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Studies on Constituents of Higher Fungi of Korea (LXXIII) Antitumor Components of the Cultured Mycelia of *Paxillus atrotomentosus*

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한국산 고등 균류의 성분 연구(제 73보)
좀우단버섯 배양 균사체의 항암 성분

곽상덕 · 복진우 · 현진원 · 최응칠 · 김병각
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ABSTRACT: To find antitumor components from higher fungi, the cultured mycelia of *Paxillus atrotomentosus* were extracted with hot water. The water soluble fraction was purified and separated by DEAE-cellulose ion exchange chromatography and Sepharose CL-4B gel filtration method. The separated fractions(Fr.) were designated CR A, B, C and D. Fr. A showed the highest inhibition ratio of 68.51% among the five fractions at a dose of 20 mg/kg/day. When Fr. A was examined for immunopotentiation activity, it increased the amount of the superoxide anion from activated macrophages to 1.1 fold and the number of plaques in hemolytic plaque assay to 2.3 fold, respectively. Otherwise, it did not show direct cytotoxity in sarcoma 180. Delayed type hypersensitivity reaction showed that the decreased footpad swelling of tumor-bearing was restored to the normal. These results indicate that antitumor activity was exerted through immunopotentiation. Its chemical analysis showed 86.36% polysaccharide, 1.52% protein and 1.64% hexosamine. The polysaccharide consisted of fucose, galactose, glucose, mannose and xylose. This component was named paxillan.

KEYWORDS: *Paxillus atrotomentosus*, antitumor activity, direct cytotoxicity, superoxide anion, hemolytic plaque assay, delayed type hypersensitivity reaction, paxillan, protein-bound polysaccharide

Antitumor polysaccharides have been isolated from natural sources such as higher plants (Nakahara *et al.*, 1964), fungi (Chihara *et al.*, 1970), yeasts (Bradner *et al.*, 1958), bacteria (Kato *et al.*, 1981) and lichens. Ringler (1957) first found the antitumor activity of from Basidiomycetes Fungi. Various kinds of Basidiomycetes preparations which include: lentinan (Chihara *et al.*, 1970), a high molecular weight β-1-1,3-glucan (Sasaki *et al.*, 1976) obtained from *Lentinus edodes* fruit bodies; a high molecular weight β-1,3 1,6-glucan prepared from *Schizophyllum commune* culture filtrates (Komatsu *et al.*, 1969); and PS-K (Tsukagoshi *et al.*, 1974), a peptide containing β-1,4 1,3 or β-1,4 1,6-glucan extracted from *Coriolus versicolor* culture mycelia, are known to exhibit antitumor activity. These polysaccharides are krown as glucans, mannans, hemicelluloses, lipopolysaccharides.

Administration of these compounds is known to inhibit the growth of various transplantable tumors in experimental animals and increases the survival. The mechanism has not been exactly elucidated, but has been suggested that the action is host mediated and not directly cytocidal (Maeda *et al.*, 1970). It was suggested that the antitumor activities were due to the excellent immune-acce-

lerators of the cell-mediated response. It has been reported that activated macrophages, NK cells, cytotoxic T cells and killer T cells usually play important roles in tumor immunity (Adachi *et al.*, 1984; Nakajima *et al.*, 1984).

More than 700 species of Basidiomycetes have been recorded in Korea but investigations on their antitumor components had been scarce. So, the authors' laboratory began to investigate on antitumor components from the basidiocarps and cultured mycelia of Korea Basidiomycetes such as *Favolus alveolarius* (Chang *et al.*, 1988) *Coriolus versicolor* (Cho *et al., 1988), Polyporus giganteus* (Kim *et al.*, 1987), *Ganoderma lucidum* (Hyun *et al.*, 1990), *Volvariella bombycina* (Kim *et al.*, 1985), *Agaricus campestris* (Park *et al.*, 1985), *Pleurotus pulmonarius* (Lee *et al.*, 1985) and *Lyophyllum decastes* (Kim *et al.*, 1984), since 1979.

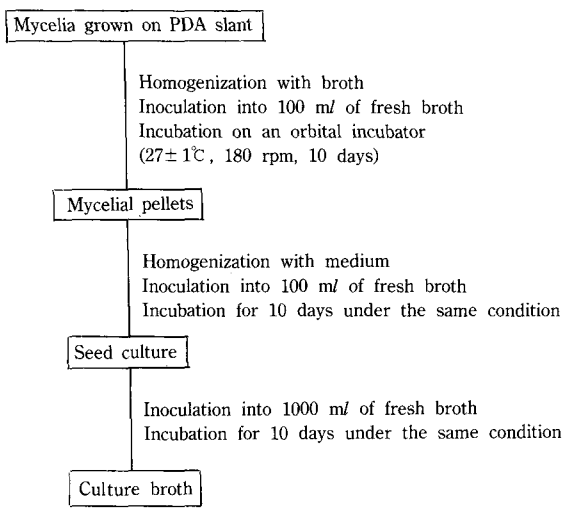
In the present study, the protein-bound polysaccharides were extracted with hot water from the cultured mycelia of *P. atrotomentosus* and examined for antitumor activity in mice. The antitumor components were purified by ion exchange chromatography and gel filtration method. Their chemical composition and molecular weight were elucidated by several analyses. In addition, their effects on immune responses in mice were examined to explain mechanisms of the antitumor activity.

