



Title	Supplementary effect of whey components on the monascin productivity of <i>Monascus</i> sp.
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1 **Supplementary effect of whey components on the monascin productivity**
2 **of *Monascus* sp.**

3 **Short running title: Effect of whey on monascin productivity**

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15 **Abstract**

16 **BACKGROUND:** *Monascus* sp. has been used for fermented foods for centuries. It can
17 synthesize yellow, red and orange pigments as secondary metabolites. Here, we focused
18 on yellow pigment monascin, responsible for anti-inflammation and antidiabetic effects,
19 and evaluated that if whey could be a suitable substrate with or without rice powder for
20 monascin production using *M. purpureus* AHU 9085, *M. pilosus* NBRC 4520 and *M.*
21 *ruber* NBRC 32318.

22 **RESULT:** Growth and monascin production of the three *Monascus* strains were
23 dependent on the three liquid media consisting of whey and/or rice. All strains showed
24 the best growth in rice and whey mixed medium where *M. ruber* NBRC 32318 exhibited
25 the highest total monascin production. Subsequent investigation on the effects of whey
26 components indicated a mineral cocktail in whey was outstandingly stimulative for the
27 monascin production efficiency of *M. ruber* NBRC 32318. However, this recipe exhibited
28 lesser stimulation or even inhibition for *M. pilosus* NBRC 4520 and *M. purpureus* AHU
29 9085, respectively. In terms of total monascin production, rice with whey provided the
30 highest level due to the growth promotion along with a relatively high production
31 efficiency.

32 **CONCLUSION:** Effect of whey on the growth and monascin production was highly
33 dependent on the *Monascus* strains. Even a mineral cocktail in whey could regulate
34 monascin productivity in a strain specific manner. Further studies are needed to elucidate
35 the mechanism behind the diverse response by the minerals on the monascin production
36 of *Monascus* species.

37 **KEYWORDS:** *Monascus* sp.; monascin; whey; simulated milk ultrafiltrate

38 Introduction

39 Some species of *Monascus*, often called red mold, have been regarded as edible
40 filamentous fungi. Rice fermented by *Monascus*, known as red yeast rice, has been
41 commercialized in East Asia since ancient times.¹ Besides, *Monascus* is recorded to have
42 a long history of fermenting grains especially beans named Tofunyu and Tofuyo in China
43 and Japan, respectively.² In recent years, red ginseng, garlic and durian seed are being
44 explored for the development of a novel type of fermented foods using *Monascus* sp..³

45 *Monascus* sp. can produce various secondary metabolites including bio-functional
46 substances and pigments.^{4,5} Monacolin K is a colorless metabolite and has been known
47 to lower cholesterol in the plasma due to the inhibitory effect of 3-hydroxy-3-
48 methylglutaryl-coenzyme A reductase.⁶ *Monascus* pigments have been favored as natural
49 pigments, and widely used in the food industry as a color intensifier and food additives.
50 Until now, more than 100 members have been identified,⁷ and yellow pigments (citrinin,
51 monascin and ankaflavin), orange pigments (rubropunctatin and monascorubrin), and red
52 pigments (rubropunctamine and monascorubramine) are representative. Among yellow
53 pigments, monascin and ankaflavin have been shown to have antioxidant, anti-cancer,
54 antimicrobial, anti-inflammation, anti-obesity and antidiabetic effects^{8,9} while citrinin is
55 thought to be hepatotoxic and nephrotoxic.¹⁰

56 Many efforts have been made to increase the secondary metabolite production of
57 *Monascus* sp..⁷ Although the review by Feng has shown that filamentous fungi can
58 produce more abundant secondary metabolites on solid culture than liquid culture,¹¹
59 liquid culture is frequently used due to its flexible applicability to the regulation of culture
60 conditions.^{12, 13} Many parameters, including carbon and nitrogen sources, pH,
61 temperature, and aeration, affect secondary metabolite production. Accordingly, a
62 synthetic medium is a convenient method for establishing the ideal composition and
63 identifying crucial factors for secondary metabolite production.

