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Influence of Agitation Intensity and Aeration Rate on Production of Antioxidative Exopolysaccharides from Submerged Mycelial Culture of *Ganoderma resinaceum*

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Abstract The present study investigated the influence of the aeration rate and agitation intensity on the production of the mycelial biomass and antioxidative exopolysaccharide (EPS) in *Ganoderma resinaceum*. In submerged cultures with varying agitation speeds and aeration rates in a stirred-tank reactor, the maximum mycelial biomass and maximum EPS concentration were achieved at 50 rpm and 300 rpm, respectively. Under varying aeration rates, the highest amount of mycelial biomass (18.1 g/l) was accumulated at the lowest aeration rate (0.5 vvm) and the maximum EPS production (3.0 g/l) obtained at 1.0 vvm. A compositional analysis revealed that the five different EPSs were protein-bound heteropolysaccharides, consisting of 87.17–89.22% carbohydrates and 10.78–12.83% proteins. The culture conditions had a striking affect on the carbohydrate composition of the EPS, resulting in different antioxidative activities. All the EPSs showed strong scavenging activities against superoxide and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, whereas no clear trend in antioxidative activity was observed against hydroxyl radicals and lipid peroxides. Although the precise reason for this difference is still unclear, the high glucose moiety of EPS is probably linked to its broad spectrum of antioxidative activity.

Key words: Antioxidative activity, *Ganoderma resinaceum*, mushrooms, polysaccharides, submerged culture

Ganoderma are basidiomycetous fungi belonging to the order Polyporales and the family Ganodermataceae. Among the numerous species of *Ganoderma*, the most commonly used is *G. lucidum* because of its wide range of biological activities [5, 20, 24]. Recently, many researchers have paid their attention on polysaccharides from natural sources, owing to the numerous side effects and toxicity resulting from current synthetic drugs [7, 16, 17, 19, 33, 36, 37]. Mushrooms are a typical source of natural medicines, yet despite the variety of biological activities related to mushrooms, there is a lack of data related to antioxidative activity. Moreover, there has been no previous report on the submerged culture conditions of *G. resinaceum*, even though it is expected to exhibit similar biological activities to *G. lucidum*.

The reactive oxygen species (ROS) meditated by cellular reactions are involved in degenerative or pathological events, such as aging, cancer, coronary heart ailments, and Alzheimer's disease [1, 13]. The oxidative damage to DNA, proteins, and other cellular determinants seems inevitable when the concentration of ROS exceeds the tolerability of cells. Thus, antioxidants could indirectly attenuate this oxidative damage to tissue by increasing the cells' natural defenses [2] and/or directly help by scavenging such free radical species [21]. As such, growing attention is being paid to antioxidative natural products, and certain polysaccharides discovered to be potent antioxidants [22, 30].

In addition to polysaccharides extracted from fruiting bodies, extracellular polysaccharides obtained from a submerged mycelial culture of mushrooms have also attracted attention [3, 6, 26]. The production of fungal exopolysaccharides (EPSs) has already been investigated using a wide range of environmental parameters, and a great number of reports documented the factors influencing fungal morphology, rheology, and product formation during the culture of many higher fungi [9, 10, 28]. The effects of

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the aeration rate and agitation speed on microbial EPS production are important factors affecting the successful progression of fermentation. Aeration can be beneficial to the growth and metabolism of microbial cells by improving the mass transfer characteristics with respect to the substrate, product, and oxygen [10, 23, 29, 31]. Agitation is also an important parameter for adequate mixing, mass, and heat transfer. Agitation creates shear forces, causing morphological changes, variation in cell growth and product formation, and also damaging the cell structure [9, 11, 38, 39].

Accordingly, the purpose of the present study was to examine the effect of the aeration rate and agitation speed on the mycelial biomass and EPS production by *G. resinaceum* in a stirred tank reactor. The morphological and rheological features between different culture conditions were also studied and the fermentation kinetics described. Finally, the antioxidative activity was evaluated for five different EPSs produced by *G. resinaceum* under different culture conditions.

