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Biotransformation of Ginsenoside Rb$_1$ to Prosapogenins, Gypenoside XVII, Ginsenoside Rd, Ginsenoside F$_2$, and Compound K by *Leuconostoc mesenteroides* DC102

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Ginsenoside Rb$_1$ is the main component in ginsenosides. It is a protopanaxadiol-type ginsenoside that has a dammarane-type triterpenoid as an aglycone. In this study, ginsenoside Rb$_1$ was transformed into gypenoside XVII, ginsenoside Rd, ginsenoside F$_2$ and compound K by glycosidase from *Leuconostoc mesenteroides* DC102. The optimum time for the conversion was about 72 h at a constant pH of 6.0 to 8.0 and the optimum temperature was about 30°C. Under optimal conditions, ginsenoside Rb$_1$ was decomposed and converted into compound K by 72 h post-reaction (99%). The enzymatic reaction was analyzed by high-performance liquid chromatography, suggesting the transformation pathway: ginsenoside Rb$_1$→ gypenoside XVII and ginsenoside Rd→ginsenoside F$_2$→compound K.

Keywords: Biotransformation, Gypenoside XVII, Ginsenoside Rd, Compound K, *Leuconostoc mesenteroides* DC102

INTRODUCTION

Ginseng, the root of *Panax ginseng* Meyer, belongs to family Araliaceae. It has been used as a medicine to treat various diseases in the Orient for several thousand. The major active components of ginseng are ginsenosides, having bioactive and pharmacological activities, including anti-cancer activities [1], anti-inflammatory [2], anti-aging activities [3]. However, these naturally occurring ginsenosides are observed to be poorly absorbed along the human intestinal tracts [4]. Previous studies demonstrated that protopanaxadiol-type ginsenosides such as Rb$_1$, Rb$_2$, and Rc are metabolized by intestinal bacteria after oral administration to their final derivative, 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol, also called compound K [5,6].

Compound K, reported to be easily absorbed by the human body, is the genuine active form of protopanaxadiol-type saponins [7], and it has recently attracted increasing interest because of its intriguing biological actions. compound K was demonstrated to anti-genotoxic activity, anti-allergic effect and the prevention of tumor invasion and metastasis, have shown to be mediated by this metabolite [8-12].

Since compound K does not exist in natural products and the natural transforming ability of human intestinal bacteria is rather limited, much attention has been paid to the preparation of compoud K. Several chemical approaches have been tried to deglycosylate ginsenosides [13]. However, none of them was suitable for preparation

of compound K, because both glycone moieties at C-20 could be cleaved nonspecifically under chemical hydrolysis conditions. Hence, enzymatic transformation, which is considered to be highly region-specific, might be a promising method.

Ginsenoside Rb_1 is the main component in ginsenosides. It is a protopanaxadiol-type ginsenoside that has a structure similar to that of compound K. By enzymatically hydrolyzing the two glucose molecules at C-3 and one of the glucose molecule at C-20, compound K can easily be transformed from ginsenosides Rb_1. In the present study, we characterized the transformation of the major protopanaxadiol ginsenoside Rb_1 into minor compound K by using cell-free extracts of the *Leuconostoc mesenteroides* DC102 strain isolated from kimchi, a traditional Korean fermented food.

MATERIALS AND METHODS

Materials

The *L. mesenteroides* DC102 strain was isolated from kimchi. Difco MRS broth was purchased from Becton Dickinson and Co. (Franklin Lakes, NJ, USA). 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 GGRB at Kyung-Hee University (Yongin, Korea). A 60 F-254 silica gel plate (Merck, Darmstadt, Germany) was used for TLC, and silica gel 60 column (70–230 mesh, Merck) was used for column chromatography. A HPLC (NS 3000i system; Futecs, Daejeon, Korea) equipped with a UV/Vis detector and gradient pump was also used for analysis.