64 In addition to a synthetic medium, agro-industrial by-products, such as orange-peel
65 waste, sugarcane bagasse, and whey, have been applied for the substrate¹⁴⁻¹⁹ to effectively
66 use resources while increasing secondary metabolite production. Kantifedaki *et al.*¹⁵ have
67 used orange peel waste as the sole source of nutrients for higher total pigment production
68 by *M. purpureus* when comparing solid, semi-solid, and submerged fermentation. Solid
69 fermentation showed **high yellow pigment production efficiency**. Velmurugan *et al.*¹⁶
70 have applied corn cobs as a substrate for *M. purpureus* in solid fermentation for red and
71 yellow pigment production and found abundant yellow pigment production, which also
72 supports the use of solid culture. Silveira *et al.*¹⁷ have used sugarcane bagasse
73 supplemented with cheese whey powder as an organic nitrogen source for the production
74 of red pigment but found that the contribution of the cheese whey powder was limited.
75 However, some studies have claimed that cheese whey could be beneficially applied to
76 *Monascus* pigment production.^{18, 19} Thus, the value of whey for *Monascus* pigment
77 production remains unclear.

78 When a solid whey culture was used, *M. purpureus* AHU 9085 produced citrinin,
79 whereas *M. pilosus* NBRC 4520 and *M. ruber* NBRC 32318²⁰ produced monacolin K;
80 however, none of these strains produced monascin (Huang and Li, unpublished results).
81 On the other hand, the monascin-producing capability of *M. purpureus* AHU 9085 and
82 *M. pilosus* NBRC 4520 has been reported in rice or potato dextrose liquid culture.^{21, 22}
83 Based on these results, the question of using whey for monascin production is whether it

84 contains unfavorable components, or a solid-state culture is unsuitable for monascin
85 production.

86 In this study, we conducted specific analysis of monascin productivity to establish the
87 value of whey as the *Monascus* substrate because a study focused on one type of total
88 pigment productivity would be too broad and vague. Since rice powder containing
89 submerged fermentation is likely to be suitable for high monascin production by
90 *Monascus* M9, as shown by Chen *et al.*,²³ we compared and evaluated the effect of whey
91 on monascin production with or without rice powder in the liquid medium using three
92 *Monascus* strains considering the strain diversity. Furthermore, the whey was fractionated
93 to identify the components responsible for a higher yield of monascin production.

94 MATERIALS AND METHODS

95 Strains and Media

96 Strains used in this study were *M. purpureus* AHU 9085 from the culture collection of
97 Hokkaido University, *M. pilosus* NBRC 4520 and *M. ruber* NBRC 32318 obtained from
98 Biological Resource Center (Chiba, Japan). Those were grown on potato dextrose agar
99 (PDA; Merck KGaA, Darmstadt, Germany) at 30°C for 10 days. Spore suspensions were
100 prepared by adding 9.0 g L⁻¹ sodium chloride solution into the grown culture and counted
101 using a hemocytometer. One hundred microliters of the spore suspension (1.3×10^5
102 spores mL⁻¹) were inoculated into a 10 g experimental liquid medium in a 100-mL flask.

103 As the basal medium, 0.5 g of commercial rice powder containing 81.9% carbohydrate,
104 6.0% protein, 0.7% lipids (Kouta Shouten Co., Ltd., Ibaraki, Japan) was mixed with Milli-
105 Q water and adjusted to pH 4.0 with hydrochloric acid to obtain 10 g of the working
106 medium. To prepare the whey medium, 0.6 g of whey powder (Meiji Co., Ltd., Tokyo,
107 Japan) was dissolved in Milli-Q water and adjusted to pH 4.0 with hydrochloric acid to
108 obtain 10 g of the working medium.

109 As the alternative to whey, a total of 10 g of the basal medium was supplemented with
110 0.52 g of whey permeate powder (Meiji Co., Ltd.), in which the lactose level was adjusted
111 to the equivalent to that of whey powder. Moreover, acid whey was prepared by
112 acidifying of raw skim milk, obtained from the experimental farm in the Field Science
113 Center for the Northern Biosphere in Hokkaido University, to pH 4.6 with 1 mol L⁻¹
114 hydrochloric acid at 20°C, followed by the recovery of the centrifuged supernatant. The
115 resulting acid whey (9.5 g) was mixed with 0.5 g of rice powder and adjusted to pH 4.0
116 with hydrochloric acid.