MATERIALS AND METHODS

Microorganism and Media

A culture of *G. resinaceum*, an isolate from a mountainous area in Egypt (Tanta, Egypt), was maintained on potato dextrose agar (PDA) slants. The slants were incubated at 25°C for 4 days, stored at 4°C, then transferred to fresh media every 4 weeks. Unless otherwise stated, the seed cultures were grown in 250 ml flasks containing 50 ml of an MCM medium (Mushroom Complete Medium: 20 g glucose, 2 g meat peptone, 2 g yeast extract, 0.46 g KH_2PO_4, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$ per liter) at 25°C and 150 rpm on a rotary shaking incubator for 4 days.

Culture Conditions

The *G. resinaceum* was initially grown on a PDA medium on a petridish, then transferred into the seed culture medium by punching out 5 mm of the agar plate culture with a house-developed cutter. The fermentation media were inoculated with 4% (v/v) of the seed culture, then cultivated at 30°C in a 5-l stirred-tank fermenter (Ko-BioTech Co., Incheon, Korea) using a six blade disc-turbine impeller. The fermentations were carried out at 30°C and pH 6.0 with a working volume 3-l and varying aeration rates and agitation speeds. The seed cultures were transferred to the fermentation medium (35 g glucose, 8 g soy peptone, 5 mM $MnCl_2$ per liter) and cultivated for 4 days. Samples were taken every 3 days to analyze the mycelial dry weight, EPS, and residual sugar concentration.

Estimation of Mycelial Biomass and EPS Concentration

The samples taken at various time intervals were centrifuged at 10,000 ×*g* for 20 min. The resulting supernatant was then filtered through Whatman filter paper No. 2 (Whatman International Ltd., Maidstone, England). The resulting culture filtrate was mixed with four volumes of absolute ethanol, stirred vigorously, and left overnight at 4°C. The EPS was dialyzed overnight against distilled water, followed by lyophilization, and then the weight was estimated. After repeated washing of the mycelium with distilled water and drying at 70°C for 24 h to a constant weight, the dry weight of the mycelium was measured. The residual sugar content was determined by the phenol sulfuric acid method using glucose as the standard [12].

Morphological and Rheological Measurements

The morphological details of the samples were evaluated using an image analyzer (Matrox Electronic System Ltd., Dorval, Quebec, Canada) with software coupled to a light microscope (Olympus Optical Co., Ltd., Tokyo, Japan) through a CCD camera (Matsushita Communication Industrial Co., Ltd., Yokohama, Japan). The CCD camera captured 512× 512 pixel images, with grayness levels from 0 (black) to 255 (white). The samples were fixed with an equal volume of fixative (13 ml of 40% formaldehyde, 5 ml glacial acetic acid with 200 ml of 50% ethanol). A 0.1-ml aliquot of each fixed sample was then transferred to a slide, air dried, and stained with methylene blue (0.3 g of methylene blue and 30 ml of 95% ethanol in 100 ml of deionized water) [27]. Rheological measurements using a Brookfield programmable LVDVII+digital viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, U.S.A.) fitted with a small sample adapter were also performed on samples collected from the bioreactor at regular intervals.

Compositional Analysis

The total sugar content of the EPS was determined by the phenol sulfuric acid method using glucose as the standard [12]. The monosaccharide components and their ratios were determined by the hydrolysis of EPS with 2 M trifluroacetic acid at 121°C for 3 h. The hydrolysates were then evaporated to dryness, followed by successive reduction with $NaBH_4$ and acetylation with Ac_2-O (1:1, v/v; 2 ml) at room temperature for 12 h [34, 35]. The sugar composition was subjected to preparation of the corresponding alditol acetate, and then analyzed by gas chromatography (Varian Co., Model: Star 3600CX, Lexington, MA, U.S.A.) using a fused silica capillary column (Na form, 300 mm×0.25 mm, Supelco Inc., Bellefonte, PA, U.S.A.) and flame ionization detector.

Assay of Scavenging Activity Against DPPH Radicals

The scavenging activity of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was assayed according to the method described by Chu *et al.* [8] with some modifications. A 1.0-ml aliquot of a 0.1 mM DPPH radical solution dissolved in methanol was mixed with 0.5 ml of the EPS solution

(10 mg/ml) or negative control (methanol). The reaction mixture was stirred vigorously and the absorbance measured at 520 nm (A_{520}). The DPPH radical-scavenging activity (%) was calculated using the following equation: {1-(A_{520} of sample/A_{520} of control)}×100.

