

Fermentative transformation of ginsenosides by a combination of probiotic *Lactobacillus helveticus* and *Pediococcus pentosaceus*

Sasikumar Arunachalam Palaniyandi¹, Bao Le², Jin-Man Kim², and Seung Hwan Yang^{2*} 

¹Department of Biotechnology, Mepco Schlenk Engineering College, Mepco Nagar, Mepco Engineering College, 626005, Sivakasi, Tamilnadu, India

²Department of Biotechnology, Chonnam National University, Yeosu 59626, Republic of Korea

프로바이오틱스 *Lactobacillus helveticus*와 *Pediococcus pentosaceus*의 조합에 의한 진세노사이드의 발효적 형질전환

Sasikumar Arunachalam Palaniyandi¹ · Bao Le² · 김진만² · 양승환^{2*} 

¹매펜솔렌크공대 공과대학 생물공학과, ²전남대학교 공학대학 생명산업공학과

(Received July 23, 2018; Revised October 8, 2018; Accepted October 10, 2018)

Ginseng are native traditional herbs, which exhibit excellent pharmacological activities. Probiotic *Lactobacillus helveticus* KII13 and *Pediococcus pentosaceus* strain KID7 were used for ginsenoside transformation by fermenting crude ginseng extract to enhance minor ginsenoside content. Thin-layer chromatography (TLC) analysis of fermented ginseng extract showed that the minor ginsenosides Rg3, Rh1, and Rh2 were main products after 5 days of fermentation. HPLC analysis was performed to quantify the major and minor ginsenosides. The Rg3 peak appeared on the 3rd day while the appearance of Rh2 peak and Rh1 peak were observed on the 5th day. The co-culture of *L. helveticus* KII13 and *P. pentosaceus* KID7 converted major ginsenosides (Rb1 and Rg1) into minor ginsenosides (Rg3, Rh2, and Rh1).

Keywords: *Lactobacillus helveticus*, *Pediococcus pentosaceus*, ginseng extract, ginsenoside, probiotics

Panax ginseng C. A. Meyer, *Panax quinquefolius* L., and *Panax notoginseng* F. H. Chen are the most common species of ginseng. Korean ginseng (*Panax ginseng* C. A. Meyer) is

native to the Asian Far East (33~48 latitude in Korea, northern Manchuria, and parts of Russia) and has excellent pharmacological efficacy (Choi, 2008). Increasing research on the major components and pharmacological effects of ginseng resulted in its recognition as a natural health food. In addition, ginseng has been mainly used in Asian countries such as Korea in the form of herbal medicine for various diseases such as psychiatric disorders, nervous system diseases and diabetes (Tang and Eisenbrand, 1992; Lee *et al.*, 2008).

Saponins, which are the main components of ginseng has the form of triterpenoid glycosides and are called ginsenosides. Minor saponins (Rg3, Rh2, Rh1, etc.) produced by hydrolysis of major saponins (Rg1, Re, Rb1, Rc, Rb2, and Rd) contained in ginseng, has been reported to exhibit excellent pharmacological activities such as inhibition of invasion of tumour cells *in vitro*, induction of cancer cell apoptosis, inhibition of cancer cell metastasis, hypotension, suppression of catecholamine secretion, analgesic activity and immunity (Christensen, 2008; Qi *et al.*, 2011; Yang *et al.*, 2015). Studies on the production of minor saponins have been actively pursued (Chi and Ji, 2005; Chi *et al.*, 2005; Han *et al.*, 2010; Kim *et al.*, 2010; Hong *et al.*, 2012;

*For correspondence. E-mail: ymichigan@jnu.ac.kr;
Tel.: +82-61-659-7306; Fax: +82-61-659-7309

Lim *et al.*, 2015; Huq *et al.*, 2016) and their pharmacological activities have been verified (Qi *et al.*, 2010; Lim *et al.*, 2015).

The present study demonstrates that fermentation with probiotic lactic acid bacteria (LAB) could result in enrichment of minor ginsenosides such as Rg3, Rh2, and Rh1. The ginseng extract was fermented with a mixture of our previously reported probiotic lactic acid bacteria such as *Lactobacillus helveticus* KII13 (Damodharan *et al.*, 2016) and *Pediococcus pentosaceus* strain KID7 (Damodharan *et al.*, 2015).

