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# Studies on Constituents of the Higher Fungi of Korea (XLVI)

## Antitumor Components Extracted from the Cultured Mycelia of *Lyophyllum decastes*

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**Abstract**—To investigate antitumor component of *Lyophyllum decastes*, the aqueous extract of its shake-cultured mycelia was subjected to antitumor test against sarcoma 180 cells implanted in ICR mice. The extract showed an inhibition ratio of 65.4% and was found to consist of a polysaccharide moiety and a protein moiety. After purification with DEAE-Sephadex A-50 ion exchange chromatography, Fraction D showed the highest inhibition ratio of 75.7%. The antitumor constituent was examined for immunoaccelerating activity and was found to increase macrophage accumulation in the peritoneal cavity and plaque forming cells of the spleen cells. It was named lyophyllan after the genus name.

**Keywords**—*Lyophyllum decastes* • Lyophyllaceae • Basidiomycetes • Antitumor component • Lyophyllan • Mycelial culture • Macrophage accumulation • Plaque-forming cells • Immunopotentiation

The polysaccharides with antitumor activity have been demonstrated in a variety of natural sources: higher plants[1-5], fungi,[6] lichens,[7] yeasts,[8] and bacteria[9]. Of the polysaccharides from higher fungi were reported many other activities such as antimicrobial, toxic, hallucinogenic and hypocholesterolemic effects.[10-17] Since Lucas reported first on the antitumor polysaccharides of basidiomycetes in 1957,[18] studies on the antitumor components of higher fungi have been extensively carried out. As a result lentinan from *Lentinus edodes*,PS-K from *Coriolus versicolor*, and schyzophyllan from *Sclerotinia libertina* and *Schyzophyllum commune* were isolated.[19-20]

The antitumor mechanism of these polysaccharides has not been completely elucidated, but recently, several scientists have suggested that the antitumor activities were due to the potentiation of the cell-mediated immunity.[21]

In recent years, our laboratory has been reporting on the antitumor components from the carpophores and cultured mycelia of Korean basidiomycetes to find antitumor components with lower toxicity.[22-24]

In the present study, the protein-bound polysaccharide fraction extracted from the cultured mycelia of *Lyophyllum decastes*, which is new to Korea, were subjected to antitumor test against sarcoma-180 cells in ICR mice. The antitumor component was purified with DEAE-Sephadex A-50 ion exchange chromatography and

analysis of its chemical composition was attempted. In addition, to study mechanisms of its antitumor activity, its effects on immune responses in the mice were examined.

## Materials and Methods

### 1. Materials

The mycelia of *Lyophyllum decastes*(the family Lyophyllaceae) used in this work were kindly provided by Agricultural Science Institute at Suwon, Gyeong-Gi Province.

### 2. Medium Composition

1) PDA slant: Bacto Potato Dextrose Agar (Difco Lab., USA) 39g/*l*

2) Culture Medium: Glucose 50g, peptone 10g, yeast ext. 10g, $KH_2PO_4$ 0.87g, $MnSO_4 \cdot 7H_2O$ 0.5g, $CaCl_2$ 0.3g, $FeSO_4 \cdot 7H_2O$ 10mg, $MnCl_2 \cdot 4H_2O$ 7mg, $ZnSO_4 \cdot 7H_2O$ 4mg, and $CuSO_4 \cdot 5H_2O$ 1mg per one liter. After filtration, the filtrate was adjusted to pH 5.5 and autoclaved at 121°C, 2 atm for 30 min.

### 3. Culture Methods

1) First Culture

The mycelia of *L. decastes* 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 a small volume of the culture medium for 10 seconds in a microblender. It was inoculated into 100 ml of the culture medium in a 500 ml flask and incubated for 15 days in an Orbital Shaking Incubator at 27±1°C, 180 rpm.

2) Second Culture

The mycelial pellets were aseptically homogenized for 10 seconds and inoculated into a 500-ml flask containing 100 ml of the culture medium. Incubation was carried out under the same condition of the first culture for 15 days.

3) Main Culture

Again, the mycelial pellets from the second

Mycelia grown on compost sugar agar slant
|
Mycelia grown on PDA slant
| Homogenization with medium
| Incubation on Gallenkamp orbital incubator (27°C, 180rpm, 15days)
Mycelial pellets
| Homogenization
| Inoculation into 100ml of fresh medium
| Incubation under the same condition
Seed culture
| Homogenization
| Inoculation into fresh medium (Inoculum size: 20v/v%)
| Incubation
Culture broth

**Scheme I.** Culture process of *Lyophyllum decastes* mycelia

culture were aseptically homogenized for 10 seconds and transferred into 500 ml of the culture medium in a two-liter flask (inoculum size: 20v/v%) and cultured for 10 days in the same condition as previously described (Scheme I).

### 4. Extraction and Separation of Fungal Metabolites

After the main culture, 18 liters of the culture broth were filtered and washed twice with distilled water. The obtained mycelia were homogenized and extracted with distilled water on a water bath at 90~100°C for five hours. After filtration, the filtrate was concentrated under vacuum and three volumes of 95% ethanol were added to the concentrate. In order to complete the precipitation, the mixture was allowed to stand at 0~4°C overnight. The precipitate was separated by centrifugation at 10,000×g for 30 min, dissolved in distilled water, and freeze-dried. A dark brownish powder was obtained with a yield of 45.9g and designated as Fraction A.

