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MINI-REVIEW

Monascus pigments

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Abstract *Monascus* pigments (MPs) as natural food colorants have been widely utilized in food industries in the world, especially in China and Japan. Moreover, MPs possess a range of biological activities, such as anti-mutagenic and anticancer properties, antimicrobial activities, potential anti-obesity activities, and so on. So, in the past two decades, more and more attention has been paid to MPs. Up to now, more than 50 MPs have been identified and studied. However, there have been some reviews about red fermented rice and the secondary metabolites produced by *Monascus*, but no monograph or review of MPs has been published. This review covers the categories and structures, biosynthetic pathway, production, properties, detection methods, functions, and molecular biology of MPs.

Keywords *Monascus* · Secondary metabolite · Pigment · Chemical structure · Molecular biology

Introduction

The genus of *Monascus* was nominated by van Tieghem in 1884 (Stchigel et al. 2004), but its fermented product of rice, named red fermented rice (RFR), also called red yeast rice, hon-chi, hongqu, red mold rice, red Chinese rice, red koji, anka, and so on, has been used as folk medicines, food colorants, and fermentation starters for more than 1,000 years in China (Li et al. 2010b; Shi and Pan 2011). Nowadays, it is proved that *Monascus* spp. can synthesize many secondary metabolites, such as *Monascus* pigments (MPs), monacolins, γ-aminobutyric acid, dimerumic acid, and so on (Chen and Hu 2005; Cheng et al. 2010; Hong et al. 2012; Jůzlová et al. 1996; Knecht et al. 2006; Liu et al. 2011; Wild et al. 2002).

MPs as natural food colorants have been widely utilized in food industries in the world, especially in China, Japan, and other Southeastern Asian countries (Dufossé et al. 2005). Moreover, MPs possess a range of biological activities, such as anti-mutagenic and anticancer properties (Akihisa et al. 2005a; Izawa et al. 1997; Su et al. 2005), antimicrobial activities (Kim et al. 2006b; Martínková et al. 1995, 1999), and potential anti-obesity characteristics (Kim et al. 2007a, b), and they could even be used for dyeing cotton yarn (Velmurugan et al. 2010b) and leather (Velmurugan et al. 2010a), sensitizing solar cells (Ito et al. 2010; Sang-aroon et al. 2012), and preparing gels (Calvo and Salvador 2002).

However, as we know, no review of MPs has been published although there have been a few reviews with regard to RFR and its production techniques (Hsu and Pan 2012; Lee and Pan 2012; Li et al. 2011; Lin et al. 2008; Shi and Pan 2011). In this review, the categories and structures, biosynthetic pathway, fermentation processes, physicochemical properties, detection methods, functions, and molecular biology of MPs will be reviewed.

Categories and structures of *Monascus* pigments

Monascus pigments (MPs) are an azaphilone mixture, which usually include yellow, orange, and red, total three kinds of constituents, even sometimes the culture conditions of *Monascus* spp. might have an impact on MPs constitutes (Domínguez-Espinosa and Webb 2003; Lin and Demain 1991; Yongsmith et al. 1993). With regard to the studies on MPs structures, it might be dated back to 1932 (Salomon and

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Karrer 1932), and a lot of studies had been done from the late 1950s to the early 1970s (Chen et al. 1969, 1971; Fielding et al. 1961; Haws et al. 1959; Kumasaki et al. 1962; Manchand and Whalley 1973). Up until 1973, six MPs compounds (Fig. 1 I a–f) including two yellow ones, monascin (Chen et al. 1969; Salomon and Karrer 1932) and ankaflavin (Manchand and Whalley 1973); two orange ones, rubropunctatin (Chen et al. 1969) and monascorubrin (Manchand and Whalley 1973); and two red ones, rubropunctamine and monascorubramine (Kumasaki et al. 1962; Sweeny et al. 1981) were identified, which are well-known as MPs fundamental compound types (Jůzlová et al. 1996; Lin et al. 1992; Pattanagul et al. 2007). Nowadays more and more attention has been paid on MPs compounds due to their various biological activities (Izawa et al. 1997; Kim et al. 2007a; Martínková et al. 1995; Velmurugan et al. 2010b). Up to June 2012, total 54 MPs compounds including two yellow compounds which had no structure shown (Zheng et al. 2009) had been reported.

Yellow constituents

Besides monascin and ankaflavin, many other yellow MPs ones have been isolated, purified, and identified from the fermented products by *Monascus* spp. In 1992, two yellow components with furanoisophthalide skeleton, xanthomonasin A and xanthomonasin B, were obtained from *Monascus anka* U-1 by Sato et al. (1992). In 1993, Yongsmith et al. (1993) purified a yellow compound Yellow II from the cassava medium of *Monascus* sp. KB 10, which had a monascin–ankaflavin–monascorubrin skeleton. And in 1996, six new yellow coumarin derivatives named monankarins A–F were isolated from culture media of *M. anka* (Hossain et al. 1996).

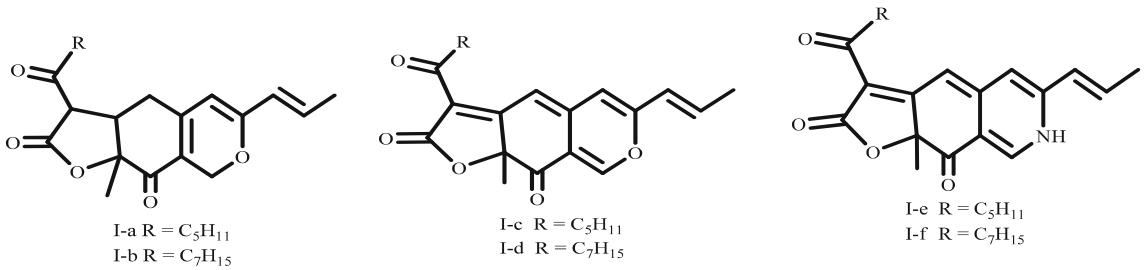
In the past decade, a total of 12 yellow MPs compounds have been investigated. In 2004, two yellow ones, monascusones A and B, together with monascin and FK17-P2B2 were achieved from the CH_2Cl_2 extract of RFR by a yellow mutant of *Monascus kaoliang* (Jongrungruangchok et al. 2004). In 2006, a yellow compound, Y3, was identified from RFR by *Monascus purpureus* IB1 (Campoy et al. 2006). In 2009, yellow-1 and yellow-2 with 356 and 384 molecular weights, respectively, were reported, but no molecular structure was shown (Zheng et al. 2009). In 2010, three yellow MPs derivatives, named monaphilones A, B, and C, were purified from RFR by *M. purpureus* NTU 568 (Hsu et al. 2010), and monapurones A, B, and C, were achieved from *M. purpureus*-fermented rice (Li et al. 2010a). At the same year, two pale yellow metabolites, named monarubrin and rubropunctin, were separated from commercially available Chinese RFR, which were supposed as the biodegraded intermediates of monascorubrin and rubropunctatin (Loret and Morel 2010). The chemical structures of MPs yellow constituents identified are presented in Fig. 1 II a–u.

Orange constituents

Investigation related to the orange compounds produced by *Monascus* spp. is less than yellow or red ones of MPs. Besides rubropunctatin and monascorubrin, only four orange MPs compounds, monapilol A–D (Fig. 1 III a–d), were purified and identified in 2011 (Hsu et al. 2011b).