One hundred gram of ginseng roots were washed and freeze dried to remove moisture. Dried roots were powdered and extracted with 30 volumes of 80% ethanol at 80°C for 1 h in water bath with shaking followed by filtering with 3 mm filter paper (Whatman). The second extraction used the remainder with 20 volumes of 80% ethanol. After an additional extraction, ginseng extract was concentrated using a vacuum evaporator and then dissolved in 10 volumes of water.

The fermentation medium contained skim milk powder, tryptic soy broth powder and ginseng extract concentrate in the ratio of 1:1.5:2.5 (W%), respectively per 100 ml of distilled water and sterilized by autoclaving. *L. helveticus* KII13 and *P. pentosaceus* strain KID7 were cultured in 10 ml MRS liquid broth at 37°C for 24 h. After incubation, cells were harvested by centrifugation at $8,000 \times g$ for 10 min and washed with sterile phosphate buffered saline (PBS) twice (at the final concentration of 10^8 cells/ml). Both the prepared LAB cells (in a ratio of 1:1) were inoculated at a concentration of 10% (v/v) of fermentation medium for transformation of ginsenoside. After inoculation, the fermentation medium was incubated statically at 37°C for 7 days. Samples were retrieved from the fermentation medium each day and analyzed for ginsenoside transformation by TLC and HPLC analysis.

A 1 ml aliquot of the fermentate was collected each day and extracted with equal volume of *n*-butanol by shaking the mixture for 1 h followed by incubation at room temperature for an additional 12 h. The *n*-butanol fraction was evaporated to dryness with a rotary vacuum evaporator (N-1000V, EYELA). Crude extract was dissolved in 50 µl of methanol, which was subjected to thin layer chromatography (TLC) analysis. TLC was conducted on silica gel 60 F₂₅₄ pre-coated plates (Merck) with chloroform:methanol: water (65:35:10, lower phase) as the developing solvent. The spots were visualized by spraying with 10% sulfuric

acid, followed by heating at 110°C for 10 min (Palaniyandi *et al.*, 2015).

The ginsenoside compositions were identified through comparison with standard ginsenoside according to our previous report (Palaniyandi *et al.*, 2015, 2017). Analysis was performed in a Waters Alliance 2695 HPLC system equipped with a Sunfire C18 column (4.5 mm × 25 cm) maintained at a constant temperature of 40°C using a column incubator. HPLC-grade acetonitrile (A) and water (B) were used as mobile phase. The analysis was performed at mobile phase flow rate of 1 ml/min using the following conditions: 0~8 min, 20~30% A; 8~12 min, 30~40% A; 12~15 min, 40~65% A; 15~20 min, 65~100% A, 20~30 min, 100% A; 30~35 min, 100~30% A; 35~40 min, 30~20% A, and column equilibration for 5 min with 20% A. Samples (20 µl) was injected into the column using an automated sample injector (Waters 2707 Autosampler) and it was monitored at wavelength of 203 nm using Waters 2996 PDA Detector. A mixture of ginsenosides Re, Rg1, Rb1, Rf, Rc, Rb2, Rg2, Rh1, Rd, Rg3, Ck, and Rh2 was used as standard for HPLC analysis. Ginsenosides such as Rb1, Rb2, Rc, Re, Rg2, and Rg3 were a kind gift from Prof. Nam-In Baek, Natural Products Chemistry Lab, Kyung Hee University, Korea. Other ginsenosides were obtained from Chromadex.

Screening of fermented ginseng extract by TLC analysis showed that fermentation of ginseng extract with strain KII13 and KID7 decreased ginsenosides Rb1 and Rg1, whereas ginsenosides Rg3, Rh2, and Rh1 were increased over an incubation period of 7 days (Fig. 1). The ginsenoside Rb1 could be hydrolysed by β -glucosidase through a series of hydrolytic pathway (Park *et al.*, 2010). Rb1 is transformed to ginsenoside Rg3, which gradually increased on 3rd day (Fig. 1). Rg3 was produced by hydrolysing the outer glycosidic linkage at C-20 position of ginsenoside Rd, which is an intermediate in the conversion of Rb1→Rg3. The content of Rg3 was increased by the 5th day with a slight increase on the 7th day (Fig. 1), as it is converted to minor ginsenoside Rh2 (Fig. 1) and other compounds by the probiotic strains. The individual and co-cultures of *Lactobacillus* and *Pediococcus* genus have been used to enhance biological efficiency of fermented ginseng. Lin *et al.* (2010) have reported that *L. helveticus* converted major ginsenoside to minor ginsenosides Rg3, Rh1 and protopanaxatriol, which can enhance anti-hepatoma and anti-cancer activities. Eom *et al.*