117 The amounts of lactose, vitamins, and minerals added to the basal medium are shown in
118 Table 1, which were decided referred to the literature.²⁴ As for the minerals, we used a
119 simulated milk ultrafiltrate (SMUF) developed by **Jenness** and Koops²⁵ to mimic the
120 mineral composition in whey. According to the instructions, SMUF should be prepared
121 by mixing SMUF I and SMUF II. Furthermore, we divided SMUF I into group i and
122 group ii. Group i stands for citrate-compounds-free SMUF I whereas group ii contains
123 SMUF I without the components of group i. We also set group iii, in which the citrates in
124 group ii are replaced by phosphates to exclude the effect of citrates. The supplemented
125 basal media were adjusted to pH 4.0 with hydrochloric acid. Ten grams of the resulting
126 media were transferred into a 100-mL flask.

127 All the media used in this study were autoclaved at 121°C for 15 min. Cultivation was
128 performed statically at 25°C for 10 days. The results of more than six preparations of each
129 culture condition were subjected to the statistical analysis.

130 **Chemicals**

131 Lactose, KH₂PO₄, K₂SO₄ and tri-potassium citrate monohydrate were purchased from
132 Kanto Chemical Co., Inc., Tokyo, Japan. Vitamins (VB1, VB2 and sodium(+)-
133 pantothenate), KCl, K₂CO₃ and tri-sodium citrate dihydrate were from FUJIFILM Wako
134 Pure Chemical Corporation, Osaka, Japan and NaH₂PO₄ was from Nacalai Tesque, Inc.,
135 Kyoto, Japan.

136 **Measurement of lactose in whey and whey permeate powder**

137 Lactose content was measured by the Lane-Eynon method.²⁶

138 **Recovery and measurement of wet biomass**

139 The culture products were filtered by a strainer to recover mycelia as the intracellular
140 fraction. The mycelia were washed by pouring 20 mL Milli-Q water. After a brief
141 absorption of excess water with a cloth, the samples were weighed to evaluate wet
142 biomass. The filtrate was freeze-dried and ground into powder to evaluate it as the
143 extracellular fraction. Intracellular and extracellular fractions were stored at -20°C until
144 use.

145 **Extraction and quantification of monascin**

146 Extraction of monascin was performed according to the study of Li *et al* with some
147 modifications.²⁷ Intracellular and extracellular fractions were suspended in 10 mL of 80%
148 ethanol. After heating at 60°C for 1 h, the samples were centrifuged at 15,000 × g at 25°C
149 for 10 min to recover the supernatant followed by filtration through filter paper (No. 5C,
150 Advantec, Tokyo, Japan). The filtrate was evaporated under a vacuum and re-dissolved
151 in acetonitrile. The suspension was filtered by a 0.5 µm syringe filter (TOSOH
152 Corporation, Tokyo, Japan) and subjected to high-performance liquid chromatography
153 (HPLC) analysis. The sum of the monascin amount in intracellular and extracellular
154 fractions was expressed as total monascin production (µg). Monascin production
155 efficiency (µg g⁻¹) was expressed as total monascin production per wet biomass.

156 **High performance liquid chromatography (HPLC)**

157 Determination of monascin was performed by HPLC according to Wu²⁸ with some
158 modifications. The stationary phase of HPLC was a TSK gel ODS-100Z reversed-phase
159 column (250 mm × 4.6 mm, particle diameter: 5 µm, TOSOH Corporation, Tokyo, Japan)
160 with temperature setting at 40°C. The mobile phase was 62.5% acetonitrile and 37.5%
161 Milli-Q water containing 0.05% trifluoroacetic acid. Ten microliters of the samples were
162 injected and eluted isocratically at the flow rate of 1.0 mL min⁻¹. Standard curve was
163 made using commercial monascin (Chengdu Biopurify Phytochemicals Ltd., Chengdu,
164 China). Detection was carried out by a UV detector at 234 nm.

165 **Statistical analysis**

166 The values were analyzed using the Tukey-Kramer multiple comparison test or student's
167 t-test. The data were analyzed by JMP software (version 16.1; SAS Institute, Inc., Tokyo,
168 Japan). Differences were considered to be statistically significant at $P < 0.05$.

169 RESULTS

170 Effects of rice and whey on growth and monascin production of three *Monascus* 171 strains

172 Table 2A shows the wet biomass, total monascin production, and production efficiency
173 of three *Monascus* strains in the three media. Despite the varied monascin amounts found
174 in these three media, rice, as well as whey, enabled *Monascus* to produce monascin while
175 growing.

176 *M. purpureus* AHU 9085 grew much better in the basal medium than in whey, but the
177 basal medium containing whey produced the highest biomass. Whey caused limited
178 monascin production and reduced the production efficiency of monascin when it was
179 supplied in the basal medium. Thus, the basal medium was the best, and the whey had a
180 negative effect on monascin production by *M. purpureus* AHU 9085.

