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Microbial Conversion of Ginsenoside Rb_1 to Minor Ginsenoside F_2 and Gypenoside XVII by *Intrasporangium* sp. GS603 Isolated from Soil

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Abstract A new strain, GS603, having β-glucosidase activity was isolated from soil of a ginseng field, and its ability to convert major ginsenoside Rb_1 to minor ginsenoside or gypenoside was studied. Strain GS603 was identified as an *Intrasporangium* species by phylogenetic analysis and showed high ginsenoside-converting activity in LB and TSA broth but not in nutrient broth. The culture broth of the strain GS603 could convert ginsenoside Rb_1 into two metabolites, which were analyzed by TLC and HPLC and shown to be the minor ginsenoside F_2 and gypenoside XVII by NMR.

Keywords: Bioconversion, ginsenoside F_2, gypenoside XVII, *Intrasporangium*, ginsenoside Rb_1

Ginseng (the root of *Panax ginseng* C.A. Meyer) is one of the most popular medicinal plants, and it has been used for strengthening immunity, providing nutrition, and recovering from fatigue. Ginseng saponins (ginsenosides) have been recognized as being responsible for the biological and pharmacological activities of ginseng. For example, many studies have focused on their antitumor effects (inhibition of tumor-induced angiogenesis and prevention of tumor invasion and metastasis) [15, 18], along with their antidiabetic [21], antifatigue [20], antistress [17], antiallergic [19], and antioxidative [8] activities.

More than 40 ginsenosides have been isolated from ginseng roots, with six major ginsenosides (ginsenosides Rb_1, Rb_2, Rc, Rd, Re, and Rg_1) constituting more than 90% of total ginsenosides [16]. In recent decades, many studies have focused on the pharmacological activities of the minor ginsenosides, as their activities were found to be superior to those of the major ginsenosides. These minor ginsenosides are present in ginseng only in small percentages and can be produced by hydrolysis of the sugar moieties of the major ginsenosides. Therefore, many studies have aimed to convert major ginsenosides to the more active minor ginsenosides with methods such as heating [16], acid treatment [7], or enzymatic conversion [1, 4, 12]. Heating and acid treatment, however, degrade other active minor ginsenosides and acidic polysaccharides by randomly hydrolyzing all glycosidic bonds, which deprive of the other pharmacological activities of ginseng. Therefore, enzymatic hydrolysis of appropriate sugar at a specific position is desirable for the production of active minor ginsenosides. This preference for enzymatic conversion has led to the development of biotransformation methods. Rb_1 is the most abundant compound (23%) [9] of the ginsenosides, and its structure can be converted to 20(S)-PPD by hydrolyses of glucose moieties. Ginsenoside Rb_1 has a total of four glucose moieties at C-3 and C-20. The conversion of ginsenoside Rb_1 to PPD begins with cleavage of a terminal sugar moiety at either the C-3 or C-20 position, and is followed by stepwise cleavage of the other sugars (Fig. 1) [2, 13, 14].

The β-glucosidase-producing bacteria were isolated from soil of a ginseng-growing field, and all the isolates were tested to verify their ginsenoside Rb_1-converting activity using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) as reported in our previous study [10]. In the present study, major ginsenoside Rb_1 was converted into minor ginsenoside F_2 and gypenoside XVII by the action of microbial enzymes.

Materials and Methods

Materials

Ginsenoside Rb_1 was obtained from *Panax quinquefolius*, and standard ginsenosides including 20(S)-Rb_1, 20(S)-Rd, 20(S)-Rg_3, 20(S)-Rh_2, and compound-K were obtained from the KT&G, Daejeon, Korea. *p*-Nitrophenyl β-D-glucopyranoside (*p*-NPG) and *p*-nitrophenol (*p*-NP) were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.).

