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Changes of Ginsenoside Content by Mushroom Mycelial Fermentation in Red Ginseng Extract

Song Hwan Bae[1#], Hyun-Sun Lee[2#], Mi-Ryung Kim[3], Sun Young Kim[4], Jin-Man Kim[5], and Hyung Joo Suh[6*]

[1]Department of Food and Biotechnology, Hankyong National University, Anseong 456-749, Korea
[2]Department of Food and Nutrition and Institutes of Health and Science, Korea University, Seoul 136-703, Korea
[3]Department of Bio-Food Biomaterials, Silla University, Busan 617-736, Korea
[4]Hongcheon Institute of Medicinal Herb, Hongcheon 250-930, Korea
[5]Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Korea
[6]Department of Food and Nutrition, Korea University, Seoul 136-703, Korea

To obtain microorganisms for the microbial conversion of ginsenosides in red ginseng extract (RGE), mushroom mycelia were used for the fermentation of RGE. After fermentation, total sugar contents and polyohenol contents of the RGEs fermented with various mushrooms were not a significant increase between RGE and the ferments. But uronic acid content was relatively higher in the fermented RGEs cultured with *Lentus edodes* (2155.6 μg/mL), *Phelllinus linteus* (1690.9 μg/mL) and *Inonotus obliquus* 26137 and 26147 (1549.5 and 1670.7 μg/mL) compared to the RGE (1307.1 μg/mL). The RGEs fermented by *Ph. linteus*, *Cordyceps militaris*, and *Grifola frondosa* showed particularly high levels of total ginsenosides (20018.1, 17501.6, and 16267.0 μg/mL, respectively). The ferments with *C. militaris* (6974.2 μg/mL), *Ph. linteus* (9109.2 μg/mL), and *G. frondosa* (7023.0 μg/mL) also showed high levels of metabolites (sum of compound K, Rh_1, Rg_5, Rk_1, Rg_3, and Rg_2) compared to RGE (3615.9 μg/mL). Among four different RGE concentrations examined, a 20 brix concentration of RGE was favorable for the fermentation of *Ph. linteus*. Maximum biotransformation of ginsneoside metabolites (9395.5 μg/mL) was obtained after 5 days fermentation with *Ph. linteus*. Maximum mycelial growth of 2.6 mg/mL was achieved at 9 days, in which growth was not significantly different during 5 to 9 days fermentation. During fermentation of RGE by *Ph. linteus* in a 7 L fermenter, Rg_3, Rg_5, and Rk_1 contents showed maximum concentrations after 5 days similar to flask fermentation. These results confirm that fermentation with *Ph. linteus* is very useful for preparing minor ginsenoside metabolites while being safe for foods.

Keywords: Red ginseng extract, Fermented red ginseng, Ginsenoside metabolites, Mushroom mycelia, *Phelllinus linteus*

INTRODUCTION

Ginseng (*Panax ginseng* of the Araliaceae family) is one of the most valuable oriental herbs. Typically, the dried root of the plant has been used as a healing drug and health tonic in countries such as China, Japan, and Korea since ancient times [1]. There are two different forms of ginseng: red ginseng is dried after steaming and is the most common form in traditional Korean medicine, while white ginseng is produced by sun dry-

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[#]The authors contributed equally to this research work.

[*]**Corresponding author**
E-mail: suh1960@korea.ac.kr
Tel: +82-2-940-2853, Fax: +82-2-940-2850

ing. More than 40 ginsenosides have been isolated and identified in Asian ginseng. Various biological activities of ginsenosides have been reported, such as antisenescence, immuno-modulatory, antitumor, antiinflammatory protein anabolic, anti-diabetic, etc. [2-5].

