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Exopolysaccharide Production and Mycelial Growth in an Air-Lift Bioreactor Using *Fomitopsis pinicola*

CHOI, DUBOK[1], JEUNG-MOO MAENG[2], JI-LU DING[2], AND WOL-SUK CHA[2]*

[1]*Biotechnology Laboratory, B-K Company Ltd., Gunsan 573-879, Korea*
[2]*Department of Chemical Engineering, Chosun University, Gwang-ju 501-759, Korea*

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Abstract For effective exopolysaccharide production and mycelial growth by a liquid culture of *Fomitopsis pinicola* in an air-lift bioreactor, the culture temperature, pH, carbon source, nitrogen source, and mineral source were initially investigated in a flask. The optimal temperature and pH for mycelial growth and exopolysaccharide production were 25°C and 6.0, respectively. Among the various carbon sources tested, glucose was found to be the most suitable carbon source. In particular, the maximum mycelial growth and exopolysaccharide production were achieved in 4% glucose. The best nitrogen sources were yeast extract and malt extract. The optimal concentrations of yeast extract and malt extract were 0.5 and 0.1%, respectively. K_2HPO_4 and $MgSO_4 \cdot 7H_2O$ were found to be the best mineral sources for mycelial growth and exopolysaccharide production. In order to investigate the effect of aeration on mycelial growth and exopolysaccharide production in an air-lift bioreactor, various aerations were tested for 8 days. The maximum mycelial growth and exopolysaccharide production were 7.9 g/l and 2.6 g/l, respectively, at 1.5 vvm of aeration. In addition, a batch culture in an air-lift bioreactor was carried out for 11 days under the optimal conditions. The maximum mycelial growth was 10.4 g/l, which was approximately 1.7-fold higher than that of basal medium. The exopolysaccharide production was increased with increased culture time. The maximum concentration of exopolysaccharide was 4.4 g/l, which was about 3.3-fold higher than that of basal medium. These results indicate that exopolysaccharide production increased in parallel with the growth of mycelium, and also show that product formation is associated with mycelial growth. The developed model in an air-lift bioreactor showed good agreement with experimental data and simulated results on mycelial growth and exopolysaccharide production in the culture of *F. pinicola*.

Keywords: Polysaccharide, *Fomitopsis pinicola*, liquid culture, air-lift bioreactor

*Corresponding author
Phone: 82-62-230-7218; Fax: 82-62-230-7262;
E-mail: wscha@mail.chosun.ac.kr

Since ancient times, mushrooms have been used in foods and folk medicines throughout the world because they contain significant amounts of proteins, carbohydrates, fiber, vitamins, and minerals and may be produced with higher biological efficiency than others. In particular, mushroom extracts are widely used as nutritional supplements and medicines, and have been touted as being beneficial for human health [28, 34, 35, 44]. *Fomitopsis* belongs to the Basidiomycetes fungus class. *Fomitopsis pinicola* is part of the *Pleurotaceae* family. *Fomitopsis pinicola* (swartz: Fr.) Karst is a saprotrophic polypore with a worldwide distribution in temperate and boreal forests. It attacks both conifers and hardwoods, causing a cubical brown-rot. It has been widely cultivated and studied in Japan and traditionally used as a health-food source for the prevention of cancer, plant growth regulating agent, and diabetes [20]. Yoshikawa *et al.* [53] have isolated the lanstane triterpenoids and lanstane triterpene glycosides from the fruit bodies of *Fomitopsis pinicola*, and examined their antiniflammatory activities against cyclooxygenase-1 and cyclooxygenase-2. Many investigators have tried to cultivate mushrooms on solid artificial media for fruit body production in order to obtain polysaccharides [1, 5, 26]. However, this method does not guarantee substituents from batch to batch. As a result, attention has been paid to the use of liquid culture for the production of mycelial biomass and polysaccharides. Recently, the mycelial growth and production of exopolysaccharide by various fungal strains have been extensively researched for potential applications, using environmental parameters as well as the composition of the medium. Although many researchers have attempted to determine the optimal culture conditions for mycelial growth and exopolysaccharide production using different fungal strains, the nutritional requirements and factors of the environment for the liquid cultures used for mycelial growth and exopolysaccharide production from the liquid culture of *F. pinicola* have not been demonstrated in an air-lift bioreactor.

In the present study, for effective production of exopolysaccharide from liquid cultures of *F. pinicola* in an air-lift bioreactor, the optimum temperature, pH, various carbon sources, nitrogen sources, and mineral sources were first investigated in a flask. Second, under the optimum conditions, batch cultures were carried out in an air-lift bioreactor. Additionally, the logistic model to describe the mycelial growth and the Leudecking-Piret model to describe exopolysaccharide production in an air-lift bioreactor were proposed.

