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Macrophage-Stimulating Activity of Exo-Biopolymer From Cultured Rice Bran With *Monascus pilosus*

K. W. Yu,[1] Y. S. Kim,[2] K. S. Shin,[3] J. M. Kim,[4] and H. J. Suh*,[5]

[1]Department of Kimchi and Food Science, Chongju National College of Science and Technology, Jeungpyeong 368-701, Korea;
[2]Korea Food Research Institute, Sungnam 463-746, Korea;
[3]Department of Food Science and Biotechnology, Kyonggi University, Suwon, 442-760, Korea;
[4]Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Korea;
[5]Department of Food and Nutrition, College of Heath Sciences, Korea University, 1 Jeongneung-dong, Sungbuk-ku, Seoul 136-703, Korea, E-mail: suh1960@unitel.co.kr

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Abstract

To find a new use of rice bran, five fungi were examined for the production of exo-biopolymer with macrophage-stimulating activity from rice bran. Among the exo-biopolymers produced from the cultures, *Monascus pilosus* had the most potent macrophage stimulating activity in a liquid culture rather than in a solid culture. In order to improve the yield of exo-biopolymer with macrophage-stimulating activity, a suitable medium for exo-biopolymer was tested in submerged culture of *M. pilosus*. The highest amount of exo-biopolymer (13.9 mg/mL) was obtained in a medium containing rice bran as an only carbon source followed by media with additional maltose and sucrose (13.8 and 13.7 mg/mL, respectively). The addition of peptone resulted in the production of high amount of exo-biopolymer (15.1 mg/mL), meanwhile the addition of ammonium chloride resulted in 264.0 μg/mL of glucosamine content. Among eight different kinds of inorganic salts tested, potassium phosphate (0.1%) was the most effective inorganic salt for the mycelial growth and exo-biopolymer production. Therefore the optimal medium composition was as follows (g/L): 20 g of rice bran, 5 g of peptone,

*Author to whom all correspondence and reprint requests should be addressed.

and 1 g of KH_2PO_4. The optimal culture pH and time for mycelial growth and exo-biopolymer production was pH 5.0 and 25°C, respectively. The maximum exo-biopolymer (20.1 mg/mL) was observed at the fourth day of cultivation. Exo-biopolymer, a crude polysaccharide fraction, mainly contained neutral sugar (81.8%) with considerable amounts of uronic acid (18.2%). Component sugar analysis showed that the active fraction consisted mainly of arabinose, galactose, glucose, which was digested from starch of rice bran during cultivation, and uronic acid (molar ratio; 0.8:1.0:0.7:0.8).

Index Entries: Macrophage-stimulating activity; *Monascus pilosus*; rice bran; submerged culture.

Introduction

Red mold rice fermented by the genus *Monascus* sp. (koji) has been used as a traditional foodstuff in Asian countries. Recent researches have shown that koji decreases blood pressure *(1,2)*, reduces plasma cholesterol levels *(3)*, and preserves food *(4)*. The koji prepared with *Monascus pilosus* IFO 4520 strain effectively reduces the elevated blood pressure and was approved as a food material for specified health use. The genus *Monascus* is a source of various secondary metabolites of polypeptide structure. Some of the compounds are applicable as food additives (the red pigments) or pharmaceuticals (mevinolin, monacolin) *(5)*.

Monascus sp. grows on rice, wheat, bran, and the red mold rice used to preserve meat, bean, and fish before the introduction of refrigeration *(6)*. Traditionally, this fungus has been produced by solid state culture process. Currently, industrial demand of most of metabolites is met by production in submerged culture. Agro-industrial residues are generally considered the best materials for the production of metabolites.

A total of 4 to 45 million tons of rice bran are produced annually, mainly in the Far East and Southeast Asia *(7)*. It is used largely as animal feed. Only a limited amount of rice bran is currently being used for food or oil extraction *(8)*. Rice bran is a valuable feedstuff because it is rich in B vitamins, fat, and protein, and compares favorably with other cereal grains in amino acid composition *(9)*. It has, however, a high content of fiber *(10)* that is rich in the hemicelluloses containing highly branched arabinoxylans *(11)*.

There are a few reports on the production of carbohydrates with immune-enhancing activities in submerged culture from rice bran. It is desirable to find new uses for rice bran, which has been used for animal feed and oil extraction. In the present work, the strain employed and studied was a newly screened strain of *M. pilosus* producing exo-biopolymers from rice bran in submerged culture. The exo-biopolymer from rice bran cultured by *M. pilosus* showed the macrophage-stimulating activity. Therefore, we have reported the factors that influence the maximization of biopolymers production from rice bran by *M. pilosus* through submerged culture.

