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Effect of ginseng residue on the growth of *Ganoderma lucidum*

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*Ganoderma lucidum*의 生育에 미치는 紅蔘粕의 影響

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The ginseng residue, a by-product of ginseng tea manufacture, was used as growth promoting substance in the submerged cultivation of *Basidiomycetes* for its effective utilization. Ginseng residue contained about 46% of total sugar, 14% of crude protein, 12% of ash, and 0.16% of crude saponin. Among inorganic substances in ginseng residue, amount of Mg, Na, K and Ca were much more than other inorganic substances. All ginsenosides existed in red ginseng residue. *Ganoderma lucidum* and *Pleurotus ostreatus* could be excellently cultured in potato dextrose broth. Most effective additional concentration of residue extract for growth of these fungi was shown to be 0.2%, and the contents of crude protein and amino acid in mycelium were increased when 0.5% of residue extract were added to the medium.

Ganoderma lucidum belongs to the the family of *Polyporaceae*, it can produce a fruit body in its life cycle. It is distributed in China, Korea and Japan. In these countries, *Ganoderma lucidum* is used as a home remedy to cure various disease, such as hepatitis, hypertension, hypercholesterolemia and gastric cancer.[1,2]

Recently, many studies reported that polysaccharide components have antitumor activities[1,3-6], and polysaccharide fraction was composed of a backbone of β-(1—3)-linked-D-glucosyl residue with a single branch of β-(1—6)-linked-D-glucosyl group in every four to six residues of backbone chain, and these polysaccharide had an average molecular weight of 1,050,000[6]. And also Nishitoba et al.[7,8] reported that they found several new bitter compounds from *G. lucidum* named lucidenic acids. Kawai[9] reported the studies on the productivity and distribution of various carbohydrolases produced by submerged culture of many kinds of *Basidiomycetes*.

We had previously reported the enzymatic characteristics of amylase from submerged culture of *G. lucidum*[10]. We have studied for a few years on the commercial use of ginseng residue, a by-product of ginseng tea manufacture, and we reported the effect of red ginseng residue on various enzyme production of alcohol fermentation koji[11].

This paper discribes the effects on mycelium growth, protein content and amino acid composition of mycelium obtained from the submerged culture of *Ganoderma lucidum*, *Pleurotus ostreatus* in potato dextrose broth with or without ginseng residue extract.

Materials and Methods

Red ginseng and red ginseng residue

Red ginseng manufactured by the Office of Monopoly in 1984 was used in this experiments. Red ginseng residue, a by-product of red ginseng tea manufacture, was prepared from red tail ginseng by 5 times extraction with 70° ethyl alcohol at 80°C for 8hrs, and the residue was dried at 60°C.

Key words: *Ganoderma lucium, Pleurotus ostreatus,* submerged culture, ginseng residue

Table 1. Analysis conditions of amino acid of *Basidiomycetes* mycelium cultured in potato dextrose broth with ginseng residue extract.

Instrument	: LKB Amino acid analyzer No. 4150 made in England
Column	: Sodium form
Injection volumn	: 40 μl
Solvent system	: pH 3.2, 4.25, 10.00 0.2M-Sodium citrate 0.4M-NaOH
Flow rate	: 40ml/min (Ninhydrin/OPA : 25ml/min)
Recorder	: LKB Intergrator
Chart speed	: 0.2 cm/min

Extract of ginseng residue

Ginseng residue was extracted with water at 80°C for 8hrs, and evaporated to oily state. It was used for the cultivation of various *Bacidiomycetes*.

Microorganisms and culture conditions

Ganoderma lucidum and *Pleurotus ostreatus* were provided by Chung-ang Agriculture Enterprise Co., *Certinellus shiitake* and *Agaricus bisporus* were provided by Agricultural Sciences Institute, Rural Development Administration. The strains were maintained on potato dextrose agar medium. The medium for submergd culture was potato dextrose broth, and the initial pH of the medium was adjusted to 5.5.

