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RESEARCH NOTE

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Solid-State Fermentation for Production of Monacolin K on Soybean by *Monascus ruber* GM011

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Abstract Monacolin K (MK) was produced on soybean using *Monascus ruber* GM011 by a two-stage-fermentation process. The optimal temperature was identified as 28°C. Higher yield was obtained by multiple-level-temperature cultivation than by single-level-temperature cultivation. The highest yield of total MK, 4.810 mg/g dry soybean product, was attained after 30 days of solid-state fermentation. No citrinin could be detected in the fermented soybean.

Keywords: *Monascus ruber*, solid-state fermentation, soybean, monacolin K, citrinin.

Introduction

Monascus fermented rice has been clearly demonstrated in clinical observations to have the capacity to lower blood-lipid levels in humans, partly due to the presence of monacolin K (MK) (1). Citrinin, a nephrotoxic and hepatotoxic mycotoxin, is also found in cultures of *Monascus* spp. (2). Therefore, the increase of MK and reduction of citrinin in *Monascus* products is of great concern (3). While steamed rice is the common traditional medium used for *Monascus* solid-state fermentation (SSF) (4), soybean was investigated in the present work as a new alternative medium for MK production.

Materials and Methods

Reagents MK (lovastatin), citrinin, and glucosamine were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). All solvents used in chromatography were of HPLC grade; all other reagents used were of analytical grade.

Microorganism and fermentation A two-stage-fermentation process was applied to ferment soybean by *M. ruber* GM011 (5), which included liquid culture of conidia and mycelium SSF. The conidia suspension (1.0×10^6 CFU/mL) was inoculated in a liquid medium (pH 5.0) containing (per liter) 40 g glucose, 40 mL glycerol, 10 g yeast extract, 1.5 g KH_2PO_4, and 0.5 g $MgSO_4\cdot7H_2O$, in shaking flasks (150 rpm) at 30°C for 18 hr. The SSF procedure was carried out by inoculating liquid seed on 60 g of steamed soybean.

Assay of monacolin K and citrinin MK and citrinin underwent reverse-phase high-performance liquid chromatography analysis by System Gold HPLC system (Beckman, Fullerton, CA, USA) according to the method of Moon *et al.* and Xu *et al.* (6, 7), respectively. Total MK (TMK) is the sum of acid form MK (AFMK) and lactone form MK (LFMK). A Ridascreen® Fast citrinin kit (R-Biopharm, Darmstadt, Germany) was used for a competitive enzyme immunoassay of citrinin.

Assay of glucosamine The glucosamine content was used to estimate the fungal biomass which was determined according to the modified method of Sakurai *et al.* (8). Sample (0.6 g) was mixed with 6 mL of H_2SO_4 (98%) for further determination of glucosamine.

Results and Discussion

Various fermented soybeans are traditional Korean foods containing physiologically active substances (9). TMK production on soybean medium was superior to that on rice medium (3.446 mg/g) in our previous work (5). No citrinin was detected in any of our samples. Ten mL of liquid seed was incubated at different single-level-temperature SSF. No significant difference of biomass could be observed after the eighth day when the temperature was above 28°C, indicating that this was the optimal temperature for MK production (Fig. 1). However, this temperature does not agree with other reported values (10, 11), possibly because different strains or culture methods may affect secondary metabolites. In order to reduce the cultivation time, the following, multiple-level-temperature process was applied in further fermentation experiments: incubation at 32°C for the initial 3 days followed by incubation at 28°C for the later SSF stage. No significant difference was observed among the biomass with different sizes of inocula after the sixth day. An inocula size of 15% gave the highest TMK yield of 4.094 mg/g after 20 days of SSF (Fig. 2). An acidic substrate can produce a higher TMK yield in the first two weeks than a natural substrate. The latter, however, appears to promote a better yield over a longer course (Fig. 3). MK was continually produced even after 20 days of SSF. Further experiments showed that TMK could reach 4.810 mg/g (4.098 mg/g of AFMK, and 0.712 mg/g of LFMK) after

