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Differential Transformation of Ginsenosides from *Panax ginseng* by Lactic Acid Bacteria

CHI, HYUN[1], BO HYUN LEE[1], HYUN JU YOU[1], MYUNG SOO PARK[2,3], AND GEUN EOG JI[1,3]*

[1]*Department of Food and Nutrition, Seoul National University, Seoul 151-742, Korea*
[2]*Department of Hotel Culinary Art, Anyang Technical College, Anyang 430-749, Korea*
[3]*Research Center, BIFIDO Co., Ltd., Hongchon 250-804, Korea*

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Abstract Ginsenosides have been regarded as the principal components responsible for the pharmacological and biological activities of ginseng. The transformation of ginsenosides with live lactic acid bacteria transformed ginsenosides Rb2 and Rc into Rd, but the reactions were slow. When the crude enzymes obtained from several lactic acid bacteria were used for transformation, those from *Bifidobacterium* sp. Int57 exhibited the most potent transforming activity of ginsenosides to compound K. In comparison, a relatively higher level of Rh2 was produced by the enzymes from *Lactobacillus delbrueckii* and *Leuconostoc mesenteroides*. These results suggest that it is feasible to develop a specific bioconversion process to obtain specific ginsenosides using the appropriate combination of ginsenoside substrates and specific microbial enzymes.

Key words: Ginsenosides, transformation, lactic acid bacteria

Ginseng (the root of *Panax ginseng* C.A. Meyer; family Araliaceae) is frequently used as a drug, taken orally, in the traditional medicines of China, Korea, Japan, and other Asian countries for the treatment of various diseases. Ginseng saponins (ginsenosides) have been regarded as the principal components responsible for the pharmacological and biological activities of ginseng, such as antioxidation in the central nervous system, cardiovascular system, antiaging, anti-inflammation [10, 19, 24], antidiabetic [5], and antitumor [20, 22] activities.

Over 30 kinds of ginsenosides have so far been isolated and identified from raw ginsengs or from various processed ginseng products. The abbreviations of the ginsenosides referred in this study are shown in Table 1. The main ginsenosides are glycosides that contain an aglycone with a dammarane skeleton, and include protopanaxadiol-type saponins such as ginsenosides Rb1, Rb2, Rc, and Rd, and protopanaxatriol-type saponins such as ginsenosides Re and Rg1.

Recently, it has been 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 [1, 2, 11–13, 23]. For example, the protopanaxadiol-type saponins including Rb1, Rb2, and Rc are metabolized to compound K by human intestinal bacteria [9, 23]. Re and Rg1, belonging to protopanaxatriol-type ginsenosides, are metabolized to Rh1 or F1 by intestinal bacteria [8, 23].

Lee *et al.* [18] also suggested that the efficiency of the conversion and transformation pathways differ greatly owing to the diversity of the resident microflora between individuals. Therefore, a more uniform and targeted biological function may be achieved by producing specifically transformed ginsenosides using microorganisms.

Previous studies have shown that transformation of ginsenosides into deglycosylated ginsenosides is required for more effective *in vivo* physiological action of ginsenosides [23]. Various transformation methods, including mild acid hydrolysis [7], enzymatic conversion [15], or microbial conversion [4, 17], have been used but the chemical methods produced side reactions, such as epimerization, hydration, and hydroxylation. In addition, most of the microorganisms used for the transformation of ginsenosides are not suitable in food.

In this study, we focused on the production of specific deglycosylated ginsenosides by using ginseng extract as substrate, and enzyme preparations obtained from selected lactic acid bacteria, and characterized the microbial transformation of the ginsenosides involved.

*Corresponding author
Phone: 82-2-880-8749; Fax: 82-2-880-6282;
E-mail: geji@bifido.com

Table 1. Abbreviations for ginsenosides.