MATERIALS AND METHODS

Strain and Cultures

Fomitopsis pinicola was obtained from the culture ground of Kaya-Backsong (Chungnam, South Korea). Plates were incubated at 25°C for 7 days and then stored at 4°C. *F. pinicola* was initially grown on PDA (potato dextrose agar) medium in a petri dish, and was then transferred into the seed medium containing malt extract 5 g/l, yeast extract 10 g/l, and glucose 3 g/l by punching out from the agar plate culture with a sterilized cork borer. Seeds were grown in a 300-ml flask containing 100 ml of seed medium at 25°C on a rotary shaker at 100 rpm for 7 days, and were then homogenized at 10,000 rpm for 30 sec. Flask cultures for exopolysaccharide production were carried out in 300-ml flasks containing 50 ml of the basal medium containing yeast extract 2 g/l, KH_2PO_4 0.5 g/l, and $MgSO_4$ 0.25 g/l. The culture medium was inoculated with 5% of the mycelial homogenate and then cultivated in a flask or an air-lift bioreactor. Air lift-bioreactor cultures were carried out in 5-l air lift-bioreactors containing 3 l of working medium comprised of optimum medium under optimum conditions at a range of 0.5 to 2.0 vvm of aeration. The pH of the culture broth in the 5-l air lift-bioreactor was controlled at 6.5. All the media were sterilized at 121°C for 20 min. The pH of the medium was adjusted to the desired value by the addition of either 1 N HCl or 1 N NaOH. All experiments were carried out in triplicate to ensure reproducibility.

Analysis

Mycelial growth was obtained by centrifuging samples at 3,000 rpm for 15 min, washing the sediment three times with distilled water, and drying to a constant weight. All supernatants were collected, and the crude exopolysaccharide was then precipitated by the addition of 95% ethanol. The precipitated exopolysaccharide was collected by centrifugation at 3,000 rpm for 10 min, and was then dried to remove residual ethanol at 60°C. Exopolysaccharide concentration in the culture broth using starch was determined by phenol-sulfuric acid assay. Residual glucose concentrations were determined by the modification to the methods of Ling *et al.* [38] and Silva *et al.* [49].

Air-Lift Bioreactor System

Based on the designs described elsewhere [31, 37, 39, 47], a 5-l air-lift bioreactor was modified for the culturing of *F. pinicola*. The air-lift bioreactor is comprised of three parts; a conical bottom holding the sparger, a cylindrical middle section, and a top portion with a degassing zone. In order to increase the mixing characteristics, the draft tube was removed and replaced with four ring spargers and wire nets. An air-lift bioreactor has several ports; for measuring the dissolved oxygen concentration and foam inside the bioreactor, for the removal of exhaust gas, and for the addition of antifoam agents, with a sampling port at the bottom of the cylindrical section. The temperature of the contents of an air-lift bioreactor can be controlled by circulating water through a jacket.

RESULTS

Effect of Culture Temperature on Mycelial Growth

In order to investigate the effect of culture temperature on mycelial growth of *F. pinicola*, various culture temperatures were tested on Petri dishes containing a solid medium (YMGA) for 12 days. The culture temperature was controlled at 20, 25, and 30°C, respectively, and results are shown in Fig. 1. The mycelial growth was found to increase with increased culture time. The optimal temperature for mycelial growth was found to be 25°C.

Effect of pH on Mycelial Growth and Exopolysaccharide Production

In general, cells can only grow within a certain pH range, and metabolite formation is also often affected by pH. To investigate the effects of the initial pH of the medium on mycelial growth and exopolysaccharide production at

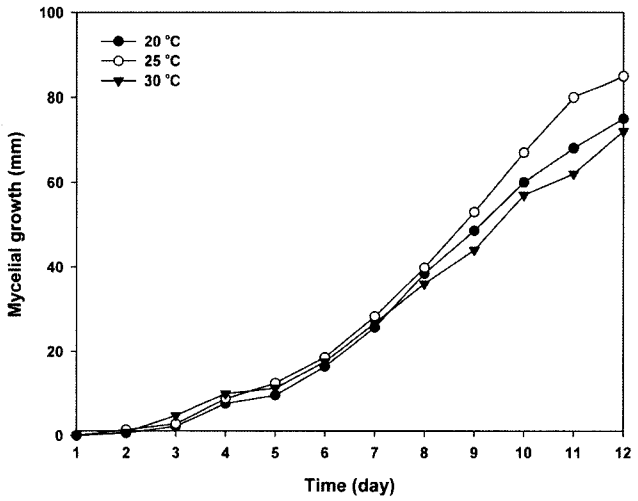

Fig. 1. Effect of temperature on mycelial growth.

Table 1. Effects of pH on mycelial growth and exopolysaccharide production.

pH	Mycelial dry weight (g/l)	Exopolysaccharide (g/l)
4.0	0.8	0.15
4.5	1.0	0.27
5.0	1.2	0.38
5.5	1.6	0.42
6.0	1.7	0.45
6.5	1.5	0.41
7.0	1.3	0.38
7.5	1.0	0.30
8.0	0.9	0.26

25°C, batch cultures were carried out within the pH range of 4.0 to 8.0 for 4 days. The results of pH testing are shown in Table 1. When cultures within the pH range of 5.5 to 6.5 were carried out, the mycelial growth and exopolysaccharide concentrations ranged from 1.5 to 1.7 g/l and 0.41 to 0.45 g/l, respectively. However, at pH levels higher than 7.0 or lower than 5.5, mycelial growth and exopolysaccharide concentrations were decreased. The maximum mycelial growth and exopolysaccharide production at pH 6.0 were found to be 1.7 and 0.45 g/l, respectively, after 4 days of culture.

