Review Article

Qinyou Wen, Xiaohua Cao, Zhiting Chen, Zixiao Xiong, Jianghong Liu, Zuxin Cheng, Zhenghuai Zheng, Chuannan Long, Baodong Zheng, Zhiwei Huang*

An overview of *Monascus* fermentation processes for monacolin K production

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Abstract: In Asia, *Monascus* has been used in food fermentation for nearly a thousand years. It has attracted increasing attention in recent years due to its ability to produce a variety of important active substances such as monacolin K (MK) and pigments. MK is an effective drug widely used for lowering human blood cholesterol that functions by inhibiting the rate-limiting enzyme in cholesterol biosynthesis. *Monascus* strains, fermentation methods and fermentation conditions have significant effects on MK yield, and much research has been undertaken to obtain higher MK yields. In this paper, the research progress of *Monascus* strain breeding for high MK yield, medium optimization for MK production during *Monascus* fermentation, and optimization of fermentation process conditions are fully reviewed. This provides reference for future research on *Monascus* fermentation and industrial production for high-yield MK production.

Keywords: *Monascus*; monacolin K; fermentation.

***Corresponding author: Zhiwei Huang,** College of Food Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China; Fujian Provincial Key Laboratory of Quality Science and Processing Technology in Special Starch, Fujian Agriculture and Forestry University, Fuzhou 350002, China; China-Ireland International Cooperation Centre for Food Material Science and Structure Design, Fujian Agriculture and Forestry University, Fuzhou 350002, China, E-mail: hzwfau@163.com
Qinyou Wen, Zhiting Chen, Zixiao Xiong, Zhenghuai Zheng, Baodong Zheng, College of Food Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China
Qinyou Wen, Zhiting Chen, Baodong Zheng, Fujian Provincial Key Laboratory of Quality Science and Processing Technology in Special Starch, Fujian Agriculture and Forestry University, Fuzhou 350002, China
Xiaohua Cao, , Jianghong Liu, Zuxin Cheng, Key Laboratory of Crop Biotechnology (Fujian Agriculture and Forestry University), Fujian Province University, Fuzhou 350002, China
Zixiao Xiong, Baodong Zheng, China-Ireland International Cooperation Centre for Food Material Science and Structure Design, Fujian Agriculture and Forestry University, Fuzhou 350002, China
Chuannan Long, Jiangxi Key Laboratory of Bioprocess Engineering, Jiangxi Science and Technology Normal University, Nanchang 330013, China

1 Introduction

Monascus is a small filamentous saprophytic fungus and can be grown by inoculation into rice for fermentation, converting the rice to 'red yeast rice'. Red yeast rice is a traditional Chinese medicine that has been used in Asian food and therapeutic applications for nearly a thousand years. Its applications include coloring, preservation of traditional foods, and treatment of indigestion, diarrhea and spleen-stomach disharmony. *Monascus* fermentation can produce functional substances such as pigments, monacolin K (MK) and γ-aminobutyric acid [1], but most *Monascus* strains can also produce citrinin that exhibits liver and kidney toxicity [2]. The presence of citrinin limits the food application of *Monascus* to a certain extent.

MK, also known as lovastatin, was first obtained by separation from *M. ruber* fermentation products in 1979 by the Japanese professor Akira Endo [3]. MK inhibits cholesterol synthesis by competitively inhibiting the activity of the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), thus lowering cholesterol levels in human and animal blood. The study found at least 14 substances with hypolipidemic function in red yeast rice, including MK, monacolin J, monacolin L, monacolin M, monacolin X and their acidic structures, dehydroMK, dihydromonacolin L and compactin [4].

Common fermentation modes for *Monascus* to produce MK include solid state fermentation (SSF) and submerged fermentation (SmF). In fermentation optimization studies for MK production by *Monascus*, most research has focused on SSF methods because they offer has many advantages compared to SmF methods. Fungi can produce more enzymes and secondary metabolites in SSF, and

some enzymes can only be produced in SSF [5]. Zhang et al. [6, 7] utilized SSF by adding agar to a liquid medium, then inoculating *Monascus* into the solid medium cut into small pieces, and used a liquid medium without agar for fermentation comparison. Compared with *Monascus* SmF, biomass accumulation, MK synthesis rate, glycerol consumption rate, cell membrane fluidity and permeability, and glycerol tolerance concentration in *Monascus* under SSF conditions were significantly higher. Furthermore, MK yield of *Monascus* under SSF is usually several to dozens of times higher than that under SmF [8, 9].

Scholars have previously reviewed the research progress in lovastatin production by *Aspergillus terreus* fermentation [10]. Herein, we comprehensively review the research progress in MK production using *Monascus* fermentation processes, from the breeding of high-yield MK strains to the optimization of fermentation medium and fermentation conditions, including SSF and SmF methods.

