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Biotransformation of Ginsenosides and Their Aglycones

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ABSTRACT

Ginseng has been used as a traditional medicine in Asian countries for thousands of years. The main molecular components responsible for the actions of ginseng are ginsenosides. It is thought that the activities are mainly carried out by the minor ginsenosides which are obtained via the hydrolysis of the sugar moieties in the major ginsenosides using hydrolytic acid, heating or microbial transformation. Due to the significant region- and stereoselectivities of biotransformation, there is a profound potential for ginsenoside structural modification. In this article, the biotransformation methods of ginsenosides by fungi and bacteria, and the enzymes involved, are reviewed.

Keywords: bacteria, enzyme, fungi, ginsenosides, ginseng, microbial biotransformation, review
Abbreviations: HPLC, High Performance Liquid Chromatography; **NMR**, Nuclear Magnetic Resonance; **PPD**, protopanaxadiol; **PPT**, protopanaxatriol

CONTENTS

INTRODUCTION

Ginseng is any one of eleven species of slow-growing perennial plants with fleshy roots, belonging to the genus *Panax* of the family Araliaceae. The major species of ginseng are distributed throughout East Asia, Central Asia, and North America (Attele *et al*. 1999; Huang 1999). Among them, *Panax ginseng* is the most widely used one. The root of *P. ginseng* has been used for more than 2,000 years as a traditional oriental medicine to strengthen immunity, provide nutrition, and reduce fatigue. Various clinical and pharmacological effects associated with its use have been reported, such as anti-cancer activity, anti-circulatory shock effects, promotion of hematopoiesis, and modulation of immune functions and cellular metabolic processes on carbohydrates, fats and proteins. Ginseng is characterized by the presence of ginsenosides (Liu and Xiao 1992; Ong and Yong 2000; Shin *et al*. 2000).

Since the beginning of the 20th century, the constituents of ginseng root have been investigated and several classes of compounds have been isolated such as triterpene saponins; essential oil-containing polyacetylenes and sesquiterpenes; polysaccharides; peptidoglycans; nitrogen-containing compounds; and various ubiquitous compounds such as fatty acids, carbohydrates, and phenolic compounds (Tang and Eisenbrand 1992).

Ginsenosides are viewed as the active compounds behind the claims of ginseng's efficacy. Because ginsenosides appear to affect multiple pathways, their effects are complex and difficult to isolate. More than 100 ginsenosides have been isolated from ginseng roots (Cheng *et al*. 2007; Jia *et al.* 2009). They are classified into four groups: oleanane-type, protopanaxadiol (PPD)-type, and protopanaxatriol (PPT)–type and ocotillol-type. The aglycone of Oleanane-type is oleanolic acid. It only contains ginsenoside Ro. The PPD- and PPT-type are all dammarane-type ginsenosides which consist of an aglycon (a dammarane skeleton) and a sugar component comprised of 1~4 molecules (glycon) such as D-glucose, L-arabinopyranoside, L-arabinofuranoside, D-xylose, and L-rhamnose. In the PPD ginsenosides, the mainly glycosidation locations are C-3 and C-20. Mostly β-D-glucopyranose and β-D-glucopyranosyl-(1→2)-β-D-glucopyranose are linked to C-3, whereas β-D-glucopyranose, α-L-arabinopyranosyl-(1→6)-β-D-glucopyranose, and α-L-arabino-furanosyl-(1→6)-β-D-glucopyranose are linked to C-20. The PPD-type ginsenosides include ginsenosides Rb_1, Rb_2, Rc, Rd, F2, Rg_3, Rh_2, Compound O, Compound Mc, Compound Y, Compound Mc, Compound K, gypenoside XVII, and gypenoside LXXV. The mainly glycosidation locations of PPT ginsenosides are C-6 and C-

