韓 國 菌 學 會 誌 第 11 卷 第 2 號
Kor. J. Mycol. Vol. 11, No. 2, 91~95, 1983

An Antitumor Constituent of the Cultured Mycelia of *Hydnum repandum**

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털수염 버섯 培養 菌絲의 抗腫瘍 成分*

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Abstract: To investigate antitumor constituents of *Hydnum repandum*, which belongs to edible basidiomycetes, the mycelia separated from the carpophore were shake-cultured and subjected to hot water extraction. The extract was concentrated under vacuum and mixed with a three-fold volume of 95% ethanol to yield precipitates. The water soluble fraction of the precipitates was dialyzed and then lyophilized to yield a water soluble protein-polysaccharide fraction (=WPPF). This exerted antitumor activity against sarcoma 180 implanted in ICR mice. When administered *i.p.* at the dose level of 20mg/kg once daily for ten consecutive days, it showed an inhibition ratio of 54.3%. WPPF was found to be composed of a polysaccharide moiety (42% of WPPF) and a protein moiety (28% of WPPF) when determined by colorimetric method using anthrone reagent and Folin's phenol reagent. The polysaccharide moiety of WPPF was found to contain glucose (57.4%), mannose (19.3%), galactose (10.8%), xylose (6.8%), and fucose (5.7%), when the methanolysate of WPPF was analysed by GLC method.

Key words: Basidiomycetes, Hydnaceae, *Hydnum repandum*, Protein-polysaccharide, Antitumor activity, Sarcoma 180.

Since Gregory and his collaborators (1966) reported the results of antitumor screening tests of basidiomycetes, many investigators have been actively worked in this field to find new antitumor components from basidiomycetes. Among them Chihara and his collaborators (1969) separated an antitumor polysaccharide fraction, LC-33, from the carpophores of *Lentinus edodes* and in the next year Chihara and his collaborators (1970) isolated and purified and antitumor $\beta(1\rightarrow3)$ glucan and named it lentinan. Recently Fujii and his collaborators (1978) isolated a new antitumor protein-bound polysaccharide, KS-2, from the cultured mycelia of *Lentinus edodes* KSLE 007.

The studies on the antitumor components of Korean basidiomycetes have been actively carried out since Kim and his collaborators (1979) reported the results of antitumor test of protein-polysaccharide fractions separated from the carpophores of *Lentinus edodes, Coriolus versicolor, and Pleurotus ostreatus.* As the results of the active studies thereafter more than 20 species of Korean Basidiomycetes, such as *Ganoderma lucidum* (Kim *et al.*, 1980), *Cryptoporus volvatus* (Kim *et al.*, 1982), *Flammulina velutipes* (Woo, 1982), and *Leatiporus sulphureus* (Kang *et al.*, 1983), were found to contain antitumor constituents.

* Part XXX of Studies on Constituents of Higher Fungi of Korea.

Since no report on an antitumor component of *Hydnum repandum*, had been found, the authors undertook this investigation and separated a protein-polysaccharide fraction from the shake-cultured mycelia of this fungus. It was examined for antitumor activity and an attempt was made to study on its chemical composition.

Materials and Methods

Fungal Strain

The strain of *Hydnum repandum* ISA-Hr-1006(the family Hydnaceae) was stored in the Institute of Agricultural Sciences, Office of Rural Development at Suweon, Korea. The mycelia of this fungus were subcultured on potato-dextrose-agar slants.

Liquid Culture Medium

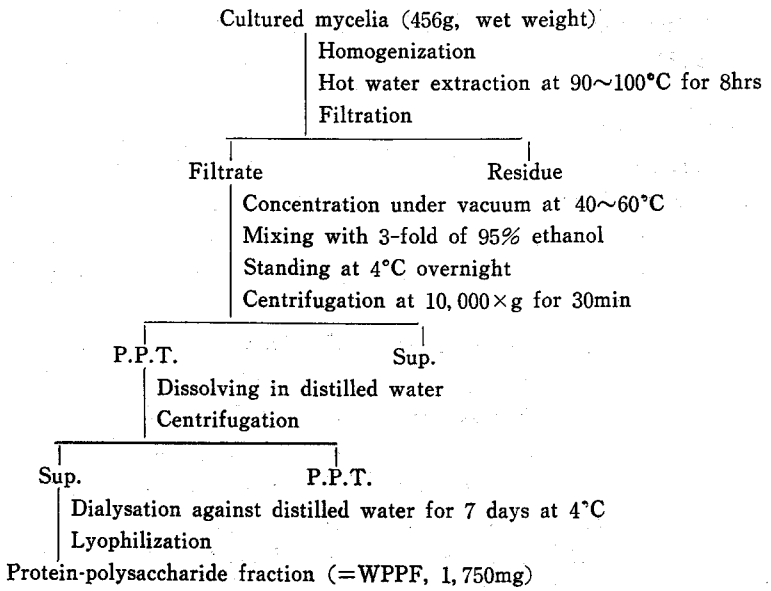
The liquid culture medium used in this study has the composition shown in Table I. It contains glucose as carbon source and peptone and yeast extract as nitrogen source.