2 *Monascus* strain breeding for high-yield MK production

Monascus strain choice has an important influence on production of MK by fermentation, and the ability of different strains to produce MK under the same culture conditions varies greatly. Usually, mutagenesis of *Monascus* is performed using separation and screening [11-13] or by physical or chemical methods to obtain strains with high yield of MK, thereby significantly increasing MK yield and reducing citrinin content. Suh et al. [14] obtained multiple mutants with high MK yields through γ-ray treatment, among which the KU609 mutant had the highest MK yield, nearly 10 times higher than the original strain. After optimization of culture conditions, MK yield of KU609 can reach 977 mg/kg. There are also studies on mutagenesis of *Monascus* strains by combining ultraviolet (UV) irradiation and LiCl treatment. UV irradiation for 45s was performed under 1.0% LiCl conditions, then five mutant strains were obtained after three rounds of irradiation. *M. purpureus* ZT35, a strain with high MK yield, was obtained through five generations of culture, which has a MK yield that is three times higher than that of the original strain [15]. In addition, MK synthesis by *Monascus* has a competitive relationship with the synthesis of red pigment. Studies have shown that treatment of *Monascus* spores with UV irradiation or ethyl methanesulfonate (EMS) can result in an albino strain that does not produce red pigment and citrinin, and its MK yield is significantly higher than that of the original strain [16].

3 Optimization of fermentation medium for MK production by *Monascus*

Due to the two fermentation modes of *Monascus*, SSF and SmF, and the significant difference in the composition of medium between SSF and SMF [5], this paper reviews the optimization of SSF and SMF medium components respectively.

3.1 Optimization of solid medium components

The types of substrates and nutrient additives were optimized to increase the MK production by *Monascus* SSF (see Table 1 for details).

3.1.1 Solid matrix

The type of solid matrix is an important factor affecting fungal growth and metabolite production. Rice is a commonly used matrix for *Monascus* fermentation [25, 26]. However, to develop a matrix more suitable for MK production than rice, researchers have used various solid matrixes for *Monascus* fermentation and compared their MK yield (see Table 2 for details). Zhang et al. [24] carried out solid-state fermentation using *M. ruber* with different kinds of grains such as rice, millet, wheat and barley. The results showed that MK yield was the highest in millet matrix (7.25 mg/g), probably due to the low viscosity and small particles of millet, while wheat (2.45 mg/g) and barley (2.69mg/g) had poor water absorption, therefore hyphae were not easy to grow, resulting in a lower MK yield.

Different rice varieties also have significant effects on MK yield from *Monascus* fermentation. Pengnoi et al. [27] used different varieties of purple rice as the matrix for *Monascus* fermentation. The results showed that MK yield (13.48 mg/kg) of purple rice variety Doi Muser as a matrix was significantly higher than that of other varieties of purple rice.

The viscosity of rice has a great influence on MK yield. Because of its relatively high viscosity and easy agglomeration, glutinous rice is not conducive to *Monascus* growth [28]. However, some researchers have studied *Monascus* fermentation with glutinous rice as a matrix, and obtained a considerable MK yield. Water content is the key control factor for fermentation with glutinous rice as matrix. Yu et al. [29] used rice and

Table 1: Optimization of the SSF Medium Components of *Monascus*.

strain	nutrients	optimal nutrients	MK production (mg/kg)	references
Monascus Mutant KU609	Nitrogen source: Histidine, Yeastextract, Glutamic, Valine Tyrosine, Alanin, Lysine Peptone, Soytone Carbon source: Glucose, Fructose, Maltose, Lactose, Glycerol, Sucrose, Galactose Inorganic salts: $MgSO_4$, $MnSO_4$, $FeSO_4$, $ZnSO_4$, $NaNO_3$, $CaSO_4$, $CuSO_4$	2.5% Soytone, 2.4% Glucose and 0.26% $MgSO_4$	977.70	Suh et al. [14]
M. purpureus KCCM 60168	Nutrients: Glucose, Peptone	1.32% glucose and 0.20% peptone	13,400	Suraiya et al. [17]
M. pilosus KMU108	Nutrients: Ganghwayakssuk, Glucose, Sucrose, Lactose, Galactose	2.2% *ganghwayakssuk* and 3.8% glucose	3,007	Lee and Lee [18]
M. purpureus TISTR 3541	Nutrients: Sodium nitrate, Methionine, Glycerol	2% glycerol, 0.14% methionine and 0.01% sodium nitrate	5,900	Jirasatid [19]
M. purpureus 9901	Nitrogen sources: peptone, yeast extract, Soybean meal, Ammonium sulfate, Sodium nitrate Carbon source: glycerol	5% soybean meal and 26% glycerol	12,900	Lu et al. [8]
M. purpureus CMU002UXX-32-44	Nutrients: NH_4Cl, $MgSO_4{\cdot}7H_2O$	4 mg/g NH_4Cl and 0.2 mg/g $MgSO_4{\cdot}7H_2O$	6,238.2	Kanpiengjai et al. [20]
M. pilosus MS-1	Nutrients: Soybean flour, Water content, Acetic acid, $MgSO_4{\cdot}7H_2O$	35% water content, 0.6% (v/w) acetic acid and 0.004 mol/kg $MgSO_4{\cdot}7H_2O$	18,733	Feng et al. [21]
M. sanguineus	Nutrients: Soybean, $CaCl_2$, Acetic acid	20 g/L soybean, 2.5 g/L $CaCl_2$ and 25 μL acetic acid	20,040	Dikshit and Tallapragada [22]
M. purpureus MTCC 369	Inorganic salts: NH_4Cl, $MgSO_4.7H_2O$, NaCl, $CaCl_2.2H_2O$	14.32 g/L NH_4Cl, 0.76 g/L $MgSO_4$, 14.65 g/L NaCl and 0.54 g/L $CaCl_2$	3,403	Kraboun et al. [23]
M. ruber	Carbon source: Glycerol Nitrogen sources: Beef extract, Peptone, Soybean powder, Yeast extract, Ammonium sulfate, Sodium nitrate	20% glycerol and 3% soybean meal	19,8100	Zhang et al. [24]