Materials and Methods

Microorganism Cultivation and Preparation of Exo-Biopolymer

Aspergillus oryzae KCCM 11530, *Aspergillus sojae* KCCM 60354 (ATCC 9362), *M. pilosus* KCCM 60029 (ATCC 22080), *Monascus purpureus* KCCM 11832 (ATCC 16360), and *Monascus anka* ATCC 36928 were used in this experiment. The medium used for a liquid culture contained the following (per liter): 20 g of rice bran, 1.5 g of $NaNO_3$, 1.0 g of $MgSO_4 \cdot 7H_2O$, 2.5 g of KH_2PO_4, and pH was adjusted to 6.0. The liquid culture was incubated in 300 mL Erlenmeyer flasks containing 50 mL medium with a rotary shaker (120 rpm, 5 d) at 25°C. After centrifugation (3000*g* for 20 min), the supernatant was mixed with four volumes of absolute ethanol, stirred vigorously, and kept overnight at 5°C. The precipitated exo-biopolymer was centrifuged at 3000*g* for 20 min, the supernatant was discarded, and exo-biopolymer was then lyophilized to be used for the assay of macrophage stimulating activity.

A solid culture was conducted in 500-mL Erlenmeyer flask containing 80 g of rice bran and 100 mL of distilled water. After sterilization by autoclaving, the flask was inoculated with a 10% inoculum level and incubated at 25°C for 15 d. The seed culture was grown in a 250-mL flask containing 50 mL of liquid medium at 30°C on a rotary shaker incubator (120 rpm for 5 d). After solid culture for 15 d, 250 mL of distilled water were added to the culture mass. The mixture was homogenized by Ultra-turrax T-50 (IKA Laboratories, Staufen, Germany; 7000 rpm, 20 min), and decocted at 100°C to a half volume (two times). After the extracts were combined and filtered through Toyo filter paper no. 2 (Toyo Roshi, Tokyo, Japan) to remove the insoluble materials, the filtrate was mixed with four volumes of absolute ethanol, stirred vigorously, and kept overnight at 5°C. Exo-biopolymer was obtained by the same method as for liquid culture.

Macrophage-Stimulating Activity

Male 6- to 8-wk-old ICR mice, purchased from Daihan-Biolink Co. (Korea) were injected interperitoneally with 1 mL of 3% thioglycolate medium. After 3 d, macrophage cells were prepared from the peritoneal cavity of mice by washing twice with 5 mL of the cold RPMI-1640 medium containing 5 m*M* HEPES, penicillin (100 U/mL), and streptomycin (100 µg/mL). An aliquot (200 µL) of the cell suspension (1×10^6 cells/mL) was seeded in a flat-bottomed 96-well microplate. After incubation for 2 h at 37°C in a humidified atmosphere of 5% CO_2-95% air, nonadherent cells were removed by washing twice with RPMI-1640 medium. The adherent macrophage monolayer was used for the following experiments *(12)*. Macrophage-stimulating activity was measured by the procedure of Suzuki et al. *(13)* with slight modification. The adherent macrophage cells were cultured in the presence of test samples in a 96-well microplate for 24 h. Macrophage monolayer in a 96-well microplate (1×10^5 cells/mL) was

solubilized by the addition of 25 µL of 0.1% Triton X-100. One hundred fifty microliters of 10.0 m*M* *p*-nitrophenyl phosphate (Sigma Chemical Co., St Louis, MO) was added to the reaction mixture, and the absorbance at 405 nm was photometrically measured using a microplate reader (Bio-Rad, Model 3550-UV).

Composition of Exo-Biopolymer and Determination of Glucosamine

Total carbohydrate, uronic acid, and protein were determined by the phenol-H_2SO_4 *(14)*, *m*-hydroxydiphenyl *(15)*, and Bradford's method *(16)* with Bio-Rad dye (Bio-Rad Co.), respectively, using galactose, galacturonic acid, and bovine serum albumin as the respective standards. Component sugars of the polysaccharides were converted into the corresponding alditol acetates after hydrolysis with 2 *M* TFA for 1.5 h at 121°C *(17)*, and analyzed by gas-liquid chromatography (GLC) according to the procedure of Zhao et al. *(18)*. GLC was carried out on a Hewlett-Packard 6890 II gas chromatograph (Hewlett-Packard) equipped with an SP-2380 capillary column (0.2 µm film, 0.25 mm i.d. × 30 m, Supelco, PA). Temperature program was 60°C for 1 min, 60 to 220°C (increasing 30°C/min), 220°C for 8 min, 220 to 250°C (increasing 8°C/min), and held at 250°C for 15 min. The molar ratios were calculated from the peak areas and response factors using the flame-ionization detector.

