Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008, Pages 61-67 藥物食品分析 第十六卷 第二期

Effect of Carbon and Nitrogen Sources on the Production and Carbohydrate Composition of Exopolysaccharide by Submerged Culture of *Pleurotus citrinopileatus*

CHIU-YEH WU[1], ZENG-CHIN LIANG[2]*, CHIAN-PING LU[3] AND SHIU-HSIUNG WU[3]

[1.] *Department of Biotechnology, Chung Chou Institute of Technology, Changhua, Taiwan (R.O.C.)*
[2.] *Department of Bioresources, Da-Yeh University, 112, Shanjiao Rd., Dacun Township, Changhua County 515, Taiwan (R.O.C.)*
[3.] *Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan (R.O.C.)*

(Received: October 4, 2006; Accepted: July 23, 2007)

ABSTRACT

This study was aimed to investigate the effect of carbon and nitrogen sources on the production of exopolysaccharides (EPS) in submerged culture of *Pleurotus citrinopileatus*, and the variation of carbohydrate compositions in EPS during various culture conditions. The most suitable carbon and nitrogen sources for mycelial biomass and EPS production by *P. citrinopileatus* were fructose and yeast peptone powder. The carbohydrate compositions of EPS were determined to be mannose, galactose, glucose, xylose and fucose, which indicated EPS as heteroglycan or a mixture of glycans. The main carbohydrate composition in EPS from *P. citrinopileatus* was mannose after incubation of 14 days under 4% fructose and 2.5% yeast peptone powder. The carbohydrate composition of EPS from *P. citrinopileatus* changed with the supplementation of various carbon and nitrogen sources under submerged culture condition.

Key words: carbohydrate composition, *Pleurotus citrinopileatus*, submerged culture, exopolysaccharide

INTRODUCTION

A number of polysaccharides or exopolysaccharides (EPS) from the fruiting body or the culture filtrate of mushrooms, such as *Ganoderma applanatum*(1), *Cordyceps* sp.(2-3), *Lentinus edodes*(4), and *Grifola frondosa*(5), have been reported with some potential pharmaceutical applications. Some kinds of mushroom polysaccharides such as lentinan, schizophyllan, krestin, and grifron-D have now commercial applications(6). Mushrooms in submerged culture to produce EPS gradually substitute for solid culture in recent years(7-9). It takes several months for the solid-culture mushrooms to grow into the fruiting bodies on solid substrates. Submerged culture gave rise to many potential advantages of higher mycelial biomass or EPS production in a compact space and shorter time with less chances of contamination(10). In fact, food manufactures have directly employed EPS of mushrooms by fermentation to prepare drinks and capsules for sale. Recently, some studies showed that the compositions of the growth medium can affect the specific rate of EPS synthesis(11-14). It has also been reported that the molecular size, degree of branching and constituents of pure EPS depended on the growth medium(15). As above, fermentative products could be changed by manipulating factors of submerged culture.

Pleurotus citrinopileatus is an edible mushroom belonging to the genus *Pleurotus*, the family Pleurotaceae. *P. citrinopileatus* was known as Yuhuangmo in Chinese and Nireohma in Japanese. Some polysaccharide compounds have been extracted from the fruiting body and mycelium of *P. citrinopileatus* and found to have antitumor(16,17), antihyperlipidemic(18), fatigue resistance, enhance immunity, delay aging(19), antigenotoxicity(20), and antioxidant activities(18). To our knowledge, the characteristics of EPS produced by *P. citrinopileatus* have not been explored now. In the previous report(21), we described the effect of environmental factors on the mycelial production of *P. citrinopileatus*. In the present study, the effects of carbon and nitrogen sources on the production of mycelial biomass and EPS, and on the variation of carbohydrate compositions of EPS from *P. citrinopileatus* in submerged culture were investigated.

MATERIALS AND METHODS

I. *Media and Chemicals*

Potato dextrose agar (PDA) and potato dextrose

* Author for correspondence. Tel: +886-4-8511888 ext. 6219; E-mail: zcliang@mail.dyu.edu.tw

