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Studies on the Constituents and Culture of Korean Basidiomycetes

Antitumor Polysaccharides from the Cultured Mycelia of Some Basidiomycetes

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Abstract □ To develop potent antitumor polysaccharides from higher fungi of Korea the alcohol precipitated polysaccharides, which were obtained from hot water extracts of shake-cultured mycelia of eight species of basidiomycetes, were subjected to antitumor screening tests, using sarcoma 180 as a tumor cell. When administered *i.p.*, at the dose level of 20mg/kg/day for ten days into the mice which were implanted with 1×10^6 cells of sarcoma 180 twenty-four hour before the start of polysaccharide injection, the polysaccharides of *Pluteus cervinus* ISA-Pc-1004, *Pleurotus pulmonarius*, *Hydnum repandum* ISA-Hr-1006, and *Laccaria laccata* ISA-Ll-1008 respectively showed inhibition ratios of 62.0%, 51.7%, 64.8%, and 57.8%. They were selected for further study, while those of *Panellus serotina* ISA-Ps-1012, *Laccaria amethystina* ISA-La-1001, *Pholiota adiposa* ISA-Pa-1010, and *Lepista* sp. ISA-Ls-1007 respectively showed inhibition ratios of −9.3%, 25.3%, 27.3%, and 33.0%.

Keywords □ Basidiomycetes, Antitumor activity against sarcoma 180, Polysaccharides, *Pluteus cervinus*, *Pleurotus pulmonarius*, *Hydnum repandum*, *Laccaria laccata*, *Panellus serotina*, *Laccaria amethystina*. *Pholiota adiposa*, *Lepista* sp.

As the results of the active studies on the antitumor components of higher fungi since 1960's, various kinds of polysaccharides and protein bound polysaccharides from basidiomycetes, for example, lentinan[1] and KS-2[2] from *Lentinus edodes*, schizophyllan[3] from *Schizophyllum commune*, PSK[4] from *Coriolus versicolor* were known to exhibit potent antitumor activities. These components, having almost no toxicity even in prolonged administrations, can be developed as potent and safe immunopotentiators in cancer treatment.

The first result of the investigation on the antitumor components of Korean higher fungi was reported by Kim[5], one of the authors, and his coworkers in 1979. Since then several other studies with this title have been carried out in this laboratory.[6-8] Those studies include not only the investigations on the antitumor polysaccharides of the carpophores of some basidiomysetes but also the studies on the antitumor components of cultured mycelia of Korean basidiomycetes. This communication contains another result of antitumor polysaccharide screening tests on the cultured mycelia of eight species of edible basidiomycetes.

EXPERIMENTAL METHODS

Fungal Strains

The following strains of fungal species were were used in this investigation: *Pluteus cervinus*

ISA-Pc-1004, *Laccaria laccata* ISA-Ll-1008, *Laccaria amethystina* ISA-La-1001, *Hydnum repandum* ISA-Hr-1006, *Pleurotus plumonarius*, *Panellus serotina* ISA-Ps-1012, *Lepista* sp. ISA-Ls-1007, *Pholiota adiposa* ISA-Pa-1010. These fungal strains were isolated from the carpophores of wild mushrooms growing in Korea and were maintained on compost-sugar-agar slants.

Submerged Culture

The medium containing glucose 50g, starch 40g, yeast extract 10g, peptone 10g, KH_2PO_4 0.87g, $MgSO_4 \cdot 7H_2O$ 0.5g, $CaCl_2$ 0.3g, $ZnSO_4 \cdot 7H_2O$ 4mg, $CuSO_4 \cdot 5H_2O$ 1mg, $MnCl_2 \cdot 4H_2O$ 7mg, $FeSO_4 \cdot 7H_2O$ 10mg per liter was used for the culture of mycelia throughout this experiment. For seed culture, the mycelia grown on compost-sugar-agar slants were homogenized and transfered to 100 ml of medium in a 500ml flask. After incubation on Gallenkamp orbital incubator for 7 to 14 days at 26° at 180 rpm, the content of the flask was homogenized and inoculated into a 500ml flask containing 100ml of the fresh medium. After another incubation under the same condition, the culture broth was homogenized and the homogenate was used as the seed culture. For production, four 500ml flasks with 100ml of medium were inoculated with the seed culture. The inoculum size was 20% (v/v) of the fresh medium. Culture was carried out for 7 to 11 days according to the growth rate of each strain.

Preparation of Polysaccharides

The mycelia were harvested and washed three times with distilled water. The washed mycelia were homogenized and extracted with distilled water on a boiling water bath. The hot water extracts were condensed and mixed with three volumes of 95% ethanol. The precipitates were separated by filtration and were dried at 60° for 1 hour after washing with acetone. The nonhygroscopic polysaccharides thus obtained were used as samples.

Antitumor Test

The antitumor test was carried out as described previously.[6] ICR mice of both sexes weighing about 20g were subcutaneously implanted with 1×10^6 cells of sarcoma 180 into the left groin 24 hour before the start of sample administration. Sample administration was carried out once daily for ten days by *i.p.* injection at a dose level of 20mg/kg/day. Twentysix days after tumor implantation, the mice were sacrificed and the solid tumors were excised and inhibition ratios were calculated from their weights.

RESULTS AND DISCUSSION

Among the tested, the polysaccharides of *P. pulmonarius*, *P. cervinus*, *H. repandum*, and *L. laccata* respectively showed the inhibition ratios of 51.7%, 62.0%, 64.8%, and 57.8%, and these strains were selected for further study, while those of *P. serotina*, *P. adiposa*, *L. amethystina*, and *Lepista* sp. were found to be of less importance. The four strains selected are newly found as antitumor polysaccharide-producing basidiomycetes by this study. When considering the fact that the samples used in this study are crude polysaccharides which are not removed of small molecules, we can hope to develop new potent antitumor polysaccharides from these strains by removal of inactive and/or immunosupressive substances with several methods.

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Table I: Results of antitumor tests.

Strain	Average body weight(g)	Average tumor weight(g)	Inhibition ratio(%)	100% Regression
Panellus serotina	27.9	4.61	24.5	0/10
Pleurotus pulmonarius	28.6	2.95	51.7	1/10
Pluteus cervinus	28.9	2.32	62.0	0/9
Control	32.6	6.11	—	0/8
Hydnum repandum	31.7	1.70	64.8	2/8
Laccaria laccata	26.4	2.71	51.3	1/9
Panellus serotina	30.0	5.27	9.3	0/9
Control	34.6	4.82	—	0/9
Pleurotus pulmonarius	30.7	2.71	51.3	0/9
Pholiota adiposa	30.8	4.05	27.3	0/8
Laccaria amethystina	30.5	4.16	25.3	0/9
Lepista sp.	31.0	3.63	33.0	0/8
Control	30.8	5.57	—	0/9

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