glutinous rice as substrates, and performed orthogonal design optimization of the fermentation conditions (initial water content, matrix size and fermentation time) of the two substrates. The results showed that initial water content had a large impact on MK yield fermented with the two substrates, and optimal MK yields of rice and glutinous rice were 2.50 mg/g and 2.71 mg/g, respectively. Meanwhile, water content is also closely related to fermentation time. During the fermentation process, water content will gradually decrease with water evaporation and utilization by microorganisms. Kanpiengjai et al. [20] fermented glutinous rice with an X-ray induced mutant strain *M. purpureus* CMU002UXX-32-44, and optimized its fermentation conditions. The results showed that MK yield could reach up to 6428 mg/kg under the conditions of 72.5% initial water content and 38 d fermentation time. Therefore, by effectively controlling water content of glutinous rice, it can also be considered an excellent matrix for MK production by fermentation using *Monascus*.

Other studies used non-cereal materials as the matrix for fermentation by *Monascus*. For example, yam can also be used as a matrix for fermentation. Yam has previously been associated with blood-lipid lowering effects, and red yeast yam after fermentation by *Monascus* has more significant blood fat-lowering effects than red yeast alone [30]. Lee et al. [31] used cassava, sweet potato, potato, yam

Table 2: Comparison of MK Yield by *Monascus* Fermentation Using Different Matrixes.

Strain	Matrix	Optimal Substrate	MK Production (mg/g)	References
M. ruber	Rice, millet, corn, barley, wheat	Millet	7.25	Zhang et al. [19]
M. purpureus CMU002U	Purple rice (Doi Muser; Doi Saked; Na; Nan; PHayao; Hom CMU)	Doi Muser	13.48	Pengnoi et al. [20]
M. purpureus NTU 301	Dioscorea, sweet potato, cassava, rice, potato	Dioscorea	2.58	Lee et al. [25]
M. purpureus MTCC-410	Raw rice, parboiled rice, wheat, barley, finger millet, germinated finger millet, maize, sorghum, njavara	Raw rice	0.64	Venkateswaran V. and Vijayalakshmi G. [28]
M. purpureus MTCC 369	Besan flour, jaggery, palm jaggery, black gram, green gram, barley, sago, ground nut cake, sesame waste, millet, ragi, wheat bran, rice bran, jack fruit seed, jong grain rice,	barley	193.70	Seraman et al. [29]
M. purpureus MTCC 410	Wheat bran, tamarind seed, jack fruit seed, rice bran	Wheat bran	0.094	Dikshit. et al. [30]

and rice as matrixes for fermentation, and found that MK content of M. purpureus strain NTU 301 grown on yam matrix can reach 2584 mg/kg, which is 5.37 times higher than MK yield from rice matrix (481 mg/kg). Suraiya et al. [17] and Hong et al. [32] used kelp and red ginseng, respectively, as matrixes for *Monascus* fermentation, and obtained high yields (13.98 mg/g, and 3.09 mg/kg, respectively)

3.1.2 Nutritional additives

Addition of carbon source (e.g. glucose, glycerol and lactose), nitrogen source (e.g. soybean meal, peptone and glutamic acid), inorganic salts and other additives to the solid matrix are effective methods to increase *Monascus* MK yields. Glucose can promote *Monascus* growth, which promotes the production of MK. Suraiya et al. [17] optimized the response surface methodology and found that the optimal glucose addition for Monascus solid medium was 1.32%. Suh et al. [14] also found during medium optimization experiments in mutant strain KU609, that adding 0.4 g of glucose to 20 g of rice could significantly increase its MK yield. Lee et al. [18] mixed 2.2% wormwood with 3.8% glucose and used it as a matrix for *Monascus* fermentation, finding that its MK yield was 6 times higher than that using rice as matrix.

