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Studies on Constituents of the Higher Fungi of Korea(XLI)

An Antitumor Fraction from the Culture Filtrate of *Lentinus edodes* DMC7

Kyeong Soo Chung, Eung Chil Choi* and Byong Kak Kim*

Laboratory of Microbial Chemistry, Department of Pharmacy, College of Science
Chungnam National University, Daejeon 300-31 and
**Department of Microbial Chemistry, College of Pharmacy*
Seoul National University, Seoul 151, Korea

韓國産 高等 菌類의 成分 硏究(第41報)

Lentinus edodes DMC7 菌株의 培養 濾液의 抗癌 成分

鄭 敬 壽·崔 應 七*·金 炳 珏*

忠南大學校 理科大學 藥學科 微生物藥品化學敎室
*서울大學校 藥學大學 微生物藥品化學敎室

Abstract: To find antitumor constituents in Korean basidiomycetes, the mycelia of *Lentinus edodes* DMC7, which had shown a good mycelial growth in shakeflasks, were cultured at 27°C on an orbital shaking incubator at 180 rev/min for 12 days. The medium was composed of glucose (50g/*l*), yeast extract (9g/*l*), peptone (9g/*l*), and seven inorganic salts. A water soluble macromolecular fraction, LF-3, was obtained from the culure filtrate by fractionation with ethanol and dialysis using a Visking tube. When LF-3 was administered i.p. at 50mg/kg/day once daily for 10 consecutive days to female ICR mice which were implanted s.c. with sarcoma 180 (10^6 cells/mouse), it exerted a highly significant antitumor activity, with the tumor inhibition ratio of 53.1%.

Koywords: *Lentinus edodes*, Polyporaceae, Basidiomycetes, Antitumor constituent, Submerged culture of mycelia, Culture filtrate.

Since Gregory and his collaborators (1966) reported on the antitumor basidiomycetes, many investigators have been actively working in this field and found out many antitumor constituents in the higher fungi. As a result, Chihara and his coworkers (1969) separated a new antitumor fraction from the carpophores of *Lentinus edodes*, which is one of the favorest edible mushrooms in Asia, and they named it as LC-33. In the next year Chihara and his collaborators (1970) isolated the potent antitumor $\beta(1\rightarrow3)$ glucan, lentinan, from this fraction. It is known that the antitumor constituents of basidiomycetes, including lentinan, exert their antitumor activity by acting as immunomodulators without any toxic effect on normal cells. However this potent antitumor polysaccharide, lentinan, has never been isolated from the cultured mycelia nor from the culture filtrate of this fungus. Recently, however, a new antitumor protein-bound polysaccharide KS-2 was isolated from the cultured mycelia of *L. edodes* KSLE007 (Fujii *et al.*, 1978). Being encouraged by this report the authors carried out a study to find out a new antitumor constituent from the culture filtrate of *L. edodes* DMC7, which had been selected as a suitable strain for the production of polysaccharide by Chung(1982), one of the authors.

This report contains the separation of a water soluble

macromolecular fraction, LF-3, from the culture of this fungus and its antitumor activity. The chemical analyses of this fraction will appear in the future reports.

Materials and Methods

Fungal Strain

The strain of *Lentinus edodes* DMC7 (the family Polyporaceae) was one of the stock cultures stored at the Department of Microbial Chemistry, College of Pharmacy, Seoul National Uiversity in Seoul, Korea. The mycelia of this strain were kept on potato-dextrose-agar slants at 4°C with succesive subculture at intervals of one month.

Culture Medium

The liquid culture medium, the composition of which is shown in Table I, was used throughout this study. It contains glucose as its cabon source, and peptone(Difco Lab., U.S.A.) and yeast extract(Difco Lab., U.S.A.) as its nitrogen sources.

Culture Process

The mycelia grown on a PDA slant were homogenized with 50 ml of the medium in a microblendor for ten seconds and divided into 500 ml Erlenmyer flasks, each of which contained 100 ml of the medium. These flasks were shaken at 27±1°C on a Gallenkamp orbital incubator at 180 rev/min for 12 days. The culture condition was all the same throughout this study. The mycelial pellets thus obtained were homogenized and used to inoculate five flasks, each of which contained 100 ml of the medium. These were shake-cultured and then the whole culture broth was used as an inoculum to obtain final culture. As for final culture, five liters of the medium were divided in ten 2-*l* Erlenmyer flasks and each of the flasks was inoculated with 50 ml of the homogenized culture broth described above. These were shake-cultured for 12 days and then the culture broths from ten flasks were combined to be used as the final culture.

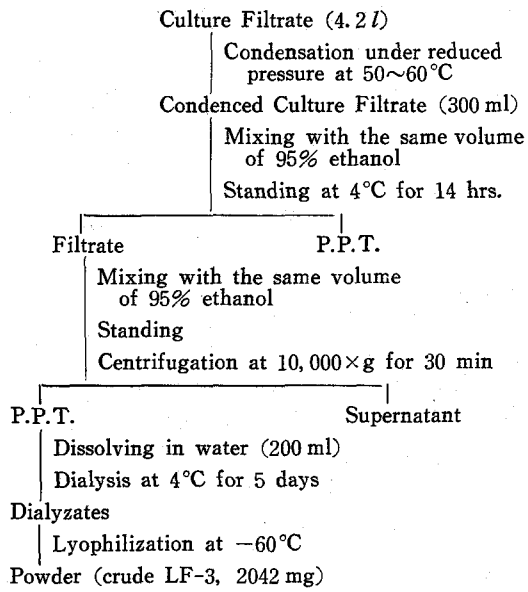
Table I. The composition of the liquid culture medium used for the shake-culture of *Lentinus edodes* DMC7.