The glucosamine content of mycelium was determined after acid hydrolysis. To 15 mg dry sample, 5 mL of 6 *N* HCl were added in glass tubes with Teflon screw caps and the tubes were incubated at 121°C for 4 h. After hydrolysis, the HCl was evaporated under vacuum at 50°C. The dry residues were dissolved in 5 mL water. The glucosamine concentration of the solutions was determined by the colorimetric method of Swift *(19)*; the condensation with acetylacetone carried out in a water bath at 96°C for 1 h. After reaction with Ehrlich's reagent, the pink color formed by the condensation products of the glucosamine was read at 530 nm.

Optimal Culture Conditions

To find the optimal culture conditions, carbon sources, nitrogen sources, pH, and temperature were studied in a basal medium. In order to select the optimal carbon source, the production of exo-biopolymer was determined after 5 d of incubation in the basal medium containing 2% of various carbon sources. The effect of the nitrogen source was investigated by incubating in the basal medium containing 2% rice bran and 0.5% nitrogen source. The effects of metal ions were investigated by incubating in the basal medium containing 2% rice bran and 0.5% peptone. The effect of temperature and pH on the production of exo-biopolymer was also investigated by incubating in the optimal medium at different temperatures and pHs.

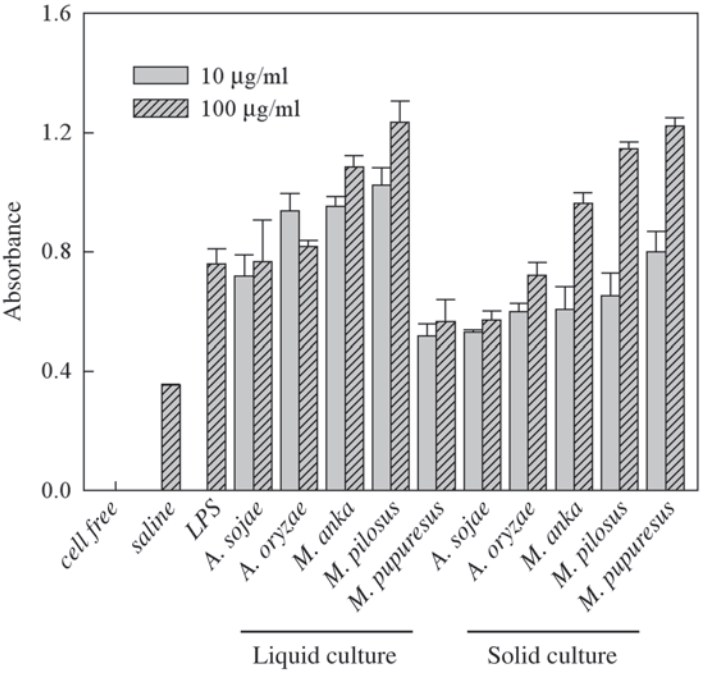

Fig. 1. Macrophage-stimulating activity of several cultured rice bran products in liquid or solid culture. The macrophage-stimulating activity of the cultured rice bran was determined after 5 d of culture in the liquid medium (20 g of rice bran, 1.5 g of $NaNO_3$, 1.0 g of $MgSO_4 \cdot 7H_2O$, 2.5 g of KH_2PO_4) and solid medium 80 g of rice bran with 100 mL of distilled water. The liquid culture was carried out at pH 6.0 and 30°C and solid culture was carried out at 25°C. Values represent the means (n = 4) ± S.D. LPS; lipopolysaccahride as positive control (100 μg/mL)

Results

Macrophage-Stimulating Activity in Liquid and Solid Culture

To find a new use for rice bran (previously used for animal feed and oil extraction), strains were applied to produce exo-biopolymers from rice bran with macrophage-stimulating activity. The results are shown in Fig. 1. In liquid cultures, *M. pilosus* and *M. anka* showed high levels of macrophage-stimulating activity. Especially, *M. pilosus* showed the highest level of macrophage-stimulating activity in liquid culture. *M. pilosus* and *M. anka* also showed the highest level of macrophage-stimulating activity in solid culture. In addition, *M. pupuresus*, which showed the lowest level of macrophage-stimulating activity in liquid culture, showed the highest level of macrophage-stimulating activity in solid culture. Therefore, *M. pilosus* was selected to produce exo-biopolymer with macrophage-stimulating activity in liquid culture, because of short cultivation time and high level of macrophage-stimulating activity in liquid culture condition.