broth (PDB) were from Himedia Laboratories (Mumbai, India). Fructose, galactose and lactose were from Archer Daniels Midland Company (Rotterdam, Netherlands). $NaNO_3$ and $(NH_4)_2SO_4$ were from BASF Aktiengesellschaft (Ludwigshafen, Germany). Sucrose and Bacto peptone were from Tai-Sugar Co. (Taipei, Taiwan) and Kyokutoseiyaku Co. (Tokyo, Japan), respectively. Hydrolyzed vegetable protein and keramine HD were obtained from Bretagne Chimie Fine (Pleucadeuc, France). Yeast peptone powder was from Bio Springer (Maisons-Alfort, France). Yeast extract, phenol, sulfuric acid, acetic anhydride (Ac_2O) and pyridine were purchased from Merck Co., Inc. (Darmstadt, Germany). KH_2PO_4, K_2HPO_4 and $MgSO_4 \cdot {}_7H_2O$ were purchased from Kanto Chemical Co. Inc (Tokyo, Japan). Methanolic HCl, hexamethyldisilazane and trimethylchlorosilane were from Sigma-Aldrich Co. (St. Louis, MO).

II. *Microorganism and Culture Condition*

(I) *Strain*

P. citrinopileatus, supplied by You-Hao Mushroom Research Institute, Heilungkiang, China, was grown on potato dextrose agar (PDA) at 25°C for regular subculture and maintained on PDA slants at 4°C for a maximum of 3 months.

(II) *Inoculum Preparation*

P. citrinopileatus was initially grown on PDA medium (2.4% potato dextrose broth and 2% agar) in a petri dish, and then transferred to the seed culture medium by punching out 5 mm of the agar plate culture with a sterilized cutter. The seed culture was grown in a 250-mL baffled flask containing 40 mL of PDB medium (2.4% potato dextrose broth) on a rotary shaker (Model LTS580, Yuh Chuen Chiou Ind. Co., Ltd., Kaohsiung, Taiwan) at 210 rpm and 25°C for 24 hr.

(III) *Tested Medium and Culture Condition*

The basal medium for the carbon sources testing contained 2.5% yeast peptone powder, 0.1% yeast extract, 0.02% KH_2PO_4, 0.04% K_2HPO_4, 0.01% $MgSO_4 \cdot 7H_2O$. As carbon sources, fructose, galactose, glucose, lactose and sucrose were tested and supplemented to the basal medium in different concentrations. The basal medium for the nitrogen sources testing contained 4% fructose, 0.1% yeast extract, 0.02% KH_2PO_4, 0.04% K_2HPO_4, 0.01% $MgSO_4 \cdot 7H_2O$. As nitrogen sources, yeast peptone powder, keramine HD, hydrolized vegetable protein, Bacto peptone, NaNO3, and $(NH_4)_2SO_4$ were tested. The cultivation was carried out in a 250-mL Erlenmeyer flask containing 30 mL of tested medium on a rotary shaker (300 rpm) at 25°C for 14 days. The initial pH was adjusted to 7.0, and the media were sterilized at 121°C for 20 min.

III. *Isolation of Exopolysaccharide*

The EPS was extracted as follows. Sample was centrifuged at 3,000 rpm for 20 min, and the supernatant was mixed with two volumes of 95% ethanol, stirred vigorously overnight at 4°C. The resultant precipitate was recovered by centrifugation (Model CN-6000, Hsiang Tai Machinery Ind. Co., Ltd., Taipei, Taiwan,) at 3,000 rpm for 20 min, washed twice with 75% ethanol, and dried to eliminate the residual ethanol in a freezing dryer.

IV. *Analytical Methods*

The culture pH was measured with a pH meter (Microprocessor SP-2200, Suntex).

The packed cell volume method (PCV)[22] was used for biomass determination. The whole-cell culture was added into a graduated centrifuge tube and centrifuged at 3,000 rpm for 10 min to read the separated cell volume.

Mycelial biomass (%) = The separated cell volume (mL)/Medium volume (mL) × 100

The residual sugar concentration of the fermentation broth except sucrose as carbon source was measured by the 3,5-dinitrosalicylic acid method[23]. While sucrose as carbon source in the medium, the residual sugar in the broth was analysed by HPLC (Hitachi Co., Tokyo, Japan) equipped with an Aminex HPX-42C column and a refractive index detector for quantitative analysis[24].

The content of EPS in the supernatant was determined by the phenol-sulfuric acid method[25] using glucose as standard.