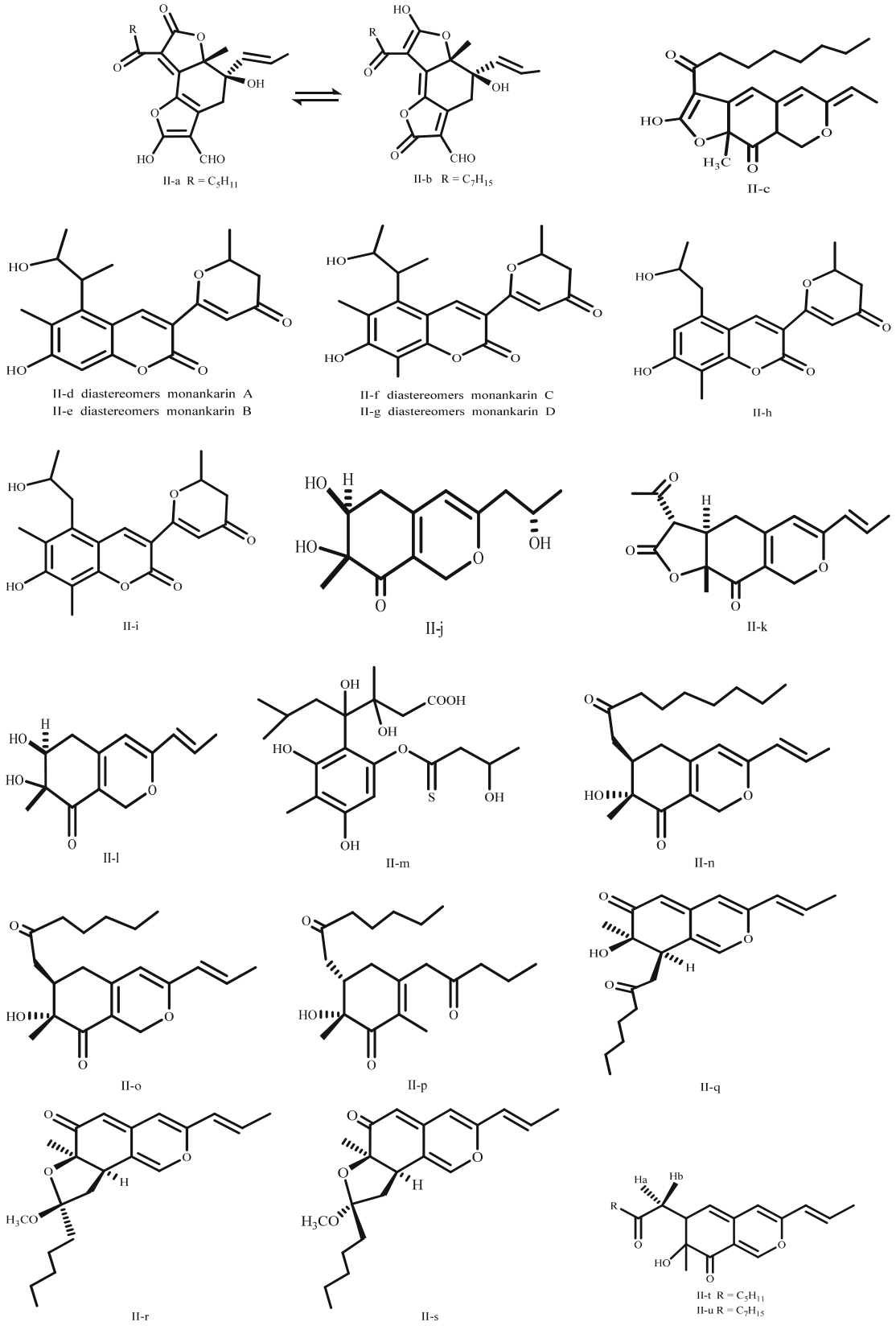
Red constituents

In 1976, two red MPs components, *N*-glucosylrubropunctamine and *N*-glucosylmonascorubramine were prepared by



I

Fig. 1 Chemical structrues of MPs compounds. *I* Six well-known MPs compounds, *II* MPs yellow compounds, *III* MPs orange compounds, *IV* MPs red compounds. *I-a* monascin, *I-b* ankaflavin, *I-c* rubropunctatin, *I-d* monascorubrin, *I-e* rubropunctamine, *I-f* monascorubramine; *II-a* xanthomonasin A, *II-b* xanthomonasin B, *II-c* yellow II, *II-d* monankarin A, *II-e* monankarin B, *II-f* monankarin C, *II-g* monankarin D, *II-h* monankarin E, *II-i* monankarin F, *II-j* monascusone A, *II-k* monascusone B, *II-l* FK17-P2B2, *II-m* Y3, *II-n* monaphilone A, *II-o* monaphilone B, *II-p* monaphilone C, *II-q* monapurone A, *II-r* monapurone B, *II-s* monapurone C, *II-t* monarubrin, *II-u* rubropunctin; *III-a* monapilol A, *III-b* monapilol B, *III-c* monapilol C, *III-d* monapilol D; *IV-a N*-glucosylrubropunctamine, *IV-b N*-glucosylmonascorubramine, *IV-c N*-glutarylrubropunctamine, *IV-d N*-glutarylmonascorubramine, *IV-(e-l)* red derivatives of alanine and aspartate from rubropunctatin and monascorubrin, *IV-m* R_3, *IV-n* unnamed red MPs compound, *IV-o* PP-V, *IV-p* unnamed red compound identified in 2011, *IV-(q-s)*, *IV-u* other isolated red MPs compounds, *IV-t* glycyl-rubropunctatin



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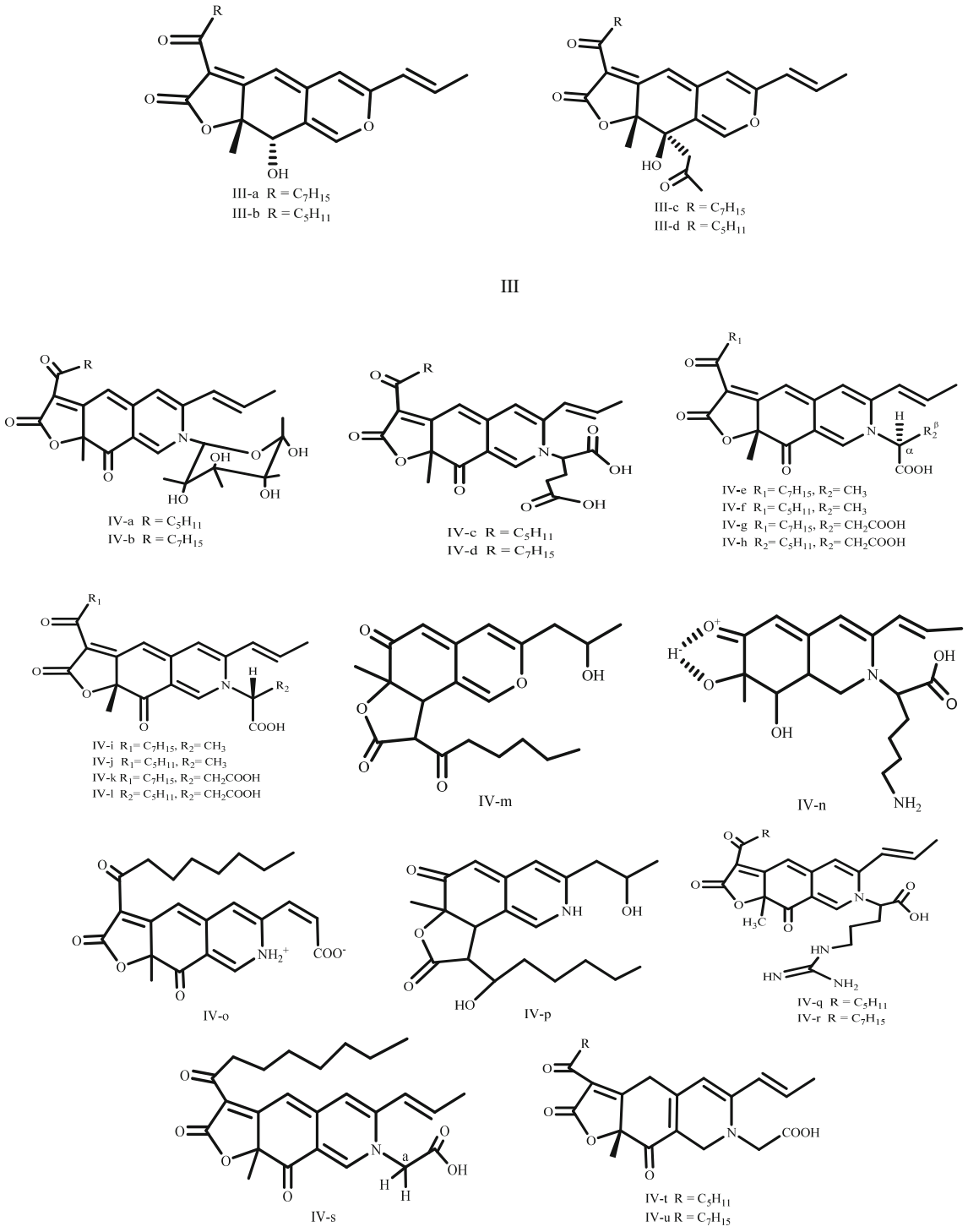

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Moll and Farr (1976). In 1992, two red compounds derived from rubropunctatin and monascorubrin, named *N*-glutaryl-rubropunctamine and *N*-glutarylmonascorubramine were produced by *Monascus* sp. TTWMB 6093 (Blanc et al. 1994; Lin et al. 1992). And in 1997, eight red derivatives of alanine and aspartate from rubropunctatin and

monascorubrin were taken from commercial RFR products by Sato et al. (1997). In 2006 and 2007, two red MPs metabolites, R_3 (Campoy et al. 2006) and another (Lian et al. 2007) were obtained, respectively. And in 2008, PP-V was purified from the fermented products by *Monascus ruber* IBT 7904, 9655 and *M. purpureus* IBT 9664 (Mapari et al. 2008). In 2011, a red MPs compound similar to rubropunctamine and monascorubramine was isolated from fermentation broth of *M. purpureus* NFCCI 1756 (Mukherjee and Singh 2011). More red MPs compounds have been isolated (Izawa et al. 1997; Jeun et al. 2008; Jung et al. 2003; Kim et al. 2007a). The chemical structures of MPs red constituents achieved among the years of 1976–2011 are listed in Fig. 1 IV a–u.

