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<https://doi.org/10.5109/23774>

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出版情報：九州大学大学院農学研究院紀要. 28 (1), pp.1-12, 1983-09. Kyushu University  
バージョン：  
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## **Productions of Intracellular Melanoidin-decolorizing Enzyme and Extracellular Antitumor Polysaccharide by *Coriolus* sp. No.20**

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(Received October 28, 1982)

*Coriolus* sp. No. 20 produced an intracellular enzyme that could decolorize the darkness of melanoidin and 4.04 g/liter of extracellular polysaccharide in the medium containing 10 % glucose and 1 % ammonium sulfate as carbon and nitrogen sources. The crude preparation of enzyme decolorized 1 % melanoidin to the extent of 72 %, at pH 1.5, 30°C as optimal conditions. Maximum production of extracellular polysaccharide was obtained between 15 and 20 days incubation. This polysaccharide consisted of glucose, galactose, mannose, xylose and fucose (50.3 : 18.7 : 15.4 : 8.0 : 7.6). When 2mg of this polysaccharide was injected 10 times, 6 out of 18 mice regressed the growth of sarcoma-180 and survived over 100 days, whereas all control mice died in 25 days after sarcoma-180 implantation. When oxidized-reduced derivative of this polysaccharide with periodic acid was injected, complete regression was observed in 11 out of 20 mice. The degradation of the polysaccharide by macerozyme and cellulase increased the antitumor activity as well and the complete regression was recognized in 11 and 14 out of 20 mice, respectively.

### **INTRODUCTION**

*Coriolus* sp. No.20 was selected by the screening of the strains which could decolorize the melanoidin in the alcohol distillery waste liquid. Under the optimal condition, *Coriolus* sp. No. 20 decolorized about 80 % of darkness of melanoidin and this decolorization was catalized by the intracellular enzyme of the strain (Watanabe *et al.*, 1982). An extracellular polysaccharide was found to be produced in the culture of this strain, and the polysaccharide showed some antitumor activity. This paper describes the optimal cultural conditions for production of extracellular polysaccharide by strain No. 20, chemical composition and antitumor activity of this polysaccharide, and the preparation of melanoidin decolorizing enzyme from the mycelia for the purpose of the treatment of distillery waste liquid.

### **MATERIALS AND METHODS**

#### **Organism**

*Coriolus* sp. No. 20 was used and it was maintained on slants of rice-bran

**Table 1.** Composition of chemically defined medium for the production of extracellular polysaccharide by *Coriolus* sp. No. 20.

Ingredients	Amount /liter
Carbon source :	
Glucose	<b>100 g</b>
Nitrogen source :	
Ammonium sulfate	10 g
Vitamins :	
Thiamine hydrochloride	<b>10 mg</b>
Riboflavin	2 mg
Pyridoxine hydrochloride	1 mg
Nicotinamide	6 mg
Calcium pantothenate	10 mg
Biotin	40 $\mu$ g
Folic acid	40 $\mu$ g
<p><i>p</i>-Aminobenzoic acid</p>	1 mg
Inositol	100 mg
Organic bases :	
Adenine sulfate	<b>10 mg</b>
Guanine hydrochloride	10 mg
Uracil	10 mg
Xanthine	10 mg
Salts :	
Potassium phosphate monobasic	2.2 g
Potassium chloride	1.7 g
Calcium chloride	250 mg
Magnesium sulfate	500 mg
Ferric chloride	10 mg
Manganese sulfate	10 mg
Zinc sulfate	10 mg
Copper sulfate	1 mg
Boric acid	100 $\mu$ g
Sodium molybdate	100 $\mu$ g
Buffer :	
Potassium citrate	4 g
Citric acid	0.8 g

