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RESEARCH NOTE

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# Mevinolin Production by *Monascus pilosus* IFO 480 in Solid State Fermentation of Soymeal

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**Abstract** Mevinolin, a fungal metabolite, is a potent inhibitor of 3-hydroxy-methyl-3-glutaryl-coenzyme A (HMG-CoA) reductase, the rate-controlling enzyme in cholesterol biosynthesis. In this investigation, the optimum factors for mevinolin production by *Monascus pilosus* IFO 480 in soymeal fermentation were studied. The highest yield of mevinolin, 2.82 mg mevinolin per g dry weight, without citrinin (a toxic fungal secondary metabolite) was obtained after 21 days of fermentation at 30°C at 65% moisture content, particle size 0.6-0.9 mm, and initial substrate pH of 6.0. Mevinolin was present in the fermentation substrate predominantly in the hydroxycarboxylate form (open lactone, 92.1-97.3%), which is currently being used as a hypocholesterolemic agent.

**Keywords:** *Monascus pilosus*, mevinolin, citrinin, hypocholesterolemic agent, soymeal fermentation

## Introduction

Hypercholesterolemia is a primary risk factor for coronary artery disease in humans. One-third of total body cholesterol comes from the diet while nearly two-thirds is synthesized in the body (1). Mevinolin (also known as lovastatin, monacolin K, Mevacor, or $C_{24}H_{36}O_5$) inhibits cholesterol synthesis by inhibiting the rate limiting step in cholesterol biosynthesis, namely the conversion of 3-hydroxy-methyl-3-glutaryl-coenzyme A (HMG-CoA) into mevalonate, catalyzed by HMG-CoA reductase (1-3). Mevinolin is a β-hydroxy lactone, and its active form is the corresponding β-hydroxy acid (4, 5).

The *Monascus* species of molds are nonpathogenic and frequently used in food processing to add to the aroma, nutrition, and color of fermentation products (6, 7). For example, red yeast rice (red *koji*, angkak), obtained from the culture of genus *Monascus* during rice fermentation, and has been used in East Asia as a natural food colorant or as medicine to improve digestion and blood circulation (8, 9). However, over the past 10 years, some researchers have discovered and demonstrated that some strains of *Monascus* also produce citrinin, a nephrotoxin, during fermentation (10-12).

Solid-state fermentation or solid-substrate fermentation (SSF) has become a very attractive alternative to submerged fermentation for specific applications due to its simplicity and lower cost (13). Many studies regarding the application of SSF are focused on the production of biologically active secondary metabolites through this process (6, 13). However, this technique has not been applied to the optimization of mevinolin production from *Monascus*-fermented soybean. Among the necessary conditions for fungal growth and activity in a particular solid substrate, particle size and moisture level are the most critical (13). The goal of the present study is to implement such factors for the optimization of mevinolin production by *M. pilosus* IFO 480 in SSF using soymeal.

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## Materials and Methods

**Chemicals** Authentic standards of citrinin and mevinolin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). To obtain mevinolinic acid, mevinolin, the standard lactone, was converted to the ß-hydroxy acid form through sodium salt according to Friedrich *et al.* (4) with minor modifications (14). Mevinolin and mevinolinic acid were identified by their retention time and UV spectra. All other reagents were of the highest grade available, unless otherwise indicated.

**Substrate** Whole soybeans (Seoritae from Pyungchang, Gangwon, Korea, 2004) were crushed into powders (soymeal) of five different particle size ranges: 0.9-1.5, 0.6-0.9, 0.3-0.6, 0.1-0.3, and less than 0.1 mm.

**Fungal strain and inoculation** *M. pilosus* IFO 480, which was previously determined to be the best strain for mevinolin production (14), was obtained from Korea Food Research Institute (KFRI, Seongnam, Korea) and maintained on petri dishes of potato dextrose agar (PDA, Difco, MI, USA). The dishes were incubated at 30°C for 7 days, subsequently stored at 4°C, and sub-cultured every two weeks. After cultivation, colonies of spores that appeared on the plates were transferred (ca.1 $cm^2$) and inoculated into 100 mL of nutrient broth (14) and incubated at 30°C for 4 days with shaking at 150 rpm.

