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ORIGINAL ARTICLE

The acyl-CoA binding protein affects *Monascus* pigment production in *Monascus ruber* CICC41233

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Abstract

The present study verified whether acyl-coenzyme A (acyl-CoA)-binding protein (ACBP) affected the production of *Monascus* pigments (MPs) in *Monascus ruber* CICC41233 (MrACBP). Phylogenetic analysis revealed that the cloned *Mracbp* gene, which encoded the MrACBP protein, exhibited the closest match (99% confidence level) to the gene from *Penicilliopsis zonata*. The MrACBP and maltose-binding protein (MBP) were simultaneously expressed in *Escherichia coli* Rosetta DE3 in the form of a fusion protein. The microscale thermophoresis binding assay revealed that the purified MBP–MrACBP exhibited a higher affinity for myristoyl-CoA (Kd = 88.16 nM) than for palmitoyl-CoA (Kd = 136.07 nM) and octanoyl-CoA (Kd = 270.9 nM). Further, the *Mracbp* gene was homologously overexpressed in *M. ruber* CICC41233, and a positive transformant *M. ruber* ACBP5 was isolated. The fatty acid myristic acid in *M. ruber* ACBP5 was lower than that in the parent strain *M. ruber* CICC41233. However, when compared with the parent strain, the production of total MPs, water-soluble pigment, and ethanol-soluble pigment in *M. ruber* ACBP5 increased by 11.67, 9.80, and 12.70%, respectively, after 6 days. The relative gene expression level, as determined by a quantitative real-time polymerase chain reaction analysis, of the key genes *acbp*, *pks*, *mppr1*, *fasA*, and *fasB* increased by 4.03-, 3.58-, 1.67-, 2.11-, and 2.62-fold after 6 days. These data demonstrate the binding preference of MrACBP for myristoyl-CoA, and its influence on MPs production.

Keywords Acyl-coenzyme A binding protein · *Monascus* pigments · *Monascus ruber* · Microscale thermophoresis binding assay · Myristoyl-CoA

Introduction

For over 1000 years, *Monascus* species have been used to ferment food in Asia, especially in China (Kalaivani et al. 2010; Patakova 2013; Chen et al. 2015). Further, *Monascus* pigments (MPs) have many functions, including anti-inflammatory, anti-atherosclerotic, anti-diabetic, and anti-cancer activities; they are also used as a food-coloring agent (Feng et al. 2012). The MPs biosynthesis in *Monascus* spp. is via polyketide and fatty acid pathways (Hajjaj et al. 2000). Acyl-coenzyme A (Acyl-CoA), the precursor of polyketide, is catalyzed by polyketide synthase (PKS) and fatty acid is catalyzed by fatty acid synthase (FAS) (Hajjaj et al. 2000). Recently, a putative gene cluster involved in the biosynthesis of MPs has been identified in *Monascus purpureus* (Balakrishnan et al. 2013) and *M. ruber* M7 (Feng et al. 2012; Chen et al. 2015). The gene cluster comprises core elements, such as PKS, FAS, dehydrogenase, transporter, regulator, and so on. Balakrishnan et al. (2014) reported that targeted inactivation of the gene *MpfasB2*, encoding the beta subunit of FAS, inhibited the biosynthesis of *Monascus* pigment; however, it promoted the accumulation of chromophoric compounds. Wang et al. (2015) reported a positive correlation between intracellular pigment production and lipid accumulation in *Monascus anka*.

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Acyl-coenzyme A binding protein (ACBP) is widespread in mammals, fungi, yeast, and bacteria (Burton et al. 2005; Færgeman et al. 2007). The protein, about 10 kDa in size, is highly conserved. It binds to medium- and long-chain saturated and unsaturated fatty acid acyl-CoA esters, and exhibits low or no affinity for free fatty acids and CoA (Frolov and Schroeder 1998; Burton et al. 2005; Færgeman et al. 2007; Xiao and Chye 2011). Many in vitro studies have revealed that ACBP is involved in the transport of intracellular acyl-CoA and formation of acyl-CoA ester pool, thus demonstrating the involvement of ACBP in intracellular lipid transport and metabolism (Knudsen et al. 1994; Færgeman et al. 2004; Xiao and Chye 2011; Yao et al. 2016).

From the *M. ruber* NRRL1597 genome database (http://genome.jgi.doe.gov/Monru1/Monru1.home.html), the key words "Acyl-coenzyme A binding protein" were used to search, and an *acbp* gene (Transcript Id 431019, designated *Mracbp*) encoding ACBP protein (Protein ID 430884, designated MrACBP) was obtained. However, there were no reports of ACBP in *Monascus* spp. Therefore, it was interesting to identify the biochemical function of ACBP in *M. ruber*. In the present study, the gene *Mracbp* was expressed in *E. coli* to test the affinity of acyl-CoA esters, and overexpressed in *M. ruber* CICC41233 via *Agrobacterium tumefaciens*-mediated transformation (ATMT) to analyze the production of MPs.

