J. Microbiol. Biotechnol. (1998), **8**(3), 277–279

JOURNAL OF
MICROBIOLOGY AND
BIOTECHNOLOGY
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NOTE

Hepatoprotective Effect of Extracellular Polymer Produced by Submerged Culture of *Ganoderma lucidum* WK-003

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Received: January 9, 1998

Abstract An extracellular polymer (exo-polymer) with hepatoprotective properties was produced after a 6-day submerged mycelial culture of *Ganoderma lucidum* WK-003. The glutamic pyruvic transaminase (GPT) activities in the serum of intoxicated Sprague-Dawley rats were decreased from 871 to 263 by the oral administration of the exo-polymer (20 mg/kg/day) for 4 consecutive days. Rhamnose, arabinose, xylose, mannose, galactose, and glucose were found in the exo-polymer along with aspartic acid, glutamic acid, histidine, serine, glycine, arginine, alanine, tryptophan, valine, phenylalanine, isoleucine, and leucine.

Key words: *Ganoderma lucidum*, hepatoprotective properties, extracellular polymer.

The medicinal effects of *Ganoderma lucidum* have been described as having anti-tumor [7], hypolipidemic [5], and anti-complement [2] actions. Basidiocarps of *Ganoderma lucidum*, which belongs to the family of Polyporaceae, are widely used in Asian countries as a folk medicine for the treatment of gastric ulcers, hypertension, hypercholesterolemia, bronchitis, insomnia, neurasthenia, asthma, and diabetes, etc. [1]. However, for the commercial use of the polymer extracted from basidiocarps of *Ganoderma lucidum*, it is difficult to obtain uniform preparations due to the variation of strains and cultural conditions. Submerged fermentation could minimize this defect and the objective of this work, therefore, was to optimize submerged mycelial culture conditions and to examine the hepatoprotective effect of extracellular (exo-) polymer.

Ganoderma lucidum WK-003 (KCTC 0179BP) was grown on a semi-synthetic medium (galactose 0.1%, sucrose 0.9%, xylose 0.1%, glucose 0.9%, yeast extract 0.05%, peptone 0.2%, potato dextrose broth 0.2%, $NH_4H_2PO_4$ 0.05%, DL-serine 0.05%, KH_2PO_4 0.1%, $CaCl_2$ 0.06%, $MgSO_4 \cdot 7H_2O$ 0.2%, $FeSO_4 \cdot 7H_2O$ 0.002%, $ZnSO_4 \cdot 7H_2O$ 0.002%, $MnSO_4 \cdot H_2O$ 0.002%) in shake cultures in 250-ml flasks containing 100 ml medium (pH 4.5, 30°C, 125 rpm, 7 days). Mycelia were harvested by centrifugation, washed, and freeze-dried. Cultivation was also carried out in a 5 l air-lift fermenter with an air flow rate of 1.0 vvm, 30°C, and pH 4.5. Exo-polymer was hydrolyzed and acetylated by the method of Jones and Albersheim [4]. Sugar composition was analyzed by gas chromatography as follows. Sugars were separated on a stainless column packed with 3% OV-225 Chromsorb WHP 100/120 in Shimadzu GC 14A fitted with FID (Flame Ionized Detector). The carrier gas was N_2 (1.75~2 kg/cm^3). The column temperature was 225°C. The injector and detector temperatures were 250°C. Exo-polymer was hydrolyzed by 6 M HCl. Amino acid composition of hydrolyzed exo-polymer was analyzed by a Gilson HPLC using a Waters Nova-pak C_{18} column (4 μm particle, 3.9× 150 mm) eluted with 50 mM sodium phosphate, 50 mM sodium acetate/tetrahydrofuran (960/40), and methanol/ DIW/acetonitrile (450/450/100). The peaks were detected with a fluorescence detector (Ex/Em=388 mm/455 mm) by comparing with the authentic standards. Exo-polymers were obtained from the culture broth as shown in Fig. 1.