**Solid substrate fermentation** Solid substrate (soymeal of five different particle sizes) was lyophilized, accurately weighed to 100 g in a baffled Erlenmeyer flask and autoclaved (121°C) for 30 min. After cooling, the substrate was inoculated with 10 mL (10%, v/w) of nutrient broth containing *M. pilosus* IFO 480 and incubated at 30°C for 5 weeks. At 7-day intervals, samples were collected aseptically from each flask to analyze mevinolin and citrinin content.

**Moisture content and pH** Various moisture levels (45, 55, 65, 75, and 85%) were attained by adding the required amount of distilled water. To study the effect of initial pH on mevinolin production, 1 N $H_2SO_4$ or 1 N NaOH was also added to the distilled water to adjust the pH to 2.0, 4.0, 6.0, 8.0, or 10.0.

**HPLC analysis** Approximately 0.1 g of fermented soymeal from each flask was extracted with 1 mL of 70% ethanol for 1 hr with sonication. The resulting extract was then centrifuged for 10 min at 10,000×g and filtered through a 0.45-μm membrane (Millipore Co., Bedford, MA, USA), after which the supernatant was directly applied to HPLC (14). Reverse-phase HPLC analysis was carried out with a JASCO system (Tokyo, Japan), using the YMC AM 303 ODS-A column (4.6×250 mm, Kyoto, Japan). A mixture of 0.1% phosphate buffer (pH 7.7) and acetonitrile (65:35, v/v) was used as the mobile phase with a flow rate of 0.8 mL/min and a detection wavelength of 238 nm. Quantitative data for mevinolin, mevinolinic acid, and citrinin were obtained by comparison to known standards.

**Statistical analysis** Results are presented as mean value ±standard deviation. Statistical analysis between experimental results was based on Student's *t* test. Differences were considered statistically significant at the level of $p<0.05$.

## Results and Discussion

**Effect of moisture content and particle size** The effects of moisture content and substrate particle size on mevinolin and citrinin production were studied separately in a series of experiments. As seen in Table 1, the highest yield of mevinolin (2.35 mg/g soymeal) without citrinin production was obtained with 65% water content. When the moisture content of the substrate was between 55 and 75%, *M. pilosus* IFO 480 produced significantly ($p<0.05$) higher concentrations of mevinolin (2.28 to 2.39 mg/g) than at other moisture contents (1.64 to 1.87 mg/g). However, as the moisture content increased, citrinin was also detected in the substrate (0.031 to 0.052 μg/g soymeal). These levels were substantially lower than those reported by Sabater-Vilar *et al.* (11), who reported detection of this mycotoxin in 12 different commercial *Monascus* samples with concentrations ranging from 0.2 to 17.1 μg/g. As shown in Fig. 1, soymeal particles ranging from 0.6-0.9 mm in size produced the highest yield of mevinolin, 2.37 mg/g, followed by the coarsest particles, which ranged from 0.9-1.5 mm (2.25 mg/g). Fine soymeal particles (<0.1 mm) yielded very little mevinolin (1.01 mg/g, $p<0.05$). This indicates that the intermediate and coarse particles provided good support for the fungal organism, which in turn produced high mevinolin yields. The data shown in Table 1 indicate that mevinolinic acid (β-hydroxy acid) was the main component, contributing 97.3% of the total, of mevinolin in the fermented soymeal. This result is similar to that of Li *et al.* (5), who showed that monacolin K hydroxy acid (mevinolinic acid) was the main component in red yeast rice powder.