Usually ginseng is administered orally, after which the ingredients are exposed to gastric juices and digestive and bacterial enzymes in the gastrointestinal tract. The characterization of the metabolism of ginseng saponins is important for explaining the pharmacological actions of ginseng [6-8]. Indeed, ginsenosides themselves have exerted various pharmacological activities, by directly being added to cell cultures *in vitro* or by being intraperitoneally or intravenously injected into experimental animals. These results have led to the misunderstanding that intact ginsenosides might be the real active principle in the body. However, Kobashi *et al*. [9] and Kobashi *et al*. [10] proposed the concept that plant glycosides act as a prodrug that is metabolized to the active form by intestinal bacterial deglycosylation. We revealed that the anticancer activities of ginsenosides after oral administration are based on their metabolites formed by intestinal bacterial deglycosylation [11,12] and fatty acid esterification [13,14].

To improve the oral absorption and bioavailability of these compounds, many different strategies have been used. Several studies have shown that the transformation of ginsenosides into deglycosylated ginsenosides (metabolites) is required in order for them to provide more effective *in vivo* physiological action [15]. Various transformation methods, including mild acid hydrolysis [16], enzymatic conversion [17], and microbial conversion [18], have been used. Chemical methods, however, produce side reactions such as epimerization, hydration, and hydroxylation, and most of the microorganisms used for the transformation of ginsenosides are not of food-grade standards.

In seeking to utilize the beneficial properties of ginsenoside metabolites using food-compatible microorganisms, we screened edible mushroom species capable of metabolizing ginsenosides from ginseng, and investigated changes in levels of total sugars, uronic acid, polyphenols, and ginsenoside metabolites during fermentation.

MATERIALS AND METHODS

Materials

Korean red ginseng extract (60 brix, RGE) was purchased at a ginseng market in Geumsan, Korea. Standard ginsenoside materials including compound K (CK), Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rg_5, Rh_1, Rh_2, and Rk_1 were purchased from Ambo Institute (Seoul, Korea). All other chemicals were of reagent grade and obtained from local suppliers.

Preculture and culture of mycelium of various mushrooms

The strains of *Cordyceps sisnensis*, *Cordyceps militaris*, *Phelllinus linteus*, *Tremella fuciformis*, *Inonotus obliquus* 26136, *I. obliquus*, *Grifola frondosa*, and *Lentus edodes* were received from Chungju University, and maintained on potato dextrose agar slants. The slants were inoculated and incubated at 25°C for 7 days, and then stored at 4°C for about 2 wk.

For the preculture, 40 mL of medium (potato dextrose broth; Difco Laboratories, Detroit, MI, USA) with an initial pH of 5.5 was prepared in a 300 mL flask, and then 10 mL of mycelium suspension from a slant culture was inoculated, followed by 7 days of incubation at 25°C on a rotary shaker (150 rpm). A 25 g portion of RGE was poured into a 500 mL flask, dissolved with 100 mL of distilled water, and sterilized at 121°C for 15 min (RGE medium). The precultured broths were inoculated into the RGE medium at 4 mL and incubated at 25°C for 7 days with mild shaking (150 rpm).

Effect of initial red ginseng extract concentration

To investigate the impact of the initial RGE concentration, RGE was used at levels of 8.3 g (5 brix), 16.7 g (10 brix), 25 g (15 brix), and 33.3 g (20 brix) per 100 mL. The medium was inoculated by transferring 4 mL of preculture broth (with ca. 300-350 mg DW of cells/L) to 100 mL of medium in a 500 mL flask and incubated at 25°C for 7 days with shaking (150 rpm).

Scaleup fermentation

Scaleup fermentation was carried out in a 7 L fermenter (Fermentec, Cheongwon, Korea) with a 5 L working volume of medium containing 333.3 g of RGE per liter. The fermentations were conducted at 25°C, with an aeration rate of 1.0 vvm, agitation speed of 150 rpm, and pH of 5.5. The seed cultures were transferred to the fermentation medium and were cultivated for 9 days.