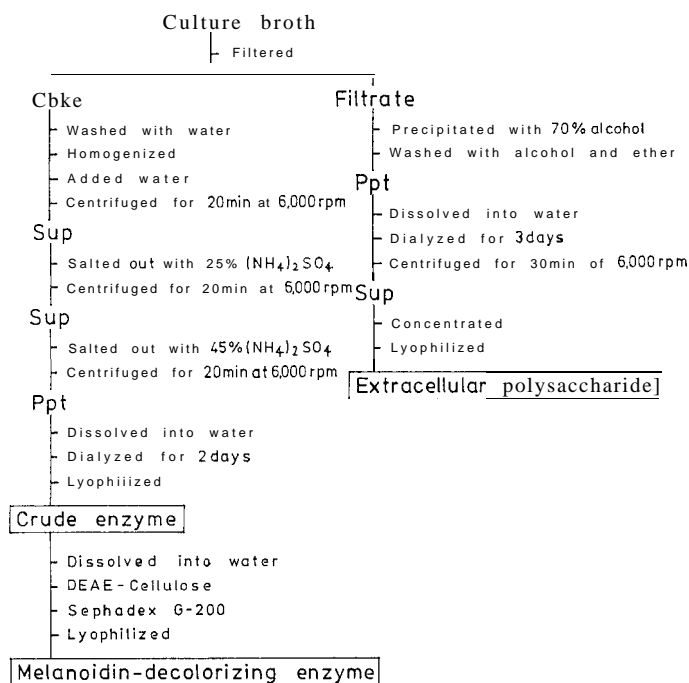
extract agar medium.

### Medium and cultural conditions

The basal synthetic medium (Table 1) in 50ml was dispensed into a 500-ml shaking flask for optimizing cultural conditions. The basal medium (1 liter) was also placed in 5-liter flask. Each culture was shaken slowly on a reciprocating shaker at 30°C for 15 to 20 days.

### Preparation of extracellular polysaccharide

Extracellular polysaccharide was precipitated with 70 % ethanol (final concentration) from culture filtrate, washed more than five times with ethanol and then with diethyl ether by centrifugation and dried under reduced pressure. The precipitate was dissolved in deionized water and dialyzed against



**Fig. 1.** Preparation of melanoidin-decolorizing activity and extracellular polysaccharide produced by *Coriolus* sp. No.20.

deionized water for 3 days. After removal of resulted precipitates by centrifugation, the supernatant was concentrated and lyophilized (Fig. 1). Weight of extracellular polysaccharide was measured before dialysis for study of cultural conditions.

#### Analysis of the neutral sugars of extracellular polysaccharide

Extracellular polysaccharides were hydrolyzed with 2 N HCl at 100°C for 5 hr and dried under reduced pressure over NaOH. Neutral sugars were converted into trimethylsilyl derivatives by the method of Sweeley *et al.* (1963), and analyzed by gas-liquid chromatography. A Hitachi model 163 gas chromatograph was employed. The stainless steel column (2 mx3 mm) was packed with 10 % silicone SE-52 on a 80~100 mesh chromosorb W-AW support. The analyses were performed by the gas chromatograph equipped with a flame ionization detector at programmed column temperature from 125 to 250°C increasing the temperature 2°C/min and the injection temperature at 300°C. N<sub>2</sub> was used as a carrier gas at a flow rate of 30 ml/min. Tentative identification of trimethylsilyl derivatives of neutral sugars were made by comparing their absolute and relative retention time to those of the authentic sugar derivatives. Quantification of all peaks was accomplished by triangulation and conversion to weight percentage using a standard curve between peak ratio (sugar/internal standard) and weight ratio (sugar/internal standard).

### Preparation of melanoidin-decolorizing enzyme

Mycelia (30g as dry matter) of *Coriolus sp. No. 20* was homogenized by Polytron (Kinematica Co.) with 1 liter of cold deionized water and centrifuged at 6,000g for 20 min. Solid ammonium sulfate was added to the supernatant to 25 % (w/v) concentration and allowed to stand overnight at 4°C. After removal of the precipitate by centrifugation at 6,000g for 20 min, additional ammonium sulfate was added to the supernatant to 45 % (w/v), and allowed to stand overnight. The precipitate thus formed was dialyzed against deionized water for 2 days, and lyophilized as crude enzyme (Fig. 1).

### Preparation of synthesized melanoidin

The mixture of 1 M glucose and glutamate was adjusted to pH 9.0 by 1 N NaOH and refluxed for 2 hr. The brown black product was dialyzed against tap water for 2 days, and then deionized water for 1 day. The non-dialyzable melanoidin was lyophilized until used (Kirigaya *et al.*, 1969).

### Determination of melanoidin-decolorizing activity

The activity was defined as the decrease in absorbance at 450 nm. The reaction mixture containing 30 mg of enzyme, 3 mg of melanoidin and 180 mg of glucose in 7 ml of 0.1 M acetate buffer (pH 4.5) was incubated at 30°C in L-type tube on a reciprocating shaker. Decolorization rate was expressed as the decrease in absorbance at 450 nm against initial absorbance.