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Invited Review

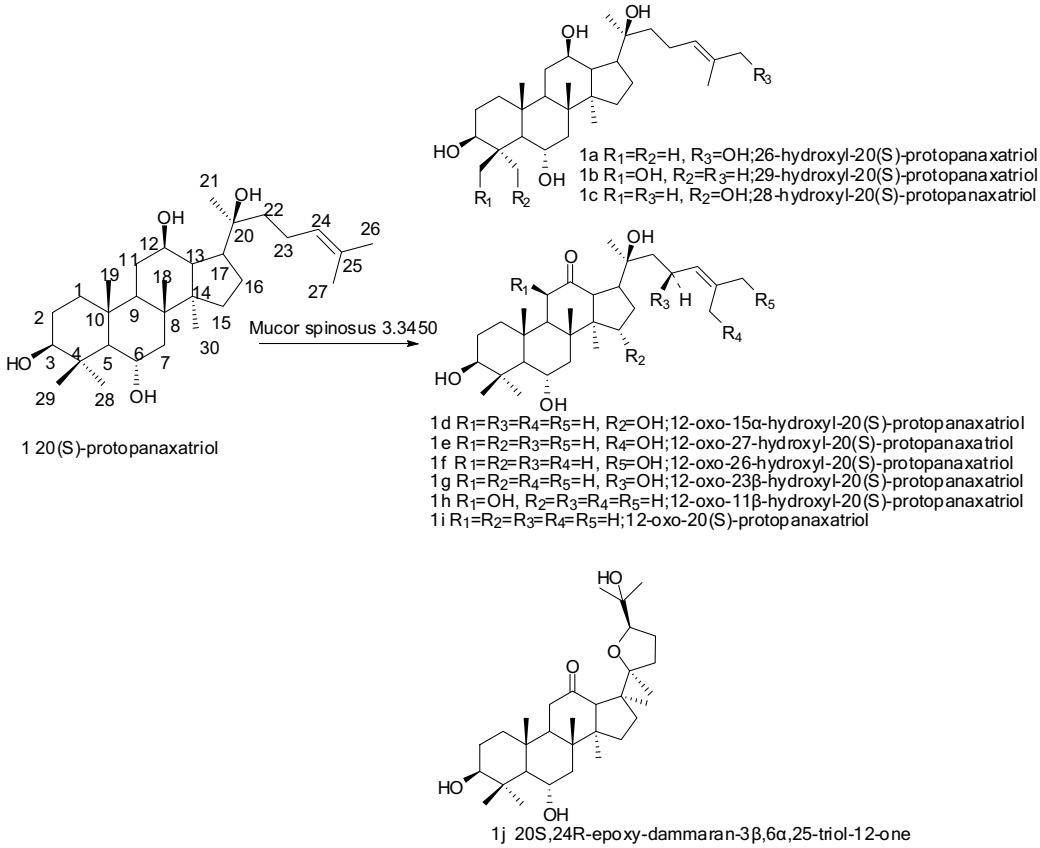


Fig. 1 Microbial transformation of 20(S)-protopanaxatriol by *Mucor spinosus*.

20. Most saccharide moieties are attached to C-6 in the PPT ginsenosides including *β*-D-glucopyranose, α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranose, *β*-D-xylopyranosyl-(1→2)-β-D-glucopyranose, and β-D-glucopyranosyl-(1→2)-β-D-glucopyranose, whereas only β-D-glucopyranose is linked to C-20. The PPT ginsenosides include ginsenosides Re, R_1, R_2, Rf, Rg_1, Rg_2, Rh_1, and F_1.

The major ginsenosides such as Rb_1, Rb_2, Rc, Re and Rg_1 comprise more than 80% in wild ginseng. However, the minor ginsenosides as deglycosylated ginsenosides are known to be more readily absorbed into the bloodstream and function as active compounds (Tawab *et al*. 2003).

As a result, many researchers have transformed the major ginsenosides to the minor ginsenosides by chemical synthesis, mild acid, hydrolysis and alkaline cleavage. But these methods are accompanied with undesirable side reactions (Han *et al*. 1982; Chen *et al*. 1987).

Biotransformation is an important tool in the structural modification of organic compounds by using microorganisms, plant cells, animal tissues or enzymes as catalyst, especially for complicated natural products due to its significant region- and stereoselectivities (Clark and Hufford 1991; Azerad 1999). Biotransformation has great potential to generate novel products or to produce known target compounds more exclusively and efficiently. This review mainly focuses on the biotransformation by fungi, bacteria and enzymes isolated from microorganisms.

BIOTRANSDORMATION USING FUNGI

Biotransformation of 20(S)-PPT and 20(S)-PPD

Microbial biotransformation of 20(S)-PPT (1) by the fungus *Mucor spinosus* (AS 3.3450) yielded 10 metabolites (1a-1j), the structures of which were determined on the basis of their chemical and spectroscopic data. The metabolites 1a, 1d-1f and 1i had cytotoxic effects on HL-60 cells (human leukemia cells) (Zhang *et al*. 2007) (**Fig. 1**).

The metabolites mentioned above had more potent cytotoxicity than substrate 1, indicating that hydroxylation at C-28 or C-29 and 12-carbonylation could increase the cytotoxicity. Especially the importance of the 12-keto group for the cytotoxicity is in good agreement with previous reports. The cyclized product 1j had no effect on the bioassay, indicating that cyclization of the side-chain would markedly reduce the activity of the substrate (Tian *et al*. 2005; Zhang *et al*. 2007).

Biotransformation of 20(S)-PPD (2) by the fungus *Mucor spinosus* AS 3.3450 yielded 8 metabolites (2a–2h). On the basis of NMR and MS analyses, 8 metabolites were identified. Among them, 2a–2e, 2g, and 2h are new compounds. Because carbonylate of C-12 are found in all metabolites of 20(S)-PPD (2), it can be speculated that *M. spinosus* could catalyze the specific C-12 dehydrogenation of 20(S)-protopanaxadiol, as well hydroxylation at different positions. These biocatalytic reactions may be difficult for chemical synthesis. However, the biotransformed products showed weaker cytotoxic activities *in vitro* than the metabolites of 20(S)-protopanaxatriol (Li *et al*. 2009) (**Fig. 2**).

Biotransformation of PPD-type ginsenosides

Some of the PPD ginsenosides are the active components of ginseng. So far, several documents have reported the studies on the biotransformation of this type and gave a series of different metabolites. Meanwhile, many researchers have been focus on increasing the yield of the major active ginsenosides as ginsenoside Rd and ginsenoside Compound K.