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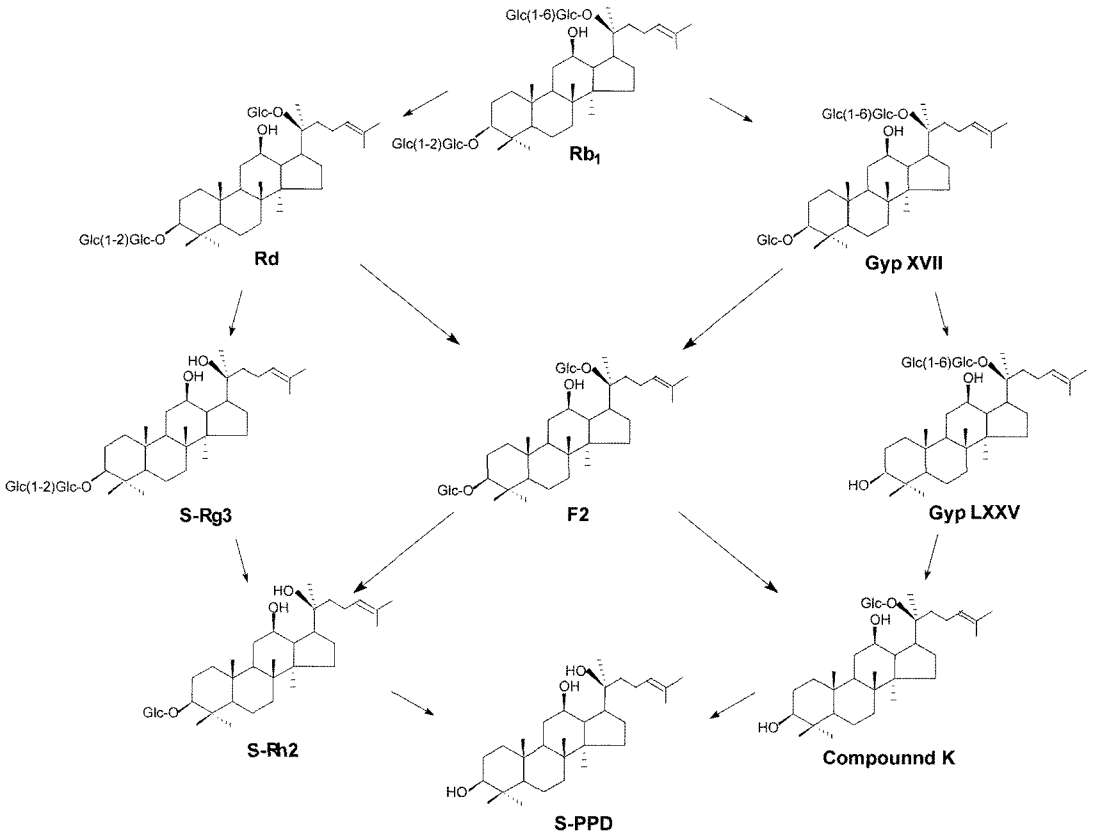

Fig. 1. Transformation pathway of ginsenoside Rb_1 to protopanaxadiols.

R2A, nutrient broth, LB (Luria-Bertani) broth, and tryptic soy broth were purchased from Difco (Miller, Becton Dickinson and Co., MD, U.S.A.). Silica gel 60 (70–230 mesh, Merck KGaA, Darmstadf, Germany) was used for column chromatography. An HPLC (NS 3000i system, FUTECS Co., Ltd., Korea) was used with a UV/Vis detector and gradient pump.

Microbial Strains and Growth Conditions

The strain GS603 was incubated in LB broth, tryptic soy broth, or nutrient broth at 27°C, 160 rpm, for 24 h. Absorbance was measured at 600 nm. Each suspension (0.5 ml) was mixed with the same volume of 1 mM ginsenoside Rb_1 dissolved in distilled water, filtered through a 0.2 μm filter and incubated at 27°C, 160 rpm, for 72 h. During the reaction, a 100-μl aliquot was taken every 12 h, extracted with *n*-butanol saturated with H_2O, and then analyzed by TLC and HPLC.