Effects of Various Carbon Sources on Mycelial Growth and Exopolysaccharide Production

The carbon source is the essential factor that acts as an energy source for cell growth and carbohydrate, protein, lipid, and nucleic acid synthesis in fungi. In order to investigate the effects of carbon sources on mycelial growth and exopolysaccharide production, glucose, maltose, dextrose, fructose, starch, galactose, arabinose, sucrose, and mannitol were used. Each carbon source was added to the basal medium at a concentration of 3%, and was tested in flasks. The results are shown in Table 2. When glucose or sucrose was used, the mycelial growth and exopolysaccharide production was favorable. In particular,

Table 2. Effects of carbon sources on mycelial growth and exopolysaccharide production.

Carbon source (0.3%)	Mycelial dry weight (g/l)	Exopolysaccharide (g/l)
Glucose	1.90	0.51
Fructose	0.93	0.24
Galactose	0.90	0.22
Arabinose	0.73	0.19
Maltose	0.89	0.001
Sucrose	1.64	0.44
Dextrin	2.12	0.001
Starch	0.79	0.002
Mannitol	0.92	0.003

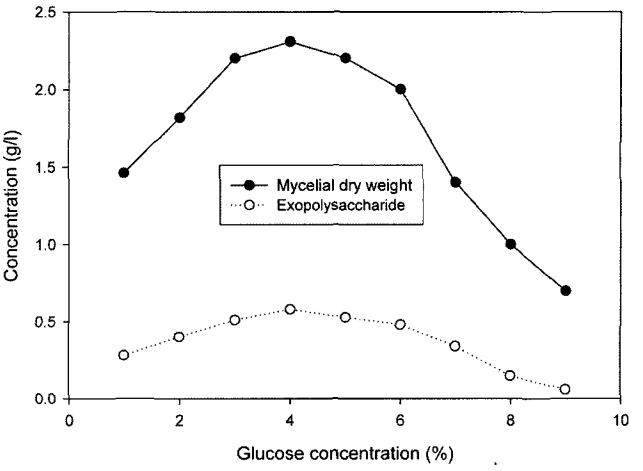

Fig. 2. Effects of glucose concentration on mycelial growth and exopolysaccharide production.

the mycelial growth and exopolysaccharide concentrations using glucose were 1.90 g/l and 0.51 g/l, respectively. Although mycelial growth was high following the addition of dextrin, the exopolysaccharide production was low. The mycelial growths using maltose, starch, and mannitol ranged from 0.79 to 0.92 g/l, but the respective exopolysaccharide productions were very poor. Based on the above results, glucose was selected as a suitable carbon source for further studies. In determining the optimal concentration of glucose for mycelial growth and exopolysaccharide production, a range of 1% to 10% was investigated in flasks. As shown in Fig. 2, the highest mycelial growth and exopolysaccharide production of *F. pinicola* were obtained when 4% glucose was used. On the other hand, when the glucose concentration was lower or higher than 4%, mycelial growth and exopolysaccharide production were decreased. Therefore, it was concluded that the optimum glucose concentration for mycelial growth and exopolysaccharide production was 4%.

Effect of Nitrogen Sources on Mycelial Growth and Exopolysaccharide Production

The nitrogen source and carbon source are two important factors that affect mycelial growth and exopolysaccharide production from microorganisms. In order to investigate the effects of nitrogen sources on mycelial growth and exopolysaccharide production, various organic nitrogen sources, including malt extract, peptone, trytophan, yeast extract, and polypeptone, and inorganic nitrogen sources such as $Ca(NO_3)_2$, $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, $NH_4H_2PO_4$, $(NH_4)_2HPO_4$, and KNO_3, were tested for 6 days. The results are shown in Table 3. Among the various nitrogen sources, when yeast extract or malt extract was used, the mycelial growth and exopolysaccharide production were favorable. More specifically, the mycelial growth and exopolysaccharide

Table 3. Effects of nitrogen sources on mycelial growth and exopolysaccharide production.