The carbohydrate compositions of EPS were determined by hydrolysis with dry methanolic HCl overnight at 80°C, followed by evaporation, re-N-acetylation of amino sugar with Ac_2O-pyridine and conversion of the residue to the trimethylsilyl (TMS) derivatives by silylation with mixture of hexamethyldisilazane, trimethylchlorosilane and pyridine[26]. The products were analysed by GC-Mass. GC-Mass was carried out on Hewlett Packard Gas Chromatographer HP6890 connected to an HP5978 Mass Selective Detector. First, the sample was dissolved in hexane prior to splitless injection into a HP-5MS fused silica capillary column (30 m × 0.25 mm I.D.) and on-column injection at 60°C. The column head pressure was maintained at around 8.2 psi to give a constant flow rate of 1 mL min^{-1} using helium as carrier gas. For sugar analysis, the oven temperature was held at 60°C for 1 min before elevating to 140°C at 25°C min^{-1} and then to 200 °C at 5°C min^{-1}, and finally to 300°C at 10 °C min^{-1}.

V. *Statistical Analysis*

Each test was performed in triplicate. Data from each test were subjected to SAS (version 8.0 by SAS Institute Inc., Cary, NC) for analysis of variance. Duncan's multiple range test was used to determine any significant difference ($p < 0.05$) among treatments.

Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008

RESULTS AND DISCUSSION

I. *Effect of Carbon Source*

The effect of carbon sources in various concentrations on mycelial biomass and EPS production was examined by employing monosaccharides (fructose, galactose and glucose) and disaccharides (lactose and sucrose). It was shown that monosaccharides yielded higher mycelial biomass than disaccharides by *P. citrinopileatus* (Table 1). The maximum mycelial biomass and EPS were achieved using fructose medium, followed by glucose medium. In all cases of cultivation, the media containing monosaccharide rather than disaccharide is preferred concerning the production of mycelial biomass and EPS by *P. citrinopileatus*. In the present study, the optimal concentration of fructose for mycelial biomass and EPS production was 4% fructose. Burns *et al.*[11] reported that glucose was the preferred carbon source rather than sucrose, galactose, lactose or xylose for optimum production of EPS in *Pleurotus florida*. Glucose was also the most suitable carbon source for *Phellinus gilvus* produced mycelial biomass and EPS[14]. However, Park *et al.*[27] revealed that the highest production of EPS in *Cordyceps militaris* was achieved in the sucrose medium. These results indicated that mushrooms tend to grow on wide range of carbon sources.

As shown in Table 1, the optimal concentrations of

Table 1. Effect of several carbon sources on mycelial biomass and EPS produced by *P. citrinopileatus* with 2.5% yeast peptone power as nitrogen source after incubation of 14 days

Carbon sources	Concentration of sugar (%)		Final pH	Mycelial biomass (%)	EPS (g/L)
	Initial	Residual			
Fructose	2	0.02 ± 0.02	7.75 ± 0.02	15.67 ± 0.23^{ef}*	1.51 ± 0.21^{bcde}
	3	0.16 ± 0.06	7.86 ± 0.19	41.67 ± 4.72^{abc}	1.76 ± 0.07^{bcd}
	4	0.11 ± 0.09	7.53 ± 0.64	45.25 ± 2.00^{a}	1.87 ± 0.05^{abc}
	5	0.11 ± 0.09	6.74 ± 1.06	44.42 ± 5.54^{ab}	1.84 ± 0.22^{a}
Galactose	2	0.02 ± 0.02	7.60 ± 0.03	12.84 ± 0.47^{f}	1.47 ± 0.10^{bcde}
	3	0.04 ± 0.03	7.79 ± 0.21	16.84 ± 0.47^{e}	1.61 ± 0.10^{bcde}
	4	0.08 ± 0.07	7.53 ± 0.18	43.09 ± 1.53^{abc}	1.74 ± 0.38^{bcd}
	5	0.07 ± 0.06	6.95 ± 1.52	30.77 ± 6.13^{d}	1.76 ± 0.40^{bcd}
Glucose	2	0.02 ± 0.00	7.79 ± 0.11	12.67 ± 0.60^{f}	1.56 ± 0.09^{bcde}
	3	0.08 ± 0.00	7.64 ± 0.17	40.94 ± 0.20^{bc}	1.77 ± 0.18^{ab}
	4	0.08 ± 0.01	7.51 ± 0.71	39.50 ± 5.42^{c}	1.79 ± 0.30^{bcd}
	5	0.03 ± 0.03	5.37 ± 0.26	39.33 ± 1.42^{c}	1.56 ± 0.46^{bcde}
Lactose	2	0.02 ± 0.02	7.75 ± 0.04	6.83 ± 0.71^{g}	1.46 ± 0.20^{bcde}
	3	0.02 ± 0.02	7.70 ± 0.01	7.34 ± 0.23^{g}	1.42 ± 0.04^{bcde}
	4	0.01 ± 0.02	7.93 ± 0.21	5.83 ± 1.25^{g}	1.46 ± 0.67^{e}
	5	0.02 ± 0.02	7.82 ± 0.08	5.83 ± 0.00^{g}	1.48 ± 0.17^{bcde}
Sucrose	2	0.02 ± 0.01	7.83 ± 0.01	5.78 ± 0.82^{g}	1.41 ± 0.32^{bcde}
	3	0.01 ± 0.01	7.81 ± 0.03	5.67 ± 0.87^{g}	1.40 ± 0.99^{cde}
	4	0.01 ± 0.01	7.99 ± 0.04	6.95 ± 0.63^{g}	1.22 ± 0.08^{e}
	5	0.04 ± 0.04	7.79 ± 0.01	6.37 ± 1.42^{g}	1.35 ± 0.03^{de}