Materials and Methods

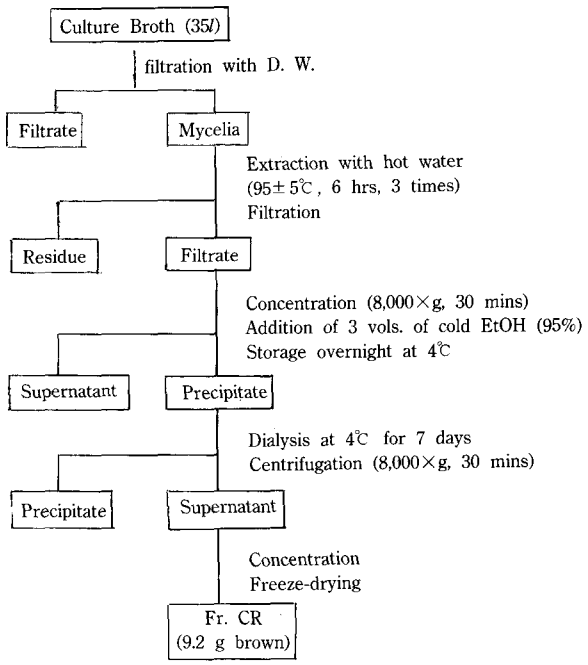
Strains: The strain of *P. atrotomentosus* 49003 was provided by Agricultural Science Institute, the Office of Rural Development at Suwon. Glucose 30g, Peptone 5g, Yeast ext. 5g, KH_2PO_4 1g, $MgSO_4 \cdot 7H_2O$ 1g, $FeSO_4 \cdot 7H_2O$ 10 mg, $MnCl_2 \cdot 4H_2O$ 7 mg, $ZnSO_4 \cdot 7H_2O$ 4 mg, $CuSO_4$ $5H_2O$ 1 mg, per 1 liter distilled water adjusted to 5.6-5.8 pH.

Culture methods: The mycelia of *atrotomentosus* were aseptically transferred into a fresh slant of seed culture medium and cultured for 10 days at 27± 1℃. The grown mycelia was homogenized with 50 m*l* of the culture broth and inoculated into 100 m*l* of the culture broth for 10 days in a 500 m*l* flask. The mycelia pellets from the above were inoculated, transferred into a 2 liter flask containing 1000 m*l* of the culture broth and cultured for 10 days under the same conditions (Scheme 1).

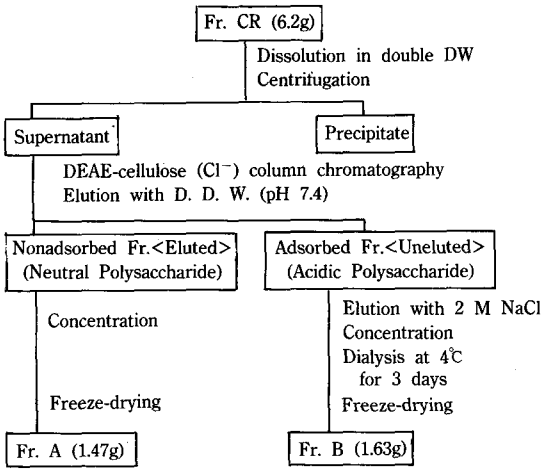
Extraction and separation of fungal metabolites: The mycelia of 35 liters of the culture broth were



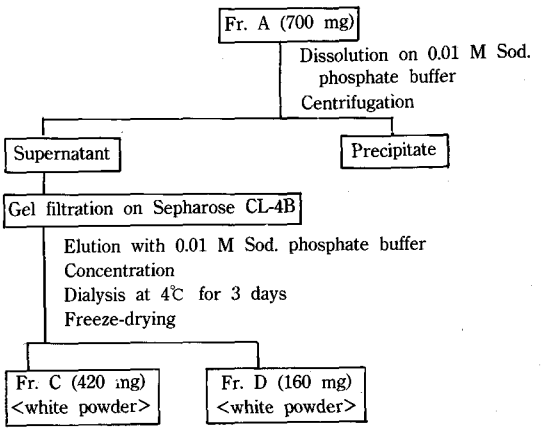
Scheme 1. Culture process of the mycelia of *paxillus atrotomentosus.*



Scheme 2. Extraction and separation of fungal metabolites from the cultured mycelia of *P. atrotomentosus.*



Scheme 3. Isolation of the neutral and acidic fraction from Fr. CR by DEAE-cellulose ion exchange column chromatography.



Scheme 4. Purification of neutral fraction by gel filtration chromatography.

