J. Microbiol. Biotechnol. (2004), **14**(5), 944–951

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

Optimization of Submerged Culture Conditions for Mycelial Growth and Exopolysaccharides Production by *Agaricus blazei*

KIM, HYUN HAN[1], JEONG-GEOL NA[2], YONG KEUN CHANG[1]*, GIE-TAEK CHUN[3], SANG JONG LEE[2], AND YEON HO JEONG[4]

[1]*Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejon 305-701, Korea*
[2]*STR Biotech CO., Ltd., Hi-Tech Venture Town, Chuncheon 200-160, Korea*
[3]*Division of Life Science, Kangwon National University, Chuncheon 200-701, Korea*
[4]*Division of Food Science and Biotechnology, Kangwon National University, Chuncheon 200-701, Korea*

Received: October 10, 2003
Accepted: December 23, 2003

Abstract The influences of inoculum size, pH, and medium composition on mycelial growth and exopolysaccharides (EPS) production were investigated in shake flasks and in a bioreactor. The optimum inoculum size for both mycelial growth and EPS production was identified to be 10% (v/v) in shake flask cultures. The optimal initial pH for mycelial growth and EPS production in shake flask cultures were found to be 5.0 and 7.0, respectively. However, the optimal pH was 5.0 for both mycelial growth and EPS production in bioreactor cultures where the pH was regulated. The optimal mass ratio of the two major carbon sources, glucose to dextrin, was 1:4. The optimal mass ratio of the two major nitrogen sources, yeast extract to soytone peptone, was 2:1. When 500 mg l^{-1} of $MnSO_4 \cdot 5H_2O$ was added to the bioreactor culture, both mycelial growth and EPS production were enhanced by approximately 10%. Under the optimized conditions, a mycelial biomass of 9.85 g l^{-1} and an EPS concentration of 4.92 g l^{-1} were obtained in 4 days.

Key words: *Agaricus blazei*, mycelial growth, exopolysaccharides, submerged culture

Various mushrooms have a long history of use in folk medicine, and higher basidiomycetes have become matters of great interest, due to their many-fold nutritional, medicinal, and pharmacological properties [25, 26, 28]. Mushroom extracts are widely used as nutritional supplements and touted as beneficial for one's health.

Agaricus blazei, a basidiomycete fungus, is native to southern Brazil. Since 1965, the strains have been exported from Brazil to Japan, where this mushroom has become popularly known as "Himematsutake" or "Kawariharatake." It has been widely cultivated and studied in Japan. It has been traditionally used as a health food source for the prevention of cancer, diabetes, hyperlipidemia, arteriosclerosis, and chronic hepatitis. Recent studies on this fungus have demonstrated many interesting biological activities, including antitumor, anticarcinogenic, and antimutagenic effects. These studies also have suggested that the antitumor substance in *A. blazei* is a polysaccharide, 1, 3-β-D-glucan [18, 23]. The most noticable feature of many 1, 3-β-D-glucans of fungal origin is their antitumor activity. They are closely related in their structure, but vary in their degree of branching, water solubility, and the nature of their side chains. The glucan from *A. blazei* has a stronger antitumor activity against Sarcroma 180 in mice than other polysaccharides from *Ganoderma lucidum*, *Lentinus edodes*, and *Coriolus versicolor* [19].

A. blazei has normally been produced in solid cultures using substrates such as grain, sawdust, or wood. It usually takes several months to cultivate the fruiting body of *A. blazei*, and product quality control is very difficult. For these reasons, submerged culture of *A. blazei* mycelia has received great interest in Asian countries as a promising alternative for efficient production of polysaccharide. Submerged culture has potential advantages of higher mycelial production in a compact space and a shorter incubation time with lesser chance of contamination [7]. In addition, exopolysaccharides (EPS), which have synergistic biological effects with the polysaccharide extracted from mycelia, can be concurrently produced and secreted.

Recently, EPS produced by various fungal strains have been extensively investigated for some potential applications [3]. The recovery of EPS from the culture broth requires

*Corresponding author
Phone: 82-42-869-3927; Fax: 82-42-869-3910;
E-mail: ychang@kaist.ac.kr

relatively simple steps and, thus, is less costly than that of polysaccharide from mycelia [2, 3]. It has been reported that the mycelial growth and EPS production are affected by a wide range of environmental parameters as well as the medium composition [21]. Although many investigators have attempted to obtain optimal submerged culture conditions for mycelial growth and EPS production with several fungi, the nutritional requirements and environmental conditions for submerged cultures have not been clearly demonstrated [5, 8, 21].

The major objective of this study was to optimize the submerged culture conditions for mycelial growth of *A. blazei* and EPS production in a bioreactor. We believe that this was the first effort for the optimization of EPS production by submerged culture of *A. blazei*.