Ginsenosides analysis with HPLC

After fermentation, the broths were transferred to centrifuge tubes and centrifuged at 4000 rpm for 10 min. The supernatants were collected and applied to an SPE C18 cartridge for sample clean-up [19]. The levels of 15 major ginsenosides were analyzed using an HPLC-based technique developed by Kim *et al*. [20]. A Varian Prostar

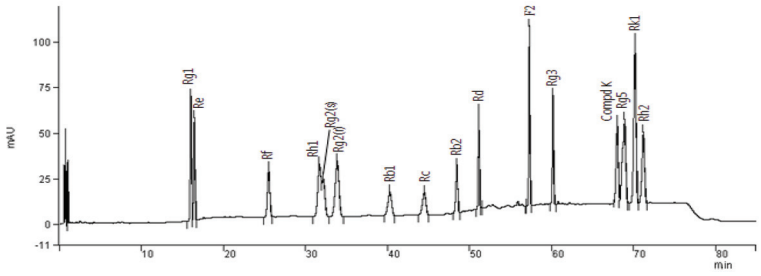

Fig. 1. Chromatogram of standard ginsenosides by HPLC assay. An IMtakt Cadenza CD-C18 (4.6×75 mm) column was used. UV absorption was measured at 203 nm. Gradient elution was employed using solvent A (10% acetonitrile) and solvent B (90% acetonitrile) at 40°C.

200 HPLC system (Varian Inc., Palo Alto, CA, USA) equipped with a quaternary solvent delivery system, an autosampler, and a UV detector was used. The column configuration consisted of an IMtakt Cadenza CD-C18 (4.6×75 mm; Imtakt Corporation, Kyoto, Japan). UV absorption was measured at 203 nm. Gradient elution was employed using solvent A (10% acetonitrile) and solvent B (90% acetonitrile) at 40°C; the gradient program was as follows: 0→11 min, 11% B (isocratic); 11→15 min, 11→16% B; 15→16 min, 16 →20% B; 16→18 min, 20→21%; 18→24 min, 21% B (isocratic); 24→25 min, 21→22% B; 25→35 min, 22% B (isocratic); 35→36 min, 22→23% B; 36→40 min, 23% B (isocratic); 40→41 min, 23→24%; 41→45 min, 24% B (isocratic); 45→53 min, 24→37% B; 53→61 min, 37→45% B; 61→66 min, 45→46%; 66→73 min, 46→48% B; 73→75 min, 48% B (isocratic); 75→77 min, 48→11%; 77→85 min, 11% B (isocratic). The flow rate was kept at 1.3 mL/min and the sample injection volume was 5 μL. The level of total ginsenosides was determined by the sum of the 15 ginsenosides. Fig. 1 shows the HPLC chromatograms of the 15 standard ginsenosides.

Analytical methods

Total polyphenol content was determined using the Folin Ciocalteu method [21] adapted to a microscale using gallic acid as a standard (50-800 μg/L). Total sugar and uronic acid levels were determined using the phenol-sulfuric acid [22] and *m*-hydroxydiphenyl methods [23], respectively, using glucose and galacturonic acid as the respective standards. In all cases, the analyses were performed in triplicate unless otherwise specified. The values were averaged and standard deviations were calculated. All data were analyzed by one-way analysis of variance and Duncan's multiple range tests using SPSS ver. 10.0 (SPSS Inc., Chicago, IL, USA). The results were considered significant at $p<0.05$.

RESULTS

Total sugar, uronic acid, and polyphenol contents in fermented red ginseng extracts

The total sugar, uronic acid, and polyohenol contents of the RGEs fermented with various mushrooms are presented in Table 1. The total sugar contents of the fermented RGEs ranged from 319.6 to 458.4 mg/mL and the total sugar content of RGE (non-fermented RGE) was 444.8 mg/mL. The submerged culture ferments of mushrooms, except that with *Ph. Linteus,* showed lower levels of total sugars than RGE. The ferment cultured with *C. militaris* showed the lowest level of total sugars (319.6 mg/mL) among the ferments.

Uronic acid (an acidic polysaccharide) content was relatively higher in the fermented RGEs cultured with *L. edodes* (2155.6 μg/mL), *Ph. linteus* (1690.9 μg/mL), *I. obliquus* 26147 (1670.7 μg/mL), and *I. obliquus* 26136 (1549.5 μg/mL) compared to the RGE (1307.1 μg/mL). In particular, the RGE fermented with *L. edodes* had the highest level of uronic acid ($p<0.05$).