Of Fraction A, 44g was re-dissolved in distilled water and dialyzed against distilled water at 0~4°C for three days using Visking tube (36/32). Insoluble materials were removed by filtration and the filtrate was concentrated and

lyophilized to obtain a brownish powder with a yield of 3.1g. It was designated as Fraction B.

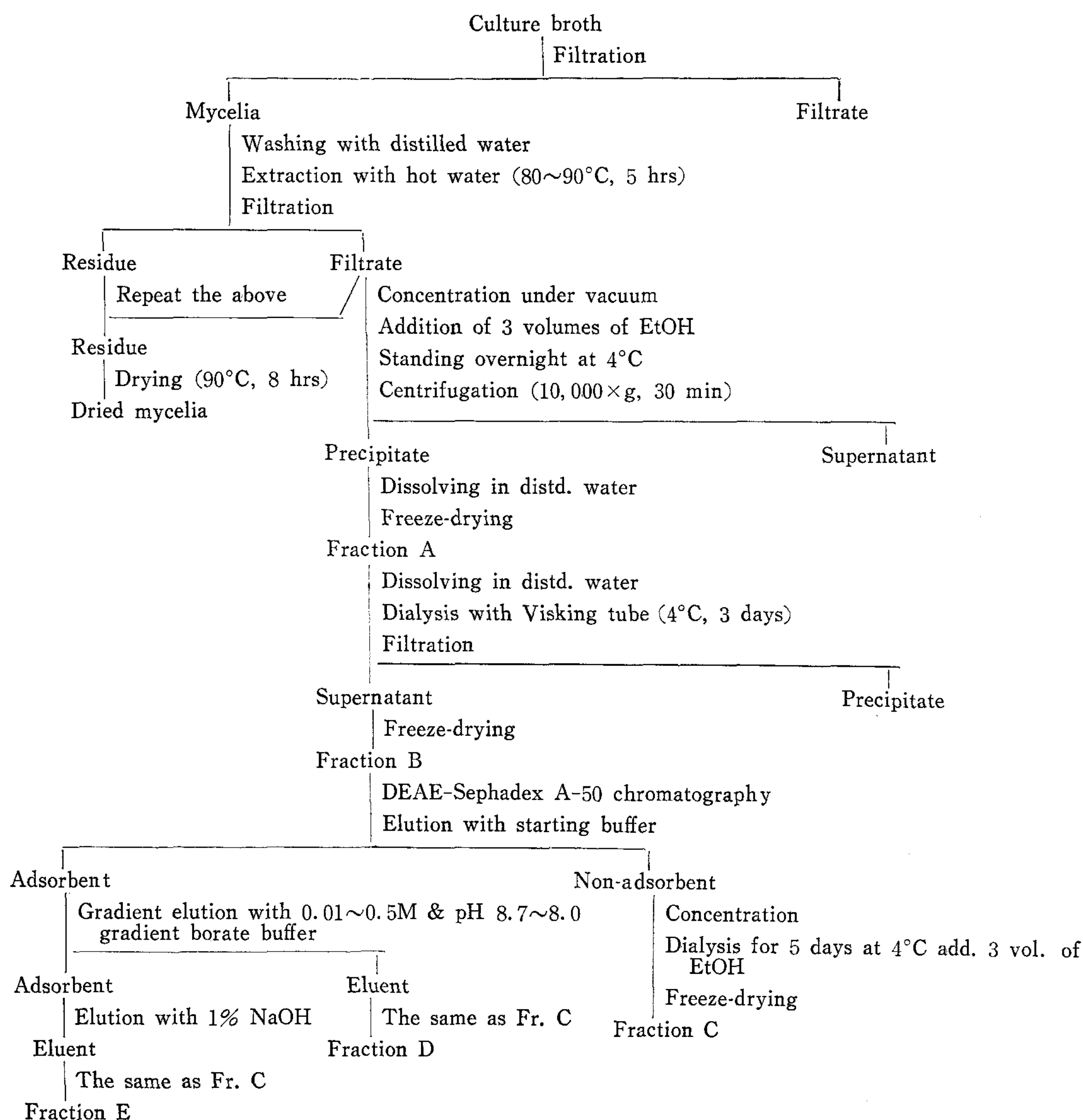
## 5. Purification of Fungal Metabolites

1) Preparation of DEAE-Sephadex A-50 Ion Exchanger

Three grams of DEAE-Sephadex A-50 (Pharmacia Fine Chemicals AB, Uppsula, Sweden, chloride form) were exchanged to borate form ($BO_2$-) with 0.1M borax. The fine particles were removed and ion exchanger was swollen with fresh 0.01M borate buffer (pH 8.7).

2) Purification of Fungal Metabolites

Of Fraction B, 2.7g was dissolved in 0.01M borate buffer (pH 8.7) and insoluble materials were removed by filtration. The filtrate was applied to pre-swollen DEAE-Sephadex A-50 column and eluted with 0.01M borate buffer of pH 8.7. The Anthrone-positive fractions detected by U.V. absorbance at 625nm were concen-




**Scheme II.** Extraction, separation and purification of the metabolites of *Lyophyllum decastes*

trated and dialyzed against distilled water for five days with Visking tube.[25~26] After dialysis, three volumes of 95% ethanol were added to the dialyzates and the mixture was allowed to stand at 0~4°C overnight. The obtained precipitate was re-dissolved in distilled water and freeze-dried. Thus non-adsorbent portion was obtained as a pale yellowish powder with a yield of 90 mg(Fraction C).