*The data in a column with different letters are significantly different at $p < 0.05$.

Table 2. Carbohydrate compositions of EPS produced by *P. citrinopileatus* under different carbon sources

Carbon sources	Monosaccharide* (%)					
	Fuc	Xyl	Man	Gal	Glc	GlcNAc
4% Fructose	0.0	0.1	95.1a**	3.2^{b}	1.6^{d}	0.0
5% Galactose	0.0	0.0	87.3^{c}	1.6^{c}	11.0^{a}	0.0
4% Glucose	0.1	0.1	86.3^{d}	6.9^{a}	6.6^{c}	0.1
5% Lactose	0.0	0.0	89.4^{b}	1.7^{c}	8.9^{b}	0.0
2% Sucrose	0.0	0.0	87.1^{cd}	1.0^{d}	11.8^{a}	0.0

*Fuc: fucose; Xyl: xylose; Man: mannose; Gal: galactose; Glc: glucose; GlcNAc: N-acetylglucosamine.
**The data in a column with different letters are significantly different at $p < 0.05$.

tested carbon sources for EPS yield appeared to be 4% of fructose, 5% of galactose, 4% of glucose, 5% of lactose and 2% of sucrose, respectively. Thus the subsequent experiments of carbohydrate compositions analysis for EPS were carried on the above five different concentrations of carbon sources. The analysis of carbohydrate compositions in the produced EPS revealed, as shown in Table 2, that mannose was the major, glucose and galactose less, fucose, xylose and N-acetylglucosamine the trace part in carbohydrate composition. The water-soluble EPS with antitumoral activity from *P. citrinopileatus* fruiting body contained glucose, mannose, arabinose and galactose[16]. Rosado *et al.*[28] isolated and purified two water-soluble extracellular polysaccharide produced by *P. ostreatoroseus* Sing. in the liquid culture medium (POL) consisted of (g/L): peptone 1.0, yeast extract 2.0, K_2HPO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.2, $(NH_4)_2SO_4$ 5.0 and glucose 60.0. One fraction was mannan, and the other was galactan. The carbohydrate compositions in crude EPS produced by *P. ostreatoroseus* and *P. ostreatus* "*florida*" in the same POL submerged culture is glucose, the major component, and the minor components, galactose, mannose, xylose and arabinose[29]. These results might indicate that compositions in EPS varied with species. Israilides *et al.*[30] reported that the pullulans produced by *Aureobasidium pullulans* grown with olive oil waste effluents, molasses and starch waste as carbon sources, contained with contents of glucose about 33, 25 and 100%, respectively. This implied that the percentage of compositions in EPS was varied with carbon sources supplement. It is possible that various carbon sources might exert effects on the catabolic repression on the secondary metabolism[31-33].

As the mycelial biomass and EPS production by *P. citrinopileatus* is concerned, fructose was the favored carbon source (Table 1). Therefore, fructose is used as carbon source in the further investigation. The concentration of fructose in the medium affected the carbohydrate composition of EPS. The mannose content reached maximum when fructose concentration was between 3% and 4% (Table 3). Perret *et al.*[34] reported that the degree of branching in β-D-glucans increased in *Phytophthora parasitica* when asparagine and high concentration of glucose were included in the medium. Thus the change of fructose concentration resulted in the change of the compositions in EPS from *P. citrinopileatus*, which might probably bring some modification in the structure of EPS.