Assay of Scavenging Activity Against Hydroxyl Radicals

The deoxyribose method was used to determine the reaction rate of hydroxyl radicals with each EPS, and performed as described by Halliwell *et al.* [15]. The reaction mixtures in a final volume of 1.0 ml contained deoxyribose (60 mM), a phosphate buffer (pH 7.4, 20 mM), $FeSO_4 \cdot H_2O$ (100 μM), EDTA (100 μM), and H_2O_2 (1 mM). The solutions of $FeSO_4 \cdot H_2O$ were prepared immediately before use. After incubation at 37°C for 1 h, the color was developed by adding 1 ml of trichloroacetic acid (TCA 2.8%, v/v) and thiobarbituric acid (TBA 1.0%, w/v), followed by heating in a boiling water bath for 10 min. The absorbance of the resulting solution was measured spectrophotometrically at 532 nm (A_{532}). The radical scavenging activity (%) was calculated using the following equation: {1-(A_{532} of sample/A_{532} of control)}×100.

Assay of Scavenging Activity Against Superoxide Radicals

The superoxide dismutase (SOD) activity was measured using the xanthine-xanthine oxidase system as the source of superoxides and nitroblue tetrazolium (NBT) as the radical scavenger. One unit of SOD was defined as the amount of enzyme responsible for a 50% inhibition of the initial rate of NBT reduction. The SOD activity was determined as described by Beauchamp and Fridovich [4] by measuring the percent inhibition of NBT reduced by SOD. Xanthine oxidase was used to generate O_2^- during the conversion of xanthine to uric acid. One ml of 0.3 mM xanthine, 500 μl of 0.6 mM EDTA, 500 μl of 0.15 mM NBT, and 100 μl of each EPS were mixed in the plate. After adding xanthine oxidase, the formazan produced was measured spectrophotometrically at 560 nm. The plates were then incubated for 20 min at 37°C. All the experiments were performed in triplicate and their mean values noted.

Assay of Lipid Peroxidation Inhibition

The inhibition of the lipid peroxidation of a rat brain homogenate was assayed according to the method described by Ng *et al.* [25] with some modifications. Brain tissue obtained from male Sprague-Dawley (SD) rats weighing 150 g was homogenized with a Polytron homogenzier in an ice-cold Tris-HCl buffer (pH 7.4, 20 mM) to produce a 1:2 (w/v) homogenate. The homogenate was then centrifuged at 3,000 ×*g* for 10 min, and an aliquot of the supernatant (0.1 ml) mixed with 0.2 ml of each EPS produced under the different culture conditions, 0.1 ml of 10 μM $FeSO_4$ added, and the mixture incubated at 37°C for 1 h. The reaction was terminated by the addition of 0.5 ml of TCA (28%, w/v), followed by 0.38 ml of TBA (2%, w/v), with heating at 100°C for 20 min. After centrifugation at 3,000 ×*g* for 10 min to remove the precipitated proteins, the absorbance of the supernatant containing the TBA-reactive substances (TBARS) was measured at 532 nm (A_{532}) using a spectrophotometer. The inhibition percentage of the lipid peroxidation in the sample was calculated using the following equation: {1-(A_{532} of sample/A_{532} of control)}×100.

RESULTS AND DISCUSSION

Effect of Agitation Intensity and Aeration Rate on Mycelial Biomass and EPS Production

In the submerged cultures under three different agitation speeds, the maximum mycelial biomass (12.7 g/l) was achieved at 50 rpm, whereas the maximum EPS concentration (2.9 g/l) was obtained at 300 rpm on day 12 (Figs. 1A and 1B). The dissolved oxygen concentration profiles were different for the three levels of agitation tested (Fig. 1D). The DO levels at 50 and 150 rpm were significantly reduced from 100% saturation at the beginning of the fermentation to around 10% at the end of the fermentation. In contrast, the DO level at 300 rpm was maintained at a level of 35% saturation on day 9, and then slowly increased to around 50% at the end of the fermentation (Fig. 1D). It is interesting to note that the oxygen limitation that occurred at 50 and 150 rpm did not result in a lower