**Table 1. Effects of moisture content on mevinolin and citrinin production by *M. pilosus* IFO 480 during soymeal fermentation (3 weeks) at 30°C**

| Moisture contents (%) | Mevinolin (mg/g soymeal) Hydroxy acid | Lactone | Citrinin (μg/g) |
|---|---|---|---|
| 45 | 1.59±0.16[a1)] | 0.05±0.01 | ND[2)] |
| 55 | 2.19±0.09[b] | 0.09±0.05 | ND |
| 65 | 2.23±0.11[b] | 0.12±0.04 | ND |
| 75 | 2.29±0.12[b] | 0.10±0.06 | 0.031±0.001 |
| 85 | 1.74±0.14[a] | 0.13±0.03 | 0.052±0.004 |

[1)]Different letters indicate significantly different values ($p<0.05$).
[2)]ND: not detected.The detection limit of citrinin is approximately 10 ng/g.

**Effect of initial pH and fermentation time** As shown in Fig. 1 and 2, mevinolin production depended on both the pH and the fermentation time. When the pH of the substrate was between 4 and 8, *M. pilosus* IFO 480 produced significantly ($p<0.05$) higher concentrations (2.30 to 2.59 mg/g) of mevinolin (Fig. 1). In the same way, Buckland *et al.* (15) reported that the pH of the growth medium played a crucial role in secondary metabolite production, as well as fungal cell growth. The maximum yield of mevinolin (2.82 mg/g) was produced at 21 days of soymeal fermentation (65% moisture content, pH 6, and particle size 0.6-0.9 mm). With increasing fermentation time, this yield decreased slightly, but the mevinolin production had not ceased at the time of termination of fermentation (35 days; Fig. 2). There have been many reports on the mevinolin production by *Monascus* sp. with submerged fermentation (16, 17). However, the production yield of mevinolin by submerged fermentation is extremely low (less than 20-131 mg/L). In comparison, this study demonstrates that the mevinolin yield (2.82 mg/g) increased significantly with the use of the solid state fermentation technique.

In conclusion, mevinolin production from soymeal fermented with *M. pilosus* IFO 480 is not only dependent upon the particle size and moisture content of the

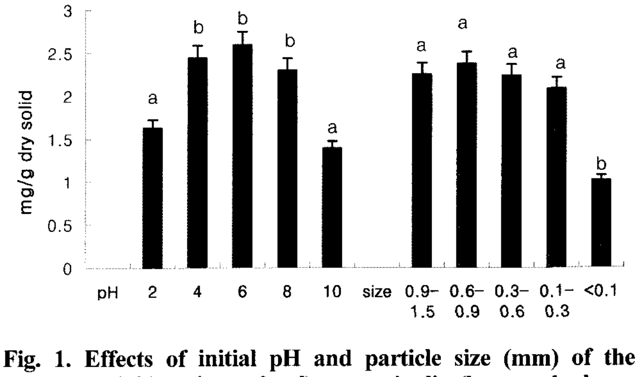


**Fig. 1. Effects of initial pH and particle size (mm) of the substrate (65% moisture level) on mevinolin (lactone+hydroxy acid) production by *M. pilosus* IFO 480 during soymeal fermentation (3 weeks) at 30°C.** Different letters indicate significantly different values ($p<0.05$).

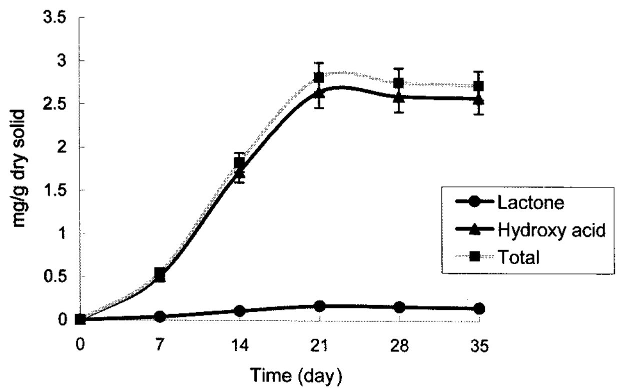


**Fig. 2. Effect of fermentation time on mevinolin production by *M. pilosus* IFO 480 during soymeal fermentation (65% moisture content, pH 6.0, particle size 0.6-0.9 mm) at 30°C.**

substrate, but also on the initial pH of the same substrate. With optimization of these conditions, a yield of 2.82 mg of mevinolin per gram of soymeal was obtained after 21 days of fermentation at 30°C.