Glycerol is also often used as a carbon source for MK fermentation of *Monascus* [6, 19]. Lu et al. [8] used bagasse as an inert carrier, and added different concentrations of glycerol (18%-34%) as a single carbon source to solid and liquid media for *M. purpureus* strain 9901. Researchers found that the optimal glycerol concentration (up to 26%) for MK production under SSF was significantly higher than under SmF, while MK yield under SmF decreased with increasing glycerol concentration and was significantly lower than that under SSF. The reason may be that cell morphology and physiological characteristics of *Monascus* under SSF are different from those under SmF, and the lower metabolic repression under SSF is more conducive to *Monascus* growth, thus the utilization rate of glycerol by thallus is also increased. Studies by Feng et al. [36] showed that when glycerol concentration was greater than 10%, metabolic repression of *Monascus* cells under SmF increased, and MK secretion was inhibited by varying degrees by increasing glycerol concentration.

Some studies have found that adding ethanol as a carbon source to medium can significantly increase MK yield and reduce citrinin content. Experimental results showed that the MK yield of *Monascus* was significantly higher than from a control group after adding 0.5% of ethanol by volume. In this study, a response surface method was used to optimize ethanol addition, culture temperature and water content. The optimal combination was: 500 g rice, 0.3% ethanol, culture temperature 30°C, 120 mL water. The MK yield under these conditions was 2.6 times higher than that of the control group, and citrinin content was reduced by nearly half [37]. However, higher concentrations of ethanol affect *Monascus* polyketide synthase (PKS) activity, resulting in polyketide synthesis inhibition (including MK, red pigment and citrinin). When the ethanol concentration reached 4%, the yield of polyketides of *Monascus* was significantly reduced. On the other hand, the biomass of *Monascus* increased at 2% and

4% ethanol concentrations relative to the control group [38].

Nitrogen is an essential nutrient for the growth of fungal hyphae. At lower concentrations, $NaNO_3$ is superior to organic nitrogen sources in increasing MK yield [39], but too high $NaNO_3$ concentration is toxic to cells, resulting in a decrease in MK yield. Soybean meal is usually added to the culture medium as a nitrogen source. Xu et al. [40] mixed rice and soybean meal as a substrate. When the additive amount of soybean meal is 20%, MK yield can reach 4.02 mg/g, which is approximately 2 times higher than MK yield (1.26 mg/g) without soybean meal. Another study was conducted to optimize the response surface of medium for MK production by strain M. pilosus MS-1 SSF. It was found that when substrate was 60% rice and 40% soybean meal, MK yielded up to 17.66 mg/g [21]. Dikshit et al. [22] also studied the additive amount of soybean meal by using wheat bran as a substrate in fermentation of strain *M. sanguineus*. The results showed that addition of 20 g/L soybean meal could significantly increase MK yield, and the highest yield was up to 20.04 mg/g. In addition, the addition of amino acids such as glutamate [41] and methionine [19] act as a nitrogen source, or precursor for MK in the medium and can increase MK yield. Kraboun et al. [23] studied the effects of varying sodium glutamate and peptone concentrations on MK yield from the *Monascus* fermentation process. Researchers found that sodium glutamate was superior to peptone in increasing MK yield under the same nitrogen content.

In many filamentous fungi, specific monovalent and divalent cations have important effects on promoting cell growth and production of metabolites. Panda *et al.* [42] used the strain *M. purpureus* MTCC 369 for SSF and optimized the inorganic salt components of the medium as a single factor. It was found that NH_4Cl, $MgSO_4$ and NaCl could greatly increase MK yield. The effects of $MnSO_4$ and $CaCl_2$ were secondary, while the effects of KH_2PO_4 and $FeSO_4$ were poor. Subsequently, the four inorganic salts (NH_4Cl, $MgSO_4$, NaCl, $CaCl_2$) were optimized using response surface methodology, and the obtained optimal combination was: NH_4Cl 14.32 g/L, $MgSO_4$ 0.76 g/L, NaCl 14.65 g/L and $CaCl_2$ 0.54 g/ L. In addition, inorganic salts also have different effects on MK yields from different strains. Dikshit et al. [35] used *M. purpureus* and *M. sanguineus* strains to optimize SSF medium by Plackett-Burman design. $MgSO_4{\cdot}7H_2O$ significantly increased MK yields of the two strains, and $MnSO_4{\cdot}7H_2O$ also had a significantly promoted MK yield from *M. sanguineus*. In addition, MK yield from the two strains significantly decrease after the addition of $CaCl_2$.

3.2 Optimization of liquid medium components

The medium components were optimized to increase the MK yield of *Monascus* SmF, such as carbon source, nitrogen source, inorganic salts, inducers and precursors (see Table 3 for details).