MATERIALS AND METHODS

Microorganism

The microorganism used in this study was *A. blazei*, which was kindly provided by STR Biotech. Co., Ltd., Chuncheon, South Korea. Its freeze dried culture was resuscitated in M1 liquid medium. The composition of M1 medium was as follows: 10 g l^{-1} glucose, 40 g l^{-1} dextrin, 4 g l^{-1} yeast extract, 2 g l^{-1} soytone peptone, 2 g l^{-1} KH_2PO_4, 0.6 g l^{-1} $MgSO_4 \cdot 7H_2O$, and 0.2 mg l^{-1} $FeCl_3 \cdot 6H_2O$. The first stock culture was grown in a 250-ml flask containing 50 ml of M1 medium at 28°C on a rotary shaker incubator at 150 rpm for 5 days. The second stock culture was inoculated with 10% (v/v) of the first stock culture broth and then cultivated in a 500-ml flask containing 100 ml of M1 medium at 28°C on a rotary shaker incubator at 150 rpm. Glycerol (20%, v/v) stock samples were made from the culture broth after 2 days of the second stock culture and maintained at - 70°C.

Inoculum Preparation

For the first seed culture, 50 ml of M1 medium in a 250-ml flask was inoculated with 5 ml of 20% (v/v) glycerol stock stored at - 70°C and incubated on a rotary shaker incubator at 150 rpm and 28°C for 5 days. The second seed culture was inoculated with 10 ml of the first seed culture broth and then cultivated in a 500-ml flask containing 100 ml of M1 medium at 28°C on a rotary shaker incubator at 150 rpm for 2 days. After the second seed cultivation, the pellet-containing culture broth was homogenized aseptically using a Waring blender (31BL92, Coleparmer Co., NH, U.S.A.) for 10 sec. The homogenized culture broth was used as an inoculum for submerged cultures in shake flasks and in a bioreactor.

Flask Culture

Flask culture experiments were performed in 250-ml flasks containing 50 ml of M1 medium. Cultures were performed on a rotary shaker incubator at 150 rpm and 28°C for 5 days. To examine the effects of inoculum size and initial pH of the medium, cultures were carried out for five different inoculum sizes of 2.5, 5, 10, 15, and 20% (v/v), and for six different initial pHs of 3, 4, 5, 6, 7, and 8. The pH was adjusted with 2 N HCl or 2 N NaOH before sterilization. All experiments were repeated at least twice.

Bioreactor Culture

Three liters of medium in a bioreactor (LK230, KoBioTech Co., South Korea) were inoculated with 10% (v/v) of the seed culture and then cultivated for 5 days at 100–150 rpm, 1 vvm, and 28°C. The composition of the medium was the same as M1 medium unless otherwise specified. The effect of pH, glucose to dextrin ratio, yeast extract to soytone peptone ratio, and the following factors were studied. The medium pH was controlled at 4, 5, 6, and 7 with 2 N HCl or 2 N NaOH. A culture with no pH control was also carried out (the control). The initial pH was 7.0 in the control. The mass ratios of glucose to dextrin were 5:0, 4:1, 3:2, 1:1, 2:3, 1:4, and 0:5 with a total carbon source concentration of 30 g l^{-1}. The mass ratios of yeast extract to soytone peptone were 3:0, 2:1, 1:1, 1:2, and 0:3 with a total nitrogen source concentration of 6 g l^{-1}, while the mass ratio of glucose to dextrin was fixed at 1:4. All experiments were performed at least in duplicates.

Analytical Methods

Samples taken from the shake flasks and bioreactor were filtered with a filter paper (Whatman #1, Whatman Inc., NJ, U.S.A.). The filtrate was further filtered using a membrane filter (0.2 μm, Millipore). The resulting filtrate was analyzed by HPLC (L6200, Hitachi Co., Japan) with

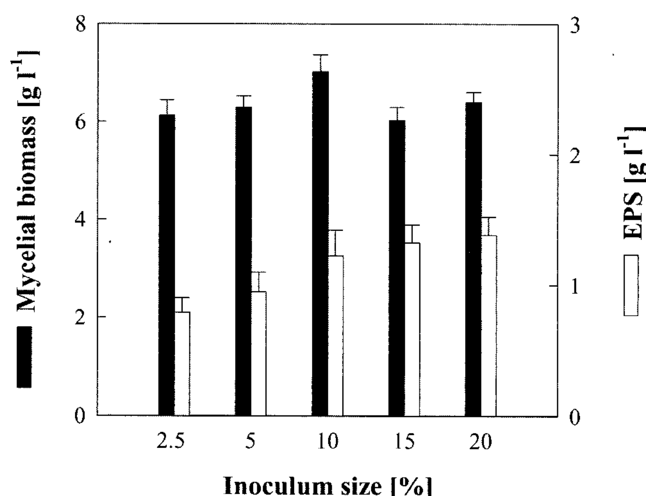

Fig. 1. Effects of inoculum size on mycelial growth and EPS production in shake flask cultures.