Chen *et al*. (2008) selected *Acremonium strictum* AS 3.2058 for the biotransformation of ginsenoside Rb_1 (3). Preparative-scale fermentation of ginsenoside Rb_1 (3) yielded 8 different metabolites from 3a to 3h. Among them, metabolites 3f, 3g and 3h were three new compounds and the reactions as selective C-3 carbonization and two side-chain oxidation -reduction generated by *Acremonium strictum* AS 3.2058 were uncommon in the micro biotransformation of ginsenosides. The other five known metabolites, 3a to 3e, are the metabolites of ginsenoside Rb_1 in both

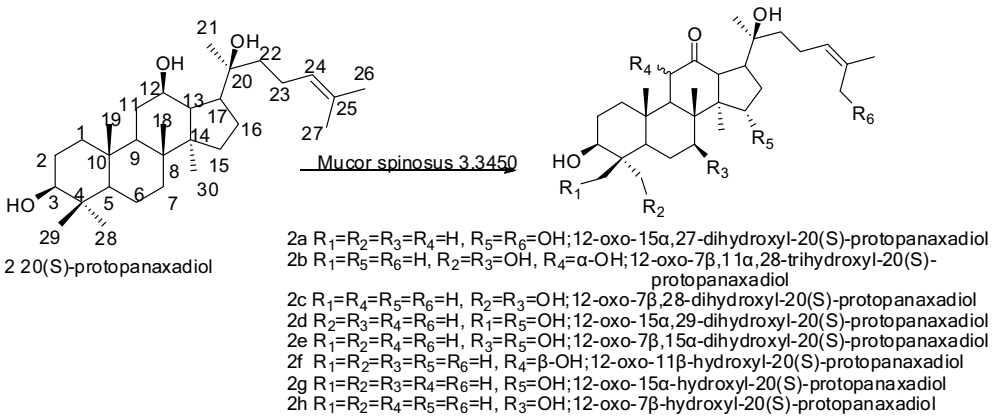


Fig. 2 Biotransformation of 20(S)-protopanaxadiol by the fungus *Mucor spinosus*.

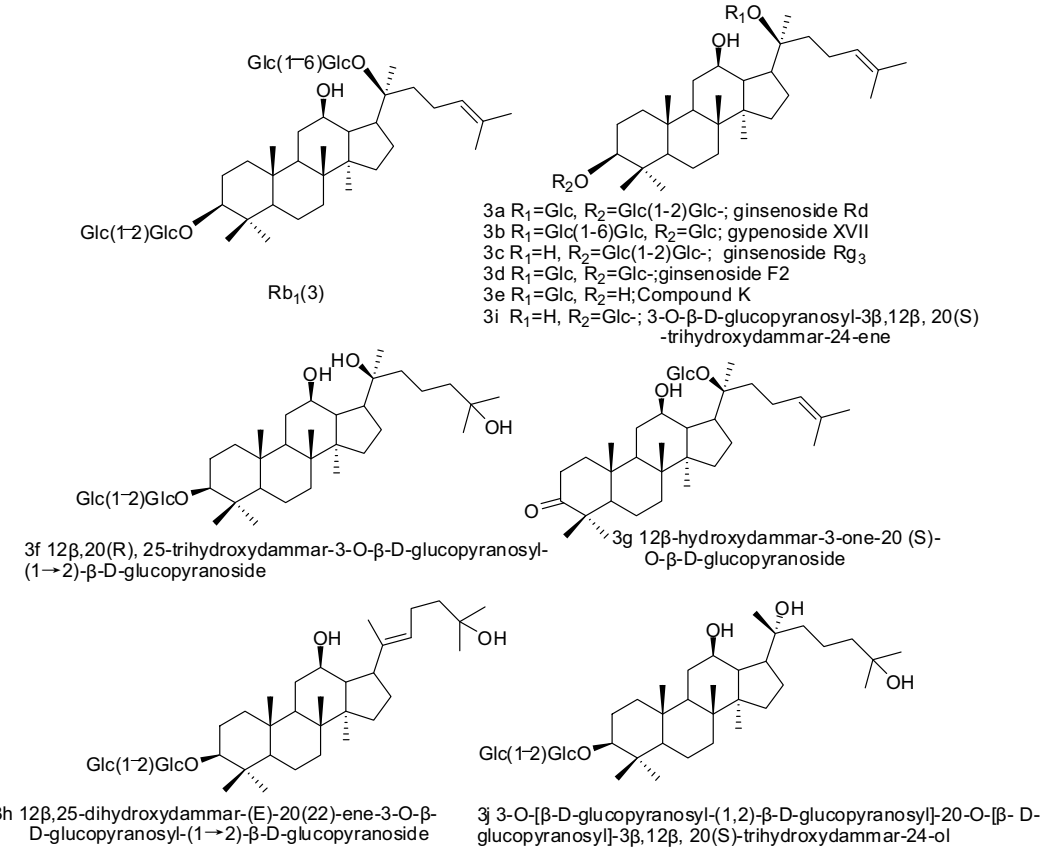


Fig. 3 Biotransformation of ginsenoside Rb_1.

mammals and this microbial model which suggested that *A. strictum* has the potential to serve as a microbial model for generating mammalian metabolites of the related analogs of ginsenosides for the structural identification and further use in investigating pharmacological and toxicological properties of this series of compounds (Chen *et al*. 2008) (**Fig. 3**).

Dong *et al*. (2003) reported the biotransformation of ginsenoside Rb_1 (3) by *Rhizopus stolonifer* and *Curvularia lunata* gave 4 products (3a, 3c, 3i, 3j) and metabolite 3j was a new compound (Dong *et al*. 2003) (**Fig. 3; Table 1**).