Sprague-Dawley rats (male, initial body weight 200~240 g) were divided into four groups as follows;

- Group 1: Saline-treated normal rats (n=4)
- Group 2: Exo-polymer (20 mg/kg, p.o.) treated normal rats (n=4)
- Group 3: Saline treated with CCl_4 (0.35 ml/kg, i.p.) intoxicated rats (n=12)
- Group 4: Exo-polymer (20 mg/kg, p.o.) treated with CCl_4 (0.35 ml/kg, i.p.) intoxicated rats (n=11)

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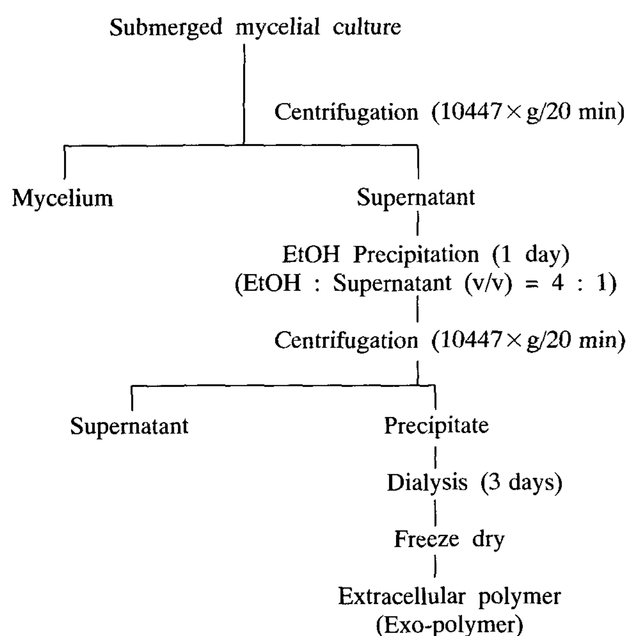

Fig. 1. Revovery of exo-polymer from submerged mycelial culture of *Ganoderma lucidum* WK-003.

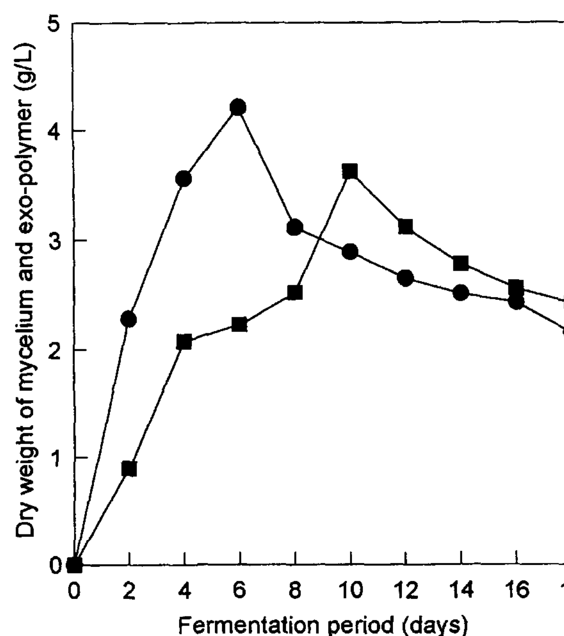

Fig. 2. Typical growth curve and production of exo-polymer by using an air-lift fermenter with synthetic medium of *Ganoderma lucidum* WK-003.

(pH 4.5, 30°C with aeration of 1.0 vvm in a 5 *l* fermenter) ●: mycelial growth, ■ : exo-polymer production.

The rats were weighed daily and they received normal chow and water *ad libitum*. They were maintained under 23±2°C, 12 h light-dark cycles throughout the experiments. The rats in groups 2 and 4 were given exo-polymer (20 mg/kg, dissolved in saline) orally using an intubation needle for 4 consecutive days. The rats in groups 1 and 3 were given equal volumes of saline for 4 consecutive days. Two hours after the last exo-polymer or saline treatment, CCl_4 (0.35 ml/kg as CCl_4, diluted in corn oil) was injected peritoneally to groups 3 and 4. Equal volumes of corn oil were injected to rats in groups 1 and 2. The rats were sacrificed 24 h after the CCl_4 or corn oil injection. Blood was obtained by cardiac puncture under ether anesthesia. Blood was kept at room temperature for 3 h to coagulate. After blood coagulation, it was centrifuged at 3,000×g for 30 min to obtain serum. The serum was kept in -20°C until processed further. Serum GPT (glutamic pyruvic transaminase) and GOT (glutamic oxaloacetic transaminase) were measured by Serum Transaminase Diagnostic Kit (Asan Pharm. Co, Kyugi-do, Korea) by the Reitman-Frankel method [9]. Results were expressed as means±S.D. Statistical differences were determined between groups by Student's *t*-test. Values of $p<0.05$ were considered to indicate a significant difference.