Also, for the other strains except *C. militaris* (1058.8 μg/g), polyphenol content (989.0-1012.2 μg/g) was lower in the fermented RGEs compared to the control

Table 1. Total sugar, uronic acid, and polyphenol contents after 7 days fermentation of red ginseng extracts by mushroom mycelia

Strain	Total sugar (mg/mL)	Uronic acid (μg/mL)	Polyphenols (μg/mL)
Red ginseng extract	444.8±37.9^{ab}	1307.1±98.6^{cd}	1052.8±23.7^{a}
Cordyceps sinensis	393.1±29.0^{cd}	1276.8±356.1^{d}	1009.1±16.6^{b}
Cordyceps militaris	319.6±24.5^{e}	973.7±38.1^{e}	1058.8±17.4^{a}
Phelllinus linteus	458.4±8.6^{a}	1690.9±181.8^{b}	998.8±10.7^{b}
Tremella fuciformis	345.3±26.1^{de}	918.2±121.2^{e}	999.2±12.0^{b}
Inonotus obliquus 26136	351.2±32.9^{de}	1549.5±53.2^{bc}	997.4±26.3^{b}
Inonotus obliquus 26147	386.2±7.9^{cd}	1670.7±38.1^{b}	989.0±16.0^{b}
Grifola frondosa	361.0±22.3^{cde}	1115.2±90.9^{de}	1012.2±25.7^{b}
Lentus edodes	405.7±27.0^{bc}	2155.6±68.3^{a}	995.75±13.2^{b}

Table 2. Total gisenoside and ginsenoside metabolite contents after 7 days fermentation of red ginseng extracts (RGEs) by mushroom mycelia

Gisenoside	Fermented RGE (μg/mL)								
	RGE	*Cordyceps sinensis*	*Cordyceps militaris*	*Phelllinus linteus*	*Tremella fuciformis*	*Inonotus obliquus 26136*	*Inonotus obliquus 26147*	*Grifola frondosa*	*Lentus edodes*
Rg_1	668.7±23.1	424.6±23.2	773.3±57.6	810.4±40.3	515.5±24.5	552.1±35.5	826.5±65.3	465.3±35.4	373.3±13.3
Re	1974.9±48.5	1348.8±83.4	1688.6±76.4	1449.1±34.1	1271.2±76.2	1312.4±67.4	1380.0±70.5	1215.8±82.1	1170.9±67.9
Rf	464.6±54.3	435.1±32.3	518.5±43.5	508.8±36.5	418.8±18.4	442.0±24.8	455.3±35.4	457.3±37.5	412.2±22.1
Rh_1+$Rg_{2(S)}$	276.4±34.5	360.0±24.3	404.5±42.3	597.1±33.3	338.7±28.7	357.0±40.2	522.6±26.4	403.0±30.0	410.8±28.5
$Rg_{2(R)}$	67.1±8.9	123.7±10.3	139.0±10.7	229.5±18.7	123.0±10.3	131.2±13.5	165.1±10.2	147.0±10.7	125.7±7.8
Rb_1	2105.4±203.4	1802.9±85.6	2208.0±105.6	2419.6±100.3	1365.1±56.8	1421.6±58.4	1627.0±67.4	2096.0±94.4	1324.5±44.5
Rc	2224.3±142.3	1867.5±105.3	2386.2±165.4	2572.4±89.5	1412.9±40.6	1468.1±68.4	1705.6±30.5	2194.0±94.1	1391.9±31.9
Rb_2	1674.2±45.2	1467.7±67.4	1841.4±50.5	2030.5±70.1	1122.8±43.3	1161.7±38.5	1352.5±32.5	1708.1±37.5	1100.8±40.8
Rd	1058.7±87.5	777.7±65.3	1109.3±37.5	1117.7±45.4	550.3±22.2	580.5±20.1	670.3±30.5	1107.2±47.3	532.2±32.3
Rg_3	459.6±25.3	844.3±34.8	1032.1±23.4	1188.1±23.1	740.8±18.9	788.0±33.5	846.6±25.4	1035.1±13.2	754.1±21.2
Rg_5	1523.5±34.2	2649.5±68.9	3141.7±94.2	3874.0±100.2	2156.5±80.4	2349.1±84.5	2712.7±57.5	3184.0±81.3	2347.0±74.3
Rk_1	1289.3±85.4	1850.8±79.8	2257.0±104.3	3220.9±59.5	1416.4±46.4	1554.5±78.4	2158.8±46.5	2253.8±83.1	1710.6±40.6
CK	0.0	108.3±13.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rh_2	0.0	0.0	1.6±0.5	0.0	0.0	0.0	0.0	0.0	0.0
Total	13786.5±792.6	14060.6±694.1	17501.6±811.9	20018.1±650.9	11431.9±466.7	12118.5±553.2	14423.1±498.1	16267.0±646.9	11654.0±425.2
Rg_1+Rb_1	2774.1±226.5	2227.5±108.8	2981.4±163.2	3230.3±140.6	1880.5±81.3	1973.8±93.9	2453.5±132.7	2561.4±129.8	1697.8±57.8
Metabolites[1)]	3615.9±228.3	5936.6±231.6	6974.2±274.9	9109.2±234.8	4775.6±184.7	5179.8±250.1	6406.0±166.0	7023.0±218.3	5348.3±172.4