181 In contrast to *M. purpureus* AHU 9085, *M. pilosus* NBRC 4520 grew better in whey
182 than in the basal medium. The highest biomass was recorded using the basal medium with
183 whey. In terms of monascin production, whey was inferior to the basal medium, as was
184 found in *M. purpureus* AHU 9085. The total monascin production increased when whey
185 was added to the basal medium. However, the increase was due to the increase in fungal
186 growth as the production efficiencies of the basal medium and the basal medium with
187 whey powder were comparable. Thus, for *M. pilosus* NBRC 4520, whey contributed to
188 growth rather than monascin production.

189 Unlike the other two strains, there was no significant difference between the wet biomass
190 of the basal medium and that of the whey for *M. ruber* NBRC 32318. The basal medium
191 with whey yielded the highest wet biomass likewise other two strains. In terms of
192 monascin production, there was no significant difference between the basal medium and
193 whey. However, the total monascin production and production efficiency in the basal
194 medium with whey increased by 11.6- and 4.1-fold, respectively, compared to the basal
195 medium. Thus, for *M. ruber* NBRC 32318, the basal medium with whey provided
196 remarkably superior monascin production.

197 Table 2B shows the distribution of monascin in the three strains. With few exceptions,
198 monascin was commonly located in the intracellular fraction. The ratio of intracellular to
199 extracellular monascin tended to increase as the total production increased and reached
200 90% under high production conditions.

201 Thus, the effect of whey on growth and monascin production depends on the strain.
202 Since adding whey to the basal medium was exclusively advantageous for *M. ruber*
203 NBRC 32318, further investigation was conducted to identify the substances in whey that
204 stimulate monascin production by this strain.

205 **Tracing stimulative substances in whey**

206 Table 3A shows the wet biomass, total monascin production, and production efficiency
207 of *M. ruber* NBRC 32318 using three different types of whey to supplement the basal
208 medium. The addition of whey permeate resulted in significantly lower biomass.
209 Nevertheless, the total monascin production and production efficiency were comparable
210 in these three groups. As acid whey and whey permeate are devoid of lactic starter and
211 proteinaceous components compared to whey powder, these components were unlikely
212 to be involved in the stimulation effect.

213 Table 3B shows the wet biomass, total monascin production, and production efficiency
214 of *M. ruber* NBRC 32318 using lactose, minerals, and vitamins in whey. The difference
215 was exclusively found in the basal medium supplemented with SMUF, which allowed
216 significant growth promotion and increased monascin production. The total monascin
217 production and production efficiency were increased 4.7- and 2.2-fold, respectively.
218 Therefore, the stimulative components were likely to be included in SMUF.

219 Table 3C shows the wet biomass, total monascin production, and production efficiency
220 of *M. ruber* NBRC 32318 using SMUF I and SMUF II. The addition of either SMUF I or
221 II did not affect the biomass compared to the basal medium. However, the addition of
222 SMUF I resulted in remarkable monascin production and the highest production
223 efficiency, whereas that of SMUF II was comparable with that of the basal medium.

224 Subsequently, Group i, Group ii, and Group iii were used to determine the stimulative
225 candidates for monascin production (Table 3D). An insignificant difference in wet
226 biomass was found in these groups. Group i showed more stimulative effects than Groups
227 ii and iii, which implied that neither citrate nor sodium ion supply affects monascin
228 productivity. Nevertheless, the stimulative extent was reduced unless whole component
229 of SMUF I was supplied. Thus, not a single component, but the mineral cocktail in SMUF
230 I is the key component for stimulating the monascin production of *M. ruber* NBRC 32318.

231 **Supply of SMUF I in the basal medium to other *Monascus* species**

232 Table 4 shows the wet biomass, total monascin production, and production efficiency of
233 the three strains in basal medium supplied with SMUF I. Adding SMUF I into the basal
234 medium reduced the growth of *M. purpureus* AHU 9085. The monascin production and
235 production efficiency of this strain also dramatically decreased. In contrast, there was no
236 significant difference in the wet biomass, irrespective of the use of SMUF I, for *M. pilosus*
237 NBRC 4520. Adding SMUF I led to a total monascin production twice as high as that in
238 the basal medium, attributed to elevated production efficiency. For *M. ruber* NBRC
239 32318, the addition of SMUF I into the basal medium induced 6.7- and 6.4-fold increases
240 in monascin production and production efficiency, respectively. Therefore, the
241 supplementary effect of SMUF I for monascin production is highly dependent on the
242 strain.