Screening of lactic acid bacteria producing β-glucosidase

Esculin-MRS agar was used to isolate β-glucosidase-producing lactic acid bacteria [14]. This growth medium contains (per 1 L) 3 g esculin and 0.2 g ferric citrate in MRS agar (Becton Dickinson and Co.). The lactic acid bacteria which produced the β-glucosidase that hydrolyzes esculin appeared on the esculin-MRS agar as colonies surrounded by a reddish-brown to dark brown zone. Subsequently, single colonies from those plates were subjected to an additional two-day incubation at 37°C.

Phylogenetic analysis

The complete sequence of the 16S rRNA gene from the *L. mesenteroides* DC102 strain was compiled through SeqMan program in the DNASTAR package, and edited using the BioEdit program [15]. The 16S rRNA gene sequences of related taxa were obtained from GenBank (National Center for Biotechnology Information; Bethesda, MD, USA), and the phylogenetic tree was constructed using the neighbor-joining method of the MEGA 4 program [16]. A bootstrap analysis with 1,000 replicates was similarly performed to obtain confidence levels for the branches. Finally, the closest type strains were included in the phylogenetic tree.

Preparation of crude microbial enzymes

The *L. mesenteroides* DC102 strain was grown in the MRS broth at a temperature of 37°C until absorbance at 600 nm reached 1.0. After culture broth centrifugation (15,000 ×g for 10 min at 4°C) using a MICRO 17R microcentrifuge (Hanil Science Industrial, Seoul, Korea), four volumes of ethanol were added to the supernatant. This solution was mixed sufficiently and placed in an ice chamber for 20 min to foster further reactions. Meanwhile, the protein pellet was collected via centrifugation (10,000×g for 40 min at 4°C) and was dissolved in a 20 mM sodium phosphate buffer (pH 7.0).

Parameter optimization for the biotransformation of ginsenoside Rb_1

The effects of temperature on the transformation of ginsenoside Rb_1 were investigated at temperatures in the range of 25°C to 60°C. Reactions were performed in sodium phosphate buffer (pH 7.0) containing ginsenoside Rb_1 (1 μmol) and the crude enzyme (250 μL) for 48 h.

The optimum reaction pH for the conversion of ginsenoside Rb_1 varied between 3.0 and 13.0. Reactions were performed in various buffers (pH 3.0–13.0) containing ginsenoside Rb_1 (1 μmol) and the crude enzyme (250 μL) at 30°C for 48 h, followed by extraction with water-saturated *n*-butanol. Subsequently, the *n*-butanol fraction was evaporated to dryness, and the methanolic extract was analyzed using HPLC.

Biotransformation of ginsenoside Rb_1 by crude enzymes

The *L. mesenteroides* DC102 strain was grown in MRS broth at 37°C until its absorbance at 600 nm reached 1.0. The crude enzymes from each culture broth were dissolved in 20 mM sodium phosphate buffer (pH 7.0) and then mixed with 1 mM ginsenoside Rb_1 dissolved in distilled water at a 1:4 ratio (v/v). Subsequently, the mixture was incubated at 30°C and stirred at 190 rpm for 72 h. During the reaction period, a 1.25-mL aliquot was collected every 12 h, extracted in water-saturated *n*-

butanol and then analyzed using both TLC and HPLC.

Analysis of ginsenosides by thin-layer chromatography

TLC was performed with silica gel plates (60F254, Merck), and $CHCl_3$-CH_3OH-H_2O (65:35:10, v/v, lower phase) was used as its developing solvent. The spots on the TLC plates were detected through spraying with 10% H_2SO_4, followed by heating at 110°C for 10 min [17].

Analysis of ginsenosides by high-performance liquid chromatography

The HPLC-grade acetonitrile and water were purchased from SK Chemicals (Ulsan, Korea). The reaction mixture was extracted in *n*-butanol saturated with H_2O and evaporated *in vacuo*. Additionally, the residue was dissolved in CH_3OH and injected for HPLC analysis. This experiment also employed a C18 (250×4.6 mm, particle size 5 μm) column using acetonitrile (solvent A) and distilled water (solvent B) mobile phases at A/B ratios of 15:85, 21:79, 58:42, 90:10, and 15:85; with run times of 0-5, 5-25, 25-80, 70-82, and 82-100 min, respectively. The flow rate was 1.6 mL/min and the sample was detected at UV 203 nm.