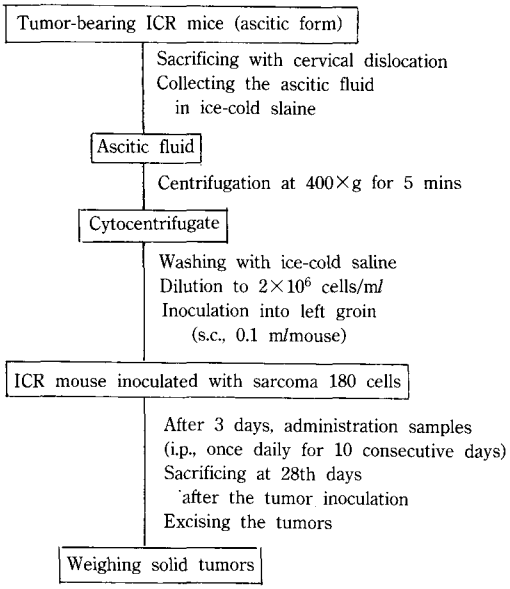
filtered and washed three times with distilled water. The obtained mycelia were homogenized and extracted with distilled water (DW) on a water bath at 85-90℃ for 6 hrs. After filtration, the mycelia were extracted twice again under the same conditions. The filtrates were concentrated under vacuum and three volumes of 95% cold ethanol were added to the concentrates and then stored at 4℃ overnight. After the supernatants were decanted, the precipitates were dissolved in DW and dialyzed at 4℃ for 7 days. The supernatant by centrifugation was concentrated and freeze-dried. A brown powder was obtained at a yield of 9.2g. It was designated "Fraction CR" (Scheme 2).

DEAE-cellulose column chromatography: A solution of Fr. CR (6.2g) in double distilled water (DDW) was applied to the column and eluted with DDW (pH 6.8) at flow rate of 48 ml/hr. The effluents were fractionated into 5 ml/fraction and were measured at both 625 nm (Anthrone test) and 540 nm (Lowry-Folin test). The anthrone-positive fraction was collected, concentrated and freeze-dried (white powder, "Fr. A"). The adsorbate on DEAE-cellulose was eluted with 2 M NaCl (brownish powder, Called to "Fr. B", see Scheme 3).

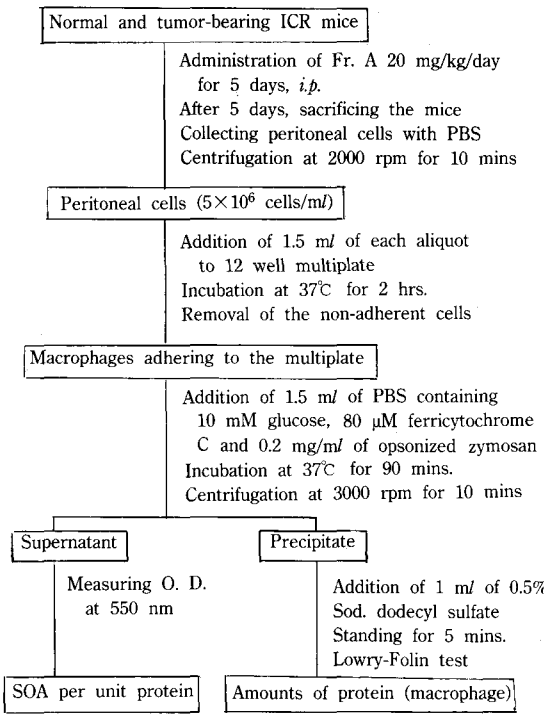
Sepharose CL-4B gel filtration chromatography: Sepharose CL-4B (Pharmacia Fine Chem., Sweden), was used to determine molecular weight. T2000 (Sigma Chem. Co., USA), T480 (Sigma Chem. Co) and T60 (Nakarai Chem. Ltd., Japan) were also used as standard dextrans. A column (2.5 $\phi \times 85$ cm) was silated with 5% dichlorodimethylsilane-$CHCl_3$ to form a silicone film in the inside of the column, and then dried. A solution of Fr. A (700 mg) in the buffer was applied to the above column. It was eluted with the same buffer at a flow rate of 12-20 ml/hr and the effluents were fractionated into 4.3 ml/fraction. The anthrone-positive fractions were collected and dialyzed at 4℃ for 3 days. This freeze dried fractions were designated to "Fr. C" and "Fr. D" (Scheme 4).

Antitumor test: Male ICR mice (20-25g) were supplied from the Experimental Animal Farm of Seoul National University. Sarcoma 180 cells maintained in the peritoneum of male ICR mice were used for antitumor test. ICR mice were sacrificed on the 7th day after 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 2×10^6 cells per 1 ml. The 0.1 ml of this suspension was subcutaneously inoculated into the left groin of the mice.

Applications of the water soluble fractions: Each (20 mg) of Fr. CR, A, B, C, D and Krestin was dissolved in 10 ml of saline for a dose of 20 mg/kg/day. They were autoclaved at 121℃, 1.1



Scheme 5. Procedure for antitumor test *in vivo*.