Abbreviation	Full name
Rb1	3-*O*-[β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl]-20(S)-protopanaxadiol
Rb2	3-*O*-[β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl]-20-*O*-[α-L-arabinopyranosyl-(1-6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol
Rc	3-*O*-[β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl]-20-*O*-[α-L-arabinofuranosyl-(1-6)-β-D-glucopyranosyl-]-20(*S*)-protopanaxadiol
Rd	3-*O*-[β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl]-20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol
Rg3	3-*O*-[β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol
F2	3-*O*-β-D-glucopyranosyl-20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol
Compound K	20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol
Rh2	3-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol
Re	6-*O*-[α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl]-20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxatriol
Rf	6-*O*-[α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl]-20(*S*)-protopanaxatriol
Rg1	6-*O*-β-D-glucopyranosyl-20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxatriol
Rg2	6-*O*-[α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl]-20(*S*)-protopanaxatriol
Rh1	6-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxatriol
F1	20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxatriol

MATERIALS AND METHODS

Materials

Standard Rb1, Rc, and Re were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); Rb2, F2, Rg2, Rh1, and Rh2 were purchased from LKT Laboratories (St. Paul, MN, U.S.A.); Rg1 was purchased from Wako Pure Chemical Industries (Osaka, Japan); Rd and Rg3 were purchased from BTGin (Chungnam, Korea); Rf was purchased from Molecular Neurophysiology Biomedical Research Center, Korea Institute of Science and Technology (Seoul, Korea); and compound K was a kind gift from the Department of Pharmaceutical Science, Kyung Hee University (Seoul, Korea). Six-year-old root hair of *Panax ginseng* was purchased from Kyoung-Dong Market in Seoul, Korea.

Microbial Strains and Growth Conditions

Four *Bifidobacterium* strains previously reported [18, 14] [*Bifidobacterium* sp. Int57 (Int57), *Bifidobacterium bifidum* BGN4 (BGN4), *Bifidobacterium* sp. SJ32 (SJ32), and *Bifidobacterium* sp. SH5 (SH5)] and *Leuconostoc paramesenteroides* PR [6] were used. *Lactobacillus delbrueckii* ssp. *delbrueckii* KCTC 1047 (*L. delbrueckii*) was purchased from the Korean Collection for Type Cultures (Daejeon, Korea). The bacterial strains were incubated in MRS medium (Hardy, Santa Maria, CA, U.S.A.) containing 0.05% (w/v) L-cysteine·HCl under anaerobic culture conditions, overnight at 37°C, and were subcultured in MRS medium and used for incubation of ginseng extract or preparation of bacterial cell extract [16].

Preparation of Crude Microbial Enzymes

Bacterial cells (10^9 CFU/ml in 1 l culture broth) were harvested by centrifugation (3,000 ×*g* for 30 min at 4°C), and washed twice with 50 mM phosphate buffer (pH 6.0). The bacteria were resuspended in 50 ml of the same buffer and disrupted in a cell disrupter (Stansted Fluid Power, Essex, U.K.). The supernatant was then used as crude enzyme solutions after centrifugation (5,000 ×*g* for 30 min at 4°C). The enzyme activities were calculated as described in Choi and Ji [6].

Preparation of Ginseng Saponin Extracts

Five g of *Panax ginseng* was extracted with 30 volumes of 80% methanol at 80°C for 1 h (shaking water bath; Lab. Companion BS-31, Jeiotech, Kimpo, Korea) and filtered through 3 MM filter paper (Whatman Ltd., Maidsone, England). The remainder was then extracted with 20 volumes of 80% methanol and filtered. After an additional extraction, ginseng extract was evaporated under reduced pressure to remove the methanol (Lab. Companion, Jeiotech) and then dissolved in 10 volumes of water.

Transformation of Ginseng Saponins by Live Bacterial Cells

Five-hundred μl of bacterial suspension (a concentration of 10^9 CFU/ml) was added to 7 ml of MRS medium containing 1 ml of ginseng extract, which was anaerobically incubated at 37°C for 72 h. A 1-ml aliquot of the microbial culture broth was extracted with 200 μl *n*-butanol. The *n*-butanol fraction was analyzed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Transformation of Ginseng Saponins by Crude Enzymes

The reaction mixtures (550 μl) containing 50 μl of ginseng extract and 500 μl crude microbial enzymes amounting to 2–20 mU pNP(p-nitrophenyl)-β-glucosidase activities

with 0.1–0.5 mU/mg proteins were incubated at 37°C for 48–72 h. The reaction mixture was extracted with 200 μl of *n*-butanol, and the *n*-butanol fraction was analyzed by TLC and HPLC.

