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitor that mediates the rate-limiting reaction of cholesterol biosynthesis (Seraman et al. 2010; Yang and Mousa 2012; Mulder et al. 2015). Citrinin is a nephrotoxin, because it can act on the kidneys and cause tumors, deformities, and mutations (Liang et al. 2019). Pigments have been used as natural food colourants for a long time (Srianta et al. 2020). Their various bioactivities, such as antioxidants, anti-inflammation, anticancer, antimicrobes, antidiabetes, and anti-obesity, have also been reported (Su et al. 2005; Srianta et al. 2017).

Supported by the Natural Science Foundation of Hebei Province (Project No. C2019408061).

https://doi.org/10.17221/4/2022-CJFS

Gu et al. (2019) showed that the acetyl-CoA synthetase (ACS) gene ChAcs1 is essential for lipid metabolism, carbon utilisation, and virulence of the hemibiotrophic fungus *Colletotrichum higginsianum*. One ACS (GenBank No. VIFY01000136.1) is found in *Monascus*. Citrinin and pigment synthesised by *Monascus* via the polyketone pathway have a common intermediate, namely, tetraketone, which is formed by the condensation of one acetyl-CoA and three malonyl-CoAs (Hajjaj et al. 1999).

In a previous study, mok E is overexpressed in *Monascus*, and genome walking is used to analyse the insertion site of the exogenous gene. The AAA adenosine triphosphate synthase (ATPase) gene located upstream, and ACS located downstream of the insertion can be obtained by genome walking. The expression levels of these genes are enhanced by the insertion of an exogenous gene (Lin et al. 2018). Therefore, ACS and AAA ATPase genes influence the yield of citrinin and MK because of the enhanced MK production and the reduced citrinin production in monk E-overexpressing transformant (T-mok E3).

In this study, the ACS expression level was inhibited by ribonucleic acid interference (RNAi) to investigate the effect of ACS on MK and citrinin production. This study was the first to examine the effects of the low ACS expression level on the colonial morphology and the morphology of mycelia and spores of the transformant in *Monascus*. The concentrations of pigment, MK, and citrinin as the main secondary metabolites were also determined in this study.

MATERIAL AND METHODS

Strains and culture conditions. As a wild-type strain, *Monascus buliginosus* M1 was isolated from red yeast rice and preserved in our laboratory. It was activated on malt extract agar at 30 °C for 7 days (HH.B11-600; Tianjin Zhonghuan Experimental Electric Furnace Co. LTD, China). Spores were harvested with sterile water (2 mL) and inoculated into 100 mL of the seed medium (6 g of glucose, 2 g of peptone, 1 g of KH_2PO_4, 1 g of $NaNO_3$, and 0.5 g of $MgSO_4{\cdot}7H_2O$) in a 250 mL flask. The cultures were incubated at 30 °C for 48 h and shaken at 180 rpm (HYG-IIa KCL-2000W; Shanghai Xinrui Automation Equipment Co., LTD, China). Afterward, 10 mL of the seed fungus liquid was inoculated in a sterilised rice medium (28 g of overnight soaked rice and 12 g of water) in a 300 mL flask incubated at 30 °C for 6 days and then at 25 °C for 15 days to produce MK and citrinin and determine their colour values. The samples were taken every 3 days.

Deoxyribonucleic acid (DNA) extraction, ACS fragment cloning, and RNAi vector construction. Total DNA was extracted using a plant DNA kit (Omega, USA). The total DNA of M1 was used as a template. The sequences of the primers used are listed in Table 1. The polymerase chain reaction (PCR) primers B/E-CoA-F and B/E-CoA-R (Bgl II and EcoR Ib restriction sites were included, respectively; F: forward primer, R: reverse primer) were designed on the basis of the ACS of M1 to amplify a 469 base pair (bp) fragment by Primer Premier 5. The ACS fragment was linked to the linearised expression vector pCAMBIA1303-TrpC-Hygro-gpdA-TEF1 under the action of T4 ligase, which was double-digested by BglII and EcoRI. The binary expression vector pCAMBIA1303-TrpC-Hygro-gpdA-TEF1 preserved in our laboratory contained the bacterial kanamycin resistance gene (KanR) and hygromycin B phosphotransferase gene (hph) as selective marker genes.