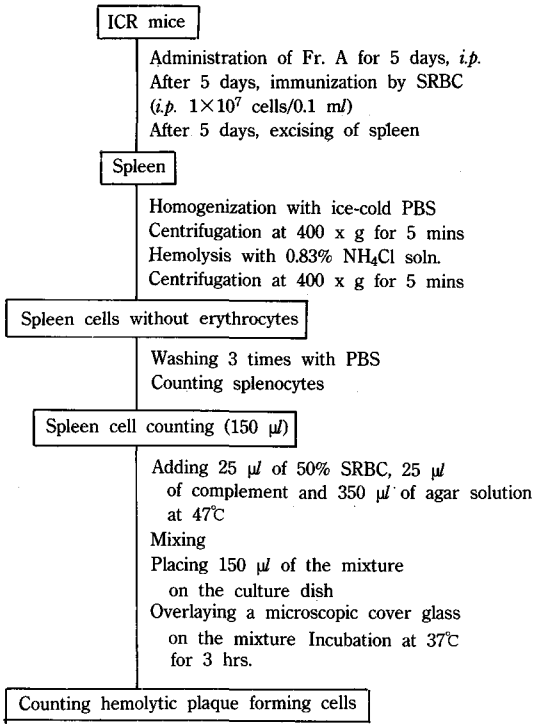


Scheme 6. Procedure of superoxide anion assay in activated macrophages.

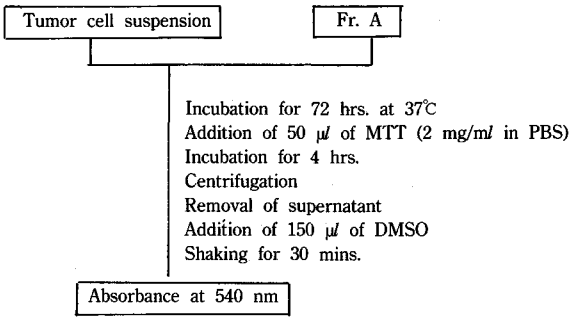
kg/cm^2 for 15 mins and stored in a refrigerator. Krestin was used as standard antitumor agent and physiological saline for control. Eight mice were used for each group. Administration of the sample was initiated on the third day after the tumor inoculation and continued for ten consecutive days once a day. Each sample solution (0.1 m*l*) was injected intraperitoneally. On the 28th day after the tumor inoculation, the mice were sacrificed and the solid tumors were excised and weighed (Scheme 5).

Calculation of inhibition ratio: The tumor inhibition ratio was calculated as follows: Tumor inhibition ratio (I.R.%)$=(C_w-T_w)/C_w\times100$, where C_{wis} was average tumor weight of the control group and T_{wis} was average tumor weight of the treated group.

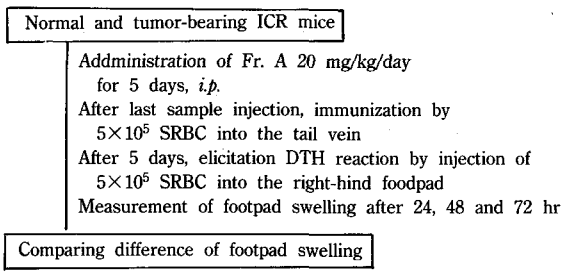
Effect on macrophage activation: The animals employed for it and all chemicals were described in detail (곽, 1991). Twelve mice were divided into four groups, two of which were prepared for control groups and the other for treated groups. To each of the control and the treated groups, 0.1 m*l* of the tumor cell suspension (2×10^6 cells/m*l*) was subcutaneously inoculated into the left groins of ICR mice. For the two treated groups, 0.1 m*l* of Fr. A solution in physiological saline was injected intraperitoneally to ICR mice at a dose of 20 mg/kg/day for 5 consecutive days from the day when the tumor cells were inoculated. Physiological saline was used for the control groups. Collection of macrophages (Mφ) in peritoneal cavity: Mφ were collected. On the fifth day after completion of injection, Peritoneal exudate cells (PEC) were obtained by washing the peritoneal cavities of ICR killed by cervical dislocation with PBS. After centrifugation at 2000 rpm for 10 mins, precipitated cells were collected and adjusted to 5×10^6 cells/m*l* with RPMI 1640 medium. Aliquots of 1.5 m*l* each were cultured in a 5% carbon dioxide incubator at 37℃ for 2 hrs so that Mφ were adsorbed selectively on the plate. The non-adherent cells were eliminated by washing three times with PBS. Measurement of Mφ and superoxide anion (SOA): To Mφ adhering to the plate, 1.5 m*l* of PBS containing 10 mM glucose, 80 μM ferricytochrome C and 0.2 mg/m*l* of opsonized zymosan were added and the plate was in-



Scheme 7. Procedure for hemolytic plaque forming cells assay.



Scheme 8. Procedure for MTT assay.



Scheme 9. Procedure for delayed type hypersensitivity assay.

cubated for 90 mins at 37℃. After incubation, the culture was centrifugated at 3000 rpm for 10 mins, the supernatant was transferred into an ice-chilled test tube and optical density was measured at 550 nm. On the other hand, 1 ml of 0.5% sodium dodecyl sulfate was added to precipitated cells, then after leaving for 5 mins, the cells were well dispersed and the amount of protein was measured by Lowry-Folin test. The amount of ferricytochrome C was obtained from the absorbance at 550 nm and free SOA per unit protein was calculated (Adachi *et al.*, 1990; Sabato *et al.*, 1988; Kyoko *et al.*, 1987; Richard *et al.*, 1975; Ito *et al.*, 1983) (Scheme 6).