Materials and methods

Strains and fermentation culture

Monascus ruber CICC41233 was procured from the China Center of Industrial Culture Collection and cultured on malt–peptone–starch (MPS) agar (10 g/L malt extract, 10 g/L peptone, 40 g/L soluble starch, and 2 g/L agar) at 30 °C for 7 days. *Escherichia coli* DH5a was used to construct vector and amplify plasmid, which cultivated in Luria–Bertani medium with antibiotics according to conditions for plasmid selection. *Escherichia coli* Rosetta DE3 was used to express target protein. *Agrobacterium tumefaciens* EHA105 was used for *M. ruber transformation*.

Monascus pigment fermentation experiments were conducted in 250 mL Erlenmeyer flasks containing 50 mL of medium (9.0% rice powder, 0.2% sodium nitrate, 0.1% potassium dihydrogen phosphate, 0.2% magnesium sulfate heptahydrate, and 0.2% acetate, pH 3.2) in triplicates. The flasks were examined at 48, 72, 96, 120, and 144 h. After 7-day cultivation, the spores of *M. ruber* were obtained (a small amount of glass beads and sterile water were added to the centrifuge tube, the mycelium was scraped in the centrifuge tube, and then oscillated and filtrated through lens wiping paper). The final concentration of freshly harvested spores used for inoculation was 10^5 conidia/mL, which was analyzed by counter technique with blood count plate. The flasks were shaken at 30 °C at a rotation speed of 180 rpm.

Construction of gene expression vectors

The cDNA of *Mracbp* gene (450 bp, MONRU_430884 of *M. ruber* NRRL1597 genome database) was cloned in *M. ruber* CICC41233 using cDNA as a template via polymerase chain reaction (PCR) using the primers ACBP-F-*Xba*I and ACBP-R-*Hin*dIII (Supplementary Table S1), and sequenced by Beijing Genomics Institute (BGI) (China). The gene fragment and pMAL-c4x (New England Biolabs, USA) were digested by *Xba*I and *Hin*dIII, and then ligated to construct the expression vector pMAL-MrACBP.

To overexpress *Mracbp* in *M. ruber* CICC41233 for MPs production, it was cloned using DNA as a template by PCR using the primers ACBP-*Hin*dIII-F and ACBP-*Sac*I-R (Supplementary Table S1), and then sequenced by BGI (China). The gene fragment and pNeo0380 (Fig. 1c) were digested by *Hin*dIII and *Sac*I, and then ligated to construct the binary expression vector pNeo0380-MrACBP.

Phylogenetic analyses

Initially, the amino-acid sequence of MrACBP was analyzed by the protein Basic Local Alignment Search Tool (BLAST) on National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/) to obtain homologous sequences. These sequences were aligned using CLUSTALX, and then constructed a phylogenetic tree by Neighbor-Joining method with MEGA 4.

Expression and purification of MrACBP protein

The vector pMAL-MrACBP was transformed in *E. coli* Rosetta DE3. One of the transformants was picked and inoculated into 10 mL Luria–Bertani broth (containing 100 µg/mL ampicillin and 34 µg/mL chloramphenicol). After overnight cultivation (37 °C with shaking), 4 mL of the culture was inoculated into 400 mL of LB broth (containing 100 µg/mL ampicillin and 34 µg/mL chloramphenicol), and then cultured at 25 °C with shaking. When optical density at 610 nm (OD_{610}) of the sample reached 0.5, isopropyl β-D-1-thiogalactopyranoside (IPTG) (final concentration of 0.5 mM) as an inducer was added and incubated for 16 h at 16 °C.