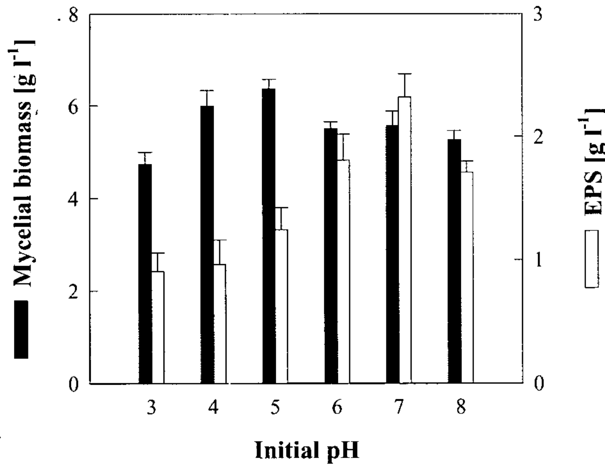

Fig. 2. Effects of initial pH on the mycelial growth and EPS production in shake flask cultures.

an Ultrahydrogel™ 1,000 column (0.78x30 cm, Waters Co., MA, U.S.A.) and an evaporated light scattering detector (ELSD) (SEDEX 75, Sedere Co., France) for quantitative analysis of EPS concentration. The residual glucose concentration was measured with a glucose analyzer (YSI 2700, Yellow Springs Instruments Co., OH, U.S.A.). Dry weight of mycelium was measured after repeated washing of the mycelial pellets with distilled water and drying overnight to a constant weight at 80°C.

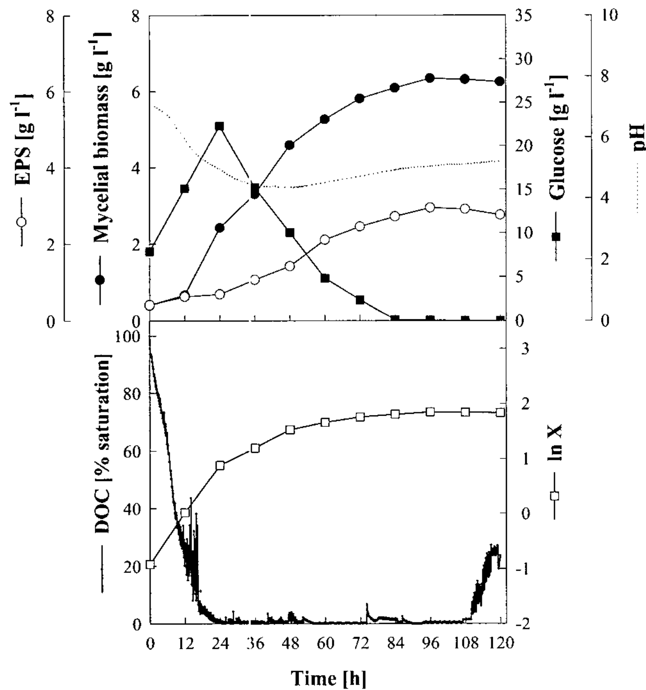

Fig. 3. Time profiles of mycelial growth and EPS production in bioreactor with no pH control (Initial pH: 7.0).

RESULTS

Effects of Inoculum Size in Shake Flask Cultures

To determine the optimal inoculum size for mycelial growth and EPS production, *A. blazei* was cultivated with different inoculum sizes in shake flask cultures. Five inoculum sizes were tested: 2.5, 5, 10, 15, and 20% (v/v). The individual pellet size did not significantly change with inoculum size. Figure 1 indicates that both the mycelial

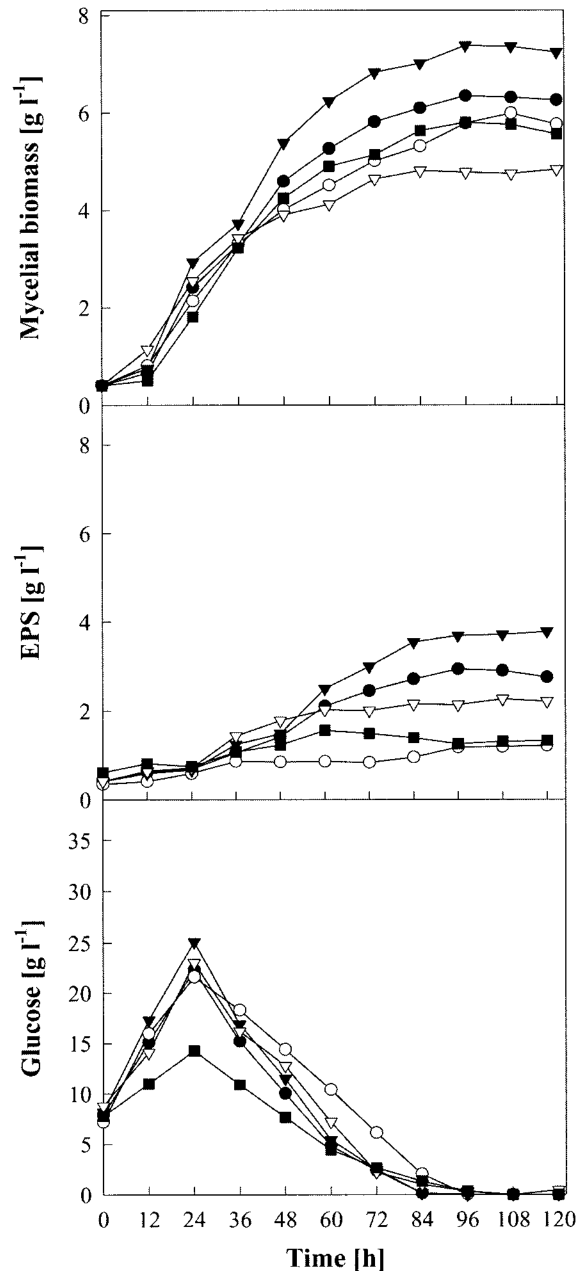

Fig. 4. Time profiles of mycelial growth and EPS production in bioreactor at different pHs.
No pH control (●); pH 4 (○); pH 5 (▼); pH 6 (▽); pH 7 (■).