Nitrogen source (0.3 %)	Mycelial dry weight (g/l)	Exopolysaccharide (g/l)
$Ca(NO_3)_2$	2.51	0.24
$NaNO_3$	2.39	-
$(NH_4)_2SO_4$	2.38	-
NH_4NO_3	2.35	-
$NH_4H_2PO_4$	2.64	0.34
$(NH_4)_2HPO_4$	2.51	0.51
KNO_3	2.55	0.58
Malt extract	3.61	0.82
Peptone	3.02	0.62
Tryptone	3.23	0.72
Yeast extract	3.74	0.97
Polypeptone	3.12	0.77

concentrations using yeast extract were 3.74 g/l and 0.97 g/l, respectively. In the case of malt extract, these values were 3.62 g/l and 0.82 g/l, respectively. In order to determine the optimum concentration of yeast extract on mycelial growth and exopolysaccharide production, ranges of 0.0 to 1.25% of yeast extract and 0.0 to 0.3% of malt extract were investigated. The results are shown in Tables 4 and 5. When the yeast extract concentrations were increased from 0.0 to 0.5%, mycelial growth was increased. However, at yeast extract concentrations higher than 0.75%, mycelial growth was not increased. In particular, when 0.5% yeast extract was used, the maximum mycelial growth and exopolysaccharide production were obtained, 3.90 and 1.07 g/l, respectively. When the malt extract concentration was greater than 0.1%, the mycelial growth did not increase, and when the malt extract concentration was above 0.125%, the exopolysaccharide production did not increase.

Effects of Mineral Sources on Mycelial Growth and Exopolysaccharide Production

In order to investigate the influences of mineral sources on the mycelial growth and exopolysaccharide production in a medium containing 4.0% glucose, 0.1% malt extract, and 0.5% yeast extract, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, $MgCl_2$, Na_2HPO_4, $FeSO_4 \cdot 7H_2O$, K_2HPO_4, and KH_2PO_4 were investigated for 7 days in flasks. The results are shown in Table 6. The mineral source was added to the

Table 4. Effect of yeast extract concentration on mycelial growth and exopolysaccharide production.

Concentration (%)	Mycelial dry weight (g/l)	Exopolysaccharide (g/l)
0	2.52	0.44
0.25	3.40	0.75
0.5	3.90	1.07
0.75	3.89	0.98
1.0	3.87	0.90

Table 5. Effect of malt extract concentration on mycelial growth and exopolysaccharide production.

Concentration (%)	Mycelial dry weight (g/l)	Exopolysaccharide (g/l)
0.00	3.30	0.50
0.025	3.48	0.62
0.05	3.52	0.72
0.075	3.58	0.75
0.10	3.60	0.79
0.125	3.58	0.68
0.15	3.50	0.68

medium at a concentration of 0.10% for each mineral. K_2HPO_4 and $MgSO_4 \cdot 7H_2O$ were found to be the best mineral sources for mycelial growth. In particular, as shown Tables 7 and 8, the maximum mycelial growth and exopolysaccharide production obtained were 5.12 g/l and 1.47 g/l, respectively, when 0.1% K_2HPO_4 was used. However, when 0.05% $MgSO_4 \cdot 7H_2O$ was used, the maximum mycelial growth and exopolysaccharide production were obtained.

Effect of Aeration on Mycelial Growth and Exopolysaccharide Production in an Air-Lift Bioreactor

In order to investigate the effect of aeration on mycelial growth and exopolysaccharide production, various aerations were tested in a 5-l air-lift bioreactor containing 3 l of working medium comprised of glucose 4%, yeast extract 0.5%, malt extract 0.1%, K_2HPO_4 0.1%, and $MgSO_4 \cdot 7H_2O$ 0.05% at 25°C. Cultures were carried out for 8 days at a range of 0.5 to 2.0 vvm of aeration, and the pH of the cultures was controlled at 6.5. The results are shown in Fig. 3. The mycelial growths were generally found to increase with increased culture time, for up to 8 days of culture. More specifically, when 0.5 vvm of aeration was used, mycelial growth increased for the first 6 days of culture. However, after 6 days of culture, mycelial growth did not increase compared with those for culture undergoing greater than 1.0 vvm of aeration. In the case of 1.0 and 2.0 vvm of aeration, the mycelial growths were somewhat similar. In contrast, when 0.5 vvm of aeration was used, the exopolysaccharide production also increased for 5 days

Table 6. Effects of mineral sources on mycelial growth and exopolysaccharide production.

Mineral source	Mycelial dry weight (g/l)	Exopolysaccharide (g/l)
$CaCl_2 \cdot 2H_2O$	4.38	1.03
$ZnSO_4 \cdot 7H_2O$	0.69	-
$MgCl_2$	5.14	0.89
$MgSO_4 \cdot 7H_2O$	5.02	1.09
Na_2HPO_4	4.39	0.76
$FeSO_4 \cdot 7H_2O$	4.58	0.62
K_2HPO_4	5.02	1.31

Table 7. Effect of K_2HPO_4 on mycelial growth and exopolysaccharide production.

Concentration (%)	Mycelial dry weight (g/l)	Exopolysaccharide (g/l)
0.0	4.13	0.90
0.025	4.36	1.1
0.05	4.84	1.33
0.075	5.07	1.40
0.100	5.12	1.47
0.125	4.98	0.42
0.150	4.89	0.31

of culture, and then did not increase after 6 days of culture. When the culture was performed at aeration higher than 1.0 vvm, the exopolysaccharide production increased with the increase of culture time up to 7 days of culture, and then after 8 days of culture, exopolysaccharide production appeared to be dependent on the level of aeration. At 1.5 vvm of aeration, the maximum mycelial growth and exopolysaccharide production were 7.9 g/l and 2.6 g/l, respectively, after 8 days of culture.