Biosynthesis of *Monascus* pigments

Although MPs biosynthesis is considered to generally follow a polyketide pathway like many other secondary metabolites (Jůzlová et al. 1996; Turner 1971), MPs biosynthesis pathway is unclear and controversial. In early 1960s, the biosynthesises of *n*-hexanoylacetyl residue is similar with β-ketoacid and chromophore (Fig. 2) of rubropunctatin and monascorubrin were examined (Birch et al. 1962; Hajjaj et al. 2000b; Holker et al. 1964; Whalley 1963), and the results revealed that chromophore was derived from acetate plus malonate by a β-ketide pathway, and biosynthesis of the *n*-hexanoylacetyl residue followed another pathway involving elaboration of hexanoate and octanoate (Hadfield et al. 1967).

In 2000, a biosynthetic route for *N*-glutarylmonascorubramine, which was similar to the results of 1960s, was proposed (Fig. 3a) according to the effects of exogenous medium-chain fatty acids on *N*-glutarylmonascorubramine yield (Hajjaj et al. 2000b). And based on the metabolic routes above the possible biosynthetic pathway of monascin, monascusones A and B, together with FK17-P2B2 was proposed in 2004 (Fig. 3b) (Jongrungruangchok et al. 2004).

In 1977, the interconversion among the three kinds (yellow, orange, red as mentioned above) of MPs compounds was proposed that only orange MPs components

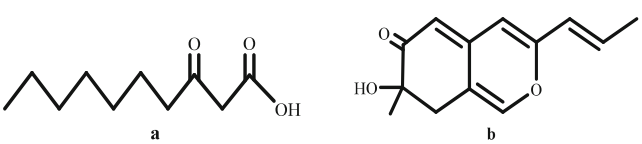


Fig. 2 The chemical structure of β-ketoacid and chromophore. **a** β-ketoacid, **b** chromophore

(rubropunctatin and monascorubrin) were biosynthetic, and the other ones were transformed from them by chemical transformations (Carels and Shepherd 1977). However, Yongsmith et al. (1993) pointed out that the orange MPs compounds could be oxidized to yellow MPs ones, and both of them were biosynthesized. And in 1996, the biosynthesis of rubropunctatin and red MPs constituents was proposed in Fig. 3c and d, respectively (Jůzlová et al. 1996; Lin et al. 1992). In 1997, formation of red MPs metabolites (*N*-glucosylrubropunctamine, *N*-glucosylmonascorubramine, *N*-glutarylrubropunctamine, and *N*-glutarylmonascorubramine) was shown in Fig. 3e (Hajjaj et al. 1997).

The production of *Monascus* pigments

Monascus strains used for *Monascus* pigments production

A lot of *Monascus* strains are used to produce MPs, which mainly include *M. pilosus*, *M. purpureus*, *M. ruber,* and *M. anka* (Cheng et al. 2012a, b). In China, seven *M. anka* strains such as As.3.913 and As.3.987, are widely used to produce MPs (Fu and Bai 1977). Other *Monascus* strains were isolated and applied for MPs production. For example, mutant R-10847 derived from *M. kaoliang* F-2 (ATCC 26264) could produce 100-fold MPs of the original strain (Lin and Iizuka 1982). *M. purpureus* FRR2190 was used to produce high-yield red and yellow MPs (Johns and Stuart 1991). *M. anka* MYM was able to produce 88.14 and 92.45 units/mL fermented broth at OD_{410} of yellow MPs in a 250-mL triangular flask and a 5-L fermenter, respectively (Zhou et al. 2009).

The *Monascus* pigments production processes

Solid-state fermentation (SSF) and liquid-state fermentation (LSF) are two major processes for MPs production. The SSF products, RFR can be directly used as food colorants (Liu et al. 2010) or materials for MPs extraction, while LSF products must be extracted to get MPs before used as colorants (Gong et al. 2002).

SSF is a classical process to produce MPs in China, in which *Monascus* strain is inoculated into the steamed rice spreaded on the wooden trays and cultured for about 20 days in an air-, moisture-, and temperature-controlled room (Dufossé et al. 2005). Compared with MPs production by LSF, MPs production by SSF possesses many advantages including simpler technique, less capital investment, lower levels of end-product inhibition and catabolite repression, lower amount of waste output, better product recovery, and

higher yield (Joshi et al. 2003; Lee et al. 2002). However, LSF is an attractive alternative owing to being easier to be managed, shorter cultivation time, lower production costs, and higher product quality (Silveira et al. 2011).

Effect factors on *Monascus* pigments production

In SSF of MPs production, the main effective parameters are moisture content and oxygen partial pressure (de Carvalho et al. 2006; Dufossé et al. 2005; Han and Mudgett 1992). For instance, maximum MPs yields by *M. purpureus* ATCC 16365 were obtained at 0.5 atm of oxygen partial pressure while MPs production was completely inhibited at 1.0 atm of carbon dioxide partial pressures (Han and Mudgett 1992). De Carvalho et al. (2006) reported that the maximum MPs production velocity (27.5 OD_{500}/g biomass h) was observed at the optimal conditions.

With regard to MPs production by LSF, there are many effective factors, such as carbon source, nitrogen source, pH, temperature, minerals, oxygen partial pressure, other microorganisms, etc, which will be reviewed as follows.

Carbon source

The carbon sources for MPs in LSF mainly include starch, oligo- and polysaccharides, various monosaccharides, ethanol, and so on, which have different and complex effects on both *Monascus* growth and its pigments production. For example, glucose and its oligo- and polysaccharides were better carbohydrates than maltose and fructose for both growth and MPs production of *Monascus* sp. TTWMB 6042 (Lin and Demain 1991). There is an effect of carbon sources on MPs constituents, too. For instance, when maltose and glucose were used as carbon sources, *M. purpureus* mainly produced dark brown MPs, while sucrose was utilized as the carbon source, it produced a light MPs (Joshi et al. 2003); and compared with glucose, maltose was a fitter material for monascorubramine production by *M. purpureus* (Tseng et al. 2000).

Ethanol or ethanol together with saccharides is a good carbon source for *Monascus* growth and its MPs production. When ethanol was used as the sole carbon source, MPs constituents by *M. purpureus* CCM8152 at 410 and 500, 470, and 410 and 470 nm, respectively, were higher than those cultivated on maltose (Jůlová et al. 1994). Two-stage cultivation using maltose and ethanol in the first and second stages, respectively, might increase the efficiency of ethanol utilization for MPs production (Jůlová et al. 1994). When glucose was used as the initial carbon source and ethanol was supplemented after exhaustion of ethanol produced from glucose, the red MPs yield by *M. purpureus* CBS 10907 could be enhanced (Hamdi et al. 1997).

The metal ion such as Zn^{2+}, and combination of amino acids (glycine, L-leucine, and L-tryptophan) could improve MPs transfering coefficient of carbon sources, when D-arabinose, D-xylose, D-glucose, D-fructose, sucrose, maltose, starch, or alcohol was the sole carbon source during *Monascus* spp. fermentation (Johnson and Mchan 1975).

Besides the above carbohydrates, some other materials, such as wheat flour, prickly pear juice, glycerol, etc, were also used for producing MPs in LSF (Domínguez-Espinosa and Webb 2003; Hamdi et al. 1996; Meinicke et al. 2012).

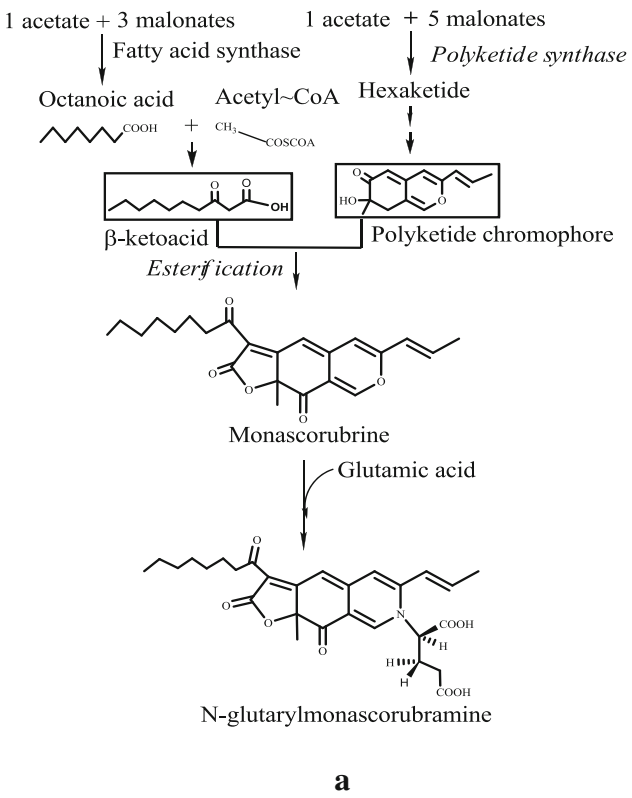
Nitrogen source

Inorganic ammonic compounds such as NH_4Cl, NH_4NO_3, and organic nitrogen sources such as urea ($CO(NH_2)_2$), peptone, monosodium glutamate (MSG), and amino acids, are good nitrogen sources for both growth and pigment production of *Monascus* spp. in LSF, and generally, organic nitrogen sources are better than inorganic ammonic compounds (Chen and Johns 1993; Lin and Demain 1991, 1994). However, Joshi et al (2003) pointed out that NH_4Cl was best to produce MPs, and followed by NH_4NO_3 and MSG. What is more, it is very interesting that some amino acids such as leucine, lysine, valine, and methionine, especially leucine, had strong inhibition on formation of water-soluble MPs when they were used as the sole nitrogen source in *Monascus* sp. TTWMB 6093 fermentation (Lin and Demain 1994, 1995).