Table 1. *A. blazei* cultures in bioreactor at different pHs.

Culture parameters	pH				
	Uncontrolled	4	5	6	7
Maximum mycelial biomass X [g l^{-1}]	6.35	5.78	7.38	4.78	5.80
Maximum EPS concentration P [g l^{-1}]	2.94	1.18	3.69	2.13	1.26
Specific growth rate [h^{-1}]	0.07	0.07	0.08	0.07	0.06
Specific consumption rate of substrate $Q_{S/X}$ [g g^{-1} d^{-1}]	1.18	1.30	1.02	1.57	1.29
Specific production rate of EPS $P_{P/X}$ [g g^{-1} d^{-1}]	0.12	0.05	0.13	0.11	0.05
Yield of mycelial biomass on substrate $Y_{X/S}$ [g g^{-1}]	0.21	0.19	0.25	0.16	0.19
Yield of EPS on substrate $Y_{P/S}$ [g g^{-1}]	0.10	0.04	0.12	0.07	0.04

growth and EPS production were affected by the inoculum sizes. The maximum mycelial biomass (7.02 g l^{-1}) was achieved with an inoculum size of 10% (v/v). In contrast, the maximum EPS concentration (1.38 g l^{-1}) was obtained with an inoculum size of 20% (v/v). However, the change in EPS production with inoculum sizes over 10% (v/v) was not significant. Therefore, an inoculum size of 10% (v/v) was chosen for the subsequent experiments.

Effects of Initial pH in Shake Flask Cultures

To investigate the effects of the initial pH on mycelial growth and EPS production, *A. blazei* was cultivated with different initial pHs (3.0-8.0) in shake flask cultures (Fig. 2). EPS production showed the highest value of 2.32 g l^{-1} with an initial pH of 7.0, whereas mycelial growth showed the highest value of 6.37 g l^{-1} with an initial pH of 5.0.

Effects of pH in Bioreactor Cultures

To investigate the effects of pH, a series of cultures at four different pHs of 4, 5, 6, and 7 were carried out in a bioreactor. As the control case, a culture was also carried out with no pH control. The initial pH was 7.0 in the control. The time profiles of mycelial growth and EPS production with no pH control are shown in Fig. 3. As shown in Fig. 3, in the control with no pH control, the pH declined from 7.0 to 4.3 during the exponential growth phase, and then returned to 5.0 at the end of the culture. Glucose concentration increased during the early 24 h of cultivation, and thereafter began to decrease rapidly. Dissolved oxygen concentration dropped rapidly to nil with active mycelial growth during the same period, and thereafter mycelial growth became slow because of oxygen limitation. Mycelial growth and EPS production continuously increased until 84-96 h of fermentation. The maximum mycelial biomass and EPS concentration were 6.35 g l^{-1} and 2.94 g l^{-1}, respectively. The results strongly suggest that EPS production in *A. blazei* is growth-associated.

As previously described, the pH of the culture medium dropped significantly in the culture with no pH control. Since the pH of the culture broth was expected to affect mycelial growth and EPS production, it was considered that pH regulation and the identification of its optimal value would be required. Fermentation data at various pHs are shown in Fig. 4 and Table 1, with the control data for comparison. The fermentation patterns in pH-controlled cultures were similar to those with no pH control. As shown in Table 1, the maximum mycelial biomass and EPS concentration were the highest at pH 5.0. Although the specific growth rates of the cells were nearly the same irrespective of pH control, the specific production rate of EPS and the yield of EPS on substrate differed significantly.

Effects of Glucose to Dextrin Ratio in Bioreactor Cultures

To identify the best mass ratio of glucose to dextrin for mycelial growth and EPS production, different combinations of these two carbon sources were tested in the bioreactor (Table 2). The total concentration of these two carbon sources was fixed at 30 g l^{-1}.

As listed in Table 2, the mycelial biomass and EPS production increased with the amount of dextrin, showing

Table 2. *A. blazei* cultures in bioreactor at different mass ratios of glucose to dextrin.

Culture parameters	Glucose:Dextrin						
	5:0	4:1	3:2	1:1	2:3	1:4	0:5
Maximum mycelial biomass X [g l^{-1}]	8.10	8.12	8.05	7.75	8.25	8.95	7.78
Maximum EPS concentration P [g l^{-1}]	2.16	2.66	3.19	3.81	3.91	4.51	4.26
Specific growth rate [h^{-1}]	0.08	0.08	0.08	0.09	0.08	0.09	0.08
Specific consumption rate of substrate $Q_{S/X}$ [g g^{-1} d^{-1}]	0.93	0.92	0.93	0.97	0.91	0.84	0.96
Specific production rate of EPS $P_{P/X}$ [g g^{-1} d^{-1}]	0.07	0.08	0.10	0.12	0.12	0.13	0.14
Yield of mycelial biomass on substrate $Y_{X/S}$ [g g^{-1}]	0.27	0.27	0.27	0.26	0.28	0.30	0.26
Yield of EPS on substrate $Y_{P/S}$ [g g^{-1}]	0.07	0.09	0.11	0.13	0.13	0.15	0.14

Table 3. *A. blazei* cultures in bioreactor at different mass ratios of yeast extract to soytone peptone.