Assay of β-Glucosidase Activity

The microbial cells were removed by centrifugation (10,000 ×*g* for 15 min at 4°C) and 80 μl of the supernatant was added to 20 μl of 10 mM *p*-NPG in 20 mM sodium phosphate buffer (pH 7.0) and then the reaction mixture was incubated at 30°C for 5 min. The reaction was stopped by adding the 100 μl of 0.5 M Na_2CO_3 and the produced *p*-NP was measured at 405 nm by a UV spectrophotometer (Amersham Biosciences Ultrospec 2100 *Pro*, Cambridge, U.K.). One unit of β-glucosidase activity was defined as the amount that liberates 1 nmol of *p*-NP per min from *p*-NPG under the above reaction condition.

Phylogenetic Analysis

The almost complete sequence of the 16S rRNA gene of the strain GS603 was compiled with the SeqMan software and edited using the BioEdit program. The 16S rRNA gene sequences of the related taxa were obtained from GenBank. The phylogenetic tree was constructed *via* the neighbor-joining method using the MEGA 3.1 program. A bootstrap analysis with 1,000 replicates was also conducted to obtain confidence levels for the branches. The closest type strains were included in the phylogenetic trees.

Analysis of Ginsenosides by Thin-Layer Chromatography

TLC was performed with silica gel plates ($60F_{254}$, Merck KGaA, Darmstadf, Germany). The following mixture was used as the developing solvent for TLC: $CHCl_3$-CH_3OH-H_2O (65:35:10 v/v, lower phase). The spots on the TLC plates were detected by spraying with 10% H_2SO_4 followed by heating at 110°C for 5 min.

Analysis of Ginsenosides by High-Performance Liquid Chromatography

HPLC-grade acetonitrile and water were purchased from SK Chemicals (Ulsan, Korea). The reaction mixture was extracted with *n*-butanol saturated with H_2O, evaporated *in vacuo*, and the residue was dissolved in CH_3OH and applied to the HPLC analysis. HPLC used a C_{18} (250× 4.6 mm, ID 5 μm) column with acetonitrile (solvent A) and distilled water (solvent B) at A/B ratios of 15:85, 21:79, 58:42, 90:10, 90:10, 15:85, and 15:85, with run times of 0–5, 5–25, 25–70, 70–72, 72–82, 82–84, and 84–100 min, respectively, at a 1.6 ml/min flow rate. Detection was done at 203 nm.

Analysis of Nuclear Magnetic Resonance Spectrum

Eighty ml of suspension of strain GS603 cultured in LB broth was mixed with the same volume of 1 mM ginsenoside Rb_1 and incubated at 27°C, 160 rpm, for 48 h. The reaction mixture was extracted with 150 ml of *n*-butanol two times and evaporated *in vacuo* at 45°C. The residue was applied to the silica gel 60 (Φ 4.5×22 cm) column and eluted with $CHCl_3$-CH_3OH-H_2O (65:35:10 v/v, lower phase). Fractions of the eluate were collected in every 20 ml. The separated metabolite transformed from ginsenoside Rb_1 was dissolved in pyridine-d_5 and identified by ^{1}H-NMR and ^{13}C-NMR using FT-NMR spectrometry (400 MHz Varion Inova AS 400, Varion, U.S.A.).

Metabolite (1)

^{1}H-NMR (pyridine-d_5, 400 MHz): δ 0.75 ppm (3H, s, H-19), δ 0.91 ppm (3H, s, H-18), δ 0.93 ppm (3H, s, H-30), δ 0.94 ppm (3H, s, H-29), δ 1.25 ppm (3H, s, H-28), δ 1.56 ppm (3H, s, H-26), δ 1.61 ppm (6H, s, H-21, H-27), δ 4.89 ppm [1H, d, j=8.0 Hz, H-3-glc (inner)-1H], δ 5.03 ppm [1H, d, j= 7.6 Hz, H-20-glc (outer)-1H'''], δ 5.08 ppm [1H, d, j=7.6 Hz, H-20-glc (inner)-1H"]. Among these signals, that of 3H in the H-21 methyl overlapped with that of the H-27 methyl. ^{13}C-NMR (pyridine-d_5, 100 MHz) data are shown in Table 1.

Table 1. ^{13}C-NMR chemical shift of metabolite (1) (100 MHz; solvent: pyridine-d_5).