Transformation and selection. *Agrobacterium tumefaciens*-mediated transformation was used in this study (Wang et al. 2011). The recombinant plasmid was transferred into *A. tumefaciens* (GV3101) competent cells through electroporation (Gene Pulser Xcell; Bio-Rad, USA). *A. tumefaciens* transformants were selected on Luria broth (LB) plates with 50 mg mL^{-1} kanamycin. The spore suspension of M1 and *A. tumefaciens* transformant suspension were cultivated in a co-fermentation-inducing medium that contained acetosyringone. *Monascus* transformants were selected on potato dextrose agar (PDA) plates supplemented with 150 mg mL^{-1} hygromycin B and incubated at 30 °C for about 2 weeks (HH.B11-600; Tianjin Zhonghuan Experimental Electric Furnace Co., LTD, China) until colonies could be observed.

PCR and gene expression analysis. Ten transformants were randomly selected. The genomic DNA of the putative transformants was screened for the presence of gpdA-TEF (present in the destination vector) through PCR (070-851; Biometra Corporation, Germany) by using a set of primers (Table 1). The PCR conditions were set as follows: 95 °C for 3 min, followed by 35 cycles each of 95 °C for 20 s, 55 °C [melting temperature (Tm)] for 20 s, and 72 °C for 30 s. After 35 cycles, the product was maintained at 72 °C for 5 min and then stored at 4 °C (Agilent; Agilent Technologies, Inc., USA).

The transformants and the wild-type strain were inoculated in the seed culture. The culture was then fil-

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Table 1. The primer used in this study

Primer name	Sequence (5'-3')	Description
B/E-CoA-F	**GGTAGATCT**CGCTGTCCACTCCGTCG[a]	amplification of ACS gene fragment production size: 469 bp
B/E-CoA-R	**ACCGAATTC**GCCGACATCACCACCGC[b]	
gpdA-TEF-F	GCTACATCCATACTCCATCCTTC	production size: 638 bp
gpdA-TEF-R	GTAGTATCTGAGCACTTCTCC	
β-actin-F	AGTCCAACAGGGAGAAGATG	used in RT-qPCR
β-actin-R	CACCAGAGTCAAGCACGATA	
ACS-F	CGCTTGTAGACCAGGACG	used in RT-qPCR
ACS-R	GGTCATTATCACCTCCGACG	

[a]Bold font of the primer B/E-CoA-F is the Bgl II restriction site and protective base; [b]bold font of the primer B/E-CoA-R is the EcoR I restriction site and protective base; B – cleavage site Bgl II; E – cleavage site EcoR I; F – forward primer; R – reverse primer; bp – base pair; CoA – coenzyme A; ACS – acetyl-CoA synthetase, RT-qPCR – quantitative real-time polymerase chain reaction

tered with gauze to obtain the mycelia. Total RNA was extracted from the mycelia by using a plant RNA kit (Omega, USA), and complementary DNA (cDNA) was synthesised using a Prime Script first-strand cDNA synthesis kit (Takara, Japan). cDNA was used as a template for quantitative real-time PCR (RT-qPCR). Gene expression was tested through RT-qPCR by using SYBR Premix Ex Taq II (Takara, Japan). The primers for ACS (GenBank No. VIFY01000136.1) and β-actin (GenBank No. HQ123045.1) designed by Primer Premier 5 are listed in Table 1. After the data were normalised, variations in gene expression data among different samples were expressed as $2^{-\Delta\Delta CT}$ (Sengupta et al. 2021). The strain with the lowest ACS expression level was selected for the subsequent analysis. All values were normalised using the expression level of β-actin as a reference and the gene expression level of the wild-type strain as a calibrator. All experiments were performed in triplicate.

Observation of colony morphology. The activated *Monascus* M1 and the transformant were inoculated into the seed medium and incubated at 30 °C for 48 h with shaking at 180 rpm (HYG-IIa KCL-2000W; Shanghai Xinrui Automation Equipment Co., LTD, China). The spore suspension was filtered with sterile gauze and used to adjust the number of spores to 10^6 mL^{-1} by adding sterile water. Then, 45 μL of suspension was placed on the center of a solid plate medium (a seed medium with 3% agar) and cultured at 30 °C for 7 days (HH.B11-600; Tianjin Zhonghuan Experimental Electric Furnace Co. LTD, China). During this period, the colony morphological characteristics of the transformant and the wild-type strain were observed. The wild-type *Monascus* M1 was used as blank control.