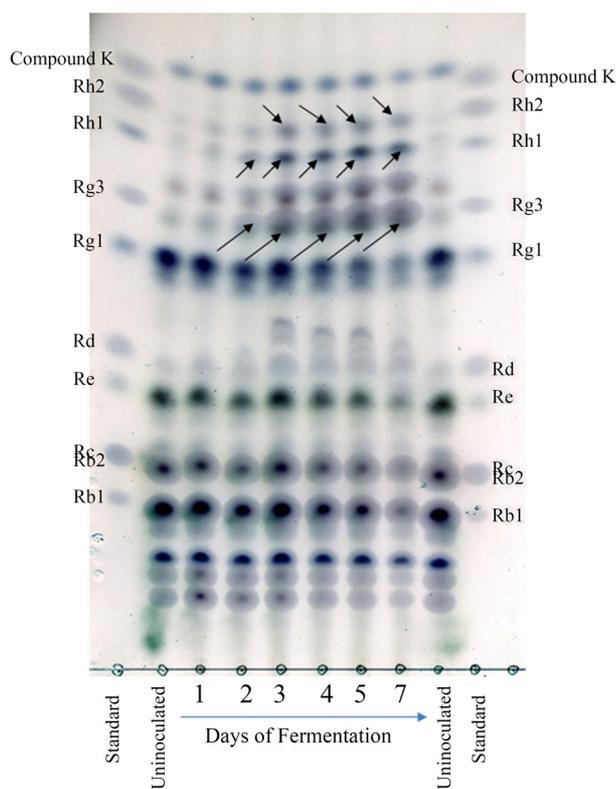


Fig. 1. TLC analysis of the fermented ginseng extract.

(2018) determined that the ginseng marc fermented with *Pediococcus acidilactici* enhanced its antioxidant and nitric oxide scavenging activities. The co-culture of *P. pentosaceus* and *L. mesenteroides* also showed that Rb1 transferred to Rg3 (Park *et al.*, 2017). In the present, a co-culture of probiotic strains *L. helveticus* KII13 and *P. pentosaceus* KID7 converted ginsenoside Rb1, Rg1 into ginsenosides Rg3, Rh1 and Rh2.

HPLC analysis of ginseng extract fermented with the combination of probiotic *L. helveticus* KII13 and *P. pentosaceus* KID7 (Fig. 2) to confirm the results of the TLC analysis. The Rb1 peak decreased but the peak of Rg3 increased on the 3rd day compared to zero day (Fig. 2). The appearance of Rh2 peak and an increase in the Rh1 peak were observed on the 5th day (Fig. 2). On the 5th day, the relative amounts of ginsenosides Rb1, Rg3, and Rh2 were 1.2%, 76.1%, and 14.7%, respectively, whereas, on the 7th day amounts were 0.7%, 75.2%, and 12.4%, respectively. Additionally, the amounts of Rg1 and Rh1 were 0.8% and 13.6%, respectively on 5th day and were 0.4% and 14.2%, respectively on the 7th day. The hydrolytic pathway of major protopanaxadiol (PPD)-type ginsenosides to minor