### Antitumor bioassay

Antitumor bioassay was made using ddN mice (♀) weighing 20 to 25 g. Seven-day-old ascites of sarcoma-180 were intraperitoneally injected in 0.2 ml doses after dilution to  $5 \times 10^5$  cells of sarcoma-180 per ml with PBS (–) (phosphate buffered saline without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) by measuring the cell population on Burkert-Turk haemocytometer. The samples were dissolved in PBS (–) (10 mg/ml unless otherwise stated) and injected intraperitoneally 2 times (-6, -3 days) before tumor implantation and 8 times (2, 3, 4, 5, 6, 7, 8, 9 days) after tumor implantation for 10 days in 0.2 ml doses. As control, only PBS (–) was injected under the same conditions. Complete regression was measured as the ratio of survivors over 100 days after sarcoma-180 implantation against test mice. Survival days were estimated as the mean survival days and standard deviation of died mice.

### Chemical conversion of extracellular polysaccharide to oxidized-reduced derivative

The oxidized-reduced derivative of extracellular polysaccharide was prepared by the slightly modified procedure described by Chihara *et al.* (1970). Extracellular polysaccharide solution was mixed with the same volume of 50 mM periodic acid solution and stirred for 14 days at 5°C. The mixture was adjusted to pH 4.5 with acetic acid and centrifuged to yield a colorless paste. This substance was suspended in deionized water and 0.2M sodium borohy-

dride was added with stirring. The mixture was stirred for 20 hr for effective reduction. A further excess sodium borohydride was added and the stirring continued for another 20 hr. The reaction mixture was adjusted to pH 6.0 with acetic acid and centrifuged. The precipitate was suspended in deionized water and dialyzed for 3 days and finally lyophilized to obtain white powder.

### Enzymatic degradation of extracellular polysaccharide

Stalase (Seishin Pharm. Co., Ltd.), cellulase and macerozyme (*Trichoderma*, Kinki Yakult MFG. Co., Ltd.) were used as enzyme. For enzymatic degradation, 5 ml of 0.5 % polysaccharide solution, 1 ml of 0.1% enzyme solution and 1 ml of 0.1 M acetate buffer (pH 4.0 for stalase, pH 5.0 for cellulase and macerozyme) were mixed and incubated at 30°C (stalase) or 37°C (cellulase and macerozyme) for 4 days. Degradation of polysaccharide was represented as the relative viscosity against buffer. Preparations of enzymatically-degraded polysaccharides for antitumor bioassay were performed as follows. Polysaccharide was dissolved in deionized water and incubated with cellulase or macerozyme at 37°C for 24 hr. The reactions were terminated by boiling for 1 hr and centrifuged. The supernatants were dialyzed against 0.05 M acetate buffer (pH 4.0) and applied to a column of DEAE-Sephadex. The non-adsorbed fractions were dialyzed against deionized water and concentrated and lyophilized.

## RESULTS

### Production of extracellular polysaccharide

#### I) Effect of carbon and nitrogen sources

Effects of carbon sources on extracellular polysaccharide production is shown in Table 2. When glucose, mannose or maltose was used as carbon source, both cell growth and production of extracellular polysaccharide were high. In particular, maximum production of extracellular polysaccharide was shown in the cultivation with maltose. In the case of saccharose, cell growth was high, but the production of extracellular polysaccharide was low. Effects

Table 2. Effects of carbon sources on extracellular polysaccharide production by *Coriolus* sp. No.20.

Carbon source*	Polysaccharide (g/liter)	Dry cell weight (g/liter)
Glucose	2.46	12.8
Fructose	0.60	6.0
Mannose	2.18	13.8
Galactose	0.16	4.4
Xylose	1.08	8.2
Arabinose	0.16	4.0
Maltose	3.20	10.8
Saccharose	1.76	14.0

\* Instead of glucose (100 g/liter) in chemically defined medium (Table 1), the carbon sources listed in the table were used.