Mycelial Growth and Exopolysaccharide Production in an Air-Lift Bioreactor

A batch culture using optimally designed medium and basal medium in an air-lift bioreactor was carried out under optimal culture conditions. The optimal culture conditions were a temperature of 25°C and an aeration rate of 1.5 vvm. The changes of mycelial growth, pH, exopolysaccharide production, and residual glucose concentrations are shown in Fig. 4. When the optimally designed medium was used, mycelial growth was increased with the increase of culture time by 11 days. However, in the case of basal medium, the increase of culture time was 8 days. The maximum mycelial growth was 10.4 g/l, which was about 1.7-fold higher than that of basal medium after 11 days of culture. Both pH values were similar for 11 days. The glucose consumption was also found to increase with increased culture time when the optimally designed medium was used. The consumed glucose was 30 g/l after 11 days of culture. However, in the case of basal medium, the glucose consumption was found to increase with an increased culture by 7 days, and did not increase after 8 days of culture. The amount of consumed glucose

Table 8. Effect of $MgSO_4 \cdot 7H_2O$ on mycelial growth and exopolysaccharide production.

Concentration (%)	Mycelial dry weight (g/l)	Exopolysaccharide (g/l)
0.0	4.37	1.08
0.025	5.04	1.24
0.05	5.50	1.31
0.075	5.30	1.24
0.10	5.00	1.15

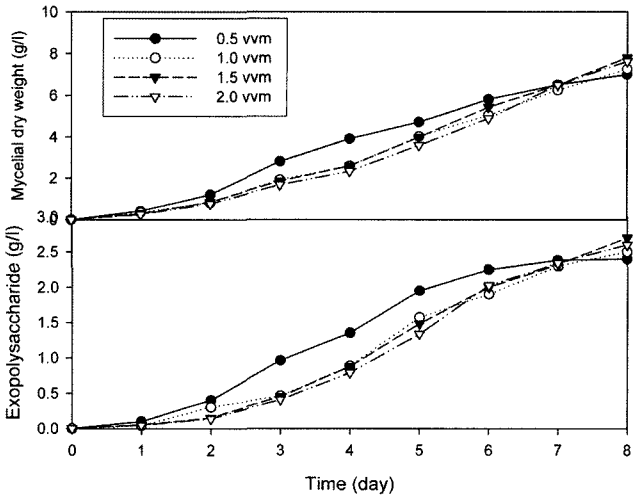

Fig. 3. Changes of mycelial growth and exopolysaccharide production in an air-lift bioreactor at various aerations.

was 18.0 g/l after 12 days of culture. Exopolysaccharide production increased with increased culture time when the optimally designed medium was used. The maximum concentration of exopolysaccharide was 4.4 g/l, which was about 3.3-fold higher than that of basal medium after 11 days of culture. These results indicate that exopolysaccharide

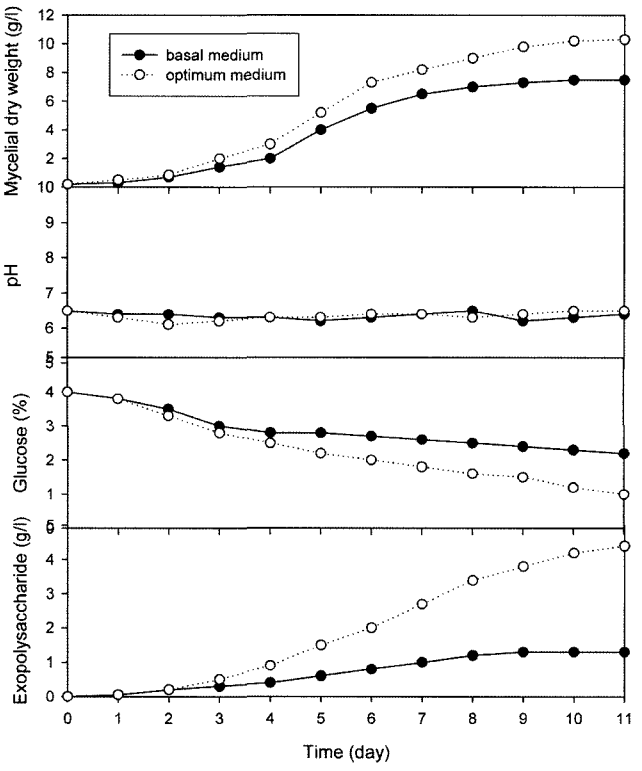

Fig. 4. Comparison of basal medium and optimal medium on mycelial growth, pH, glucose consumption, and exopolysaccharide production in an air-lift bioreactor.