Scanning electron microscopy (SEM). The wild-type strain and the transformant were cultured on a solid plate medium (seed medium with 3% agar) for 12 days. The hyphae of the wild-type strain and the transformant were collected, placed in 2.5% glutaraldehyde, and fixed for 6 h. The hyphae of the wild-type strain and the transformant were rinsed with 0.1 mol L^{-1} phosphate buffer (pH 7.2) thrice, dewatered with 50, 70, 90, and 100% ethanol for 15 min each time, and freeze-dried (FD-1A-80; Shanghai Siyu Instrument Co., LTD, China). Then, the hyphae of the wild-type strain and the transformant were sprayed with gold by using an EIKO IB-3 ion sputtering instrument (EIKO CO., LTD, Japan) and scanned with an XL-3 SEM (20 kV; Philips Co., TLD, Netherlands).

Extraction and high performance liquid chromatography (HPLC) analysis of MK. The samples were dried at 50 °C (XMTD-1000; Tianjin Tianyu Experimental Instrument Co., LTD, China), ground into powder, weighed at 0.40 g, extracted with 10 mL of 75% ethanol thrice, subjected to ultrasonic (KQ5200DE; Kunshan, China) for 30 min each time, and centrifuged at 2 150 × g for 15 min (TDL-5-A; Shanghai Anting Scientific Instrument Factory, China). The total suspension was merged and filtered through a 0.45 μm filter membrane.

MK was analysed by HPLC. An Eclipse XDB-C18 column (5 μm, 4.6 mm × 250 mm; Agilent, USA) was used at 25 °C, and isocratic elution was performed using 0.1% phosphoric acid in water/acetonitrile (40/60, v/v) as the mobile phase at 1 mL min^{-1} for 30 min. The samples were monitored using a diode-array detection (DAD) detector at 273 nm. Standards from Sigma were used, and all samples were determined at least thrice.

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HPLC analysis of citrinin. The samples were extracted in the same way as the determination of MK. Citrinin was analysed using the same HPLC column (Eclipse XDB-C18 column; Agilent, USA), and isocratic elution was performed using 0.1% phosphoric acid in water/acetonitrile (50/50, v/v) as the mobile phase at 1 mL min^{-1} for 30 min. The samples were monitored with a DAD detector at 330 nm. Standards from Fermentek LTD. (Israel) were used, and all samples were determined at least thrice.

Determination of colour value. The samples were extracted in the same way as the determination of MK. Their colour value at 505 nm ($E^{1\%}_{1\,cm}$ 505 nm) was analysed by using a spectrophotometer (Agilent 8453; Agilent, USA), and 75% ethanol was used as the blank control. $E^{1\%}_{1\,cm}$ 505 nm was calculated using Equation (1). All samples were determined at least thrice.

$$E^{1\%}_{1\,cm}\,505\,nm = \frac{\left(A - A_0\right) \times f}{m \times 100} \quad (1)$$

where: 505 nm – maximum absorption wavelength of pigments dissolved in 75% ethanol; A – absorbance of the sample; A_0 – absorbance of the blank control; f – dilution multiple; m – weight of the sample.

RESULTS AND DISCUSSION

Construction and selection of the ACS RNAi strains. A total of 100 transformants were preliminarily screened with hygromycin. Ten transformants were selected to measure the ACS expression level through RT-qPCR. The results showed that the ACS expression levels in T5 and T6 transformants were higher (1.154 and 1.162 times) than that of the wild-type strain. The ACS gene expression levels in other transformants (T1–T4 and T7–T10) were lower than that of the wild-type strain. This finding indicated that the RNAi vector was not successfully expressed in T5 and T6 transformants but successfully expressed in other transformants. The ACS expression level in T7 transformant was the lowest, which was 0.401 times as much as that of the wild-type strain. T7 transformant was selected for the follow-up analysis.