Table 3. Carbohydrate compositions of EPS produced by *P. citrinopileatus* under different fructose concentrations and 2.5% yeast peptone powder

Fructose concentrations (%)	Monosaccharide*(%)				
	Fuc	Xyl	Man	Gal	Glc
2	0.1	0.1	93.8^{b}**	4.4^{b}	1.6^{b}
3	0.2	0.1	95.1^{a}	3.5^{bc}	1.0^{c}
4	0.0	0.1	95.1^{a}	3.2^{c}	1.6^{b}
5	0.1	0.0	89.8^{c}	7.2^{a}	2.8^{a}

*Fuc: fucose; Xyl: xylose; Man: mannose; Gal: galactose; Glc: glucose.
**The data in a column with different letters are significantly different at $p < 0.05$.

II. *Effect of Nitrogen Sources*

The effect of nitrogen, source on the production of mycelial biomass and EPS of *P. citrinopileatus* in submerged culture, is shown in Table 4. Among all nitrogen sources tested, the highest mycelial biomass and EPS production were achieved in the medium containing yeast peptone powder. In comparison with organic nitrogen sources, inorganic nitrogen sources gave rise to relatively lower mycelial biomass and EPS production. This result was in accordance with that for other higher fungi, such as *Paecilomyces sinclairii*[13], *Phellinus gilvus*[14], *Cordyceps militaris* NG3[35], *Ganoderma lucidum*[36] and *Antrodia cinnamomea*[37]. It was suggested that certain essential amino acids could not be synthesized from inorganic nitrogen sources[8] and proteolytic activity for

Table 4. Effect of several nitrogen sources on mycelial biomass and EPS produced by *P. citrinopileatus* with 4% fructose as carbon source after incubation of 14 days

Nitrogen source (2.5%)	Residual sugar (%)	Final pH	Mycelial biomass (%)	EPS (g/L)
Yeast peptone powder	0.11 ± 0.09	7.53 ± 0.64	45.25 ± 2.00[a]*	1.87 ± 0.05[a]
Keramine HD	0.32 ± 0.04	5.26 ± 0.50	26.50 ± 2.12[b]	0.14 ± 0.02[c]
Hydrolyzed vegetable protein	0.19 ± 0.01	5.93 ± 0.06	20.40 ± 0.57[c]	0.11 ± 0.02[c]
Bacto peptone	0.06 ± 0.00	6.55 ± 0.26	18.35 ± 1.91[d]	0.47 ± 0.07[b]
$NaNO_3$	0.14 ± 0.01	5.74 ± 0.11	15.95 ± 0.86[e]	0.12 ± 0.04[c]
$(NH_4)_2SO_4$	0.04 ± 0.00	3.63 ± 0.03	5.17 ± 0.94[f]	0.11 ± 0.04[c]

*The data in a column with different letters are significantly different at $p < 0.05$.

Table 5. Carbohydrate compositions of EPS produced by *P. citrinopileatus* with different nitrogen sources

Nitrogen source	Monosaccharide* (%)						
	Rha	Fuc	Xyl	Man	Gal	Glc	GlcNAc
Yeast peptone powder	0.0	0.0	0.1	95.1[a]**	3.2[d]	1.6[d]	0.0
Keramine HD	0.0	0.1	0.1	56.2[d]	10.2[c]	33.4[a]	0.0
Hydrolized vegetable protein	0.0	0.0	0.1	61.3[c]	12.0[c]	26.5[ab]	0.0
Bacto peptone	0.1	2.5	0.9	38.8[e]	37.1[a]	14.4[c]	6.3
$NaNO_3$	0.0	0.1	0.1	64.9[b]	21.7[b]	13.2[c]	0.0
$(NH_4)_2SO_4$	0.0	0.1	0.1	63.7[bc]	11.0[c]	25.2[b]	0.0

*Fuc: fucose; Xyl: xylose; Man: mannose; Gal: galactose; Glc: glucose; GlcNAc: N-acetylglucosamine
**The data in a column with different letters are significantly different at $p < 0.05$.

protein hydrolysis existed in the fermentation of higher fungi. Aslim *et al*.[38] reported that the highly protease-secreting strains can produce higher amount of EPS. The analysis of carbohydrate compositions in the above EPS revealed that the percentage of carbohydrate composition was significantly changed with different nitrogen sources (Table 5). Except for Bacto peptone medium, mannose was still the main composition in EPS. However, the relationship between the proteolytic activity and the variation of sugar composition in EPS was not clear at present.