Carbon	Gypenoside XVII	Metabolite (1)	Carbon	Gypenoside XVII	Metabolite (1)
Aglycon moiety			Sugar moiety		
C-1	39.3	39.1	3-O-inner-Glc		
C-2	26.7	26.6	C-1'	107.0	106.8
C-3	88.9	88.8	C-2'	75.8	75.6
C-4	39.7	39.6	C-3'	78.8	78.6
C-5	56.5	56.3	C-4'	72.0	71.8
C-6	18.5	18.4	C-5'	78.4	78.2*
C-7	35.2	35.1	C-6'	63.2	63.0
C-8	40.1	40.0	20-O-inner-Glc		
C-9	50.3	50.1	C-1"	98.2	98.0
C-10	37.0	36.9	C-2"	74.8	74.8
C-11	30.9	30.7	C-3"	79.3	79.0
C-12	70.2	70.1*	C-4"	71.6	71.4
C-13	49.6	49.4	C-5"	77.1	76.9
C-14	51.5	51.3	C-6"	70.2	70.1*
C-15	30.8	30.7	20-O-outer-Glc		
C-16	26.8	26.7	C-1'''	105.4	105.1
C-17	51.7	51.6	C-2'''	75.3	75.1
C-18	16.1	16.0	C-3'''	78.4	78.2*
C-19	16.3	16.3	C-4'''	71.8	71.6
C-20	83.5	83.4	C-5'''	78.4	78.2*
C-21	22.5	22.4	C-6'''	62.9	62.7
C-22	36.3	36.2			
C-23	23.3	23.2			
C-24	126.0	125.8			
C-25	131.1	130.9			
C-26	25.8	25.8			
C-27	18.0	17.9			
C-28	28.2	28.1			
C-29	16.8	16.8			
C-30	18.0	17.4			

*Overlapped with other signals.

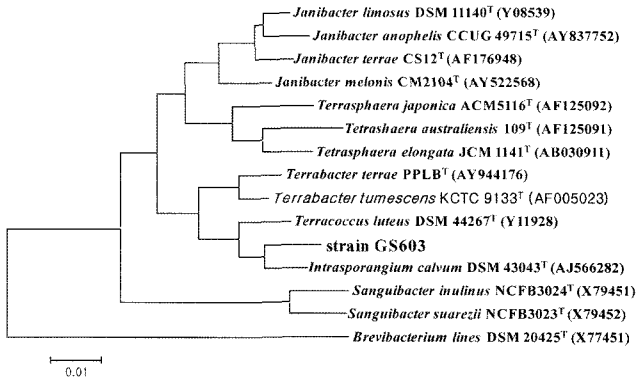

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic relationships among strain GS603 and related species.

RESULTS

Phylogenetic Study

The 16S rRNA gene sequences of the strain GS603 was aligned with those of the type strains found to have the closest taxonomic relationships. The phylogenetic tree is shown in Fig. 2. Strain GS603 was grouped with an *Intrasporangium* species, and the highest degrees of 16S rRNA gene sequence identities were to *Intrasporangium calvum* DSM 43043^T (AJ566282) (98.2%), *Terracoccus luteus* DSM 44267^T (Y11928) (96.6%), and *Terrabacter tumescens* KCTC 9133^T (AF005023) (95.4%). Therefore, the strain GS603 should be classified as an *Intrasporangium* species.

Transformation of Ginsenoside Rb_1 by Strain GS603 and TLC Assay

The growth of the strain GS603 cultured in LB broth, tryptic soy broth, and nutrient broth at 27°C for 24 h was measured by absorbance at 600 nm. The strain GS603 grew well in LB broth (Absorbance=2.231) and tryptic soy broth (Absorbance=2.482) but not in nutrient broth (Absorbance=0.292). Rb_1-transforming activities of the strain GS603 cultured in LB broth and tryptic soy broth were higher than that in nutrient broth. The enzyme activity of the strain GS603 cultured in LB broth was 1.94 U/ml and that in tryptic soy broth was 1.48 U/ml. No activity was detected in the nutrient broth. The TLC results are shown in Fig. 3. Ginsenoside Rb_1 was transformed into two types of metabolites: (1) and (2). The R_f value of metabolite (1) was slightly lower than that of ginsenoside Rd, and the R_f value of metabolite (2) was slightly above that of ginsenoside Rg_3. Reaction products (1) and (2) were separated on a silica gel column (see Materials and Methods).

