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Studies on Constituents of the Higher Fungi of Korea (LIV) Antitumor Components of *Favolus alveolarius*

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Abstract □ To find antitumor components from the higher fungi of Korea, the mycelia of *Favolus alveolarius* (Fr.) Quelet were cultured in a liquid medium. The cultured mycelia were extracted with hot water twice, and a high molecular weight fraction was obtained by adding two volumes of ethanol to the extract. Two grams of Fraction A were obtained by dialyzing it. It was further separated into four fractions by gel filtration with Sepharose CL-4B, and they were designated Fractions B, C, D, and E. The results of the antitumor test showed that Fractions A, B, C, D and E had tumor inhibition ratios of 92.3, 78.5, 59.6, 77.4 and 62.2%, respectively. Anthrone test was carried out to determine the contents of total polysaccharide of the five fractions, and they had 46.3, 27.3, 65.3, 64.6, and 46.1%, respectively. The contents of the total protein of the five fractions were 29.4, 13.9, 14.3, 14.3, and 29.1%, respectively. Monosaccharide subunits of each fraction were analyzed with gas chromatography, and glucose, xylose, mannose, galactose and fucose were identified. Fraction A was examined for immunological effects. It increased the count of hemolytic plaque forming cells 12.8 times to that of the control group, and the population of macrophage in peritoneal cavity 3.2 times to that of the control group.

Keywords □ *Favolus alveolarius,* antitumor activity, hemolytic plaque-forming cells, peritoneal exudate cells, macrophage, lymphocytes, PMNs.

Polysaccharides with antitumor activity have been isolated from various natural sources, including higher plants, fungi, bacteria, and yeast.[1-4] Since Lucas reported the antitumor activity of basidiomycetes, studies on the antitumor polysaccharides of basidiomycetes have been extensively carried out.[5-11]

The antitumor polysaccharides differ widely in their sugar composition and consequently differ greatly in chemical structure. And they can be extracted with various solvents, but extracted components can differ in variety with the choice of solvents. One common point of the active polysaccharides is their relatively high molecular weight. They include lentinan, a β-1,3 glucan, schizophyllan, a β-1,3; 1,6 glucan, and PSK, a peptide bound β-1,4; 1,3- or β-1,4; 1,6-glucan.[12-14] The physicochemical properties of the antitumor β-(1,3)-D-glucans, such as lentinan and schizophyllan, have been extensively studied. The X-ray crystallography strongly suggested the existence of triple helix structure.[15] No correlation can be drawn between sugar composition and structure of the polysaccharides and their antitumor activity from the information presently available.

These compounds are considered to exert their antitumor activity through potentiation of the host-mediated immunity rather than direct cytotoxicity to tumor cells. It is widely accepted that activated macrophages, cytotoxic T cells, natural killer cells, and killer T cells usually play important roles in tumor immunity.[16-18]

More than 700 species of basidiomycetes have been recorded in Korea, but investigations on their antitumor components had been scarce. So our laboratory began to investigate on the antitumor components from the carpophores and cultured mycelia of Korean basidiomycetes, such as *Lentinus edodes, Ganoderma lucidum, Coriolus versicolor, Pluteus cervinus, Laccaria laccata, Ramaria formosa, Pleurotus pulmonarius, Lyophyllum decastes* and so on, since 1979.[19-24]

As *Favolus alveolarius* was new to Korea, attempts were made to investigate the components of this fungus. *Favolus alveolarius* is a fungus belonging to the family Polyporaceae of the Eubasidio-

mycetes. In the present study, the protein bound polysaccharides were extracted with hot water from the cultured mycelia of this fungus and examined for antitumor activity in mice. The antitumor components were purified by gel filtration method. They were also analyzed to find their chemical compositions. In addition, their effects on immune responses in the mice were examined to study mechanisms of their antitumor activities.

EXPERIMENTAL METHODS

Materials

The strain of *Favolus alveolarius* (Fr.) Quelet ASI-51001 (the family Polyporaceae) used in this work was kindly provided by Agricultural Science Institute at Suwon, Gyeong-Gi Province, Korea (Fig. 1).

Medium composition

1) PDA slant

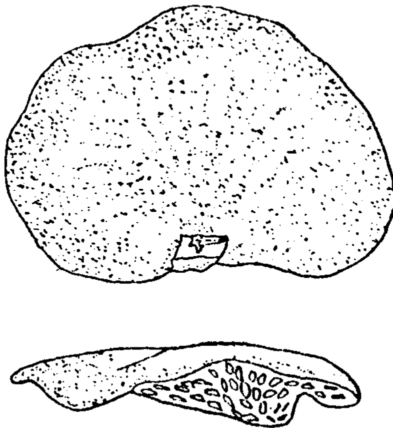


Fig. 1-a. The carpophores of *Favolus alveolarius*.