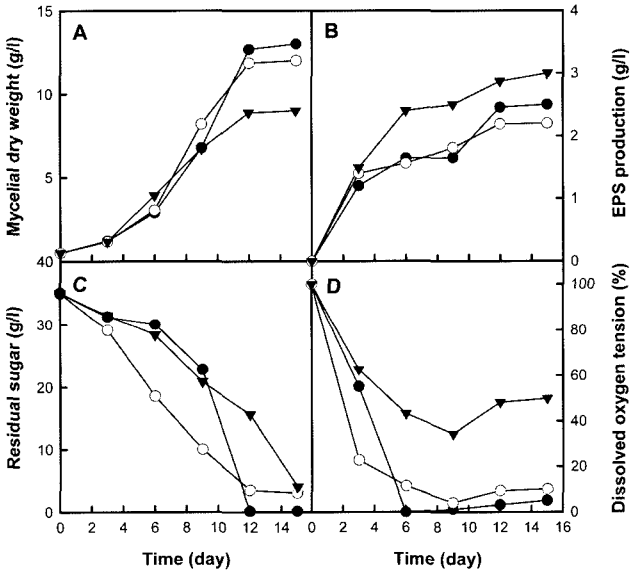

Fig. 1. Time profiles of mycelial biomass (**A**), exopolysaccharide production (**B**), residual sugar (**C**), and dissolved oxygen (**D**) during submerged mycelial culture of *Ganoderma resinaceum* in a 5-l stirred-tank fermenter under 2 vvm and varying agitation speeds.
50 rpm (●), 150 rpm (○), 300 rpm (▼).

Table 1. Fermentation results of *Ganoderma resinaceum* under different conditions of agitation and aeration in a 5-l stirred-tank reactor.

Kinetic parameters	Agitation speed (rpm)[a]			Aeration rate (vvm)[b]		
	50	150	300	0.5	1.0	2.0
Maximum biomass concentration, X (g/l)[c]	12.7(d 14)	11.9(d 14)	8.9(d 14)	18.1(d 12)	17.9(d 12)	8.9(d 12)
Maximum EPS concentration, P (g/l)[d]	2.5(d 14)	2.2(d 14)	2.9(d 14)	2.5(d 9)	3.0(d 9)	2.9(d 12)
Specific growth rate, μ (d^{-1})[e]	0.29	0.33	0.29	0.47	0.42	0.27
Specific consumption rate of substrate, $Q_{S/X}$ (g/g/d)[f]	0.25	0.45	0.32	0.23	0.20	0.23
Specific production rate of EPS, $P_{P/X}$ (g/g/d)[g]	0.01	0.01	0.03	0.01	0.02	0.05
Yield of EPS on substrate, $Y_{P/S}$ (g/g)[h]	0.05	0.02	0.09	0.03	0.11	0.21

[a]Aeration was fixed at 2.0 vvm at 30°C. [b]Agitation was fixed at 300 rpm at 30°C. [c,d]Data was obtained on a specified day (d). [e]μ (d^{-1})=(1/t) ln(X/X_0). [f]$Q_{S/X}$ (g/g/d)=(dS/dt)(1/X). [g]$P_{P/X}$ (g/g/d)=(dP/dt)(1/X). [h]$Y_{P/S}$ (g/g)=(dP/dt)(dS/dt). X, S, and P refer to concentrations of cells, substrate, and EPS at time t, respectively. [e,f,g,h]Values were calculated from the slopes of data obtained from day 4 to day 12 at 30°C.

mycelial growth and shorter log-growth phase. Conversely, a higher fungal growth was achieved at the low agitation speeds because of the prevention of mycelial fragmentation mediated by a severe shearing effect.

Table 1 shows the growth kinetic data for *G. resinaceum* under different agitation speeds. The specific growth rate (μ) of the cells was not significantly altered by varying the agitation speed, and the maximum yield of EPS formation from the substrate ($Y_{P/S}$) was 0.09 g/g/d at an agitation speed of 300 rpm.

In general, vigorous agitation is beneficial for mycelial growth in a submerged culture, as it increases the uptake rate of oxygen and nutrients. However, in the present study, a really high rotating speed had a detrimental effect on the formation of mycelial pellets. Yang and Liau [38] reported that the maximum mycelium concentration of *G. lucidum* was observed at 100 rpm when testing a range of 50–250 rpm. Furthermore, the mycelial growth of *Tremella mesenterica* in a submerged culture is severely hampered at high shear rates mediated by high agitation speeds [11]. Conversely, a high agitation speed (400 rpm) results in the rapid formation of EPS in the early stage of *G. lucidum* fermentation [39], as in the present study.