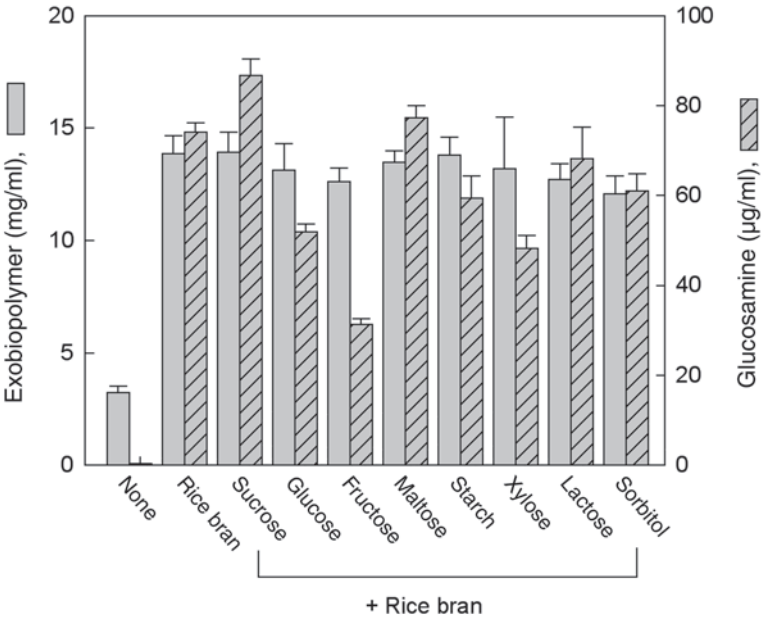

Fig. 2. Exo-biopolymer production by the submerged culture of *M. pilosus* in various carbon sources. The production of exo-biopolymer was determined after 5 d of incubation in the basal medium (20 g/L of rice bran, 1.5 g/L of $NaNO_3$, 1.0 g/L of $MgSO4{\cdot}7H_2O$, and 2.5 g/L of KH_2PO_4) containing 2% of various carbon sources. The submerged culture was carried out at initial pH 6.0 and 30°C.

Optimal Culture Conditions

The selection of a suitable carbon source for a fermentation process is a critical factor and thus involves the screening of a number of carbon sources for microbial growth and exo-biopolymer formation. In the present studies, rice bran alone and eight carbon sources (sucrose, glucose, fructose, maltose, starch, xylose, lactose, and sorbitol) with rice bran were used for the growth of *M. pilosus* and its exo-biopolymer production. The results were shown in Fig. 2. Rice bran alone produced as much exo-biopolymer as rice bran with carbon sources. However, single uses of the other carbon sources without rice bran did not rise to higher exo-biopolymer production compared to the combination with rice bran (data not shown). A high amount of exo-biopolymer (13.9 mg/mL) was obtained in a medium containing rice bran followed by maltose and sucrose (13.8 and 13.7 mg/mL, respectively). But there was not a significant difference between substrates in exo-biopolymer amount after 5 d of cultivation. The addition of sucrose and maltose resulted in the high level of mycelial growth. After 5 d of cultivation, the amounts of glucosamine in the addition to sucrose and maltose were 86.7 and 77.2 μg/mL, respectively. Rice bran was a more economical carbon source than sucrose and maltose. The order of carbon suitability was rice bran > sucrose + rice bran > maltose + rice bran.