III. *Growth Curve*

The time course of residual sugar contents, pH values, mycelial biomass and EPS production by *P. citrinopileatus* were shown in Figure 1. The samples were collected at various intervals from the submerged cultures with 4% fructose and 2.5% yeast peptone powder. Note that the residual sugar decreased rapidly to near zero at the 6th day. Initial pH value of the fermentation broth decreased from around 6.5 to 5.0 during 6th to 12th day, thereafter increased to 7.5. The decline pattern of pH, caused by the production of organic acid resulting from high consumption of carbon source for cell growth,

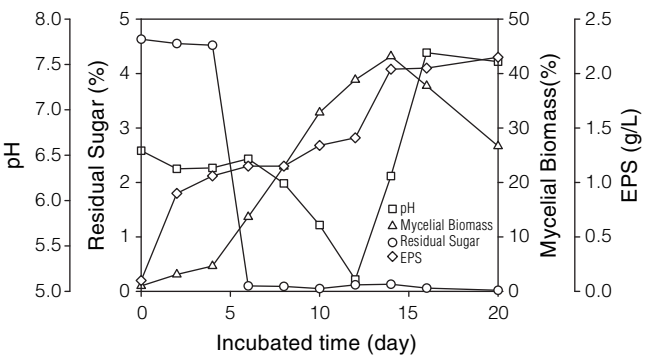

Figure 1. Time course of pH, sugar consumption, mycelial biomass and EPS produced by *P. citrinopileatus* under 4% fructose and 2.5% yeast peptone powder as carbon and nitorgen sources, respectively.

has been observed in mushroom fermentation[37], and the reason for the pH increase after 12th day was attributed to the low consumption rate of carbon source at low residual sugar levels[36]. The EPS concentration reached maximum at 14th day, so did the maximum of biomass. These results indicated that EPS production in *P. citrinopileatus* was growth-associated.

The analyzing results of the carbohydrate compositions in EPS from time-course samples are shown in Table 6. The percentage of carbohydrate compositions varied with the incubated days. Among the carbohydrate compositions, the percentage of mannose and galactose reached maximum at 20th and 8th day, respectively. Lee *et al*.[32] reported that the molar ratio of carbohydrate compositions in pullulan produced by *A. pullulans*, such as glucose and mannose, remained almost constant as a function of culture time, but glucosamine decreased with culture time. Further research is needed to clarify the physiological meaning of the percentage variation of carbohydrate compositions in EPS from *P. citrinopileatus*.

Table 6. Change of carbohydrate compositions of EPS produced by *P. citrinopileatus* with 4% fructose and 2.5% yeast peptone powder as carbon and nitrogen sources during 20 days of growth

Incubated days	Monosaccharide (%)				
	Fuc	Xyl	Man	Gal	Glc
4	0.0	0.0	92.4^{c}**	2.4^{b}	5.2^{a}
8	0.0	0.0	92.3^{c}	3.6^{a}	4.0^{b}
12	0.0	0.1	94.5^{b}	2.7^{b}	2.6^{c}
16	0.1	0.1	96.3^{a}	1.6^{c}	1.9^{d}
20	0.0	0.2	97.2^{a}	1.3^{c}	1.3^{e}

*Fuc: fucose; Xyl: xylose; Man: mannose; Gal: galactose; Glc: glucose.

**The data in a vertical column with different letters are significantly different at $p < 0.05$.

ACKNOWLEDGMENTS

This work was supported by the Research Grant, Tung Fang Institute of Technology and Academia Sinica, Taipei, Taiwan.

