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Effect of Different pH Processes on Branched β-1,3-Glucan Production from Submerged Culture of *Ganoderma lucidum*

영지(*G. lucidum*)의 액체배양에 의한 β-1,3-Glucan 생산에 미치는 서로 다른 pH Process의 영향

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Abstract

A submerged cultivation of *Ganoderma lucidum* was carried out in an air-lift fermenter system, and the effects of different pH processes on extracellular branched β-1,3-glucan(EPS) production and mycelial growth(MDW) were investigated. The controlled pH process improved the production of branched β-1,3-glucan and biomass in comparison to the uncontrolled pH process. However, the maximum production of branched β-1,3-glucan were obtained by the bi-staged pH process. From these results, we confirmed that the bi-staged pH process was the most effective for improving the production of branched β-1,3-glucan from submerged culture of *G. lucidum*.

키워드 : *영지버섯, β-1,3-글루칸, 액체배양, pH 공정*
Keywords : *Ganoderma lucidum,* β-1,3-Glucan, submerged cultivation, pH process

1. Introduction

Mushrooms, a fungus of the class of Basidiomycete, have long been esteemed for their medicinal properties(1-4). Especially, the polysaccharides having anti-tumor activity were found in many mushrooms, and the structure of anti-tumor active polysaccharide is mainly known to β-1,3-glucan(1,3). So far, four fungal polysaccharides, Schizophyllan(Katen Tokyo), Krestin(Sankyo, Tokyo), Lentinan(Ajinomoto, Tokyo) and PSP(Shanghai Teachers University, Shanghai) are being used in the treatment of various cancers as an immuno-modulator(1).

Ganoderma lucidum belonging to the family of *Polyporaceae* has been known to have many medicinal benefits such as curing chronic hepatitis, gastric ulcer, hyperlipemia, hypertension etc. as a panacea(4). Recently, it also was reported that cultured mycelium as well as fruit body could be used as functional foods and medicinal substances(2,5,6). Such polysaccharides from *G. lucidum* were generally obtained by solvent extraction of cultured mycelium or fruit body. However, these processes are not commercially feasible, since the yield of extraction is very low and the mass production

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is impractical. For convenient, inexpensive and effective production of anti-tumor polysaccharide, an exo-polysaccharide(EPS) production from submerged culture could be chosen for the most promising process(7).

During the past several years, we carried out a series of study for achieving the above purpose(8-13). According to this effort, it was found that an EPS secreted by *Ganoderma lucidum* ASI 7004 had a structure of β-1,3 glucan with 1,6 branch (Mwt=1.2x10^6)(9,12), and this EPS had a potential industrial importance because its high anti-tumor activity and induction of differentiation of HL-cells(9, 13). More recently, we concluded that submerged cultivation with air-lift fermenter system was more beneficial for the efficient production of the EPS from *G. lucidum*. This system was suitable for more economical production of EPS from higher yields in less time(11). We also found that the optimum pHs for the production of EPS and biomass were different each other(11).

By this time, the effects of pH on the EPS production were well examined in pullulan production of *Aureobacidium pullulans*. It was found that pH of the culture broth influenced on the pullulan production, mycelial growth, morphology of mycelium and rheology of culture fluid. Especially, by pH shift, the improved production of EPS was obtained(14-16).

Therefore, we attempted the effects of the uncontrolled, controlled and bi-staged pH processes to establish a more effective process for producing EPS from submerged culture of *G. lucidum* with air-lift fermenter system.

2. Materials and Methods

2.1. Strain and maintenance

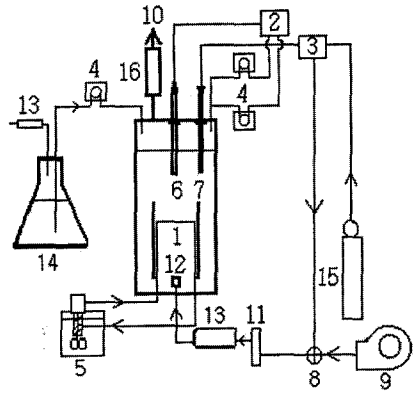
The strain used in this study was *Ganoderma lucidum* ASI 7004, which was a stock culture of this laboratory. Stock culture was maintained on PDA(potato dextrose agar) plate, and subcultured every 3 months(8-10).