Adding Zn^{2+} togethering with nitrogen sources could also increase *Monascus* spp. growth as mentioned in "carbon source" when carbohydrates or glycolytic-pathway intermediates were supplied as carbon source, which might indicate that Zn ion was probably required as a co-factor for a metal-dependent enzymes involved in the inter-relationships between carbohydrate and nitrogen metabolism of *Monascus* spp. for optimum growth (Johnson and Mchan 1975; Mchan and Johnson 1979).

pH

Generally, the suitable pH for growth and MPs production of *Monascus* spp. is 5.5–6.5 (Chen and Johns 1993; Joshi et al. 2003). However, different pH values in media may affect MPs constituents. For example, Chen and Johns (1993) found that ankaflavin synthesis by *M. purpureus* 192F was favored at pH4.0, while the other MPs synthesis was independent of pH. Red MPs were observed in media at pH6.5, whereas orange MPs were produced in media at pH2.5 (Carels and Shepherd 1979; Orozco and Kilikian 2007). Yongsmith et al. (1993) also found that an orange–red MPs mixture was produced at the initial pH7.0 of media, a light golden MPs mixture was obtained at initial pH below 4, and a monascin–ankaflavin–monascorubrin skeleton was received at initial pH2.5 by *Monascus* sp. KB 10.



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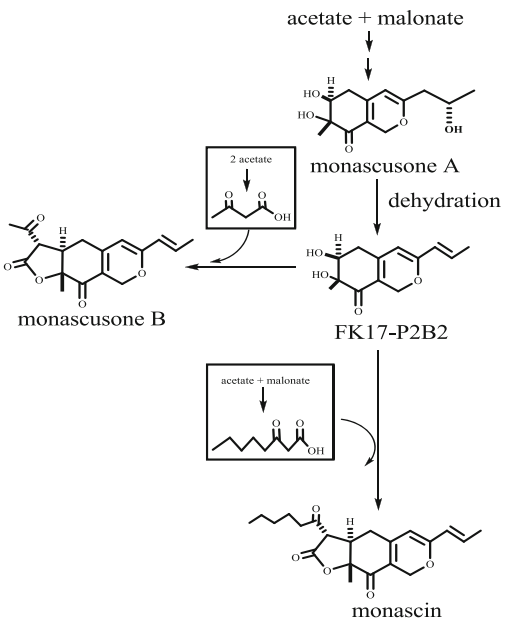

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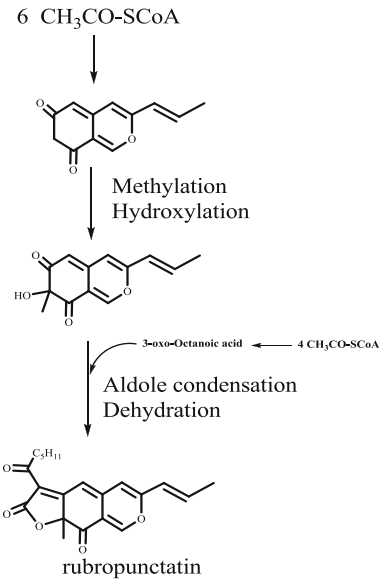

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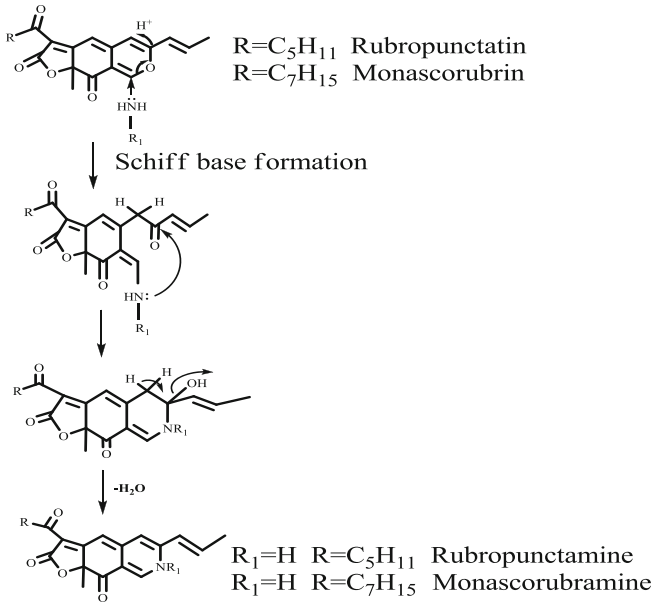

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Fig. 3 Some published biosynthetic pathways of MPs. **a** scheme of the hypothetic metabolic routes of *N*-glutarylmonascorubramine, **b** possible biosynthetic pathway of monascusone A, monascusone B, monascin, and FK17-P2B2, **c** probable mechanisms of the biosynthesis of rubropunctatin, **d** formation of red pigments, **e** possible reaction mechanism for the formation of extracellular red pigments (*N*-glucosylrubropunctamine, *N*-glucosylmonascorubramine, *N*-glutarylrubropunctamine, and *N*-glutarylmonascorubramine) from 1-D-glucosamine or monosodium glutamate and rubropunctatin and monascorubrin

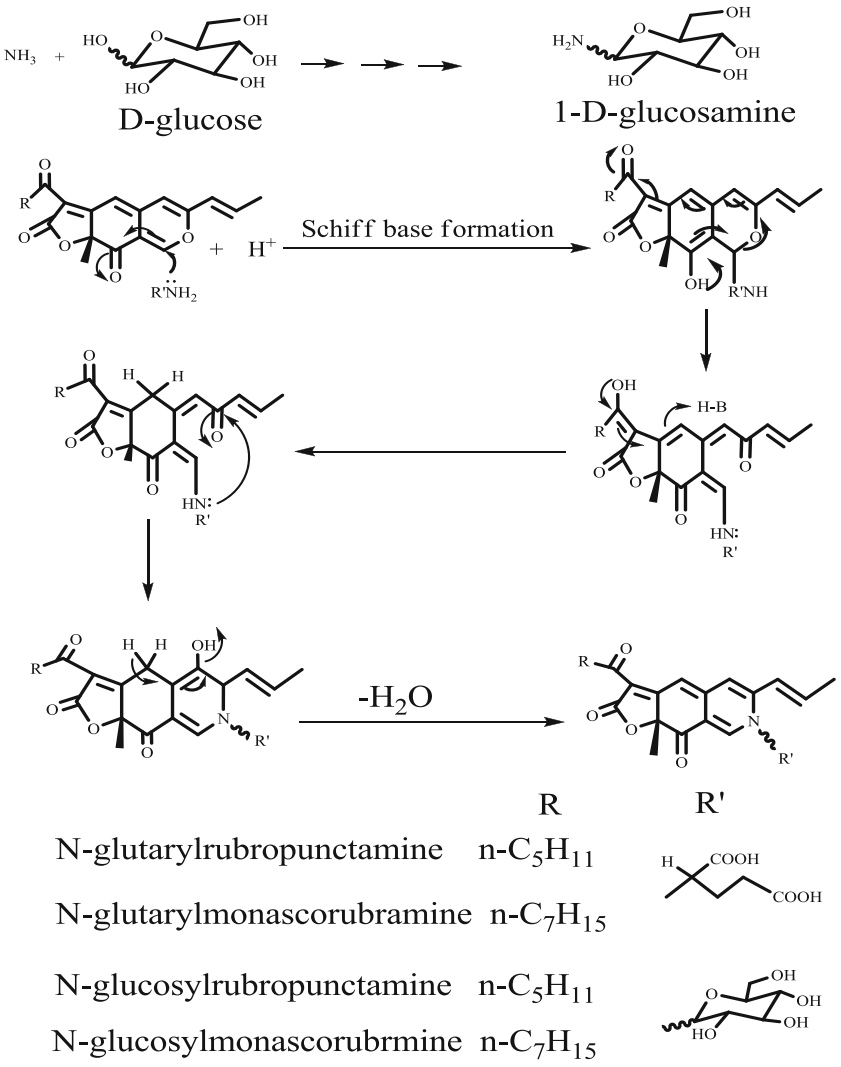

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Fig. 3 (continued)

Temperature

As a general rule, *Monascus* spp. were cultured at 25–30 °C for MPs production (Hu et al. 2012; Joshi et al. 2003), whilst *Monascus* spp. could also grow at higher temperature, such as 31 °C (Zhou et al. 2009), 32 °C (Liu et al. 2007; Mohamed et al. 2009). There is a great difference of MPs produced at various culture temperatures. Ahn et al. (2006) reported that when *Monascus* sp. J101 was cultured at 25 °C, the MPs yield was 10 times more than that at 30 °C. This may be due to the longer time cell growth (120 h) and lower broth viscosity at 25 °C than those at 30 °C (48 h).