Culture Process

The mycelia grown on a PDA slant were homogenized for ten seconds in a microblendor and transferred into two 500ml Erlenmyer flasks, each of which contained 100ml of liquid culture medium and was shaken for 15 days at 28±1°C and 180rev/min. The mycelial pellets were obtained by decanting off excess medium and these were homogenized and used to inoculate ten flasks, each of which contained 100 ml of the liquid culture medium. These were shake-cultured for another 15 days and then the whole culture broth was used as inocula for main culture. Ten liters of fresh liquid culture medium delivered in twenty two-liter-Erlenmyer flasks were inoculated with the homogenated culture broth. These were shake-cultured for 15 days.

Extraction and Separation of a Protein-polysaccharide Fraction

The mycelia (456g, wet weight) were harvested by filtration, washed with distilled water and then homogenized for five minutes in a blendor. The homogenized mycelia were extracted two times on boiling water bath using distilled water as extracting solvent. The residue was filtered off and the filtrate (6 liters) was concentrated to one liter at 40~60°C under reduced pressure. After being cooled the concentrated extract was mixed with three-fold volume of 95% ethanol and let to stand at 4°C overnight to precipitate the protein-polysaccharides. The precipitates were separated by centrifugation at 10,000xg for 30min, and then dissolved in 500ml



Scheme I. Separation of protein-polysaccharide fraction from the cultured mycelia of *Hydnum repandum*.

of distilled water. After centrifugation under the same condition the supernatant was dialysed in a Visking tube for seven days at 4°C against distilled water. The dialysates yielded a water soluble protein-polysaccharide fraction (=WPPF) as brownish powder when lyophilized. The procedure was depicted in Scheme I.

Antitumor Test

ICR mice of female sex, weihning 18~22g were used as a test animal. They were supplied from the Experimental Animal Farm of Seoul National University. As a tumor cell line, sarcoma 180 cells, which were maintained by weekly passage into the peritoneum of mice in our laboratory, were used.

1) Tumor cell implantation

Each of the test animals was subcutaneously implanted with 0.1ml of tumor cell suspension (1×10^7 cells/ml, hemacytometer count) at the left axillary region. The detailed procedure was described previously (Kim *et al.*, 1982b).

2) Treatment and Evaluation of Antitumor Activity

WPPF in physiological saline was administered i.p. at the dose level of 20mg/kg once daily for ten consecutive days starting 24 hr after tumor implantation. The control mice received only physiological saline. Injection volume was 0.1ml. Twenty-six days after the tumor implantation, the mice were sacrificed and the tumors were excised and weighed. The antitumor activity was expressed as percent tumor inhibition ratio.

Chemical Assay

1) Total Polysaccharide Content

Total polysaccharide content of WPPF was determined by colorimetric method using anthrone reagent as described previously (Kim *et al.*, 1982b).

2) Total Protein Content

Total protein content of WPPF was determined by colorimetric method using Folin's phenol reagent (Lowry *et al.*, 1951).

3) Monosaccharide Analysis

The monosaccharides which constitute the polysaccharide moiety of WPPF were analysed as described previously (Kim *et al.*, 1982b). In brief, WPPF was methanolysed by reacting with 3% HCl in methanol at 80°C for 20 hours and the resulting methylmonosaccharides were trimethylsililated and then analyzed by GLC method.

4) IR Spectrum

The IR spectrum of WPPF was obtained by KBr disc method.

Results

Yield of WPPF

The mycelia (456g, wet weight), which were harvested from ten liters of mycelial culture broth, yielded 1,750mg of WPPF as freeze-dried powder.

Antitumor Activity

WPPF showed a tumor inhibition ratio of 54.3% at the dose level of 20mg/kg as shown in Table II.

Chemical Composition

The total polysaccharide content of WPPF was 42% and the total protein content was 28% as

Table I. The composition of the liquid culture medium.

Ingredient	Quantity (g/liter)
Glucose	50
Peptone	10
Yeast extract	10
KH_2PO_4	0.87
$MgSO_4 \cdot 7H_2O$	0.50
$CaCl_2$	0.30
Mineral solution*	10ml

*Ten ml of mineral solution contains $ZnSO_4 \cdot 7H_2O$ 4mg, $CuSO_4 \cdot 5H_2O$ 1mg, $MnCl_2 \cdot 4H_2O$ 7mg, $FeSO_4 \cdot 7H_2O$ 10mg.

Table II. The results of antitumor test of WPPF separated from *Hydnum repandum*.