The cultivation was carried out in 500ml Erlenmeyer flask containing 100ml of the medium on rotary shaker (200rpm) at 30°C for 14 days or a 3000ml sakaguchi flask containing 500ml of the medium on reciprocal shaker (9cm, 88 strokes/min) at 30°C for 14 days.

HPLC pattern of ginseng saponin

Saponins of ginseng residue extract were extracted by methods of Namba and Shibata group[12, 13] and each ginsenoside (ginsenoside-Rg_1, -Re, -Rd, -Rc, -Rb_2, -Rb_1 and Ro) was analyzed by high performance liquid chromatograph (HPLC).

Protein and amino acid analysis

Protein contents of mycelium was determined by Kjeldahl method[14] and amino acid composition was analyzed with amino acid autoanalyzer LKB 4150. Sample solution for amino acid analysis was prepared as follows: Mycelium cultured for 14 days was dried and 50mg of dried mycelium was transferred to ample. 2ml of 6N-HCl were added and sealed in vacuum state, and hydrolyzed at 120°C for 20hrs. The hydrolyzates was evaporated and 4ml of buffer solution (Sodium citrate 19.78g, phenol 1g, 35%-HCl 16.5ml in 100ml of distilled water and adjusted to pH 2.2) was added and dissolved, filterred subsequently. The filtrate was used for determination of amino acid compositions, and analysis conditions were shown in Table 1.

Table 2. General components of red ginseng residue.

(Unit: % dry basis)

Reducing sugar	Total sugar	Crude fat	Crude protein	Ash	Crude saponin	Water extract
0.82	46.21	0.42	14.51	12.48	0.16	12.48

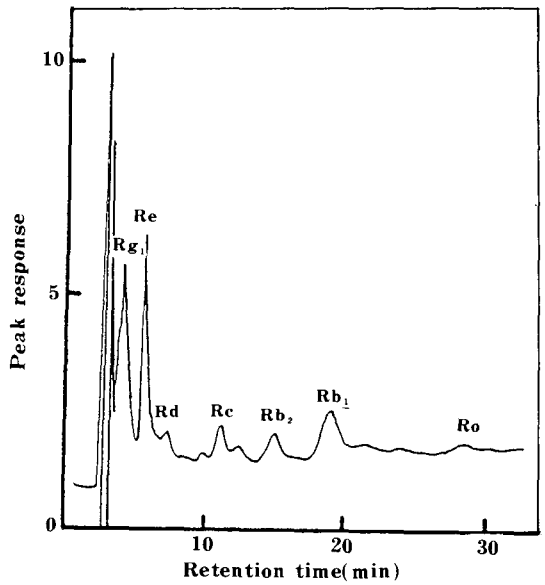

Fig. 1. HPLC chromatogram of ginseng saponin in water extract from red ginseng residue.

Model: Waters associate model 244, column: μ-Bondapak carbohydrate analysis (4mm×30cm), Solvent : Acetonitril/H_2O/BuOH (80/20/15), Detector: Differential refractometer (RI), Sensitivity: 8x, Flow rate: 1.5ml/min., Chart speed: 1cm/min., Injection volume: 20μl (5 % solution)

Estimation of mycelium growth

Mycelia cultured were harvested by filtration, and washed with distilled water 3 times. Thereafter they were dried at 105°C to constant weight and weighed.

Results and Discussions

General composition of red ginseng residue

General composition of red ginseng residue used in this

Table 3. Content of inorganic compound of red ginseng residue. (ppm)

Mg*	Zn	Fe	Mn	Cu	Ca	Na*	K*
2.4	61.3	122.5	35.0	19.7	1770	0.92	22.3

*mg/g

experiments was shown in Table 2. The residue contained 46% of total sugar, 14% of crude protein, 12.5% of ash and 12.5% of water extract, and crude saponin content was about 0.16%. But though the content of saponin was of little quantity, it was found that all ginsenosides exist in ginseng residue (Fig. 1). And inorganic substances affect the growth of microorganisms, so the contents of inorganic substances in ash were investigated. The results were summarized in Table 3, very large amounts of Mg, Ca, Na and K were existed in ash of the ginseng residue.