Structural identification

Reaction products 1, 2, 3 and 4, from the ginsenoside Rb_1 bacterial biotransformation by the *L. mesenteroides* DC102 strain, were separated on a ODS flash column chromatography using the gradient method (10% MeOH to 100% MeOH). Their structures were assessed using the proton and carbon nuclear magnetic resonance (NMR; ^{1}H-NMR, ^{13}C-NMR) spectrum method with a Fourier transform NMR spectrometer (Inova AS 400 MHz NMR system; Varian, Vernon Hills, IL, USA) and pyridine-d_5 as the solvent.

Metabolite 1: white powder; mp 185-187°C; ^{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'']. ^{13}C-NMR (pyridine-d_5, 100 MHz) data are shown in Table 1.

Metabolite 2: white powder; mp 204-206°C; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ 0.73 (3H, s, H-19), 0.88 (3H, s, H-30), 0.89 (3H, s, H-18), 1.05 (3H, s, H-29), 1.22 (3H, s, H-28), 1.56 (6H, s, H-26, H-27), 1.58 (3H, s, H-21), 4.87 [1H, d, J=7.6 Hz, 3-O-Glc (inner) H-1], 5.14 [1H,

d, J=7.6 Hz, 20-O-Glc H-1], 5.34 [1H, d, J=7.6 Hz, 3-O-Glc (outer) H-1]; ^{13}C-NMR (pyridine-d_5, 100 MHz) data see Table 1.

Table 1. ^{13}C-NMR chemical shifts of material Rb_1 and metabolites 1 and 2 (100 MHz, solvent: pyridine-d_5)

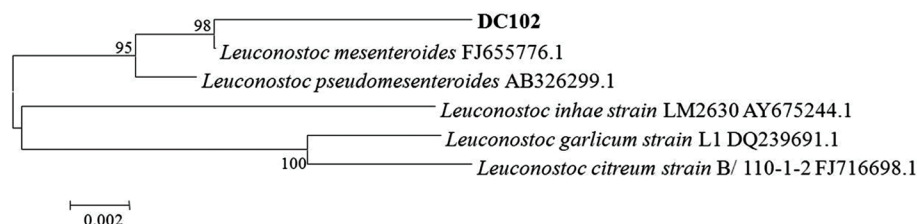| Carbon site	Gypenoside XVII		Ginsenoside Rd	
	Reference	Metabolite 1	Reference	Metabolite 2
Aglycone moiety				
C-1	39.3	39.1	39.1	39.1
C-2	26.7	26.6	26.7	26.6
C-3	88.9	88.8	88.5	88.9
C-4	39.7	39.6	39.6	39.7
C-5	56.5	56.3	56.4	56.3
C-6	18.5	18.4	18.5	18.4
C-7	35.2	35.1	35.2	35.1
C-8	40.1	40.0	40.0	40.0
C-9	50.3	50.1	50.2	50.1
C-10	37.0	36.9	36.9	36.8
C-11	30.9	30.7	30.8	30.8
C-12	70.2	70.1	70.2	70.2
C-13	49.6	49.4	49.4	49.3
C-14	51.5	51.3	51.4	51.4
C-15	30.8	30.7	30.8	30.8
C-16	26.8	26.7	26.7	26.7
C-17	51.7	51.6	51.7	51.7
C-18	16.1	16.0	16.3	16.3
C-19	16.3	16.3	15.9	15.9
C-20	83.5	83.4	83.8	83.3
C-21	22.5	22.4	22.4	22.5
C-22	36.3	36.2	36.0	36.0
C-23	23.3	23.2	23.2	23.3
C-24	126	125.8	125.9	125.8
C-25	131.1	130.9	130.9	130.8
C-26	25.8	25.8	25.8	25.8
C-27	18.0	17.7	17.8	17.8
C-28	28.2	28.1	28.0	28.1
C-29	16.8	16.8	16.6	16.6
C-30	18.0	17.5	17.3	17.3
Sugar moiety				
3-O-inner-Glc				
C-1	107.0	106.8	105.0	105.0
C-2	75.8	75.6	83.3	83.1
C-3	78.8	78.6	78.1	77.9
C-4	72.0	71.8	71.6	71.6
C-5	78.4	78.2	78.1	78.2
C-6	63.2	63.0	62.7	62.6
3-O-outer-Glc				
C-1	–	–	105.9	105.8
C-2	–	–	77.0	77.0
C-3	–	–	79.1	79.1
C-4	–	–	71.6	71.4
C-5	–	–	78.1	78.0
C-6	–	–	62.7	62.6
20-O-inner-Glc				
C-1	98.2	98.0	98.2	98.2
C-2	75.8	75.5	75.0	75.1
C-3	78.8	78.7	78.1	78.2
C-4	72.0	71.9	71.6	71.6
C-5	78.4	78.3	78.1	78.2
C-6	63.2	62.9	62.7	62.8
20-O-outer-Glc				
C-1	105.4	105.2	–	–
C-2	75.3	75.1	–	–
C-3	78.4	78.2	–	–
C-4	71.8	71.6	–	–
C-5	78.4	78.2	–	–
C-6	62.9	62.6	–	–