Group	Average tumor weight (g)	Inhibition ratio(%)	Complete regression
WPPF Treated (20mg/kg)	4.45±1.11[a]	54.3	1[b]/10[c]
Control	9.74	—	0/8

a : mean ± standard error.
b : the number of mice in which the tumor was completely regressed.
c : the number of mice used.

Table III. The composition of WPPF separated from *Hydnum repandum*.

Moiety	Content
Protein	28%
Polysaccharide	42%

Table IV. Monosaccharide contents in the polysaccharide moiety of WPPF separated from *Hydnum repandum*.

Monosaccharide	Content
Fucose	5.7%
Xylose	6.8%
Mannose	19.3%
Galactose	10.8%
Glucos	57.4%

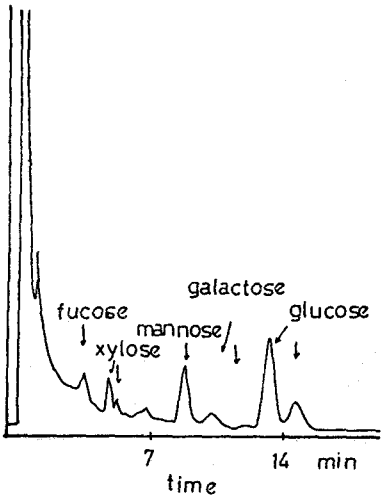


Fig. 1. GLC pattern of monosaccharides in the polysaccharide moiety of WPPF separated from *Hydnum repandum*.

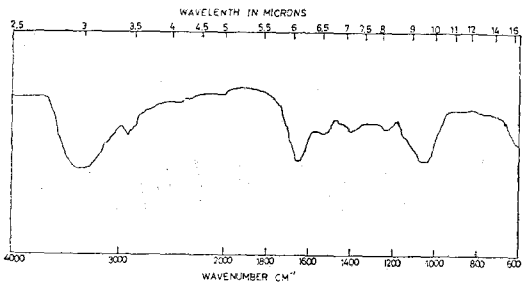


Fig. 2. IR spectrum of WPPF of *Hydnum repandum*.

shown in Table III. The polysaccharide moiety of WPPF was found to be composed of five monosaccharides including glucose as its main sugar and mannose, galactose, xylose, and fucose (Table IV). The GLC chromatogram of these monosaccharides was shown in Fig. 1. The IR spectrum of WPPF in KBr disc was shown in Fig. 2.

Discussion

Hydnum repandum is newly recognized as an antitumor basidiomycete, whose protein-polysaccharide fraction, WPPF, showed moderate antitumor activity (inhibition ratio=54.3%). But there remains a possibility of isolating more potent antitumor substance(s) from WPPF because WPPF is thought to be composed of a variety of polysaccharides and proteins. The fact that WPPF is separated from the cultured mycelia indicates the possibility of mass production of the antitumor constituent of *Hydnum repandum* by growing the mycelia in artificial media. Further studies on the separation and purification of the antitumor substance(s) from WPPF are now being carried out in our laboratory.

Conclusion

A water soluble protein-polysaccharide fraction (WPPF) was separated from the shake-cultured mycelia of *Hydnum repandum*. WPPF showed antitumor activity against sarcoma 180 implanted in ICR mice (inhibition ratio=54.3%). WPPF was found to consist of a polysaccharide moiety (42% of WPPF) and a protein moiety (28% of WPPF). The polysaccharide moiety of WPPF was found to consist of five monosaccharides including glucose (57.4%), galactose (10.8%), mannose (19.3%), xylose (6.8%), and fucose (5.7%).

요 약

한국산 담자균류의 항종양성 성분을 탐색하기 위하여 턱수염버섯의 균사를 분리하여 인공배지에서 진탕배양후, 그 배양균사체로부터 수용성 단백성 다당류를 추

출하였다. 이 성분이 ICR 마우스에 이식한 육종 180에 대하여 54.3%의 종양억제율을 나타내었다. 이 성분은 다당류 42%와 단백질 28%로 구성되어 있었다. 그 중 다당류는 glucose (57.8%), mannose(19.3%), galactose (10.8%), xylose (6.8%), 및 fucose (5.7%)를 함유하고 있었다.

Acknowledgments

This investigation was supported in part by a research grant from the Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University for 1983 and a grant from the Ministry of Education, Republic of Korea. The authors acknowledge with gratitude the supports and wish to express gratitude to Dean Varro E. Tyler and Dr. James E. Robbers, School of Pharmacy and Pharmacal Sciences, Purdue University, W. Lafayette, Indiana, U.S.A. for their encouragement and advice.

The authors thank Mr. Chong Kil Lee and other members of the Department of Microbial Chemistry, College of Pharmacy, Seoul National University, for their kind assistance throughout this study. K.S. Chung (one of the authors) gives the greatest thanks to Dr. Chang Kyu Moon for his deep interest in and encouragement for this study.

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〈Received March 13, 1983〉