The critical effect of the aeration rate on the mycelial biomass and EPS production is shown in Fig. 2. Unexpectedly, a high level of mycelial biomass (18.1 g/l) was accumulated at the lowest aeration rate (0.5 vvm), whereas the maximum EPS production (3.0 g/l) was obtained at 1.0 vvm (Figs. 2A, 2B, and Table 1).

The concentration of residual sugar decreased rapidly from the beginning of the fermentation, with corresponding increases in the mycelial biomass and EPS production. An almost complete depletion of sugar was observed after 14 days for all the aeration rates tested (Fig. 2C). The DO levels at all the aeration rates tested were reduced from 100% saturation at the beginning of the fermentation to around 20–35% saturation on days 6–9. The DO level at an aeration rate of 2.0 vvm increased slowly as the growth shifted to the stationary phase, and thereafter a high DO level (about 50%) was maintained towards the end of the fermentation (Fig. 2D). Thus, when taken together, the results in this study indicate that the agitation intensity and aeration influenced the DO concentration significantly, which in turn affected the cell growth, EPS formation, and substrate utilization. There was no significant difference in the maximum EPS production at the aeration rates of 1.0 and 2.0 vvm, and since more power is needed for a higher level of aeration, 1.0 vvm was considered to be the optimum aeration rate in this study.

Many investigators claim that relatively high aeration rates (mostly 2–4 vvm) are required for an enhanced production of the mycelial biomass in submerged cultures of higher fungi [10, 31]. However, the optimal aeration condition for the production of EPS and other metabolites is closely linked to other factors, including the substrate

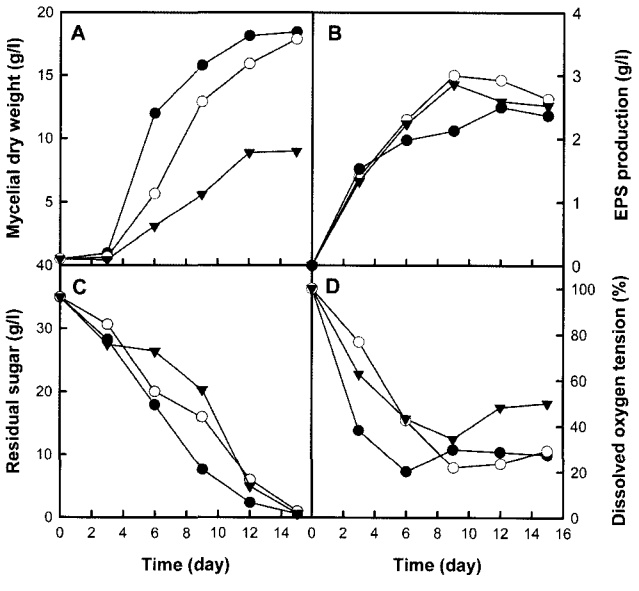

Fig. 2. Time profiles of mycelial biomass (**A**), exopolysaccharide production (**B**), residual sugar (**C**), and dissolved oxygen (**D**) during submerged mycelial culture of *Ganoderma resinaceum* in a 5-l stirred-tank fermenter under 300 rpm and varying aeration rates.
0.5 vvm (●), 1.0 vvm (○), 2.0 vvm (▼).

employed, favorable morphological forms, and the strain. Tang and Zhong [31] conducted an extensive study on the role of oxygen in the production of the mycelial biomass of *G. lucidum* and its two valuable products (EPS and ganoderic acid). An interesting result was that a high dissolved oxygen tension in *G. lucidum* fermentation resulted in an increased production of intracellular polysaccharides and ganoderic acid, yet a decreased production of EPS. Yang and Liau [39] also reported a controversial result in that a relatively higher aeration rate (1.5 vvm) was favorable for EPS formation in *G. lucidum* fermentation.