Biotransformation of ginsenoside Rb_1 in time course was investigated. As shown in Fig. 3, ginsenoside Rb_1 was almost hydrolyzed after 48 h, where the level of metabolite (1) reached a maximum after 48 h and then gradually decreased, whereas metabolite (2) appeared from 36 h to 72 h. This indicated that metabolite (1) was an intermediate of metabolite (2). That is to say, ginsenoside Rb_1 hydrolyzed to metabolite (1), and then the metabolite (1) further hydrolyzed to metabolite (2).

Isolation of Ginsenosides and HPLC Assay

The enzymatic conversion products [metabolites (1) and (2)] from ginsenoside Rb_1 by the strain GS603 were separated and purified using a silica gel column. A mass of 13.9 mg (24.5%) of metabolite (2) was obtained and 48.6 mg (71.1%) of metabolite (1) was obtained after purification. As shown in Fig. 4, the peak for Rb_1 disappeared and two peaks for ginsenosides appeared. The identities of the peaks were established by comparison of retention time (t_R) values with those of standards. The presence of metabolite (1) (t_R 47 min) and metabolite (2) (t_R 54 min) was confirmed in the HPLC chromatogram. As shown in

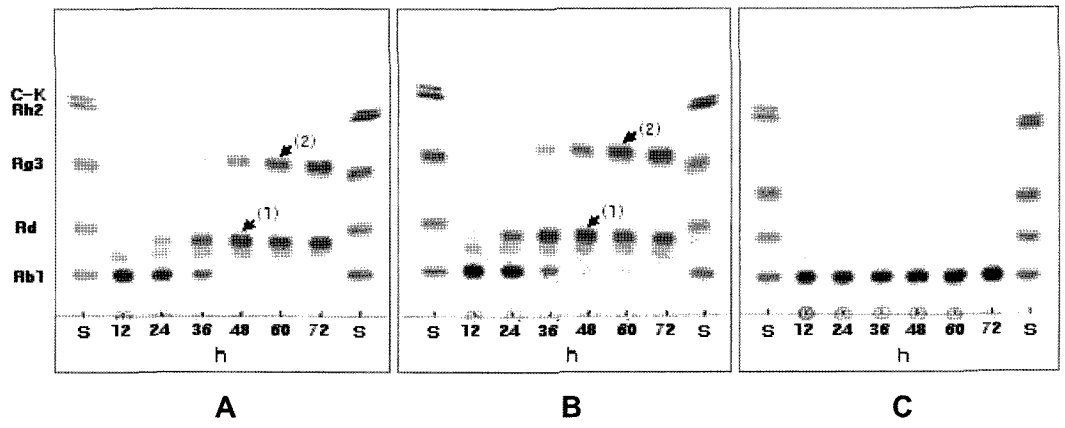

Fig. 3. TLC analysis of the reaction mixture of ginsenoside Rb_1 and strain GS603.
(**A**) LB broth; (**B**) Tryptic Soy broth; and (**C**) Nutrient broth.