The adsorbent portion was eluted with 0.01~0.5M linear gradient and pH 8.7~8.0 linear gradient borate buffer. The Anthrone-positive fractions were collected by the previous method and yielded 400mg of a yellow brownish powder. It was designated as Fraction D.

The non-eluants with gradient borate buffer were eluted with one percent NaOH solution and the eluants were treated in the same method as previously described. Fraction E was obtained as a brownish powder with a yield of 1050 mg(Scheme II).

### 6. Antitumor Test

1) Animals

Female ICR mice weighing 14~18g were supplied from the Experimental Animal Farm of Seoul National University.

2) Transplantation of Tumor Cells

Sarcoma 180 cells maintained in the peritoneum of ICR mice were used for antitumor test of samples. An ICR mouse with ascitic forms of sarcoma 180 cells was killed on the seventh day after transplantation of tumor cells and the ascitic fluid was collected. After the peritoneal cells were washed several times with ice-cold saline, the peritoneal cell suspension was adjusted to $1\times10^7$ tumor cells with a hemacytometer and 0.1 ml of this suspension was inoculated into the left groin of ICR mice subcutaneously.

3) Administration of the Metabolites

Intraperitoneal administration of the samples was initiated 24 hours after tumor transplantation and continued for consecutive 10 days

An ICR mouse with sarcoma 180 cells(ascitic forms)
| Sacrificing with $CHCl_3$ vapor
| Collecting the sarcoma 180 cells with ice-cold saline
| Centrifugation (400×g, 5 min)

Cytocentrifugate
| Washing with ice-cold saline (×3)
| Adjustment to $1\times10^7$ cells/ml with hemacytometer
| Inoculation with 0.1 ml of sarcoma 180 cells into the left groin

ICR mice implanted with sarcoma 180 cells
| Sample injection (i.p., once daily for consecutive 10 days)
| Sacrificing 28 days after tumor transplantation
| Excising the tumors

Weighing the solid tumors

**Scheme III.** Antitumor test

once a day. To prepare a sample solution at a dose of 20mg/kg, 40 mg each of Fractions A, B, C, D, and E were dissolved in 10 ml of physiological saline. Physiological saline was used for control and injection volume was 0.1 ml. All of the solutions were autoclaved at 121°C and 2 atm for 20 min and stored in a refrigerator(Scheme III).

4) Calculation of Inhibition Ratio

On the 30th day after tumor implantation the mice were sacrificed and the solid tumors were dissected and weighed. The tumor inhibition ratio was calculated by the following formulus:

$$\text{Tumor inhibition ratio} = \frac{Tw - Cw}{Cw} \times 100(\%)$$

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

5) Life Span

Sarcoma 180 cells ($1\times10^6$ cells/0.1ml/mouse) were inoculated into the peritoneal cavity of ICR mice and samples were injected intraperitoneally by starting 24 hours after tumor inoculation. Sample injection was carried out once a day for consecutive 10 days at a dose of 20mg/kg and only physiological saline was injected to the control group.

The numbers of survival mice were observed

for 25 days.

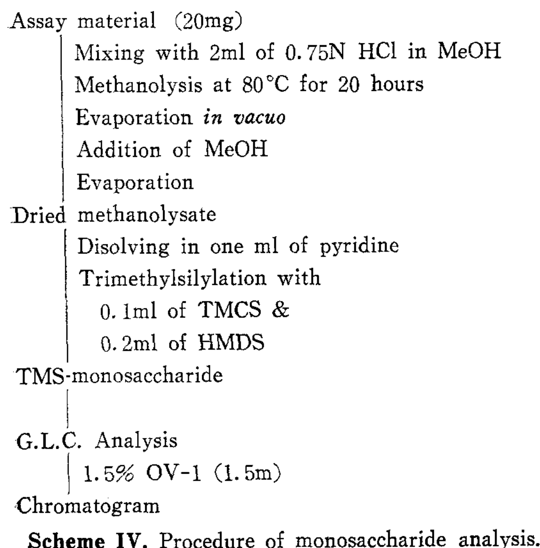
## 7. Chemical Analysis

1) Total Polysaccharide Content

Determination of the polysaccharide content was carried out according to Herbert *et al.*[27] After Anthrone test, the polysaccharide content was calculated from U.V. absorbance at 625nm using glucose as a standard sugar.

2) Monosaccharide Analysis

Monosaccharide analysis was performed according to Mitruka.[28] Twenty milligrams of each sample and 10mg of each standard monosaccharide were dissolved in two milliliters of three percent HCl-methanol and methanolyzed at 80 ±5°C for 20 hours in a cap tube filled with nitrogen gas. After filtration, the filtrate was evaporated in vacuum and re-dissolved in absolute methanol. Again, the solution was evaporated in vacuum and dissolved in one milliliter of pyridine. Trimethylsilylation was carried out with 0.2ml of hexamethyldisilazane and 0.1ml of trimethylchlorosilane. After vigorous shaking for 30 seconds, gas chromatography was performed under the usual conditions. To identify the monosaccharide, retention times of each peak were compared with those of standard monosaccharides. The content of each monosaccharide was calculated from the chromatograms by measuring the peak area (Scheme IV).