The cells containing the target protein in LB broth were harvested by centrifugation at 8000×*g* for 20 min. The precipitate of the harvested cells was resuspended in binding buffer (20 mM Tris-hydrochloride, 200 mM sodium chloride, 1 mM ethylenediaminetetraacetic acid, pH 7.4). The harvested cells were purified to obtain the target protein

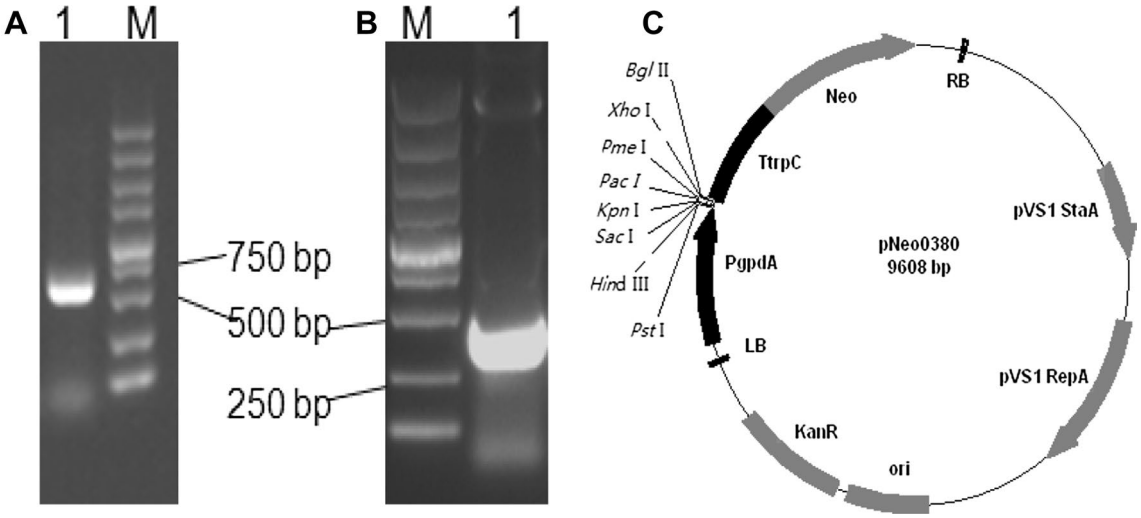

Fig. 1 Polymerase chain reaction (PCR) amplification of the gene *Mracbp* and the schematic map of pNeo0380. (**a** *Mracbp* gene from DNA fragment; **b** *Mracbp* gene from cDNA fragment; **c** pNeo0380)

(MBP–MrACBP fusion protein) according to the previous method (Hao et al. 2016). The target protein was stored at − 80 °C for further analysis.

The protein concentration was determined using a Bradford protein assay kit (Sangon Biotech Co. Ltd., Shanghai, China). The protein was denatured by incubating in boiling water for 10 min and directly analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% polyacrylamide gel stained with Coomassie brilliant blue.

Affinity testing of MrACBP by microscale thermophoresis binding assay

The affinity of MrACBP and fatty acid acyl-CoA esters (from C4 to C22) was measured by the method of microscale thermophoresis (MST) with a NanoTemper monolith NT.115 (CA, USA). Test ligands at a wide concentration range (62.5–0.0153 μM) were, respectively, incubated with the purified target protein (72 nM) for 5 min. These samples were loaded into the NanoTemper glass capillaries. The microthermophoresis conditions included a light emitting diode (LED) power of 20% and a microscale thermophoresis power of 40%. The Kd value was calculated using the mass action equation in the NanoTemper software from duplicate reads of an experiment (Jerabek-Willemsen et al. 2011; Parker and Newstead 2014).

Screening positive transformants

Agrobacterium tumefaciens EHA105, containing a binary vector (pNeo0380-MrACBP), was introduced into *M. ruber* CICC41233 via ATMT with G418 (80 μg/mL) as a screening marker (Balakrishnan et al. 2013). The transformants were picked from the MPS agar (containing G418) plate and then cultured thrice on MPS agar plates without G418. Single strains were transferred to MPS plates containing G418 to determine the stability. Total genomic DNA was extracted from the mycelia by the method of Balakrishnan et al. (2017). Positive transformants were identified by PCR amplification using the primers PgpdA-OE-F and TtrpC-OE-R.

Analysis of *Monascus* pigment production

After fermentation, 25 mL of culture broth was centrifuged at 16 °C for 30 min at 9000×*g*. The supernatant was the extracellular pigments (water-soluble pigment). The precipitate was resuspended in 25 mL of 70% (v/v) ethanol and incubated at 60 °C for 1 h with shaking at 90×*g*, and then centrifuged at 16 °C for 15 min at 9000×*g*. The supernatant contained the intracellular pigment (ethanol-soluble pigment). The residual mycelial precipitate was dried to a constant weight at 60 °C to determine its biomass.

The absorbance spectrum of the pigment sample was adjusted to 0.1–1.0 using a spectrophotometer at 510 nm. The result was expressed in units of absorbance (U) at a given wavelength, multiplied by the dilution factor (Lopes et al. 2013; Shi et al. 2015). The total MPs comprised water-soluble and ethanol-soluble pigments.