**Table 3.** Effects of nitrogen sources on extracellular polysaccharide production by *Coriolus* sp. No.20.

Nitrogen source*	(%)	Polysaccharide (g/liter)	
		Glucose**	Malt extract**
Casamino acid	0.1		
	0.5	1.50 1.62	4.30 4.60
	1:0	1.56	3.96
Ammonium citrate	0.5	1.54	2.60
	1.0	2.46	5.50
	1.5		
	2.0	1.98 1.76	3.14 5.38
Ammonium oxalate	0.5	1.46	1.84
	1.0	0	0
	2.0	0.0	0.0
Ammonium sulfate	0.5	3.02	4.54
	1.0	4.04	4.96
Ammonium acetate	0.5	1.06	3.36
	1.0	0	0

\* Instead of ammonium sulfate in chemically defined medium (Table 1), the nitrogen sources listed in the table were used.

\*\* Glucose or malt extract (100 g/liter) in chemically defined medium (Table 1) was used as carbon source with each nitrogen source.

**Table 4.** Sugar compositions of extracellular polysaccharides produced by *Coriolus* sp. No.20 with various carbon sources.

Carbon source*	Sugar composition (% w/w)				
	Fucose	Xylose	Mannose	Galactose	Glucose
Glucose	7.6				
Fructose	22.4	8.0	15.4	18.7	50.3
Mannose		19.0	13.4	10.3	34.9
Maltose	13:1	11.4	30.9	12.3	32.3
Saccharose	9.2	9.2	20.5	19.1	42.0
Lactose	9.4	10.5	18.0	16.7	45.4
	11:5	8:3	16.7	25.4	38.1

\* Instead of glucose (100 g/liter) in chemically defined medium (Table 1), the carbon sources listed in the table were used.

of nitrogen sources on the production of extracellular polysaccharide are shown in Table 3. Ammonium citrate and ammonium sulfate were excellent as nitrogen sources. The yield of extracellular polysaccharide was 4.04/liter with 10 % glucose and 1 % ammonium sulfate as carbon and nitrogen sources. When 10 % malt extract was used as carbon sources, the yield of extracellular polysaccharide increased with almost any nitrogen sources.

## 2) Relation of carbon source to sugar composition of polysaccharide

Sugar composition of extracellular polysaccharides produced with various carbon sources were studied by gas-liquid chromatography (Table 4). The sugar composition of extracellular polysaccharide produced in the presence of

### Production of Decolorizing Enzyme and Antitumor Activity

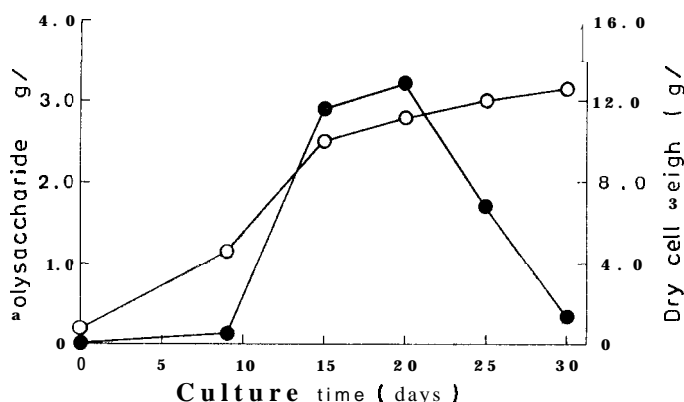


Fig. 2. Time course of extracellular polysaccharide production by *Coriolus* sp. No.20. Cultivation was carried out in chemically defined medium (Table 1) at 30°C. ●—●, extracellular polysaccharide (g/liter); ○—○, dry cell weight (g/liter).

glucose as carbon source was fucose, xylose, mannose, galactose and glucose (7.6 : 8.0 : 15.4 : 18.7 : 50.3). The content of mannose or galactose increased in the cultivation with mannose or lactose as carbon source, respectively.

#### 3) Cultures of *Coriolus* sp. No.20 by selected medium for polysaccharide EX-1 production

The production of extracellular polysaccharide with 10 % glucose and 1 % ammonium sulfate as carbon and nitrogen sources, respectively, is shown as a function of incubation time in Fig. 2. Production of extracellular polysaccharide increased from about 10 days after incubation and reached the maximum amount between 15 and 20th days and then the amount of extracellular polysaccharide decreased.