3.2.1 Carbon source

In SmF of *Monascus*, medium composition is crucial, and different nutrients have different effects on MK production in the thallus. Regarding the influence of carbon sources, there are inhibitory carbon sources (e.g. glucose), moderately inhibitory carbon sources (e.g. fructose and maltose) and non-inhibitory carbon sources (e.g. glycerol) [43]. Glucose plays an important role in fermentation, and it can be rapidly absorbed and utilized by the thallus in a short time, thereby promoting mycelial growth of filamentous fungi. However, when glucose is used as a single carbon source, higher concentrations of glucose (>70g/L) will inhibit the expression of MK-related genes, resulting in a decrease in MK yield [49]. Nevertheless, by combining glucose with different carbon sources, the inhibition of MK synthesis by glucose can be alleviated, thereby significantly increasing MK yield. Chang et al. [44, 50] combined higher doses of glucose (129.2 g/L) with appropriate levels (26.4 ml/L and 36.4 ml/L) of glycerol as a carbon source for *Monascus* fermentation, finding that MK yield was up to 131mg/L. Miyake et al. [43] used different carbon source combinations for a *M. pilosus* SmF process. The results showed that MK yield from fermentation with combined carbon sources was significantly greater than for a single carbon source. MK yield was significantly higher (444mg/L) when using a combination of maltose and glycerol 1:7 (v/v), than other combinations. A high glucose and glycerol condition (glucose/glycerol = 7:7) or high glycerol ratio (glucose/glycerol = 1:7) were not effective for *Monascus* production of MK, yielding 3.74 mg/L and 8.62 mg/L, respectively. An optimized glucose/glycerol ratio (3:7) could significantly increase MK production (108.43 mg/L) however. This highlights that the combination ratio of glucose and glycerol as a carbon source can significantly reduce the inhibitory effect of glucose on MK synthesis.

Table 3: Optimization of the SmF Medium Components of *Monascus*.

strain	nutrients	optimal nutrients	MK production (mg/L)	references
M. pilosus MK-1 *M. pilosus*	Carbon sources: Glucose, Maltose, Fructose, Glycerol, Lactose Nitrogen sources: Peptone	*M. pilosus* MK-1: 3% glucose, 7% glycerol and 3.8% peptone *M. pilosus*: 1% Maltose, 7% glycerol and 3.8% peptone	725 (*M. pilosus* MK-1) 444 (*M. pilosus*)	Miyake et al. [43]
M. ruber CCRC 31535	Rice powder, Peptone, Glycerin, Glucose	34.4 g/L rice powder, 10.8 g/L peptone, 26.4 ml/L glycerin and 129.2 g/L glucose	131	Chang et al. [44]
M. purpureus 410	Carbon sources: Glucose, Maltose, Fructose, Lactose, Sucrose Nitrogen sources: Peptone, Yeast extract, Ammonium chloride, Ammonium sulphate, Ammonium nitrate	4.4% maltose and 0.14% peptone	81.27	Mohan-Kumari et al. [45]
M. purpureus MTCC 369	Dextrose, NH_4Cl, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}H_2O$	29.59 g/L dextrose, 3.86 g/L NH_4Cl, 1.73 g/L KH_2PO_4, 0.86 g/L $MgSO_4{\cdot}7H_2O$ and 0.19 g/L $MnSO_4$	351	Sayyad et al. [46]
M. purpureus MTCC 369	Glucose, Peptone, NH_4Cl, KH_2PO_4, Yeast extract, K_2HPO_4, KNO_3, $MgSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}H_2O$, NaCl, $CaCl_2{\cdot}2H_2O$, FeSO4·$7H_2O$	52.61 g/L glucose, 16.65 g/L peptone, 1 g/L NH_4Cl, 1 g/L KH_2PO_4, 3 g/L yeast extract, 1 g/L K_2HPO_4, 0.5 g/L KNO_3, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.418 g/L $MnSO_4{\cdot}H_2O$, 0.5 g/L NaCl, 0.1 g/L $CaCl_2{\cdot}2H_2O$ and 0.001 g/L $FeSO_4{\cdot}7H_2O$	88.54	Seraman et al. [47]
M. purpureus 9901	Precursors: Ethanol, Sodium acetate, Sodium citrate, Trisodium citrate, Methionine, Phenylalanine Surfactants: PEG-400, PEG-4000, Tween-20, Tween-80, Span-40, Span-80 and Trition X-100	4.0 g/L sodium citrate (supplemented at 48 h) and 40.0 g/L Triton X-100	2,026.0	Zhang et al. [48]

3.2.2 Nitrogen source

Nitrogen source is another important limiting factor that affects MK yield by regulating *Monascus* growth. Peptone is usually a suitable nitrogen source for MK production by *Monascus* SmF processes [43]. Mohan-Kumari et al. [45] compared the effects of different nitrogen sources on MK yield from fermentation by *Monascus*, and found that MK yield where peptone was used as a nitrogen source was significantly greater than that of other nitrogen sources. However, MK yield is usually related to nitrogen source limitation in the stable phase. When microorganism growth is limited by the consumption of nitrogen source, redundant carbon sources are used for the production of secondary metabolites, thereby improving yield of secondary metabolism [10]. Therefore, it is necessary to limit the nitrogen source. Miyake et al. [43] combined carbon source and nitrogen source in different proportions for fermentation by *M. pilosus*. The results showed that under different carbon source combinations, even though glucose that has a strong inhibitory effect on MK yield, the optimal carbon-to-nitrogen (C/N) ratio is between 7-9.