CK, compound K.
[1)]Sum of CK, Rh_1, Rg_5, Rk_1, Rg_3, and Rg_2.

(1052.8 μg/g, $p<0.05$).

Changes of ginsenoside composition in fermented red ginseng extracts

The ginsenoside compositions of the RGEs fermented by mushroom mycelia are shown in Table 2. The ginsenoside content of RGE was 13786.5 μg/mL and the total ginsenoside contents of the fermented RGEs were in a range of 11431.9-20018.1 μg/mL. The RGEs fermented by *Ph. linteus*, *C. militaris,* and *G. frondosa* showed particularly high levels of total ginsenosides (20018.1, 17501.6, and 16267.0 μg/mL, respectively). The ginsenosides Rg_1 and Rb_1 in the ferments with *Ph. linteus* (3230.3 μg/mL) and *C. militaris* (2981.4 μg/mL) also showed high levels compared to the RGE (2774.1 μg/mL).

In recent decades, many studies have focused on the pharmaceutical activities of minor ginsenosides such as ginsenosides Rd, Rg_3, Rh_2, and ginsenoside K (CK), as their activities are found to be superior to those of major ginsenosides. These minor ginsenosides are present in ginseng only in small percentages and are known to be produced by the hydrolysis of sugar moieties of major ginsenosides. In addition, ginseng and its derived products are orally administered in most cases, and a number of metabolites are produced via the degradation of ginsenosides by acid or intestinal bacteria in the gastrointestinal tract. Ginsenoside metabolites (sum of CK, Rh_1, Rg_5, Rk_1, Rg_3, and Rg_2) were found in the RGE fermented using mushroom mycelia (Table 2). In particular, the ferments with *Ph. linteus* (9109.2 μg/mL), *G. frondosa* (7023.0 μg/mL), and *C. militaris* (6974.2 μg/mL), showed high levels of metabolites compared to the RGE (3615.9 μg/mL).

Ginseng is usually administered orally, after which its components are exposed to gastric juices and digestive and bacterial enzymes in the gastrointestinal tract. The intestinal bacteria population is variable, depending on the conditions of the host, including diet, health, and even stress. To overcome such variations, a microorganism that can produce ginsenoside metabolites would be deemed valuable. Therefore, the *Ph. linteus* strain was selected for the production of ginsenoside metabolites.