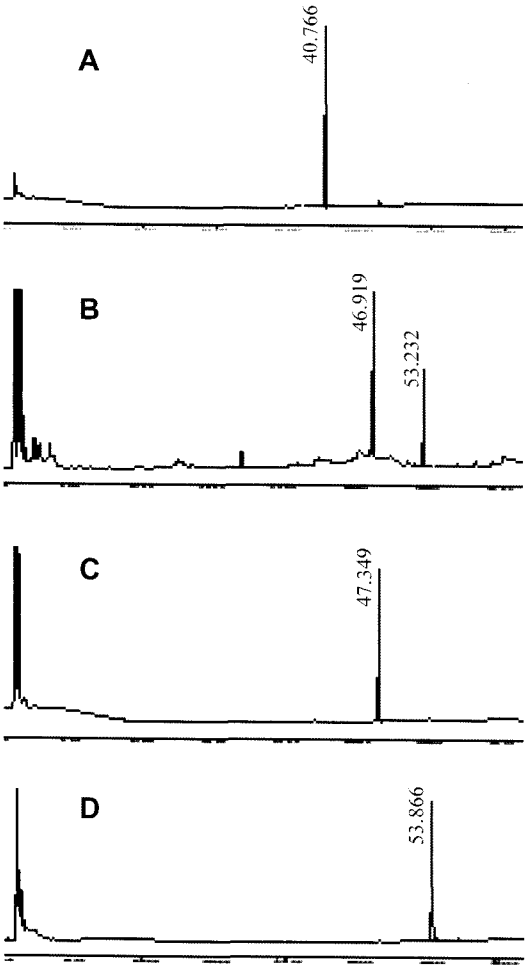

Fig. 4. HPLC analysis of ginsenoside. The reaction mixture of ginsenoside Rb_1 and strain GS603 was analyzed.
(**A**) Substrate Rb_1; (**B**) Reaction mixture; (**C**) Purified metabolite (1); and (**D**) Purified metabolite (2).

Fig. 4C and Fig. 4D, it was verified that pure metabolites (1) and (2) could be isolated through column chromatography.

Structural Identification of Metabolites

The structure of metabolite (2) (=ginsenoside F_2) has already been identified and confirmed in our previous study [3]. In the ^{1}H-NMR spectrum of metabolite (1), the anomeric proton signals for the H-1 of the 3-O-inner-glucopyranosyl moiety, 3-O-outer-glucopyranosyl moiety, and 20-glucopyranosyl moiety appeared at δ 4.89 ppm (1H, d, J=8.0 Hz), δ 5.03 ppm (1H, d, J=7.6 Hz), and δ 5.08 ppm (1H, d, J=7.6 Hz), respectively, showing that the aglycon of metabolite (1) harbored three β-D-glucoses. The anomeric proton signals of metabolite (1) showed that there was a loss of one terminal glucose at C-3 compared with that of ginsenoside Rb_1, whose anomeric proton signals appeared at 4.88 ppm [1H, d, J=7.6 Hz, H-3-glc(inner)-1'], 5.06 ppm [1H, d, J=7.7 Hz, H-20-glc(inner)-1'''], 5.09 ppm [1H, d, J=7.7 Hz, H-20-glc(terminal)-1''''], and 5.34 ppm [1H, d, J=7.6 Hz, H-3-glc(outer)-1''] [6]. ^{13}C-NMR (pyridine-d_5, 100 MHz) data are shown Table 1. A comparison of the ^{13}C-NMR spectrum of metabolite (1) with that of ginsenoside Rb_1 [6] showed that the signal for the C-2' of the 3-inner-glucose was shifted upfield, from 83.5 ppm to 75.6 ppm, but the other signals were similar to those of ginsenoside Rb_1. It is believed that the terminal glucose on C-3 of the aglycon of Rb_1 was hydrolyzed by the strain GS603, and this hypothesis is consistent with the ^{1}H-NMR data. Therefore, the metabolite (1) produced by strain GS603 from ginsenoside Rb_1 is 3-*O*-[β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl-(6,1)-β-D-glucopyranosyl]-20(S)-protopanaxadiol, identical to gypenoside XVII.

Discussion

Ginsenoside Rb_1 can be converted to gypenoside XVII by loss of a glucose moiety at the C-3 position of the ginsenoside aglycon. Ginsenoside F_2 can also be produced from gypenoside XVII by additional hydrolysis of a single glucose moiety.

Of the six major ginsenosides, only four ginsenosides (Rb_1, Rb_2, Rc, and Rd) are the protopanaxadiol (PPD)-type and can be converted to minor ginsenosides. The aglycon of ginsenosides Rb_1, Rb_2, Rc, and Rd is identical; these compounds differ only in the presence of 20-*O*-β-D-glucopyranoside, arabinopyranoside, and arabinofuranoside, respectively. Many enzymes that can hydrolyze the above glycosidic bonds are known, with β-glucosidase regarded as being the most useful [2, 11]. In the previous study, a large-scale screening for active β-glucosidase-producing bacteria was performed [10]. The Esculin-R2A agar was specially designed to screen β-glucosidase-producing bacteria. Esculin is needed to detect the β-glucosidase activity, and R2A agar is optimal for the cultivation of the bacteria dominant in soil, as this agar has a low concentration of nutrients.