Analysis of Ginsenosides by Thin-Layer Chromatography

TLC chromatography was performed on a silica gel plate ($60F_{254}$, Merck, Darmstadt, Germany). The following mixture was used as the developing solvent for TLC: $CHCl_3$-CH_3OH-H_2O (63:35:10, v/v, lower phase). Spots on TLC plates were detected by spraying with 10% H_2SO_4 followed by heating at 110°C for 10 min. Ginsenosides and transformed ginsenosides were identified and assayed by comparison with known ginsenoside standards.

Analysis of Ginsenosides by High-Performance Liquid Chromatography

HPLC-grade acetonitrile, isopropanol, and water were obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.). HPLC analyses were conducted using an HPLC pump (model 526, Alltech Associates, Deerfield, IL, U.S.A.), a normal-phase column (5 μm, 250 mm×4.6 mm; Prevail Carbohydrate ES, Alltech Associates), an evaporative light scattering detector (ELSD; model 800, Alltech Associates), and Allchrom Plus software. Elution was performed at a flow rate of 0.8 ml/min using a solvent gradient consisting of (A) acetonitrile-isopropyl alcohol-water (80:15:5) and (B) acetonitrile-isopropyl alcohol-water (67:12:21) with the following temporal profiles: 0–28 min, linear gradient from 75% A and 25% B to 15% A and 85% B; 28–35 min, from 15% A and 85% B to 20% A and 80% B; 35–45 min, from 20% A and 80% B to 25% A and 75% B; 45–50 min, from 25% A and 75% B to 10% A and 90% B; 50–51 min, from 10% A and 90% B to 0% A and 100% B; and 51–57 min, from 0% A and 100% B to 75% A and 25% B. The *n*-butanol fraction of the reaction mixture was evaporated *in vacuo* (Speed Vacuum Concentrator 4080 C; Biotron, Bucheon, Korea), and the residue was dissolved in CH_3OH that was applied to the HPLC analysis after filtration (Millex SLLHR04NL, 0.45 μm PTFE, 4-mm LH; Millipore, Bedford, MA, U.S.A.). The reaction mixture was monitored using an ELSD. Quantitative data for ginsenosides were obtained by comparison with known standards. Triplicate samples were used throughout the experiment.

RESULTS

Transformation of Ginsenosides by Live Bacterial Cells

The transformations of the ginsenosides by live bacterial cells were analyzed by TLCs, as shown in Fig. 1. HPLC analysis confirmed the TLC results (data not shown). Rb2 and Rc were converted to Rd when ginseng extract was incubated with Int57 at 37°C for 72 h (Fig. 1). The resultant Rd was not hydrolyzed further. Interestingly, a new spot below Rb1 was observed on TLC. SJ32 showed almost the same TLC profile as Int57 even though Rb2 was weakly converted to Rd. Rc was hydrolyzed at a greater level than Rb2 in SJ32. There was no significant transformation of ginsenosides when ginseng extract was incubated with SH5, *Leu. paramesenteroides*, or *L. delbrueckii* at 37°C for 72 h.

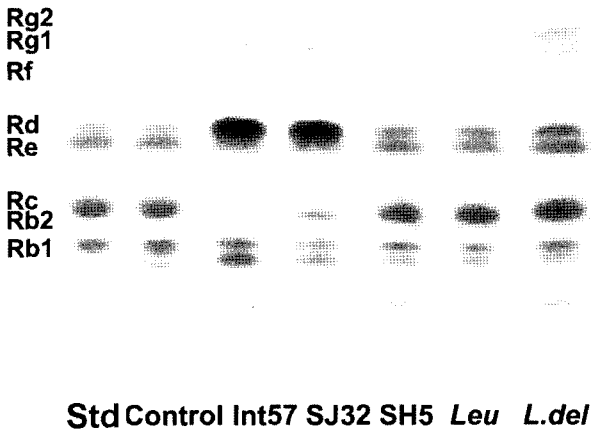

Fig. 1. The TLC (thin-layer chromatography) profiles of ginsenosides transformed by live lactic acid bacterial cells. Control indicates the non-transformed ginsenosides.

