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Microbial Conversion of Ginsenoside from the Extract of Korean Red Ginseng (*Panax ginseng*) by *Lactobacillus* sp.

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Abstract

Thirty-four strains of *Lactobacillus* species were isolated from soil and eight of these isolates (M1-4 and P1-4) were capable of growing on red ginseng agar. The M1 and P2 strains were determined to be *L. plantarum* and other strains (M2, M3, M4, P1, P3 and P4) were determined to be *L. brevis*. Fermentation of red ginseng extract (RGE) with strains M1, M2, P2 and P4 resulted in a low level of total carbohydrate content (174.3, 170.0, 158.8 and 164.8 mg/mL, respectively). RGE fermented by M3 showed a higher level of uronic acid than the control. The polyphenol levels in RGE fermented by M1, P1 and P2 (964.9, 941.7 and 965.3 μg/mL, respectively) were higher than the control (936.8 μg/mL). Total saponin contents in fermented RGE (except M1) were higher than the control. RGE fermented by M2 and M3 had the highest levels of total ginsenosides (31.7 and 32.7 mg/mL, respectively). The levels of the ginsenoside Rg_3 increased from 2.6 mg/mL (control) to 3.0 mg/mL (M2) or 3.1 mg/mL (M3). RGE fermented by M2 and M3 also had the highest levels of Rg5＋Rk1 (7.7 and 8.3 mg/mL, respectively). Metabolite contents of ginsenoside (sum of CK, Rh1, Rg5, Rk1, Rg3 and Rg2) of M2 (13.0 mg/mL) and M3 (13.9 mg/mL) were also at a high level among the fermented RGE. Protopanaxadiol and protopanaxatriol content of ginsenoside of M2 (10.9 and 5.4 mg/mL, respectively) and M3 (11.0 and 5.7 mg/mL, respectively) were at higher levels than other fermented RGE.

Key words: *Lactobacillus* sp., red ginseng, ginsenoside, microbial conversion

INTRODUCTION

Ginseng (the root of *Panax ginseng* C.A. Meyer, Araliaceae) is frequently taken orally as a traditional herbal medicine in Asian countries. The major components of ginseng are ginsenosides, which are glycosides with a dammarane skeleton aglycone (1-3). These ginsenosides are reported to have anti-inflammatory activity (4), and anti-tumor effects (inhibition of tumor-induced angiogenesis and the prevention of tumor invasion and metastasis) (5,6).

Ginsenosides, glycosides with steroids or triterpenes as aglycons, are an important class of physiologically active compounds found in many herbs. In recent years, the specific type of sugar chain of each ginsenoside was found to be closely related to that compound's biological activity. Modification of the sugar chains of these ginsenosides appears to markedly change their biological activity (7-9). The pharmacological activities of ginsenosides have been explained by the biotransformation of ginsenosides by human intestinal bacteria (10,11). Several studies have shown that the transformation of ginsenosides into deglycosylated ginsenosides is required for them to have more effective *in vivo* physiological actions (12,13). Therefore, the deglycosylation process of ginsenoside is crucial for its pharmacological expression. Intestinal bacteria, however, are very changeable in dependence of host conditions, including diet, health, and even stress. Indeed, bacterial ginsenoside-hydrolyzing potentials have been shown to differ among humans and experimental mice (14).

Various transformation methods of ginsenosides, including mild acid hydrolysis (15), enzymatic conversion and microbial conversion (16), have been used; however, these chemical methods produce side reactions such as epimerization, hydration and hydroxylation, and most of the microorganisms used for the transformation of ginsenosides are not of a food-grade standard. Ko et al. (17) and Bae et al. (18) reported the biological activities of fermented ginseng with *Bifidobacterium*. This species requires the strictest anaerobic organisms. Therefore, we screened the *Lactobacillus* species, which are aerobic bacteria capable of metabolizing ginsenosides, and investigated changes in total carbohydrate content, uronic

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acid levels, and polyphenol and ginsenoside levels during fermentation.