2.2. Medium and inoculum

The inoculum for all experiments was prepared in a optimum medium reported previously(8-10). The seed was incubated at 30°C on rotary shaker (150rpm) for 5 days.

2.3. Cultivation

Batch culure was performed in 2.5L and 10L air-lift fermenter system with the working volumes of 2.0 and 7.5L (Fig.1).



1. Air-lift fermenter
2. pH controller
3. DO controller
4. Peristaltic pump
5. Temp. controller
6. pH sensor
7. DO sensor
8. Three-way valve
9. Air compressor
10. Gas outlet
11. Flow meter
12. Sparger
13. Air filter
14. Feed reservoir
15. Oxygen tank
16. Condenser

Fig. 1. Schematic diagram of air-lift fermenter for submerged cultivation of *G. lucidum*.

Five percent(v/v) of inoculum was transferred into fermenter, cultivated for 7 days under the temperature of 25°C and the aeration rate of 2.5 vvm. Air was supplied through the sparger at 2.5 vvm and aeration rate was controlled at constant level by the gas regulator of the compressed air, and the foaming was removed by anti-foaming agent (Antifoam 289, Sigma Chemical Co.).

The pH of culture was adjusted to the pH desired in the range of 3 to 5 by three pH procedures: uncontrolled, controlled, and bi-staged pH processes. Initial pH was not adjusted during cultivation in uncontrolled pH process, while initial pH was maintained to a constant pH with pH controller by adding 5N NaOH solution in controlled pH process. In bi-staged pH process, initial pH was maintained constantly for the early periods of

cultivation, and then was shifted to different pHs.

2.4. Analytical methods

Twenty five mL of culture broth was sampled and analyzed at the time intervals of 24 hours in all experiments. Biomass and culture medium were separated by centrifugation at 10,000xg for 30min.

The biomass harvested were washed three times with distilled water and then were dried to a constant weight at 105°C.

The supernatant was collected and followed by the addition of two volumes of acetone. The above mixtures was whirled with the glass rod and then the viscous aggregates to be wound by rod was collected as the EPS(8,9). The EPS were dried to a constant weight at 105°C.

The viscosity of the culture supernatant fraction was measured with Ostwald viscometer at 30°C. Morphology of mycelium was observed by light microscope (Olymphus CHS-213E).

3. Results and Disscusion

3.1. The uncontrolled pH process

The initial pH effect on the EPS(β-1,3-glucan with 1,6 branch) production and the mycelial growth is shown in Fig. 2(a).

As shown in Fig. 2, the initial pH of the culture broth was decreased rapidly from its optimum initial pH of 3.0 or 5.0 to final value of pH 2.4 and 2.6 within 48 hours, respectively. It also was observed the optimal pH for the EPS was different from that of the mycelial growth.

The higher mycelial growth was obtained at the lower pH(pH 4) of culture broth, while the higher production of EPS was obtained at the higher pH(pH 5) than that of mycelial growth.

The maximum mycelial growth, 17.37g/l, was obtained at the initial pH 4.0, and the maximum EPS production, 4.91g/l, was obtained at initial pH 5.0. However, the optimal pH of the mycelial growth and EPS production under uncontrolled pH process might not be coincided with the above results, because the pH of the culture broth changed during the cultivation.

3.2. The controlled pH process

G. lucidum was cultivated in 2.5L air-lift fermenter under the controlled pH, and its results are shown in Fig. 2(b). EPS production increased with the increase of pH, and the maximum EPS production of 5.69g/l was obtained at constant pH 5.0. This EPS yield was