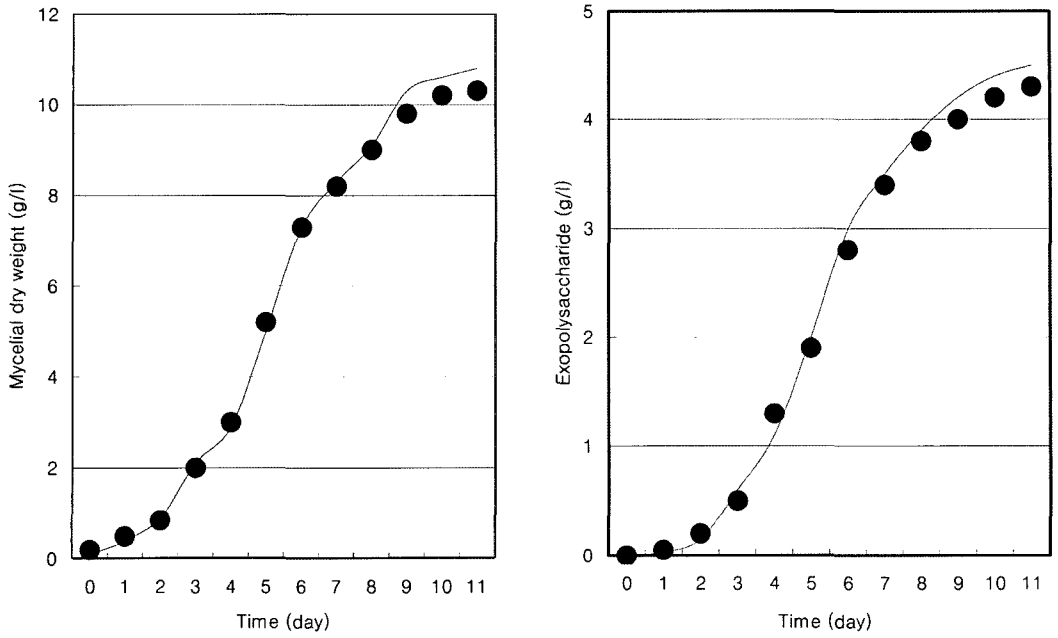

Fig. 5. Comparison of experimental data and simulation data of mycelial growth and exopolysaccharide concentration.
The symbol (●) represents experimental data and the line (—) represents results of the simulation.

production increased in parallel with the growth of mycelium, and also showed that product formation is associated with mycelial growth in an air-lift bioreactor using *Fomitopsis pinicola.*

Empirical Kinetic Modeling of Mycelial Growth and Exopolysaccharide Production in an Air-Lift Bioreactor

The logistic model to describe the mycelial growth and Leudecking-Piret model for exopolysaccharide production in an air-lift bioreactor using *F. pinicola* were proposed.

$$dX/dt=\mu X \quad (1)$$

$$=_{max}(1-X/X_m) \quad (2)$$

$$dP/dt=dX/dt+X \quad (3)$$

where X is mycelial growth, P is exopolysaccharide concentration, μ is the specific mycelial growth rate, α is the growth-associated product formation coefficient, and α is the nongrowth-associated product formation coefficient. The specific mycelial growth rate decreased with the increase of mycelial growth, and their correlation can be expressed using Eq. (2).

DISCUSSION

A liquid culture using mushrooms has potential advantages in that it allows higher mycelial production in a compact space and requires a shorter incubation time with a lower chance of contamination. We previously studied liquid cultures for effective polysaccharide production from *Pleurotus ferulae*, *Lentinus* sp., *Pholiota nameko*, *Pleurotus nebrodensis*, and *Agrocybe aegerita* in jar fermentors [7, 8, 14, 16, 48]. Additionally, in order to determine the nutritional values such as crude fat, carbohydrate, protein, amino acid, vitamin, and mineral compositions, we studied the mycelia of *Cordyceps militaris*, *Morchella esculenta*, and *Lentinus edodes* [6, 9, 10]. We are attempting to confirm the possibility of enhancing the production of mycelial biomass and polysaccharide using an air-lift bioreactor, because air-lift bioreactors have many economical implications in regard to reactor construction, maintenance, and scale-up, as reported previously [15, 17]. However, the ability of air-lift bioreactors to supply oxygen is generally lower than that of the conventional type of bioreactor, *i.e.*, aeration and stirred tank bioreactor. Therefore, it is necessary to determine the optimal culture conditions that make suitable operation of an air-lift bioreactor. Recently, we found that biological activities of *F. pinicola* extracts *in vitro* were much higher that that of other mushrooms. Therefore, the optimization of mycelial growth and exopolysaccharide production using *F. pinicola* was required.