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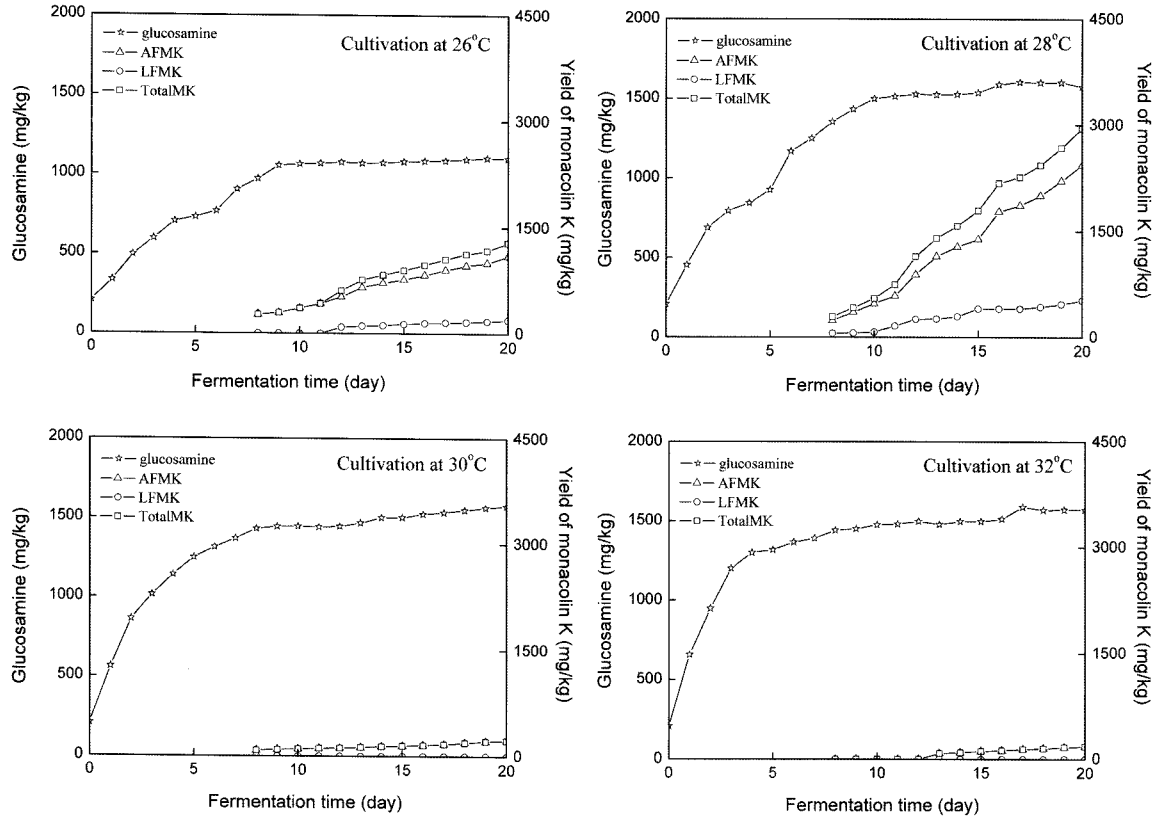