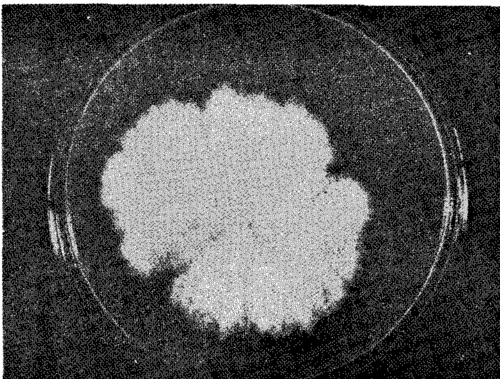


Fig. 1-b. The ten-day cultured mycelia of *F. alveolarius* on PDA medium.

Potato dextrose agar

2) Culture medium

Glucose 50 g, peptone 10 g, KH_2PO_4 0.87 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCl_2$ 0.3 g, $FeSO_4 \cdot 7H_2O$ 10 mg, $MnCl_2 \cdot 4H_2O$ 7 mg, $ZnSO_4 \cdot 7H_2O$ 4 mg, and $CuSO_4 \cdot 5H_2O$ 1 mg per one liter. The pH of this medium was adjusted to 5.5 and autoclaved at 121 °C, 2 atm for 15 min.

Culture methods

1) First culture

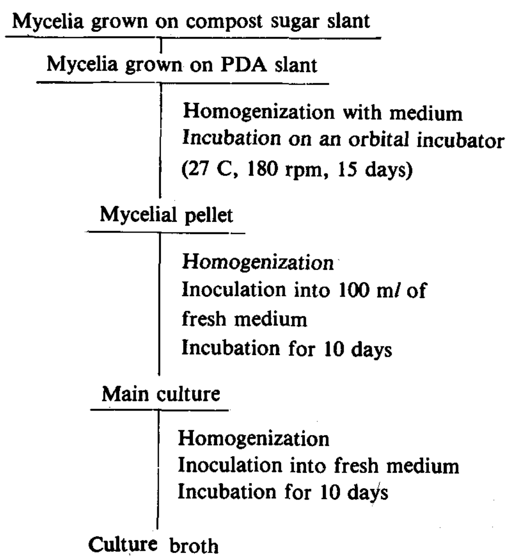
The mycelia of *F. alveolarius* were aseptically transferred into a fresh PDA slant and cultured for seven days at 27 ± 1 °C. The fully grown mycelia were separated aseptically and homogenized with 50 m*l* of the culture medium for 10 seconds in a microblender. it was inoculated into 50 m*l* of the culture medium in a 500-m*l* flask and incubated for 15 days in an orbital shaking incubator at 27 ± 1 °C, 180 rpm.

2) Second culture

The pellets of mycelia were aseptically homogenized for the seconds and inoculated into a 500-m*l* flask containing 100 m*l* of the culture medium. Incubation was carried out under the same condition of the first culture for ten days.

3) Main culture

The mycelial pellets from the second culture were aseptically homogenized for ten seconds and transferred into 500 m*l* of the culture medium in a two liter flask (inoculum size: 20 v/v %) and cultured for ten days under the same codition as pre-



Scheme I. Culture process of *Favolus alveolarius*

viously described (Scheme I).

Extraction and separation of fungal metabolites

The mycelia from 18 liters of the culture broth were filtered and washed twic with distilled water. The obtained mycelia were homogenized and extracted with distilled water on water bath at 95 °C for six hours. After filtration, the mycelia were extracted again, and the filtrates were concentrated under vacuum, and two volumes of 95% ethanol were added to the concentrate. To complete the precipitation the mixture was allowed to stand at 4 °C overnight. The precipitate was obtained by centrifugation at 8.000 × g for 20 min, dissolved in distilled water, and dialyzed at 4 °C for seven days using Visking tube (36/32). The precipitate was removed by filtration and the filtrate was concentrated and lyophilized to obtain a brownish powder with yield of 2.0 g. It was designated as Fraction A.

