Biotechnology Letters **24:** 1319–1325, 2002.

Hypolipidemic effect of an exo-biopolymer produced from submerged mycelial culture of *Auricularia polytricha* in rats

Byung-Keun Yang[1], Ji-Young Ha[1], Sang-Chul Jeong[1], Young-Jae Jeon[2], Kyung-Soo Ra[3], Surajit Das[1], Jong-Won Yun[1] & Chi-Hyun Song[1,*]

[1]*Department of Biotechnology, Taegu University, Kyungbuk 712-714, South Korea*
[2]*Department of Biotechnology, New South Wales University, Sydney 2052, Australia*
[3]*Department of Food & Nutrition, Technical Junior College, Daegu City 704-350, South Korea*
[*]*Author for correspondence (Fax: 82-53-850-6559; E-mail: chsong@taegu.ac.kr)*

Received 15 April 2002; Revisions requested 2 May 2002; Revisions received 30 May 2002; Accepted 30 May 2002

Key words: Auricularia polytricha, exo-biopolymer, hypolipidemic effect, submerged mycelial culture

Abstract

The hypolipidemic effect of an exo-biopolymer (EBP) produced from a submerged mycelial culture of the mushroom fungus, *Auricularia polytricha*, was investigated in the dietary-induced hyperlipidemic rats. In a dose-dependent study, the EBP was fed at 50–100 mg kg^{-1} body weight and significantly decreased the concentrations of the plasma triacylglycerols, total cholesterol, and low-density lipoprotein (LDL) cholesterol. The plasma LDL cholesterol concentration was decreased up to 70%. Production of *A. polytricha* biomass and EBP were optimal at pH 4 with maximum growth at 20 °C and EBP production at 30 °C. Gel chromatography of the EBP revealed a single peak of a glycoprotein with a molecular size of 32 kDa. It contained 77.5% carbohydrate and 22.5% protein. The sugar and amino acid compositions of the EBP were analyzed.

Introduction

The search for natural substances capable of exhibiting hypolipidemic effect is ongoing in the field of nutritional research. Various mushrooms have proved themselves as an ideal food for the dietetic prevention of hyperlipidemia due to high content of fibers, proteins, microelements, and low fat content (Cheung 1996). Kaneda & Tokuda (1966) have already investigated the hypolipidemic effect of the fruiting body of *Auricularia polytricha*. However, knowledge on hypolipidemic or hypocholesterolemic effect of EBP produced by submerged mycelial culture of various mushrooms is limited. Most of the researches on hypolipidemic effect have been carried out either with the fruiting bodies or the mycelia. Many investigators have documented that, apart from fruiting bodies or mycelia, bioactive polymeric compounds can also be obtained from the culture broth of the submerged mycelial culture. There are number of reports available on the isolation of various bioactive components from the culture precipitate of *Lentinus edodes* (Suzuki *et al.* 1988).

The present study was designed to examine the hypolipidemic effect of the EBP produced from submerged mycelial culture of *A. polytricha*. The study reports the isolation and purification of water-soluble EBP from a culture broth of *A. polytricha*, a dose-dependent analysis of EBP by oral administration to normolipidemic rats, optimization of fermentation condition for the production of EBP and mycelia in a 5-l jar fermenter, and an analysis of the sugar and amino acid compositions of EBP.

Materials and methods

Organism and preparation of inoculum

Auricularia polytricha, from Rural Development Administration in Korea, was grown on containing a potato/dextrose broth on a rotary shaker (120 rpm,

Table 1. Effect of initial culture pH on mycelial growth (dry wt) and exo-biopolymer (dry wt) production in submerged culture of *Auricularia polytricha.*

pH	Mycelia (g l^{-1})	Exo-biopolymer (g l^{-1})
3	8.5	0.5
4	11.1	1.2
5	10.4	0.5
6	10.3	0.5
7	10.3	0.6
8	10.2	0.9
9	9.5	0.8
10	8.9	0.8

The submerged culture was performed in 500 ml flasks containing 200 ml medium on a rotary shaker (120 rpm, 30 °C, 25 d). The initial medium pH was adjusted between 3–10 before sterilization. All the values are given as the mean based on results of triplicate experiments.