MATERIALS AND METHODS

Materials

Six-year-old red ginseng extract (RGE) was purchased at a ginseng market in Geumsan, Korea. Standard ginsenosides including CK, Rh2, Rh1, Rg5, Rk1, Rg2, Rg3, Rg1, Rf, Re, Rd, Rb2, Rc and Rb1 were purchased from Embo Laboratory (Daejeon, Korea). All other chemicals were obtained from local suppliers and were of analytical grade.

Screening and identification of microorganisms for the fermentation of RGE

Microorganisms were isolated from the commercial ginseng products. The strains were isolated from colonies cultured on PDA and MRS agar plates. Next, the strains with high activity on these two substrates were subjected to further selections.

The next round of screening employed various procedures, including the use of total saponins of ginseng as the sole carbon source. To isolate the most efficient red ginseng-fermentation strains, red ginseng agar (5 g of red ginseng powder and 2 g of agar in 100 mL) was used. The strains were serially diluted (from 10^5 to 10^7), spread to red ginseng (5%) agar plates and incubated at 37°C for 48 hr. The strains were isolated from colonies cultured on these plates.

After isolating the strains used for further analysis, the 16S rRNA gene sequences of the bacteria were sequenced by Genotec (Daejeon, Korea). Sequencing was performed using the Applied Biosystems (Foster City, CA, USA) Prism™ Bigdye™ Terminator Cycle Sequencing Ready Reaction Kit V.3.1 in combination with an Applied Biosystems 3730XL Capillary DNA Sequencer machine with primer sets p27F and p1492R (19). The phylogenic relationship of the isolates was determined by comparing the sequencing data with the related 16S rRNA gene sequences in the GenBank database of the National Center for Biotechnology Information, via BLAST search. The phylogenetic trees were constructed by using the Jukes and Cantor algorithm and neighbor-joining method (20). In order to determine the stability of our phylogenetic tree, the sequence data were sampled 1,000 times for bootstrap analysis using Mega version 3.1 with Kimura 2-parameter distances.

Carbohydrate fermentation

Ten carbohydrates were used to investigate the responses of 8 lactobacilli-like isolates to carbohydrate fermentation. Cells were grown on MRS agar for 24 hr at 37°C, harvested using a sterile loop and resuspended in MRS sugar-free broths to which to the individual sugars of API 50 CH strips (API System, Montalieu, Vercieu, France) were added and incubated at 37°C. Observations of color changes were made at 24, 48 and 72 hr. A positive fermentation reaction occurred when the color changed from purple to yellow.

Fermentation of RGE

Red ginseng extract (60 brix) was diluted with water (1:2, v/v) and adjusted to a pH of 6.0. Next, 50 mL each of diluted ginseng extract was poured into a 250 mL Δ-flask and sterilized at 121°C for 15 min. *Lactobacillus* sp. (CFU of 10^9) isolated from the ginseng extracts were then inoculated in a 1.0 mL suspension. Finally, the diluted extracts were incubated at 37°C for 5 days with mild shaking.

Bacterial strains were precultured in MRS broth (Difco) containing 0.05% (w/v) L-cysteine·HCl under mild aerobic conditions overnight at 37°C, subcultured in MRS broth and used for the fermentation of ginseng extract.

HPLC analysis of ginsenosides

A round-bottom flask fitted with a cooling condenser was used to perform saponin extraction according to the method described by Ando et al. (21). Extraction with 70% ethanol was carried out 3 times at 70°C. Each extraction was performed for eight hours. The extract obtained was evaporated using a rotary evaporator under vacuum at 45°C. The evaporated residue was dissolved in 100 mL of distilled water and washed with 100 mL of diethyl ether. The aqueous layer was extracted 3 times with 100 mL of water-saturated *n*-butanol. The butanol solution was washed with 100 mL of distilled water to remove impurities and to yield crude saponins. The remaining butanolic solution was transferred to the tared round bottom flask for evaporation using a rotary evaporator under vacuum at 60°C.