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequence showing the phylogenetic relationships between the *Leuconostoc mesenteroides* DC102 strain and related *Leuconostoc* species.

RESULTS AND DISCUSSION

Phylogenetic study

The 16S rRNA gene sequence of DC102 was aligned with other neighboring strains, confirming the taxonomic relationships. As shown in Fig. 1, the phylogenetic tree revealed that DC102 was actually grouped with the *Leuconostoc* species. Moreover, the highest degree of 16S rRNA gene sequence identities were with *L. mesenteroides* FJ655776.1 (99%). Hence, based on the phylogenetic tree and homology analysis, the DC102 strain can be designated as *L. mesenteroides* DC102.

Biotranformation of ginsenoside Rb$_1$

After *L. mesenteroides* DC102 was cultured until A600 reached 1.0, the crude enzyme solution of the strain was used to transform ginsenoside Rb$_1$ to both metabolite 1, metabolite 2, metabolite 3 and 4, as shown in Fig. 2. Evidently, this indicated that metabolite 1, metabolite 2 and metabolite 3 was an intermediate metabolite, and that metabolite 4 was the final product. The Rf values of metabolite product 1, 2, 3 and 4 on TLC were similar to those of **gypenoside XVII, ginsenoside Rd, ginsenoside F$_2$ and compound K**. This result suggested that Rb$_1$ was converted by crude enzyme from *L. mesenteroides* DC102. Reaction products 1, 2, 3 and 4, were separated on a ODS flash column chromatography structure.

The structure of metabolite 3 (=ginsenoside F$_2$) and metabolite 4 (=compound K) has already been identified and confirmed in our previous study [18].

Structure of metabolites 1 and 2

In the ^{1}H-NMR spectrum of metabolite 1, the anomeric proton signals for the H-1 of the 3-O-innerglucopyranosyl 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)
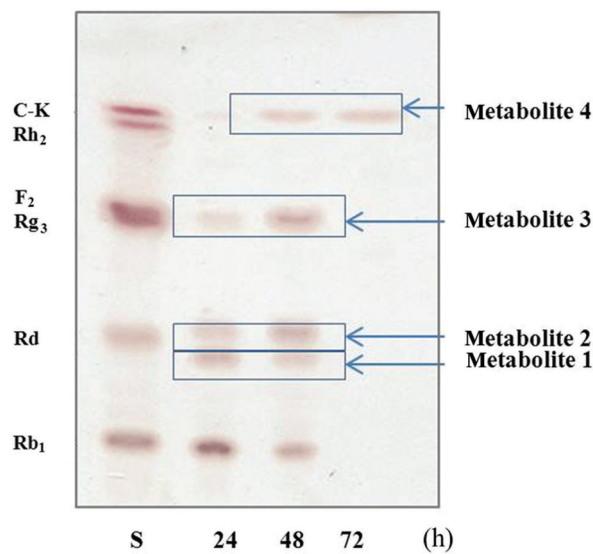