Transcription analysis

Approximately, 20 mL of the remaining broth was taken from the flask and centrifuged at 4 °C for 30 min at 9000×*g*. The precipitate was frozen immediately with liquid nitrogen

to extract RNA using RNAiso Plus reagent (TaKaRa, Dalian, China). The quality of total RNA was evaluated by electrophoresis and was measured by a spectrophotometer (Nanodrop 2000; Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription was performed using the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China). Relative expression was determined using 10.0 µL of SYBR® *Premix Ex Taq*™ (Tli RNaseH Plus) (TaKaRa, Dalian, China), 1.0 µL of 10.0 µM forward primer, 1.0 µL of 10.0 µM reverse primer, 6 µL of water, and 2.0 µL of template cDNA (tenfold dilution). Thermal cycling conditions comprised an initial denaturation at 95 °C for 3 min, followed by 40 amplification cycles at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s with a final extension step at 72 °C for 5 min using a real-time PCR system (CFX Connect; Bio-Rad, Hercules, CA, USA). The gene *GAPDH* was used as an endogenous control gene. The gene *acbp* encodes acyl-CoA binding protein. *pks*, *mppr1*, *fasA*, and *fasB* are the key genes in the biosynthetic gene cluster of MPs coding polyketide synthase, positive regulatory factor, fatty acid synthase alpha, and beta subunits, respectively (Feng et al. 2012; Balakrishnan et al. 2013; Chen et al. 2015). The primers used have been listed in Supplementary Table S1.

Fatty acid analysis

Extraction of total lipids and estimation of the percentage of individual lipids were performed according to the protocol of Wu et al. (2011), which was conducted by GuangZhou Chemical Union Quality Testing Technology Co., Ltd (China).

Results

Cloning of *Mracbp* gene and phylogenetic tree analysis of MrACBP protein

A full-length *Mracbp* DNA and cDNA fragments were cloned by PCR (Fig. 1a, b) and sequenced by BGI. The sequence *Mracbp* DNA and cDNA showed the presence of two introns located at positions 265–341 and 411–512 bp. The deduced 149 amino-acid sequence of MrACBP was analyzed by the protein BLAST on NCBI to obtain homologous sequences (Supplementary Sequence S1). These sequences were used for the phylogenetic analysis, which has been presented in Fig. 2. The phylogenetic analysis revealed that the MrACBP from *M. ruber* CICC41233 was the closest match (99% confidence level) to *Penicilliopsis zonata* CBS 506.65. The *Mracbp* sequence (MG520638) has been deposited in the GenBank database.

Heterologous expression and purification of MrACBP protein

In the LB broth without IPTG, MrACBP was not expressed in *E. coli* Rosetta DE3 (Fig. 3, lane 1). However, with the addition of IPTG as an inducer, MrACBP was highly expressed (Fig. 3, lane 2). The crude protein after ultrasonication has been represented in Fig. 3, lane 3. The predicted molecular weight of MrACBP was 16.38 kDa and the molecular weight of MBP-tag was 42.0 kDa. Thus, the purified fusion protein MBP–MrACBP was approximately 60.0 kDa. This was consistent with the expected size by SDS-PAGE analysis (Fig. 3, lane 5).

The *MrACBP* showed binding preference for myristoyl-CoA

The acyl-CoA esters from C4 to C22 were tested for affinity with the purified MBP–MrACBP fusion protein using MST (Supplementary Figure S1). As shown in Fig. 4, the Kd value of MBP–MrACBP for myristoyl-CoA (C14-CoA) was 88.16 nM, which was the lowest value among the acyl-CoA esters. The MrACBP clearly showed a binding preference for myristoyl-CoA. Furthermore, it exhibited a good binding preference for esters from C12-CoA to C22-CoA, which was better than that for esters from C4-CoA to C10-CoA. Thus, it was concluded that MrACBP from *M. ruber* CICC41233 was a long-chain acyl-CoA binding protein.

Comparison of *Monascus* pigments production in parent strain and *Monascus ruber* ACBP5

The gene *Mracbp* was overexpressed in the parent strain *M. ruber* CICC41233 via ATMT, and nine transformants were isolated. After PCR detection, three transformants were tested positive. They all promoted MPs production. Further, one of the transformants *M. ruber* ACBP5 was selected and used for further analysis.

As shown in Fig. 5a, the production of MPs in *M. ruber* CICC41233 and *M. ruber* ACBP5 was enhanced with increment of incubation time. Although the difference between the two strains was low, *M. ruber* ACBP5 always produced higher MPs than the parent strain, except for 5 days. Compared with the parent strain, the total MPs, water soluble pigment, and ethanol soluble pigment production in *M. ruber* ACBP5 increased by 11.67, 9.80, and 12.70%, respectively, after 6 days. The biomasses of both the strains are shown in Fig. 5b. After 6 days of cultivation, the biomass of *M. ruber* ACBP5 was greater than that of the parent strain *M. ruber* CICC41233.