Ingredient	Quantity(g/liter)
Glucose	50
Peptone	9
Yeast extract	9
KH_2PO_4	0.87
$MgSO_4 \cdot 7H_2O$	0.50
$CaCl_2$	0.30
Mineral solution*	10ml

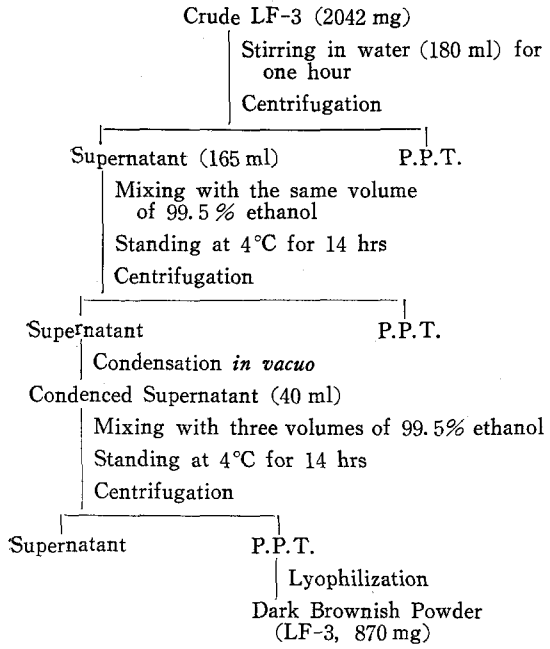
*Ten ml of mineral solution contains $ZnSO_4 \cdot 7H_2O$ 4mg, $CuSO_4 \cdot 5H_2O$ 1mg, $MnCl_2 \cdot 4H_2O$ 7mg, $FeSO_4 \cdot 7H_2O$ 10mg.

Preparation of LF-3

The culture filtrate (4.2*l*) was obtained by filtering the mycelia from the final culture and then condensed to 300 ml under reduced pressure at 50~60°C and mixed with the same volume of 95% ethanol. After the mixture had been kept at 4°C for 14 hours the precipitates were filtered. The filtrate was mixed with the same volume of 95% ethanol and let to stand at 4°C for 14 hours. The precipitates were obtained by centrifugation at 10,000×g for 30 minutes, and then dissolved in 200 ml of distilled water. This was dialyzed using a Visking tube to remove small



Scheme I. Separation of crude LF-3 from the culture filtrate of *Lentinus edodes* DMC7.



Scheme II. Separation of LF-3 from crude LF-3.

molecules at 4°C for five days. The dialyzates were lyophilized at −60°C to yield brownish powder(crude LF-3) (Scheme I).

Crude LF-3 was stirred in 180 ml of distilled water for one hour and the insoluble precipitates were centrifugated at 10,000×g for 30 minutes. The supernatant (165 ml) was mixed with the same volume of 99.5% ethanol and kept at 4°C overnight. The precipitates were removed by centrifugation and the supernatant was condensed *in vacuo* to 40 ml. This was mixed with 120 ml of 99.5% ethanol and kept at 4°C overnight. The precipitate was separated by centrifugation and lyophilized to yield a dark brownish powder (LF-3) (Scheme II).

Antitumor Test

The detailed procedure of antitumor test used was described previously (Chung *et al.*, 1983). But in brief, ICR mice of female sex, weighing 18~22g, were used as a test animal, and sarcoma 180 was used as a tumor cell line. Each of the test animals was implanted s.c. with 0.1 ml of tumor cell suspension (1×10^7 cells/ml, hemacytometer count) at the left axilliary region. LF-3 in phisiological saline was administered i.p. at 50mg/kg/day once daily for 10 consecutive days starting 24 hours after tumor implantation. As for a control group the tumor-implanted mice received only physiological saline. The injection volume was 0.1 ml for each mice. In 25 days after tumor implantation the mice were sacrificed and the tumors were excised and weight.

Results and Discussion

The mycelia of *Lentinus edodes* DMC7 showed full growth in 12 days so that the final culture broth gave almost no supernatant layer when it was let stand for one hour. From 4.2*l* of the culture filtrate, 2042 mg of the crude LF-3 were obtained. The crude LF-3 contained both water-soluble and practically insoluble macromolecular components. From the water-soluble portion of the crude LF-3, 870 mg of LF-3 were prepared as a dark brownish powder which dissolved clearly in distilled water and physiological saline.

LF-3, the water-soluble macromolecular fraction from the culture filtrate of *Lentinus edodes* DMC7, showed a tumor inhibition ratio of 53.1% (highly significant) when administered i.p. for 10 consecutive days at 50mg/kg/day (Table II).

Table II. The antitumor activity of LF-3 separated from the culture filtrate of *Lentinus edodes* DMC7.

Group	Average tumor weight (g)	Inhibition ratio(%)	Complete regression
LF-3 treated (50mg/kg/day)	2.61±0.66[a] ($p<0.01$)	53.1	0[b]/9[c]
Control	5.57±1.85	—	0/8

a : mean±standard error.
b : the number of mice in which the tumor was completely regressed.
c : the number of mice used.

Conclusion

A water-soluble macromolecular fraction (LF-3) was separated from the culture filtrate of *Lentinus edodes* DMC7. LF-3 showed antitumor activity against sarcoma 180 implanted in ICR mice.

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적 요

한국산 담자균류로부터 새로운 항종양 성분을 개발하기 위하여 표고의 일개 균주인 ***Lentinus edodes*** DMC7을 인공배지에서 진탕배양하여 그 균사 배양물을 얻었다. 그중 배양 여액으로부터 수용성 고분자 분획을 분리하여 LF-3라고 명명하였다. LF-3는 ICR 마우스에 이식된 Sarcoma 180 세포에 대하여 항암작용을 나타내었으며 그 저지율은 53.1%였다.

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