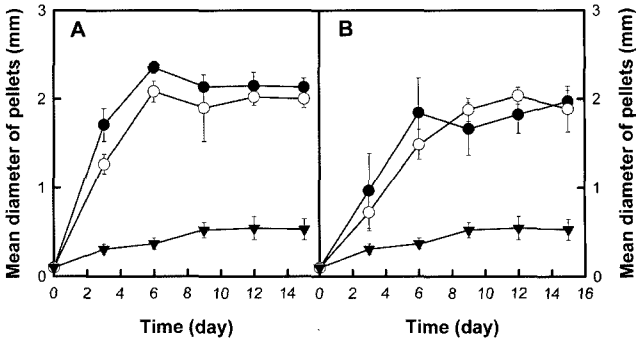

Fig. 3. Variances in mean diameter of mycelial pellets during submerged culture of *Ganoderma resinaceum* in a 5-l stirred-tank fermenter under different agitation speeds (**A**) and different aeration rates (**B**).
50 rpm and 0.5 vvm (●), 150 rpm and 1.0 vvm (○), 300 rpm and 2.0 vvm (▼). All data are mean±SD of triplicate experiments.

Changes in Mycelial Morphology and Broth Rheology

The morphological properties of a fungus play an important role in their metabolism during the fermentation process [28]. Many investigators have reported that the morphology of individual mycelia is significantly affected by the aeration rate and agitation speed in a submerged culture, as vigorous mixing is closely linked to the modification of morphology and transport phenomena within a bioreactor [9, 10].

Figure 3 shows the changes in the pellet mean diameter of *G. resinaceum* under the different agitation speeds and aeration rates. The lowest pellet size was observed at an extremely high agitation rate (300 rpm) with a high EPS production, whereas the largest pellet size was observed at a low agitation rate (50 rpm) with an increased mycelial biomass (Fig. 3A). Likewise, the largest pellet size was observed at a low aeration rate (0.5 vvm) with an increased mycelial biomass and EPS production (Fig. 3B).

The broth rheology in fungal fermentation is frequently related to the morphology and yield of the mycelial biomass and EPS production [28]. The apparent viscosity and rheological parameters were measured for the whole broth throughout the fermentation period under the different aeration rates and agitation intensities (Figs. 4 and 5). As in many fungal fermentations, the culture broth displayed a pseudoplastic behavior (Figs. 4B and 5B). The highest apparent viscosity for the fermentation broth was observed with the lowest agitation and aeration (*e.g.*, 50 rpm, 0.5 vvm), which was associated with a high accumulation of mycelial biomass rather than the amount of EPS. The consistency index, K, continued to rise during the early fermentation period; however, a significant decline was observed at the end of the fermentation (Figs. 4C and 5C). The flow behavior index, n, decreased until day 12, and then increased again towards the end of the fermentation (Figs. 4C and 5C). These results were closely related with the morphological variance, in that the size of the mycelial pellets was reduced in the later period of the fermentation (Fig. 3). Martin and Bailey [23] observed that higher agitation speeds caused an increase in the mycelial biomass concentration linked to a high frequency of filamentous mycelia, yet a decrease in the pellet size of the *Agaricus* mycelium.

The pellet size, hair length of the pellets, and free filamentous mycelial fraction in the total biomass were found to be independent of the dissolved oxygen tension

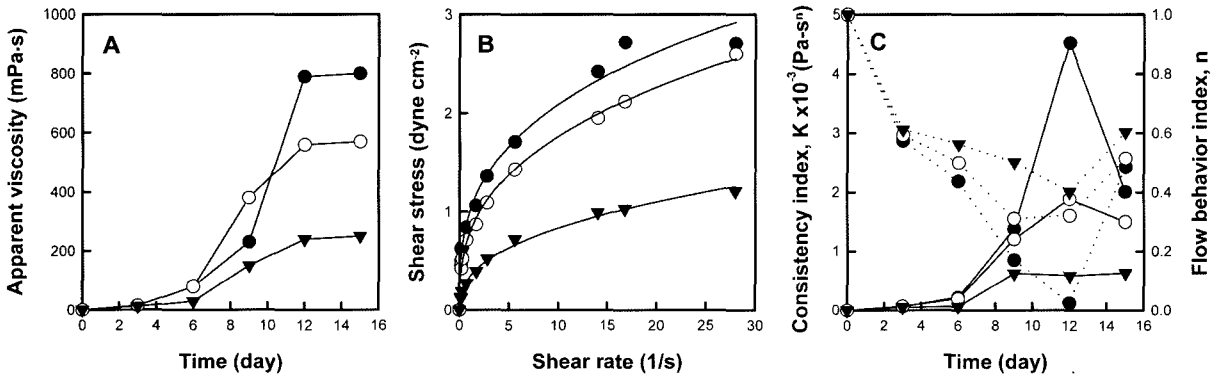

Fig. 4. Apparent viscosity (**A**), shear stress rate diagrams (**B**), and rheological parameters (**C**) of whole fermentation broth in a 5-l stirred-tank fermenter under different agitation speeds at 2 vvm.
50 rpm (●), 150 rpm (○), 300 rpm (▼). Rheograms were obtained at a shear rate of 8 S^{-1} with the sample taken on day 10.