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## References

1. Goldstein JL, Brown MS. Progress in understanding the LDL receptor and HMG-CoA reductase, two membrane proteins that regulate the plasma cholesterol. J. Lipid Res. 25: 1450-1461 (1984)
2. Endo A. Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. J. Antibiotechnol. 32: 852-854 (1979)
3. Manzoni M, Rollini M. Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. Appl. Microbiol. Biot. 58: 555-564 (2002)
4. Friedrich J, Zuzek M, Bencina M, Cimerman A, Strancar A, Radez I. High performance liquid chromatographic analysis of mevinolin as mevinolinic acid in fermentation broths. J. Chromatogr. A 704: 363-367 (1995)
5. Li YG, Zhang F, Wang ZT, Hu ZB. Identification and chemical profiling of monacolins in red yeast rice using high-performance liquid chromatography with photodiode array detector and mass spectrometry. J. Pharmaceut. Biomed. 35: 1101-1112 (2004)
6. Juzlova P, Martinkova L, Kren V. Secondary metabolites of the fungus *Monascus*: a review. J. Ind. Microbiol. 16: 163-170 (1996)
7. Chung SH, Suh HJ, Choi YM, Noh DO, Bae SH. Enzyme activities and inhibitory effect on angiotensin converting enzyme of *Monascus-koji* for *kochujang* production. Food Sci. Biotechnol. 8: 179-183 (1999)
8. Ma J, Li Y, Ye Q, Li J, Hua Y, Ju D, Zhang D, Cooper R, Chang M. Constituents of red yeast rice, a traditional Chinese food and medicine. J. Agr. Food Chem. 48: 5220-5225 (2000)
9. Rhyu MR, Kim EY, Kim HY, Ahn BH, Yang CB. Characteristics of the red rice fermented with fungus *Monascus*. Food Sci. Biotechnol. 9: 21-26 (2000)
10. Wang YZ, Ju XL, Zhou YG. The variability of citrinin production in *Monascus* type cultures. Food Microbiol. 22: 145-148 (2005)
11. Sabater-Vilar RFM, Fink-Gremmels J. Mutagenicity of commercial *Monascus* fermentation products and the role of citrinin contamination. Mutat. Res. 444: 7-16 (1999)
12. Norlha T, Lee IH. Protoplast preparation and regeneration from young hyphae of the citrinin producing fungus *Monascus ruber*. Food Sci. Biotechnol. 14: 543-546 (2005)
13. Pandy A, Soccol CR, Mitchell D. New developments in solid state fermentation: I - bioprocesses and products. Process Biochem. 35: 1153-1169 (2000)
14. Pyo YH. Optimum conditions for production of mevinolin from the soybean fermented with *Monascus* species. Korean J. Food Sci. Technol. 38: 256-261 (2006)
15. Buckland B, Gbewonyo K, Hallada T, Kaplan L, Masurekar P. Novel microbial products for medicine and agriculture. Elsevier, Amsterdam, Netherlands. pp. 161-169 (1989)
16. Lai LST, Tsai TH, Wang TC, Cheng TY. The influence of culturing environments on lovastatin production by *Aspergillus terreus* in submerged cultures. Enzyme Microb. Tech. 36: 737-748 (2005)
17. Chang YN, Hwang JC, Lee CC, Shih IL, Tzeng YM. Use of response surface methodology to optimize culture medium for production of lovastatin by *Monascus ruber*. Enzyme Microb. Tech. 30: 889-894 (2002)