PPD-type ginsenosides conversion happens through several intermediates as follows: Rb1→Rd→Rg3→Rh2 or Rb1→Rd→F2→Ck and/or Rh2; Rb2→Rd→Rg3 or F2→Ck or Rh2, Rb2→C-O→F2 or C-Y→Ck or Rh2; Rc→Rd→Rg3 or F2→Ck or Rc→C-Mc1→F2 or C-Mc→Ck or Rh2 (Park *et al.*, 2010). Similarly, the hydrolytic pathway of major protopanaxatriol (PPT)-type ginsenosides to minor PPT-type ginsenosides conversion also happens through several intermediates as follows: Re→Rg1 or Rg2→F1 or Rh1; R1→R2 or Rg1→F1 or Rh1; and Rf→Rh1 (Park *et al.*, 2010). Various microbial enzymes have been shown to hydrolyze the glycosides of major PPD and PPT-type ginsenosides and produce a variety of minor compounds, which has been reviewed by Park *et al.* (2010). Since, we used a combination of probiotic strains and crude ginseng extract for fermentation, it is observed in the HPLC chromatogram of 5th and 7th day the major ginsenoside peaks disappeared and several new peaks appeared, indicating hydrolysis of major ginsenosides to form minor ginsenosides (Fig. 2). The results of our study suggested that hydrolytic pathway of ginsenoside Rb1 and Rg1 by the probiotic strains is Rb1→Rg3→Rh2 and Re→Rg1→Rh1 (Fig. 3). A similar pathway was observed for a *L. paracasei* subsp. *tolerans* MJM60396 in our previous report (Palaniyandi *et al.*, 2016). The peak of Rg3 appeared on the 3rd day while Rh2 appeared on the 7th day. The recombinant *L. lactis* produced minor ginsenoside Rg3 as follows: Rb1→Rd→Rg3 and Rg3 was produced after 24 h (Li *et al.*, 2017). Production of minor ginsenosides using β -glucosidase-producing bacteria has been described in previous reports. *P. pentosaceus* WiKim20 and *L. mesenteroides* WiKim19 transformed ginsenoside Rb1 into the ginsenoside Rg3, although *P. pentosaceus* WiKim20 lacked β -glucosidase activity (Park *et al.*, 2017). *Weissella hellenica* DC06 could also convert major ginsenoside Rb1 into pharmacologically active ginsenoside Rg3 (Huq *et al.*, 2016). The peak for ginsenoside Rg3 was observed on the 3rd day and Rb1 was no longer present within 7 days fermentation.

To our knowledge, this is the first report on biotransformation of ginsenoside Rb1 to non-natural ginsenosides Rg3, Rh2 and Rh1 using a combination of two different probiotic strains. By using food grade bacteria (GRAS), there is potential of preparing a higher biofunctional ginseng extract by fermentation for food and pharmaceutical-applications.