Observation of colony morphology. The colony morphology observations of the wild-type strain and T7 transformant are shown in Figure 1. The wild-type strain colony was grown on the culture on the 1st day. However, the colony of T7 transformant was grown on the culture on the 4th day. The colony of T7 transformant on the 4th day was small and white, while the

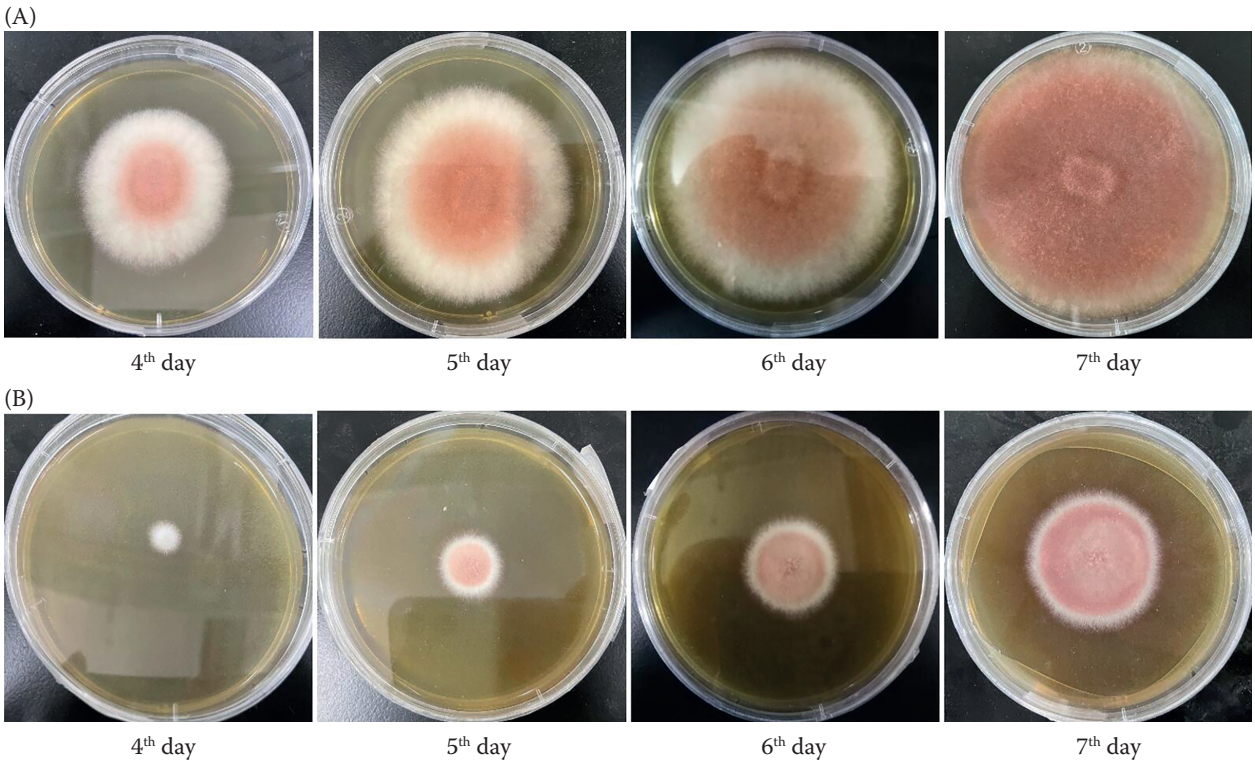

Figure 1. The colony morphology observations for the wild-type strain and T7 transformant: (A) the colony morphology of wild-type strain at the 4th day to the 7th day, (B) the colony morphology of T7 transformant at the 4th day to the 7th day

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colony of the wild-type strain was larger and had red pigments (Figure 1). On the 6th day, the whole colony of the wild-type strain had a wool blanket shape, and its aerial hyphae were obvious. However, the growth of the aerial hyphae of T7 transformant was not obvious. Therefore, the low ACS expression level could affect the mycelial growth of *Monascus* M1. It was observed that mitochondrial acetyl-CoA is necessary to produce energy sources for the survival and normal growth of eukaryotic cells.