Changes of ginsenosides in RGE fermented with *Phelllinus linteus* under various RGE concentrations

The osmotic pressure caused by a high RGE concen-

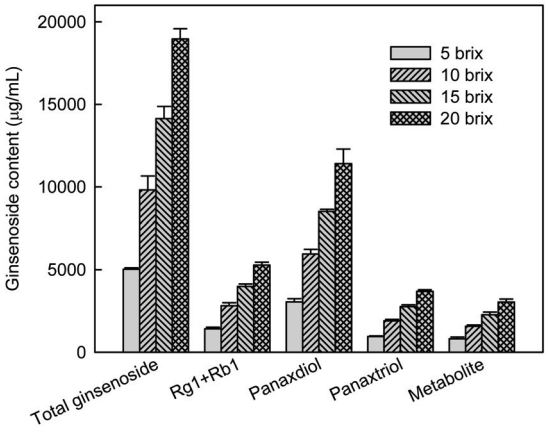

Fig. 2. Ginsenoside contents after 7 days fermentation with *Phellinus linteus* under various red ginseng extract (RGE) concentrations. RGE was used at levels of 8.3 g (5 brix), 16.7 g (10 brix), 25 g (15 brix), and 33.3 g (20 brix) per 100 mL. The medium was inoculated by transferring 4 mL of preculture broth to 100 mL of medium in a 500 mL flask and incubated at 25°C for 7 days with shaking (150 rpm).

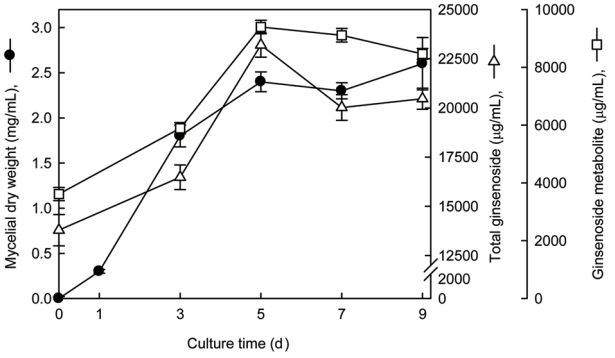

Fig. 3. Time profiles of mycelia growth, total ginsenoside, and their metabolite changes in submerged culture of *Phelllinus linteus* in a 7 L fermenter. Fermentations were conducted at 25°C, an aeration rate of 1.0 vvm, agitation speed of 150 rpm, and pH of 5.5. The seed cultures were transferred to fermentation medium and were cultivated for 9 days.

tration may be detrimental to fermentation. To find a suitable RGE concentration for ginsenoside transformation by *Ph. linteus,* RGE was added at different concentrations ranging from 8.3 g (5 brix) to 33.3 g (20 brix) per 100 mL. After 7 days fermentation, the ginsenoside contents (total ginsenosides, Rg_1 and Rb_1, protopanaxadiols, protopanaxatriols, and metabolites) in the ferment increased with an increase in concentration (Fig. 2). Ginsenoside content showed a similar fold increase with the fold increase in RGE concentration until 20 brix. However, mycelial growth was inhibited at the above 20 brix because of decrease of mycelial dry weight at the concentration (data not shown). Therefore, the 20 brix concentration of RGE was favorable for the fermentation of *Ph. linteus*. Using a high concentration of RGE may have some advantages such as decreased production time and cost, and easier control of procedures.

Mycelial growth, total gisenosides, and metabolite changes in a submerged culture of *Phelllinus linteus* in a 7 L fermenter

Fig. 3 shows the typical time course of mycelial growth and ginsenoside changes in the 7 L fermenter. Maximum mycelial growth of 2.6 mg/mL was achieved at 9 days, in which growth was not significantly different during 5 to 9 days of fermentation. The total ginsenoside and metabolite contents of the RGE fermented by *Ph. linteus* increased with increasing fermentation time until 5 days.

Table 3 shows changes in ginsensides during fermentation of RGE by *Ph. linteus*. Rg_1 and Rb_1 content increased with increasing fermentation time. In particular, both Rb_1 and Rg_1 increased with increasing fermentation time (Table 3). Rg_3 and Rk_1 contents showed maximum concentrations after 5 days, similar to flask fermentation. A comparable result was observed in our previous flask culture (Table 3). After 5-days fermentation, contents were 140.1% to 261.1% higher than those of non-fermented RGE.