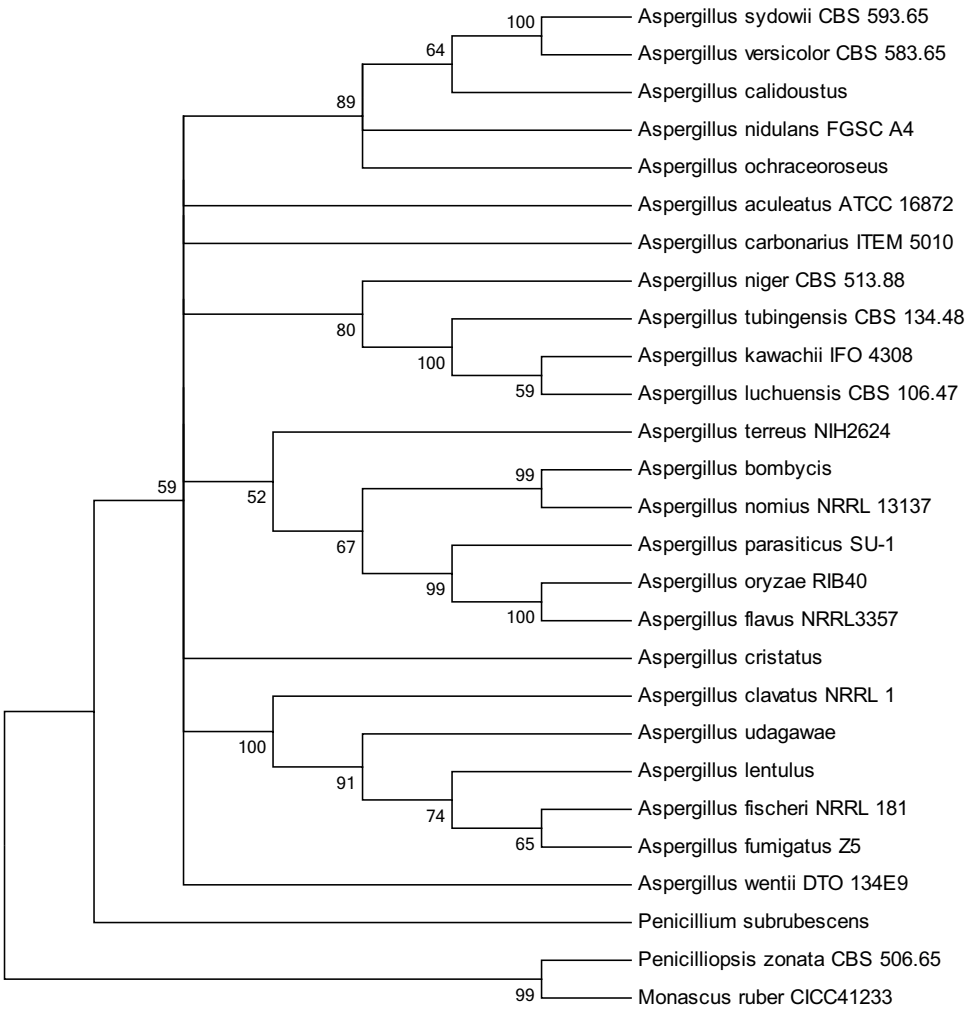

Fig. 2 Phylogeny analysis of MrACBP protein

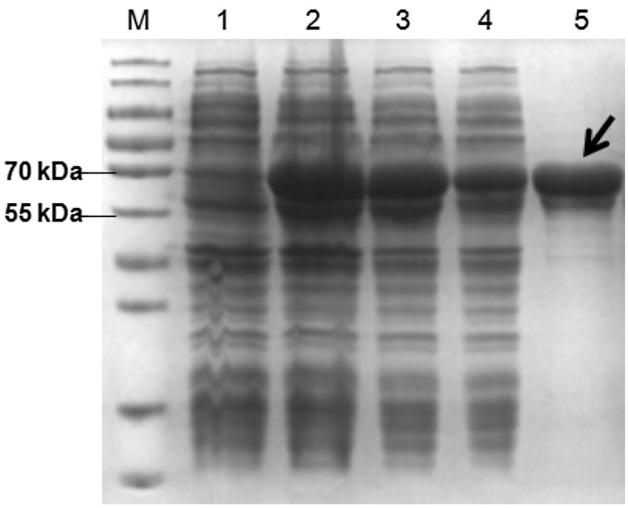

Fig. 3 SDS-PAGE analysis of MBP–MrACBP protein. (1. No IPTG inducer; 2. IPTG inducer; 3. The supernatant of cells broken by ultrasonication; 4. Purified target protein)