#### Procedure for crude preparation of melanoidin-decolorizing enzyme from mycelia

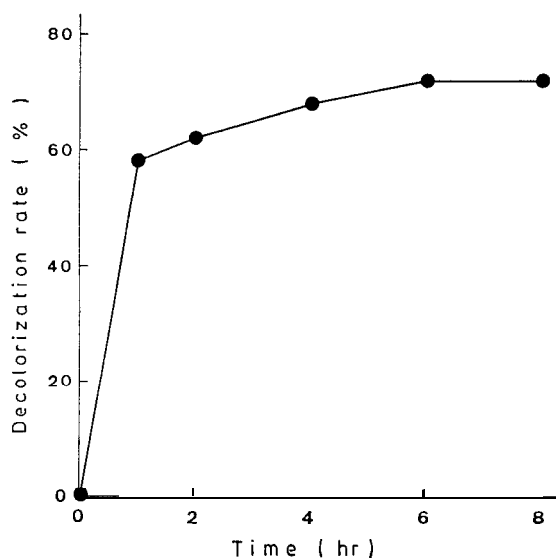
At the same time of the production of extracellular polysaccharide in the culture, melanoidin-decolorizing enzyme could be prepared from the mycelia of *Coriolus* sp. No. 20 (Fig. 1). Melanoidin-decolorizing enzyme was prepared as follows. Mycelia of *Coriolus* sp. No.20 was homogenized to extract the enzyme. The proteins were precipitated with 25-45 % (w/v) ammonium sulfate and after dialyzed, they were lyophilized as crude enzyme. The darkness of 1 % melanoidin solution was decolorized by this crude enzyme to the extent of 72 % in the presence of sugars and by aeration of the reaction mixture. The time course of decolorization by this crude enzyme is shown in Fig. 3.

#### Antitumor activity of extracellular polysaccharide

##### 1) Native polysaccharide

Antitumor activity of extracellular polysaccharide produced with 10% glucose and 1% ammonium sulfate as carbon and nitrogen sources, respec-





**Fig. 3.** Time course of enzymatic decolorization of melanoidin. The assay mixture containing 30mg of crude enzyme, 3 mg of melanoidin and 180 mg of glucose in 7 ml of 0.1 M acetate buffer (pH 4.5) was incubated at 30°C. Decolorization rate was calculated from the decrease in absorbance at 450nm against the initial absorbance.

**Table 5.** Effect of extracellular polysaccharide produced by *Coriolus* sp. No. 20 on sarcoma-180.

Dose* (mg/mouse)	Dose schedule	Complete** regression	Survival days*** (mean&SD)
Control	-6, -3, 2, 3, 4, 5, 6, 7, 8, 9	0/18	18.0, 3.1
2x10	"	6/18	32.8±26.6
4x10	"	3/15	28.4±17.8
Control	-15, -12, -8, -7, -6, -5, -4, -3, -2, -1	0/20	18.6±2.0
2x10	"	1/20	17.1±2.8

\* ddN mice were given intraperitoneal injection of 100 and 200 mg/kg/day of sample in PBS (-) Only PBS (-) was injected as control. Sarcoma-180 cell were also injected intraperitoneally.

\*\* Complete regression was measured as the ratio of survivors over 100 days after sarcoma-180 implantation against test mice.

\*\*\* Survival days were estimated as the mean survival days and standard deviation of died mice.

tively, is shown in Table 5. All control mice died within about 18 days after tumor implantation. When 2 mg of this polyaccharide was administered 10 times, 6 out of 18 mice did not get cancer and survived over 100 days. In this case, the other died mice lived for 33 days after tumor implantation in average. Accordingly, effect of delayed death was recognized on these died

### ***Production of Decolorizing Enzyme and Antitumor Activity***

Table 6. Effect of oxidized-reduced derivative and enzymatic degraded derivatives of extracellular polysaccharide produced by *Coriolus* sp. No. 20 on sarcoma-180.

Material	Dose* (mg/mouse)	Complete** regression	Survival days*** (mean $\pm$ SD)
Control	—	0/18	18.0 $\pm$ 3.1
Native polysaccharide	2x10	6/18	32.8 $\pm$ 26.6
Oxidized-reduced derivative of polysaccharide	2x10	11/20	28.8 $\pm$ 23.6
Polysaccharide degraded by macerozyme	2x10	11/20	35.7 $\pm$ 21.7
Polysaccharide degraded by cellulase	2x10	14/20	44.2 $\pm$ 22.5

\* ddN mice were given intraperitoneal injections of 100 mg/kg/day of sample in PBS (—) for 10 days. The other conditions were the same as those in Table 5.

\*\* , \*\*\* see Table 5.

mice. When this polysaccharide was administered only before tumor implantation, antitumor activity was very low.