Culture parameters	Yeast extract:Soytone peptone				
	3:0	2:1	1:1	1:2	0:3
Maximum mycelial biomass X [g l^{-1}]	8.01	8.95	8.13	7.51	6.85
Maximum EPS concentration P [g l^{-1}]	4.33	4.51	4.37	4.12	3.81
Specific growth rate [h^{-1}]	0.08	0.09	0.08	0.08	0.07
Specific consumption rate of substrate $Q_{S/X}$ [g g^{-1} d^{-1}]	0.94	0.84	0.92	1.00	1.10
Specific production rate of EPS $P_{P/X}$ [g g^{-1} d^{-1}]	0.14	0.13	0.13	0.14	0.14
Yield of mycelial biomass on substrate $Y_{X/S}$ [g g^{-1}]	0.27	0.30	0.27	0.25	0.23
Yield of EPS on substrate $Y_{P/S}$ [g g^{-1}]	0.14	0.15	0.15	0.14	0.13

its maximum value at the mass ratio of glucose to dextrin of 1:4. However, the effect of the carbon source composition on mycelial growth was relatively insignificant. At this mass ratio, the specific production rate of EPS, yield of EPS on substrate, and yield of mycelial biomass on substrate were higher than any other mass ratio.

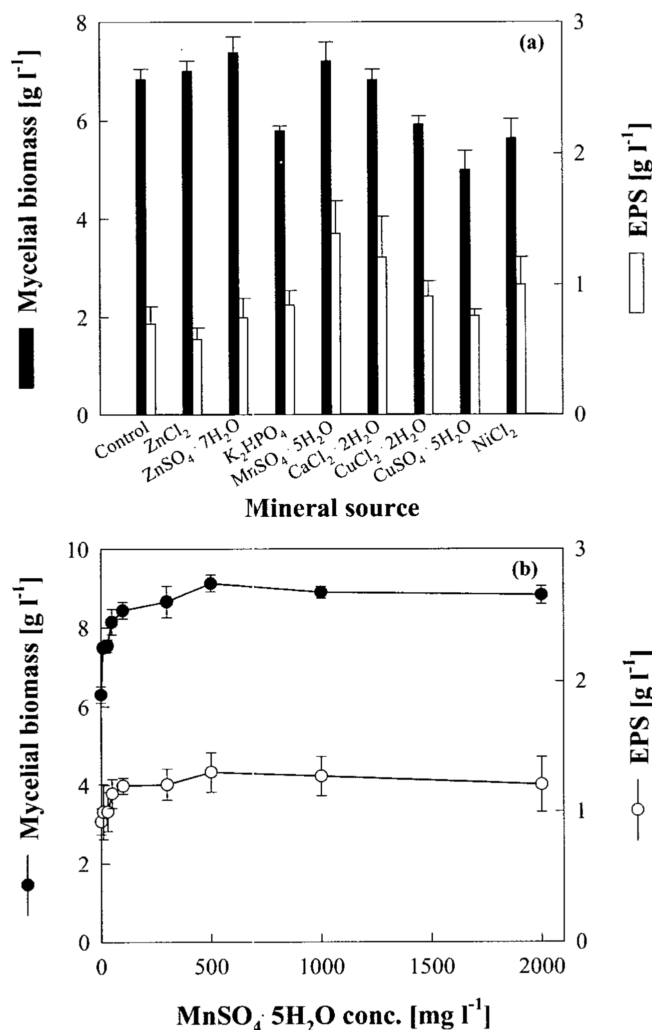

Fig. 5. Effects of mineral sources on mycelial growth and EPS production in shake flask cultures.

Effects of Yeast Extract to Soytone Peptone Ratio in Bioreactor Cultures

To determine the optimal mass ratio of yeast extract to soytone peptone for mycelial growth and EPS production, different combinations of these two nitrogen sources were investigated at a total nitrogen content of 6 g l^{-1} in the bioreactor by changing the mass ratio to be 3:0, 2:1, 1:1, 1:2, and 0:3. As summarized in Table 3, in the case where soytone peptone was the sole nitrogen source, the mycelial growth and EPS production were relatively low. However, when yeast extract was supplied, EPS production and especially mycelial growth were much improved. When the mass ratio of yeast extract to soytone peptone was 2:1, the highest mycelial growth and EPS production were obtained, i.e. 8.95 g l^{-1} and 4.51 g l^{-1}, respectively.