Selection of excellent strain for growth

To select the excellent strain for the submerged culture of mycelium, 5 strains collected were cultured at 30°C for 14 days (200rpm) in potato dextrose broth, and dry weight of mycelium was estimated. As shown in Table 4, amounts of mycelia of *G. lucidum* (red strain) and *P. ostreatus* were much more than those of other strains. So we selected *G. lucidum* and *P. ostreatus* as excellent strain for submerged culture.

Effect of ginseng residue extract on submerged culture

To investigate the effect of the residue extract concentration on submerged culture of two kinds of *Bacidiomycetes*, they were incubated at 30°C for 14 days (9cm, 88 stroke/min) in potato dextrose broth added residue extract. In case of *G. lucidum*, the addition of 0.2% of residue extract to potato dextrose both increased the mycelium amount of 98%, but only increased the growth of 26% in *P. ostreatus*. And the addition of 0.5% residue extract decreased the amount of mycelium in both strains (Table 5). It seems that ginseng residue had the growth promoting substances or nutrition for growth. High concentration of ginseng extract or ginseng saponin in media caused the decrease of cell growth in case of *Saccharomyces cerevisiae*,[15, 16] *Lactobacillus acidophilus* and *Streptococcus thermophilus*,[17] *Saccharomyces uvarum*.[18] As same as above reports, our experimental result was similier to them.

Table 4. Mycelium amounts of various strains on submerged culture.

Strains	Amounts of mycelium (mg/100 ml)
Gonoderma lucidum (red)	691
Ganoderma lucidum (white)	20
Pleurotus ostreatus	970
Certinellus shiitake	41
Agaricus bisporus	16

The strains were incubated at 30℃ for 14 days with 200 rpm in potato dextrose broth.

Table 5. Effect of concentration of red ginseng residue extract on the growth of mycelium.

Strains	Conc. of residue extract (%)			
	0	0.05	0.2	0.5
Ganoderma lucidum	100(%)	179	198	118
Pleurotus ostreatus	100	113	127	115

The strains were incubated at 30℃ for 14 days with shaking incubator (9 cm, 88 strokes/min) in potato dextrose broth enhanced 0.5% yeast extract.

Table 6. Effect of ginseng residue extract on the protein content of *Basidiomycetes* mycelium. (Unit: % dry basis)

Strains	Concentration of GRE* (%)		
	0	0.2	0.5
Ganoderma lucidum	45.3	45.2	46.8
Pleurotus ostreatus	33.3	34.0	36.2

*GRE: concentration of ginseng residue extract in potato dextrose broth.
The strains were incubated at 30℃ for 14 days with reciprocal shaking incubacor (9cm, 88 strokes/min).

Effect of ginseng residue extract on protein conent of *Basidiomycetes*

In order to investigate the effect of ginseng residue extract on protein content of *G. lucidum* and *P. ostreatus*, the residue extract was added to potato dextrose broth with the concentration of 0.2 and 0.5%. After incubation, the mycelia were filtered and washed with distilled water and weighed. As shown in Table 6, the content of crude protein of *G. lucidum* and *P. ostreatus* were slightly increased with an increase in the addition of ginseng reside extract, and the increasing range in *P. ostreatus* was higher than that in *G. lucidum*.

Oura et al.[19] reported that ginseng had the acceleration

Table 7. Composition of amino acid of *Basidiomycetes* cultured in potato dextrose broth with ginseng residue extract.