REFERENCES

1. Usui, T., Iwasaki, Y., Hayashi, K., Mizuno, T., Tanaka, M., Shinkai, K. and Arakawa, M. 1981. Antitumor activity of water-soluble β-D-glucan elaborated by *Ganoderma applanatum*. Agric. Biol. Chem. 45: 323-326.
2. Kiho, T., Yamane, A., Hui, J., Usui, S. and Ukai, S. 1996. Polysaccharide in fungi. XXXVI. Hypoglycemic activity of polysaccharide (CF-F30) from the cultural mycelium of *Cordyceps sinensis* and its effect on glucose metabolism in mouse liver. Biol. Pharm. Bull. 19: 294-296.
3. Yang, B. K., Ha, J. Y., Jeong, S. C., Das, S., Yun, J. W., Lee, Y. S., Choi, J. W. and Song, C. H. 2000. Production of exo-polymers by submerged mycelial culture of *Cordyceps militaris* and its hypolipidemic effect. J. Ind. Microbiol. Biotechnol. 10: 784-788.
4. Chihara, G., Himuri, J., Maeda, Y. Y., Arai, Y. and Fukuoka, F. 1970. Fractionation and purification of the polysaccharides with marked antitumor activity, especially, lentinan, from *Lentinus edodes* (Berk) SING. Cancer Res. 30: 2776-2781.
5. Adachi, Y., Ohno, N., Ohsawa, K., Oikawa, S. and Yadomae, T. 1990. Change of biological activities of (1→3)-β-D-glucan from *Grifola frondosa* upon molecular weight reduction by heat treatment. Chem. Pharm. Bull. 37: 1838-1843.
6. Kidd, P. H. 2000. The use of mushroom glucans and proteoglycans in cancer treatment. Alt. Med. Rev. 5: 4-27.
7. Yang, F. C. and Liau, C. B. 1998. The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged cultures. Process Biochem. 33: 547-53.
8. Hwang, H. J., Kim, S. W., Choi, J. W. and Yun, J. W. 2003. Production and characterization of exopolysaccharides from submerged culture of *Phellinus linteus* KCTC 6190. Enzyme Microb. Technol. 33: 309-319.
9. Xiao, J. H., Chen, D. X., Liu, J. W., Liu, Z. L., Wan, W. H., Fang, N., Xiao, Y., Qi, Y. and Liang, Z. Q. 2004. Optimization of submerged culture requirements for the production of mycelial growth and exopolysaccharide by *Cordyceps jiangxiensis* JXPJ 0109. J. Appl. Microbiol. 96: 1105-1116.
10. Lee, B. C., Bae, J. T., Pyo, H. B., Choe, T. B., Kim, S. W., Hwang, H. J. and Yun, J. W. 2004. Submerged culture conditions for the production of mycelial biomass and exopolysaccharides by the edible Basidiomycete *Grifola frondosa*. Enzyme Microb. Technol. 35: 369-374.
11. Burns, P. J., Yeo, P., Keshavarz, T., Roller, S. and Evans, C. S. 1994. Physiological studies of exopolysaccharide production from the Basidiomycetes *Pleurotus* sp. *florida*. Enzyme Microb. Technol. 16: 566-572.
12. Kanari, B., Banik, R. R. and Upadhyay, S. N. 2002. Effect of environmental factors and carbohydrate on gellan gum production. Appl. Microbiol. Biotechnol. 102-103: 129-140.
13. Kim, S. W., Hwang, H. J., Xu, C. P., Na, Y. S., Song, S. K. and Yun, J. W. 2002. Influence of nutritional conditions on the mycelail growth and exopolysaccharide production in *Paecilmyces sinclairii*. Lett. Appl. Microbiol. 34: 389-393.
14. Hwang, H. J., Kim, S. W., Xu, C. P., Choi, J. W. and Yun, J. W. 2003. Production and molecular characteristics of four groups of exopolysaccharides from submerged culture of *Phellinus gilvus*. J. Appl. Microbiol. 94: 707-719.
15. Rouhier, P. M., Bruneteau, M. and Nichel, G. 1995. Structure analysis on β-glucans from *Phytophthora capsici*. J. Carbohydr. Chem. 14: 247-254.
16. Zhang, J., Wang, G., Li, H., Zhuang, C., Mizuno, T.,

Ito, H., Suzuki, C., Okamoto, H. and Li, J. X. 1994. Antitumor polysaccharides from a Chinese mushroom, "Yuhuangmo", the fruitung body of *Pleurotus citrinopileatus*. Biosci. Biotechnol. Biochem. 58: 1195-1201.