243 **DISCUSSION**

244 To determine the effect of whey on the secondary metabolite production, for monascin
245 in particular, we surveyed three strains belonging to three representative *Monascus*
246 species and applied three culture media that consisted of rice powder, whey powder, or

247 its mixture. Although the growth preference of the three strains toward rice and whey
248 depended on the strains, it was equally promoted in the mixed culture. Monascin was
249 predominately found in the intracellular fraction with variable yields depending on the
250 strain and substrate. In this study, a remarkable increase of monascin production
251 efficiency in the basal medium with whey was exclusively recognized in *M. ruber* NBRC
252 32318. Application of three types of whey or representative whey components to the basal
253 medium revealed that the minerals in SMUF I were responsible for the stimulation of
254 monascin production efficiency. The effect of SMUF I was stimulatory to some extent
255 for *M. pilosus* NBRC 4520 as well. However, it was rather inhibitory for *M. purpureus*
256 AHU 9085. Therefore, whey can be advantageous for some *Monascus* strains.

257 Many environmental factors affect the growth and secondary metabolite biosynthesis
258 profiles of *Monascus*. Since yellow pigment production is likely to be promoted below
259 pH 4,²⁹ we seeded the tested strains into acidic cultures. Although it was already known
260 that the three strains used in this study could grow on whey solid substrate, the catabolic
261 capability toward whey components depends on the strain, as shown in the biomass
262 resulting from liquid whey cultivation. Consistent with previous studies, *M. purpureus*
263 AHU 9085 and *M. pilosus* NBRC 4520 exhibited monascin production, and the former
264 preferred the rice liquid medium to the whey liquid medium. In contrast, *M. ruber* NBRC
265 32318 showed comparable monascin production in rice and whey liquid cultures, which
266 suggested that liquid cultivation is more suitable for monascin production than solid
267 cultivation because monascin was undetectable when this strain was seeded on the whey
268 solid medium (Huang and Li, unpublished results). It was noteworthy that applying whey
269 to the basal medium yielded outstanding monascin production and production efficiency
270 by *M. ruber* NBRC 32318. Thus, the effect of whey on growth and monascin production
271 was proven to depend on the strain.

272 Further studies regarding the whey component responsible for monascin production
273 were performed on *M. ruber* NBRC 32318. As the first step, the basal medium was
274 supplied with whey of three different origins. Compared with acid whey, commercially
275 available whey powder is often prepared from cheese whey, which contains additional
276 components, such as C-terminal region peptide from κ -casein and lactic starter
277 metabolites.³⁰ Meanwhile, whey permeate is devoid of the majority of whey proteins,
278 which comprise 13% of the solid component of whey.^{31, 32} No significant monascin
279 productivity difference was found between the three types of whey; therefore, whey
280 protein, the C-terminal region peptide from κ -casein, and metabolites of lactic starter
281 bacteria are not involved in the stimulation of monascin production. Furthermore, lactose,
282 which accounts for around 75% of the solid component of whey, and vitamins did not
283 assist in monascin production. In contrast, the minerals in SMUF I exhibited a stimulative
284 effect on monascin production and were associated with the highest monascin production
285 efficiency. Accordingly, the components in whey responsible for the promotion of *M.*
286 *ruber* NBRC 32318 monascin production were not specific to dairy products but to well-
287 known minerals.

288 Further studies were conducted on the mineral component in whey related to monascin
289 production by *M. ruber* NBRC 32318. Although many studies on the effect of minerals
290 on *Monascus* yellow pigment production have been conducted using spectrophotometric
291 analysis,^{12, 13, 27} few studies have been concerned with specific monascin production
292 analysis. Lung *et al.*³³ have reported that the addition of Mg, Ca, Zn, or Fe to water
293 increased monascin production when dioscorea was subjected to solid-state fermentation