Table 2. Effect of temperature on mycelial growth (dry wt) and exo-biopolymer (dry wt) production in submerged culture of *Auricularia polytricha.*

Temperature (°C)	Mycelia (g l^{-1})	Exo-biopolymer (g l^{-1})
15	8.7	0.6
20	9.7	0.9
25	9.5	0.9
30	8.9	1.2
35	8.3	0.9

The submerged culture was performed in 500 ml flasks containing 200 ml medium on a rotary shaker (120 rpm, pH 4, 25 d). The temperature was varied between 15–35 °C. All the values are given as the mean based on results of triplicate experiments.

pH 4) at 30 °C. After an incubation for 7 d, 100 ml culture including with mycelia were homogenized for 3 min in an ice bath. One percent of mycelial suspension was used as inoculum for the subsequent experiments.

Optimization of culture conditions and production of EBP

The composition of potato malt peptone (PMP) medium for the production of EBP was as follows (g l^{-1}): potato/dextrose broth 24, malt extract 10, peptone 1, the pH was adjusted to 4 before sterilization. Optimum temperature and pH was determined at the 15–35 °C and pH of 3–10 before sterilization. The submerged mycelial culture was carried out in a 5-l jar fermenter for 21 d. The recovery procedure for EBP and mycelia from submerged culture is shown in Figure 1 (Yang *et al.* 2000).

Animal experiments

Sprague–Dawley male rats, obtained Korean Research Institute of Chemical Technology, 120–130 g, were housed individually in stainless steel cage in a room with controlled temperature (22 ± 0.5 °C), humidity (55 ± 5%) and a 12-h cycle of light and dark. The rats were fed with modified AIN-76 diet for 4 weeks. The diet consisted of 55.5% carbohydrates (including 40.5% sucrose), 14.5% fats (30% of total energy) and 20% casein by dry weight. The rats of each group were administered either with saline (control) or EBP at the level of 50 mg kg^{-1}, 75 mg kg^{-1}, 100 mg kg^{-1} body wt (BW) using an oral zonde daily for 2 weeks. Food intake and BW were recorded daily. At the end of oral administration, the animals were fasted for 14 h and then sacrificed.

Analysis of plasma and liver lipids

Blood samples were collected in heparinized tubes and plasma was separated by centrifugation (1110 × *g* for 10 min). Livers were perfused with cold saline, excised, and kept frozen at −70 °C. Liver lipid was extracted by the method of Folch *et al.* (1957). The plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and triacylglycerol of plasma were measured by using enzymatic kits (Sigma). LDL cholesterol was calculated by the equation as follows:

total cholesterol − HDL cholesterol − (triacylglycerol/5).

Liver total cholesterol was assayed using the same method as for the plasma total cholesterol after the treatment with Triton X-100.

Gel filtration and molecular weight

The EBP obtained from culture broth was dissolved in 0.2 M NaCl and subjected to gel filtration, on a column (2.6 × 99 cm) of Sepharose CL-6B equilibrated with 0.2 M NaCl at 0.34 ml min^{-1}. Molecular weights of purified EBP were determined by HPLC (Asahipak GS-520, 320, and 220 column). Standard pullulan series (P1600, 800, 400, 200, 100, 50, 20, 10, and 5) were used for the determination of molecular weight.