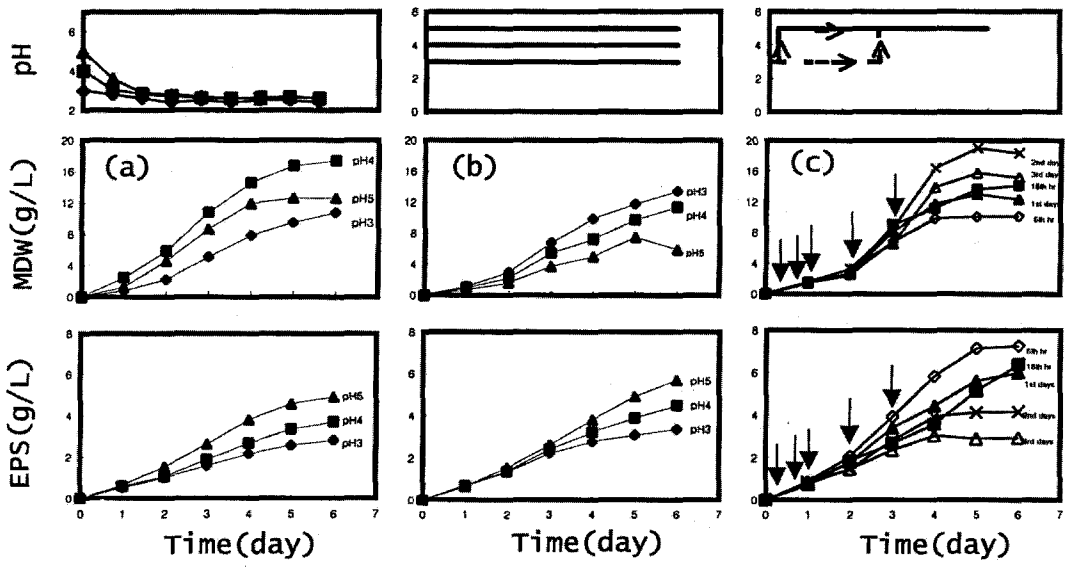


Fig. 2 Effects of pH process on the branched β-1,3-glucan production from *G. lucidum* under 2.5L air-lift fermenter system (Arrow(↓) shows the time of pH shift from 3 to 5).
(a) uncontrolled, (b) controlled, (c) bi-staged pH process.

slightly higher than that of obtained at the uncontrolled pH 5.0(4.69g/l). However, maximum biomass production of 12.45g/l was obtained at controlled pH 3, and this mycelial growth at controlled pH 5.0 was very low at the end of the culture. This phenomenon was considered to be cell lysis araised by the physiological change due to adding the large amount of NaOH solution added.

The similar but enhanced results were obtained when 10L air-lift fermenter was used. EPS yield in 10L fermenter was higher than that in 2.5L fermenter(data not shown).

Therefore, it was expected that the bi-staged pH process would be effective for the more EPS production, which the 1st stage was carried out at the low pH 3 showing the maximum mycelial growth, and then the second stage of cultivation was initiated by adjusting the medium pH to a higher value of pH 5.

3.3. The bi-staged pH process

Fig. 2(c) represents the time course of pH shift from pH 3.0 to pH 5.0 at different shift periods. pH shift from 3 to 5 after 6 hrs of culture gave the best result for EPS, however, maximum mycelial growth was observed at pH shift after 2 days of culture. The EPS obtained at pH shift from 3 to 5 after 6 hrs of culture was higher than those of the uncontrolled and controlled pH processes, and was about 1.5-3 times higher than those of the uncontrolled and controlled pH 4 or 5.0(Fig. 3).

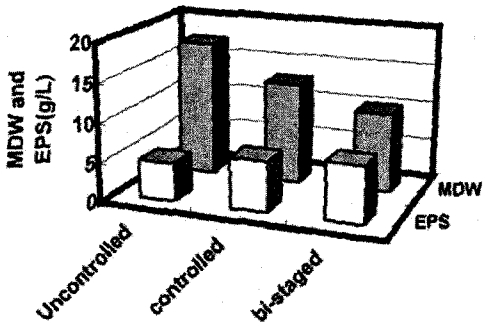


Fig. 3. Comparisons of branched β-1,3-glucan (EPS) and biomass (MDW) produced by the uncontrolled, controlled and bi-staged pH processes.