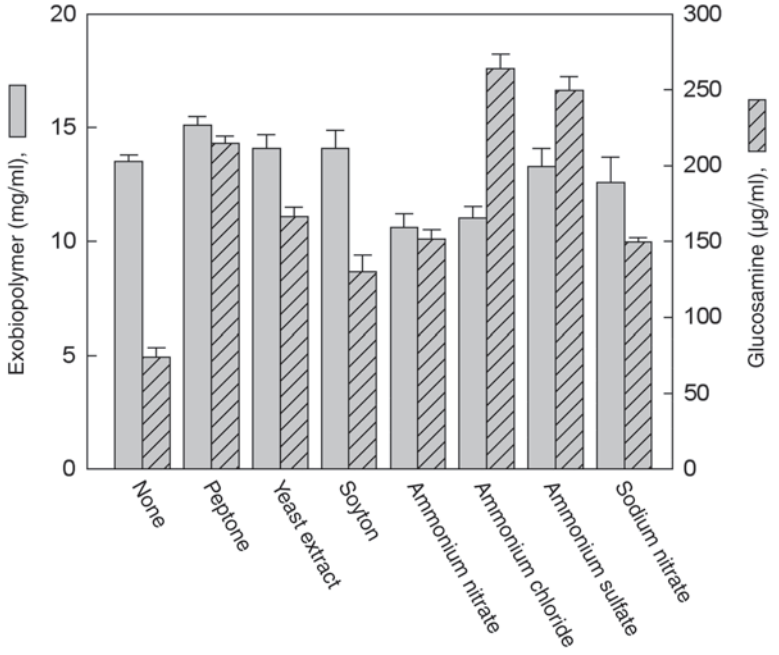

Fig. 3. Exo-biopolymer production by the submerged culture of *M. pilosus* in various nitrogen sources. The production of exo-biopolymer was determined after 5 d of incubation in the basal medium (20 g/L of rice bran, 1.0 g/L of $MgSO4{\cdot}7H_2O$, and 2.5 g/L of KH_2PO_4) containing 0.5% of various nitrogen sources. The submerged culture was carried out at initial pH 6.0 and 30°C.

The effect of nitrogen source on mycelial growth and exo-biopolymer production was studied by incubating the medium containing rice bran (20 g/L) and nitrogen source (5 g/L). As shown in Fig. 3, organic nitrogen sources, such as peptone, yeast extract, and soytone, gave rise to higher exo-biopolymer production compared to inorganic nitrogen sources, ammonium nitrate, ammonium chloride, ammonium sulfate, and sodium nitrate. Especially the amount of exo-biopolymer in addition to inorganic nitrogen sources showed lower results than no addition of nitrogen source. The addition of peptone resulted in high amounts of exo-biopolymer (15.1 mg/mL), meanwhile the addition of ammonium chloride resulted in glucosamine content (264.0 μg/mL).

Potassium phosphate (0.1%) was the most effective inorganic salt for mycelial growth and exo-biopolymer production among the eight different kinds of inorganic salts tested (Fig. 4). After 5 d of cultivation, the amount of glucosamine and exo-biopolymer in the addition of potassium phosphate were 181.7 μg/mL and 16.3 mg/mL, respectively. No addition of inorganic salts showed a higher amount of exo-biopolymer than the other inorganic salts. Based on the results, the optimal medium composition obtained in this study was as follows (per liter): 20 g of rice bran, 5 g of peptone, and 1 g of KH_2PO_4.

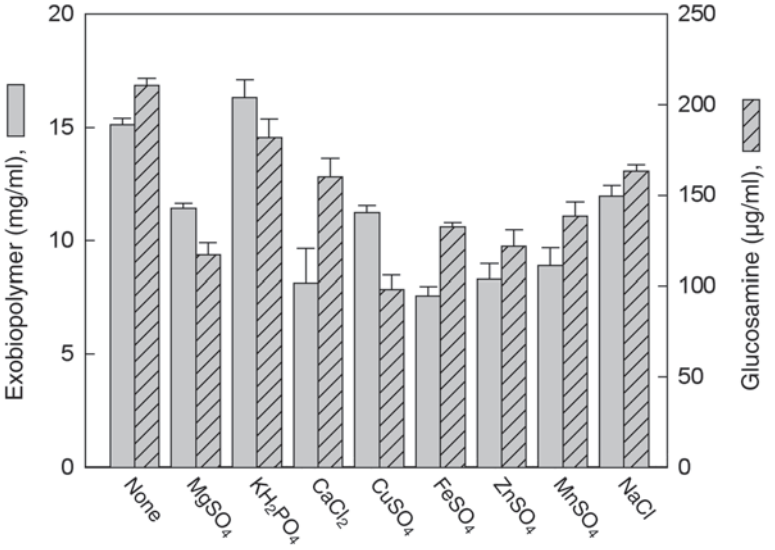

Fig. 4. Exo-biopolymer production by the submerged culture of *M. pilosus* in various mineral sources. The production of exo-biopolymer was determined after 5 d of incubation in the medium (20 g/L of rice bran and 5 g/L of peptone) containing 0.1% of various metal ion sources. The submerged culture was carried out at initial pH 6.0 and 30°C.