The levels of 14 major ginsenosides were analyzed using an HPLC-based technique developed by Kim et al. (22). The HPLC system used was an ACME 9000 HPLC (Young Lin Instrument Co., Ltd., Anyang, Korea) with an ELSD detector. A Prevail carbohydrate ES column (4.6×250 mm, Alltech Associates, Inc., Illinois, USA) was also used. The solvent flow rate was held constant at 0.8 mL/min. A column oven was used to fix the column temperature at 35°C. The mobile phase used for the separation consisted of solvent A (acetonitrile : water : IPA = 80:5:15) and solvent B (acetonitrile : water : IPA = 67:21:12). A gradient elution procedure was used as follows: 0～28 min 90% A, 28～35 min 15% A, 35～45 min 20% A, 45～50 min 25% A, 50～51 min 10% A,

51～57 min 0% A, 57～58 min 75% A, and 58～65 min 90% A. The injection volume used for analysis was 20 μL. Peak identifications were based on retention times and comparisons with injected standard samples. All solutions were filtered through 0.45 μm membrane syringe filters (Millipore Co.) prior to analysis. To determine calibration curves, the ginsenoside standards, CK, Rh2, Rh1, Rg5, Rk1, Rg2, Rg3, Rg1, Rf, Re, Rd, Rb2, Rc and Rb1, were dissolved individually in HPLC-grade methanol and adjusted to the appropriate concentrations and quantities. The level of total ginsenosides was determined by summing the levels of the 14 ginsenosides.

Analytical methods

Total polyphenol (TP) content was determined using the Folin-Ciocalteu method (23) adapted to a microscale using gallic acid as standard (50～800 μg/L). Total carbohydrate and uronic acid levels were determined using the phenol-H_2SO_4 (24) and *m*-hydroxydiphenyl methods (25), respectively, using glucose and galacturonic acid as the respective standards.

In all cases, analyses were performed in triplicate, unless otherwise specified. These values were averaged and standard deviations (SD) were calculated. All data were analyzed by one-way analysis of variance and Duncan's multiple range tests using the SPSS version 10.0 software (SPSS, Chicago, IL). Results were considered significant at $p<0.05$.

RESULTS

16S rRNA analysis and carbohydrate fermentation of the isolated strain

Thirty-four strains of LAB were isolated from soil and eight of these isolates were capable of growing on red ginseng agar. Growth on this agar is a requirement of bacteria capable of fermentating RGE. The isolates were a Gram-positive strain of a coccus-type cell, and formed creamy, opaque and circular colonies on these plates. The isolates were determined to be *Lactobacillus* sp. by examination of their metabolic characteristics (data not shown). Also, the 16S rRNA gene sequences of the isolates showed 99% or 100% homology with *Lactobacillus* sp. The M1 and P2 strains were determined to be *L. plantarum*. All other strains (M2, M3, M4, P1, P3 and P4) were determined to be *Lactobacillus brevis*.

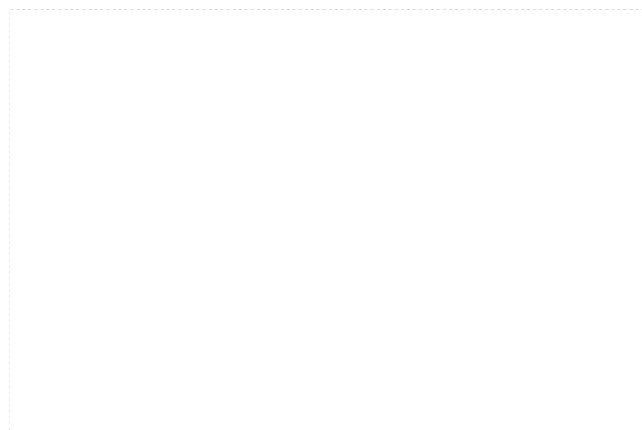

Fig. 1. Phylogenetic placement of 16S rDNA sequences from sample M (M1-M4) and P (P1-P4). The branching pattern was generated by the neighbor-joining method. Bootstrap values are expressed as percentage per 1,000 replicates. Bar = 0.01 substitutions per nucleotide position.