Effects of Mineral Sources in Bioreactor Cultures

The influence of mineral sources on mycelial growth and EPS production was examined by supplement of various mineral sources at a concentration level of 50 mg l^{-1} in shake flask cultures. As shown in Fig. 5(a), a high level of mycelial growth and EPS production was obtained when $CaCl_2 \cdot 2H_2O$, $MnSO_4 \cdot 5H_2O$, or $ZnSO_4 \cdot 7H_2O$ was added. Among these mineral sources, $MnSO_4 \cdot 5H_2O$ yielded the best EPS production, where the optimal concentration of $MnSO_4 \cdot 5H_2O$ for mycelial growth and EPS production was 500 mg l^{-1} (Fig. 5(b)).

When 500 mg l^{-1} of $MnSO_4 \cdot 5H_2O$ was added to the bioreactor under the optimal culture conditions, both the mycelial biomass and EPS concentration were enhanced by approximately 10% (Table 4).

DISCUSSION

Fungal morphology is an important parameter that affects the rheological properties of the fermentation broth, and the control of the morphology is highly desired in industrial fungal fermentation [9, 20]. In general, two growth forms, the filamentous and the pelleted forms, are observed in most fungal fermentations, and the pelleted form is usually less viscous than the filamentous form [22]. The pelleted growth form depends on obtaining uniform pellets of a

Table 4. Enhancement of mycelial growth and EPS production by addition of $MnSO_4 \cdot 5H_2O$ in bioreactor.

Culture parameters	$MnSO_4 \cdot 5H_2O$	
	None added	Added
Maximum mycelial biomass X [g l^{-1}]	8.95	9.85
Maximum EPS concentration P [g l^{-1}]	4.51	4.92
Specific growth rate [h^{-1}]	0.09	0.09
Specific consumption rate of substrate $Q_{S/X}$ [g g^{-1} d^{-1}]	0.84	0.84
Specific production rate of EPS $P_{P/X}$ [g g^{-1} d^{-1}]	0.13	0.12
Yield of mycelial biomass on substrate $Y_{X/S}$ [g g^{-1}]	0.30	0.33
Yield of EPS on substrate $Y_{P/S}$ [g g^{-1}]	0.15	0.16

desired size and mechanically stable structure, and is influenced by a variety of factors such as inoculum size, type and age, medium composition, the biosynthesis or the addition of polymers, the addition of surfactants and chelators, shear force, temperature, pressure, medium viscosity, and oxygen tension [22]. Among these factors, the pellet size is mainly influenced by the inoculum size. Yang and Liau [27] suggested that an increase in inoculum size increased the yield of mycelial growth and the number of pellets but decreased the size of mycelial pellets. In this study, however, inoculum size had no obvious effect on the size of mycelial pellets, while it had significant effect on the number of pellets and mycelial biomass. Mycelial growth did not monotonically increase, while EPS production monotonically increased with the inoculum size (Fig. 1).

Many investigators claimed that the different morphology of fungal mycelia under a different initial pH was the critical factor in biomass accumulation and metabolite formation [6, 16, 24]. The initial medium pH may affect cell membrane function, cell morphology and structure, the solubility of salts, the ionic state of substrate, the uptake of various nutrients, and product biosynthesis. In general, cells can only grow within a certain pH range, and metabolite formation is also often affected by pH. For, example, EPS production in cultures of *Sclerotium glucanicum* was greatly affected by culture pH [24]. Fang and Zhong [5] reported that lowering the initial pH from 6.5 to 3.5 gradually led to a higher production of EPS and higher specific production of intracellular polysaccharide in a popular basidiomycete, *Ganoderma lucidum*. Lee *et al.* [16] suggested that the bistage pH control technique was applied to enhanced EPS production in an airlift fermentor, by which one pH value for optimal mycelial growth was shifted to another value in the liquid-culture of *Ganoderma lucidum*.

It should be stressed that the initial pH 7.0, which is an optimal initial pH for EPS production in shake flask cultures (Fig. 2), may not be the optimum for bioreactor cultures where the pH is regulated for the entire culture period. In this work, the optimal pH for both mycelial growth and EPS production in the bioreactor appeared to be 5.0, which was close to the final pH in the culture with no pH control, starting at an initial pH of 7.0 (Table 1 and Fig. 3).

In batch cultures of *A. blazei* in the bioreactor, as shown in Figs. 3 and 4, glucose concentration was observed to increase because of the dextrin decomposition to glucose during the early 24 h of cultivation, and thereafter began to decrease rapidly. Such a pattern of carbon source consumption is quite different from that in the case of many other microorganisms. In general, microbial cells consume glucose first, and the production of enzymes involved in the uptake of other carbon sources is repressed while glucose is consumed. On the other hand, in the case of *A. blazei* cultivation used in this study, both glucose consumption and dextrin decomposition to glucose seemed to occur simultaneously.

As also shown in Fig. 4, the faster the decomposition rate of dextrin to glucose during the early 24 h of cultivation was, the higher the mycelial biomass and EPS concentration were. And the faster the consumption rate of glucose after 24 h of cultivation was, the higher the EPS concentration was. These results suggest that the pH of the culture broth might have affected the activity of enzymes hydrolyzing dextrin to glucose, such as amylase. Further studies are needed to understand the relationships among activity, mycelial growth, and EPS production.