The PPD type ginsenoside Rd has been reported to protect neurons from neurotoxic chemicals, enhance the differentiation of neural stem and prevent the contraction of blood vessels. Rd might be a new promising drug. However, it is difficult to separate Rd from ginseng because of its low concentration (< 0.4%) and to prepare it by chemical synthesis because of its complex structure. A possible pathway for preparation of Rd is through transforming structurally related compounds to it. Rb_1 is the major component of ginsenosides and it has the same aglycone (PPD) as Rd. Therefore it can be transformed to Rd by removing the glucose residue from position C-20. Chemical method usually has poor selectivity and generates more by-products. Biotransformation has more potential for conversion because of its region- and stereoselectivities. Some studies have looked for suitable microbes or enzymes that can transform Rb_1 into Rd (**Fig. 3**). Zhao *et al*. (2009) have done some experimental studies on optimization of culture conditions of transforming ginsenoside Rb_1 to Rd as the sole product by *Cladosporium fulvum*. As a result, the maximum yield was 86% (molar ratio). Further, a preparative scale transformation with *C. fulvum* was performed at a dose of 100 mg of Rb_1 by a yield of 80%. This fungus has potential to be applied on the preparation for Rd in pharmaceutical industry (Zhao *et al*. 2009) (**Fig. 3; Table 1**).

Besides, Ye *et al*. (2010) obtained a highly substrate-tolerant strain *Paecilomyces bainier* 229-7 by UV irradiation for 8 min in the presence of 0.4% LiCl. The mutant produced ginsenoside Rd (3a) from ginsenoside Rb_1 with a bioconversion rate as high as 94.9% under optimized cul-

Table 1 Biotransformation of ginsenoside Rb_1 by molds.

Substrate	Strains	Products	Reference
Rb_1(3)	*Acremonium strictum*	3a-3h	Chen *et al*. 2008
	Rhizopus stolonifer	3a, 3c,3i	Dong *et al*. 2003
	Curvularia lunata	3a, 3j	Dong *et al*. 2003
	Paecilomyces Bainier sp. 229	3a	Ye *et al*. 2010
	Cladosporium fulvum	3a	Zhao *et al*. 2009
	Paecilomyces Bainier sp. 229-7	Compound K (3e)	Zhou *et al*. 2008
	Fusarium sacchari	Compound K (3e)	Han *et al*. 2007

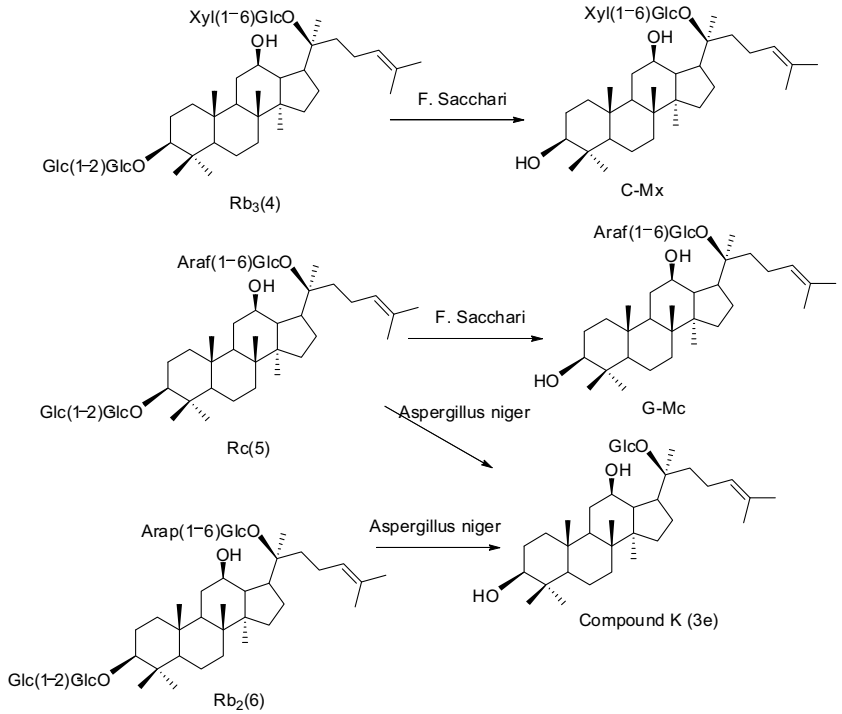


Fig. 4 Biotransformation of ginsenoside Rb_3, Rb_2 and Rc.

ture conditions in shake flask. Scale-up in a 10-L fermenter resulted in an 89% bioconversion rate. Ginsenoside Rd was purified from the culture medium by a macroporous resin with a chromatographic purity of 92.6% (Yan *et al*. 2010; Ye *et al*. 2010) (**Fig. 3; Table 1**).

The major ginsenosides account for 90% (w/w) of the total saponins in ginseng. However, several studies have shown that these abundant ginsenosides are poorly absorbed along the human intestinal tract. They could be transformed to more potent bioactive compounds *in vivo* by intestinal bacteria after oral ingestion. Compound K (3e), is the main pharmacologically active metabolites detected in blood after the oral administration of ginsenosides Rb_1, Rb_2, or Rc. It has been shown that Compound K possesses various biological activities, such as anti-tumor, anti-allergy, and anti-inflammatory activities. However, Compound K is not present in most natural ginseng plants. Therefore, the study of converting major ginenosides to Compound K is of great significance. And microbial biotransformation is shown to be the most effective way to produce Compound K, via the cleavage of sugar moieties at the C-3 or C-20 positions. It has been reported that *P. bainier* sp. 229, which was also gained from the UV irradiation is the best candidate strain showing a high conversion capacity with few by-products. After the optimization of the cultural conditions, The *Mol* conversion quotient of ginseng saponins increased from 21.2% to 72.7%. Scale-up fermentation, under conditions of controlled pH and continuous air supply in the optimal medium, resulted in an 82.6% yield of ginsenoside Compound K (Zhou *et al*. 2008) (**Table 1**).