Transformation of Ginsenosides by Crude Microbial Enzymes

The transformations of the ginsenosides by crude microbial enzymes were analyzed by TLCs, as shown in Fig. 2. HPLC analysis confirmed the TLC results. One example of the HPLC results is shown in Fig. 3, where the HPLC profiles of the ginsenosides transformed by the crude enzymes from Int57 are shown. Ginseng extracts were incubated with crude enzymes from various lactic acid bacteria at 37°C for 48 or 72 h (Fig. 2A). The enzyme from Int57 transformed Rb1, Rb2, Rc, Rd, and Rf to F2 or compound K. SJ32 showed similar results to Int57, except that Rd was only weakly hydrolyzed. SH5, *Leu. paramesenteroides*, and *L. delbrueckii* exhibited weaker ginsenoside-transforming activities than did Int57 and SJ32. Most of the Rb1 and Rc and some of the Rb2 disappeared, and Rd was newly formed, but further hydrolysis was not observed in SH5. When the amount of SH5 enzyme was increased four-fold,

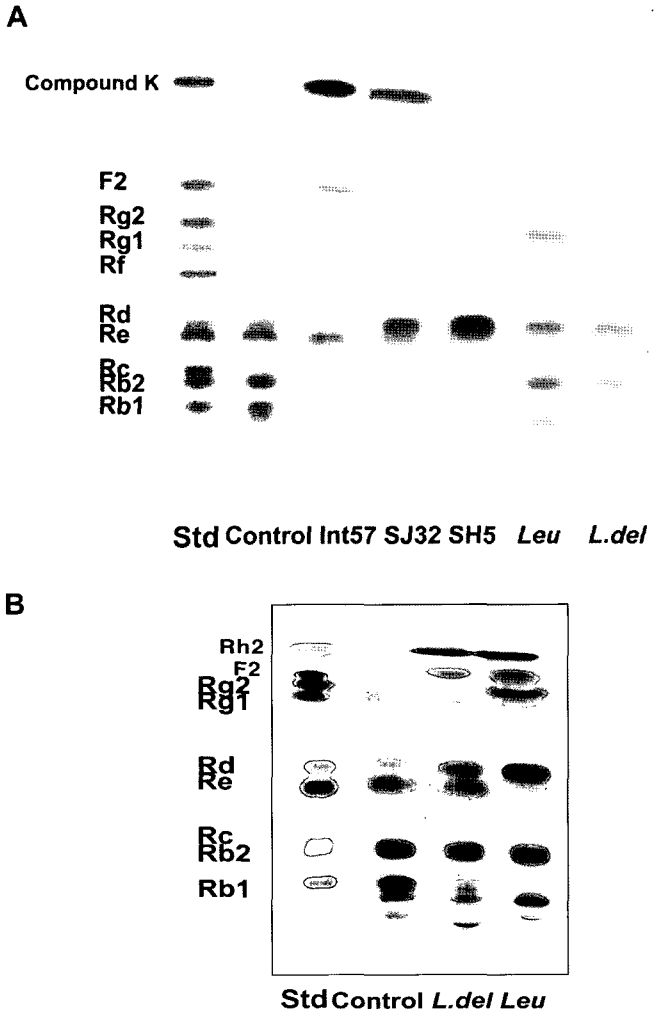

Fig. 2. The TLC profiles of ginsenosides transformed by crude enzymes from lactic acid bacteria. Control indicates the non-transformed ginsenosides.

Rb1, Rb2, and Rc were completely converted to Rd, but Rd was not transformed further (data not shown). When the amounts of enzymes from *Leu. paramesenteroides* and *L. delbrueckii* were increased four-fold, the Rb1 bands almost completely disappeared and the amount of transformed F2 and Rh2 were increased (Fig. 2B).

Discussion

Ginseng is widely used as a dietary herbal supplement worldwide. Its major components include protopanaxadiol ginsenosides such as Rb1, Rb2, and Rc, and protopanaxatriol ginsenosides such as Re and Rg1. Rb1, Rb2, and Rc have previously been shown to be metabolized by human intestinal bacteria to their final derivative, compound K, with the degree of the transformation of the ginsenosides and production of compound K differing between *Eubacterium* sp., *Streptococcus*