Purification of fungal metabolites

Preswollen Sepharose CL-4B (Pharmacia Co.) was washed with two volumes of 0.01 M sodium phosphate buffer twice and degassed under vacuum. The gel was packed into a column (3.0 × 72 Cm) with 0.01 M sodium phosphate buffer in 0.3 M NaCl (pH 7.2). Fraction A (1.5 g) was dissolved in the eluent and applied to the column. It was eluted with 0.01 M sodium phosphate buffer (pH 7.2) in 0.3 M NaCl at the flow rate of 30 m*l* an hour.

Optical densities of the fractions were measured at 625 nm (anthrone test) and 540 nm (Lowry-Folin method). Anthrone positive fractions were collected and concentrated. They were dialyzed at 4 °C for 3 days and lyophilized. Each of the obtained fractions was designated as Fr. B (82.5 mg), Fr. C (53.6 mg), Fr. D (120 mg), and Fr. E (58.5 mg). They were white or brownish white powder (Scheme II).

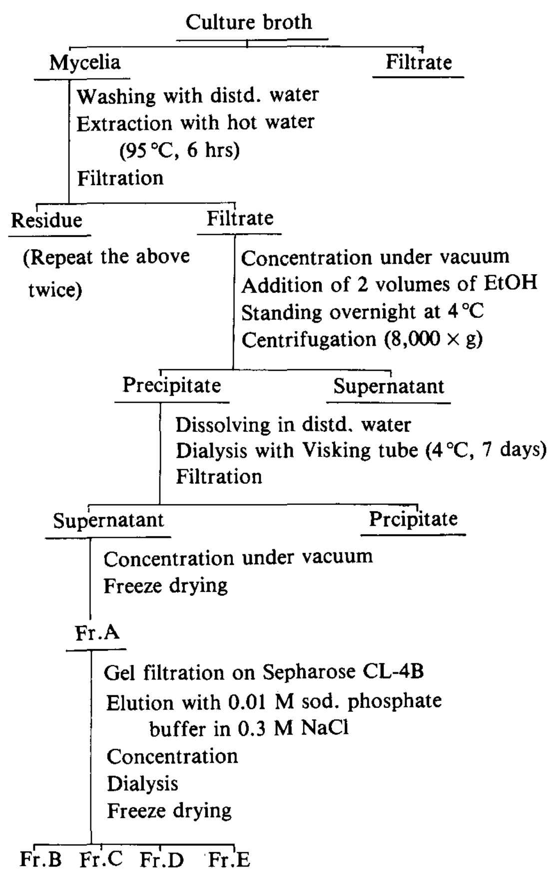
Antitumor test

1) Animals

Female ICR mice (18-22 g) were supplied from the Experimental Animal Farm of Seoul National University.

2) Tumor cells

Sarcoma-180 cells maintained in the peritoneum of female ICR mice were used for antitumor test. An ICR mouse was sacrificed on the seventh day after the inoculation of tumor cells and the ascitic fluid was collected. After the cells were washed three times with ice-cold saline, the cell suspension was diluted to 1×10^7 cells per milliliter. And 0.1 m*l* of this suspension was inoculated into the left groin of ICR mice subcutaneously.



Scheme II. Extraction, separation and purification of the polysaccharides from the cultured mycelia of *Favolus alveolarius*

3) Administration of fungal metabolites

Each 35 milligrams of Fractions A, B, C, D, E, and Krestin were dissolved in 20 m*l* of saline for a dose of 20 mg/kg, autoclaved at 121 °C, 2 atm for 15 min and stored in a refrigerator. Krestin was used as standard antitumor agent, and physiological saline for control. Eight mice were used for each group.

Administration of the samples was started on the third day after the tumor inoculation and continued for consecutive ten days once a day. Each sample solution (0.2 m*l*) was administrated intraperitoneally.

4) Calculation of inhibition ratio

On the 30th day after the tumor inoculation the mice was sacrificed, and the solid tumors were excised and weighted. The tumor inhibition ratio was calculated as follows (Scheme III):

Tumor inhibition ratio (%) Cw-Tw Cw × 100

ICR mouse with sarcoma-180 cells (ascitic form)

sacrificing with $CHCl_3$ vapor
Collecting the ascites fluid with ice-cold saline
Centrifugation at $400 \times g$ for 3 min

Cytocentrifugate

Washing with ice cold-saline (3x)
Dilution to 1×10^6 cells/ml
Inoculation into right groin (S.C., 0.1 ml/mouse)

ICR mice inoculated with sarcoma-180 cells

After three days, sample administration
(i.p., once daily for 10 consecutive days)
Sacrificing 30 days after the tumor inoculation
Excising the tumors

Weighing solid tumors

Scheme III. Procedure of antitumor test *in vivo*

Cw: Average tumor weight of the control group
Tw: Average tumor weight of the treated group

Chemical analysis

1) Total polysaccharide content

Total polysaccharide contents were quantitatively determined by Anthrone test.[26] The optical densities of each fraction and standard slution solution were measured at 625 nm. A standsrd curve was made to calculate the total polysaccharide content of each fraction using glucose.