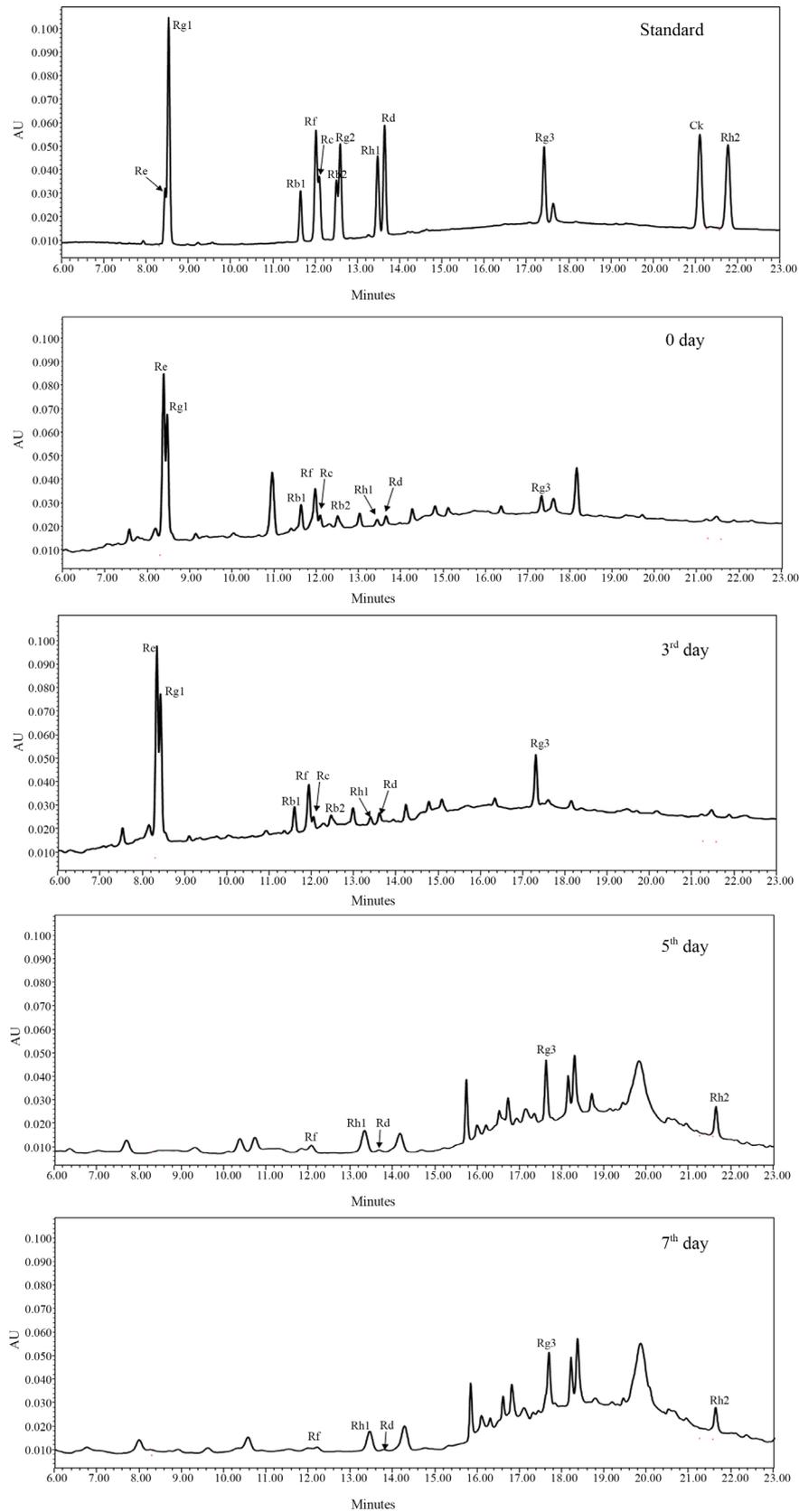


Fig. 2. Time course HPLC analysis of the composition of major and minor ginsenoside in ginseng extract fermented by the probiotic strains combination.