#### ***2) Oxidized-reduced polysaccharide***

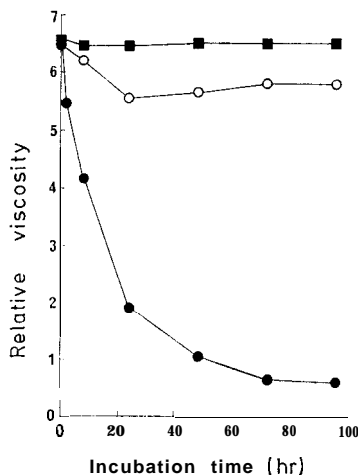
Antitumor activities of native extracellular polysaccharide and oxidized-reduced derivative of this polysaccharide were compared as shown in Table 6. Survivors over 100 days after tumor implantation (complete regression) were 6 out of 18 mice with the administration of native polysaccharide, whereas with the administration of oxidized-reduced derivative of this polysaccharide, complete regression was recognized in 11 out of 20 mice.

#### ***3) Partially hydrolyzed polysaccharide***

The degradation curves of extracellular polysaccharide by stalase, macerozyme and cellulase are shown in Fig. 4. This extracellular polysaccharide was not degraded by stalase, but degraded by macerozyme or cellulase (*Trichoderma*) to decrease the viscosity. This extracellular polysaccharide was degraded by macerozyme or cellulase for 24 hr and the antitumor activity was studied (Table 6). In this case, all control mice died within about 18 days after implantation. Compared to 6 out of 18 mice of complete regression with native polysaccharide, polysaccharide degraded by macerozyme or cellulase inhibited the tumor growth in 11 or 14 out of 20 mice, respectively.

### **DISCUSSION**

*Coriolus* sp. No. 20 decolorized the darkness of melanoidin to the extent of about 80 % under an optimal condition. This decolorization was performed by the intracellular enzyme with melanoidin-decolorizing activity. On the other hand, this strain produced an extracellular polysaccharide in the culture. Consequently, parallel preparation of melanoidin-decolorizing enzyme from mycelia and extracellular polysaccharide from the culture was carried out.



**Fig. 4.** Enzymatic degradation of extracellular polysaccharide produced by *Coriolus* sp. No. 20. Reaction mixtures consisting of 5 ml of 0.5 % polysaccharide solution, 1 ml of 0.1 M acetate buffer (stalase pH 4.0, macerozyme and cellulase pH 5.0) and 1 ml of enzyme solution were incubated at 30°C (stalase) or 37°C (macerozyme and cellulase). ■—■, stalase; ○—○, macerozyme ; ●—●, cellulase.

The crude preparation of enzyme decolorized 1 % melanoidin to the extent of 72 % at pH 4.5, 30°C as optimal conditions. The extracellular polysaccharide was produced with the maximum amount for 15 to 20 days of incubation and the yield was 4 g/liter with 10 % glucose and 1 % ammonium sulfate as carbon and nitrogen sources.

As Schizophyllan, an extracellular polysaccharide produced by *Schizophyllum commune*, showed the yield of 3 to 8 g/liter (Kikumoto *et al.*, 1970), the yield of extracellular polysaccharide produced by *Coriolus* sp. No. 20 was almost the same level as Schizophyllan and high compared to the other extracellular polysaccharides produced by basidiomycetes.

The extracellular polysaccharide produced by *Coriolus* sp. No. 20 consisted of fucose, xylose, mannose, galactose and glucose. Rhavandan *et al.* (1964) reported the intracellular polysaccharide of *Polyporus giganteus* which belongs to the same family with *Coriolus* sp. and this polysaccharide consisted of fucose, mannose, galactose and glucose. Those two polysaccharides were considered to the similar kind of polysaccharide.

Ikekawa *et al.* (1968, 1969) reported the antitumor activity of several polysaccharides extracted from basidiomycetes, especially Lentinan from *Lentinus edodes*. Antitumor activity of PS-K extracted from *Coriolus versicolor* was reported by Tsukagoshi (1974 a, b), and PS-K is now in clinical use (Kaibara *et al.*, 1977; Mikuriya *et al.*, 1977; Kida *et al.*, 1977; Miwa, 1977). The extracellular polysaccharide EX-1 produced by *Coriolus* sp. No. 20 also showed antitumor activity, and this activity increased in oxidized-reduced derivative with periodic acid and enzymatically-degraded polysaccharides. PS-K is proteo-

polysaccharide and Lentinan is  $\beta$ -1,3-; $\beta$ -1,6- glucan, and both materials have strong antitumor activity to solid-type tumor, but weak to ascite-type tumor. The extracellular polysaccharide EX-1 showed antitumor activity to ascite-type tumor. Particularly, the extracellular polysaccharide degraded by *Trichoderma* cellulase represented strong antitumor activity to sarcoma-180 ascite-type tumor, compared to the previously reported antitumor polysaccharides, and did not have toxicity.