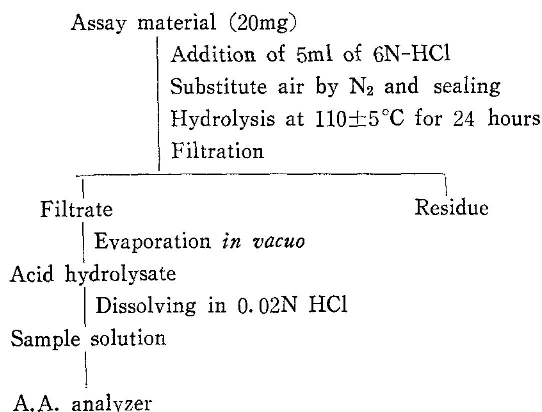

**Scheme IV.** Procedure of monosaccharide analysis.

3) Total Protein Content

The protein content was determined by U.V. absorbance at 540 nm according to Lowry-Folin method[29] using Bovine Serum Albumine (=BSA, Sigma, USA) as a standard protein.

4) Amino Acid Analysis

To analyze amino acids of the protein moiety, twenty milligrams of each sample were dissolved in five milliliters of 6N-HCl and hydrolyzed at 110±5°C for 24 hours in a cap tube filled with nitrogen gas. After filtration, the filtrate was evaporated in vacuum and re-dissolved in two milliliters of 0.02M-HCl. The sample solutions were injected into Hitachi KLA-5 Amino Acid Analyzer with an injecting volume of 0.5ml under the usual conditions. The contents of each amino acid were calculated from the chromatograms by peak height method (SchemeV)




**Scheme V.** Sample preparation for amino acid analysis

5) Infrared Spectrum

One milligram of each sample was analyzed by KBr disc method.

## 8. Effects of Fraction B on Immune Responses

1) Effects on Peritoneal Cell Population

a) Animals

Female ICR mice weighing 16~20g were

provided by the Experimental Animal Farm of Seoul National University.

b) Reagents

i) PBS=Phosphate Buffered (0.01M) Saline: pH7.2~7.4

ii) BSS (=Balanced Salt Solution)

Stock Solution I: Dextrose 10g, $KH_2PO_4$ 0.6 g, $Na_2HPO_4 \cdot 7H_2O$ 3.58g and 0.5% Phenol red solution 20ml per one liter

Stock Solution II: $CaCl_2$·anhyd. 1.86g, KCl 4.0g, NaCl 80g, $MgCl_2$·anhyd. 2.0g per one liter

BSS Working Solution: 800ml of demineralized water was added to 100ml of stock solution I and 100ml of stock solution II and the mixed solution was adjusted to pH 7.3.

iii) NSE Staining Solution

Stock Solution III: one gram of $\alpha$-naphthyl acetate was dissolved in 50ml of demineralized water and 50ml of acetone.

NSE Working Solution: two milliliters of stock solution III and 15ml of 0.1M phosphate buffer (pH7.3) were added into 15ml of demineralized water and then 20mg of fast red TR salt was added to the solution. Before use, the mixture was filtered and used immediately.

iv) Giemsa Staining Solution

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

c) Methods

i) Sample Administration[30]

ICR mice were injected intraperitoneally with one milligram of Fraction B per one milliliter of phosphate buffered saline and all of the injecting solutions were autoclaved at 121°C, 2 atm, for 20 min.

ii) Peritoneal Exudate Cell Count

After 5 hours, one day, two days, three days and five days of sample administration, the ICR mice were sacrificed and the ascitic fluid was collected with ice-cold BSS. The numbers of PEC were counted directly by a hemacytometer.

iii) Lymphocyte and PMN Count

After PEC count, the peritoneal fluid was centrifugated at 4°C, 400×g for 10min and the cytocentrifugates were suspend into 0.2ml of ice-cold BSS. The PEC suspension was smeared on the glass slides, air-dried, fixed in MeOH for five min. and stained with Giemsa working solution for 30min. The slides in 90% ethanol to remove the excess dye were rinsed and examined using cedar oil as a mounting solution. The cells which showed even blue color and round shape were counted as lymphocytes and the cells which contained a multi-lobbed and/or doughnut-type nucleus were counted as PMNs (Polymorphonuclear leucocytes).

iv) Macrophage Count

The specimen on the glass slides were air dried, fixed in cold acetone for five min and incubated with NSE working solution at 37°C for 30min. The slides were backwashed with tap water and counter-stained with one percent Malachite green staining solution for 15 seconds and backwashed with tap water again. The slides were examined using the glycerol jelly as a mounting solution. The cells which contained reddish granules were identified as nonspecific esterase-positive macrophage (Scheme VI).

2) Effects on Hemolytic Plaque-Forming Cells

a) Animals

Male ICR mice weighing 16~20g were supplied from the Experimental Animal Farm of Seoul National University.