2) Total protein content

Total protein contents were quantitatively determined by Lowry-Folin method.[27] The optical desities of each fraction and standard solution were measured at 540 nm. A standard curve was made to calculate the total protein content of each fraction using boving serum albumin.

3) Monosaccharide analysis

Two milligrams of each fraction and standard monosaccharide were dissolved in two milliliters of 3% HCl-methanol. The air in the tube was substituted with nitrogen gas and the tube was sealed. They were methanolized at 80 ± 5 °C for 20 hours. The methanolysate was filtered, evaporated and dissolved in one milliliter of pyridine. For trimethylsilylation 0.2 ml of hexamethyl disilazane and 0.1 ml of trimethyl chlorosilane were added to the solutions and shaked vigorously. The sample solutions were injected into Shimazdu gas chromatography RIA and analyzed under the standard conditions. Retention time of each peak was compared with that of standard monosaccharide to identify the monosaccharide. Glucose, galactose,

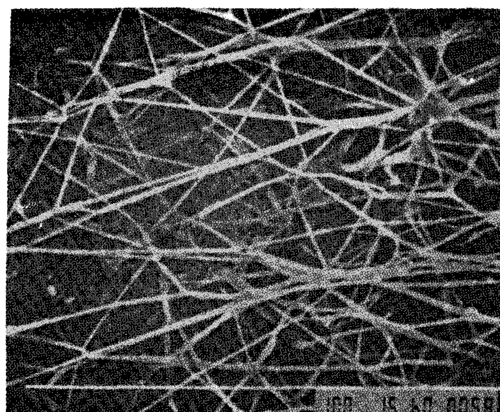


Fig. 2. Scanning electron micrograph of the mycelia of *F. alveolarius* after gold coating.
Each bar means 100 um.

mannose, xylose, fucose and arabinose were used as standards. The content of each monosaccharide was calculated from the chromatograms by measuring the peak area.

4) Amino acid analysis

To analyze the amino acids of the protein moiety, two milligrams of each sample were disolved in five milliliters of 6 N-HCl. The air in the tubes was substituted with nitrogen gas, and the tubes were sealed. The samples were hydrolyzed at 110 ± 55 °C for 24 hours. After filtration, the filtrate was evaporated in vacuum and re-dissolved in two milliliters of 0.02 N-HCl. The sample solutions were injected into hitachi amino acid analyzer 835 and analyzed. The contents of each amino acid were calculated from the chromatograms by peak height method.

5) Infrared spectroscopy

Each sample was analyzed by KBr disc method.

Effects of Fraction A on immune responces

1) Effects on hemolytic plaque-forming cells

a) animals

Male ICR mice (20-22 g) were provided by the Experimental Animal Farm of Seoul National University.

b) Reagents

i) PBS (=Phosphate Buffered Saline, 0.01 M)

ii) BSS (=Balanced Salt Solution)

Stock solution I: Dextrose 10 g, KH_2PO_4 0.6 g, Na_2HPO_4 H_2O 3.58 g and 0.5% Phenol red solution 20 ml per liter.

Stock solution II: $CaCl_2$·anhyd. 1.86 g, KCl 4.0 g, NaCl 80 g, and $mgCl_2$·anhyd. 2.0 g per liter.

BSS working solution: Demineralized water was added to 100 ml of stock solution I and 100 ml of

stock solution II and the mixed solution was adjusted to pH 7.3.

iii) 0.83% NH_4Cl Solution

iv) Microchamber

After cleansing glass slides with 95% ethanol, two strips of double side scotch tape were placed across both ends of the slides. A slide was placed on the top of the taped slide in such way that a microchamber was formed between the two slides.

v) Sheep Red Blood Cell (=SRBC)

SRBCs were kindly supplied from Korea National Health Institute.

iv) Complement

Guinea pig serum as a source of complement was provided by Green Cross Company.