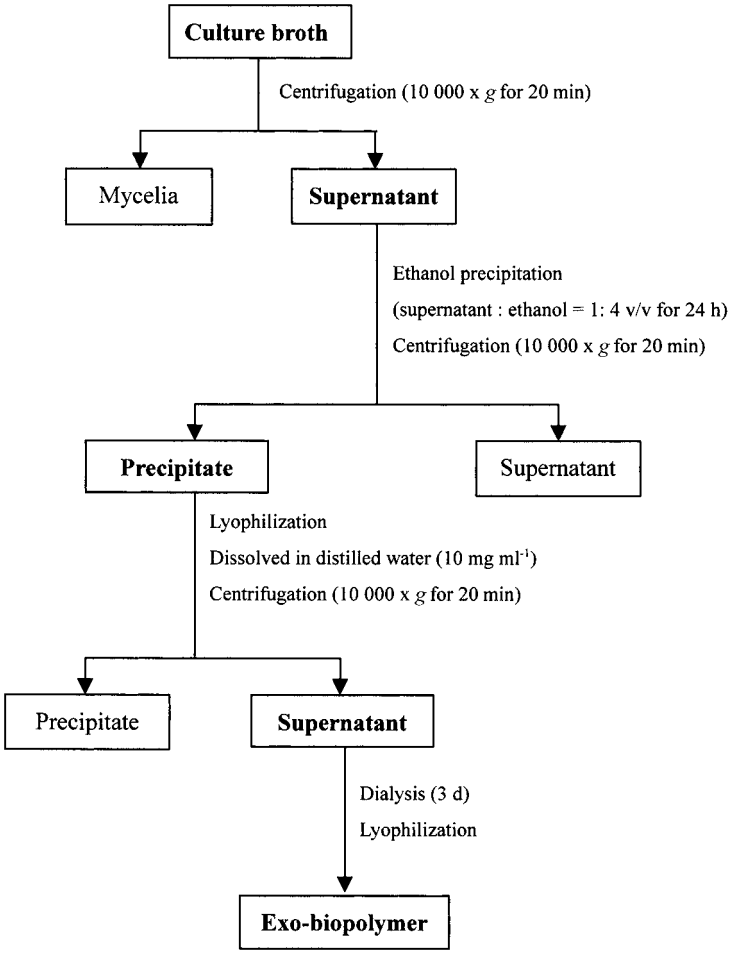

Fig. 1. Schematic diagram depicting the separation of the exo-biopolymer from submerged mycelial culture of *Auricularia polytricha.*

Analysis component sugars and amino acids of EBP

Total protein content of EBP was determined by the method of Lowry with BSA as a standard. The amino acid composition was analyzed by a Biochrom 20 (Pharmacia Biotech. Ltd., USA) amino acid auto-analyzer with a Na-form column after hydrolysis of the protein. Total sugar content of EBP was determined by the phenol/sulfuric acid method, using a mannose/galactose (1:1, w/w) as standard. The sugar composition was analyzed by a GC 3600 gas chromatograph based on the hydrolysis and acetylation method described by Jones & Albersheim (1972).

Statistical analysis

Each data value is expressed as the mean ± SE. Group means were compared by a one-way analysis of variance and Duncan's multiple-range test. Statistical differences were considered significant at $p < 0.05$.

Results and discussion

Effect of pH and temperature for the production of EBP

Auricularia polytricha grew best at pH 4 (Table 1) at which pH EBP production was also maximal.

Maximum growth yield was at 20 °C but yield of EBP was maximal at 30 °C (see Table 2).

Mycelial growth curve and production of EBP

Maximum biomass yield (10.3 g l^{-1}) was after 15 d in a 5-l fermenter but the yield of EBP (1.9 g l^{-1}) was maximal after 18 d (Figure 2).

Table 3. Effect of *Auricularia polytricha* exo-biopolymer on the BW gain, liver weight, and food intake in the dietary-induced hyperlipidemic rats for 2 weeks.

Experimental groups (exo-biopolymer, mg kg^{-1} BW)	BW gain (g d^{-1})	Food intake (g d^{-1})	Liver weight (g 100 g^{-1} BW)
Control (saline)	4.2 ± 0.3^{NS}	13.5 ± 0.3^{NS}	5.9 ± 0.2^{NS}
50	3.9 ± 0.3	12.6 ± 0.3	5.7 ± 0.2
75	4.1 ± 0.2	13.2 ± 0.2	5.5 ± 0.2
100	4.1 ± 0.2	13.6 ± 0.2	5.4 ± 0.3

The rats of each experimental group were administered orally with either saline (control) or exo-biopolymer at the level of 50–100 mg kg^{-1} BW daily for 2 weeks.
Each value is the mean ± SE for 8 rats.
^{NS}Not significant.

Table 4. Effect of *Auricularia polytricha* exo-biopolymer on the plasma HDL, LDL, and total cholesterol levels in the dietary-induced hyperlipidemic rats for 2 weeks.