Nutritional requirements for mycelial growth and EPS production in basidiomycetes and ascomycetes depend on strains and culture conditions. Moreover, different carbon sources can result in different carbohydrate compositions in the polysaccharides produced. In this study, glucose and dextrin were selected as the most suitable carbon sources through a series of preliminary experiments of flask cultures. The optimal mass ratio of glucose to dextrin was found to be 1:4 (Table 2). These results are rather different to the nutritional requirements of other mushrooms in submerged cultures. Bae *et al.* [2] observed a high level of mycelial growth of *P. japonica* in media containing maltose as a carbon source in shake flask cultures. Jonathan and Fasidi [11] reported that the highest mycelial growth of *Psathyerella atroumbonata* was obtained in a glucose medium. Park *et al.* [21] reported that the highest mycelial growth and EPS production in *Cordyceps militaris* were obtained in a sucrose medium. On the other hand, Manzoni and Rollini [17] reported that glucose and dextrin were the best carbon

sources for stimulating mycelial growth and EPS production of *Daedalea quercina*.

It is known that several complex organic nitrogen sources such as polypeptone and corn steep powder are desirable, while inorganic nitrogen sources are inefficient for the production of EPS in submerged cultures of mushrooms [1, 14, 15]. It has been frequently suggested that certain essential amino acids could scarcely be synthesized from inorganic nitrogen sources in the fermentation of higher fungi. Thus, it is probable that most basidiomycetes prefer complex organic nitrogen sources [12]. In this study, the most suitable nitrogen sources for mycelial growth and EPS production were identified to be yeast extract and soytone peptone, which are similar to the results obtained by several groups mentioned above. The optimal mass ratio of yeast extract to soytone peptone was found to be 2:1 (Table 3).

Mineral ions are frequently recognized as essential trace elements for mycelial growth and the production of secondary metabolites like polysaccharides in fungal fermentations [21]. Jonathan and Fasidi [10] reported that Mg^{2+}, K^{+}, and Ca^{2+} promoted mycelial growth of two species of mushroom, *L. subnudus* (Berk) and *S. commune*. It is also reported that KH_2PO_4 and $CaCl_2$ were the most effective mineral sources for mycelial growth of *Phellinus* sp. [13]. In this work, higher mycelial growth and EPS production were achieved in the culture with $MnSO_4{\cdot}5H_2O$ (Fig. 5 and Table 4). A similar observation has been reported by another group. Choi *et al.* [4] reported that production of methylan, an exopolysaccharide produced by *Methylobacterium organophilum* from methanol, was suppressed by the deficiency of mineral sources such as Mn^{2+} or Fe^{2+} ions.

In order to enhance the mycelial growth and EPS production, a further study on the effects of dissolved oxygen concentration on mycelial growth and EPS production of *A. blazei* in bioreactor cultures is needed. In addition, apart from varying the operational conditions, a more sophisticated culture strategy should be developed. Taking into account that carbon source limitation occurred at around 4 days, fed-batch culture appears to be a promising culture mode to improve both mycelial biomass and EPS production.

Acknowledgment

This work was supported by the Biogreen21 Programs of Rural Development Administration of Korea.

REFERENCES

1. Bae, J. T., J. P. Park, C. H. Song, C. B. Yu, M. K. Park, and J. W. Yun. 2001. Effect of carbon source on the mycelial growth and *exo*-biopolymer production by submerged culture of *Paecilomyces japonica*. *J. Biosci. Bioeng.* **91:** 522-524.
2. Bae, J. T., J. Sinha, J. P. Park, C. H. Song, and J. W. Yun. 2000. Optimization of submerged culture conditions for exo-biopolymer production by *Paecilomyces japonica*. *J. Microbiol. Biotechnol.* **10:** 482-487.
3. Cavazzoni, V. and A. Adami. 1992. Exopolysaccharides produced by mycelial edible mushrooms. *Ital. J. Food Sci.* **1:** 9-15.
4. Choi, J. H., S. Y. Kim, D. K. Oh, and J. H. Kim. 1998. Optimization of culture conditions for production of a high viscosity polysaccharide, methylan, by *Methylobacterium organophilum* from methanol. *Kor. J. Appl. Microbiol. Biotechnol.* **26:** 244-249.
5. Choi, K. H. 1999. Development of a new synthetic medium composition for the submerged culture of *Phellinus linteus*. *Kor. J. Biotechnol. Bioeng.* **14:** 167-173.
6. Fang, Q. H. and J. J. Zhong. 2002. Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. *Process Biochem.* **37:** 769-774.
7. Friel, M. T. and A. J. McLoughlin. 2000. Production of a liquid inoculum/spawn of *Agaricus bisporus*. *Biotechnol. Lett.* **22:** 351-354.
8. Ha, T. M., J. H. Choi, Y. H. Kim, and J. H. Eum. 1997. Submerged culture of *Phellinus linteus* with food by-products. *J. Ind. Crop Sci.* **39:** 22-30.
9. Jimenez-Tobom, G. A., M. J. Penninckz, and R. Lejeunne. 1997. The relationship between pellet size and production of Mn (II) peroxidase by *Phanerochate chrysosporium* in submerged culture. *Enzyme Microbiol. Biotechnol.* **21:** 537-542.
10. Jonathan, S. G. and I. O. Fasidi. 2001. Studies on phytohormones, vitamins and mineral element requirements of *Lentinus subnudus* (Berk) and *Schizophyllum commune* (Fr. Ex. Fr) from Nigeria. *Food Chem.* **75:** 303-307.
11. Jonathan, S. G. and I. O. Fasidi. 2001. Effect of carbon, nitrogen and mineral sources on growth of *Psathyerella atroumbonata* (Pegler), a Nigerian edible mushroom. *Food Chem.* **72:** 479-483.
12. Jung, I. C., S. H. Kim, Y. I. Kwon, S. Y. Kim, J. S. Lee, S. Park, K. S. Park, and J. S. Lee. 1997. Cultural condition for the mycelial growth of *Phellinus igniarius* on chemically defined medium and grains. *Kor. J. Mycol.* **25:** 133-142.
13. Kang, T. S., D. G. Lee, and S. Y. Lee. 1997. Isolation and mycelial cultivation submerged of *Phellinus* sp. *Kor. J. Mycol.* **25:** 257-267.
14. Kim, S. W., H. J. Hwang, C. P. Xu, Y. S. Na, S. K. Song, and J. W. Yun. 2002. Influence of nutritional conditions on the mycelial growth and exopolysaccharide production in *Paecilomyces sinclairii*. *Lett. Appl. Microbiol.* **34:** 389-393.
15. Kim, S. W., H. J. Hwang, C. P. Xu, J. M. Sung, J. W. Choi, and J. W. Yun. 2003. Optimization of submerged culture process for the production of mycelial biomass and exo-polysaccharides by *Cordyceps militaris* C738. *J. Appl. Microbiol.* **94:** 120-126.
16. Lee, K. M., S. Y. Lee, and H. Y. Lee. 1999. Bistage control of pH for improving exopolysaccharide production from