The primary structures of the 16S rDNA sequences were compared with each other and with those of closely related reference strains. A tree depicting the phylogenetic position is shown in Fig. 1. All of the sequences were placed within the *Lactobacillu*s species. P4 clone clustered with the *L. brevis* B 5401 and P1 clone clustered with the *L. brevis* NRIC 0137. M4 sequence was related to the *L. brevis* BFE 8359. The sequence of clone M2 and P3 were associated with the *L. brevis* ATCC 14687. One clone (M3) was related to the *L. brevis* NRIC 0138. Two clones (M1 and P2) were associated with the *L. plantarum* Ru2-1i.

Homology values were determined for a continuous stretch of 1530 nucleotides (Table 1). Very high sequence homologies (ca. 99%) were also displayed between the following pairs of species: M1 and *L. plantarum* Ru2-li; P2 and *L. plantarum* Ru2-li; the other strains (M2, M3,

Table 1. Percentage homologies for a 1530-nucleotide region of 16S rRNAs of *Lactobacillus* isolates

Strain	Percentage homologies					
	L. brevis					*L. plantarum*
	B5401	NRIC0137	BFE8359	ATCC14687	NRIC0138	Ru2-li
M1	91.5	90.6	92.4	92.6	91.3	99.8
M2	99.5	98.8	98.9	99.9	99.9	92.4
M3	99.2	99.2	99.0	98.7	99.5	90.2
M4	98.6	98.8	99.9	99.3	98.9	93.3
P1	99.9	99.9	99.0	99.1	98.8	91.3
P2	90.7	93.2	94.3	93.7	92.3	99.9
P3	98.9	99.1	99.3	98.8	99.3	93.9
P4	99.8	99.9	99.5	99.2	99.0	90.2

Table 2. Carbohydrate utilization profiles of *Lactobacillus* isolates

Strain	Carbohydrate utilization									
	Glucose	Mannose	Starch	Raffinose	Lactose	Mannitol	Arabinose	Ribose	Xylose	Galactose
M1	+	+	−	−	+	−	−	+	−	+
M2	+	+	−	+	+	−	+	+	+	+
M3	+	+	−	+	+	−	+	+	+	+
M4	+	+	−	+	+	−	+	−	+	+
P1	+	+	−	−	+	−	+	+	+	+
P2	+	+	−	+	+	−	−	+	+	+
P3	+	+	−	+	+	−	+	+	+	+
P4	+	+	−	+	+	−	+	+	−	+

Table 3. Broth pH and viable cell number of *Lactobacillus* sp. following the fermentation of RGE

Strain	Control	M1	M2	M3	M4	P1	P2	P3	P4
pH	5.6	3.67	4.29	4.31	4.28	4.04	3.57	4.29	4.03
Viable cell number (CFU/mL)	not found	4.2×10^3	1.3×10^7	1.6×10^2	1.0×10^3	9.8×10^6	2.4×10^5	7.3×10^6	7.2×10^6

M4, P1, P3 and P4) and *L. brevis* strains (B5401, NRIC0137, BFE8359, ATCC 14687 and NRIC0138).

Ten carbohydrates were used to investigate the responses of 8 lactobacilli-like isolates to carbohydrate fermentation. The results indicated that all the isolates could use glucose, galactose and lactose, while almost all could not use starch and mannitol (Table 2). M1 and P2 could and not use arabinose, but the other strains could use arabinose. M1 and P2 strain showed a different utilization of carbohydrate with the other strains.