In this study, in order to achieve high productivity of mycelial growth and exopolysaccharide using *F. pinicola* in the air-lift bioreactor, various environmental factors that affect these productivities were first researched in flask cultures. In order to investigate the effect of culture temperature on mycelial growth of *F. pinicola*, various temperatures were tested. The mycelial growths were increased with increased culture time, up to 8 days of

culture. After 9 days of culture, mycelial growths were dependent on culture temperature. The maximum mycelial growth was obtained at 25°C after 12 days of culture. A similar phenomenon was also observed in the culture of *Pleurotus eryngii*, *Lentinus lepideus*, and *Phellinus linteus* [5, 23, 54]. In the case of *Flammulina veltipes*, the optimal temperature for mycelial growth was 30°C, but the production of angiotensin-converting enzyme inhibitor at 30°C was lower than that of the culture at 20°C [27]. In the case of fed-batch culture of *Ganoderma resinaceum* DG-6556, the optimal temperature for mycelial growth was at 28°C, whereas the maximum exopolysaccharide production was achieved at 31°C [26]. In the case of the mycelial growth and exobiopolymer production of *Cordyceps militaris*, the optimum temperature was found to be 20°C [43].

Many researchers reported that cell membrane function, cell growth, cell morphology, and cell structure, the solubility of salts, the ionic state of substrate, the uptake of various nutrients, and product biosynthesis were affected by the pH of the medium. For example, the mycelial and exopolysaccharide production in a culture of *Sclerotium glucanicum* was greatly affected by low or high pH [51]. Fang and Zhong [18] reported that lowering the initial pH from 6.5 to 3.5 gradually led to a high production of exopolysaccharide and high specific-production of intracellular polysaccharide in a culture of *Ganoderma lucidum*. Lee *et al.* [33] suggested that the pH control technique could be applied to enhance exopolysaccharide production, by which the pH value can be altered in the liquid culture of *Ganoderma lucidum*. In our case, when culture was carried out in pH 6.0, the exopolysaccharide was 0.45 g/l, which was about 3-fold higher than that of pH 4.0. However, in the case of pH values above 8.0 or below 4.0, these values were greatly decreased. A similar phenomenon was also observed in *Formitella fraxinea* [11]. In the case of *Sphaeropsis pyriputrecens*, mycelia grew at pH 3.0–6.0, and optimum growth was observed at pH 3.2–4.2. However, no mycelial growth was seen at pH values above 8.0 after 10 days of culture [29]. The mycelial growth and exopolysaccharide production from *Cordyceps jiangxiensis* JXPJ 0109, *Phellinus linteus*, *Poria cocos*, *Coriolus versicolor,* and *Grifola frondosa* were optimal at pH 5.0 [23, 30, 45, 52].

Nutritional requirements for mycelial growth and exopolysaccharide production in basidiomycetes and ascomycetes depend on the strains and culture conditions used. In addition, different carbon sources can result in different carbohydrate compositions in the polysaccharides produced. In our case, when glucose or sucrose was used, the mycelial growth and exopolysaccharide production were favorable. Similar results were also obtained in cultures of *Coriolus versicolor*, *Psathyrella atroumbonata*, *Cordyceps militaris*, and *Lentinus edodes* [21, 43, 45]. However, in the case of *Paecilomyces japonica*, maltose was selected as the most suitable carbon source for mycelial growth and exopolysaccharide production [3]. A white mutant culture of *Ganoderma lucidum* using xylose, sorbitol, and mannitol showed good mycelial growth [13]. In the case of *Phellinus linteus*, the maximum mycelial growth was obtained in the glucose medium, and the maximum yield of exopolysaccharide in the mannose medium [32]. Manzoni and Roillini [40] reported that dextrin was the best carbon source for stimulating mycelial growth and exopolysaccharide production in the culture of *Daedalea quercina*. Kim *et al.* [25] also reported that the mycelial growth and exopolysaccharide production from *Agaricus blazei* increased with the amount of dextrin, showing its maximum value at a glucose to dextrin mass ratio of 1:4. However, the effect of the carbon source composition in mycelial growth was relatively insignificant. Bae *et al.* [2] reported that a significant difference was observed in the production pattern of exobiopolymer and broth rheology during the culturing of *Paecilomyces japonica* when maltose and sucrose were used. The highest mycelial growth and exopolysaccharide production of *F. pinicola* were obtained when 4% of glucose was used. These results indicate that a high glucose concentration was unfavorable for exopolysaccharide production. Here, it appears that the osmotic pressure caused by a high glucose concentration may be detrimental to metabolite biosynthesis, although cell growth was not inhibited. Kim *et al.* [26] reported that the greatest exopolysaccharide concentrations were observed when 70 g/l of an initial glucose was used after 12 days, and about 50% was increased after 18 days of fed-batch culture of *Ganoderma resinaceum* DG-6556.