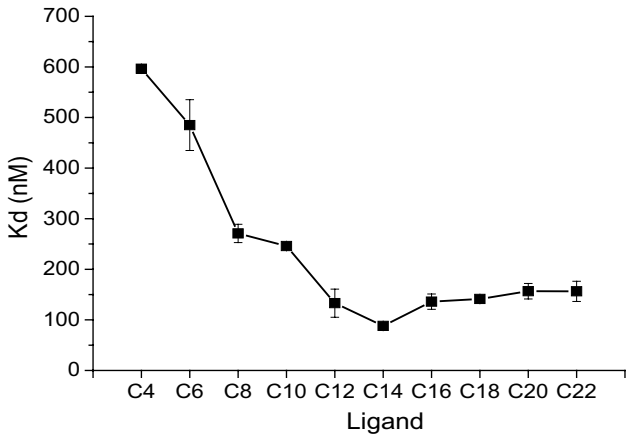

Fig. 4 The affinity of Kd value between MBP–MrACBP and fatty acyl-CoA tested by MST. (Error bars represent SD from duplicate replicates)

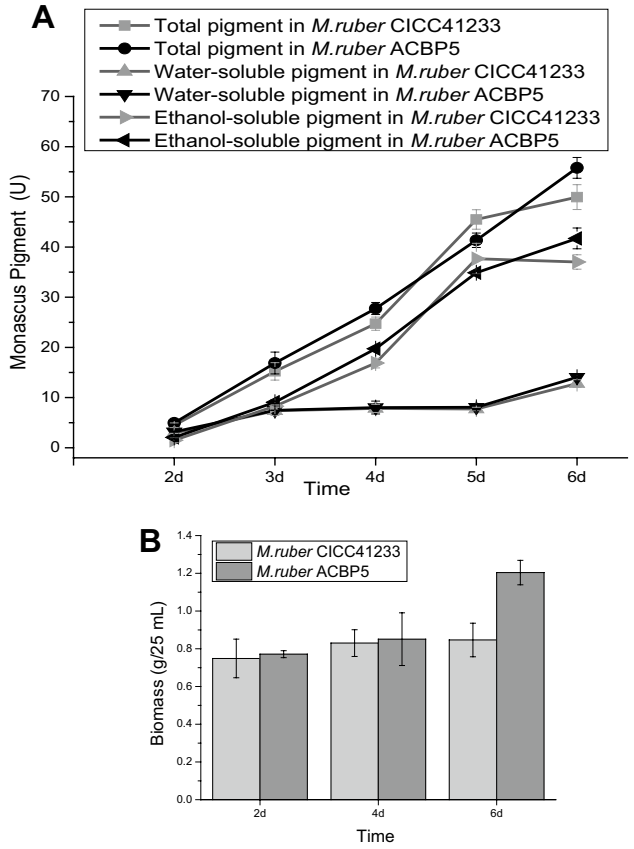

Fig. 5 *Monascus* pigment production (**a**) and biomass (**b**) of *M. ruber* CICC41233 and *M. ruber* ACBP5. (Error bars represent SD from three replicates)

Analysis of fatty acid composition

The major fatty acid components of *M. ruber* were myristic acid (C14:0), palmitic acid (C16:0), stearic acid C18:0, oleic acid (C18:1n9), and linoleic acid (C18:2n6) (Table 1). Compared with 48 h, the content of fatty acid C14:0 and C18:2n6 decreased in *M. ruber* Amy9 and *M. ruber* ACBP5 after 120 h, while the content of fatty acid C16:0 and C18:0 increased in *M. ruber* Amy9 and *M. ruber* ACBP5 after 120 h. There was no significant difference in the content of fatty acid C18:1n9 in both the strains. However, the content of fatty acid C14:0 in *M. ruber* ACBP5 was lower than that in *M. ruber* CICC41233 not only for 48 h but also for 120 h.

Increased expression level of key genes correlates with increased *Monascus* pigments production in *M. ruber* ACBP5

The expression level of genes has been presented in Fig. 6. The expression of the gene *acbp* was up-regulated in *M. ruber* ACBP5, which showed 77.39- and 4.03-fold increase in expression than that of the parent strain after 2 and 6 days, respectively. In comparison with the parent strain *M. ruber* CICC41233, the expression of key genes *pks*, *mppr1*, *fasA*, and *fasB* in the biosynthetic gene cluster of *Monascus* pigments production in *M. ruber* ACBP5 increased by 6.41-, 3.21-, 3.42-, and 4.41-fold, respectively, after 2 days, and by 3.58-, 1.67-, 2.11-, and 2.62-fold, respectively, after 6 days.