294 with *M. purpureus*. However, for monascin production of *M. ruber* NBRC 32318, the Ca
295 and Mg in SMUF II showed no effect, or rather canceled the stimulation effect of SMUF
296 I to yield production levels equal to the whole SMUF. Lin *et al.*³⁴ have found that
297 removing KH₂PO₄ from a rice-containing liquid medium caused a marked decline in the
298 growth and pigmentation of *M. pilosus*, which implies potassium has a crucial role.
299 Although the working basal medium in this study was assumed to contain 0.75, 2.3, and
300 5.7 μmol of calcium, magnesium, and potassium, respectively³⁵ as the background,
301 SMUF provided as much as 85, 30, and 353 μmol of calcium, magnesium, and potassium,
302 respectively. Taking this difference into account, it is possible that potassium is crucial
303 for monascin production by *M. ruber* NBRC 32318, whereas divalent metal ions are
304 important for *M. purpureus*. Continuous studies are needed where minerals crucial to a
305 secondary metabolite are present in the target substance and the strain applied.

306 To elucidate the species- or strain-dependence on monascin production resulting from
307 minerals, molecular analysis, as well as synthetic media-based studies, are required. The
308 biosynthetic pathways of monascin of both *M. ruber* and *M. purpureus* have been
309 proposed with related pigment gene clusters.³⁶ Adjusting the culture condition leading to
310 up-regulation of *mppE*, the gene encoding an enoyl reductase in *M. purpureus* related to
311 the final conversion toward monascin formation could increase monascin production.^{37,}
312 ^{38, 39} In contrast, the orthologue of *mppE*, *mrpigH* in *M. ruber*, has been proposed as less
313 essential for monascin production.⁴⁰ Furthermore, in addition to the proposed
314 biosynthesis pathways, the involvement of isozymes encoded on other gene clusters to
315 provide some intermediate products in the monascin synthesis pathway has not been ruled
316 out. For instance, *M. ruber* encodes **MrPigA**, whose function is predicted as polyketide
317 synthase that assembles a hexaketide intermediate³⁶ at the early stage of *Monascus*
318 pigment production, while four polyketide biosynthetic systems were found in a *M. ruber*
319 strain.²¹ Thus, it should be investigated that if the regulation of these alternative genes in
320 the presence of some minerals to take part in monascin production.

321 Finally, the genetic background of the three edible strains used in this study should be
322 discussed. Some studies have claimed *M. ruber* and *M. pilosus* could belong to the same
323 clade,^{41, 42} based on the high homology of the nucleotide sequence of the ITS region and
324 partial β-tubulin genes, while *M. purpureus* could be classified in another. Higa *et al.*²¹
325 have analyzed the secondary metabolite biosynthetic gene clusters and concluded that *M.*
326 *purpureus* and *M. pilosus* are chemotaxonomically different, while *M. pilosus* and *M.*
327 *ruber* have similar biosynthetic and secondary metabolite gene clusters. This was
328 supported by another study that claimed the genetic organization of the *M. purpureus*
329 pigment cluster is significantly different from that of *M. pilosus* and *M. ruber* in one
330 region.⁴³ The results were consistent with our study: *M. pilosus* and *M. ruber* exhibited
331 different degrees of stimulatory response, while *M. purpureus* showed a negative
332 response to the same substrate for monascin production. Similar trends were also found
333 in mycotoxin citrinin production, as citrinin was undetectable in all culture products of
334 *M. pilosus* NBRC 4520 and *M. ruber* NBRC 32318 in our study, while *M. purpureus*
335 AHU 9085 produced it under most of the culture conditions tested (data not shown). Thus,
336 *M. purpureus* often exhibits discriminated traits from *M. pilosus* and *M. ruber*, along with
337 the opposite trend of monascin production brought by supplementation of whey
338 constituents.

339 In conclusion, the applicability of whey as the substrate component for monascin
340 strongly depends on the strain. Although monascin production efficiency was higher in

341 rice liquid medium supplied with SMUF I than that with whey, whey provided the highest
342 biomass resulting in the highest total monascin production. Thus, whey is a feasible
343 supplementary component for monascin production for some *Monascus* strains.

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348 CONFLICT OF INTEREST

349 The authors declare that they have no conflict of interest.

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461 Table 1 Components and the amount added in the basal medium

Components			Amount per 10 g medium	
Whey constituents				
lactose			0.45 g	
vitamins	vitamin B1		30 µg	
	vitamin B2		15 µg	
	sodium (+)-pantothenate		40 µg	
SMUF	SMUF I	Group i	KH ₂ PO ₄	110 µmol
			K ₂ SO ₄	9.8 µmol
			K ₂ CO ₃	21 µmol
			KCl	76 µmol
	SMUF I	Group ii	tripotassium citrate monohydrate	35 µmol
			trisodium citrate dihydrate	58 µmol
	SMUF II	CaCl ₂ ·2H ₂ O		85 µmol
		MgCl ₂ ·6H ₂ O		30 µmol
Others				
Group iii	KH ₂ PO ₄		105 µmol	
	NaH ₂ PO ₄		173 µmol	

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Table 2A Growth and monascin production of the three *Monascus* strains.