To determine the best nitrogen source, various nitrogen sources were investigated. As shown in Table 3, when organic nitrogen sources were used, the mycelial growth and exopolysaccharide production were increased compared with inorganic nitrogen sources. In particular, the highest mycelial growth and exopolysaccharide production were achieved in media containing yeast and malt extracts. The lower mycelial growth in the medium containing inorganic nitrogen was similar to a common situation in fungi [19]. Park *et al.* [46] reported that the mycelial growth was closely related to exopolysaccharide production in the culture of *Coriolus versicolor*. These results suggest that yeast extract and malt extract might contain the necessary components for mycelial growth and exopolysaccharide production. However, when inorganic nitrogen sources were used in cultures of *Ganoderma lucidum*, the mycelial growth and exopolysaccharide production were similar to those found when using organic nitrogen sources [36]. In the case of *Cordyceps militaris*, corn steep powder was the most suitable carbon source for mycelial growth and exopolysaccharide production [43]. In the case of *F. pinicola*, of the various amino acids, when using organic

nitrogen sources such as malt extract, peptone, trytophan, yeast extract, and polypeptone, it was found that alanine, lysine, leucine, glycine, serine, methionine, glutamic acid, and arginine were consumed to a much greater extent than the other amino acids. On the other hand, when using inorganic nitrogen sources, the consumption of these amino acids was decreased (data not shown). This indicates that the exopolysaccharide production was affected by alanine, lysine, leucine, glycine, serine, methionine, glutamic acid, and arginine.

These mineral ions are recognized as favorable bioelements for mycelial growth and exopolysaccharide production. In the case of *F. pinicola*, when $CaCl_2 \cdot 2H_2O$, $MgCl_2$, Na_2HPO_4, and $FeSO_4 \cdot 7H_2O$ were used, the mycelial growths were similar but the exopolysaccharide production was significantly decreased. In addition, the effect of $ZnSO_4 \cdot 7H_2O$ on the mycelial growth and exopolysaccharide production was very poor. The best result of the mycelial growth and exopolysaccharide production in the medium containing K_2HPO_4 and $MgSO_4 \cdot 7H_2O$ was also similar to the effects of various inorganic salts on enzyme production by *Aspergillus japonicus* [12]. Phosphate has been used as a buffering reagent, and potassium is an important mineral involved in cell structure; the magnesium cation may stimulate biosynthesis of the fungal cell wall and affect its permeability. Kim *et al.* [25] reported that a high level of mycelial growth and exopolysaccharide production was obtained when $CaCl_2 \cdot 2H_2O$, KH_2PO_4, $MnSO_4 \cdot 5H_2O$, or $ZnSO_4 \cdot 7H_2O$ was used. In particular, when 500 mg of $MnSO_4 \cdot 5H_2O$ was added to the culture broth, both mycelial growth and exopolysaccharide production were increased by approximately 10%. Jonathan and Fasidi [21] also reported that Mg^{2+}, K^+, and Ca^{2+} promoted the mycelial growth of *L. subnudus* and *S. commune*. In the case of *Pellinus* sp., KH_2PO_4 and $CaCl_2$ were the most effective mineral sources for mycelial growth [22].

In order to investigate the effects of aeration on the mycelial growth and exopolysaccharide production in a 5-l air-lift bioreactor containing a 3 l working volume, various aerations were investigated under the optimum medium and culture conditions. The mycelial growth and exopolysaccharide production were increased when aeration was within a range of 0.5 to 1.5 vvm. On the other hand, in the case of aerations higher than 2.0 vvm, mycelial growth and exopolysaccharide production were not increased. This result indicates that the mycelial growth and exopolysaccharide production were affected by aeration in an air-lift bioreactor. Using the optimum culture conditions, we conducted comparisons of optimal medium and basal medium on mycelial growth, pH, exopolysaccharide production, and residual glucose concentrations in an air-lift bioreactor. The mycelial growth was found to be increased with increased culture time. When the optimum medium was used, the yield of mycelial growth was 0.3 g/g, which was similar to that of basal medium after 11 days of culture. However, the yield of exopolysaccharide was 0.15 g/g, which was about 2.1-fold higher than that of basal medium after 11 days of culture.

Kinetic studies supported by mathematical models are a vital component of the overall investigation of cell growth and product formation in the culture process. Models allow for easy data analysis and provide strategies for solving problems encountered at the design stage. Knowledge and understanding of the kinetics of exopolysaccharide production are of great economic importance in view of the fact that exopolysaccharide production is a major industrial issue in many culture processes. Kinetic studies for several extracellular microbial polysaccharides have been reported [41, 50]. However, few studies have been carried out on the unstructured kinetic model for exopolysaccharide production in fungi, even though the suitable predictive tools are required for understanding their prolonged and complicated culture processes [42]. In order to evaluate the model, we compared the calculated values from the kinetic model with experimental data obtained in an air-lift bioreactor using *F. pinicola* (Fig. 5). The results for mycelial growth and exopolysaccharide production were found to fit well to the model. This result suggests that the model may potentially describe the liquid culture process of *F. pinicola* in an air-lift bioreactor.

It is difficult to culture aerobic fungi in an air-lift bioreactor because of mycelial morphology, apparent increased viscosity due to mycelial growth or medium, and unplanned mixing. However, the mycelial growth and exopolysaccharide production in the culture of *Fomitopsis pinicola* through the development of the medium and culture conditions was similar to those of a jar fermentor (data not shown). This suggests that an air-lift bioreactor has potential use in exopolysaccharide production and mycelial growth using *F. pinicola*. The combination of a high-yield exopolysaccharide producer, a simple and less expensive production medium, and an air-lift bioreactor may lead to the realization of a large-scale production of exopolysaccharide production.

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