Table 1 Cellular fatty acid composition of *Monascus ruber*

Strain				
Fatty acid	*M. ruber* CICC41233 48 h (%)	*M. ruber* ACBP5 48 h (%)	*M. ruber* CICC41233 120 h (%)	*M. ruber* ACBP5 120 h (%)
14:0	12.06	1.63	0.61	0.52
15:0	0.08	0.08	0.22	0.19
16:0	23.98	26.76	36.29	35.17
16:1n9	0.08	0.07	0.66	0.46
16:1n7	0.27	0.31	ND	ND
17:0	0.11	0.13	0.31	0.53
18:0	4.56	5.25	16.78	19.65
18:1n7	0.48	0.39	ND	ND
18:1n9	17.07	20.08	17.89	19.01
18:2n6	39.95	43.49	25.26	23.35
18:3n3	0.65	0.64	0.44	0.18
20:0	0.15	0.15	0.11	0.12
20:1n9	0.12	0.60	ND	ND
24:0	0.31	0.42	ND	ND
Others	0.13	0	1.43	0.82

ND not detected

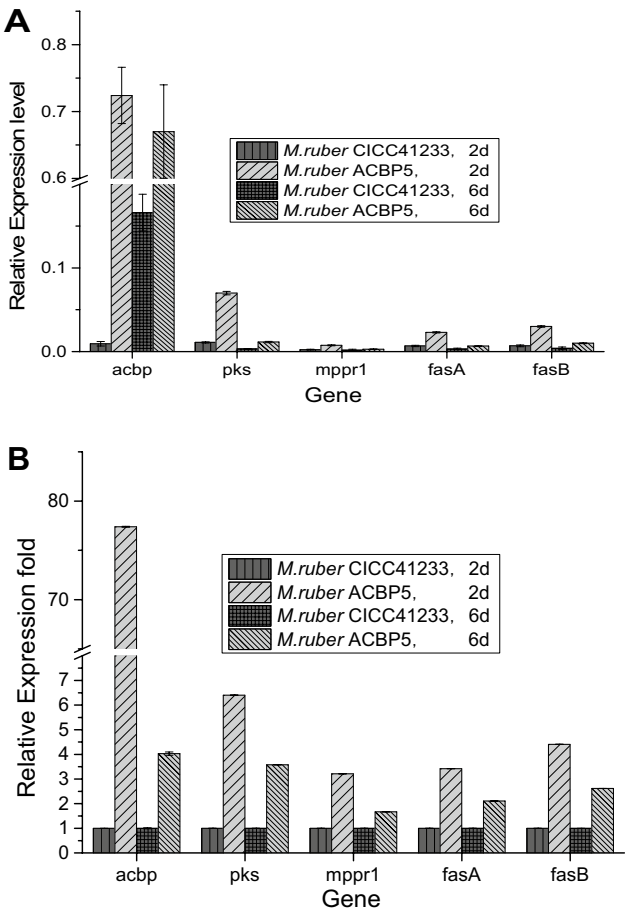

Fig. 6 Gene expression analysis of *M. ruber* CICC41233 and *M. ruber* ACBP5. (**a** Relative expression level of the genes *acbp*, *pks*, *mppr1*, *fasA*, and *fasB* in *M. ruber* CICC41233 and *M. ruber* ACBP5; **b** relative expression fold increase in the expression of the genes *acbp*, *pks*, *mppr1*, *fasA*, and *fasB* in *M. ruber* ACBP5; Error bars represent SD from three replicates)

Discussion

The biosynthesis and metabolism of fatty acids in *Monascus* spp. are important for *Monascus* pigments production (Hajjaj et al. 2000; Wang et al. 2015). In the present study, the deduced MrACBP according to the cDNA sequence of the gene *Mracbp* in *M. ruber* CICC41233 was the closest match to the ACBP of *P. zonata* CBS 506.65 (synonymy *Aspergillus zonatus*) (Kocsubé et al. 2016). However, the function of ACBP from *P. zonata* was not clear. In the present study, the MST assay revealed that MBP–MrACBP exhibited a binding preference for C14:0-CoA. Furthermore, it showed a binding preference for myristoyl-CoA (Kd = 88.16 nM) over palmitoyl-CoA (Kd = 136.07 nM). Similar results have been demonstrated in *Plasmodium falciparum* (van Aalten et al. 2001). However, it was different from the AoACBP of *A. oryzae* 3.042, which showed higher binding affinity for palmitoyl-CoA than for myristoyl-CoA (Hao et al. 2016). Further, in the present study, MBP–MrACBP exhibited a binding preference for myristoyl-CoA (Kd = 88.16 nM) over octanoyl-CoA (Kd = 270.9 nM).