Medium/ Strain	<i>M. purpureus</i> AHU 9085	<i>M. pilosus</i> NBRC 4520	<i>M. ruber</i> NBRC 32318
	Wet biomass (g)		
basal medium	2.5 ± 0.1 ^{xa}	0.9 ± 0.1 ^{xb}	1.1 ± 0.1 ^{xb}
whey	0.9 ± 0.1 ^{ya}	1.5 ± 0.1 ^{yb}	1.3 ± 0.1 ^{xab}
basal medium + whey	4.3 ± 0.2 ^{za}	3.0 ± 0.1 ^{zb}	3.1 ± 0.1 ^{yb}
	Total monascin production (µg)		
basal medium	496.0 ± 43.2 ^{xa}	283.3 ± 19.0 ^{xb}	268.3 ± 13.2 ^{xb}
whey	4.9 ± 1.3 ^{ya}	30.1 ± 7.6 ^{xa}	206.6 ± 22.5 ^{xb}
basal medium + whey	335.8 ± 55.1 ^{za}	1023.9 ± 338.2 ^{ya}	3125.3 ± 243.9 ^{yb}
	Monascin production efficiency (µg g ⁻¹)		
basal medium	198.8 ± 19.9 ^{xa}	312.8 ± 34.5 ^{xb}	252.9 ± 14.2 ^{xab}
whey	5.3 ± 1.3 ^{ya}	20.3 ± 5.2 ^{ya}	176.1 ± 27.6 ^{xb}
basal medium + whey	77.1 ± 10.9 ^{za}	338.5 ± 110.3 ^{xa}	1028.6 ± 94.2 ^{yb}

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Table 2B Distribution of monascin in the three *Monascus* strains.

Medium/ Location	Intracellular (µg)	Extracellular (µg)	Ratio (Intracellular/Extracellular)
	<i>M. purpureus</i> AHU 9085		
basal medium	463.5 ± 39.0 ^x	32.5 ± 4.9 ^x	93: 07
whey	1.3 ± 0.9 ^y	3.6 ± 0.8 ^y	27: 73
basal medium + whey	315.7 ± 56.3 ^z	20.1 ± 2.1 ^z	94: 06
	<i>M. pilosus</i> NBRC 4520		
basal medium	214.3 ± 21.0 ^x	68.9 ± 8.0 ^{xy}	76: 24
whey	18.5 ± 4.5 ^x	11.6 ± 3.1 ^x	61: 39
basal medium + whey	916.0 ± 312.1 ^y	107.9 ± 31.8 ^y	89: 11
	<i>M. ruber</i> NBRC 32318		
basal medium	214.3 ± 13.5 ^x	54.0 ± 3.6 ^x	80: 20
whey	158.3 ± 20.0 ^x	48.2 ± 4.3 ^x	77: 23
basal medium + whey	2812.8 ± 232.9 ^y	312.5 ± 22.6 ^y	90: 10

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Value is expressed as mean ± SE (n = 6, except n = 26 of the basal medium in *M. ruber* NBRC 32318 and n = 15 of the basal medium with whey in *M. ruber* NBRC 32318).

x, y and z in a column indicate a significant difference between the media for the strains (Tukey-Kramer; P < 0.05).

a, b and c in a row indicate a significant difference between the strains for the media (Tukey-Kramer; P < 0.05).