Sample administration and immunization: Twelve mice were divided into two groups. For a treated group, 0.1 ml of Fr. A solution in physiological saline was injected, *i.p.*, to the mice at a dose of 20 mg/kg/day for 5 consecutive days. Physiological saline was used as control. On the seventh day after completion of injection, the mice were immunized by intraperitoneal injection of 1×10^7 cells/0.1 ml of SRBC (Cunningham *et al.*, 1973; Jerne *et al.*, 1963; Klaus, 1987). On the fifth day after immunization, the mice were sacrificed with cervical dislocation and the spleen were excised. They were homogenized with ice0cold PBS and centrifugated at $400\times g$ for 5 mins. Cytocentrifugates were collected and hemolyzed with 0.83% NH_4Cl solution at 37℃ for 1 min. After hemolysis, the cells were washed 3 times and centrifugates were resuspended in ice-cold PBS. The spleen cells were counted directly with a hemacytometer and then diluted 1×10^7 cell/ml. SRBC were washed 3 times with PBS and the pellets were diluted with equal volume of PBS. Growing cells were harvested, counted, and 10^4 cells/ml of sarcoma 180 inoculated into 96-well plates. Concurrently, Each of Fr. A (50, 100, 200 and 1000 μg/ml) was applied to triplicate culture wells. After the third day incubation, 50 μl of a 2 mg/ml solution of MTT in PBS were added to each well and the plates incubated for a further 4 hrs. Following this the plates were centrifugated for 5 mins at

the 450×g and reinverted to remove unconverted MTT leaving the formazan crystals at the bottom of the well. These crystals were dissolved in 150 μl of dimethylsulphoxide (DMSO) by agitating on a plate shaker for 30 mins. The absorbance of the wells was measured using a ELISA Reader at wave length 540 nm (Wilson *et al.*, 1990, Jabbar *et al.*, 1989, Weisenthal *et al.*, 1983) (Scheme 8).

Delayed type hypersensitivity (DTH) reaction: Twenty four mice were divided into four groups. For treated groups, 0.1 ml of Fr. A solution in PBS was injected, *i.p.*, to the mice at a dose of 20 mg/kg/day for 5 consecutive days. After completion of injection, the mice were immunized by injection of 5×10^5 SRBC into the tail vein. Five days after immunization, injection of 5×10^5 SRBC in PBS in a volume of 0.1 ml into the right-hind footpad. The same volume of PBS was injected into the left footpad as control. The increase in thickness of footpad swelling was measured 24, 48 and 72 hrs later with a dial thickness gauge. Results were expressed as the difference in thickness between right and left footpads (Cher *et al.*, 1987, Ohmori *et al.*, 1986) (Scheme 9). DTH seaction (%)=thickness of right foot−thickness of left foot/thickness of left foot.

Chemical analysis: Total polysaccharide contents were quantitatively determined by anthrone test (Carney, 1987). Total protein contents of the fraction were quantitatively determined by Lowry-Folin test. Free hexosamine and N-acetylhexosamine derived from each fraction were quantitatively determined by Elson-Morgan method (Chaplin *et al.*, 1987).

Monosaccharide analysis: Each (5 mg) of fractions and standard monosaccharides was dissolved in 2 ml of anhydrous 3% HCl-methanol. The HCl-methanol solution was obtained by adsorbing the gaseous HCl generating from the reaction of NaCl and conc. H_2SO_4 into absolute methanol. The air in the tube containing each saccharide and 3% HCl-methanol was substituted with nitrogen gas and the tube was sealed. They were methanolized at 80± 5℃ for 20 hrs. The methanolysate was filtered, evaporated and dissolved in 1 ml of pyridine. For trimethylsilylation, 0.2 ml of hexamethyldisilazane and 0.2 ml of trimethylchlorosilane were added to the solution and shaken vigorously. The sample solution was injected into Shimadzu gas chromatography RIA and analyzed under the standard conditions. Retention time of each peak was compared with that of standard monosaccharide. Fucose, galactose, glucose, mannose and xylose were used as a standard. The contents of each monosaccharide were calculated from the chromatograms by measuring the peak area (Scheme 11).

Amino acid analysis: To analyze amino acids of the protein moiety of the antitumor components, 5 mg of each fraction was dissolved in 5 ml of 6 N HCl. The air in the tube was substituted with nitrogen gas and the tubes were sealed. The samples were hydrolyzed at 100± 5℃ for 24 hrs. After filtration, the filtrate was evaporated under reduced pressure and redissolved in 2 ml of 0.02 N HCl. The sample solutions were injected into Hitachi amino acid analyzer 835 and analyzed under the standard conditions. The content of each amino acid was calculated from the chromatograms by peak area method.

Elemental analysis: Contents of elements in each fraction were quantified by Perkin-Elmer elemental analyzer. One milligram of each sample was burned at 240℃. Oxygen percentage was calculated by subtracting the values of carbon, hydrogen and nitrogen from 100. The weight percentage was reduced to mole percentage. Each sample was analyzed by KBr disc method, using Perkin-Elmer IR 20.