Experimental groups (exo-biopolymer, mg kg^{-1} BW)	Plasma cholesterol (mg ml^{-1})		
	Total	HDL	LDL*
Control (saline)	0.64 ± 0.02^{c}	0.26 ± 0.01^{a}	0.3 ± 0.01^{c}
50	0.57 ± 0.03^{b}	0.28 ± 0^{ab}	0.21 ± 0.01^{b}
75	0.5 ± 0.02^{ab}	0.3 ± 0.01^{bc}	0.13 ± 0.01^{ab}
100	0.47 ± 0.03^{a}	0.32 ± 0.01^{c}	0.09 ± 0.01^{a}

The rats of each experimental group were administered orally with either saline (control) or exo-biopolymer at the level of 50–100 mg kg^{-1} BW daily for 2 weeks.
*Total cholesterol − HDL cholesterol − (Triacylglycerol/5).
Each value is the mean ± SE for 8 rats.
^{a,b,c}Values with different superscript letters in the same row are significantly different ($p < 0.05$).

Table 5. Effect of *Auricularia polytricha* exo-biopolymer on the plasma triacylglycerol, ratio of HDL-cholesterol to total cholesterol, atherogenic index, and liver total cholesterol levels in the dietary-induced hyperlipidemic rats for 2 weeks.

Experimental groups (exo-biopolymer, mg kg^{-1} BW)	Plasma			Liver total cholesterol (mg g^{-1})
	Triacylglycerol (mg ml^{-1})	HDL/Total cholesterol*	Atherogenic index**	
Control (saline)	0.41 ± 0.01^{c}	0.41 ± 0.02^{a}	1.46 ± 0.02^{c}	7.43 ± 0.49^{b}
50	0.38 ± 0.02^{bc}	0.49 ± 0.03^{ab}	1.04 ± 0.03^{bc}	6.5 ± 0.6^{ab}
75	0.35 ± 0.01^{ab}	0.6 ± 0.03^{bc}	0.67 ± 0.02^{ab}	5.98 ± 0.49^{a}
100	0.31 ± 0.02^{a}	0.68 ± 0.03^{c}	0.47 ± 0.01^{a}	5.85 ± 0.1^{a}

The rats of each experimental group were administered orally with either saline (control) or exo-biopolymer at the level of 50–100 mg kg^{-1} BW daily for 2 weeks.
*HDL cholesterol/total cholesterol.
**(Total cholesterol-HDL cholesterol)/HDL cholesterol.
Each value is the mean ± SE for 8 rats.
^{a,b,c}Values with different superscript letters in the same row are significantly different ($p < 0.05$).

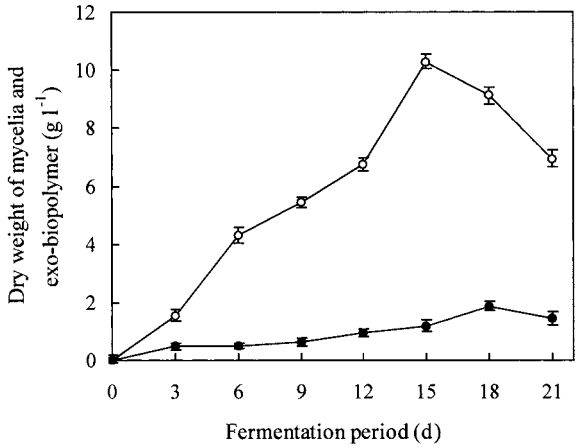

Fig. 2. Time course of the mycelia (○) and exo-biopolymer (●) production by *Auricularia polytricha* in a 5-l jar fermenter (working volume, 3-l; agitation speed, 100 rpm; aeration rate, 1 vvm; pH 4; temperature, 30 °C). The data recorded every 3 d interval up to 30 d.

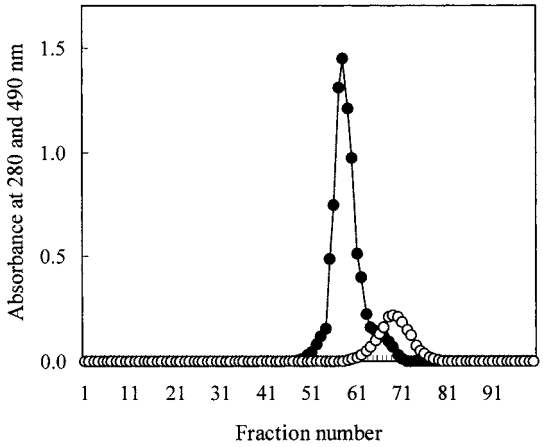

Fig. 3. Elution profile of the exo-biopolymer produced from the submerged mycelial culture of *Auricularia polytricha* by using Sepharose CL-6B chromatography. The volume of each fraction was 5 ml, and the eluates were checked by measuring the absorbance at 490 nm after a phenol-sulfuric acid reaction for sugar (●), and at 280 nm after Lowry reaction for protein (○).