SEM observation. The hyphae and spores of the wild-type strain and T7 transformant were observed under SEM (Figure 2). Figure 2 show the wild-type strain at 500×, the wild-type strain at 1 000×, T7 transformant at 500×, and T7 transformant at 1 000×. Cleistothecia formed singly at the top of the stalk-like hyphae and scattered throughout the mycelium (Figure 2), which was consistent with the description of Ji et al. (2010). The comparison of Figures 2A and 2C revealed that the number of the asexual conidia of T7 transformant was significantly less than that of the wild-type strain. Figures 2B and 2D illustrate that the spores of the wild-type strain were plump and round, whereas the spores of T7 transformant were more shriveled. Son et al. (2011) indicated that acetyl-CoA is an important metabolite for the sexual development of *Gibberella zeae*, and ACS1 deletion results in defect in sexual development. In the present study, the low ACS expression level by RNAi could affect the formation and morphological characteristics of *Monascus* M1 spores.

HPLC analysis of MK. MK, first discovered by Endo (1979), is one of the main secondary metabolites in *Monascus*. According to the concentration and the

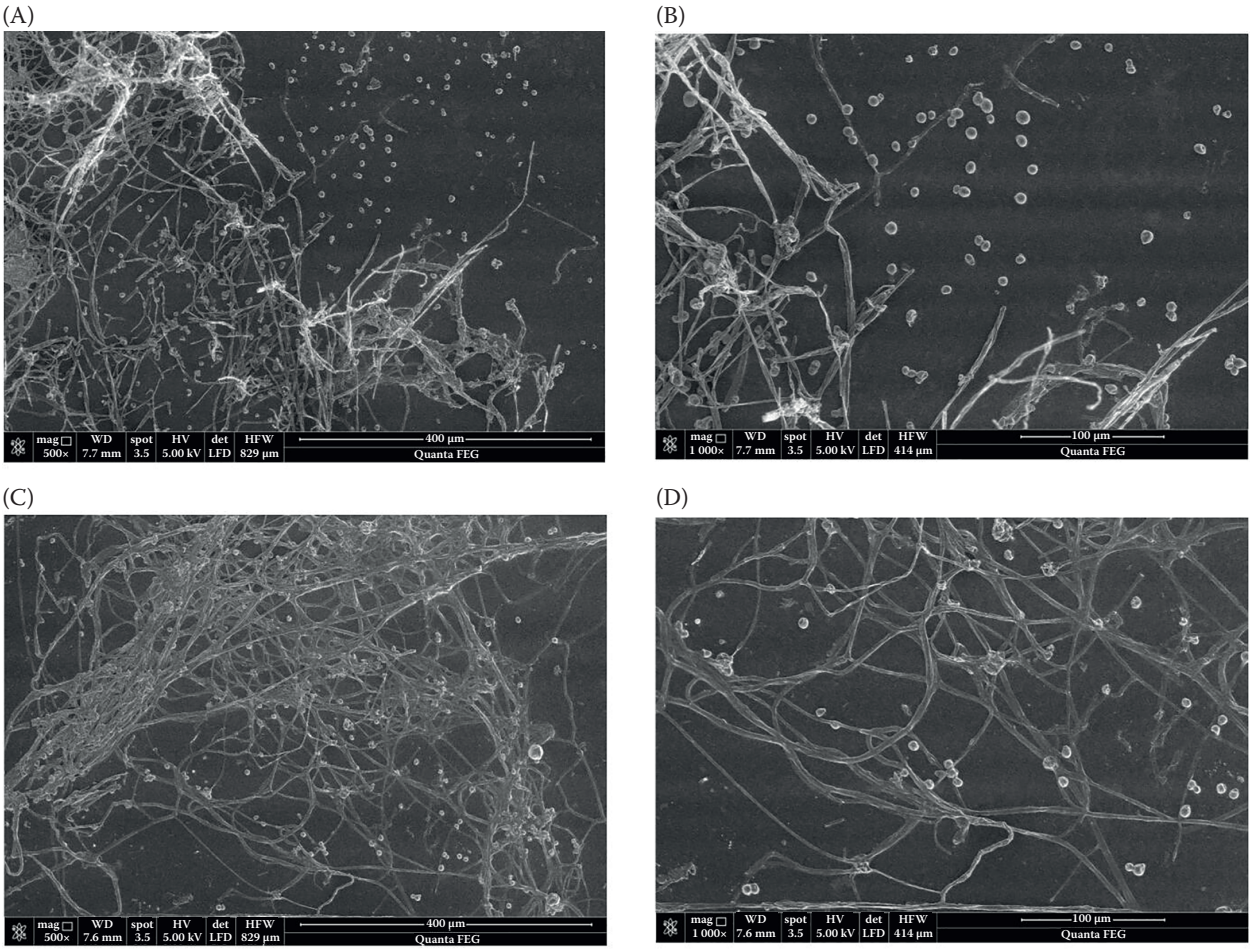

Figure 2. The scanning electron microscope observation of (A) wild-type strain at 500×, (B) wild-type strain at 1 000×, (C) T7 transformant at 500×, and (D) T7 transformant at 1 000×

mag – magnification; WD – working distance; HV – high voltage; det – detector; LFD – large field detector; HFW – horizontal field width; FEG – field emission gun