Fig. 2. Time course TLC analysis of metabolites of ginsenoside Rb$_1$ bioconverted by *Leuconostoc mesenteroides* DC102. Ginsenoside Rb$_1$ was used as the substrate to yield gypenoside XVII (1), ginsenoside Rd (2), ginsenoside F$_2$ (3) and compound K (4). Developing solvent: CHCl$_3$/MeOH/H$_2$O (65:35:10, by vol., lower phase). C-K, compound K; S, saponin standards; Rb$_1$, ginsenoside Rb$_1$; Rd, ginsenoside Rd; F$_2$, ginsenoside F$_2$; Rg$_3$, ginsenoside Rg$_3$; Rh$_2$, ginsenoside Rh$_2$.

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''] . ^{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$_1$ 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$_1$ [19]. It is believed that the terminal glucose on C-3 of the aglycon of ginsenosides Rb$_1$ was hydrolyzed by the from *L. mesenteroides* DC102, and this hypothesis is consistent with the 1HNMR data. Therefore, the metabolite (1) produced by from *L. mesenteroides* DC102 from
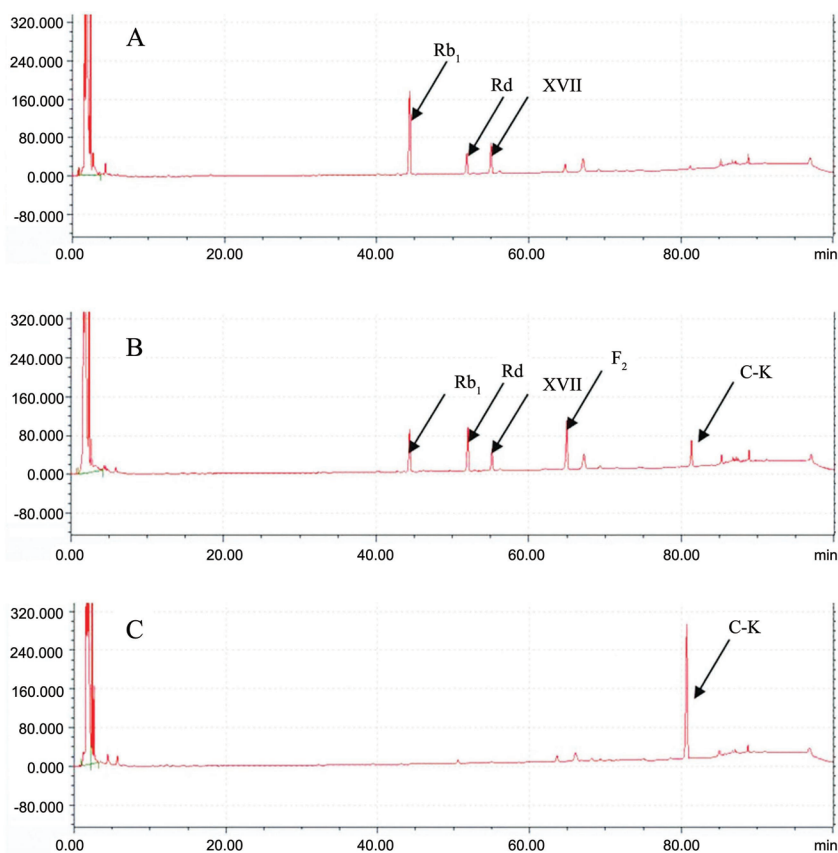
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Fig. 3. HPLC analysis of the bioconversions of ginsenosides. The reaction time-course transformation of ginsenoside Rb$_1$ by the crude enzyme *Leuconostoc mesenteroides* DC102. Incubation time: A, 24 h; B, 48 h; C, 72 h. Rb$_1$, ginsenoside Rb$_1$; Rd, ginsenoside Rd; XVII, gypenoside XVII; F$_2$, ginsenoside F$_2$; C-K, compound K.