This study is the first one to obtain aerobic bacteria from soil that are capable of converting ginsenoside Rb_1 to gypenoside XVII. There are reports on microbial sources able to convert the major ginsenoside Rb_1 to minor ginsenosides. β-Glucosidases from the human intestinal bacteria, *Bifidobacterium* spp., *Eubacterium* spp., and *Fusobacterium* spp. hydrolyzed ginsenoside Rb_1 to ginsenoside Rd [2]. Fungal conversion, using the fungi *Rhizopus stolonifer* and *Curvularia lunata,* has also been reported [6]. Because aerobic bacteria grow faster and produce enzymes in greater quantities than human intestinal bacteria [5, 22], aerobic bacteria can be more effectively 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.

At present, no evidence exists that the β-glucosidases from the strain GS603 play a significant role in the conversion of ginsenoside Rb_1, as other enzymes secreted from the β-glucosidase-producing microorganism can also hydrolyze ginsenoside Rb_1. Purification of the ginsenoside Rb_1-converting enzyme would verify the enzymatic reaction. In fact, most researches on the conversion of ginsenoside have been performed with purified enzymes. In China, ginsenoside-β-arabinofuranase hydrolyzing ginsenoside Rc to Rd has been purified from fresh ginseng root [24] and gypenoside-β-rhamnosidase hydrolyzing gypenoside-5 to ginsenoside Rd has also been purified and characterized [23].

The ginsenoside Rb_1-converting enzymes from the strain GS603 can be applied in industry for the production of gypenoside XVII and ginsenoside F_2 after the target enzymes for the conversion have been purified and characterized, a goal that should be included in future studies. The substrate specificity of the enzymes for the other major ginsenosides Rb_2 and Rc must also be determined. Furthermore, a kinetic analysis of the conversion over a longer period of time will be required. For example, it is assumed that the strain GS603 would be able to produce a higher amount of compound K with a longer incubation time, as compound K can be produced by further hydrolysis of a sugar from ginsenoside F_2 (Fig. 1).