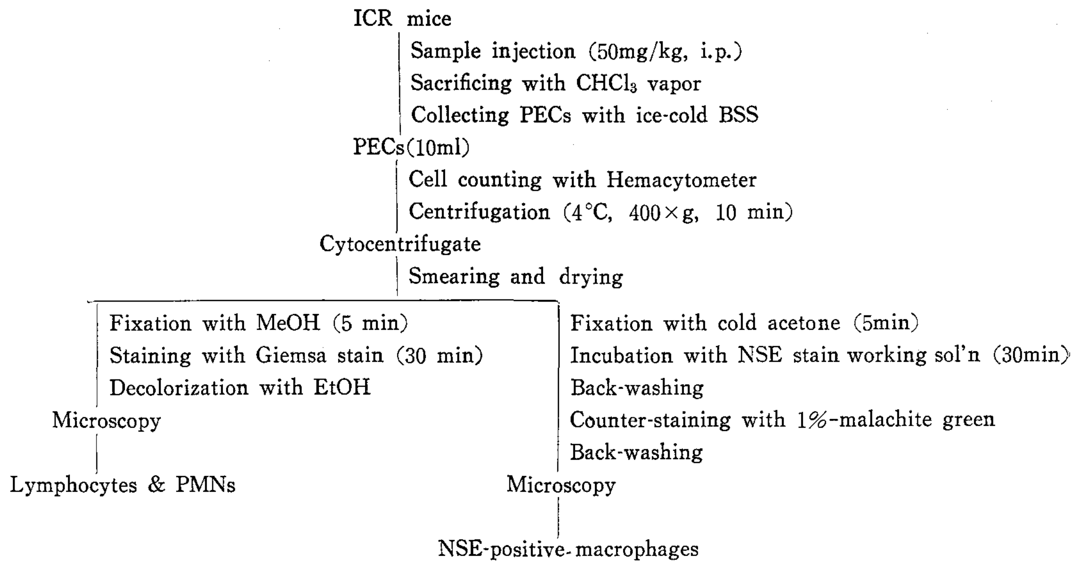
b) Materials and Reagent

i) BSS

ii) 0.83% $NH_4Cl$ solution

iii) Microchamber

After glass slides were cleaned with 95% ethanol, two strips of double-sided Scotch tape was placed across both ends of the slides. The




**Scheme VI.** Method for examining effects of antitumor component on peritoneal cell population.

slides was placed on the top of the taped slides in such way that a microchamber was formed between the two slides.

iv) Sheep Red Blood Cells (=SRBC)

SRBC was kindly supplied from Korea National Institute of Health.

v) Complement

Guinea pig serum as a source of complement was also cordially provided by Korea National Institute of Health.

c) Methods

i) Sample Administration and Immunization[31]

Ten mice were divided into two groups. For a treated group, 50 mg of Fraction B was dissolved in five milliliters of physiological saline and 0.1ml of this solution was injected to ICR mice once a day for consecutive five days. Physiological saline was used for control group. On the seventh day after the last sample injection, immunization was performed by injecting $1\times10^7$ cells of SRBC.

ii) Preparation of Spleen Cell Suspension

After five days of immunization, mice were sacrificed and the spleens were dissected. 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 suspension was centrifugated under the same condition and the cytocentrifugates were resuspended in ice-cold BSS. The spleen cells were counted directly by hemocytometer.

iii) Preparation of Complement-SRBC

SRBC was centrifugated and resuspended in physiological saline to adjust the concentration into 20v/v%. And then, 500 $\mu$l of 20v/v% SRBC was mixed with 1,000 $\mu$l of guinea pig serum in the microwell and the fixation on the ice bath was carried out.

iv) Preparation of Incubation Mixture

Incubation mixture consisted of 150 $\mu$l of complement-SRBC solution and 650 $\mu$l of spleen cell suspension, and 100 $\mu$l 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 VII).

$$\text{PFC}/10^6 \text{ spleen cells} = \frac{N}{C \cdot Vm \cdot a} \times 10^6$$

ICR mice
- Sample injection (50 mg/kg, 5 days, i.p.)
- After 7 days, immunization with by injecting $1\times10^7$ SRBC
- After 5 days, excising of spleen

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

Spleen cells without erythrocytes
- Washing with BSS (×4)
- Dilution with BSS

Spleen cell suspension
- Addition of 150 μl of prepared suspension of indicator SRBC (100 μl) containing complement from guinea pig serum (50 μl)
- Mixing
- Filling the microchamber
- Sealing
- Incubation at 37°C for one hour

Counting hemolytic PFCs

**Scheme VII.** Procedure of hemolytic plaque assay

PFC/total spleen cells

$=\text{PFC}/10^6 \text{ spleen cells} \times C \times Vs$

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

$N$: number of plaque observed in one microchamber

$C$: count of spleen cells in one ml of spleen cell suspension

$Vm$: volume of incubation mixture filled into one microchamber (ml)

$Vs$: total volume of spleen cell suspension (ml)

## Results

### 1. Antitumor Activity

Antitumor activity of the metabolites against sarcoma 180 cells was shown in Table I. Of the five fractions tested, Fraction D showed the most effective tumor inhibition ratio of 75.7%. Figure 1 showed the life span of the treated group and it was longer than that of the control group.

### 2. Chemical Analysis

The contents of total polysaccharide and total protein of the five samples were shown in Table II.

As shown in Table III and Figure 2, the major monosaccharide subunits of the five fractions were glucose and mannose.