c) Methods

i) Sample administration and immunization

Ten mice were divided into two groups. For the treated group, 0.1 m*l* of Fraction A solution (20 mg/5 m*l*) in saline was injected intraperitoneally once a day for five consecutive days. Physiological saline was used for the control group. On the seventh day after the last sample injection, the mice were immunized by intraperitoneal injection of 1×10^7 cells of SRBC.

ii) Preparation of spleen cell suspension

On the fifth day after immunization, mice were sacrificed with $CHCl_3$ vapor and the spleens were excised. The spleens were homogenized with ice-cold BSS and centrifugated at 400 × g for five min. Cytocentrifugates were collected and hemolyzed with 0.83% NH_4Cl solution at 37 °C for five min. After hemolysis the cells were washed three times and centrifugated under the same condition. The cytocentrifugates were resuspended in ice-cold BSS. The spleen cells were counted directly by a hemacytometer.

iii) Preparation of complement-SRBC

SRBC was centrifugated and resuspended in physiological saline to adjust the concentration to 20 v/v %. And then, 500 μl of 20 v/v % SRBC was mixed with 1,000 μl of guinea-pig serum in the microwell and fixed on the ice bath.

iv) preparation of Incubation Mixture

To make the incubation mixture, 150 μl of complement-SRBC solution and 650 μl of spleen cell suspension wre mixed. And 50 μl of this mixture was placed into the microchamber.

v) Incubation and reading of results

After sealing the microchamber with vaselin and wax (1:1), incubation was carried out at 37 °C for an hour and the numbers of hemolytic plaques were counted (Scheme IV):

PFC/10^6 spleen cells = c Vm a N × 10^6

ICR mice

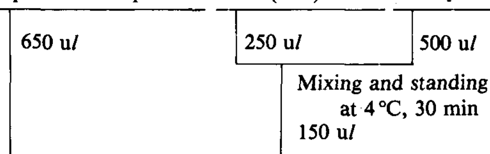
Sample injection (20 mg/kg, 5 days, i.p.)
After 7 days, immunized by injecting 1×10^7 SRBCs
After 5 days, excising spleen

Spleen

Homogenization with ice-cold BSS
Centrifugation (400 × g, 5 min)
Hemolysis with 0.83% NH_4Cl soln
Centrifugation

Spleen cells without erythrocytes

Washing with BSS (× 3)
Dilution with BSS



Mixing and standing at 4 °C, 30 min
150 ul

Mixing
Filling the microchamber
sealing
Incubation at 37 °C for 1 hr

Counting PFCs

Scheme IV. Assay procedure of hemolytic plaque-forming cells

PFC/total spleen cells = PFC/10^6 spleen cells × c × Vs

$a = \frac{800 \text{ (vol. of incubation mixture)}}{600 \text{ (vol. of spleen cell suspension)}}$

N : number of plaque observed in one microchamber

c : count of spleen cells in one m*l* of spleen cell suspension

Vm : volume of incubation mixture filled into one microchamber

Vs : total volume of spleen cell suspension (m*l*)

2) Effects on peritoneal cell population

a) Animals

Male ICR mice (19-23 g) were provided by the Experimental Animal Farm of Seoul National University.

b) Reagents

i) PBS

ii) BSS

iii) NSE Staining Solution

Stock solution III: One gram of α-naphtyl acetate was dissolved in 50 m*l* of demineralized water and 50 m*l* of acetone.

NSE working solution: Two milliliters of stock solution III, 15 m*l* of 0.1 M phosphate buffer (pH 7.3) and 15 m*l* of demineralized water were mixed, and then 20 mg of fast red TR salt was added to the solution. Before use, the mixture was filtered and used immediately.

iv) Giemsa Staining Solution

Stock IV: Giemsa stain powder (0.38 g) was added into 20 m*l* of glycerol and dissolved on a water bath at about 60 °C for two hours. Then 31 m*l* of absolute methanol was added. It was stored in brown bottle at room temperature.

c) Methods

i) Sample administration

ICR mice were injected intraperitoneally with Fraction A solution at a dose of 50 mg/kg.

ii) Peritoneal exudate cell count

On the first day, third day, fifth day after the sample administration, the ICR mice were sacrificed and the ascitic fluids were collected with ice-cold BSS. The numbers of PEC were couted directly by a hemacytometer.