Mycelial Growth and Production of Exo-polymer Using 5 *l* Fermenter

Maximum productions of exo-polymer and mycelia were obtained by 10 and 6 day, respectively, batch fermentation (Fig. 2). It is generally known that microbial exo-polymer accumulates on the cell surface in the precursor form during logarithmic growth and is subsequently released in the stationary phase [8]. Reduction of exo-polymer in the phase of decline (after 6 days cultivation) could be due to the production of a hydrolytic enzyme (endogenous depolymerase) that hydrolyzes its own products [3]. During a period of about 2~5 days after inoculation, the small pellets began to aggregate into loose flocculent masses. After about 6 days the aggregates break up and individual mycelial pellets were dispersed. The viscosity of culture broth began to increase at this stage.

Sugar and Amino Acid Composition of Exo-polymer

Six kinds of neutral sugars were detected by gas chromatographic analysis. The amounts were, in the order, glucose, mannose, arabinose, rhamnnose, galactose, and xylose (Table 1). However, acidic sugars were not detected. There were compositional and quantitative differences between endo- and exo-polymers. Ribose was present in the endo-polymer [6] whereas our result showed that rhamnose was present instead of ribose in the exo-polymer. Twelve kinds of amino acids were detected by HPLC (Table 1). However, Lee *et al.* [6] reported that sixteen kinds of amino acids were present in the endo-polymer. Consequently, it can be concluded that the produced exo-polymer could be a peptidoglycan which is composed of sugars and amino acids.

Table 1. Sugar and amino acid compositions of exo-polymer produced from *Ganoderma lucidum* WK-003.

Sugars	Molar ratio	Amino acids	Composition (%)[a]
Rhamnose	1.7±0.057[b]	Aspartic acid	7.7±1.07
Arabinose	2.9±0.057	Glutamic acid	7.3±0.43
Xylose	1.0±0.000	Histidine	9.8±1.05
Mannose	4.2±0.050	Serine	14.9±1.25
Galactose	1.5±0.050	Glycine	8.8±0.57
Glucose	6.7±1.987	Arginine	5.2±0.80
		Alanine	10.8±0.96
		Tryptophan	2.2±0.70
		Valine	11.9±2.28
		Phenylalanine	4.4±0.01
		Isoleucine	8.6±1.60
		Leucine	7.6±0.52

[a]Percentages were calculated on the basis of total amino acids.
[b]Mean±SD (three replications).

Table 2. Hepatoprotective effects of exo-polymer on CCl_4 intoxicated rats.

Group	GPT (Karmen unit)	GOT (Karmen unit)
1. Normal rats	30±7	101±9
2. Exo-polymer-treated rats	28±5	105±11
3. CCl_4 intoxcated rats	871±609[a]	1705±753[a]
4. CCl_4 intoxcated rats treated with Exo-polymer	263±088[b]	1245±865

[a]Significantly different from normal rats (p<0.001).
[b]Significantly different from CCl_4 intoxicated rats (p<0.01).

Hepatoprotective Effect of Exo-polymer

Serum GPT and GOT activities in exo-polymer-treated rats are shown in Table 2. The levels of serum GPT and GOT were markedly increased to 871 and 1705 Karmen units, respectively, in CCl_4 intoxicated rats, showing that acute hepatic injury was induced. In exo-polymer treated CCl_4 intoxicated rats, the GPT levels were significantly reduced to 31% compared with that of CCl_4 intoxicated rats without exo-polymer treatment ($p<0.01$). The GOT levels of rats intoxicated with exo-polymer-treated CCl_4 were reduced to 73% of that of CCl_4 intoxicated-rats, but there was no significance. It is well known that serum GPT is more sensitive than GOT as an index of hepatocellular damage [9]. These results show that the exo-polymer produced from *Ganoderma lucidum* WK-003 had a marked hepatoprotective effect on acute liver injury by carbon tetrachloride, while there was no hepatoprotective effect in endo-polymer treated rats (data not shown).

Acknowledgments

This work was supported by academic research fund (1997) of the Ministry of Education (97-H-7), Republic of Korea, in part by the Choongwae Pharmaceutical Co., and the Medicinal Resources Research Center (95B-16-02-01-3) sponsored by the Korea Science and Engineering Foundation.

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