3.2.3 Inorganic salts

Inorganic salts are also essential in SmF by *Monascus*, such as metal ions such as Fe^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Mg^{2+} and Mn^{2+}, which have been shown to promote cell growth and MK synthesis of *Monascus* in SmF [51]. Studies have shown that Mg^{2+} and Mn^{2+} have the most significant positive effect on MK production by *Monascus* SmF compared with other inorganic salts [52], and these two inorganic salts can even orchestrate their positive effects on MK synthesis when both are present [46]. In addition, Plackett-Burman design was used in other studies to analyze the effects of different inorganic salts on MK yield from *Monascus*. The

results showed that Mn^{2+} had the most significant effect on yield [47].

3.2.4 Inducers and precursors

The addition of linoleic acid also significantly increased MK yield from *Monascus* in SmF. As a quorum sensing molecule, linoleic acid can stimulate the cAMP-PKA pathway, thereby increasing the transcription level of MK synthesis-related genes, thus increasing MK yield. The addition of 512 μmol/L and 256 μmol/L linoleic acid significantly up-regulated the expression of mok A and mok H genes. When 512 μmol/L linoleic acid was added, the yield of MK was 1.35 times greater than that of the control group [53].

Studies have shown that the addition of precursors and surfactants can also increase MK yield from *Monascus* SmF. In the process of secondary metabolite biosynthesis, the precursor substance directly participates as a substrate in the synthesis of MK. Surfactant can alter the permeability of cell membranes and promote nutrient absorption and discharge of intracellular metabolites, thereby increasing MK yield [48]. Zhang et al. [48] compared the effects of different precursors (ethanol, sodium acetate, sodium citrate, trisodium citrate, methionine and phenylalanine) on MK yield, and found that various precursors had different extents of promoting effects on mycelial growth of *M. purpureus* 9901 and its MK yield. Sodium citrate had the most significant positive effect on MK yield, and the optimal promoting effect was achieved when the concentration of sodium citrate was 4 g/L and addition time was 48 h after fermentation. In this study, the effects of different surfactants on cell growth of strain *M. purpureus* 9901 and MK synthesis were also investigated. The results showed that Triton X-100 could simultaneously increase biomass and MK yield from hyphae, and the effect was optimal when the concentration was 40 g/L.

4 Fermentation process optimization for MK production by *Monascus*

Figure 1 shows that the main parameters affecting the MK production of *Monascus* fermentation products are as follows: initial moisture content of the solid matrix, initial pH of the medium, inoculum size, culture temperature and fermentation time. The optimization of these parameters

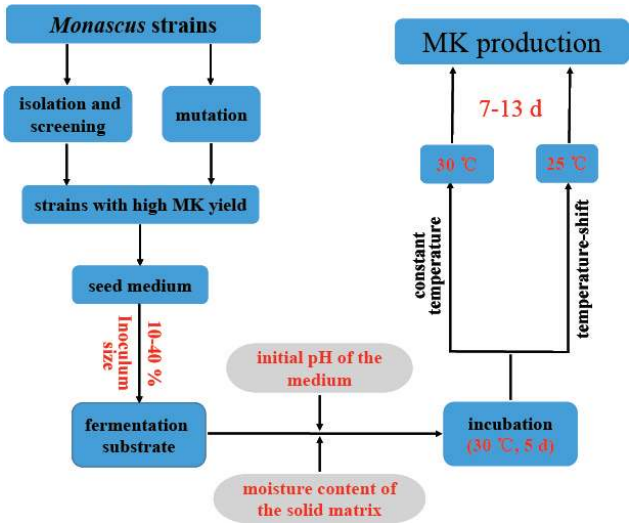

Figure 1: The Process Chart of *Monascus* Fermentation for MK Production.

was one of the research hotspots for scholars to increase the MK production of *Monascus* (Table 4).

4.1 Culture temperature

Regardless of SmF or SSF, culture temperature is critical for the growth of filamentous fungi and the production of secondary metabolites. Usually *Monascus* can grow at 18-45°C, and its optimum culture temperature is 30-38°C [57]. Studies have shown that the strain *M. purpureus* MTCC369 and *M. ruber* MTCC 1880 can obtain the highest MK yield under the constant temperature culture conditions of 29.46°C [54]. However, some reports have shown different results. For example, Suraiya et al. [17] showed that the strain *M. purpureus* KCCM 60168 had the highest MK yield at a constant temperature of 25.64°C.