Mineral

Metal ions, especially Zn^{2+} and Mg^{2+} greatly affect growth and MPs production of *Monascus* spp. (Bau and Wong 1979; Lin and Demain 1993). For example, growth and MPs production of *M. purpureus* (both wild type and it mutational strain NIIS) were nearly stopped when Zn^{2+} concentrations were between 2×10^{-3} and 3×10^{-3} M in liquid media, but the conditions on solid media were opposite with strain NIIS, which growth and pigmentation were inhibited and promoted respectively at 5×10^{-5} of Zn^{2+} (Bau and Wong 1979). Zn^{2+} has been considered to be

involved in inter-relationships between carbohydrate and nitrogen of *M. purpureus*, too (Mchan and Johnson 1970). Mg^{2+} and phosphate showed negative effects on MPs production by *M.* sp. TTWMB 6093 at over 0.5 and 2 mM, respectively, owing to inhibition of MPs synthases by them (Lin and Demain 1993). Fe^{2+} and Mn^{2+} showed stimulatory effect on MPs production by *M. purpureus* ATCC 16365 (Lee et al. 2001).

Oxygen partial pressure

Oxygen is an electronic acceptor of oxidative phosphorylation and the substrate of monooxygenase, which is involved in metabolite synthesis. It is proven that monooxygenases act much more in secondary metabolism than those in primary metabolism. But the role of monooxygenases in MPs synthesis has not been well-known (Han and Mudgett 1992).

The MPs quantities produced by *M. anka* M-9 in batch submerged, agar surface, and roller bottle cultures were compared by Mak et al. (1990). In roller bottles, the MPs yield was higher than that of batch submerged and agar surface cultures, the optimum time reached at maximum pigment production was shorter, and the ratio of red to yellow pigments was greatly increased. These might result from a combination of factors, including better gas exchange, efficient pigment secretion, higher medium pH, retarded conidiation, and solid support for mycelium. The growth and MPs production of *M. purpureus* FTC5391 were also affected by the impeller design and configuration which had effects on oxygen transfer and uptake (Mohamed et al. 2009).

In order to increase MPs and decrease citrinin produced by *Monascus* spp., the effects of oxygen on both have been done. Hajjaj et al. (1999) reported that the biomass and secondary metabolites including MPs and citrinin, were increased by improving oxygen supply, especially the dissolved oxygen in media, but MPs were increased less than citrinin (Pereira et al. 2008). So a fit oxygen supply was needed to reduce citrinin and increase MPs in final products by *M. ruber* ATCC 96218 (Hajjaj et al. 2000a).

Other microorganisms

The effects of other microorganisms co-cultured with *Monascus* spp. on MPs production were investigated. When *Monascus* sp. J101 was incubated together with either *Saccharomyces cerevisiae* KCCM 11991 or *Aspergillus oryzae* KCCM 11371, MPs yield was increased comparing with the *Monascus* sp. J101 monocultures (Shin et al. 1998), and *S. cerevisiae* KCCM 11991 was more effective than *A. oryzae* KCCM 11371. The hydrolytic enzymes, such as amylase, chitinase, and protease secreted by *S. cerevisiae* and *A. oryzae* might be the effectors (Ju et al. 1999; Lim et al. 2000; Shin et al. 1998).

Lights

MPs production is greatly influenced by various lights such as white, red, blue, yellow, and green light. Generally *Monascus* spp. produce the maximum MPs in darkness and minimum ones in white light, and the effects of other lights on MPs are quite different. Velmurugan et al. (2010c) reported that the maximum extracellular red pigment (36.75±2.1 OD_{500}/g dry substrate) was observed when *M. purpureus* was cultured in darkness, the minimum (5.9±1.1 OD_{500}/g dry substrate) in white unscreened light and the maximum intracellular red pigment (18.27±0.9 OD_{500}/g dry substrate) was obtained when *M. purpureus* was cultured in darkness, the minimum (8.03±0.6 OD_{500}/g dry substrate) in yellow light. And MPs produced by *M. purpureus* LPB97 was improved in total darkness, but totally suppressed in direct illumination (Babitha et al. 2008). However, Miyake et al. (2005) reported that red light could stimulate MPs production by *M. pilosus* IFO4520, but blue light might decrease MPs. This reason might be that red light could increase MPs synthesis, while blue light could degrade MPs (Miyake et al. 2005).

The others

Besides the aforementioned factors, other factors such as initial moisture content (Teng and Feldheim 2000), mycelial morphometrics of *Monascus* spp. (Kim et al. 2002), etc, could also influence MPs production. The effects of cyclic adenosine monophosphate (cAMP) on MPs produced by *M. ruber* M-7 were studied during LSF and SSF processes (Lai et al. 2011), MPs contents were increased and decreased at low cAMP concentrations (0.5–1.0 mmol/L in LSF, 0.5 mmol/kg in SSF) and high cAMP concentrations (2–10 mmol/L in LSF, 1–10 mmol/kg in SSF), respectively.

The properties of *Monascus* pigments

Solubility

The six well-known MPs (Fig. 1 I a–f) are insoluble in water, but dissolved in ethanol, acetic acid, hexane, etc. (Lin et al. 1992; Sweeny et al. 1981). The MPs solubility in water could be promoted by adding glutamate, leucine, glycine to the media of *Monascus* spp. (Jeun et al. 2008; Jung et al. 2003; Kim et al. 2007a; Lin et al. 1992) or by chemical modification through introducing –COOH or $–NH_3$ groups of amino acids into the MPs (Wong and Koehler 1983).

Stability

Temperature and pH

Usually, MPs are very stable at 30–60 °C and pH6.0–8.0 (Silveira et al. 2011). But some MPs are still stable even at higher temperatures and extreme pH values. For example, Li et al. (2003) reported that MPs from *M. anka* were still relatively stable at pH11.0 or 150 °C. Huang et al. (2011) found that MPs by *M. purpureus* JR were more stable at the basic pH (9–11) than at the acidic pH (3–5).

Lights

The MPs are sensitive to lights, especially to sunlights and ultraviolet lights, and the yellow MPs constituents are more photostable than the red MPs ones (Fabre et al. 1993; Mapari et al. 2009). Amino acid derivatives of MPs and water-soluble MPs are always more stable than the original MPs (Jung et al. 2011; Lin et al. 1992; Sheu et al. 2000). Jung et al. (2011) proved that L-Phe derivatives of rubropunctamine and monascorubramine were more stable than the original MPs in presence of sunlight irradiation, and the half-lives of MPs amino acid derivatives were 1.45–5.58 h while original MPs' ones were only 0.22 h under sunlight. Sheu et al. (2000) reported that when nata (a bacterial cellulose produced by *Acetobacter aceti* ssp. *xylinum*) was fermented by *M. purpureus* CCRC3150, MPs in *Monascus*-nata fermentation complex were more stable than ones in nata dyed by MPs under ultraviolet irradiation at 366 nm for 36 h. And 1,4,6-trihydroxynaphthalene could also inhibit *N*-glucosylrubropunctamine and *N*-glucosylmonascorubramine to be faded under sunlights owing to forming a complexe (Sweeny et al. 1981).

The others

Metal ions can also affect the MPs stability to some extent. Frequently, MPs are stable in the appearance of a small quantity of Na^{+}, Mg^{2+}, K^{+}, AL^{3+}, Ca^{2+}, Cu^{2+}, and Zn^{2+}, but the Fe^{3+} and Fe^{2+} exerted an obvious negative effect on stability of MPs at the concentrations of 20, 40, 100 ppm (Li et al. 2003; Song et al. 1995; Zhang et al. 2005).

Safety

As natural food colorants, MPs have been used in food industries in Asian countries such as China and Japan, for more than 10 centuries (Jia et al. 2010; Jůzlová et al. 1996; Lin et al. 2008; Mohan Kumari et al. 2009). In China, MPs are used as colorants in more than 20 kinds of foods and no adverse effect has been reported (Gheith et al. 2008; Mohan Kumari et al. 2009; Wang et al. 2007). However, after citrinin, a kind of mycotoxin (Pattanagul et al. 2007), was found in *Monascus*-fermented products in 1995 (Blanc et al. 1995), the permited limited quantity of citrinin in MPs in China and Japan are 1 and 0.2 mg/kg, respectively, in order to control the harmful impact of citrinin (Li et al. 2008; Yang et al. 2007).

Extract, isolation, purification, and identification of *Monascus* pigments

Monascus pigments extraction and detection

MPs include water-soluble and water-insoluble constituents according to their solubility in water, most of which are water-insoluble (Hajjaj et al. 1997; Qian and Wu 2010). The total MPs were usually extracted by ethanol at various concentrations (Babitha et al. 2006, 2007; Johns and Stuart 1991; Lai et al. 2011; Vidyalakshmi et al. 2009b), the water-insoluble MPs constituents were achieved by organic solvents such as *n*-hexane, benzene, methanol, ethanol, etc, and the water-soluble MPs ones were taken by distilled water (Lin and Iizuka 1982; Sato et al. 1997; Sweeny et al. 1981).