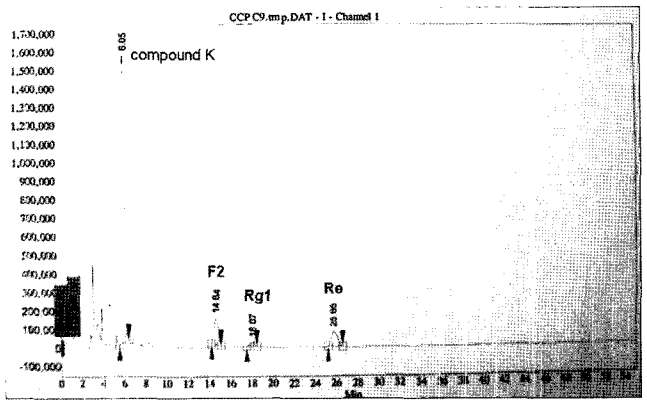

Fig. 3. The HPLC profiles of ginsenosides transformed by crude enzymes from *Bifidobacterium* sp. Int57.

sp., and *Fusobacterium* K-60 [3, 23]. Rb1 was metabolized in the rat digestive tract to Rd, F2, compound K, product VIII, and gypenoside XVII [1, 12]. In addition, Tawab *et al.* [23] reported that the protopanaxatriol ginsenosides Re and Rg1 were metabolized to F1 or Rh1, which reached the systemic circulation after oral administration in humans.

Here, we have focused on the use of lactic acid bacteria to produce and develop specific final products that would be acceptable food components after minor processing.

Transformation of ginsenosides with various live bacteria showed that *Bifidobacterium* sp. Int57 possessed relatively greater ginsenoside-transforming activity than other experimental lactic acid bacteria. In comparison with the microbial transformations with live microorganisms, a relatively large amount of compound K was produced by the crude enzymes from *Bifidobacterium* sp. Int57 and *Bifidobacterium* sp. SJ32. In comparison, crude enzymes from SH5 showed weak Rd-hydrolyzing activity, even though SH5 showed a relatively high pNPG β-glucosidase activity. In addition, commercial β-glucosidase from almond did not produce noticeable transformation of ginsenosides (data not shown). Unlike *Bifidobacterium* sp. Int57, crude enzymes from *Leu. paramesenteroides* and *L. delbrueckii* produced Rh2 as one of the major final products. These results suggest that glucosidases of various food microorganisms show different specificity on the transforming pathways of the ginsenosides. Our experimental demonstration that various lactic acid bacteria produce specific forms of ginsenosides may indicate that it is feasible to develop a specific bioconversion process to obtain specifically designed functional products by the appropriate combination of ginsenoside substrates and specific microbial enzymes.