478 Table 3A Growth and monascin production in the basal medium with different types of whey of
479 *M. ruber* NBRC 32318

Component added in basal medium	n	wet biomass (g)	Total monascin production (μg)	Monascin production efficiency ($\mu\text{g g}^{-1}$)
whey	15	3.1 \pm 0.1 ^x	3125.3 \pm 243.9 ^x	1028.6 \pm 94.2 ^x
acid whey	6	3.1 \pm 0.1 ^x	2998.3 \pm 126.7 ^x	987.4 \pm 74.6 ^x
whey permeate	6	2.3 \pm 0.1 ^y	2438.6 \pm 199.9 ^x	1077.3 \pm 83.4 ^x

480 Table 3B Growth and monascin production in the basal medium with different components in
481 whey of *M. ruber* NBRC 32318
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Component added in basal medium	n	wet biomass (g)	Total monascin production (μg)	Monascin production efficiency ($\mu\text{g g}^{-1}$)
basal medium (control)	26	1.1 \pm 0.1 ^x	268.3 \pm 13.2 ^x	252.9 \pm 14.2 ^x
lactose	6	1.1 \pm 0.1 ^x	341.4 \pm 42.2 ^x	332.0 \pm 48.9 ^x
SMUF	12	2.3 \pm 0.1 ^y	1256.9 \pm 82.2 ^y	553.6 \pm 41.3 ^y
Vitamins	6	1.0 \pm 0.1 ^x	282.9 \pm 18.5 ^x	301.8 \pm 20.6 ^x

483 Table 3C Growth and monascin production in the basal medium with different components in
484 SMUF of *M. ruber* NBRC 32318
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Component added in basal medium	n	wet biomass (g)	Total monascin production (μg)	Monascin production efficiency ($\mu\text{g g}^{-1}$)
basal medium (control)	26	1.1 \pm 0.1 ^x	268.3 \pm 13.2 ^x	252.9 \pm 14.2 ^x
SMUF	12	2.3 \pm 0.1 ^y	1256.9 \pm 82.2 ^y	553.6 \pm 41.3 ^y
SMUF I	12	1.1 \pm 0.1 ^x	1786.1 \pm 143.2 ^z	1612.4 \pm 90.8 ^z
SMUF II	6	1.1 \pm 0.1 ^x	222.1 \pm 19.5 ^x	206.1 \pm 20.1 ^x

487 Table 3D Growth and monascin production in the basal medium with different components in
488 SMUF I *M. ruber* NBRC 32318
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Component added in basal medium	n	wet biomass (g)	Total monascin production (μg)	Monascin production efficiency ($\mu\text{g g}^{-1}$)
basal medium (control)	26	1.1 \pm 0.1 ^x	268.3 \pm 13.2 ^x	252.9 \pm 14.2 ^x
SMUF I	12	1.1 \pm 0.1 ^x	1786.1 \pm 143.2 ^y	1612.4 \pm 90.8 ^y
Group i	6	1.3 \pm 0.1 ^x	971.8 \pm 60.4 ^z	763.2 \pm 68.2 ^z
Group ii	6	1.0 \pm 0.1 ^x	292.0 \pm 18.6 ^x	292.7 \pm 17.2 ^x
Group iii	6	1.1 \pm 0.1 ^x	133.3 \pm 26.2 ^x	124.2 \pm 15.9 ^x

490 Value is expressed as mean \pm SE.
491 x, y and z in a column indicate a significant difference (Tukey-Kramer; P < 0.05).
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495 Table 4. Growth and monascin production in the basal medium with SMUF I of the three
 496 *Monascus* strains

Medium/strain	<i>M. purpureus</i> AHU 9085	<i>M. pilosus</i> NBRC 4520	<i>M. ruber</i> NBRC 32318
	Wet biomass (g)		
basal medium	2.5 ± 0.1 ^x	0.9 ± 0.1 ^x	1.1 ± 0.1 ^x
basal medium + SMUF I	1.9 ± 0.0 ^y	1.0 ± 0.1 ^x	1.1 ± 0.1 ^x
	Total monascin production (µg)		
basal medium	496.0 ± 43.2 ^x	283.3 ± 19.0 ^x	268.3 ± 13.2 ^x
basal medium + SMUF I	19.7 ± 7.9 ^y	594.5 ± 75.0 ^y	1786.1 ± 143.2 ^y
	Monascin production efficiency (µg g ⁻¹)		
basal medium	198.8 ± 19.9 ^x	312.8 ± 34.5 ^x	252.9 ± 14.2 ^x
basal medium + SMUF I	10.5 ± 4.3 ^y	631.8 ± 81.8 ^y	1612.4 ± 90.8 ^y

497 Value is expressed as mean ± SE (n = 6, except n = 26 of the basal medium in *M. ruber* NBRC
 498 32318 and n = 12 of the basal medium with SMUF I in *M. ruber* NBRC 32318).
 499 x and y in a column indicate a significant difference among the media for the strains (Student's t-
 500 test; P < 0.05).
 501