17. Wang, J. C., Hu, S. H., Liang, Z. C. and Yeh, C. J. 2005. Optimization for the production of water-soluble polysaccharide from *Pleurotus citrinopileatus* in submerged culture and its antitumor effect. Appl. Microbiol. Biotechnol. 67: 759-766.
18. Hu, S. H., Liang, Z. C., Chia, Y. C., Lien, J. L., Chen, K. S., Lee, M. Y. and Wang, J. C. 2006. Antihyperlipidemic and antioxidant effects of extracts from *Pleurotus citrinopileatus*. J. Agric. Food Chem. 54: 2103-2110.
19. Wang, Q., Yan, L. L., Wang, Y. L. and Zhang, Y. J. 2001. Submerged culture of *Pleurotus citrinopileatus* and its determination. In "Proceedings of the 5th Cross-Strait Conference on Mycology". pp. 8-12. Taipei, Taiwan.
20. Wang, J. C., Hu, S. H., Liang, Z. C. and Lee, M. Y. 2005. Antigenotoxicity of extracts from *Pleurotus citrinopileatus*. J. Sci. Food Agric. 85: 770-778.
21. Liang, Z. C. and Wu, C. Y. 2003. Effect of environmental factors on the mycelium production of *Pleurotus citrinopileatus*. In "Biochemical Engineering, Proceedings of the 6th Conference". Kaohsiung, Taiwan.
22. Lee, W. Y., Park, Y., Ahn, J. k., Ka, K. H. and Park S. Y. 2007. Factors influencing the production of endopolysaccharide and exopolysaccharide from *Ganoderma applanatum*. Enzyme Microb. Technol. 40: 249-254.
23. Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428.
24. Xu, C. P., Kim, S. W., Hwang, H. J. and Yun, J. W. 2002. Application of statistically based experimental designs for the optimization of exo-polysaccharide production by *Cordyceps militaris* NG3. Biotechnol. Appl. Biochem. 36: 127-131.
25. Dubois, M., Gilles, K., Hamilton, Y., Robers, P. and Smith, F. 1956. Colorimetric method for the determination of sugars. Anal. Chem. 28: 350-356.
26. Chaplin, M. F. 1994. Monosaccharides. In "Carbohydrate Analysis: a Practical Approach". 2nd ed. pp. 30-32. Chaplin, M. F. and Kennedy, J. F. ed. Oxyford. New York, U. S. A.
27. Park, J. P., Kim, S. W., Hwang, H. J. and Yun, J. W. 2001. Optimization of submerged culture condition for the mycelial growth and exo-biopolymer production by *Cordyceps militaris*. Lett. Appl. Microbiol. 33: 76-81.
28. Rosado, F. R., Carbonero, E. R., Kemmelmeier, C., Tischer, C. A., Gorin, P. A. J. and Iacomini, M. 2002. A partially 3-*O*-methylated (1→4)-linked α-D-galactan and α-D-mannan from *Pleurotus ostreatoroseus* Sing. FEMS Microbiol. Lett. 212: 261-265.
29. Rosado, F. R., Germano, S., Carbonero, E. R., Costa, S. M. G. D., Iacomini, M. and Kemmelmeier, C. 2003. Biomass and exopolysaccharide production in submerged cultures of *Pleurotus ostreatoroseus* Sing. and *Pleurotus ostreatus* "*florida*" (Jack.: FR.) Kummer. J. Basic Microbiol. 43: 230-237.
30. Israilides, C., Scanton, B., Smith, A., Harding, S. E. and Jumel, K. 1994. Characterization of pullulans from agro-industrial wastes. Carbohydr. Polym. 25: 203-209.
31. Lee, J. W., Yeomans, W. G., Allen, A. L., Deng, F., Gross, A. and Kaplan, D. L. 1997. Compositional consistency of heteropolysaccharide-7 by *Beijerinckia indica*. Biotechnol. Lett. 19: 799-802.
32. Lee, J. W., Yeomans, W. G., Allen, A. L., Deng, F., Gross, A. and Kaplan, D. L. 1999. Biosynthesis of novel exopolymers by *Aureobasidium pullulans*. Appl. Environ. Microbiol. 65: 5265-5271.
33. Kai, A., Kikawa, M., Hatanaka, K., Matsuzaki, K., Mimura, T. and Kaneko, Y. 2003. Biosynthesis of hetero-polysaccharide by *Pestalotiopsis microspora* from various monosaccharides as carbon. Carbohydr. Polym. 54: 381-383.
34. Perret, J., Bruneteau, M., Michel, G., Marais, M. F., Joseleau, J. P. and Ricci, P. 1992. Effect of growth conditions on the structure of β-D-glucans from *Phytophthora parasitica* Dastur, a phytopathogenic fungus. Carbohydr. Polym. 17: 231-236.
35. Kim, S. W., Xu, C. P., Hwang, H. J., Choi, J. W., Kim, C. W. and Yun, J. W. 2003. Production and characterization of exopolysaccharides from an enthomopathogenic fungus *Cordyceps militaris* NG3. Biotechnol. Prog. 19: 428-435.
36. Fang, Q. H. and Zhong, J. J. 2002. Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. Process Biochem. 37: 769-774.
37. Shih, I. L., Pan, K. and Hsieh, C. 2006. Influence of nutritional components and oxygen supply on the mycelial growth and bioactive metabolites production in submerged culture of *Antrodia cinnamomea*. Process Biochem. 41: 1129-1135.
38. Aslim, B., Beyatli, Y. and Yuksekdag, Z. N. 2006. Productions and monomer compositions of exopolysaccharides by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains isolated from traditional home-made yoghurts and raw milk. Int. J. Food Sci. Technol. 41: 973-979.