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.

The ^{1}H-NMR spectrum of metabolite 2, the proton signals for the H-1 of the 3-O-inner-glucopyranosyl moiety, 3-O-outer-glucopyranosyl moiety, and 20-glucopyranosyl moiety appeared respectively, at δ 4.87 ppm [1H, d, J=7.6 Hz, 3-O-Glc (inner) H-1], δ 5.34 ppm [1H, d, J=7.6 Hz, 3-O-Glc (outer) H-1], and δ 5.14 ppm [1H, d, J=7.6 Hz, 20-O-Glc H-1]. Metabolite 2 was shown to harbor three β-D-glucoses. According to the results of comparisons of the ^{13}C-NMR spectrum of metabolite 2 with the material ginsenosides Rb$_1$, the signal for the C-6 of the 20-innerglucopyranosyl moiety was shifted upfield, from δ 71.6 ppm to δ 62.8 ppm, and the signal for the C-5 of the 20-inner-glucopyranosyl moiety was shifted downfield, from δ 77.1 ppm to δ 78.2 ppm. However, the other signals were similar to those of ginsenoside Rb$_1$. It is believed that the terminal glucose combining C-20 of aglycon of ginsenosides Rb$_1$ was hydrolyzed by the from *L. mesenteroides* DC102. Therefore, metabolite 2 was identified as 3-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-O-[β-D-glucopyranosyl]-20(S)-protopanaxadiol, identical to ginsenoside Rd.

Biotransformation by pathway

The process through which ginsenoside Rb$_1$ was decomposed by the strain DC102 was analyzed using HPLC, as were any changes in reaction time. As is shown in Fig. 3, the concentrations of ginsenosides Rb$_1$ and those of the decomposition products gypenoside XVII, ginsenosides Rd, ginsenosides F$_2$ and compound K exhibited regular changes with reaction time. After 24 h of reaction, ginsenoside Rb$_1$ was simultaneously transformed into gypenoside XVII (metabolite 1) and ginsenoside Rd (metabolite 2). And the majority of ginsenoside Rb$_1$ had been decomposed into gypenoside XVII (metabolite 1), ginsenoside Rd (metabolite 2) and ginsenosides F$_2$ (metabolite 3) after 48 h of reaction. From 48 to 72 h, all the intermediates were continuously transformed into compound K (metabolite 4). Generally, there are two pathways for the transformation of ginsenoside Rb$_1$ into compound K, one is ginsenosides Rb$_1$→ginsenosides Rd→ginsenosides F$_2$→compound K, the other is
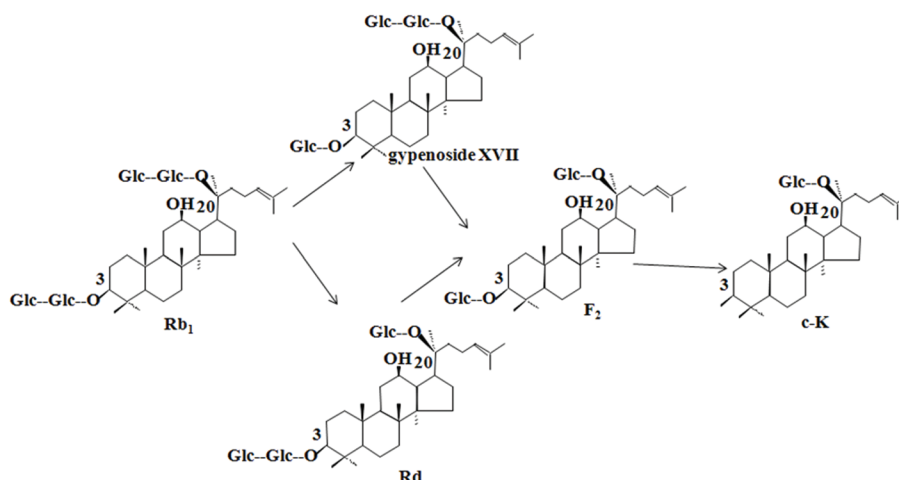
Fig. 4. Proposed bioconversion pathway of ginsenoside Rb_1 to compound K by *Leuconostoc mesenteroides* DC102. Rb_1, ginsenoside Rb_1; Rd, ginsenoside Rd; XVII, gypenoside XVII; F_2, ginsenoside F_2; C-K, compound K.