Also, Han (2007) *et al* reported that ginsenoside Compound K can be obtained from biotransformation of ginsenosides Rb_1 by *Fusarium sacchari* (**Fig. 3; Table 1**). Otherwise, PPD-type ginsenosides Rb_3 (4) and Rc (5) can be transformed to ginsenosides Compound K and Mc by *Fusarium sacchari* respectively (Han *et al*. 2007, 2010) (**Fig. 4**). In addition, Chi *et al.* (2005) reported that both ginsenosides Rc (5) and Rb_2 (6) were transformed by *Aspergillus niger* to ginsenoside Compound K (Chi *et al*. 2005) (**Fig. 4**).

Biotransformation of PPT-type ginsenosides

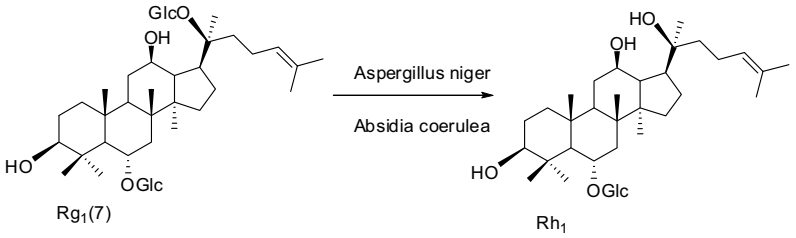
Ginsenoside Rg_1 (7) is one of the major PPT-type saponins from the genus *Panax* which possesses various pharmacological activities. In order to find more diversified microbiological transformation reactions and products, some microbial conversions have been done. Dong *et al.* (2001) reported that ginsenoside Rg_1 was transformed by *Aspergillus niger* and *Absidia coerulea* to the same metabolites Rh_1. And the biotransformation curves have been shown in the article (Dong *et al*. 2001) (**Fig. 5**).

For the purpose of expanding the chemical diversity of ginsenoside derivatives for further pharmaceutical research, and attaining a better understanding of microbial models of ginsenoside metabolism, Chen *et al.* (2007) studied the microbial transformation of three major 20(S)-protopanaxatriol-type saponins:ginsenosides-Rg_1 (7) and -Re (9) and notoginsenoside-R_1 (8) (Chen *et al*. 2007) (**Fig. 6**). The bioconversion reactions mainly took place at the side chain as the hydrolysis the glucose from C-20 or the hydroxylation of double bond and so on.

BIOTRANSFORMATION USING BACTERIA

Biotransformation of PPD and PPT derivatives

Transformation of the dammaranes (20R)-dihydroprotopanaxadiol (10), a mixture of (20S)-and (20R)-dihydroprotopanaxatriol (11, 12) and (20R)-hydroxydammarane-



$Rg_1(7)$ Rh_1

Fig. 5 Biotransformation of ginsenoside Rg_1.