iii) Lymphocyte and PMN Count

After PEC count, the peritoneal fluid was centrifugated at 4 °C, 400 × g for 10 mins and the cytocentrifugates were suspended into 0.2 m*l* of ice-cold BSS. The PEC suspension was smeared on the glass slides, air-dried, fixed in methanol for five min and stained with Giemsa working solution for 30 mins. The slides were washed in 90% ethanol to remove the excess dye and examined using Cedar oil as a mounting solution. The cells which showed even blue color and round shape were counted as lymohocytes, and the cells which contained a multilobbed and/or doughnut-type nucleus were counted as polymorphonuclear leucocytes (=PMNs).

iv) Macrophage Count

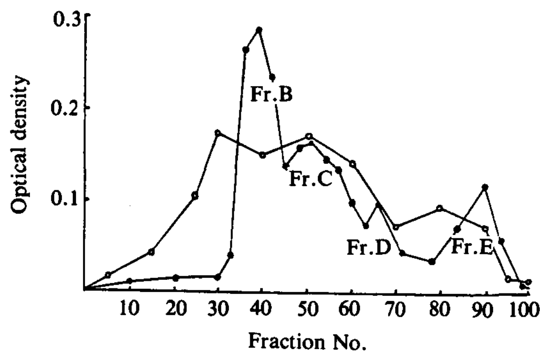


Fig. 3. The elution profile of Fraction A obtained from the mycelia of *F. alveolarius* by Sepharose CL-4B gel filtration.

●—● 625 nm; ○—○ 540 nm

The specimen on the glass slides were air-dried, fixed in ice-cold acetone for five min and incubated with NSE working solution at 37 °C for 30 mins. The slides were back-washed with tap water, counter-stained with one percent Malachite green staining solution for 15 seconds and back-washed with tap water again. The slides were examined using glycerol as a mounting solution. The cells which contained reddish granules were identified as non-specific esterase-positive macrophages.

RESULTS

Extraction and purification of polysaccharides from the cultured mycelia

The cultured mycelia of *Favolus alveolarius* were extracted with distilled water, and 2.0 g of a brownish powder was obtained (Fr. A). Fraction A was applied to Sepharose Cl-4B column and eluted. The elution profile was shown in Fig. 3. After gel filtration, four anthrone-positive peaks were collected, concentrated, dialyzed and lyophilized. And they were designated Fractions B (82.5 mg), C (53.6 mg), D (120 mg), and E (58.5 mg).

Antitumor test

Tumor inhibition ratios of each fraction and the standard agent Krestin against sarcoma-180 cells in

Table I. Antitumor activities of the fractions obtained from *F. alveolarius*

Group	Dose (mg/kg/day, i.p.)	Average Tumor Wt. (g)	Inhibition Ratio (%)	Complete Regression
Control	Saline	4.65±0.50*	—	0/7
Krestin	20	0.25±0.10**	94.6	3/7
A	20	0.36±0.17	92.3	2/7
B	20	1.00±0.23	78.5	0/7
C	20	1.88±0.29	59.6	0/7
D	20	1.05±0.40	77.4	1/7
E	20	1.76±0.34	62.2	0/7

* Mean ± standard error
** $P<0.01$

Table II. Polysaccharide and protein contents of the antitumor components

Fraction	A	B	C	D	E
Polysaccharide	46.3*	57.3	65.3	64.6	46.1
Protein	29.4	13.9	14.3	14.3	29.1

* Weight percentage

Table III. Monosaccharide contents of the polysaccharide moiety of the antitumor components

	Fr.A	Fr.B	Fr.C	Fr.D	Fr.E
Glucose	67*	89	90	83	44
Xylose	16	4	6	10	34
Mannose	10	3	3	5	15
Galactose	5	2	1	2	7
Fucose	2	2	—	—	—

* Area percentage

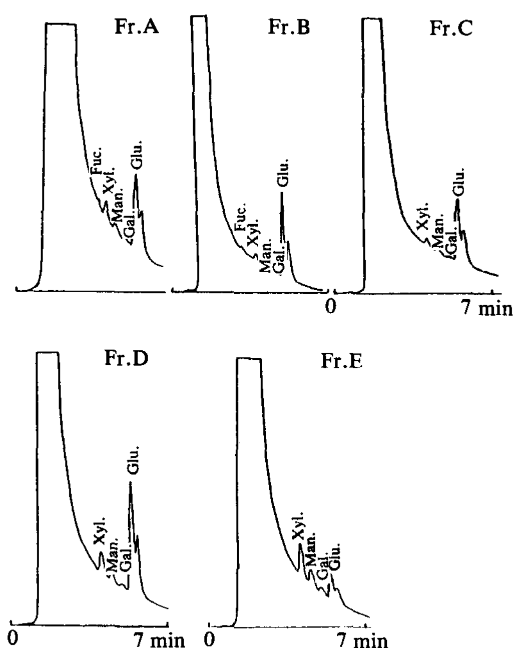


Fig. 4. GLC patterns of the antitumor components of *F. alveolarius*.

mice were shown in Table I. Fraction A showed the most effective inhibition ratio of 92.3% among the five fractions at the dose of 20 mg/kg. And two of the mice showed complete regression.