ginsenosides $Rb_1 \rightarrow$ gypenoside XVII $\rightarrow$ ginsenosides $F_2 \rightarrow$ compound K. However, these findings indicated that gypenoside XVII, ginsenosides Rd and ginsenosides F_2 were intermediate metabolites, and that compound K was the final product after 72 h of reaction time. This result suggested that strain DC102 exerts potent β-glucosidase activity, and that ginsenoside Rb_1 was converted in the following sequence: ginsenosides $Rb_1 \rightarrow$ gypenoside XVII and ginsenosides Rd $\rightarrow$ ginsenosides $F_2 \rightarrow$ compound K (Fig. 4) by the enzymes produced from strain DC102, consecutively hydrolyzing 3-C β-(1→2) glucoside, 20-C β-(1→6) glucoside and 3-C β-glucose of ginsenoside Rb_1.

The effect of temperature on enzyme activity

This experiment was performed at various temperatures (25°C, 30°C, 37°C, 50°C, 60°C) in order to determine the crude enzyme's optimum temperature. The results shown in Fig. 5 revealed that both the *L. mesenteroides* DC102 and ginsenosides Rb_1 strains' crude enzyme reactions were greatly influenced by temperature. In addition, the TLC analysis showed that the crude enzyme activity of *L. mesenteroides* DC102 was the highest at 30°C. The optimum temperature was further confirmed by quantitative HPLC analysis. It was also observed that ginsenoside Rb_1 started degrading at 25°C, reached its maximum concentration at 30°C and 37°C, and suddenly decreased after 37°C. Hence, the enzyme could not perform catalytic reactions under temperatures lower than the optimum, because the energy released was insufficient to power the reaction. Under high temperatures, enzymes lose their activities due to denaturalization.

The effect of pH on enzyme activity

The ginsenoside conversion activity of the crude enzyme was tested at various pH (4.0-10.0) levels. TLC analysis revealed that the optimum pH range was pH 6.0 to 8.0. Similarly, quantitative HPLC analysis also explained that ginsenoside Rb_1 started degrading at pH 5.0. Moreover, it sustained maximum enzyme activities at pH 6.0 to 8.0 and suddenly decreased at pH 8.0 (Fig. 6).

Ginsenoside Rb_1 can be converted to gypenoside XVII and ginsenoside Rd by loss of a glucose moiety at the C-3 and C-20 position of the ginsenoside aglycon, respectively. Compound K can be produced from ginsenoside F_2, which was transformed from gypenoside XVII and ginsenoside Rd, by hydrolysis of additional single glucose moiety at the same positions described above (Fig. 4).