In microbial cells, the optimal temperature for different enzymes varies. With ongoing deepening studies on temperature, researchers have found that variable temperature culture has a significant effect on MK yield from Monascus. Tsukahara et al. [13] separated the growth stage of *Monascus* from the production stage of MK. The screened strain *M. pilosus* NBRC4520 with high-yielding MK, was first cultured at 30°C for 4 days, and then placed at 30°C or 25°C for culture. The results showed that MK productivity was low during solid state constant temperature incubation at 30°C, but MK yield was nearly 20 times higher than that of constant temperature culture after the culture temperature was reduced from 30°C to 25°C. Pengnoi et al. [27] also found that variable

Table 4: Comparison of MK Yield by *Monascus* Fermentation Under Different parameters.

strain	fermentation parameters	optimal parameters	MK production (mg/kg)	references
M. purpureus MTCC 369 and *M. ruber* MTCC 1880 (co-culture)	Temperature, Fermentation time, Inoculum volume, pH of the solid medium	30°C, 14 d, 10% inoculum volume, pH=6	2,830	Panda et al. [54]
M. sp. KB9	Temperature, Initial moisture, Initial pH	30°C, initial moisture content 38% (w/w), initial pH=7	13,536	Subsaendee et al. [55]
M. purpureus	Initial Moisture content, Particle size, Fermentation time	initial moisture content 50%, particle size 4 cm, 13 d	2,500	Yu et al. [29]
M. purpureus MTCC 369	Temperature, Fermentation time, Inoculum volume, pH of the solid medium	29.46°C, 14.43 d, inoculum volume 5.11 mL, pH=6	3,422	Panda et al. [56]

temperature culture contributed to MK production by *Monascus* in SSF. Under the tested temperature combinations, the yield of MK cultured at 30°C and then cultured at 25°C was the highest, which was 2.8 times and 1.4 times of that cultured at constant temperature of 30°C and 25°C respectively. Studies have found that constant temperature culture at higher temperature (30°C) is more conducive to the growth of strain *M. fuliginosus* CG-6 mycelium, and dry weight of cells under constant temperature culture is significantly higher than under variable temperature culture. At lower temperatures (25°C), MK synthetic gene cluster-related genes had higher expression levels than that at higher temperatures. Under variable temperature conditions, the expression level of efflux pump protein encoded by the *mok I* gene showed the same upward trend as the *mok I* transcription level in variable temperature culture. Hence, it is more favorable for *Monascus* to secrete MK at 25°C [58].

Mohan-Kumari et al. [45] also increased MK yield of *Monascus* SmF by using a variable-temperature culture. *Monascus* was first incubated at 30°C, and then transferred to different temperatures for culture. Results indicated that in variable temperature culture, *Monascus* cultured at 25°C in the late stage had the highest MK content in the fermentation broth, but the MK content in *Monascus* cells was lower, which affected total yield of MK. When cells were cultured at 28°C in the later stage, there was greater MK concentration in both in intracellular and extracellular domains, which increased total MK yield most significantly, and MK yield was increased by nearly 60% compared with a constant temperature culture.

4.2 Unitial water content of the solid matrix

In SSF, water content of the matrix is also an important factor. Water plays an important role in the nutrient utilization and physiological activities of *Monascus*. Substrates with lower water content are not conducive to *Monascus* growth, so MK yield is adversely affected in these conditions [59]. Water content between 60%-70% can be optimal for the MK yield and biomass of the filamentous fungus. This may be due to the presence of sufficient oxygen and water in the substrate molecular layers, which supports fungal growth and metabolic heat removal. However, when the water content is increased from 80% to 90%, the oxygen in the matrix is replaced by water, leading to insufficient oxygen supply, so the biomass and MK yields from the thallus are reduced [60].

It noteworthy that the matrix particle size is an important factor affecting matrix water content. Generally, smaller substrate particles will provide a larger surface area for the attachment of microorganisms, which supports mycelial growth and metabolite accumulation [10]. However, too small a substrate size often leads to substrate aggregation, thereby causing a decrease in inter-particle voids and an increase in oxygen transfer resistance, which is disadvantageous for mycelial growth and MK production [6, 61].

In addition, different strains have different tolerances to water content. When using rice as a substrate, the optimum water content of the strain *Monascus* sp. KB9 is 38% [55], and the optimal water content of the strain *M. purpureus* is 50% [29], while the optimum water content of strain *Monascus* sp. M12-69 is 55-75% [62].

4.3 Initial pH of the medium

In *Monascus* SSF, research shows differing results for optimum pH for MK production. Lee et al. [63] prepared yam as a solid substrate of varying pH (3, 7 and 9) for Monascus fermentation, and found that strain M. purpureus NTU 301 produced more MK under acidic conditions (pH=3), and MK yield decreased as the pH increased. However, it has also been reported that when rice is fermented by Monascus sp. KB9, MK yield is highest when the substrate pH is between 5-7 [55]. Panda et al. [56] found that when rice was fermented by strain *M. purpureus* MTCC 369, the optimal pH of the matrix for MK production was 6. Therefore, the choice of optimal pH for the substrate may vary depending on the interaction between different strains, different substrates or other fermentation conditions.