**Table I.** Antitumor activities of various fractions of the metabolites of *Lyophyllum decastes*

| Group | Dose mg/kg/day, i.p. | Average Tumor Wt.(g) | Inhibition Ratio (%) | Complete Regresaion |
|---|---|---|---|---|
| Control | Saline | 3.12±0.55* | — | 0/9** |
| Fr. B | 30 | 1.08±0.42*** | 65.4 | 1/9 |
| Control | Saline | 2.55±0.54 | — | 0/9 |
| Fr. A | 20 | 1.42±0.38**** | 44.3 | 0/9 |
| Fr. B | 20 | 1.04±0.49 | 59.2 | 1/9 |
| Fr. D | 20 | 0.62±0.13 | 75.7 | 2/9 |
| Fr. E | 20 | 1.54±0.25 | 39.6 | 0/9 |
| Control | saline | 3.37±0.50 | — | 0/9 |
| Fr. C | 20 | 2.80±0.63 | 16.9 | 0/9 |

*Mean±standard error
**Numbers of mice used
***P<0.01
****P<0.001

**Table II.** Polysaccharide and protein contents of the antitumor components of *Lyophyllum decastes*

| | Fr. A | Fr. B | Fr. C | Fr. D | Fr. E |
|---|---|---|---|---|---|
| Polysaccharide | 33(%) | 44 | 41 | 71 | 54 |
| Protein | 47(%) | 37 | 17 | 5 | 5 |

**Table III.** Monosaccharide contents of the polysaccharide moiety of the antitumor components of *Lyophyllum decastes*

| Fraction | Glucose | Fucose | Xylose | Mannose | Galactose |
|---|---|---|---|---|---|
| A | 77.0(%) | 6.0 | 3.0 | 14.0 | + |
| B | 56.0 | 11.0 | 4.0 | 19.0 | 10.0 |
| C | 16.0 | 8.0 | 18.0 | 48.0 | 11.0 |
| D | 67.0 | 3.0 | + | 30.0 | + |
| E | 61.0 | + | + | 29.0 | 10.0 |

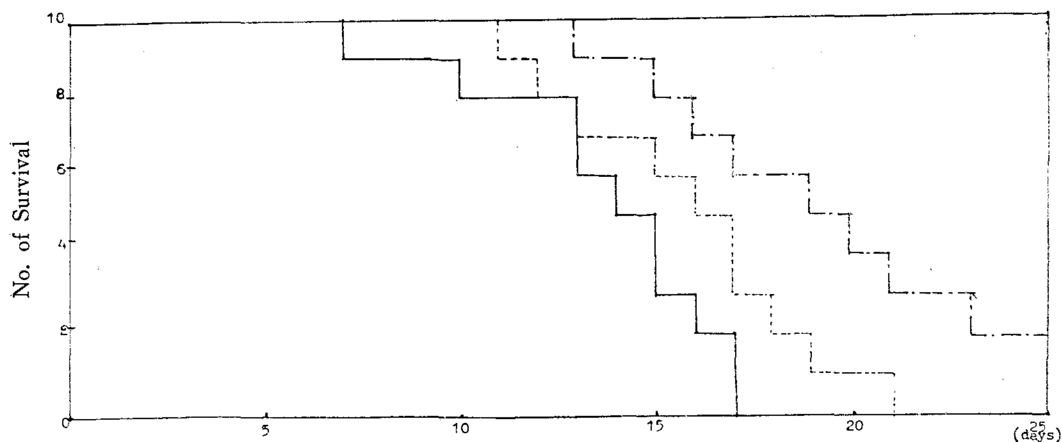



**Fig. 1.** Effects of the antitumor fractions of *L. decastes* on the life span after intraperitoneal implantation of sarcoma 180 cells in mice ($1 \times 10^6$ cells/mouse) ——Control, ······ Fraction B (20mg/kg), -·-·- Fraction D (20mg/kg).