Hypolipidemic effect of EBP

The effect of *A. polytricha* EBP on BW gain of rats, liver weight, and food intake are presented in Table 3. The administration of EBP did not influence significantly either the gain in BW or food intake. The liver weight remained unchanged in all the EBP administration groups. Moreover, oral administration of EBP caused no changes in gross behavior and none of the animals died. Therefore, there were no harmful effects in rats following oral administration of EBP of *A. polytricha*.

The effect of EBP on the plasma level of triacylglycerol, total cholesterol, LDL and HDL cholesterol, ratio of HDL cholesterol to total cholesterol, atherogenic index, and liver total cholesterol of the experimental animals are summarized in Tables 4 and 5. The levels of plasma triacylglycerol, total cholesterol, and LDL cholesterol steadily decreased with the increasing concentration of EBP. Maximum decrease of their values appeared to be at the highest level at 100 mg kg^{-1} body wt (BW) dose. The EBP dose beyond 100 mg kg^{-1} BW could not be given because of the high viscous nature of the EBP and limited volume to be given orally. At this level, it lowered the plasma total cholesterol and triacylglycerol by 27% and 24%, respectively. A substantial decrease of plasma LDL level was evidenced in the present investigation. The EBP even at the dose of 50 mg kg^{-1} BW reduced the LDL value to 30% and could lower the LDL level by 70% at the dose of 100 mg kg^{-1} BW.

The hypolipidemic effect exerted by the EBP of *A. polytricha* probably resulted from the high viscous nature of the EBP. The hypolipidemic effect was steadily enhanced with the increasing concentration of EBP. This is consistent with the increased viscosity with the increasing dose of the EBP. Perhaps for this property it could lower the triacylglycerol and total cholesterol absorption by inhibiting the formation of micelles in the small intestine and by altering the physical characteristics of the intestinal mucosa of rats (Ebihara & Schneeman 1989). Yang *et al.* (2000) also propounded the similar fact while studying the hypocholesterolemic effect of *Cordyceps militaris* EBP. However, this fact cannot exclude other mechanisms involved in exhibiting hypolipidemic effect by the EBP.

Chemical analysis of the EBP

The EBP obtained from the mycelial culture broth yielded only one peak when passed through the Sepharose CL-6B column (Figure 3).

The molecular weight of the purified EBP was determined by HPLC. The standard curve was obtained by using pullulan series, and the molecular weight of this purified EBP was estimated to be 32 kDa (Figure 4).

The EBP contained 77.5% carbohydrate and 22.5% protein, no acidic sugar was detected. Detail chemical analysis of the EBP is summarized in Table 6. Eight different kinds of sugar constituted the

Table 6. Amino acid and sugar compositions of exo-biopolymer produced from submerged mycelial culture of *Auricularia polytricha*.

Sugar	Composition (%)[a]	Amino acid	Composition (%)[b]
Rhamnose	1	Aspartic acid	13.2
Fucose	3.5	Threonine	9.3
Ribose	Trace	Serine	10.3
Arabinose	1.5	Glutamic acid	9.1
Xylose	2.4	Proline	Trace
Mannose	51.2	Glycine	12.2
Galactose	32.9	Alanine	7.7
Glucose	7.3	Cysteine	2.7
		Valine	7
		Methionine	1.6
		Isoleucine	4.1
		Leucine	5
		Tyrosine	1.3
		Phenyalanine	9
		Histidine	1.6
		Lysine	3.4
		Arginine	2.2
Total sugar content	77.5	Total protein content	22.5

[a]Percentages were calculated on the basis of total sugar.
[b]Percentages were calculated on the basis of total amino acids.