(unit : nM/0.33 mg)

Strain	*Ganoderma lucidum*			*Pleurotus ostreatus*		
Conc. of GRE*(%) / Amino acid	0	0.2	0.5	0	0.2	0.5
Asp	54.95	54.30	58.02	42.39	42.52	50.84
Thr	41.95	41.73	46.83	34.33	33.61	39.07
Ser	40.98	40.86	43.80	31.78	32.92	38.15
Glu	9.36	9.55	10.28	6.99	6.98	8.37
Pro	88.35	86.92	91.23	52.78	51.92	81.31
Gly	62.27	62.27	66.38	47.49	47.88	58.07
Ala	63.95	62.24	65.28	50.09	51.28	59.67
Cys	1.41	2.45	4.20	0.22	0.76	0.91
Val	54.99	55.28	56.36	41.48	41.30	48.76
Met	30.79	31.17	32.13	11.17	12.08	25.26
Ile	35.29	35.59	37.21	27.32	28.39	32.26
Leu	44.74	44.58	45.28	34.86	34.41	41.83
Tyr	21.98	23.21	24.07	18.02	17.92	20.29
Phe	39.46	39.50	41.26	33.08	32.93	36.57
His	26.77	26.71	27.18	21.52	22.05	25.41
Lys	36.16	36.65	38.66	27.42	28.52	34.03
Arg	43.37	44.41	45.27	34.41	34.24	41.03
Total	696.77	697.78	733.44	515.35	519.71	642.13

*GRE: ginseng residue extract.
The strains were incubated at 30℃ for 14 days with shaking (9 cm, 88 strokes/min) in potato dextrose broth enhanced 0.5% yeast extract. After mycelia were washed with distilled wafer 3-times, hydrolyzed at 30℃ for 20 hrs. Amino acids were analyzed by amino acid autoanalyzer LKB 4150.

similiar to the result of Oura group.

Effect on amino acid content of *Basidiomycetes* cultured in potato dextrose broth with ginseng residue extract

Amino acid and protein content of a edible and medical mushroom is very significant in a point of view of ingesting them. Most of *Basidiomycetes* have protein of 30-40% or more in their living cells. So, after cultivation in the medium added the residue extract, amino acid contents in mycelium were determined with amino acid analyzer LKB 4150. As shown in Table 7, the contents of amino acids were increased, when the residue extract of 0.5% were added to the medium. And the increasing range in *P. ostreatus* was slightly higher than that in *G. lucidum*. Particularly, cystein content, S-containing amino acid, was increased with the degree of 4-folds in both *G. lucidum* and *P. ostreatus*. These results indicate that some components in ginseng residue extract activated the reaction related to amino acid synthsis.

Organic acids, 3-carbon sugars and amino acid in glycolysis and tricarboxylic acid cycle are reacted as amino acid precurssor in biosynthesis of amino acid[20], and serine, cystein is transformed into pyruvate by deamination of amino acid dehydrogenase, alanine also become precurssor of pyruvate[21]. And in other many amino acid metabolism, amino acid is transformed into organic acid and amino compound by decarboxylation, Strickland reaction. It seems that these interactions, the activation of enzymes related to glycolysis and tricarboxylic acid cycle, increased the content of amino acids in mycelium of *Bacidiomycetes* by the residue extract.

From above results, we suggest that ginseng residue could be use for the cultivation of various microorganisms as growth promoting substance. And we knew that the increased ratio of amino acids are more than that of protein.

요 약

홍삼제품 제조 과정중의 부산물인 홍삼박의 활용 가능성을 조사하기 위하여 여러가지 담자균의 배양에 홍삼박 추출물을 첨가하여 배양한 후 균체 증식량, 단백질 및 amino acid 함량 등을 조사하였으며 그 결과를 보고하는 바이다.

수집된 담자균중 *G. lucidum*과 *P. ostreatus*가 액체배양에서 균체증식이 양호한 균주로 선발되었으며, 홍삼박 추출물을 0.2% 첨가하였을때 균체증식량이 가장 많았으며 단백질 함량은 0.5% 첨가하였을때 1~3%가 증가하였다. Amino acid 함량은 인삼박 추출물을 0.2% 첨가하였을 때는 거의 변화가 없었으나 0.5% 첨가하였을 때에는 *G. lucidum*, *P. ostreatus* 모두 상당히 증가하였으며 그 증가폭 중 cysteine이 가장 컸다.

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