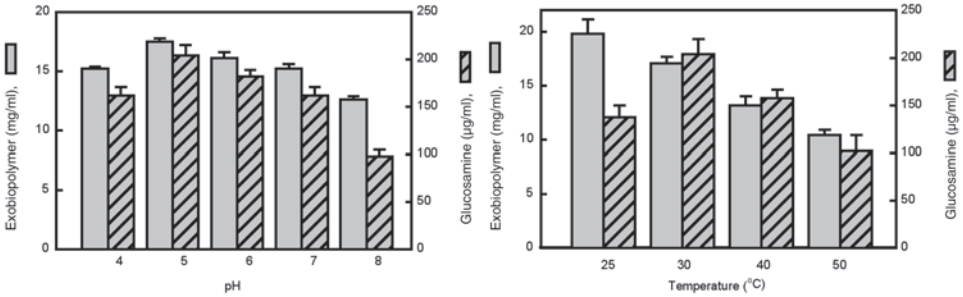

Fig. 5. Exo-biopolymer production by the submerged culture of *M. pilosus* in various pHs and temperature. The production of exo-biopolymer was determined after 5 d of incubation in the optimal medium (rice bran 20 g/L, peptone 5 g/L, and KH_2PO_4 1.0 g/L).

Effect of pH and Temperature

In order to investigate the effect of pH on mycelial growth and exo-biopolymer production, *M. pilosus* was cultivated in the optimal medium with different initial pHs in shaking culture conditions (Fig. 5). The optimal culture pH for mycelial growth and exo-biopolymer production was pH 5.0. The amounts of exo-biopolymer and mycelial growth were 17.4 mg/mL and 204.1 μg/mL after 5 d of cultivation under optimal medium. The amount of exo-biopolymer and mycelial in weak acid condition (pH 4.0–6.0) were higher than in neutral condition.

Table 1
Physicochemical Properties
of the Immunostimulating and Crude Polysaccharide Fraction
From the Fermented Rice Bran Products

Chemical content	Immunostimulating fraction (%)
Carbohydrate	81.8
Uronic acid	18.2
Protein	N.D.
Component sugar	(mol. %)
Rhamnose	3.2
Fucose	0.6
Arabinose	19.6
Xylose	8.7
Mannose	9.3
Glucose	17.2
Galactose	23.2
Uronic acid (GalA + GlcA)	18.2

N.D.; not detected.

To find optimal culture temperature, *M. pilosus* was cultivated at four different temperatures (25°C, 30°C, 40°C, and 50°C) and the optimal temperature for exo-biopolymer was found to be 25°C (Fig. 5), even though the mycelial growth (137.2 µg/mL) was lower than at 30°C.

Physicochemical Properties of Exo-Biopolymer Under Optimal Culture Conditions

Exo-biopolymer, a crude polysaccharide fraction, mainly contained neutral sugar (81.8%) with considerable amounts of uronic acid (18.2%) (Table 1). Component sugar analysis showed that the active fraction consisted mainly of arabinose, galactose, glucose (which was digested from starch of rice bran during cultivation), and uronic acid (molar ratio; 0.8:1.0:0.7:0.8) (Table 1). In the present study, we focused the abundant active macromolecules in carbohydrates. The results suggest that the active fraction is a pectic arabinogalactan-type polysaccharide.

Figure 6 shows mycelial growth in a flask culture under liquid culture conditions. The amount of glucosamine reached the maximum of 137.2 µg/mL after 5 d of cultivation. Exo-biopolymer production showed the same trend as mycelial growth. The maximum amount of exo-biopolymer production (20.1 mg/mL) was observed at the 4 d of cultivation. There was not a significant difference in exo-biopolymer production after 4 d of cultivation. The results strongly suggest that exo-biopolymer production in *M. pilosus* is growth-associated.

Control group (no addition of sample) had a low level of activity (0.38), whereas 10 and 100 µg/mL of lipopolysaccharide (LPS)-treated groups