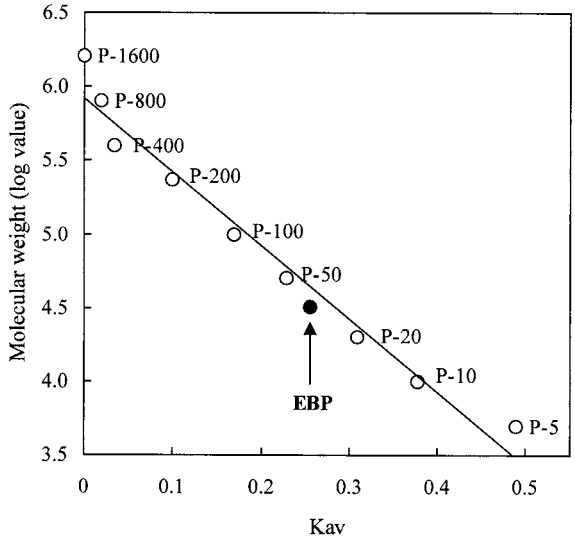

Fig. 4. Measurement of molecular weight of purified exo-biopolymer (EBP). P-1600, P-800, P-400, P-200, P-100, P-50, P-20, P-10, and P-5 are standard pullulans of 1600, 800, 400, 200, 100, 50, 20, 10, and 5 kDa, respectively. Kav = (Ve − Vo)/(Vt − Vo) (Vo; void volume, Vt; total volume, Ve; elution volume)

carbohydrate moiety of which a mannose and galactose were detected as major sugars. Aspartic acid, serine, and glycine were the major amino acids of the protein part (Table 6). It is likely that the EBP is a glycoprotein.

The present investigation demonstrated the potential of *A. polytricha* EBP in reducing the level of cholesterol-rich LDL (which are quantitatively the most significant lipoprotein class in the control of serum cholesterol levels) and preserving the HDL at relatively high level. All of the above effects would help to reduce the risk of atherosclerosis. Although the exact mechanism of the *A. polytricha* EBP in exhibiting hypocholesterolemic effect was not clear to us, the possibility of combined effect of the EBP in exerting hypocholesterolemia cannot be ruled out. The combined effect possibly involved were (1) the inhibition of cholesterol absorption and/or biosynthesis, (2) inhibition of biosynthesis of very-low-density lipoprotein (VLDL), the precursor of LDL and acceleration of fractional turnover of LDL (Tokuda *et al.* 1974) and (3) increased excretion of bile acids (Vahouny *et al.* 1987). The present investigation also determined the optimum culture condition for the production of EBP, which provides valuable information to scale-up its

production and gives an insight to the chemical nature of the hypolipidemic EBP.

Acknowledgement

This research was supported by a Daegu University research grant, 2001.

References

Cheung PCK (1996) The hypocholesterolemic effect of two edible mushrooms: *Auricularia auricula* (tree-ear) and *Tremella fuciformis* (white jelly-leaf) in hypercholesterolemic rats. *Nutr. Res.* **16**: 1721–1725.

Ebihara K, Schneeman BO (1989) Interaction of bile acid, phospholipids, cholesterol and triglyceride with dietary fiber in the small intestine of rats. *J. Nutr.* **119**: 1100–1106.

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226**: 497–509.

Jones TM, Albersheim PO (1972) A gas chromatography method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharide. *Plant Physiol.* **49**: 926–936.

Kaneda T, Tokuda S (1966) Effect of various mushroom preparations on cholesterol levels in rats. *J. Nutr.* **90**: 371–376.

Suzuki H, Okubo A, Yamazaki S, Toda S (1988) Immunopotentiating substances in *Lentinus edodes* mycelial extract (LEM)-activation of macrophage and proliferation of bone marrow cell. *Med. Microbiol. Immunol.* **177**: 235–244.

Tokuda S, Tagiri A, Kano E, Sugawara Y, Suzuki S, Sato H, Kaneda T (1974) Reducing mechanism of plasma cholesterol by shiitake. *Mushroom Sci.* **9**: 445–462.

Vahouny GV, Khalafi R, Satchithanandam S, Watkins DW, Story JA, Cassidy ME, Kritchevsky D (1987) Dietary fiber supplementation and fecal bile acids, neutral steroids and divalent cations in rats. *J. Nutr*. **117**: 2009–2015.

Yang BK, Ha JY, Jeong SC, Das S, Yun JW, Lee YS, Choi JW, Song CH (2000) Production of exo-polymer by submerged mycelial culture of *Cordyceps militaris* and its hypolipidemic effect. *J. Microbiol. Biotechnol.* **10**: 784–788.