However, the mycellial growth of 18.4g/l was obtained at pH shift from 3 to 5 after the 2nd days of culture, and this result was 1.5 times lower than that of the constant pH 3.0. The differences of morphology in pH 3 and 5 were not observed and both morphologies were pellet forms(data not shown). However, Catley(14) and then Ono et al.(15) reported that the morphology was changed from initial mycelial to yeast-like form, and subsequently showed that yeast-like pellet form was the predominant producer of pullulan. However, pellet size (2-3.5mm) at pH 5 was larger than that of pellet(1-2.5mm) at pH 3.

This result was similiar to that obtained in the shaken flask reported previously(8). pH shift also influenced on the rheological properties of broth. Culture filtrate viscosity decreased with the increase of pH as shown in Fig. 4.

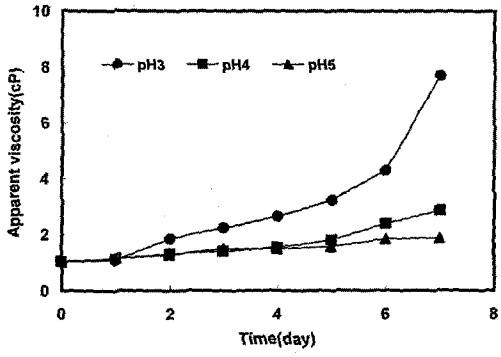


Fig. 4. Apparent viscosity of culture filtrate from submerged culture of *G. lucidum* with at different pHs.

It was suggested that the enhancement of EPS production by the increase of controlled pH was due to the improvement of mass transfer in culture broth by viscosity reduction. As the viscosity reduction means the decrease in molecular weight of EPS, this was similar to the result of Lacaroix et al.(17), who reported that the pullulan of *A. pullulans* obtained by the pH shift had relatively small molecular weight in comparison to that from the culture without controlled pH procedure. From the above pH shift experiments, we successfully elaborated the larger amounts of EPS and biomass, especially EPS. The maximum EPS and biomass production obtained by pH shift from 3 to 5 in

2.5L fermenter were 7.27 and 18.4g/l.

However, as shown in Fig. 5, the similar but enhanced results were obtained in 10L air-lift fermenter.

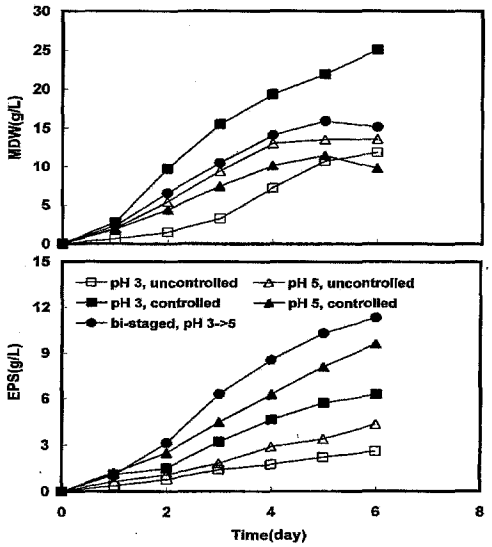


Fig. 5. Effect of pH process on the branched β-1,3-glucan (EPS) and biomass (MDW) production from *G. lucidum* under 10L air-lift fermenter system.

Both EPS and biomass yields in 10L fermenter was higher than those in 2.5L fermenter, and their values were 11.33 and 15.11g/L, respectively.

From above results, it was confirmed that the bi-staged pH process was more effective for improving the production of branched β-1,3-glucan from submerged culture of *G. lucidum*.

4. Conclusions

The extracellular branched β-1,3-glucan production and mycelial growth from submerged cultivation of *G. lucidum* under different pH processes were compared to each other. Under uncontrolled and controlled pH processes, the mycelial growth was maximized at the lower initial pH of 3-4, while the production of branched β-1,3-glucan was maximized at higher initial pH of 4-5, indicating that the optimum pH for mycelial growth and branched β-1,3-glucan production. The pH shift process, which a low pH of 3 is maintained to accelerate mycelial growth and then increased to pH 5 to obtain the highest branched β-1,3-glucan production, was more effective than those of the uncontrolled and controlled pH processes. The maximum productions of branched β-1,3-glucan of 7.27 and 12.65g/L were obtained in 2.5L and 10L air-lift fermenter, respectively.

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