The *L. mesenteroides* DC102 strain is an aerobic and edible lactic acid bacteria. The enzyme activity of this strain was found to be greatest at 30°C and decreased at temperatures higher than 37°C. According to these results, the optimum temperature determined in this study was slightly lower than those reported for other species, such as 45°C for *Rhizopus japonicas* derivative β-glucosidase [20], 40°C for *Aspergillus niger* 48 g and *A. niger* 848 g derivative β-glucosidase [21], and 40°C to 50°C for general β-glucosidase [22,23]. In addition, the crude enzyme showed a high activity rate at pH 5.0 to 9.0, while the maximum hydrolysis activity was found at pH 6.0. These pH values were slightly higher than those reported for other species, such as pH 5.0 for *A. niger* 48 g and *A. niger* 848 g derivative β-glucosidase [21], pH 4.8-5.0 for *R. japonicas* derivative β-glucosidase [20], pH 6.0 for *Fusobacterium* K-60 derivative β-glucosidase [24],
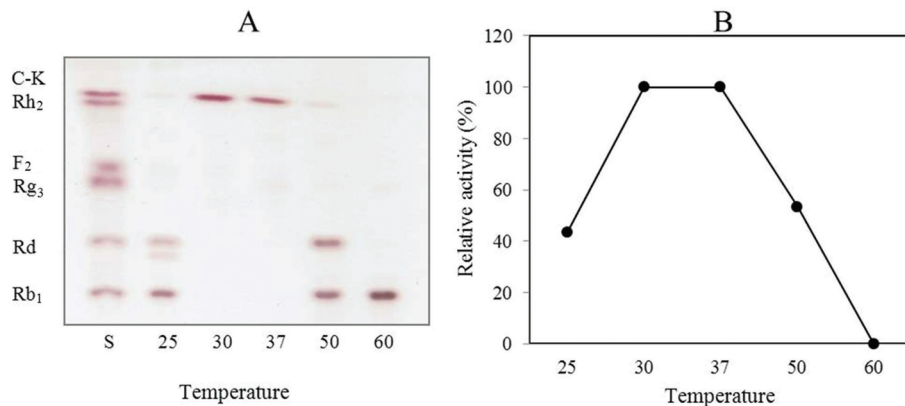
Fig. 5. Effects of temperature on the enzymatic conversion of ginsenoside Rb$_1$ according to TLC analysis (A) and production of compound K from Rb$_1$ by *Leuconostoc mesenteroides* DC102 (B). C-K, compound K; S, saponin standards.
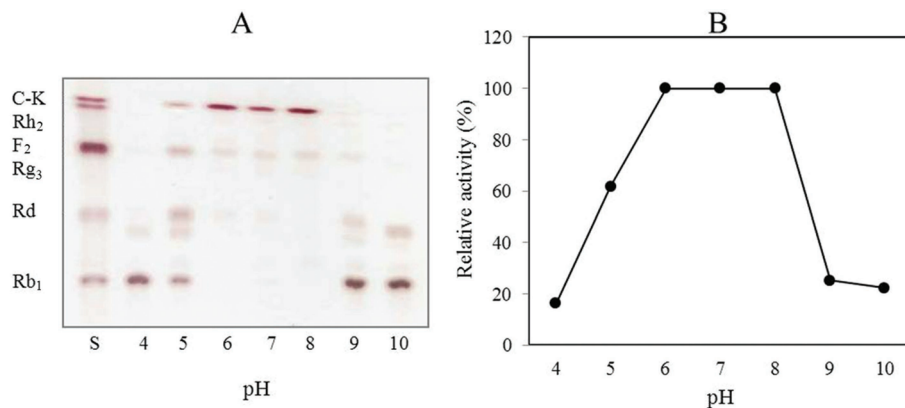

Fig. 6. Effects of pH on the enzymatic conversion of ginsenoside Rb$_1$ according to TLC analysis (A) and production of compound K from Rb$_1$ by *Leuconostoc mesenteroides* DC102 (B). C-K, compound K; S, saponin standards.

and pH 5.0 for ginseng derivative β-glucosidase [25].

This study is the first report on the aerobic bacteria, which is capable of converting ginsenoside Rb$_1$ into compound K via simultaneous bioconversion of gypenoside XVII and ginsenoside Rd. There are reports on microbial sources able to convert the major ginsenoside Rb$_1$ to minor ginsenosides. As such, enzymes secreted by microbes such as *A. niger* and *A. usamii*, and *Caulobacter leidyia* GP45 transformed ginsenoside Rb$_1$ into compound K via ginsenoside Rd and ginsenoside F$_2$ [26,27]. Additionally, *Intrasporangium* sp. GS603 transformed ginsenoside Rb$_1$ into ginsenosides F$_2$ via gypenoside XVII [28]. The ginsenoside Rb$_1$-converting enzymes from the strain DC102 can be applied in industry for the production of gypenoside XVII, ginsenoside Rd, ginsenoside F$_2$ and compound K after the target enzymes for the conversion have been purified and characterized, a goal that should be included in future studies.

ACKNOWLEDGEMENTS

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