The fine chemical structure of the extracellular polysaccharide degraded by *Trichoderma* cellulase will be discussed in the following papers.

#### ACKNOWLEDGEMENTS

The authors wish to thank Prof. K. Maekawa (Fukuoka Junior College of Social Work and Nursery) and Dr. A. Momii (Otsuka Pharm. Co., Ltd. at present) for their technical guidance about the antitumor bioassay. Dr. Y. Tanaka is acknowledged for his kind supply of the strain of *Coriolus* sp. No. 20. Thanks are due to Mr. N. Nanri and Mr. F. Hirayama (Yoshitomi Pharm. Co., Ltd. at present) for their technical assistance in part of this work.

#### REFERENCES

- Bhavandan, V. P., H. O. Bouveng and B. Linderberg 1964 Polysaccharides from *Polyporus giganteus*. *Acta Chem. Scand.*, **18**: 504-512
- Chihara, G., J. Hamuro, Y. Maeda, Y. Arai and F. Fukuoka 1970 Antitumor polysaccharide derived chemically from natural glucan (Pachyman). *Nature*, **225**: 943-944
- Ikekawa, T., M. Nakanishi, N. Uehara, G. Chihara and F. Fukuoka 1968 Antitumor action of some basidiomycetes, especially *Phellinus linteus*. *Gann*, **59**: 155-157
- Ikekawa, T., N. Uehara, Y. Maeda, M. Nakanishi and F. Fukuoka 1969 Antitumor activity of aqueous extracts of edible mushrooms. *Cancer Res.*, **29**: 734-735
- Kaibara, N., F. Inoue, K. Togi, H. Takihara, R. Jindai and K. Iguchi 1977 PS-K no Rinsho Chiken Seiseki. *Yakuri to Chiryô*, **5**: 2297-2301
- Kida, Y., N. Shibuya, C. Okada, S. Uemura, T. Kobayashi and N. Kageyama 1977 Noshuyô ni taisuru PS-K Meneki Ryôhō. *Gan to Kagakuryôhō*, **4**: 863-867
- Kikumoto, S., T. Miyajima, S. Yoshizumi, S. Fujimoto and K. Kimura 1970 Polysaccharide produced by *Schizophyllum commune* 1. Formation and some properties of an extracellular polysaccharide. *Nippon Nôgeikagaku Kaishi*, **44**: 337-342
- Kirigaya, N., H. Kato and M. Fujimaki 1969 Studies on antioxidant activity of nonenzymic browning reaction products 2. Antioxidant activity of nondialyzable browning reaction products. *Nippon Nôgeikagaku Kaishi*, **43**: 484-491
- Mikuriya, S., K. Matsumoto, T. Seto and E. Noda 1977 Three cases with metastatic lung cancer from the testicular tumor being obtained good results by various treatments. *J. Jap. Soc. Cancer Ther.*, **12**: 343-350
- Miwa Y. 1977 PS-K ga Chôkô o shimeshita Akuseishuyô no Ichishôrei. *Yakuri to Chiryô*, **5**: 3200-3204
- Sweeley, C. C., R. Bentley, M. Makita and W. W. Wells 1963 Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *J. Am. Chem. Soc.*, **85**: 2497-2507
- Tsukagoshi, S. 1974 a Host-mediated antitumor activity of polysaccharides, with special

- reference to the effect of PS-K. A protein-bound polysaccharide isolated from basidiomycetes. **Gan to Kagakuryôhō**, **1**: 251-257
- Tsukagoshi, S. 1974 b Tatôru o Riyô suru kotonyoru Menekikagakuryôhō no Kisoteki-kenkyu. **Igaku no Ayumi**, 91: 505-510
- Watanabe, Y., R. Sugi, Y. Tanaka and S. Hayashida 1982 Enzymatic decolorization of melanoidin by **Coriolus** sp. No. 20, *Agric. Biol. Chem.*, **46**: 1623-1630