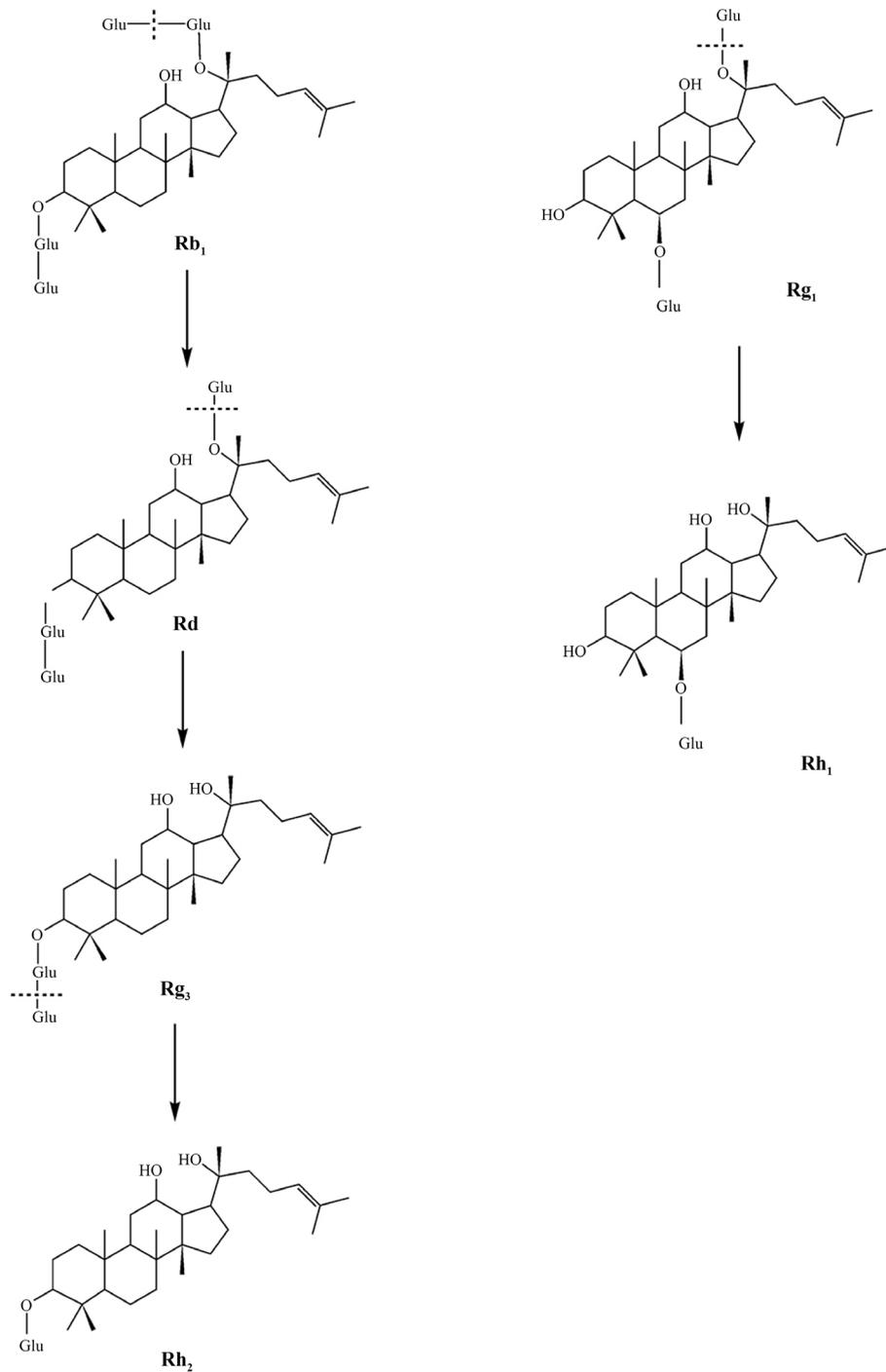


Fig. 3. Schematic representation of the possible hydrolytic pathway of ginsenoside Rg3, Rh2, and Rh1 production from Rb1 and Rg1.

적 요

인삼은 우수한 약리 활성 작용을 보이는 전통적인 약초이다. 본 연구에는 프로바이오틱스 *Lactobacillus helveticus* KII13과 *Pediococcus pentosaceus* KID7 균주를 진세노사이드(ginsenoside)

함량을 증가시키기 위해 조 인삼 추출물을 발효시켜 진세노사이드를 형질전환 시키는데 사용되었다. 발효삼 추출물의 TLC (Thin-layer chromatography) 분석 결과, 5일간의 발효 후 주요 사포닌인 진세노사이드 Rg3, Rh1 및 Rh2로 변환되는 것으로 나타났다. HPLC 분석을 수행하여 주요 및 미량 진 세노사이드

드를 정량화하였다. 3일째에는 Rg3가 나타나고, 5일째에는 Rh2 및 Rh1이 나타난다. *L. helveticus* KII13과 *P. pentosaceus* KID7의 공동 배양은 주요 진세노사이드(Rb1과 Rg1)를 미량 진세노사이드(Rg3, Rh2, Rh1)로 전환시키는 것을 확인하였다.

Acknowledgements

This study was financially supported by Chonnam National University (Grant number: 2016-2846).