Fig. 1. Effects of temperature on biomass and MK productivity

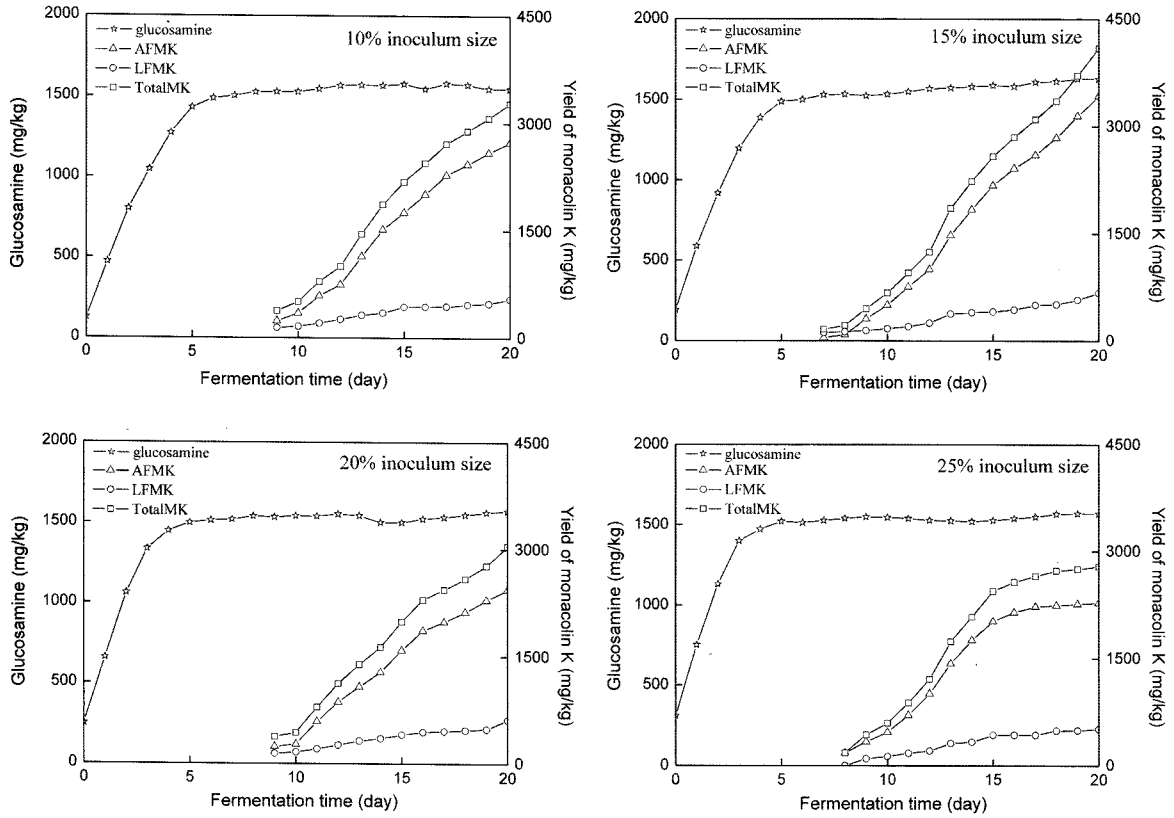

Fig. 2. Effects of inoculum size on biomass and MK productivity

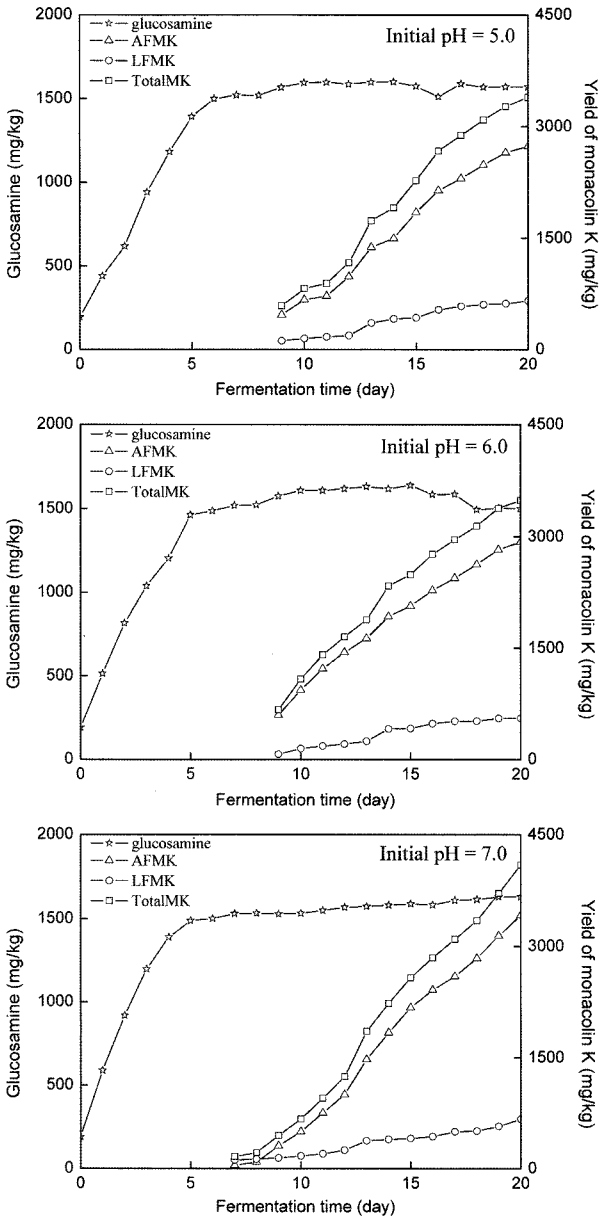

Fig. 3. Effects of initial pH on biomass and MK productivity

30 days of SSF.

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