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corresponding peak area of the MK standard, the regression equation and correlation coefficient were calculated as follows:

$$y = 57.321x + 18.71,\ R^2 = 0.9999 \quad (2)$$

The MK concentrations of the wild-type strain and T7 transformant at different time points are presented in Figure 3. The MK concentrations increased first and then decreased in the wild-type strain and T7 transformant. On the 12th day, the MK concentrations of the wild-type strain and T7 transformant peaked at 11.18 μg g^{-1} and 6.75 μg g^{-1}, respectively. The MK concentrations of the wild-type strain and T7 transformant were 11.18 μg g^{-1} and 6.75 μg g^{-1} on the 12th day. The MK concentration of T7 transformant was lower than that of the wild-type strain during cultivation. The MK of T7 transformant could not be detected by HPLC from days 15 to 21. Therefore, the low ACS expression level could inhibit MK synthesis. On the basis of the biosynthetic pathway of MK reported by Manzoni and Rollini (2002), we could speculate that this low expression level caused a decrease in CoA production, thereby decreasing the number of the MK synthesis precursors monacolin J and 2-methyl butyryl-CoA; consequently, MK production decreased.

HPLC analysis of citrinin. The biosynthesis of citrinin and pigments has a common precursor, and many studies have focused on citrinin and pigments (Hajjaj et al. 2015; Zhen et al. 2019; Liu et al. 2021). According to the concentration and the corresponding peak area of the citrinin standard, the regression equation and correlation coefficient were calculated as follows:

$$y = 29.804x - 2.786,\ R^2 = 0.9992 \quad (3)$$

The citrinin concentrations of the wild-type strain and T7 transformant at different time points are illustrated in Figure 4. On day 15, the citrinin concentrations of the wild-type strain and T7 transformant were 3.42 μg g^{-1} and 2.37 μg g^{-1}, respectively (Figure 4). Then, the citrinin concentrations of the wild-type strain and T7 transformant increased on days 18 and 21. However, the citrinin concentrations of T7 transformant were lower than those of the wild-type strain. Therefore, the low ACS expression level could inhibit citrinin synthesis.

Colour value. Colour value is one of the main quality indices of natural pigments, which can reflect the content of pigments. The colour values of the wild--type strain and T7 transformant at different time points are shown in Figure 5. The colour value of the wild-type strain did not increase significantly from days 9 to 15 (Figure 5). However, the colour value of T7 transformant was higher than that of the wild--type strain on days 9 and 12, respectively. On day 18, the colour value (17.88) of the wild-type strain markedly increased and reached 41.10 on day 21. This finding suggested that the wild-type strain began to produce pigments largely on day 18. However, the colour values of T7 transformant on days 18 and 21 did not increase significantly. Therefore, the low ACS expression

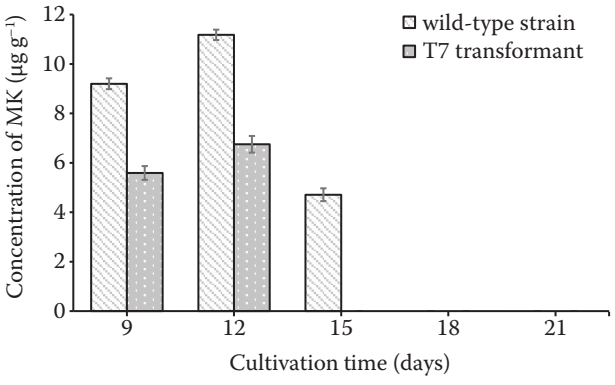

Figure 3. The monacoli K (MK) concentration of wild-type strain and T7 transformant in different time (mean ± SEM; n = 3)