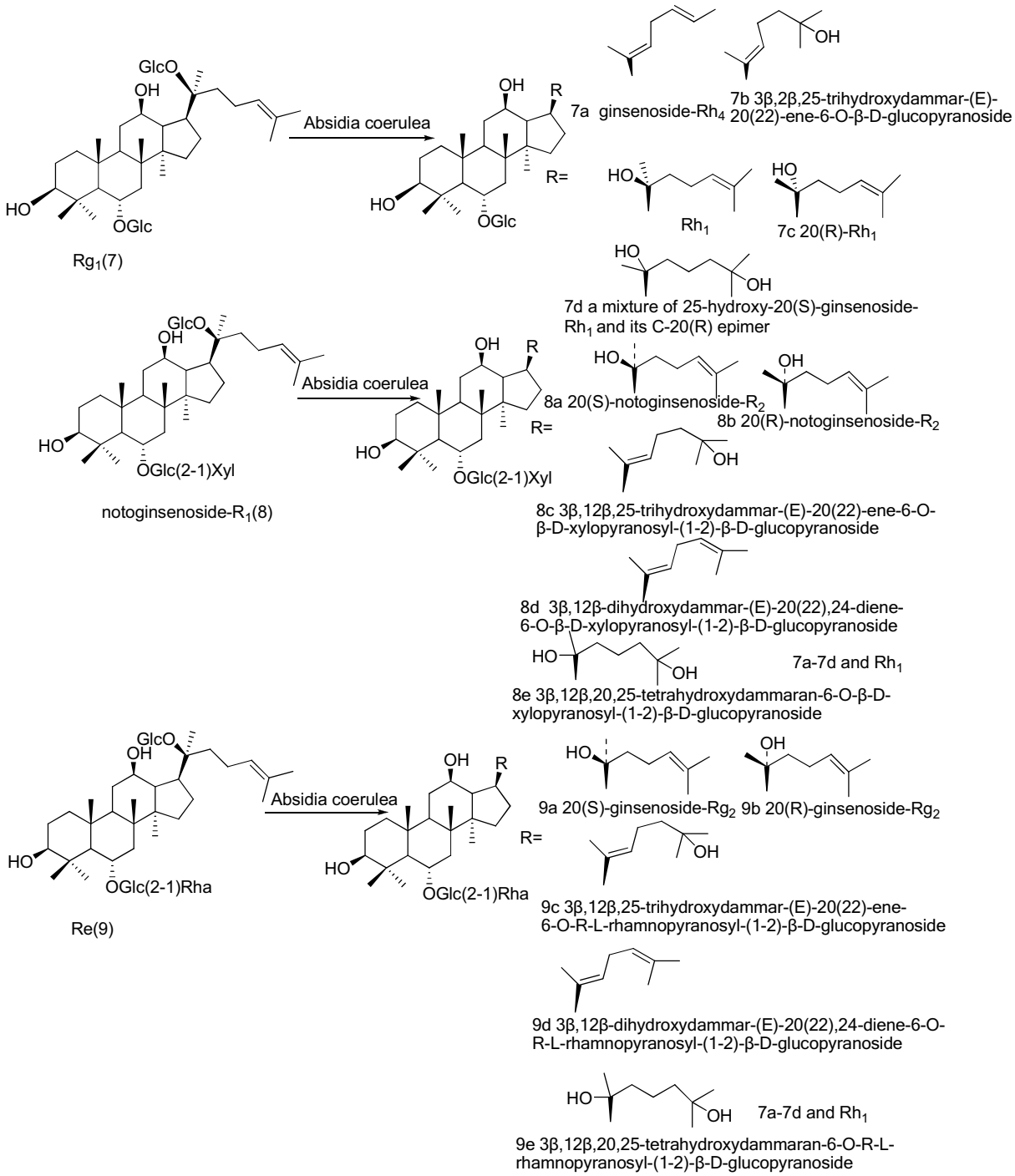


Fig. 6 Biotransformation of ginsenoside Rg_1, notoginsenoside-R_1 and ginsenoside Re.

3,12-dione (13, a pyridinium chlorochromate oxidation product of 10), by *Mycobacterium* sp. (NRRL B-3805) yielded 7 metabolites (**Fig. 7**). The author's lab has revealed that *Mycobacterium* sp. (NRRL B-3805) can oxidatively cleaved the 4,4,14α-trimethyl groups in lanosterol, cycloartenol, and 24-methylenecycloartanol, together with their C-17 side chains. These findings attracted the attention to the microbial transformation of structurally related triterpenes. Protopanaxatriol and protopanaxadiol are the corresponding aglycones of ginsenosides. They possess the dammarane skeleton, which is similar to lanostane except for the replacement of Me-13 by Me-8. They wanted to confirm that whether transformation of the derivatives of protopanaxatriol and protopanaxadiol with this same microorganism would follow a similar oxidative degradation pathway (Wang *et al.* 1997).

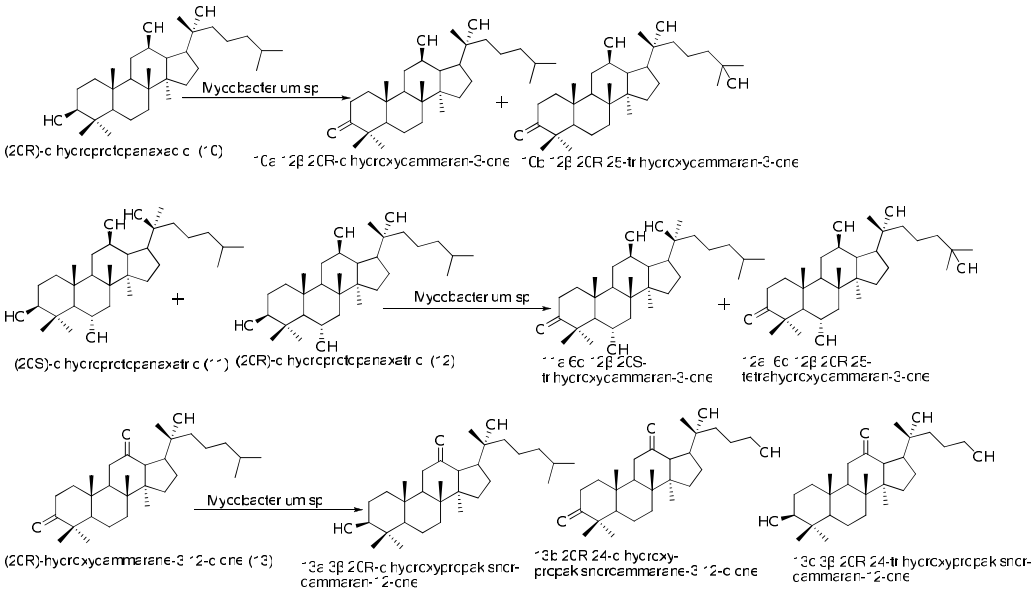


Fig. 7 Microbial transformation of protopanaxadiol and protopanaxatriol derivatives.

Table 2 Biotransformation of PPD-type saponins by bacteria.