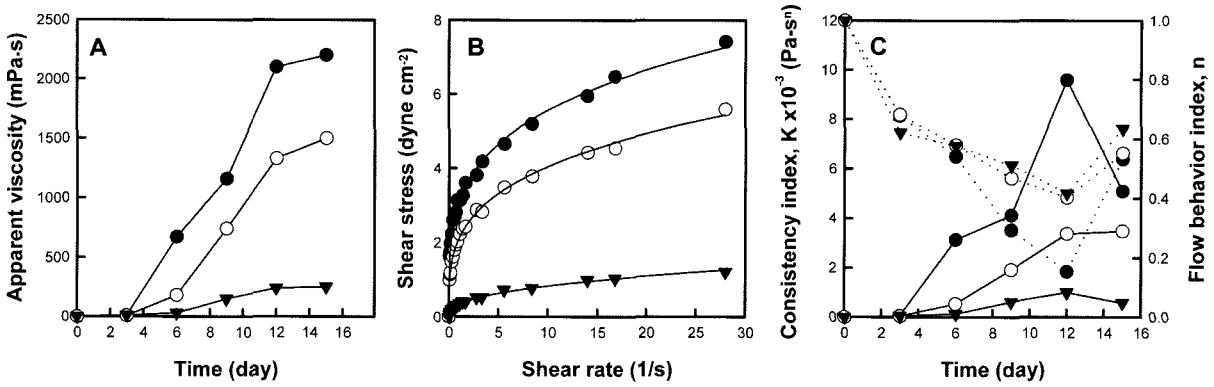

Fig. 5. Apparent viscosity (**A**), shear stress rate diagrams (**B**), and rheological parameters (**C**) of whole fermentation broth in a 5-l stirred tank reactor under different aeration rates at 300 rpm.
0.5 vvm (●), 1.0 vvm (○), 2.0 vvm (▼). Rheograms were obtained at a shear rate of 8 S^{-1} with the sample taken on day 10.

provided that the dissolved oxygen tension was neither too low nor too high [10]. Cui *et al.* [9] also suggested that the pellet size was scarcely affected by the dissolved oxygen tension, whereas the fraction of mycelia decreased slightly with an increase in the dissolved oxygen tension.

Variations in Chemical Composition of EPS

To investigate the relationship between the culture conditions and the chemical features of the EPS, the constituents of each EPS were analyzed by gas chromatography. A compositional analysis revealed that all six EPSs, produced under different agitation and aeration conditions, were protein-bound heteropolysaccharides consisting of mainly glucose (Table 2). It should be mentioned that the different culture conditions, varying the aeration and agitation, markedly affected the chemical composition of the EPS. Although the protein contents of the EPS were not significantly changed, there was a clear variance in the carbohydrate composition when varying the culture conditions. It is probable that the EPS produced from the high DO levels had a lower glucose moiety and higher galactose moiety, which deserves further study to be elucidated. In this study, the EPSs produced under the conditions of 300 rpm and 2 vvm were identical, since they were produced under the same culture condition (*i.e.*, one combination of aeration rate and agitation speed). The results of the fermentation kinetics (Table 1) and chemical composition (Table 2) also matched well, indicating a good experimental reproducibility.

Antioxidative Activity of EPS

To study the biological activity of EPS, its antioxidative activity was examined using four different evaluation methods. As shown in Fig. 6, no significant difference was observed in the scavenging activities of the five different EPSs against DPPH and superoxide radicals (Figs. 6A and 6C). However, there were distinct differences in the hydroxyl radical scavenging activity and lipid peroxide inhibitory activity (Figs. 6B and 6D). Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of lipid peroxidation. The five different EPSs obtained from *G. resinaceum* had a markedly different inhibitory effect on rat brain microsomal peroxide, where the EPS obtained from 150 rpm exhibited the strongest activity.