As mentioned in "the *Monascus* pigments production processes", MPs could be got from SSF or LSF *Monascus* products (Gong et al. 2002). And MPs from SSF products and mycelia of LSF products of *Monascus* could be extracted through solid–liquid extraction (Hu et al. 2012; Kongruang 2010; Mohamed et al. 2009) and microextraction (Mapari et al. 2008, 2009; Smedsgaard 1997); MPs in supernatant of LSF products of *Monascus* could be extracted through liquid–liquid extraction (Hu et al. 2012; Lai et al. 2011; Li et al. 2010b; Velmurugan et al. 2010c; Zhou et al. 2009).

The total MPs contents, namely color values which were defined as total optical density values at a given wavelength per milliliter or gram of MPs, might be measured at 500 nm (Evans and Wang 1984; Lin and Demain 1991), 505 nm (Ding et al. 2008), and 480 nm (Santerre et al. 1995), respectively. MPs of different color could be detected at different wavelengths (Hajjaj et al. 2012; Hu et al. 2012; Lai et al. 2011; Silveira et al. 2011; Wongjewboot and Kongruang 2011). The extractant or extract-liquor of unfermented substrate was always used as the blank.

Isolation and purification of *Monascus* pigments components

MPs components were isolated and purified by column chromatography (CC), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and high-speed counter-current chromatography. In this section, their key parameters to isolate and purify MPs constituents and components will be listed.

Column chromatography

The column chromatography (CC) has been widely used for MPs isolation and purification and its MPs isolated fractions usually need to be further purified by TLC and HPLC (Kim et al. 2006b; Vidyalakshmi et al. 2009a). Some key parameters of CC used in MPs isolation are summaried in Table 1.

Thin layer chromatography

In 1973, monascin and ankaflavin from mycelia of *M. anka* were isolated through thin layer chromatography (TLC) using 25 % ether in benzene as developing agent (Manchand and Whalley 1973). However, TLC was often used for preliminary isolation of MPs due to its relatively low sensitivity (Jung et al. 2003; Sun et al. 2005). And after TLC of MPs, HPLC is applied to further purify MPs compounds. Some process parameters of TLC utilized in MPs isolation are listed in Table 2.

High-performance liquid chromatography

HPLC is utilized to isolate, purify, and analyze various MPs constituents and components owing to its high sensitivity and multiple detection systems including ultraviolet, fluorescent, photodiode array detectors, and mass spectrograph (Lin et al. 1992; Turner et al. 2009). The main analytical parameters of HPLC used in MPs isolation, purification, and analysis are presented in Table 3.

Capillary electrophoresis

CE is fitter for analyzing MPs constituents and components due to its requiring just a little amount of sample and less time and solvent compared to HPLC. However, we can find few literatures about CE used in MPs analysis (Watanabe et al. 1997, 1999). For example, micellar electrokinetic chromatography (MEKC) of CE was utilized to separate and analyze the yellow MPs using 50 mM phosphate buffer (pH7.0) as mobile phase at 10 kV (Watanabe et al. 1997). In another study, xanthomonasin A, glycyl-rubropunctatin, and 3-hydroxyamino-1-methyl-5 H-pyrido[4,3-b] indole, were analyzed by MEKC using 25 mM phosphate buffer (pH7.0) as running separation solution (Watanabe et al. 1999).

The others

In addition to the methods listed above, high-speed counter-current chromatography was used for purification of the six well-known MPs (shown in Fig. 1 I a–f),

Table 1 *Monascus* pigments isolation by column chromatography

Sample	Column	Eluant	Reference
MPs mixture	Sephadex G-100 column (2×100 cm)	0.1 M potassium phosphate buffer (pH7.0)	Yoshimura et al. (1975)
	Silica gel (70–230 mesh)	Chloroform–methanol 90:10 Chloroform–methanol 50:50	Hajjaj et al. (1997)
	Silica gel 60 (220–400 mesh), 200 g Octadecyl silica chromatorex-ODS (100–200 mesh), 200 g	Increasing polarity (*n*-hexanes–EtOAc=1:0, 9:1, 4:1, 1:1, 3:7, 1:9, 0:1; *v*/*v* EtOAc–MeOH=9:1, 1:1, 0:1; *v*/*v*)	Akihisa et al. (2005b)
	SephadexG-50 column (1.5×120 cm)	Milli-Q water at 0.8 mL/min	Campoy et al. (2006)
	Silica gel column (7×30 cm)	Chloroform–methanol 100:0–80:20 30 mL/min	Kim et al. (2006b)
	Silica gel (60–120 mesh) column (1×21 cm)	Chloroform–ethanol 9:1, *v*/*v*	Vidyalakshmi et al. (2009a)
	SiO_2 (230–400 mesh)	*n*-Hexane–EtOAc (8:1)	Cheng et al. (2010)
	Silica gel 60 (70–230 mesh and 230–400 mesh) column	Gradient solvent systems, *n*-hexane-ethyl/acetate, 10:0, 9:1, 8:2, 7:3, 6:4, 1:1, 4:6, 0:10 Dichloromethane–ethyl acetate, 95:5, 90:10, 85:15	Hsu et al. (2011b)
	Silica gel (80–120 mesh) column (3×80, 4.5×55, and 3×35 cm)	Ethyl acetate/petroleum ether 1:1, *v*/*v*	Li et al. (2011)
Brownish-yellow MPs	Silica gel column (3×5 cm)	Benzene–acetone 9:1, 8:2, 7:3, 6:4, 2:8	Yongsmith et al. (1994)
Yellow MPs	Silica gel column	CH_2Cl_2/acetone 99:1	Jongrungruangchok et al. (2004)

Table 2 *Monascus* pigments isolation and purification by thin layer chromatography

Sample	Gel	Developing agent	Reference
MPs mixture	Silica gel G	*n*-Hexane/ethyl acetate 9:1	Yoshimura et al. (1975)
	Silica gel	Benzene/chloroform/methanol 85:12:3	Wong and Bau (1977)
	Polygram Silica G	Chloroform/methanol/acetic acid 285:21:9	Jůlová et al. (1994)
	Silica60 Kieselguhr F254	*n*-Hexane/ethyl acetate 7:3 (first dimension) *n*-Hexane/acetone 2:1 (second dimension)	Teng and Feldheim (1998)
	Silica gel 60 F254	Chloroform/methanol/water 90:25:4	Jung et al. (2003); Nimnoi and Lumyong (2009)
	Silica gel 60	Chloroform/methanol/water 90:25:4	Babitha et al. (2006)
	Silica gel 60F254	Benzene/methanol/chloroform 30:10:9	Vidyalakshmi et al. (2009b)
	Silica gel 60F254 Silica gel 60F254	Chloroform/acetone 9:1 Petroleum ether/diethyl ether 5:5	Loret and Morel (2010)
	Silica gel 60	*n*-Hexane/ethyl acetate 7:3	Hsu et al. (2011b)
	Silica gel 60, 50	Chloroform/methanol/water 90:25:4	Jung et al. (2011)
Yellow MPs	Silica gel cc	CH_2Cl_2/acetone 99:1	Jongrungruangchok et al. (2004)
	Silica gel G	*n*-Hexane/acetone 1:1	Yoshimura et al. (1975)
	Silica gel	Benzene/methanol/chloroform 30:10:9	Wong and Bau (1977)
	Silica gel	Chloroform/methanol/water 65:25:4	Hajjaj et al. (1997)
Red MPs	Silica gel G	Ethyl acetate/formic acid/acetic acid/water 100:11:11:26	Mukherjee and Singh (2011)
	–	Chloroform/ethanol/water 65:25:4	Kim et al. (2006b)
	–	Chloroform/ethanol/water 65:25:4	Vidyalakshmi et al. (2009a)

"–" Did not mention in the literatures

xanthomonasin A and xanthomonasin B (Inoue et al. 2010).

Identification of *Monascus* pigments components

In the early 1970s, nuclear magnetic resonance (NMR) was used for MPs components identification (Yoshimura et al. 1975). Nowadays, although NMR is still a good method for identification of MPs compounds, it is normally combined with ultraviolet–visible (UV–vis) spectra, infrared (IR) spectra, mass spectrum (MS), gas chromatography (GC)-MS, liquid chromatogram (LC)-MS, high-resolution mass spectrometry (HRMS), fluorescence spectra, and/or electron paramagnetic resonance (EPR) to identify MPs (Akihisa et al. 2005b; Loret and Morel 2010; Mukherjee and Singh 2011). Some MPs compounds which are confirmed by NMR or NMR together with other methods aforementioned are listed in Table 4.

The functions of *Monascus* pigments

Anticancer activities

The extracts of RFR by *Monascus*, MPs constituents and MPs compounds all have anti-mutagenic and anticancer activities at a certain extent.

Both of whole extract and pigment-rich fractions of commercial Chinese RFR had the effects on proliferation and apoptosis of colon cancer cells (Hong et al. 2008). In the Ames *Salmonella* assay, red and yellow MPs extracted from *M. anka* and *M. purpureus* inhibited mutagenicity of heterocyclic amines such as Trp-P-2 (3-amino-1-methyl-5H-pyrido [4,3-b] indole) due to acceleration of its decomposition (Izawa et al. 1997). And other researches also proved that RFR coarse extracts possessed potential anti-mutagenic activities (Ho et al. 2010; Hsu et al. 2011a; Hsu and Pan 2012).