The pH value of liquid medium also plays an important role in MK production by *Monascus* SmF. pH has a significant effect on the catalytic process of many enzymes and the transport of various components of the cell membrane, thus affecting the use of substrates by fungi. As reported in the literature, *A. terreus* has accelerated metabolism at higher pH (7.5) and accelerated the absorption and utilization of carbon sources, which increases MK yield [64]. Mohan-Kumari et al. [45] found that acidity of liquid medium had a significant effect on the biomass and MK yield of strain *M. purpureus* MTCC 410, and the yield of MK under low acid fermentation conditions (pH=5) was significantly higher than that at other pHs. The reason is that too-high or too-low pH affects the production of key enzymes required for MK production, and under specific pHs, some enzymes that degrade MK may be activated, resulting in decreased final MK yield. In addition, comparison between biomass and MK yields at different pHs reveals that higher biomass also affects MK production. Therefore, MK yield can only be increased only by reducing excessive accumulation of *Monascus* biomass.

4.4 Inoculum size

The inoculum size of *Monascus* also has an effect on its MK yield, and the degree of influence varies for different strains. Studies have found that when the inoculum size of *Monascus* seed solution is 10-40% of the SSF medium, there is no significant difference in MK yield by fermentation of strain *M. purpureus* 9901 [8], while MK yield of strain *M. ruber* is significantly affected. When the inoculum size exceeds 20%, MK yields of the two strains decrease to varying degrees. The reason is that *Monascus* biomass accumulates rapidly under the big inoculum size, and MK yield decreases due to premature consumption of nutrients [24]. Therefore, it's better to use an inoculum size of *Monascus* up to 20%.

4.5 Fermentation time

The substrate concentration and fermentation conditions will affect the peak time of secondary metabolites. The MK yield of *Monascus* stabilizes when it is fermented to 12-18d [13, 24, 40], and the optimum fermentation time is usually 14-15d [17, 54, 56]. As the fermentation time goes on, MK content will reach the peak value followed by a decreasing trend [15, 62]. Therefore, it is recommended to control the fermentation time of MK production by *Monascus* to about 15d.

5 Conclusion and outlook

MK is a secondary metabolite produced by filamentous fungus *Monascus*, which can significantly lower cholesterol levels in the human body. The fermentation modes of *Monascus* include SSF and SmF. Generally, MK yield under SSF is several to ten times higher than that under SmF. For an increase of MK yield from fermentation by *Monascus*, methods such as strain selection and optimization of fermentation conditions are generally used. The most commonly used method for strain selection is to adopt a physical method such as UV irradiation or chemical method such as a mutagenic agent (e.g. LiCl) to induce mutagenesis, thereby obtaining a *Monascus* strain with high MK yield.

For SmF medium of *Monascus*, the choice and addition of carbon and nitrogen source is a key factor in MK yield. The combination of different carbon sources for fermentation helps reduce the inhibition effect from single carbon sources, such as glucose on MK synthesis, thereby increasing MK yield. MK yield is usually associated with nitrogen source limitation during the stable phase. When *Monascus* growth is inhibited by nitrogen source limitation, redundant carbon sources can be used for secondary metabolite production, thereby improving secondary metabolite yield. Therefore, it is necessary to effectively control C/N ratio of the medium, and the optimal ratio is 7-9. In addition, appropriate exogenous inducing substances (metal ions, linoleic acid,

etc.), precursors (sodium citrate, sodium acetate, etc.) or surfactants can also increase MK yield of *Monascus* to some extent.

For SSF medium of *Monascus*, in addition to containing sufficient nutrients, the matrix should meet conditions such as low viscosity, low agglomeration, easy moisture absorption, appropriate water content and have small particles. In addition, nutrients such as glycerin and glucose as a carbon source, soybean meal and peptone as a nitrogen source, and metal ions such as Mg^{2+} and Zn^{2+} can significantly increase MK yield from *Monascus*.

In terms of fermentation conditions of *Monascus*, variable temperature cultures are useful for an increase of MK yield. The reason is that the growth and development of *Monascus* cells differs from MK synthesis-related enzymes by their optimum temperature, and growth and development and MK synthesis are subject to subsection adjustment by variable temperature cultures. First, *Monascus* are incubated at 30°C for 3-5 d, and then adjusted to approximately 25°C, which significantly increases MK yield. For initial pH of the medium, it is better to be controlled at 4~6. For optimum conditions the inoculum size of *Monascus* seed solution should not exceed 20% of the SSF medium, fermentation time should be maintained for approximately 15d, and initial water content of the solid matrix in SSF should be controlled at 50~70%.

Nowadays, the research and industrial production technology of MK production by *Monascus* SSF is mature, but the research and industrial application of SmF is relatively scarce. Compared with SSF, *Monascus*' SmF has the advantages of lower labor intensity, better automation technology, stable product quality, short production cycle and low cost. Furthermore, SmF is more conducive to large-scale factory production and market competitiveness of fermentation products. Therefore, use of SmF for MK production will be an inevitable trend in the future production of MK by *Monascus* fermentation processes.

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