Results

Extraction and separation of antitumor components: The cultured mycelia of *P. atrotomentosus* were extracted with hot distilled water (90-95℃), precipitated with cold ethanol and freeze-dried. 7.2g of brown powder was obtained from 35 liters of the culture broth. It was designated Fr. CR

Purification of the water soluble fraction: Fr. CR was applied to DEAE-cellulose ion exchange column. The unadsorbed fraction eluted by the double DW was designated Fr. A (1.47g) and it

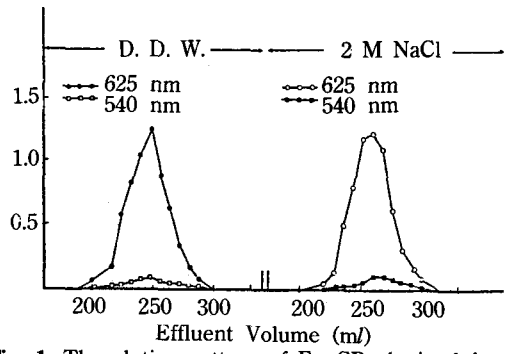


Fig. 1. The elution pattern of Fr. CR obtained from the mycelia of *P. atrotomentosus* by EDAE-cellulose ion exchange chromatography.

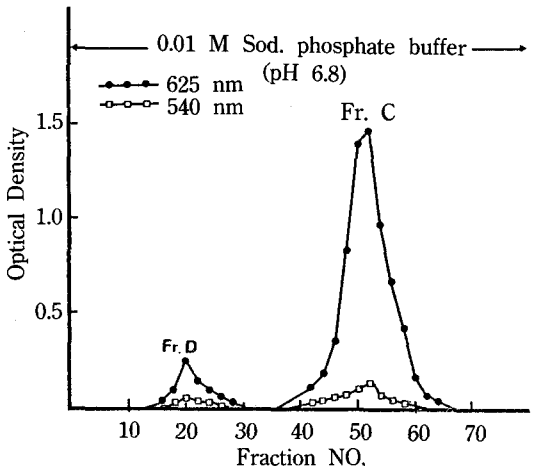


Fig. 2. The elution pattern of Fr. A obtained from the mycelia of *P. atrotomentosus* by Sepharose CL-4B gel filtration chromatography.

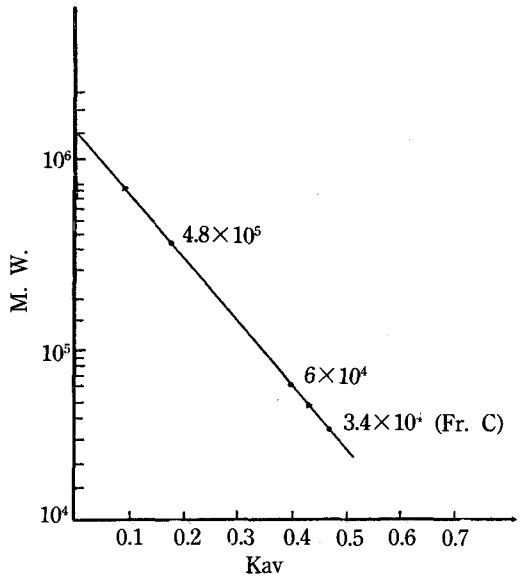


Fig. 3. determination of molecular weight of Fr. C by elution volume.

was white powder of neutral polysaccharides. The adsorbed fraction eluted by 2 M NaCl solution was designated Fr. B (1.63g). Fr. B was brownish powder of acidic polysaccharides. The elution pattern of Fr. A was shown in Fig. 1. Fr. A (700 mg) was applied to Sepharose CL-4B gel filtration column and eluted by 0.01 M sodium phosphate buffer in pH 6.8. The obtained fractions were designated Fr. C (420 mg) and Fr. D (160 mg).

Molecular weight determination of the antitumor fraction: Gel filtration method with Sepharose CL-4B was adopted to determine molecular weights. T2,000, T480 and T60 were standard dextrans. When Fr. A was applied to Sepharose CL-4B column, the elution pattern was obtained as shown in Fig. 2. The molecular weight of Fr. C was 34,000 dalton (Fig. 3).

Antitumor activity: Tumor inhibition ratios of each fraction and krestin against sarcoma 180 growth in ICR mice were shown in Table 1. Fr. A showed the highest inhibition ratio of 68.51% among the five fractions at a dose of 20 mg/kg /day.

Effects of antitumor component on immune responses: The released amount of superoxide anion from activated macrophages of the treated group was 1.1 times higher than that of the control group. The results were shown in Table 2. The counts of PFC in the treated group showed 2.3 times higher than that of the control group and the results were summarized Table 3. The number of grown cell at the 1000 μg/ml of Fr. A decreased 32% to that of the control and the results were summarized in Fig. 4. The decreased footpad swelling of tumor-bearing mice was restored to the normal level by administration of 20 mg/kg /day of Fr. A, the result was shown in Fig. 5.

Chemical analysis: The contents of polysaccharide and protein of five fractions were shown in Table 4. The content of hexosamine in the antitu-

Table 1. Antitumor activity of the protein-bound polysaccharides obtained from *P. atrotomentosus*.