**Fig. 2.** G.L.C. patterns of the five fractions of the antitumor components of *Lyophyllum decastes*

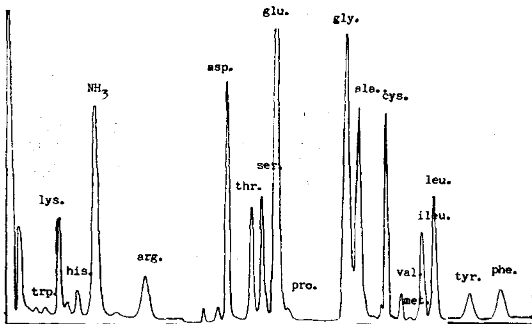



**Fig. 3-a.** Chromatogram of Fraction A

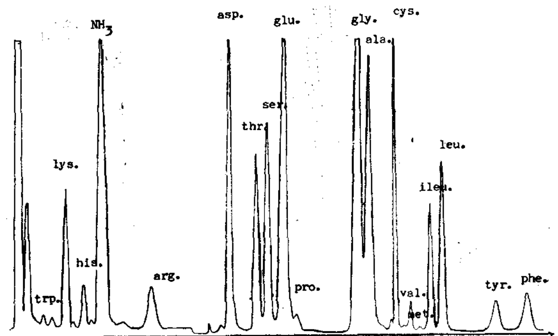



**Fig. 3-b.** Chromatogram of Fraction B

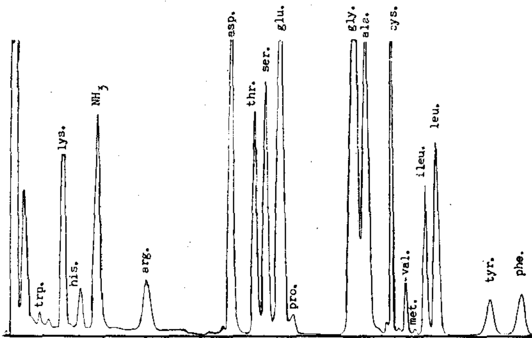



**Fig. 3-c.** Chromatogram of Fraction C

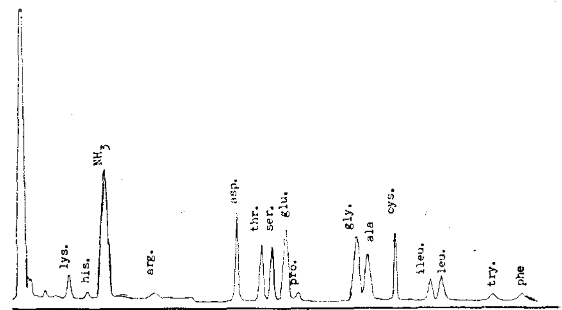



**Fig. 3-d.** Chromatogram of Fraction D

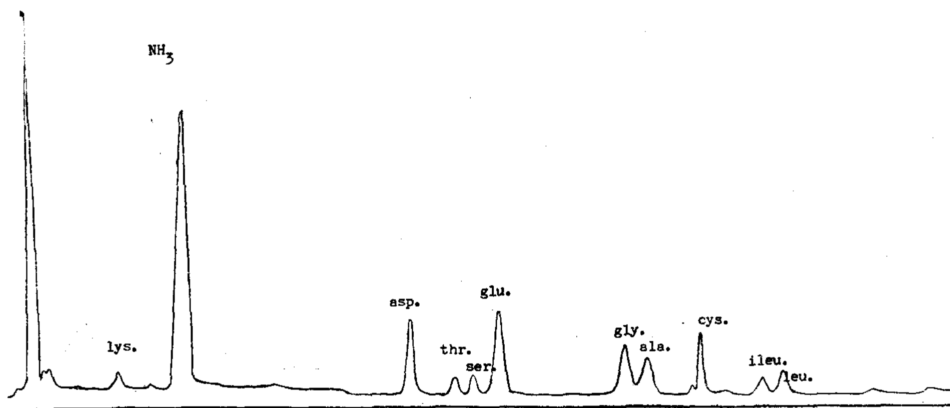



**Fig. 3-e.** Chromatogram of Fraction E

Table IV and Figure 3 showed the composition of amino acids contained in the protein moiety.

Infrared spectra of the samples were depicted

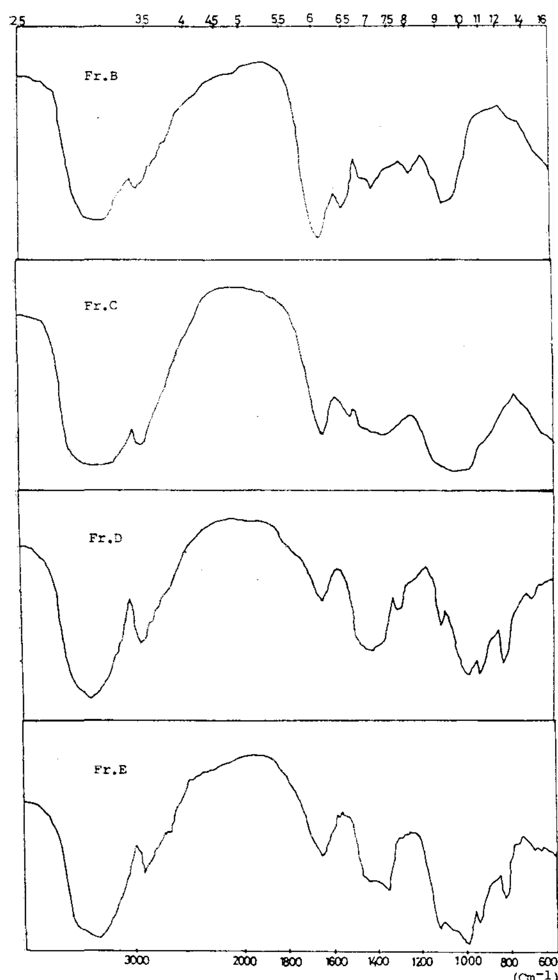



**Fig. 4.** IR spectra of the fractions of antitumor component of *Lyophyllum decastes*

in Figure 4.

### 3. Effects on Immune Responses

1) Effects on PEC population

The counts of PECs and NSE-positive macrophages showed the maximum on the 24th hour after sample administration and decreased slowly. PMNs also showed the highest count after 24 hours of sample injection. The results of accumulation of cells in the peritoneum were depicted in Figure 5.

2) Effects on Plaque Forming Cells

The PFC counts of the treated group showed about twenty three times greater than those of