Total carbohydrate, uronic acid and polyphenols content of the fermented RGE

The pH and viable cell numbers were determined after RGE fermentation and are shown in Table 3. Broth pH dropped from 5.60 to 3.57～4.31 (Table 3), which may be the result of lactic acid production during fermentation. The number of viable cells at the end of fermentation increased from 0 to 1.6×10^2～1.3×10^7 (Table 3). Especially, strain M1, M2 and M3 showed pH 3.67, 4.31 and 4.28 and low levels of viable cell numbers after fermentation of ginseng. The strains might have weak acid-tolerance.

Total carbohydrate and uronic acid contents of the RGEs fermented with the various isolates are presented in Fig. 2. Control (non-fermentation) conditions showed a high level of total carbohydrate content (224.3 mg/mL). Total carbohydrate contents of fermented RGE ranged from 158.8 to 200.9 mg/mL. The growth of *Lactobacillus* sp. causes a decrease of total carbohydrate contents after fermentation. Fermentation with strains M1, M2, P2 and P4 resulted in a low level of total carbohydrate content (174.3, 170.0, 158.8 and 164.8 mg/mL, respectively). The total carbohydrate content of fermented RGE was not significantly different among the strains ($p<0.05$).

The levels of uronic acid (an acidic polysaccharide) following RGE fermentation are presented in Fig. 2. M3-fermented RGE and M4-fermented RGE had higher levels of uronic acid than the control, although the difference between M4-fermented RGE and the control was

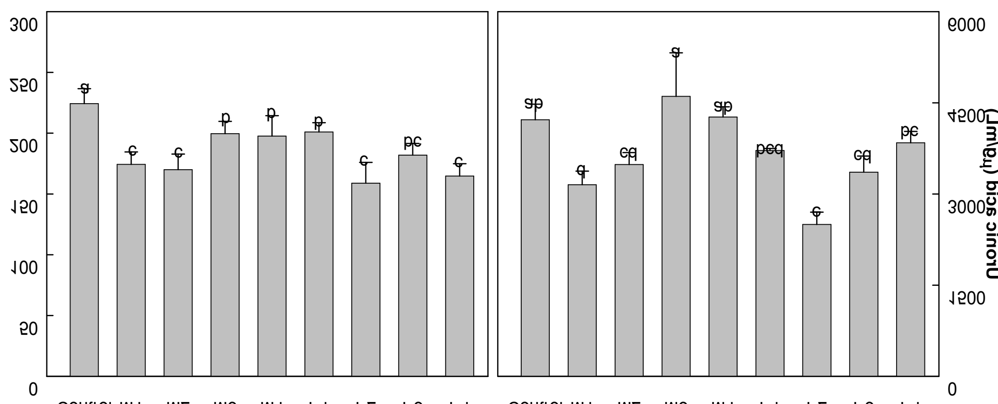

Fig. 2. Total carbohydrate and uronic acid content of the fermented RGE. Results are shown as mean±SD of the carbohydrate and uronic acid content. Means with the same letters are not significantly different at $p<0.05$.

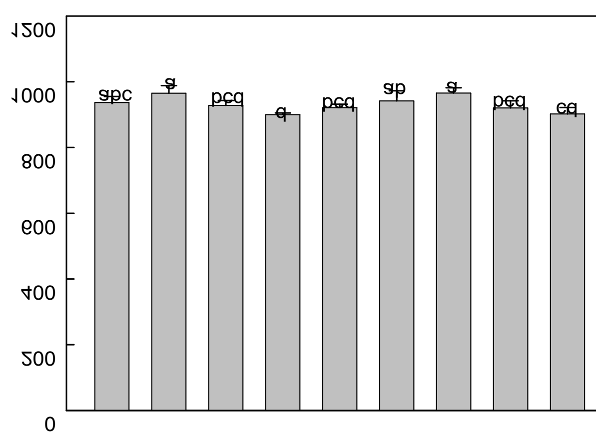

Fig. 3. Polyphenol contents of the fermented RGE. Results are shown as mean ± SD of the polyphenol contents. Means with the same letters are not significantly different at $p<0.05$.

not significant. RGE fermented by all other strains had lower levels of uronic acid than the control.