Group	Dose[1]	Average tumor weight(g)[2]	IR (%)[3]	Complete regression
Control	Saline	5.97± 1.21		0/8[4]
Krestin	20	2.52± 0.97	57.79	0/8
	50	2.23± 0.72	62.65	0/8
Fr. CR	20	2.99± 0.40	49.86	0/8
	50	2.72± 1.02	54.44	0/8
Fr. A	20	1.88± 0.57*	68.51	0/8
	50	3.09± 0.73	48.24	0/8
Fr. B	20	2.78± 0.91	53.43	0/8
	50	3.45± 0.99	42.21	0/8
Control	Saline	4.13± 0.24	–	0/8
Fr. C	20	2.15± 0.31	47.94	0/8
	50	1.98± 0.43	52.06	0/8
Fr. D	20	2.34± 0.23	43.34	0/8

[1] mg/kg/day, i.p.

[2] Mean± standard deviation (*$p<0.05$).

[3] $IR=\frac{\text{Control tumor weight-Treated tumor weight}}{\text{Control tumor weight}}\times 100$

[4] Number of ICR mice used

Table 2. Effect of the antitumor component on the release of SOA by induced macrophages from normal and tumor-bearing mice.

Group		Macrophage contents (μg/ml)	SOA released (nmol/mg protein)	Ratio (t/c)
Normal	Control	89.00± 0.73[1]	30.26± 0.45	1.00
	Treated	93.72± 0.69	30.37± 0.63	
Tumor	Control	101.10± 0.93	27.63± 0.37	1.11
	Treated	105.81± 0.84*	30.92± 0.42	

[1] Mean± standard deviation (*$p<0.05$).

Table 3. Effect of the antitumor component on the hemolytic plaque forming cells (PFC) in the spleen of ICR mice immunized with SRBC.

	Treated group	Control group
Body weight(g)	25.97± 1.57[1]	23.95± 0.80
Spleen weight(mg)	174.20± 15.60	172.23± 20.5
Spleen cell count (1×10^7)	1.43± 0.32	1.38± 0.15
PFC/10^6 spleen cells	106.75± 6.43*	46.06± 2.73

[1] Mean± standard deviation (*$p<0.05$)

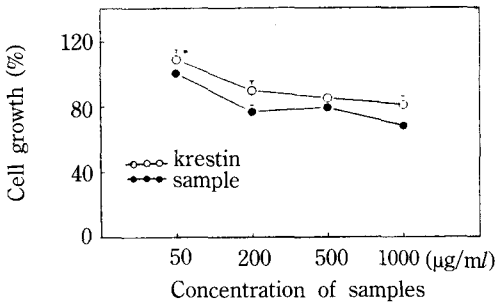


Fig. 4. Direct effect of Fr. A on sarcoma 180 cells in the tissue culture (*$p<0.01$).

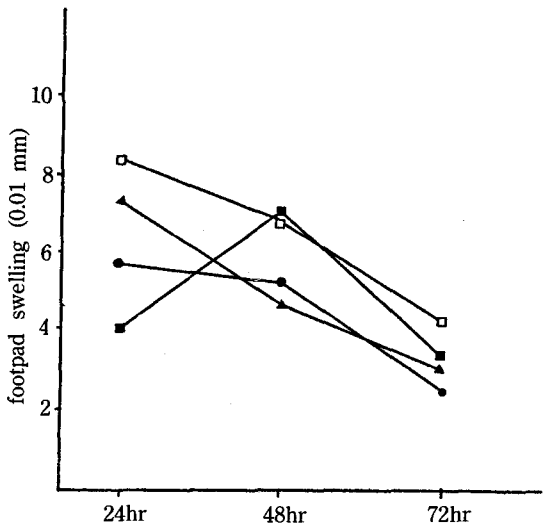


Fig. 5. Effect of Fr. A on the delayed type hypersensivity responses. ▶—◀: Normal, □—□: Normal+Fr. A (20 mg/kg/day), ●—●: Tumor, ■—■: Tumor+Fr. A (20 mg/kg/day), (*p<0.05).

mor fractions was shown in Table 5. As shown above, these fractions were protein-bound polysaccharides. As shown in Table 6, the monosaccharide composed of the polysaccharide moiety in the fractions were fucose, galactose, glucose, mannose and xylose. The composition of amino acids contained in the protein moiety was shown in Table 7. The protein moiety of Fr. A contained 14 amino acids.

Instrumental analysis: As shown in Table 8, the elements of each fraction were carbon, oxygen, hydrogen and nitrogen. Infrared spectra of the fractions showed the typical characteristics of carbohydrates.

Discussion

The polysaccharides obtained from the cultured mycelia of *Paxillus atrotomentosus* suppressed the growth of implanted sarcoma 180 when they were injected into ICR mice. Fr. A exhibited the highest inhibition ratio of 68.51% among the five fractions at a dose of 20 mg/kg/day. For mechanisms of the antitumor activity, the effect on macrophage activation was examined. Fr. A potentiated the superoxide anion release from the activated macrophages. To study the effect on B cell activation, the hemolytic plaque assay was carried out. It was found that Fr. A increased the production of hemolytic plaque forming cells in the spleen. To study direct cytotoxity, MTT assay was used. It was found that the number of cell growth at a dose of 1000 μg/ml of Fr. A. decreased 32% to that of the control.