**Table IV.** Amino acid contents of the protein moiety of the antitumor component of *Lyophyllum decastes*

| Amino Acid \ Fraction | A | B | C | D | E |
|---|---|---|---|---|---|
| Tryptophan | 1.0 | 0.8 | 0.3 | — | — |
| Lysine | 4.9 | 5.0 | 7.3 | 4.3 | 6.9 |
| Histidine | 2.0 | 2.0 | 1.6 | 3.1 | — |
| Arginine | 4.1 | 3.0 | 3.0 | 3.1 | — |
| Aspartic acid | 8.6 | 9.5 | 9.6 | 11.0 | 13.9 |
| Threonine | 4.7 | 7.0 | 6.0 | 7.3 | 4.0 |
| Serine | 5.3 | 7.2 | 7.2 | 7.3 | 5.1 |
| Glutamic Acid | 17.4 | 14.1 | 11.2 | 20.9 | 19.8 |
| Proline | 1.0 | 0.8 | 0.7 | 3.1 | — |
| Glycine | 16.8 | 15.9 | 16.7 | 13.5 | 13.9 |
| Alanine | 11.5 | 12.4 | 12.9 | 9.8 | 11.9 |
| Cysteine | 7.0 | 6.8 | 6.3 | — | 13.8 |
| Valine | 1.4 | 1.1 | 1.7 | — | — |
| Methionine | 0.1 | 0.1 | 0.1 | — | — |
| Isoleucine | 4.1 | 4.7 | 4.6 | 4.9 | 5.0 |
| Leucine | 5.5 | 5.7 | 6.8 | 5.5 | 5.7 |
| Tyrosine | 2.3 | 1.8 | 1.7 | 2.5 | — |
| Phenylalanine | 2.3 | 2.1 | 2.3 | 3.7 | — |

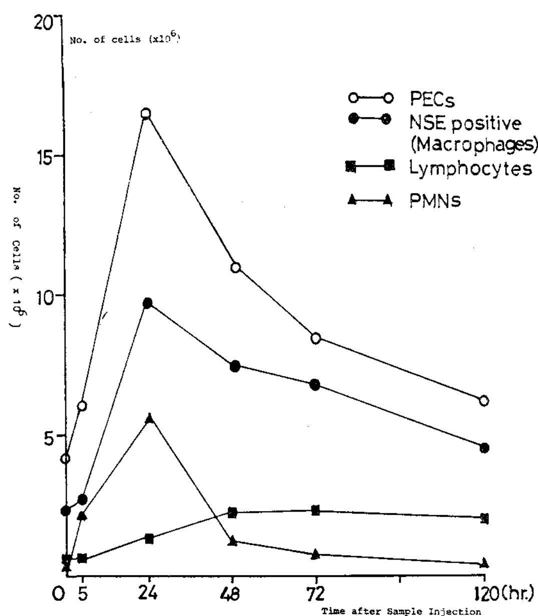



**Fig. 5.** Kinetics and composition of peritoneal exudate cells after injection of the protein-bound polysaccharide of *Lyophyllum decastes.*

**Table V.** Effects of the antitumor component on hemolytic plaque forming cells (=PFCs) in the spleen of ICR mice immunized with sheep red blood cells ($1\times10^7$ cells)

| | Control | Treated |
|---|---|---|
| Body Weight (g) | 26.0±1.0* | 27.9±0.1 |
| Spleen Weight (mg) | 150.1±28.3 | 180.0±16.2 |
| Spleen Cell Count ($1\times10^7$) | 10.8±0.6 | 27.6±1.2 |
| PFC/$10^6$ Spleen Cells | 3.9±0.9 | 88.9±6.9 |
| PFC/Spleen ($\times10^2$) | 4.2±1.0 | 245.4±2.5 |

* Mean ± standard deviation

the control group and the result was summarized in Table V.

## Discussion

As indicated in the results previously described, the protein-bound polysaccharides obtained from the cultured mycelia of *Lyophyllum decastes* showed relatively good antineoplastic activities. Especially Fraction D showed the tumor inhibition ratio of 75.7%.

This fraction contained 71% polysaccharide and 5% protein. The major monosaccharide of its polysaccharide fraction was glucose (67 %), and mannose and fucose were also contained. Its protein moiety contained 14 amino acids including glutamic acid (20.9%), glycine (13.5%) and aspartic acid (11.2%). In the IR spectra of Fractions B, C, D and E, O-H stretching frequency at 3300~3400cm$^{-1}$ and C-H stretching frequency at 2900cm$^{-1}$ and C-H, C-O bending frequency in 1000~1100cm$^{-1}$ and C-O streching frequency at 1630cm$^{-1}$ were observed and these characteristics were common to all of the polysaccharides.

In order to study mechanism of the antitumor activity, effects on immune responses were examined. Peritoneal exudate cells and NSE-positive macrophages reached the maximum on the 24th hour after sample administration. Also, polymorphonuclear lymphocytes showed the greatest count on the 24th hour. Although the mechanism of antineoplastic action was not fully understood, it was considered that the effect exerted through a cell-mediated immunity on the basis of the effects on macrophage accumulation. In additon, the increase of plaque forming cells indicated that this sample also stimulated the humoral immunity and that it inhibited the tumor growth in the early stage of neoplasm. Thus the action of this fraction was regarded as an immuno-accerlerating activity, not as a direct cytotoxic activity against sarcoma 180 cells.

More detailed studies on the immunomodulating activity of the T- and B-lymphocytes will be required to elucidate the action mechanism of the antitumor component of *Lyophyllum decastes.*

## Conclusions

The protein-bound polysaccharide from the cultured mycelia of *Lyophyllum decastes* was found to have antitumor activity against sarcoma 180 cells in ICR mice. Of its five fractions, Fraction D showed the highest tumor inhibition ratio of 75.7% at the dose of 20mg/kg/day. Fraction D contained 71% polysaccharide and 5% protein. The polysaccharide of Fraction D consisted of glucose, mannose and fucose. And its protein moiety contained 14 amino acids including glutamic acid, glycine and aspartic acid. This antitumor component was named lyophyllan after the genus name.

Fraction B stimulated the production of peritoneal exudate cells and the accumulation of NSE-positive macrophages. In addition, it also increased the number of plaque-forming cells of the spleen.

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