SEM – scanning electron microscopy; the MK contents were assessed by high performance liquid chromatography (HPLC) and given in absolute values dividing by the dry weight of red yeast rice

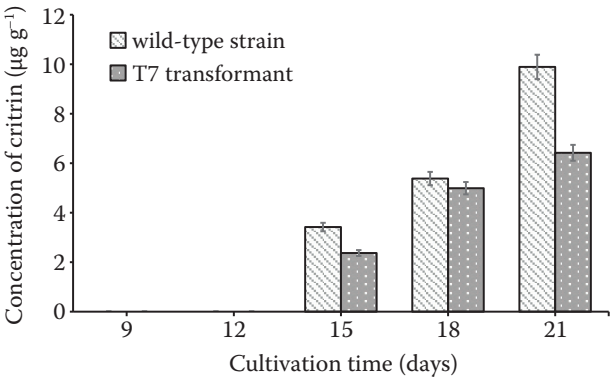

Figure 4. The citrinin concentration of wild-type strain and T7 transformant in different time (mean ± SEM; n = 3)

SEM – scanning electron microscopy; the citrinin contents were assessed by high performance liquid chromatography (HPLC)

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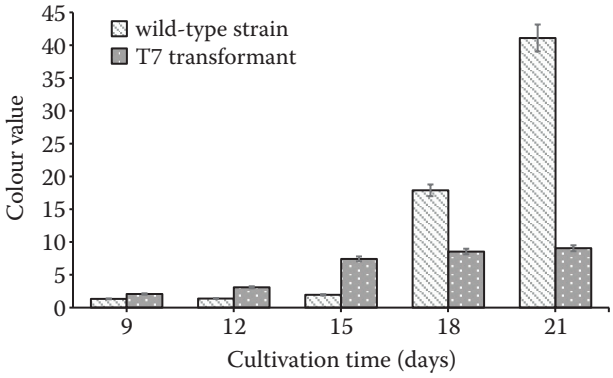

Figure 5. The colour values of wild-type strain and T7 transformant in different time (mean ± SEM; n = 3)

SEM – scanning electron microscopy; colour value was analysed by spectrophotometer at 505 nm; it can reflect the pigments content in wild-type strain and T7 transformant

level could inhibit the production of pigments. Hynes and Murray (2010) showed that cytosolic acetyl-CoA is an essential building block for the biosynthesis of fatty acids and numerous secondary metabolites. The low ACS expression level inhibited acetyl-CoA production in *Monascus*, resulting in slower growth and reducing the production of MK, citrinin, and pigment. However, ACS is not the branching site of MK and citrinin production.

CONCLUSION

In this study, the ACS expression was inhibited by RNAi. T7 transformant was selected for the follow-up analysis of the lowest ACS expression, which was 0.401 times higher than that of the wild strain. The colony of the wild-type strain grew on the culture on the 1st day. However, the colony of the T7 transformant grew on the culture on the 4th day. On the 6th day, the aerial hyphae of the wild-type strain could be observed, but the growth of aerial hyphae of T7 transformant was not obvious. The SEM of the morphological characteristics of the mycelia and spores revealed that the asexual conidia of the T7 transformant were significantly fewer and more shriveled than those of the wild-type strain. The concentrations of MK and citrinin were detected through HPLC, and colour values were measured with a spectrophotometer. The results showed that the MK and citrinin concentrations of the T7 transformant were lower than those of the wild-type strain during cultivation. The wild-type strain largely produced pigments on the 18th day. However, the colour values of the T7 transformant did not increase significantly on the 18th day and 21st day. The low ACS expression could lead to the low production of MK, citrinin, and pigment as the main secondary metabolites of *Monascus*. The low ACS expression also resulted in the slow growth of mycelia.

REFERENCES

Endo A. (1979): Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. Journal of Antibiotics, 32: 852–854.

Gu Q., Yuan Q., Zhao D., Huang J., Hsiang T., Wei Y., Zheng L. (2019): Acetyl-coenzyme A synthetase gene Chacs1 is essential for lipid metabolism, carbon utilization and virulence of the hemibiotrophic fungus *Colletotrichum higginsianum*. Molecular Plant Pathology, 20: 107–123.