Polyphenolic compounds are considered to be secondary metabolites that are synthesized in plants and which function as defense mechanisms in response to various stress conditions (26). The observed increase of total polyphenols in the roots of *P. ginseng* was accompanied by an increase in flavonoids, total proteins and antioxidant activity. This indicates that CO_2 not only induces the synthesis of polyphenolic compounds but also maintains higher enzymatic activities. Induced polyphenol accumulation may also have an impact on the biological activities of ginseng.

As shown in Fig. 3, the polyphenol contents of RGE fermented by M1, P1 and P2 (964.9, 941.7 and 965.3 μg/mL, respectively) were at higher levels than control (936.8 μg/mL). However, there were no significant differences ($p<0.05$) among the strains. RGE fermented by the other strains showed low levels of polyphenols compared to the control.

Changes in the ginsenoside composition of fermented RGE

Ginseng saponins (ginsenosides) are the principal components in RGE having pharmacological and biological functions, such as anti-diabetic and anti-tumor activities (5,27). To date, more than 30 different ginsenosides have been isolated and identified from ginseng saponins.

The ginsenoside compositions of RGEs are shown in Table 4 and Fig. 4. Total ginsenoside contents in fermented RGE (except M1) were higher than the control. RGE fermented by M2 and M3 had the highest levels of total ginsenosides (31.7 and 32.7 mg/mL, respectively). The levels of the ginsenoside Rg3 increased from 2.6 mg/mL (control) to 3.0 mg/mL (M2) or 3.1 mg/mL (M3). RGE fermented by M2 and M3 also had the highest levels of Rg5 + Rk1 (7.7 and 8.3 mg/mL, respectively). Metabolite contents of ginsenoside (sum of CK, Rh1, Rg5, Rk1, Rg3 and Rg2) of M2 (13.0 mg/mL) and M3 (13.9 mg/mL) were also at high levels among the fermented RGE (Table 4 and Fig. 4). Protopanaxadiol ginsenoside levels are reflected in the sum of Rb1, Rd and Rg3. The levels of protopanaxatriol ginsenosides are found by summing Rg1, Rg2, Re, Rf and Rh1. Protopanaxadiol and protopanaxatriol content of the ginsenosides of M2 (10.9 and 5.4 mg/mL, respectively) and M3 (11.0 and 5.7 mg/mL, respectively) were found to be at higher levels than other fermented RGE.

Table 4. Ginsenoside compositions of RGE fermented by *Lactobacillus* sp.

Ginsenoside	Concentration (mg/mL)[1]								
	Control	M1	M2	M3	M4	P1	P2	P3	P4
CK[2]	0	0	28.2	28.6	19.6	0	0	24.7	0
Rh2	0.6	0.6	0.7	0.7	0.6	0.6	0.7	0.7	0.7
Rh1	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.4	0.3
Rg5+Rk1	6.7	6.8	7.7	8.3	7.2	6.9	7.4	7.3	7.2
Rg2	1.7	1.8	2.0	2.2	1.9	1.8	2.0	1.9	1.8
Rg3	2.6	2.5	3.0	3.1	2.7	2.6	2.9	2.8	2.7
Rg1	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.4	0.4
Rf	0.8	0.8	0.9	0.9	0.8	0.8	0.8	0.9	0.8
Re	1.5	1.4	1.7	1.7	1.4	1.5	1.5	1.6	1.5
Rd	2.8	2.7	3.2	3.2	2.8	2.8	3.0	3.0	2.9
Rb2+Rc	5.9	5.8	7.0	6.9	5.9	5.9	6.3	6.4	6.2
Rb1	3.9	3.7	4.7	4.8	4.1	4.1	4.0	4.2	4.2
Metabolite[3]	11.3	11.4	13.0	13.9	12.1	11.6	12.7	12.4	12.2
Total	27.2	26.7	31.7	32.7	28.1	27.6	29.4	29.6	29.0

[1]The unit of ginsenoside concentration was mg/mL except CK.
[2]CK was compound K and its unit was μg/mL.
[3]Metabolite = CK + Rh1 + Rg5 + Rk1 + Rg3 + Rg2.