Although the major fatty acid components of *M. ruber* were C14:0, C16:0, C18:0, C18:1n9, and C18:2n6, the gene *Mracbp* was overexpressed in the parent strain. Therefore, the content of fatty acid C14:0 in *M. ruber* ACBP5 was lower than that in the parent strain *M. ruber* CICC41233. However, the content of fatty acid C16:0, C18:0, C18:1n9, and C18:2n6 in *M. ruber* ACBP5 was higher than that in *M. ruber* CICC41233 after 48 h (Table 1). This indicated that ACBP was responsible for the transport of intracellular acyl-CoA and formation of acyl-CoA ester pool (Knudsen et al. 1994; Færgeman et al. 2004; Xiao and Chye 2011; Yao et al. 2016). The MST analysis revealed that the binding preference of MrACBP for long-chain acyl-CoA, especially for myristoyl-CoA.

The gene *Mracbp* was overexpressed in the parent strain, whereas there was a little increase in the *Monascus* pigment production in *M. ruber* ACBP5, except for 5 days (Fig. 5a). Although at 5 days, the amount of total MPs and ethanol-soluble pigments in *M. ruber* CICC41233 were more than that *M. ruber* ACBP5, it was like an inflection point. The ethanol-soluble pigments in *M. ruber* CICC41233 were on the plateau. However, the ethanol-soluble pigments in *M. ruber* ACBP5 were increasing. We speculated that it was related to biomass. From the Fig. 5b., there was no significant increase in biomass for *M. ruber* CICC41233. But for *M. ruber* ACBP5, there was still a lot of growth from 4 to 6 days. Duran et al. (2010) reported that the *acb1* (encoding acyl-CoA binding protein) gene mutant strain of *Saccharomyces cerevisiae* exhibited retarded growth and produced less spores. Kwon et al. (2017) reported that the Aoacb2 encoding acyl-CoA binding protein from *Aspergillus oryzae* was an essential gene for growth. The MrACBP from *M. ruber* CICC41233 showed 32.0% homology with *acb1* protein and 40.5% homology with AoAcb2 protein. Therefore, this meant that when the *Mracbp* was overexpressed in *M. ruber* CICC41233, there was an increase of biomasses in *M. ruber* ACBP5.

Knudsen et al. (1994) reported that the overexpression of yeast ACBP in *S. cerevisiae* resulted in an expansion of the intracellular acyl-CoA pool. Furthermore, in the present study, overexpression of the gene *Mracbp* in *M. ruber* resulted in the expansion of the intracellular acyl-CoA pool. Acyl-CoA is the precursor of polyketide and fatty acid in the synthesis of MPs in *Monascus* spp. Further, PKS and FAS have been reported to play a role in MPs biosynthesis (Hajjaj et al. 2000). The data on relative gene expression level analysis showed that the expression of the key genes *pks*, *mppr1*, *fasA*, and *fasB* increased in *M. ruber* ACBP5 in comparison with that of the parent strain *M. ruber* CICC41233 (Fig. 6). The genes *fasA* and *fasB* encoding FAS were involved in the

MPs biosynthetic gene cluster in *M. purpureus* and *M. ruber* (Feng et al. 2012; Chen et al. 2015). Presently, to the best of our knowledge, there are no reports on the function of the gene *fasA* in the MPs biosynthetic gene cluster. However, the gene *fasB*—encoding short-chain fatty acid synthase—in *M. purpureus* supplied short-chain (C8 and C10) fatty acyl moieties and modified azaphilone polyketides during pigment biosynthesis. However, it has not been determined whether the octanoic acid methyl esters are derived from the product of MpFAS2 (*fasB*) (Balakrishnan et al. 2014). Hajjaj et al. (2000) reported that the medium-chain fatty acids, such as octanoic acid, when supplemented to the medium showed a 30–50% increase in the production of red pigment. However, the addition of hexanoic acid decreased the production of red pigment. Furthermore, addition of fatty acids, such as decanoic acid, dodecanoic acid, myristic acid, stearic acid, and oleic acid, did not affect the production of red pigment. This indicates that expansion of the intracellular acyl-CoA pool in *M. ruber* ACBP5 might enhance the syntheses of octanoic acid methyl esters and ecanoyl acid methyl esters and promote the production of MPs.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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