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REFERENCES

1. Akao, T., M. Kanaoka, and K. Kobashi. 1998. Appearance of compound K, a major metabolite of ginsenoside Rb1 by intestinal bacteria, in rat plasma after oral administration---Measurement of compound K by enzyme immunoassay. *Biol. Pharm. Bull.* **21:** 245–249.
2. Akao, T., H. Kida, M. Kanaoka, M. Hattori, and K. Kobashi. 1998. Intestinal bacterial hydrolysis is required for the appearance of compound K in rat plasma after oral administration of ginsenoside Rb1 from *Panax ginseng*. *J. Pharm. Pharmacol.* **50:** 1155–1160.
3. Bae, E. A., S. Y. Park, and D. H. Kim. 2000. Constitutive beta-glucosidases hydrolyzing ginsenoside Rb1 and Rb2 from human intestinal bacteria. *Biol. Pharm. Bull.* **23:** 1481–1485.
4. Bae, E. A., M. J. Han, M. K. Choo, S. Y. Park, and D. H. Kim. 2002. Metabolism of 20(*S*)- and 20(*R*)-ginsenoside Rg3 by human intestinal bacteria and its relation to *in vitro* biological activities. *Biol. Pharm. Bull.* **25:** 58–63.
5. Chang, H. M. 1986. *Pharmacology and Application of Chinese Material Medica.* Vol 1. World Scientific, Singapore.
6. Choi, E. K. and G. E. Ji. 2005. Food microorganisms that effectively hydrolyze O-glycoside but not C-glycoside isoflavones in *Puerariae radix*. *J. Food Sci.* **70:** C25–C28.
7. Han, B. H., M. H. Park, Y. N. Han, L. K. Woo, U. Sankawa, S. Yahara, and O. Tanaka. 1982. Degradation of ginseng saponins under mild acidic conditions. *Planta Med.* **44:** 146–149.
8. Hasegawa, H., J. H. Sung, S. Matsumiya, and M. Uchiyama. 1996. Main ginseng saponin metabolites formed by intestinal bacteria. *Planta Med.* **62:** 453–457.
9. Hasegawa, H., J. W. Sung, and Y. Benno. 1997. Role of human intestinal *Prevotela oris* in hydrolyzing ginseng saponins. *Planta Med.* **63:** 436–440.
10. Huang, K. C. 1999. *The Pharmacology of Chinese Herbs.* CRC Press, Florida.
11. Kanaoda, M., T. Akao, and K. Kobashi. 1994. Metabolism of ginseng saponins, ginsenosides, by human intestinal bacteria. *J. Trad. Med.* **11:** 241–245.
12. Karikura, M., T. Miyase, H. Tanizawa, T. Taniyama, and Y. Takino. 1991. Studies on absorption, distribution, excretion and metabolism of ginseng saponins. VII. Comparison of the decomposition modes of ginsenoside-Rb1 and -Rb2 in the digestive tract of rats. *Chem. Pharm. Bull.* **39:** 2357–2361.
13. Karikura, M., H. Kato, F. Shimada, and S. Yano. 1992. Studies on the enzyme immunoassay of bioactive constituents contained in oriental medicinal drugs. VI. Enzyme immunoassay of ginsenoside Rb1 from *Panax ginseng*. *Chem. Pharm. Bull.* **40:** 314–317.
14. Kim, H. Y., J. H. Yang, and G. E. Ji. 2005. Effect of bifidobacteria on production of allergy-related cytokines from mouse spleen cells. *J. Microbiol. Biotechnol.* **15:** 265–268.
15. Ko, S. R., K. J. Choi, K. Uchida, and Y. Suzuki. 2003. Enzymatic preparation of ginsenosides Rg2, Rh1, and F1 from protopanaxatriol-type ginseng saponin mixture. *Planta Med.* **69:** 285–286.
16. Lee, B. H. and G. E. Ji. 2005. Effect of *Bifidobacterium* cell fractions on IL-6 production in RAW 264.7 macrophage cells. *J. Microbiol. Biotechnol.* **15:** 740–744.
17. Lee, B. H., H. J. You, M. S. Park, B. Kwon, and G. E. Ji. 2006. Transformation of the glycosides from food materials by probiotics and food microorganisms. *J. Microbiol. Biotechnol.* **16:** 497–504.
18. Lee, D. S., Y. S. Kim, C. N. Ko, K. H. Cho, H. S. Bae, K. S. Lee, J. J. Kim, E. K. Park, and D. H. Kim. 2002. Fecal metabolic activities of herbal components to bioactive compounds. *Arch. Pharm. Res.* **25:** 165–169.
19. Lee, F. C. 1992. *Facts About Ginseng, the Elixir of Life.* Hollyn International, New Jersey.
20. Mochizuki, M., C. Y. Yoo, K. Matsuzawa, K. Sato, I. Saiki, S. Tono-oda, K. Samukiwa, and I. Azuma. 1995. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside Rb2, 20(*R*)- and 20(*S*)-ginsenoside Rg3, of Red ginseng. *Biol. Pharm. Bull.* **18:** 1197–1202.
21. Park, S. Y., G. E. Ji, Y. T. Ko, H. K. Jung, Z. Ustunol, and J. J. Pestka. 1999. Potentiation of hydrogen peroxide, nitric oxide, and cytokine production in RAW 264.7 macrophage cells exposed to human and commercial isolates of *Bifidobacterium*. *Int. J. Food Microbiol.* **46:** 231–241.
22. Sato, K., M. Mochizuki, I. Saiki, Y. C. Yoo, K. Samukawa, and I. Azuma. 1994. Inhibition of tumor angiogenesis and metastasis by a saponin of *Panax ginseng*-ginsenoside Rb2. *Biol. Pharm. Bull.* **17:** 635–639.
23. Tawab, M. A., U. Bahr, M. Karas, M. Wurglics, and M. Schubert-Zsilavecz. 2003. Degradation of ginsenosides in humans after oral administration. *Drug Metab. Dispos.* **31:** 1065–1071.
24. Wu, J. Y., B. H. Gardner, C. I. Murphy, J. R. Seals, C. R. Kensil, J. Recchia, G. A. Beltz, G. W. Newman, and M. J. Newman. 1992. Saponin adjuvant enhancement of antigen-specific immune responses to an experimental HIV-1 vaccine. *J. Immunol.* **148:** 1519–1525.