Chemical composition

The contents of the polysaccharide and the protein of the fractions were shown in Table II, They were protein-bound polysaccharides. As shown in Table III and Fig. 4, the monosaccharide subunits of the five fractions were glucose, mannose, xylose, galactose and fucose. The composition of amino acids contained in the protein moiety was shown in Table IV and Fig. 5. Infrared spectra of the samples were depicted in Fig. 6.

Table IV. Amino acid contents of the protein moiety of the protein moiety of the antitumor components

Fraction / Amino acid	A	B	C	D	E
L-Aspartic acid	9.1*	9.9	8.0	8.1	10.1
L-Threonine	3.8	4.6	4.2	5.4	6.0
L-Serine	3.4	4.9	4.2	7.0	7.2
L-Glutamic acid	13.9	14.2	12.5	12.3	13.9
Glycine	9.1	9.6	8.7	9.8	10.1
L-Alanine	10.6	11.6	10.5	11.6	11.3
L-Custine	1.0	0.6	0.7	0.5	0.5
L-Valine	9.1	8.7	8.0	8.4	8.1
L-Methionine	1.0	1.2	2.1	0.2	0.5
L-Isoleusine	4.8	5.2	4.5	4.2	4.5
L-Leucine	8.2	8.7	7.7	6.7	7.2
L-Tyrosine	1.4	1.7	1.4	1.2	1.2
L-Phenylalanine	4.3	4.6	4.2	3.5	3.6
L-Lysine	5.8	4.9	4.5	3.5	3.8
L-Histidine	6.7	4.6	5.2	3.5	3.1
L-Arginine	2.9	3.5	2.4	2.1	3.6
L-Proline	4.8	1.5	11.1	12.1	5.3

* Expressed as the mole %

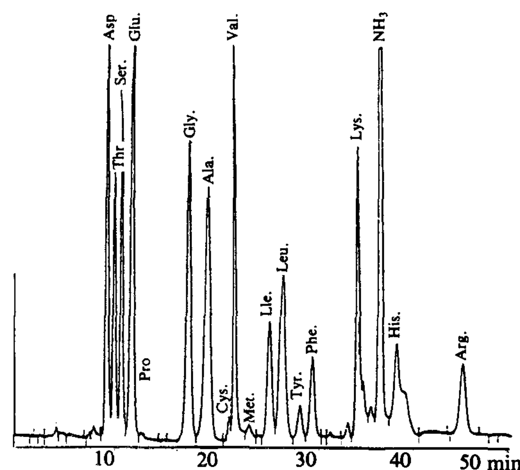


Fig. 5. Chromatogram of Fraction A.

Effects on immune responses

1) Effects on hemolytic plaque-forming cells

The PFC counts of the treated group were 12.8 times higher than those of the control group, and the result was summarized in Table V.

2) Effects on PEC population

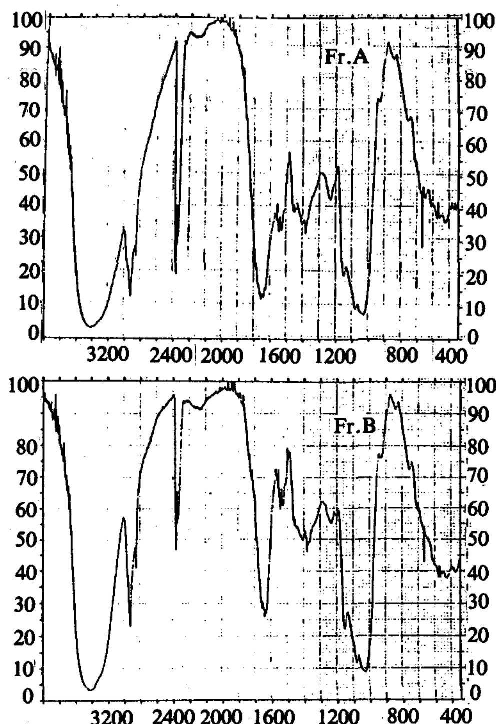


Fig. 6. IR spectra of the antitumor components of *F. alveolarius*.