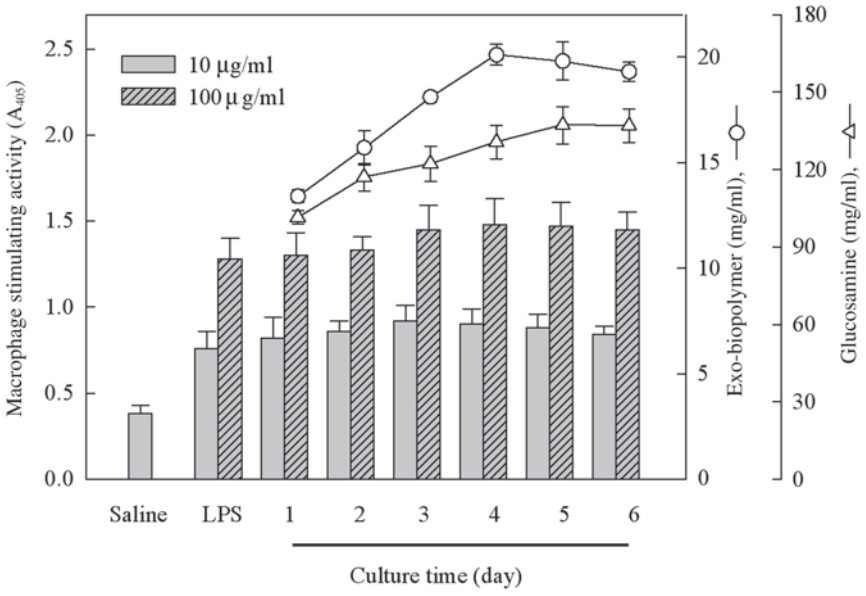

Fig. 6. Changes of exo-biopolymer, glucosamine contents, and macrophage stimulating activity in the liquid culture of *M. pilosus*. The liquid culture carried out at pH 5.0 and 25°C in the medium (20 g/L of rice bran, 5 g/L of peptone, 1 g/L of KH_2PO_4). Values represent the means ($n = 4$) ± S.D. LPS; lipopolysaccahride as a positive control.

had 0.76 and 1.28 of macrophage-stimulating activity, respectively. As cultivation time went on, the macrophage stimulating activity was gradually increased in a dose-dependent manner. Maximum macrophage-stimulating activity of 100 μg/mL (1.48) was also observed after 4 d of cultivation and was similar at 5 d of incubation.

Discussion

In Korea, rice bran, by-products obtained by milling brown rice, could be a promising foodstuff because it contains high content of fiber that is rich in the hemicelluloses containing highly branched arabinoxylans. In Fig. 1, rice bran was a good nutrient for the production of exo-biopolymer with macrophage-stimulating activity by *Aspergillus* sp. and *Monascus* sp. *Monascus* sp. especially showed a high level of macrophage-stimulating activity. During the fermentation process, amylases released from rice koji, the molded rice *Aspergillus* sp., act to liquefy and saccharify the starch materials contained in the rice endosperm cells. Yoshizawa et al. *(20)* reported that the endosperm cell wall, which composed a material of rice bran, prevents the amylase to attack on starch in the rice endosperm cells. Therefore, *Aspergilllus* sp. showed a lower level of macrophage-stimulating activity than *Monascus* sp. because the growth of *Aspergillus* sp. might have been inhibited by the components of rice endosperm cell wall in rice bran.

Monascus pigment fermentations have been also performed mainly in solid cultures *(21)*; however, production yields have been too low to allow industrial scale production. As a means of increasing the pigment yield, many researches have focused on liquid cultures *(22)*. Liquid culture obviously gives rise to potential advantages of higher mycelial production in a compact space and shorter time without significant problem of contamination. In Fig. 1, liquid culture of *M. pilosus* also showed a higher level of macrophage-stimulating activity than solid culture.

The substitution of agricultural wastes for other substrates depends initially on the ability of the microbial species to utilize them for polymer production. Several of the exo-biopolymer producing microorganisms are relatively limited in their nutritional capabilities and will grow only satisfactorily on relatively complex media such as rice bran, yeast hydrolysates, corn-steep liquor, and appropriate carbohydrate carbon sources such as sucrose, starch hydrolysates, or molasses. The single use of rice bran has found wide acceptance, because the substrate is available in most parts of the Far East and Southeast Asia, and is relatively cheap. Therefore rice bran was a good carbon source for the production of exo-biopolymer by *M. pilosus*. Glucose concentration of 2 to 5% (w/v) is usually the preferred carbon source, because it is utilized by a very wide range of microbial species with wide availability and the production cost depends on the degree of purity. The usual source is starch hydrolysates. However, as reported in this study, glucose and starch were not effective for the production of exo-biopolymer by *M. pilosus* (Fig. 2). For some microorganisms, either of these carbohydrates will provide good yields of exo-biopolymers, but other species may be more restrictive. Lin and Demain *(23,24)* indicated that the utilization of carbon sources for growth appeared to be strain-specific; glucose and its oligo- and polysaccharide were better than other carbon sources both for growth and pigment production.

Among the nitrogen sources tested, the addition of peptone resulted in the maximum exo-biopolymer production (Fig. 3). The relatively poor growth in the medium containing nitrate was similar to a common situation in fungi *(25)*. The toxicity of nitrite and nitrate could be its ability to deaminate amino acid and interfere with sulphur metabolism owing to its similarity to the sulphite ion *(26)*. But Lin *(22)* reported that *Monascus* sp. grew in medium containing 3.5% starch and that sodium nitrite or potassium nitrate gave a maximum yield of the pigments but that peptone and yeast extract were not appropriate sources for the pigment production. Lin and Demain *(24)* also reported that sodium nitrate and ammonium sulphate used as nitrogen sources were found to be the most suitable for pigment production, so they suggested that organic nitrogen was optimal for growth, but not for pigment production. Although there was relatively poor growth in the medium containing rice and peptone, they were the best carbon and nitrogen sources for the production of exo-biopolymer.