Hajjaj H., Blanc P.J., Groussac E., Goma G., Loubiere P. (2015): Improvement of red pigment/citrinin production ratio as a function of environmental conditions by *Monascus ruber*. Biotechnology and Bioengineering, 64: 497–501.

Hajjaj H.A., Klaébé Loret M.O., Goma G., Blanc P.J., Francois J. (1999): Biosynthetic pathway of citrinin in the filamentous fungus *Monascus ruber* as revealed by 13c nuclear magnetic resonance. Applied and Environmental Microbiology, 65: 311–314.

Hynes M.J., Murray S.L. (2010): ATP-citrate lyase is required for the production of cytosolic acetyl-CoA and development in *Aspergillus nidulans*. Eukaryotic Cell, 9: 1039–1048.

Ji Y.K., Kim H.J., Oh J.H., Lee I. (2010): Characteristics of *Monascus* sp. isolated from *Monascus* fermentation products. Food Science and Biotechnology, 19: 1151–1157.

Liang G., Hong Z., Li T., Ming G.F., Duan X.W., Wang J.S., Jiang Y.M. (2019): Molecular signatures of cytotoxic effects in human embryonic kidney 293 cells treated with single and mixture of ochratoxin A and citrinin. Food and Chemical Toxicology, 123: 374–384.

Lin L., Wu S., Li Z., Ren Z., Wang C. (2018): High expression level of mok E enhances the production of monacolin K in *Monascus*. Food Biotechnology, 32: 35–46.

Liu H.Q., Huang Z.F., Yang S.Z., Tian X.F., Wu Z.Q. (2021): Inducing red pigment and inhibiting citrinin production by adding lanthanum(III) ion in *Monascus purpureus* fermentation. Applied Microbiology and Biotechnology, 105: 1905–1912.

Manzoni M., Rollini M. (2002): Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. Applied Microbiology and Biotechnology, 58: 555–564.

Mulder K.C., Mulinari F., Franco O.L., Soares M.S., Magalhães B.S., Parachin N.S. (2015): Lovastatin production:

https://doi.org/10.17221/4/2022-CJFS

From molecular basis to industrial process optimization. Biotechnology Advances, 33: 648–665.

Sengupta S., Bhattacharya S., Karmakar A., Ghosh S., Datta S.K. (2021): RNAi-mediated down-regulation of itpk-2 enhanced inorganic phosphorus and minerals in the transgenic rice. Journal of Biosciences, 46: 32–45.

Seraman S., Rajendran A., Thangavelu V. (2010): Statistical optimization of anticholesterolemic drug lovastatin production by the red mold *Monascus purpureus*. Food and Bioproducts Processing, 88: 266–276.

Son H., Lee J., Park A.R., Lee Y.W. (2011): ATP citrate lyase is required for normal sexual and asexual development in *Gibberella zeae*. Fungal Genetics and Biology, 48: 408–417.

Srianta I., Zubaidah E., Estiasih T., Iuchi Y., Harijono, Yamada M. (2017): Antioxidant activity of pigments derived from *Monascus purpureus*-fermented rice, corn, and sorghum. International Food Research Journal, 24: 1186–1191.

Srianta I., Ristiarini S., Nugerahani I. (2020): Pigments extraction from *Monascus*-fermented durian seed. IOP Conference Series Earth and Environmental Science, 443: 1–7.

Su N.W., Lin Y.L., Lee M.H., Ho C.Y. (2005): Ankaflavin from *Monascus* fermented red rice exhibits selective cytotoxic effect and induces cell death on Hep G2 cells. Journal of Agricultural and Food Chemistry, 53: 1949–1954.

Wang L., Wang W., Xu G. (2011): Promotion of monacolin K production by *Agrobacterium tumefaciens*-mediated transformation in *Monascus albidus* 9901. Current Microbiology, 62: 501–507.

Yang C.W., Mousa S.A. (2012): The effect of red yeast rice (*Monascus purpureus*) in dyslipidemia and other disorders. Complement Therapies in Medicine, 20: 466–474.

Zhen Z., Xiong X., Liu Y., Zhang J., Wang S., Li L., Gao M. (2019): NaCl inhibits citrinin and stimulates *Monascus* pigments and monacolin K production. Toxins, 11: 118–128.

Received: January 9, 2022
Accepted: September 26, 2022
Published online: November 24, 2022