mycelia of *Ganoderma lucidum* in an air-lift fermentor. *J. Biosci. Bioeng.* **88:** 646-650.
17. Manzoni, M. and M. Rollini. 2001. Isolation and characterization of the exopolysaccharide produced by *Daedalea quercina. Biotechnol. Lett.* **23:** 1491-1497.
18. Menoli, R. C. R. N., M. S. Mantovani, L. R. Ribeiro, G. Speit, and B. Q. Jordão. 2001. Antimutagenic effects of the mushroom *Agaricus blazei* Murrill extracts on V79 cells. *Mutat. Res.* **496:** 5-13.
19. Mizuno, T., T. Hagiwara, T. Nakamura, H. Ito, K. Shimura, T. Sumiya, and A. Asakura. 1990. Antitumor activity and some properties of water-soluble polysaccharides from 'Himematsutake', the fruiting body of *Agaricus blazei* Murill. *Agr. Biol. Chem.* **54:** 2889-2896.
20. Moreira, M. T., A. Sanroman, G. Feijoo, and J. M. Lema. 1996. Control of pellet morphology of filamentous fungi in fluidized bed bioreactors by means of a pulsing flow. Application to *Aspergillus niger* and *Phanerochate chrysosporium. Enzyme Microbiol. Biotechnol.* **19:** 261-266.
21. Park, J. P., S. W. Kim, H. J. Hwang, and J. W. Yun. 2001. Optimization of submerged culture conditions for the mycelial growth and exo-biopolymer production of *Cordyceps militaris. Lett. Appl. Microbiol.* **33:** 76-81.
22. Sinha, J., J. T. Bae, J. P. Park, K. M. Kim, C. H. Song, and J. W. Yun. 2001. Changes in morphology of *Paecilomyces japonica* and their effect on broth rheology during production of *exo*-biopolymers. *Appl. Microbiol. Biotechnol.* **56:** 88-92.
23. Takaku, T., Y. Kimura, and H. Okuda. 2001. Isolation of an antitumor compound from *Agaricus blazei* Murill and its mechanism of action. *J. Nutr.* **131:** 1409-1413.
24. Wang, Y. C. and B. McNeil. 1995. pH effects on exopolysaccharide and oxalic acid production in cultures of *Sclerotium glucanicum. Enzyme Microb. Technol.* **17:** 124-130.
25. Yang, B. K., S. C. Jeong, and C. H. Song. 2002. Hypolipidermic effect of exo- and endo-biopolymers produced from submerged mycelial culture of *Ganoderma lucidum* in rats. *J. Microbiol. Biotechnol.* **12:** 872-877.
26. Yang, B. K., J. B. Park, and C. H. Song. 2002. Hypolipidermic effect of exo-polymer produced in submerged mycelial culture of five different mushrooms. *J. Microbiol. Biotechnol.* **12:** 957-961.
27. Yang, F. C. and C. B. Liau. 1998. Effects of cultivating conditions on the mycelial growth of *Ganoderma lucium* in submerged flask cultures. *Bioprocess Eng.* **19:** 233-236.
28. Zhang, X. H., H. B. Hu, Y. L. Tang, R. S. Huang, J. F. Luo, and B. K. Hur. 2002. The hepatoprotective effects of polysaccharides isolated from submerged fermentation of *Ganoderma lucidum. J. Microbiol. Biotechnol.* **12:** 367-370.