Generally, glucose or sucrose is used as a carbon source in submerged culture. Nitrogen sources used include peptone, yeast extract, yeast pow-

der, peanut flour, soybean flour, and soy sauce. Phosphates are used for buffering; magnesium sulfate is commonly the only other inorganic nutrient used. Fermentation is carried out at 24 to 28°C under highly aerobic conditions. Bioreactors or fermenters used were similar to those described for culturing filamentous microfungi such as *Penicillium chrysogenum (27)*. The optimal medium composition obtained in this study was as follows (per liter): 20 g of rice bran, 5 g of peptone, and 1 g of KH_2PO_4. Under the culture condition (pH 5.0 and 25°C), exo-biopolymer production showed the same tendency of the mycelial growth. And macrophage-stimulating activity was also consistent with the amount of exo-biopolymer. Park et al. *(28)* also reported that mycelial growth was closely related to the polysaccharide production in *Coriolus versicolor*. However, the profile of polysaccharide production was not consistent with that of mycelial growth of *Phellinus linteus*, which is often the case in fermentation kinetics of higher fungi *(29)*.

Exo-biopolymer mainly contained neutral sugar (81.8%) with considerable amounts of uronic acid (18.2%) (Table 1). The results suggest that the active fraction is a pectic arabinogalactan-type polysaccharide. Arabinogalactans are polysaccharides rich in galactosyl and arabinosyl residues, and in some cases occur in covalent association with protein as proteoglycans (arabinogalactan-properties). Arabinogalactans are typically found in higher plants and in liverworts, and are components of cell membrane, extracellular matrices, and in gum exudates. Clarke et al. *(30)* classified plant arabinogalactans and arabinogalactan-proteins into type I, type II, and other types according to the structure of the arabinogalactan portion. Because the crude active fraction mostly comprised arabinose and galactose (which can be the components of arabinogalactan, and is rich in uronic acid and rhamnose), it is reasonable to assume that it is another arabinogalactan (pectic polysaccharides with arabinogalactan side chains). In addition, the active fraction is considered to comprise mainly pectic polysaccharides, which contained a galacturonan region [poly- or oligomerized α-(1–4)-galacturonic acid] and a "ramified" region (rhamnogalacturonan core with side chains) *(31)* because they contained a considerable uronic acid. However, studies on the structure and structure-activity relation of the purified active polysaccharide must await further study.

In liquid culture conditions, exo-biopolymer production showed a similar tendency of the mycelial growth (Fig. 6). Also macrophage stimulating activity was also consistent with the amount of exo-biopolymer. Park et al. *(32)* also reported that mycelial growth was closely related to the polysaccharide production in *C. versicolor*. However, the profile of polysaccharide production was not consistent with that of mycelial growth of *P. linteus*, which is often the case in fermentation kinetics of higher fungi *(33)*.

Figure 6 shows typical mycelial growth and exo-biopolymer production. Exo-biopolymer and mycelial content reached in the maximum level after 4 and 5 d, respectively. In this study, results showed that a maximum

level of exo-biopolymer was followed a maximum level of mycelial growth. In shake flask cultures, the exo-biopolymer production by *Paecilomyces japonica* reached after 6 d of fermentation, whereas the mycelial dry weight reached in a maximum level after 7 d of fermentation *(34)*. A maximum level of exo-biopolymer production in *P. japonica* was also followed a maximum level of mycelial growth. But Park et al. *(35)* reported that the exo-biopolymer concentration reached a maximum level after 9.5 d of fermentation, whereas maximum mycelial content indicated 13.3 g/L after 7.5 d. Exo-biopolymer production in *Cordyceps militaris* reached a maximum level after a maximum mycelial growth. Therefore, it is possible to enhance the exo-biopolymer production in the liquid culture of *M. pilosus* after the optimization of nutrients and culture conditions.

Although many workers have attempted to obtain exo-biopolymer with immune enhancing activity by liquid culture process, little information is available regarding the nutritional factors affecting mycelial growth and exo-biopolymer production in a liquid culture. For the large-scale exo-biopolymer production, further studies are ongoing including the characterization of exo-biopolymers produced from *M. pilosus*.

Acknowledgments

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