Acknowledgments

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References

1. Bae, E. A., N.-Y. Kim, M. J. Han, M.-K. Choo, and D.-H. Kim. 2006. Transformation of ginsenosides to compound K (IH-901) by lactic acid bacteria of human intestine. *J. Microbiol. Biotechnol.* **13:** 9–14.
2. Bae, E. A., S. Y. Park, and D. H. Kim. 2000. Constitutive β-glucosidases hydrolyzing ginsenoside Rb_1 and Rb_2 from human intestinal bacteria. *Biol. Pharm. Bull.* **23:** 1481–1485.
3. Cheng, L. Q., M. K. Kim, J. W. Lee, Y. J. Lee, and D. C. Yang. 2006. Conversion of major ginsenoside Rb_1 to ginsenoside F_2 by *Caulobacter leidyia*. *Biotechnol. Lett.* **28:** 1121–1127.
4. Chi, H., B. H. Lee, H. J. You, M. S. Park, and G. E. Ji. 2006. Differential transformation of ginsenosides from *Panax ginseng* by lactic acid bacteria. *J. Microbiol. Biotechnol.* **16:** 1629–1633.
5. Coskun, H. and E. Öndül. 2004. Free fatty acid accumulation by mesophilic lactic acid bacteria in cold-stored milk. *J. Microbiol.* **42:** 133–138.
6. Dong, A. L., M. Ye, H. Z. Guo, J. H. Zheng, and D. Guo. 2003. Microbial transformation of ginsenoside Rb_1 by *Rhizopus stolonifer* and *Curvularia lunata*. *Biotechnol. Lett.* **25:** 339–344.
7. Han, B. H., M. H. Park, Y. N. Han, W. S. Woo, U. Sankawa, S. Yahara, and O. Tanaka. 1982. Degradation of ginseng under mild acidic condition. *Plant Med.* **44:** 146–149.
8. Kim, D. J., G. S. Seong, D. W. Kim, S. L. Go, and J. C. Jang. 2004. Antioxidative effects of red ginseng saponins on paraquat-induced oxidative stress. *Korean J. Ginseng Res.* **28:** 5–10.
9. Kim, M.-W., S.-R. Ko, K.-J. Choi, and S.-C. Kim. 1987. Distribution of saponin in various sections of *Panax ginseng* root and changes of its contents according to root age. *Korean J. Ginseng Sci.* **11:** 10–16.
10. Kim, M. K., J. W. Lee, K. Y. Lee, and D.-C. Yang. 2005. Microbial conversion of major ginsenoside Rb_1 to pharmaceutically active minor ginsenoside Rd. *J. Microbiol.* **43:** 456–462.
11. Kim, S.-J., C.-M. Lee, M.-Y. Kim, Y.-S. Yeo, S.-H. Yoon, H.-C. Kang, and B.-S. Koo. 2007. Screening and characterization of an enzyme with β-glucosidase activity from environmental DNA. *J. Microbiol. Biotechnol.* **17:** 905–912.
12. Lee, B. H., H. J. You, M. S. Park, B. K., and G. E. Ji. 2006. Transformation of the glycosides from food materials by probiotics and food microorganisms. *J. Microbiol. Biotechnol.* **16:** 497–504.
13. 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 metabolite activities of herbal components to bioactive compounds. *Arch. Pharm. Res.* **25:** 165–169.
14. Lin, M. C., K. C. Wang, and S. S. Lee. 2001. Transformation of ginsenosides Rg_1 and Rb_1 and crude Sanchi saponins by human intestinal microflora *J. Chin. Chem. Soc.* **48:** 113–120.
15. Mochizuki, M., Y. C. Yoo, and K. Matsuzawa. 1995. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside Rb_2, 20(R)- and 20(S)-ginsenoside Rg_3, of Red ginseng. *Biol. Pharm. Bull.* **18:** 1197–1202.
16. Park J. H. 2004. Sun ginseng - a new processed ginseng with fortified activity. *Food Ind. Nutr.* **9:** 23–27.
17. Saito, H., Y. Yoshida, and K. Takagi. 1974. Effect of *Panax ginseng* root on exhaustive exercise in mice. *Jpn. J. Pharmacol.* **24:** 119–127.
18. 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 Rb_2. *Biol. Pharm. Bull.* **17:** 635–639.
19. Shin, Y.-W., E.-A. Bae, M. J. Han, and D.-H. Kim. 2006. Metabolism of ginsenoside Rg5, a main constituent isolated

from red ginseng, by human intestinal microflora and their antiallergic effect. *J. Microbiol. Biotechnol.* **16:** 1791–1798.

20. Wang, B. X., J. C. Cui, A. J. Liu, and S. K. Wu. 1983. Studies on the anti-fatigue effect of the saponins of stems and leaves of *Panax ginseng* (SSLG). 1983. *J. Tradit. Chin. Med.* **3:** 89–94.
21. Xie, J. T., S. R. Mehendale, X. Li, R. Quigg, X. Wang, C. Z. Wang, J. A. Wu, H. H. Aung, P. A. Rue, G. I. Bell, and C. S. Yuan. 2005. Anti-diabetic effect of ginsenoside Re in ob/ob mice. *Biochim. Biophys. Acta* **1740:** 319–325.
22. Yoon, K. Y., E. E. Woodams, and Y. D. Hang. 2004. Probiotication of tomato juice by lactic acid bacteria. *J. Microbiol.* **42:** 315–318.
23. Yu, H., H. Liu, C. Zhang, D. Tan, M. Lu, and F. Jin. 2004. Purification and characterization of gypenoside-α-L-rhamnosidase hydrolyzing gypenoside-5 to ginsenoside Rd. *Process Biochem.* **39:** 861–867.
24. Zhang, C., H. Yu, Y. Bao, L. An, and F. Jin. 2002. Purification and characterization of ginsenoside-α-arabinofuranase hydrolyzing ginsenoside Rc to Rd from the fresh root of *Panax ginseng*. *Process Biochem.* **37:** 793–798.