Substrate	Strain	Metabolite	Reference
Rb_1(3)	intestinal bacteria	Compound K (3e)	Lee *et al*. 2000
	human fecal microflora	Compound K (3e)	Kim *et al*. 2008
	Burkholderia pyrrocinia GP16	Rd (3a)	Kim *et al*. 2005
	Bacillus megaterium GP27	Rd (3a)	Kim *et al*. 2005
	Sphingomonas echinoides GP50	Rd (3a)	Kim *et al*. 2005
	Caulobacter leidyia GP45	Rd (3a), F_2(3d), Compound K (3c)	Cheng *et al*. 2006
	Microbacterium sp. GS514	Rg_3 (3c)	Cheng *et al*. 2007
	Intrasporangium sp. GS603	Gypenoside XVII (3b)	Cheng *et al*. 2008
Rc(5)	*Bacteroides* HJ-15, *Bifidobacterium* K-506	Mb, Mc, Compound K (3e)	Bae *et al*. 2002
	Bacteroides HJ-15, *Bifidobacterium* K-506	20(S)-protopanaxadiol	Bae *et al*. 2002
	Bifidobacterium K-103, *Eubacterium* A-44	Rd(3a), F_2 (3d), Compound K (3e)	Bae *et al*. 2002
	Bifidobacterium sp. Int57	Compound K (3e)	Chi *et al*. 2005
	Bifidobacterium sp. SJ32	Compound K (3e)	Chi *et al*. 2005
	Lactobacillus delbrueckii	Rh_2 (3i)	Chi *et al*. 2005
	Bifidobacterium sp. SH5	F_2 (3d)	Chi *et al*. 2005
Rb_2(6)	*Bifidobacterium* sp. Int57	Compound K (3e)	Chi *et al*. 2005
	Bifidobacterium sp. SJ32	Compound K (3e)	Chi *et al*. 2005
	Lactobacillus delbrueckii	Rh_2 (3i)	Chi *et al*. 2005
	Bifidobacterium sp.SH5	F_2 (3d)	Chi *et al*. 2005
PPD-saponins	*Pythium irregulare*	F_2 (3d)	Yousef and Bernards 2006

Biotransformation of PPD-type ginsenosides

Ginseng saponins (ginsenosides) have been recognized as being responsible for the biological and pharmacological activities of ginseng. Ginsenoside Rb_1 is the predominant ginsenoside in *Panax ginseng*, *P. quinquefolium*, *P. japonicum*, and *P. notoginseng*. In last section, we have already mentioned the microbial transformations of Rb_1 by a series of fungi giving a lot of metabolites. Although, the biotransformation by bacteria of Rb_1 could not produce so many structural similarities, these studies have aimed to convert Rb_1 to the more active minor ginsenosides Rg_3, F_2 and Compound K, etc. The conversion of ginsenoside Rb_1 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.

Lee *et al*. (2000) reported that after the oral administration of ginseng extract in human and rats, ginsenoside Compound K (3e) was isolated as a metabolite which was formed from ginsenoside Rb_1 (3) by intestinal bacteria. Compound K is of particular interest in cancer chemoprevention and treatment. They investigated the effects of Compound K on the human myeloid leukemia cell line HL-60 in terms of inhibition of proliferation and induction of apoptosis. Compound K showed a significant cytotoxic activity in HL-60 cells (IC50 5 24.3 mM) following a 96-hr incubation. Treatment of HL-60 cells with IH-901 resulted in the formation of internucleosomal DNA fragments (Lee *et al*. 2000) (**Table 2**).

In addition, it has been reported that ginsenoside Rb_1 (3) can be transformed to Compound K by human fecal microflora (Kim *et al*. 2008) (**Table 2**).

Furthermore, other minor ginsenosides have been isolated and identified from the biotransformation of ginsenoside Rb_1 (3) by NMR or HPLC. Kim *et al*. (2005) selected 70 strains for screening test. Among them, three strains, *Burkholderia pyrrocinia* GP16, *Bacillus megaterium* GP27 and *Sphingomonas echinoides* GP50, were observed to be most potent at converting ginsenoside Rb_1 to ginsenoside Rd (3a) almost completely within 48 h by HPLC (Kim *et al*. 2005) (**Table 2**). Cheng *et al*. (2006) reported that biotransformation of ginsenoside Rb_1 (3) by *Caulobacter leidyia* GP45 gave three products: ginsenoside Rd (3a), F_2 (3d) and Compound K (3c) (Cheng *et al*. 2006) (**Table 2**). In addition 20(S)-ginsenoside Rg_3 (3c) and gypenoside XVII (3b) were gained from the microbial transformations of ginsenoside Rb_1 (3) by *Microbacterium* sp. GS514 and *Intrasporangium* sp. GS603, respectively (Cheng *et al*. 2007, 2008) (**Table 2**).

Besides ginsenoside Rb, PPD-type ginsenosides ginsenoside Rc and Rb_2 have been shown to be metabolized by

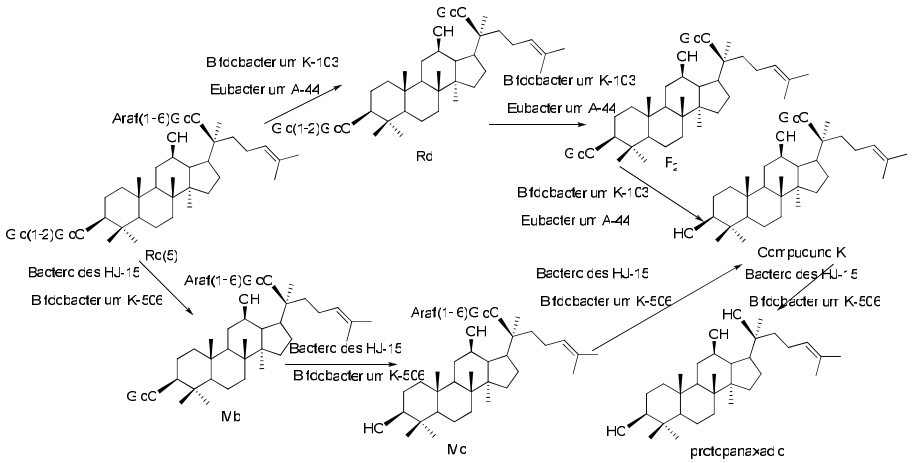


Fig. 8 Proposed metabolic pathway of ginsenoside Rc by human intestinal bacteria.