Tsipali *et al.* [32] demonstrated the free radical scavenging activity of glucan and nonglucan polymers from *Laminaria japonica*. The antioxidative effect of

Table 2. Composition of exopolysaccharides produced by submerged culture of *Ganoderma resinaceum* under different culture conditions in a 5-l stirred-tank reactor.

Composition (%)		Agitation speed (rpm)[a]			Aeration rate (vvm)[b]		
		50	150	300	0.5	1.0	2.0
Protein content		11.82	11.88	10.80	12.83	10.96	10.78
Carbohydrates[c]	Fuc	0.55	0.93	2.30	1.54	1.58	1.88
	Xyl	0.91	1.16	3.57	2.14	2.30	3.10
	Man	3.93	4.40	8.72	5.66	5.89	8.78
	Gal	0.70	0.92	11.95	4.00	4.16	11.61
	Glu	92.50	91.58	73.01	86.66	85.29	73.43

[a]Aeration was fixed at 2.0 vvm at 30°C. [b]Agitation was fixed at 300 rpm at 30°C. [c]Fuc, fucose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose.

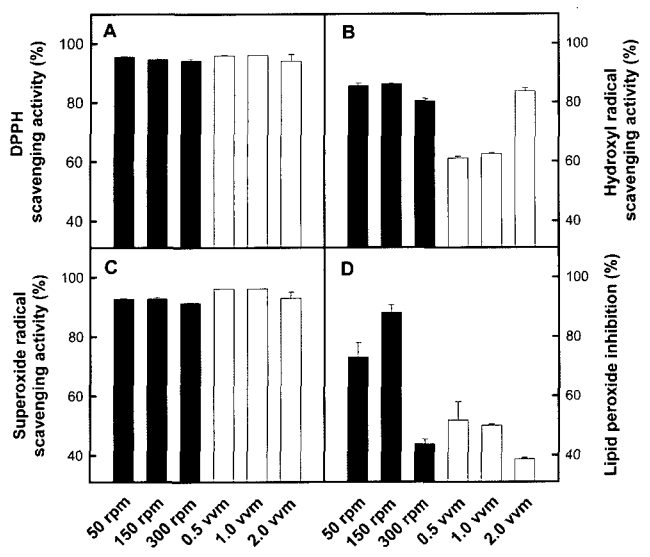

Fig. 6. Antioxidative activities of six different EPSs produced by *Ganoderma resinaceum* under different culture conditions.
1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (**A**), hydroxyl free radical scavenging activity (**B**), superoxide radical scavenging activity (**C**), and inhibition of lipid peroxide in rat brain microsome (**D**). All data are mean±SD of triplicate experiments.

these carbohydrates was not correlated with the type of intrachain linkage, molecular weight, or degree of polymer branching, yet linked to the monosaccharide composition of the polymer. They observed that phosphate and sulfate glucan exhibited a greater antioxidative activity than glucan and other neutral polysaccharides, indicating that polyelectrolytes, such as glucan sulfate or phosphate, may have increased free radical scavenging activities. The free radical scavenging activities of polysaccharides and protein-bound polysaccharides from the fruiting bodies of mushrooms have already been reported by Liu *et al.* [22], and they suggested that the free radical activity of mushroom polysaccharides was dependent on the amount of protein (peptide) present as polysaccharide-protein complexes.

In this study, it was found that agitation and aeration significantly affected the chemical composition of the EPS produced, thereby resulting in different antioxidant activities. This result suggests that the polysaccharide production by higher fungi should be carefully controlled in fermentation processes. In addition, it should be noted that the culture medium, including the carbon source, nitrogen source, and other elements, can also cause a compositional difference in the polymeric metabolites produced from microbial cultures [14, 18].

Therefore, precise control of the agitation and aeration is important to ensure the quality of the biological activity of the resulting polysaccharides. Although it is still unclear, the variance in the EPS characteristics resulting from different environmental conditions was presumably linked to the cell metabolic activity associated with oxygen availability. Thus, overall, there would seem to be an appropriate aeration rate for each strain, although a high oxygen transfer would appear to be desirable for most mushrooms in submerged cultures for EPS production.

Acknowledgments

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