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Isolation and Characterization of Polysaccharides from *Ganoderma lucidum*

Chuan-huai Wang, Chun-hui Wang, Min-hua Shen, Xiao-ying Yan
College of Chemical Engineering of Nanjing Forestry University, Nanjing 210037, China

ABSTRACT: *Ganoderma* spp. known as wood rotting fungi are noted for their medicinal values and tonic qualities in China and abroad. Polysaccharides with a yield of 1.38% were isolated from the hot water soluble fraction of sporocarps of *Ganoderma lucidum*. These polysaccharides, after purification and determination by TLC and GC consisted of xylose, mannose, arabinose, galactose and glucose in a ratio of 1:1:1.7:5:20. Their molecular weights were about 23,000 and 34,000 as measured by gel filtration chromatography. The IR spectrum showed absorptions at 1050, 1140, 1420 and 3400 cm. An effective method for isolation and purification of the polysaccharides from sporocarps of *Ganoderma* spp. is proposed. The sequence for *G. lucidum* polysaccharide production, IR spectrum of *G. lucidums* extract liquor and some lignin-cellulose degradation enzymes were all studied. Element analysis of extracted liquor from *G. lucidum* by ICP showed the mushroom contains Ca, K, Mg, Zn and other elements favoring health.

1 INTRODUCTION

Ganoderma is a famous medicinal fungus which was described as a cure for many unspecified illness in China for more than two thousand years. As reported in many papers, this fungus was used as a nontoxic medicine which was beneficial to viscera and could improve intelligence by enhancing memory, hearing, vision and smell. Many papers have indicated the pharmacology and clinical uses of the fruit body and the mycelium of *Ganoderma* cultivated by submerged fermentation. *Ganoderma* is reported to have effects on immunofunctions which can stimulate the

activity of acid phosphtase and β-glucuronidase of peritoneal macrophages in mice (Liu 1993). It was also showed that there was a significant increase in PFC and agglutination titer of anti-SRBC antibody as well as the activity of interferon γ of mice (Li 1994). However, a few papers describe the formation of enzymes during the culture period and the analysis of the preparation of *Ganoderma*. The present paper reports the results of our studies on polysaccharides isolated from *Ganoderma*.

2 MATERIALS AND METHODS

2.1 *Strains and cultivation*

The organism used in this study was *Ganoderma lucidum* (Leyss ex Fr.) Karst kindly provided by the Institute of Microbiology, Academia Sinica, Beijing. The fungus was cultivated on artificial logs. Sawdust was the major cultivation material, which was mixed with 25% wheat bran. Water was added to obtain a moisture content of 70%.

The medium for production of lignin cellulose degrading enzymes for *G. lucidum* was as follows: 80% corn cob powder, 8% corn meal mixed with 9% wheat bran and 3% KH_2PO_4 and water, for a final content of 70%. The culture filtrate used for the experiments was obtained by water extraction at 30°C for 2 h and then centrifuged.

2.2 *Enzyme assays and element analysis*

Methods for the determination of CM-cellulase and xylanase activities were the same as those described by Mandels et al. (1976) except for a buffer solution of citric acid and its salt that was used instead of acetate salt. Units were expressed as micromoles of reducing sugar produced min^{-1}. Element analysis was determined by ICP.

2.3 *Extract and isolation of polysaccharides*

A sequence for *G. lucidum* polysaccharides production was followed which is summarized in Figure 1.

2.1 *Analysis of the polysaccharides component*

Polysaccharides isolated from the hot water soluble fraction of the fruit body of *G. lucidum* was determined by TLC and GC. Molecular weights were measured by gel filtration chromatography.

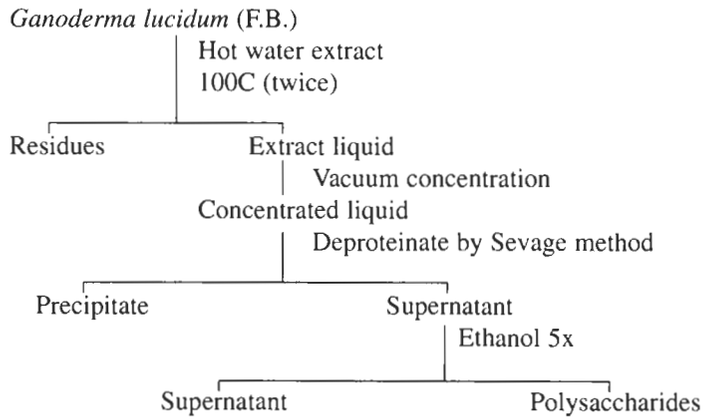

Figure 1. Sequence for *G. lucidum* polysaccharide production.

3 RESULTS

A group of lignocellulose degrading enzymes including cellulase (endo β 1.4 glucanase), xylanase and laccase were extracted and are shown in Table 1.

Table 1. Enzyme yields in the *G. lucidum* solid state medium.

Duration of cultivation (days)	Yield of enzyme (u/g)		
	CM-Celullase	Xylanase	Laccase
7	15.6	18.0	102.5
11	19.3	14.0	57.5
15	6.1	6.8	32.5
19	0.7	0.9	40.2
23	1.1	1.2	10.0
27	1.7	2.4	5.0

After extraction with hot water, the residues were reextracted for polysaccharides with 4N NaOH solution but no polysaccharides were detected. Our study shows that the polysaccharides, after pruification and determination by TLC and GC, consisted of xylose, mannose, arabinose, and galactose in a ratio of 1:1:1.7:5:20. Their molecular weights were about 23,000 and 34,000 as measured by gel filtration chromatography. The IR spectrum showed absorption at 1050, 1140, 1420 and $34cm^{-1}$. Element analysis of the extracted liquor of *G. lucidum* by ICP, as shown in Table 2, indicates a substantial content of Ca, Mg, P, Mn and Zn.

Table 2. Element analysis of extracted liquor from *G. lucidum* by ICP (μg/ml)[a].

Al	Ba	Be	Ca	Cd	Cu	Fe	K	Mg
1.09	0.15	N.D.	26.4	N.D.	0.13	0.10	240.1	37.0
Mn	Na	P	Ni	Si	Sr	Zn	Ge	
0.95	47.2	107.8	N.D.	3.75	0.11	0.64	N.D.	

[a]The sample was diluted with water by 5 times.

4 DISCUSSION

As we know, cellulase and hemicelullase activities are closely related to the degradation rate of cultivation substrates and yield of fruitbodies. Our studies indicated the fungus has the highest level of cellulase, xylanase and laccase activities during the period of mycelial growth and then the activities of these enzymes are all reduced rapidly. These results may not facilitate the formation of fruitbodies.

The M.W. of polysaccharides isolated from *G. lucidum* fruitbodies is more than $1x10^4$ daltons and its IR spectrum shows strong absorption at 1149 1429 cm^{-1}. These polysaccharides may inhibit the growth of tumors (Qinyan 1994). More studies are obviously needed.

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