References

- Chi H and Ji GE.** 2005. Transformation of ginsenosides Rb1 and Re from *Panax ginseng* by food microorganisms. *Biotechnol. Lett.* **27**, 765–771.
- Chi H, Kim DH, and Ji GE.** 2005. Transformation of ginsenosides Rb2 and Rc from *Panax ginseng* by food microorganisms. *Biol. Pharm. Bull.* **28**, 2102–2105.
- Choi KT.** 2008. Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* CA Meyer. *Acta Pharmacol. Sin.* **29**, 1109–1118.
- Christensen LP.** 2008. Ginsenosides: chemistry, biosynthesis, analysis, and potential health effects. *Adv. Food Nutr. Res.* **55**, 1–99.
- Damodharan K, Lee YS, Palaniyandi SA, Yang SH, and Suh JW.** 2015. Preliminary probiotic and technological characterization of *Pediococcus pentosaceus* strain KID7 and *in vivo* assessment of its cholesterol-lowering activity. *Front. Microbiol.* **6**, 768.
- Damodharan K, Palaniyandi SA, Yang SH, and Suh JW.** 2016. Functional probiotic characterization and *in vivo* cholesterol-lowering activity of *Lactobacillus helveticus* isolated from fermented cow milk. *J. Microbiol. Biotechnol.* **26**, 1675–1686.
- Eom SJ, Hwang JE, Kim KT, and Paik HD.** 2018. Increased antioxidative and nitric oxide scavenging activity of ginseng marc fermented by *Pediococcus acidilactici* KCCM11614P. *Food Sci. Biotechnol.* **27**, 185–191.
- Han Y, Sun B, Jiang B, Hu X, Spranger M, Zhang Y, and Zhao Y.** 2010. Microbial transformation of ginsenosides Rb1, Rb3 and Rc by *Fusarium sacchari*. *J. Appl. Microbiol.* **109**, 792–798.
- Hong H, Cui CH, Kim JK, Jin FX, Kim SC, and Im WT.** 2012. Enzymatic biotransformation of ginsenoside Rb1 and gypenoside XVII into ginsenosides Rd and F2 by recombinant β -glucosidase from *Flavobacterium johnsoniae*. *J. Ginseng. Res.* **36**, 418.
- Huq MA, Akter S, Kim YJ, Farh MEA, and Yang DC.** 2016. Biotransformation of major ginsenoside Rb1 to pharmacologically active ginsenoside Rg3 through fermentation by *Weissella helvetica* DC06 in newly developed medium. *Bangladesh J. Sci. Ind. Res.* **51**, 271–278.
- Kim BG, Choi SY, Kim MR, Suh HJ, and Park HJ.** 2010. Changes of ginsenosides in Korean red ginseng (*Panax ginseng*) fermented by *Lactobacillus plantarum* M1. *Process Biochem.* **45**, 1319–1324.
- Lee ST, Chu K, Sim JY, Heo JH, and Kim M.** 2008. *Panax ginseng* enhances cognitive performance in Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* **22**, 222–226.
- Li L, Lee SJ, Yuan QP, Im WT, Kim SC, and Han NS.** 2017. Production of bioactive ginsenoside Rg3 (S) and compound K using recombinant *Lactococcus lactis*. *J. Ginseng Res.* **42**, 412–418.
- Lim TG, Lee CC, Dong Z, and Lee KW.** 2015. Ginsenosides and their metabolites: a review of their pharmacological activities in the skin. *Arch. Dermatol. Res.* **307**, 397–403.
- Lin YW, Mou YC, Su CC, and Chiang BH.** 2010. Antihepatocarcinoma activity of lactic acid bacteria fermented *Panax notoginseng*. *J. Agric. Food Chem.* **58**, 8528–8534.
- Palaniyandi SA, Damodharan K, Lee KW, Yang SH, and Suh JW.** 2015. Enrichment of ginsenoside Rd in *Panax ginseng* extract with combination of enzyme treatment and high hydrostatic pressure. *Biotechnol. Bioprocess Eng.* **20**, 608–613.
- Palaniyandi SA, Son BM, Damodharan K, Suh JW, and Yang SH.** 2016. Fermentative transformation of ginsenoside Rb1 from *Panax ginseng* CA Meyer to Rg₃ and Rh₂ by *Lactobacillus paracasei* subsp. *tolerans* MJM60396. *Biotechnol. Bioprocess Eng.* **5**, 587–594.
- Palaniyandi SA, Suh JW, and Yang SH.** 2017. Preparation of ginseng extract with enhanced levels of ginsenosides Rg1 and Rb1 using high hydrostatic pressure and polysaccharide hydrolases. *Pharmacogn. Mag.* **13**, S142.
- Park B, Hwang H, Lee J, Sohn SO, Lee SH, Jung MY, Lim HI, Park HW, and Lee JH.** 2017. Evaluation of ginsenoside bioconversion of lactic acid bacteria isolated from kimchi. *J. Ginseng Res.* **41**, 524–530.
- Park CS, Yoo MH, Noh KH, and Oh DK.** 2010. Biotransformation of ginsenosides by hydrolyzing the sugar moieties of ginsenosides using microbial glycosidases. *Appl. Microbiol. Biotechnol.* **87**, 9–19.
- Qi LW, Wang CZ, and Yuan CS.** 2010. American ginseng: potential structure–function relationship in cancer chemoprevention. *Biochem. Pharmacol.* **80**, 947–954.
- Qi LW, Wang CZ, and Yuan CS.** 2011. Ginsenosides from American ginseng: chemical and pharmacological diversity. *Phytochemistry* **72**, 689–699.
- Tang W and Eisenbrand G.** 1992. *Panax ginseng* CA Mey, pp. 711–737. In Chinese drugs of plant origin, Springer.
- Yang XD, Yang YY, Ouyang DS, and Yang GP.** 2015. A review of biotransformation and pharmacology of ginsenoside compound K. *Fitoterapia* **100**, 208–220.