To elucidate the effect on T cell activation, delayed type hypersensitivity reaction was performed and the decreased footpad swelling of tumor-bearing mice was restored to the normal level by injection at a dose of 20 mg/kg/day of Fr. A.

The elemental analysis of the antitumor Fr. A showed that the average ratio of C:H:O:N was 6.00:3.36:51.81:0.37. Fr. A contained 86.36% polysacharide and 1.52% protein. The detection of 1.64 % hexosamine in Fr. A suggests that it should be protein-bound polysaccharides. The main monosaccharides constituiting the polysaccharides were fucose, galactose, glucose, mannose and xy-

Table 4. Polysaccharide and protein contents of each fraction.

Fraction	Polysaccharide contents(%)	protein contents
CR	87.17± 2.31[1)]	7.24± 0.71
A	86.36± 3.21	1.52± 0.21
B	80.77± 2.07	8.75± 0.94
C	88.85± 1.92	3.34± 0.31
D	88.04± 2.93	7.42± 0.43

[1)] Mean± standarde deviation (p<0.05)

Table 5. The contents of free hexosamine and N-acetylhexosamine of each fraction.

Fraction	Hexosamine (%W/W)
CR	1.98± 0.28[1)]
A	1.64± 0.17
B	1.21± 0.20
C	1.42± 0.13
D	1.37± 0.18

[1)] Mean± standard deviation p<0.05)

Table 6. Monosaccharide contents of the polysaccharide moiety of the each fraction by G. L. C. analysis.

Fraction	Fucose	Galactose	Glucose	Mannose	Xylose
CR	1.27*	18.21	35.04	37.25	3.42
A	1.34	16.62	35.12	35.07	6.97
B	1.46	2.91	68.27	16.59	2.47
C	1.83	19.68	29.52	39.36	6.41
D	N.D**	N.D	85.10	10.67	N.D

*Expressed as the area percentage, **Not detected

Table 7. Amino acid contents of the protein moiety of the each fraction.

Amino acids	Fr. CR	Fr. A	Fr. B	Fr. C	Fr. D
L-Aspartic acid	8.03*	3.00	8.94	5.71	8.80
L-Threonine	5.17	7.38	4.82	4.13	14.95
L-Serine	10.39	17.00	11.76	11.75	N.D
L-Glutamic acid	8.36	3.58	10.05	7.15	8.54
L-Glycine	14.91	8.93	15.02	11.26	13.31
L-Alanine	11.39	11.60	8.37	8.99	5.70
L-Valine	5.10	5.23	5.15	6.56	2.58
L-Methionin	1.10	1.26	1.23	N.D	N.D
L-Isoleucine	1.30	1.35	1.23	1.79	N.D
L-Leucine	4.85	2.57	2.52	3.40	N.D
L-Tyrosine	1.94	0.75	0.72	N.D	N.D
L-Phenylalanine	5.53	3.00	2.41	5.59	N.D
L-Lysine	4.69	1.78	2.32	2.39	N.D
L-Histidine	1.87	1.54	1.23	5.16	6.32
L-Arginine	2.98	N.D**	1.03	N.D	N.D

*Area percentage, **Not detected

Table 8. Elemental compositions of the each fraction of *P. atrotomentosus*.

Fraction	C	H	O	N
CR	6.00*	9.92	5.54	0.33
A	6.00	3.36	51.81	0.37
B	6.00	3.86	49.28	0.56
C	6.00	2.02	55.91	0.46
D	6.00	9.63	48.53	0.11

lose. And the protein moiety contained 14 amino acids. The IR spectra showed the typical characteristics of polysaccharides, *i.e.*, O-H streching frequency at 3300-3400 cm^{-1}, C-H streching frequency at 2900 cm^{-1}, C-O streching frequency at 1630 cm^{-1}, C-H and C-O bending frequency at 1000-1100 cm^{-1}.

摘 要

담자균류인 좀우단버섯 *Paxillus atrotomentosus*의 항암성분을 구명하기 위하여 그 균사체를 열수 추출하여 단백 다당체를 분리하였고, 이를 DEAE-cellulose ion 교환수지와 Sepharose CL-4B gel filtration chromatography로 정제하였다. 분획들을 각각 20 또는 50 mg/kg/day의 용량으로 마우스의 복강내에 투여하였을 때, sarcoma 180 고형암에 대하여 Fr. A가 68.51%의 가장 높은 억제율을 나타내었다. 항암기전을 밝히기 위하여 마우스에 대한 면역학적

실험을 한 결과, Fr. A는 대조군에 비해 활성화된 macrophage에서 분비되는 superoxide anion 양을 1.1배, hemolytic plaque assay에서의 용혈반 형성 세포수를 2.3배 증가시켰고 감소된 DTH 반응을 정상수준으로 회복시켰다. 암세포에 대한 직접적인 영향을 조사하기 위하여 MTT assay를 한 결과 대조군에 비해 세포의 viability가 약 32% 감소됨을 나타내었다. 화학분석에 의해, 이 성분은 fucose, galactose, glucose, mannose 및 xylose로 구성된 86.36%의 다당체와 14종의 아미노산으로 구성된 1.52%의 단백질 및 1.64% hexosamine으로 구성된 protein-bound polysaccharide이었다. 이 성분을 paxillan으로 명명한다.

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