Monascin showed marked inhibitory activity on mouse skin carcinogenesis induced by peroxynitrite and ultraviolet light B (Akihisa et al. 2005a), but no cytotoxicity on Hep G2 (human cancer cell lines) cells (Su et al. 2005). The inhibitory concentration at 50 % (IC_{50}) of ankaflavin on Hep G2 and A549 (human cancer cell lines) cells was 15 μg/mL, but no significant toxicity on normal diploid fibroblast cell lines such as MRC-5 and WI-38 cells at the same concentration (Su et al. 2005). Two yellow MPs compounds, monaphilone A and monaphilone B, exhibited antiproliferative effect against HEp-2 (human laryngeal carcinoma cell line) and WiDr (human colon adenocarcinoma cell line), but no toxicity to normal MRC-5 and WI-38 cells at 70 μM (Hsu et al. 2010). Rubropunctatin could induce the apoptosis mediated by tumor necrosis factor (TNF) and inhibit proliferation of human gastric adenocarcinoma

Table 3 *Monascus* pigments purification and analysis by HPLC

Sample	Column	Detector	Wavelength	Mobile phase	Reference
MPs mixture	μ Bondapak C_{18} column	Tunable UV absorbance	400, 470, and 500 nm	Acetonitrile/water (70:30, *v*/*v*) at 1.0 mL/min	Chen and Johns (1993, 1994)
	L-column ODS packed column	Programmable solvent module 125 and programmable detector module 166	460 nm	Sol.A distilled water containing 0.05 % trifluoroacetic acid (TFA), Sol.B acetonitrile containing 0.05 % TFA, the linear-gradient program from 30 to 60 % of sol.B in 30 min at 1.0 mL/min	Watanabe et al. (1997)
	C_{18} column	481 Spectrophotometer	233 nm	Acetonitrile/water (80:20, *v*/*v*), at 0.5 mL/min	Teng and Feldheim (1998); Teng and Feldheim (2000)
	μ Bondapak C_{18} column	Tunable UV absorbance	405, 470, and 495 nm	Acetonitrile/water (75:25, *v*/*v*) at 1.0 mL/min	Domínguez-Espinosa and Webb (2003)
	ODS C_{18} column	UV-120-2 detector	425 nm	Elution gradient of distilled water/methanol from 100:0 to 30:70 in 40 min at 0.8 mL/min	Jung et al. (2003)
	LiChrospher 100RP-18 pore column	Photodiode array detector	390, 470, and 520 nm	Water (A) and acetonitrile (B), the conditions were adapted for optimal resolution of the different MPs components	Campoy et al. (2006)
	Cosmosil 5 C_{18}-MS	–	490 and 380 nm	Linear gradient, acetonitrile/water containing 0.1 % HCOOH (60:40, *v*/*v*) to acetonitrile–water containing 0.1 % HCOOH (100:0, *v*/*v*) in 20 min at 0.5 mL/min	Miyake et al. (2008)
	Cosmosil C_{18}	Photodiode array detector, RF-10AXL fluorescence detector	390 nm	Eluent A H_2O/HAC (100:10, *v*/*v*), eluent B acetonitrile/HAC (100:10, *v*/*v*), elution gradient: 0 min, 80 % A and 20 % B; 25 min, 50 % A and 50 % B; 26 min, 15 % A and 85 % B, flow rate: 1 mL/min	Zheng et al. (2009)
	5 μm Nucleosil C_{18}	Fluorimetric detector	–	Isocratic elution of acetonitrile/water (8:2, *v*/*v*) at 2 mL/min	Loret and Morel (2010)
	C_{18} column	2487 Dual absorbance detector	400 and 500 nm	Acetonitrile/H_2O (8:2, *v*/*v*) at 0.5 mL/min	Mukherjee and Singh (2011)
	Cosmosil 5C_{18} packing column	–	–	85 % MeOH as mobile phase solvent at 7 mL/min	Hsu et al. (2011b)
	Luna C_{18}	Photodiode array detector, Lintelligent fluorescence detector FP-2020 plus	234 nm	Isocratic elution using 0.05 % TFA in acetonitrile–water (62.5:37.5, *v*/*v*) at 1.0 mL/min	Wu et al. (2011)
Yellow MPs	Pegasil ODS IIC_{18} silica column	Refractive index detector	–	CH_3OH/H_2O/HAc 75:25:3(*v*/*v*/*v*) at 2.5 mL/min	Akihisa et al. (2005b)
Orange MPs	Pegasil ODS IIC_{18} silica column	Refractive index detector	–	CH_3OH/H_2O/HCOOH 75:25:0.1 (*v*/*v*/*v*) at 2.5 mL/min	Akihisa et al. (2005b)
Red MPs	C_{18} column (Techsphere 5ODS 4 μm)	2487 Dual absorbance detector	400 and 500 nm	A cetonitrile/H_2O (80:20, *v*/*v*) at 0.5 mL/min	Mukherjee and Singh (2011)
	μ Bondapak C_{18} column	Model 450	500 nm	CH_3CN/H_2O (45:55, *v*/*v*) at 1.0 mL/min	Sweeny et al. (1981)
	μ Bondapak C_{18} column	481 LC spectrophotometer	500 nm	Initial 35 % aqueous solution of acetonitrile gradually increasing to 70 % within 15 min at 1.0 mL/min	Lin et al. (1992)
	Pegasil ODS II C_{18} silica column	Refractive index detector	–	CH_3OH/H_2O/AcOH 60:40:3(*v*/*v*/*v*) at 2.5 mL/min	Akihisa et al. (2005b)

"–" Did not mention in the literatures

Table 4 Identification methods of MPs compounds

Sample	Method	MPs compound name	Molecular weight	Molecular formula	Reference
MPs mixture	NMR	Monascin	358	$C_{21}H_{26}O_5$	Yoshimura et al. (1975)
		Monascorubrin	382	$C_{23}H_{26}O_5$	
	MS, NMR	Y3	430	$C_{20}H_{30}O_8S$	Campoy et al. (2006)
		R3	374	$C_{21}H_{26}O_6$	
	UV–vis, MS, NMR	Monascin	358	$C_{21}H_{26}O_5$	Akihisa et al. (2005b)
		Ankaflavin	386	$C_{23}H_{30}O_5$	
		Rubropunctatin	354	$C_{21}H_{22}O_5$	
		Monascorubrin	382	$C_{23}H_{26}O_5$	
		Rubropunctamine	354	$C_{21}H_{23}NO_4$	
		Xanthomonasin A	388	$C_{21}H_{24}O_7$	
		Xanthomonasin B	416	$C_{23}H_{28}O_7$	
	LC-MS	Yellow 1	356	—	Zheng et al. (2009)
		Yellow 2	384	—	
	UV, LC-MS, HRMS, IR, NMR	Monarubrin	330	$C_{20}H_{26}O_4$	Loret and Morel (2010)
		Rubropunctin	358	$C_{22}H_{30}O_4$	
	UV–vis, LC-DAD-MS	Monascin	358	$C_{21}H_{26}O_5$	Mapari et al. (2008)
		Ankaflavin	386	$C_{23}H_{30}O_5$	
		Rubropunctatin	354	$C_{21}H_{22}O_5$	
		Monascorubrin	382	$C_{23}H_{26}O_5$	
	UV–vis, IR, GC-MS, NMR	Unnamed	375	$C_{21}H_{29}NO_5$	Mukherjee and Singh (2011)
Yellow MPs	IR, MS, NMR	Monascin	358	$C_{21}H_{26}O_5$	Yongsmith et al. (1993, 1994)
		Ankaflavin	386	$C_{23}H_{30}O_5$	
		Yellow II	372	$C_{22}H_{26}O_5$	
	ESI-MS, MEKC-ESI-MS	Xanthomonasin A	388	$C_{21}H_{24}O_7$	Watanabe et al. (1997)
		Xanthomonasin B	416	$C_{23}H_{28}O_7$	
	Polarimeter, IR, UV, ESI-TOF-MS, NMR	Monascusone A	254	$C_{13}H_{18}O_5$	Jongrungruangchok et al. (2004)
		Monascusone B	302	$C_{17}H_{18}O_5$	
Orange MPs	UV, IR, polarimeter, fluorescence spectra, ESI-MS, HR-ESI-MS, NMR	Monapilol A	384	$C_{23}H_{28}O_5$	Hsu et al. (2011b)
		Monapilol B	356	$C_{21}H_{24}O_5$	
		Monapilol C	440	$C_{26}H_{32}O_6$	
		Monapilol D	412	$C_{24}H_{28}O_6$	
Red MPs	UV, MS, NMR	*N*-glucosylrubropunctamine	515	$C_{27}H_{33}O_9N$	Hajjaj et al. (1997)
		N-glucosylmonascorubramine	543	$C_{29}H_{37}O_9N$	
		N-glutarylrubropunctamine	483	$C_{26}H_{29}O_8N$	
		N-glutarylmonascorubramine	511	$C_{28}H_{33}O_8N$	
	IR, UV, ESI-MS, NMR	Unnamed	364	$C_{19}H_{28}O_5N_2$	Lian et al. (2007)