The number of PECs was maximum on the 24th hour after the sample administration, and decreased slowly. The number of NSE-positive macrophages also was maximum on the 24th hour after the sample administration. The composition of PECs was depicted in Fig. 7.

DISCUSSION

The polysaccharides obtained from the cultured mycelia of *Favolus alveolarius* showed a potent antitumor activity against sarcoma-180 cells implanted in mice. The total extract (Fraction A) showed the inhibition ratio of 92.3% when administered at a dose of 20 mg/kg/day for 10 days. And it was more effective than the purified antitumor components.

Fraction A contained 46.3% polysaccharide and 29.4% protein. The polysaccharide moiety of Fraction A consisted of 67% glucose, 16% xylose, 10% mannose, 5% galactose and 2% fucose. The added value of the total contents of the polysaccharide and protein of Fraction A was not 100% as the total content of the polysaccharide was measured by Anthrone test using glucose as standard. Other polysaccharides showed lower optical densities than glucose when measured by anthrone test.

Table V. Effects of Fraction A on hemolytic plaque-forming cells in the spleen of ICR mice immunized with SRBCs (1×10^7)

	Control	Treated
Body weight (g)	21.3 ± 1.2*	21.5 ± 1.7
Spleen weight (mg)	163.3 ± 22.6	185.6 ± 8.9
Spleen cell count (1×10^7)	10.8 ± 1.3	17.8 ± 1.6
PFC/10^6 spleen cells	5.9 ± 0.2	45.74 ± 1.64
PFC/spleen ($\times 10^2$)	6.4 ± 0.8	81.48 ± 8.72

* means ± S.D.

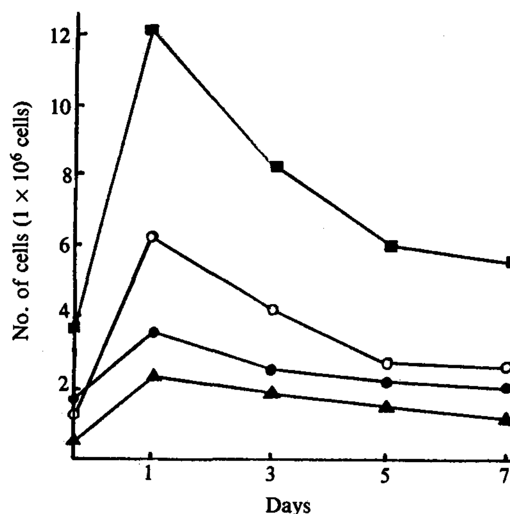


Fig. 7. Kinetics and composition of peritoneal exudate cells after the administration of Fraction A of *F. alveolarius*.

■—■ PECs
○—○ Macrophages
●—● Lymphocytes
▲—▲ PMNs

The IR spectra of the five fractions showed the common characteristics of polysaccharide, O-H stretching frequency at 3300-3400 cm^{-1}, C-H stretching frequency at 2900 cm^{-1}, C-O stretching frequency at 1630 cm^{-1}, and C-H and C-O bending frequency at 1000-1100 cm^{-1}

To elucidate mechanisms of the antitumor activity, effects of Fraction A on the immune response of mice immunized with SRBC were studied. It was found that the fraction increased the production of hemolytic plaque-forming cells in the spleen. In addition, to examine the effects of Fraction A on cell-mediated immunity, peritoneal exudate cell (PEC) population in mice administered with this fraction

was studied. The number of PECs was increased and showed maximum on the 24th hour after the sample administration. And macrophage was the major cell in PECs.

These results suggested that this fraction showed the antitumor activity through the host immunopotentiation.

CONCLUSION

The protein-bound polysaccharide extracted from the cultued mycelia of *Favolus alveolarius* showed a potent antitumor activity against sarcoma-180 cells in ICR mice. Fraction A showed the highest inhibition ratio of 92.3% at a dose of 20 mg/kg/day.

Fraction A was a protein-bound polysaccharide, containing 46.3% polysaccharide and 29.4% protein. And the polysaccharide moiety of this fraction consisted of 67% glucose, 16% xylose, 10% mannose, 5% galactose and 2% fucose.

Fraction A increased the number of plaque-forming cell and peritoneal exudate cells.

These results indicated that Fraction A showed the antitumor activity through the host immunopotentiation.

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