The combined content of ginsenosides Rb_1, Rb_2, Rc, Rd, Rg_3, CK, and Rh_2, which are major ginsenosides (protopanaxadiol type), only changed from 7522.2 μg/mL to 9791.2 to 12271.4 μg/mL during fermentation. On the other hand, the content of protopanaxtriols (Rg_1, Re, Rf, Rh_1, and Rg_2) was slightly change from 3451.7 μg/mL to 3430.8-3875.5 μg/mL throughout fermentation.

DISCUSSION

Ginsenosides, glycosides with steroids or triterpenes as aglycones, are an important class of physiologically active compounds occurring in many herbs. In recent years, the sugar chains of saponins have been found to be closely related to the biological activity of ginsenosides, and modification of the sugar chains may markedly change the biological activity of ginsenosides [24,25]. Recently, it was suggested that orally ingested ginsenosides are metabolized by human intestinal bacteria, and deglycosylated ginsenoside metabolites are known to be more readily absorbed into the bloodstream and act as active compounds [15,17]. Lee *et al*. [26] also suggested that the efficiency of the conversion and transformation pathways differs greatly owing to the diversity of resident microflora between individuals. Metabolites are generally prepared via the biotransformation of

Table 3. Total gisenoside and ginsenoside metabolite contents in fermentations of red ginseng extracts by *Phelllinus linteus*

Ginsenoside (μg/mL)	Culture time (d)				
	0	3	5	7	9
Rg_1	668.7±23.1	698.9±48.2	703.5±35.7	810.4±40.3	819.3±39.8
Re	1974.9±48.5	1576.6±66.7	1660.8±60.8	1449.1±34.1	1705.0±57.0
Rf	464.6±54.3	456.7±26.7	508.9±29.8	508.8±36.5	491.8±28.5
Rh_1+$Rg_{2(S)}$	276.4±34.5	506.2±44.6	696.5±55.6	597.1±33.3	675.7±47.5
$Rg_{2(R)}$	67.1±8.9	192.4±12.4	217.5±17.5	229.5±18.7	183.7±13.7
Rb_1	2105.4±203.4	2130.1±90.1	2542.3±85.4	2419.6±100.3	2640.3±64.2
Rc	2224.3±142.3	2197.0±67.5	3515.6±65.4	2572.4±89.5	2077.3±77.3
Rb_2	1674.2±45.2	2108.6±68.2	2381.1±51.8	2030.5±70.1	2142.3±42.3
Rd	1058.7±87.5	2149.8±49.7	2397.6±39.7	1117.7±45.4	2327.7±27.7
Rg_3	459.6±25.3	1205.7±20.5	1434.8±34.8	1188.1±23.1	954.0±25.4
Rg_5	1523.5±34.2	2456.8±68.5	3268.9±62.3	3874.0±100.2	3345.8±54.8
Rk_1	1289.3±85.4	1530.8±60.8	3778.0±78.4	3220.9±59.5	3305.8±58.4
CK	0.0	0.0	0.0	0.0	0.0
Rh_2	0.0	0.0	0.0	0.0	0.0
Total	13786.5±792.6	16480.0±623.9	23197.4±617.2	20018.1±650.9	20466.9±536.6
Protopanaxdiols[1)]	7522.2±503.7	9791.2±296.0	12271.4±277.1	9328.3±328.4	10141.6±236.9
Protopanaxtriols[2)]	3451.7±169.3	3430.8±198.6	3787.2±199.4	3594.9±162.9	3875.5±186.5
Metabolite[3)]	3615.9±228.3	5891.9±194.4	9395.5±231.1	9109.2±234.8	8465.0±186.1

[1)]Sum of Rb_1, Rb_2, Rc, Rd, Rg_3, Rh_2 and CK.
[2)]Sum of Re, Rf, Rg_1, Rg_2 and Rh_1.
[3)]Sum of CK, Rh_1, Rg_5, Rk_1, Rg_3, and Rg_2.

ginsenosides in the presence of human intestinal bacteria [6,18], soil fungi [27], or certain commercial enzymes [28,29]. Therefore, ginsenosides with more uniform and targeted biological functions may be attained by using specially isolated microorganisms. In this study, we screened for available microorganisms using RGE as a substrate and produced fermented RGE using *Ph. linteus*.