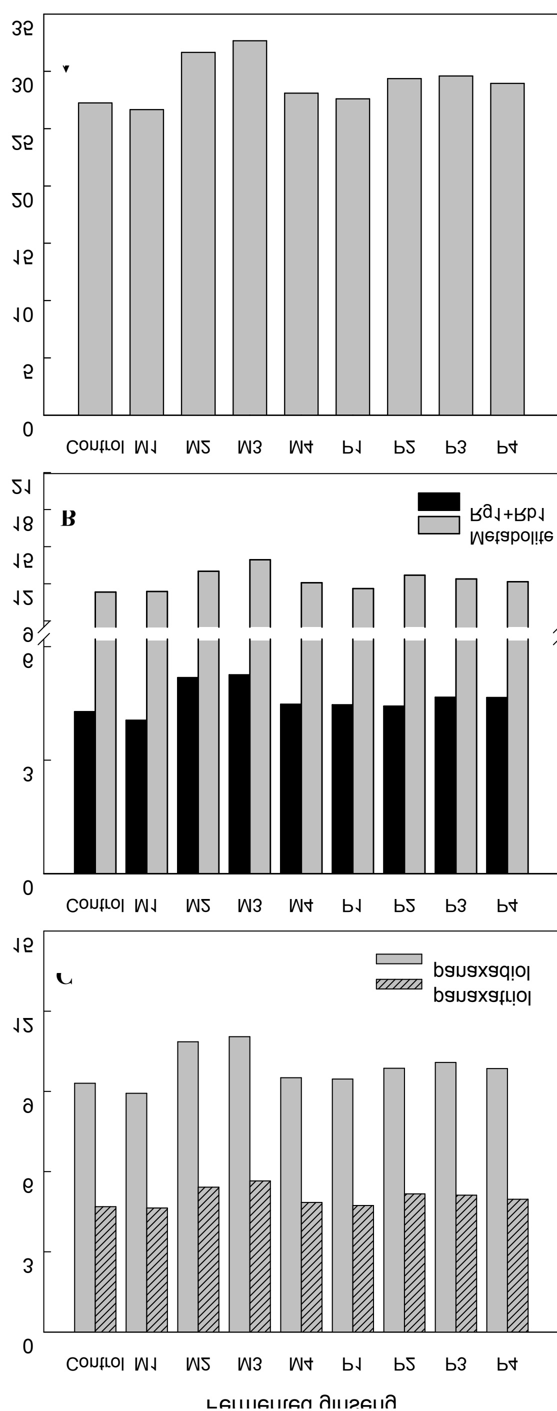

Fig. 4. Ginsenoside contents of the fermented RGE by *Lactobacillus* sp.

Protopanaxadiol ginsenosides such as Rb1, Rb2 and Rc have previously been shown to be metabolized by human intestinal bacteria to their final derivative, CK. CK levels in RGE fermented by M2, M3, M4 and P3 were 28.2, 28.6, 19.6 and 24.7 μg/mL, respectively.

DISCUSSION

Orally ingested ginsenoside passes through the stomach and small intestine without decomposition by either gastric juice or liver enzymes into the large intestine, where ginsenoside is deglycosylated by colonic bacteria followed by transit to the circulation. Therefore, the deglycosylation process of ginsenoside is crucial for its pharmacological expression (14). The decomposition by colonic bacteria into the large intestine led to efforts to use microbial conversion (12,15,25) and enzymatic conversion (14,26) of ginsenosides to create viable nutrition products. Although several cases of ginsenosides being transformed by microorganisms have been identified, investigations of the transformation pathways have been limited to only a few microorganisms. In addition, most of the microorganisms used for the transformation of ginsenosides in previous studies were inedible, and therefore could not be used for nutritional purposes. To determine which microorganisms might be applied for the bioconversion of ginsenoside in the creation of nutritional products, we isolated eight potential microorganisms that were involved in the fermentation of RGE. The eight microorganisms were identified as either *L. brevis* or *L. plantarum* strains.