NMR nuclear magnetic resonance, *UV–vis* ultraviolet–visible, *IR* infrared, *TOF-MS* time-of-flight mass spectrometer, *LC-MS* liquid chromatogram and mass spectrometry, *GC-MS* gas chromatography and mass spectrometry, *HRMS* high-resolution mass spectrometry, *MEKC-ESI-MS* micellar electrokinetic chromatography electron-impact mass spectrometry, *LC-DAD-MS* high-resolution liquid chromatography-diode array detection-mass spectrometry

BGC-823 both in vivo and vitro at 12.57 μM of IC_{50}, but no significant toxicity to normal gastric epithelial cell GES-1 at this concentration (Zheng et al. 2010a). Rubropunctatin could offer a similar effect as taxol at the same dose according to the in vivo experimental data (Zheng et al. 2010a), and its tricyclic structure was considered to be a necessary moity to its activity (Zheng et al. 2010b). Rubropunctamine and monascorubramine displayed strong cytotoxicity and antimitotic effects on IHKE (immortalized human kidney epithelial) cells (knecht and Humpf 2006). However, two yellow pigments (Fig. 1 I a, b) and two orange pigments (Fig. 1 I c, d) showed no significant cytotoxic activity towards rat hepatocytes in vitro (Martínková et al. 1999). And monascusone A

exhibited no cytotoxicity against breast cancer and human epidermoid carcinoma of cavity cell lines (Jongrungruangchok et al. 2004).

Antimicrobial activities

Rubropunctatin and monascorubrin had antibiotic action not only against bacteria but also against yeasts and filamentous fungi (Martínková et al. 1995). The MPs derivatives of amino acids possess higher antimicrobial activities than the original MPs since the derivatives are easier to be adsorbed onto the bacterial cell surface and resulted in oxygen transfere limited (Kim et al. 2006b; Martínková et al. 1999). For example, the red MPs derivatives of L-Phe, D-Phe, L-Tyr, and D-Tyr exhibited much higher antimicrobial activities against G^+ and G^- bacteria with minimal inhibitory concentration (MIC) values of 4–8 μg/mL than the original red MPs with MIC of 32 μg/mL, and the red MPs derivatives of L-Asp, D-Asp, L-Tyr, and D-Tyr could be against *Penicillium citrinum*, *Aspergillus niger,* and *Candida albicans*, but the original MPs could not (Kim et al. 2006b). The MPs derivatives of hydrophobic amino acids have stronger inhibition of microorganisms than ones of hydrophilic amino acids. For instance, L-Tyr and L-Phe derivatives of MPs exhibited higher antimicrobial activities (MIC, 8 and 16 mg/L, respectively) while L-Glu and L-Asn derivatives exhibited lower activities (MIC, 64 and 128 mg/L, respectively) when *Escherichia coli* was tested (Kim et al. 2006a).

Potential anti-obesity activities

The extracts of RFR by *Monascus* spp. and MPs compounds exhibited potential anti-obesity characteristics by inhibit the activities of lipases (Kim et al. 2007a, b), adipocyte cell proliferation, adipogenesis, and so on (Choe et al. 2012; Jou et al. 2010).

Some literatures showed that RFR coarse extracts and MPs could prevent obesity development by inhibiting cell proliferation, adipogenesis, lipolysis, and heparin-releasable lipoprotein lipase (HR-LPL) of 3T3-L1 preadipocyte (Choe et al. 2012; Jou et al. 2010). The L-Leu-OEt (L-leucinethylester) derivatives of MPs exhibited some specifical inhibition to porcine pancreatic lipase but not to other digestive enzymes (Kim et al. 2007a). When mice were fed with L-Trp and L-Leu-OEt derivatives of MPs, the average body weights and the intraperitoneal adipose tissues weights were reduced by 13.6–50.9 % and 16.7–30.5 %, respectively (Kim et al. 2010). Monascin and ankaflavin might reduce triglyceride accumulation and suppress expression of adipocyte-specific transcription factors to decrease proliferation and differentiation of preadipocyte related with obesity (Jou et al. 2010). Both of monascin and ankaflavin might also promote mature adipocyte delipidation by releasing glycerol and downregulating the HR-LPL activities (Jou et al. 2010).

Anti-inflammation

The MPs compounds such as the six well-known MPs (Fig. 1 I a–f), exhibited potent inhibitory effects on inflammation induced by 12-*O*-tetradecanoylphorbol-13-acetate, tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) (Akihisa et al. 2005b). Lin et al. (2011) reported that monascin and ankaflavin reduced endothelial adhesiveness which were induced by TNF-α. And monascin could significantly degenerate TNF-α and interleukin 6 (IL-6) (inflammation-associated cytokines) at both the protein and mRNA levels, it might also bind peroxisome proliferator activated receptor-γ (PPAR-γ) and regulate expression of anti-inflammatory genes (Hsu et al. 2012; Lin et al. 2011). So, monascin and ankaflavin may be potential anti-inflammation agents and beneficial for reducing the risk of vascular disease associated with inflammation (Lin et al. 2011).

Regulation of cholesterol levels

The threonine derivative of total MPs and orange MPs significantly decreased the low-density lipoprotein (LDL) level, increased the high-density lipoprotein (HDL) level and the ratio of HDL to LDL in mice sera (Jeun et al. 2008). And the inhibitory activity against HMG (hydroxy methylglutaryl)-CoA reductase and lipoprotein lipase of L-Leu-OEt of MPs was higher than that of its L-Trp derivative in vitro (Kim et al. 2010).

Anti-diabetes

Monascin was able to improve insulin sensitivity through the AKt (serine/threonine protein kinases) pathway by stabilizing PPAR-γ structure, preventing its phosphorylation, and inhibiting JNK (c-Jun *N*-terminal kinase) activation (Lee et al. 2011). Monascin also conferred several treatment-oriented properties on diabetic rats through reducing hyperglycemia, improving antioxidant ability, and protecting tissue, and a resistance against thermotolerance and oxidative stress on nematoden *Caenorhabditis elegans* by regulating the FOXO/DAF-16-dependent insulin signaling pathway (Shi et al. 2012). These findings suggest that monascin has a therapeutic potential on diabetes and diabetes-associated oxidative stress complications (Shi et al. 2012).

Molecular biology of *Monascus* pigments

Monascus spp. can produce a variety of bioactive substances including MPs, monacolins (Endo 1979), γ-amino butyric acid (Kohama et al. 1987) and so on, so structural genes coding for these metabolites attract extensive attention from worldwide researchers. Up to now, PKSs (polyketide syntheses) genes responsible for the biosynthesis of monacolins and citrinin have been cloned and identified in *Monascus* spp. (Chen et al. 2008; Shimizu et al. 2005), but there is no any report about genes of MPs synthesis. In our research group, we have cloned a PKS gene cluster with 8.1 kb of its DNA length from a T-DNA insertion library (Shao et al. 2009) of *M. ruber* M-7, and targeted-deletion of this PKS gene resulted in MPs depletion. On this basis, a 53-kb flanking DNA sequences of the PKS was cloned, and a putative gene cluster related to MPs synthesis was achieved, which consisted of PKS gene, fatty acid synthases gene, esterase gene, dehydrogenase gene, transport protein, and regulator (The data will be published in the next future).

It is well-known that G-protein-mediated signaling pathway (G-protein pathway) is conserved in filamentous fungi and plays a crucial role in transferring external signals into the cells to elicit correspondingly morphological and secondary metabolite responses (Li et al. 2007). Li et al. (2010b) cloned the *mga1* gene conding G-protein alpha subunit in *M. ruber* M-7 and constructed *mga1* deletion mutant which resulted in MPs increase, but the MPs amounts produced by *mrflbA* (encoding a regulator of G-protein alpha subunit) deletion mutant were significantly less than those produced by the wild-type M-7 in PDB medium (Yang et al. 2012). The investigation also showed beta and gamma subunit of G-protein had regulatory functions to the MPs synthesis.

Conclusion and outlook

Monascus pigments (MPs) as food colorants have been utilized in oriental countries for more than ten centuries (Li et al. 2010b). In the past two decades, more than 50 MPs compounds have been characterized due to their possessing a range of biological activities, even though the first structure of MPs component might be dated back to 1932 (Salomon and Karrer 1932). However, the investigations on the biological activity mechanism of MPs compounds, which will promote more MPs compounds to be discovered, are very limited. According to existing research results about MPs biosynthesis, the MPs synthesis should follow the polyketide pathway as other secondary metabolites (Jůzlová et al. 1996; Turner 1971), but it is still unclear and controversial. Fortunately, the genomics of *M. pilosus* and *M. ruber* M-7 (the data will be published in the next future) have been sequenced in Taiwan Food Industry and Research Institute and our laboratory, respectively, which will greatly promote the aforementioned relative investigations using the obtained genetic information.

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