For the utilization of Korean ginseng (*Panax ginseng*) in a functional drink, Park *et al.* [30] prepared fermented Korean ginseng with mushroom mycelia (*Ganoderma lucidum*, *Hericium erinaceum*, and *Ph. linteus*) by solid culture. The Korean ginseng fermented with mushroom mycelia by solid culture contained chemical ingredients different from Korean ginseng, and it might provide beneficial immunostimulating activity. Through two screening steps, a strain of fungus (ECU2042) capable of selectively transforming ginsenoside Rg_3 into ginsenoside Rh_2 was isolated from soil samples and identified as *Fusarium proliferatum*. In the preparation of ginsenoside Rh_2 using the crude cell-free extract of *F. proliferatum* ECU2042, a higher conversion of 60.3% was obtained as compared to that previously reported. Therefore, this method was considered to be potentially useful for the practical preparation of ginsenoside Rh_2 [31].

The fermented RGE had increased total ginsenoside and metabolite contents, especially for Rh_1, Rh_2, Rg_5, Rk_1, Rg_2, and Rg_3 (Table 2). Red ginseng mainly contains ginsenosides such as Rg_3, Rb_1, Rb_2, and Rc. Rh_1, Rh_2, and Rg_3, which are representative constituents in red ginseng, and are produced from protopanaxadiol ginsenosides by steaming raw ginseng. Table 3 shows the changes in ginsensides during fermentation of RGE by *Ph. linteus*. Rg_3, Rg_5, and Rk_1 contents showed maximum concentrations after 5 days, similar to flask fermentation.

Through several studies, investigators have reported that the ginsenosides Rb_1, Rb_2, and Rc are metabolized to CK, and that this metabolite induces antimetastatic or anticarcinogenic activity. It has also been reported that a deglycosylation process is required for ginsenosides to exhibit their greatest effects *in vivo,* and that their clinical efficacy varies with the hydrolyzing potential of the components of the intestinal microflora. Ginsenosides may be degraded by a variety of methods, including mild acid hydrolysis, enzymatic activity, or microbial

activity [17]. However, each of these methods has demonstrated some defects when used routinely. Many kinds of bacteria have been used in an attempt to overcome these obstacles, including *Prevotella oris*, *Eubacterium* sp. A-44, *Bifidobacterium* sp. K506, *Bacteroides* sp. JY6, and *Fusobacterium* sp. K-60, all of which can cooperatively metabolize ginsenosides [18,32].

Ginsenoside Rh_1 is produced from Re via Rg_1 and Rg_2 by *Bifidobacterium* sp. Int57, *Bifidobacterium* Sp. SJ32, *Aspergillus niger,* and *Aspergillus usamii* [33]. Rg_2 has been found to reduce the acetylcholine-evoked secretion of catecholamines from cultured bovine adrenal chromaffin cells [34]. Rh_1 is known to possess antiallergic and anti-inflammatory activity [35]. Therefore, fermentation may be a means to simultaneously obtain red ginseng components that have immunopotentiating activity and potent cytotoxicity against tumor cells, and that are easily absorbed in the human intestinal tract.

We attempted to identify a suitable mushroom mycelium that could replace the need for human intestinal bacteria in the biotransformation of ginsenosides into their metabolites. For the production of fermented RGE, this study used *Ph. linteus*, whose enzymes are increasingly being used as an effective means of structure modification, as well as for the metabolism study of natural and synthetic organic compounds. The RGE underwent many changes during fermentation in terms of total sugar, uronic acid, and ginsenoside composition, and had increases in absorbable ginsenosides such as Rh_1, Rg_3, Rg_5, and Rk_1.

Collectively, our results indicate that *Ph. linteus* is a very useful tool in the structure modification and metabolism study of ginseng, as well as for the preparation of minor ginsenosides and intestinal bacterial metabolites from ginseng extract, which possess both selectivity and efficiency.

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