Kim et al. (28) were the first to identify aerobic bacteria from soil that were capable of converting the ginsenoside Rb1. There have been reports on microbial sources capable of converting the major ginsenoside Rb1 to ginsenoside Rd. β-Glucosidases from the human intestinal bacteria, *Bifidobacterium* sp., *Eubacterium* sp. and *Fusobacterium* sp., hydrolyzed ginsenoside Rb1 to ginsenoside Rd (29,30). Fungal conversion, using *Rhizopus stolonifer* and *Curvularia lunata*, has been also reported (31). Because aerobic bacteria grow faster and produce enzymes in greater quantities than human intestinal bacteria (32,33) and fungi, aerobic bacteria can more effectively be used for large scale enzyme preparations. Furthermore, the cultivation of human intestinal bacteria requires anaerobic space and a medium with a high concentration of nutrients, and their cultivation is not as simple as that of aerobic bacteria. However, the isolated strains were facultative aerobes and could be used more effectively for the fermentation of RGE.

Acidic polysaccharides obtained from plant sources have been shown to exhibit a variety of biological activities, including immunostimulatory, antioxidant, antitumor and antiviral properties (34). After fermentation, total carbohydrate and uronic acid contents showed a slight decrease in fermented RGE (Fig. 2); however, the RGE fermented by M3 showed a slight increase in uronic acid contents.

After fermentation, the M2 and M3 strains showed effective bioconversion of ginsenoside. In particular, levels of Rh1, Rh2 Rg2, and Rg3 were evident in RGE

fermented by M2 and M3 (Table 4).

Previously, the biotransformation of Rb1 into Rh2 via Rd and Rg3 by the fungus *Rhizopus stolonifer* was reported (31). Bae et al. (29) reported that Rb1 and Rb2 were not metabolized to Rh2 by human intestinal bacteria. They suggested that Rb1 and Rb2 were transformed into Rg3 in the stomach prior to absorption in the form of Rh2. They base this claim on the observation that human intestinal bacteria such as *Bacteroides* sp., *Fusobacterium* sp. and *Bifidobacterium* sp. were able to transform Rg3 into Rh2. Otherwise, Rb1 and Rb2 were presumed to be metabolized to CK in the human intestine. Since *L. delbreuckii* is a safe bacterium, *L. delbreuckii*-treated ginsenosides would be an effective source for providing Rh2 when the intake of Rh2 is desired.

The functions of Rh1, Rh2 and Rg2, which are the specific final products for the enzymes characterized in this study, have been reported. The major activities of Rh2 include inhibition of the initiation and progression of tumor cells (35). Rh1 possesses anti-allergic and anti-inflammatory activities (36). The production of nitric oxide by IFN-gamma plus LPS-treated macrophages is markedly reduced by Rh1 or Rh2 (in a dose-dependent manner), but is not inhibited by Rb1, Rc or Re (37).

CK induces an anti-metastatic or anti-carcinogenic effect by blocking tumor invasion or by preventing chromosomal activation and tumorigenesis (38). Kudo et al. (39) suggested that Rg2 decreases the acetylcholine-evoked secretion of catecholamines from cultured bovine adrenal chromaffin cells.

According to the above results, the M2 and M3 strains showed high levels of Rh1, Rh2, Rg2, Rg3 and CK (Table 4 and Fig. 4). According to the above results, the M2 and M3 strains showed high levels of metabolite ginsenoside (sum of CK, Rh1, Rg5, Rk1, Rg3 and Rg2) (Table 4 and Fig. 4). The isolated strains (M2 and M3) showed an effective bioconversion of ginsenoside. Therefore, the M2 and M3 strains were suitable for the fermentation of RGE.

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