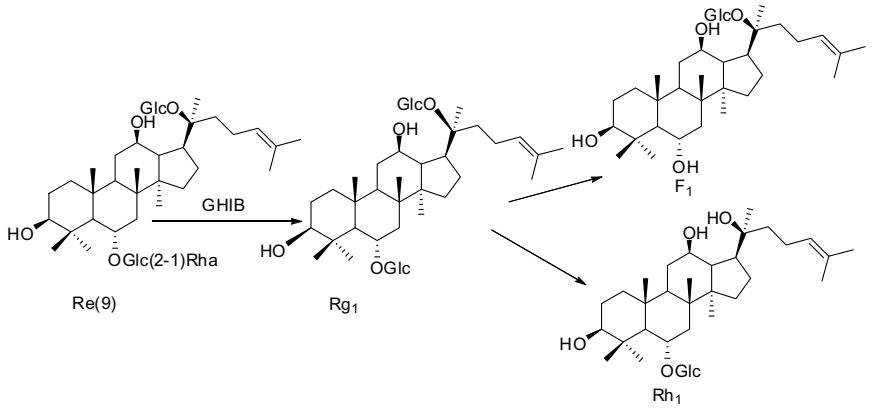


Fig. 9 Biotransformation of ginsenoside Re by GHIB.

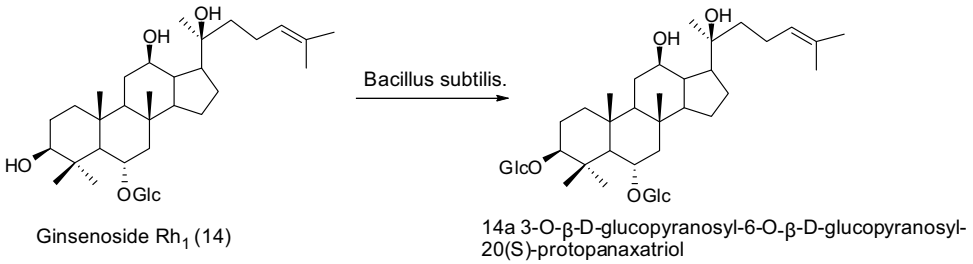


Fig. 10 Biotransformation of Ginsenoside Rh_1 by *Bacillus subtilis*.

bacteria to their final derivatives, Compound K as well. Bae *et al.* (2002) isolated ginsenosides Mb, Mc, Compound K and 20(S)-PPD from the biotransformation of ginsenoside Rc by *Bacteroides* HJ-15 and *Bifidobacterium* K-506. Meanwhile, Ginsenosides Rd, F_2 and Compound K were isolated as metabolites by *Bifidobacterium* K-103 and *Eubacterium* A-44. They have speculated metabolic pathway of Ginsenoside Rc by these human intestinal bacteria above (Bae *et al*. 2002) (**Fig. 8; Table 2**).

Chi *et al*. (2005) reported that ginsenoside Rc (5) and Rb_2 (6) were transformed into Compound K (3e) via Ginsenoside Rd (3a) and F_2 (3d) by *Bifidobacterium* sp. Int57 and *Bifidobacterium* sp. SJ32, *Lactobacillus delbrueckii* transformed Rb_2 and Rc into ginsenoside Rh_2 (3i) and *Bifidobacterium* sp.SH5 transformed Rb_2 and Rc into F_2 (3d) (Chi *et al*. 2005). In addition, 20(S)-protopanaxadiol ginsenosides mixture (containing Rb_1, Rb_2, Rc, Rd, and to a limited extent G-XVII) were metabolized into the minor ginsenoside F_2, by *Pythium irregulare* (Yousef and Bernards 2006) (**Table 2**).

Biotransformation of PPT-type ginsenosides

Ginsenoside Re, a main PPT-type saponin in *Panax ginseng*, its metabolic pathway by human intestinal microflora were investigated. All human fecal specimens metabolized ginsenoside Re, mainly to ginsenoside Rh_1 and ginsenoside F_1, via ginsenoside Rg_1. Almost all isolated ginsenoside re-metabolizing intestinal bacteria (GHIB) also metabolized ginsenoside Re, mainly to ginsenoside Rh_1 and ginsenoside F_1, via ginsenoside Rg_1 (Bae *et al*. 2005) (**Fig. 9**).

Li *et al*. (2006) reported that Ginsenoside Rh_1 (14) was microbial transformed by *Bacillus subtilis* to yield a metabolite 14a (**Fig. 10**).

BIOTRANSFORMATION USING ENZYMES

The Biotransformation of ginsenosides by microorganisms almost depends on the enzymes contained in these strains. The microbial transformation of ginsenosides is characterized by low selectivity, yield, and productivity, and the enzymes involved in the transformation remain unknown. Thus, enzymatic transformation has been proposed as a method with some potential, owing to its high specificity, yield, and productivity. The method is increasingly being recognized as a useful tool in structural modification and metabolism studies. Until now, several enzymes have been extracted from the microorganisms such as *β*-glucosidase,

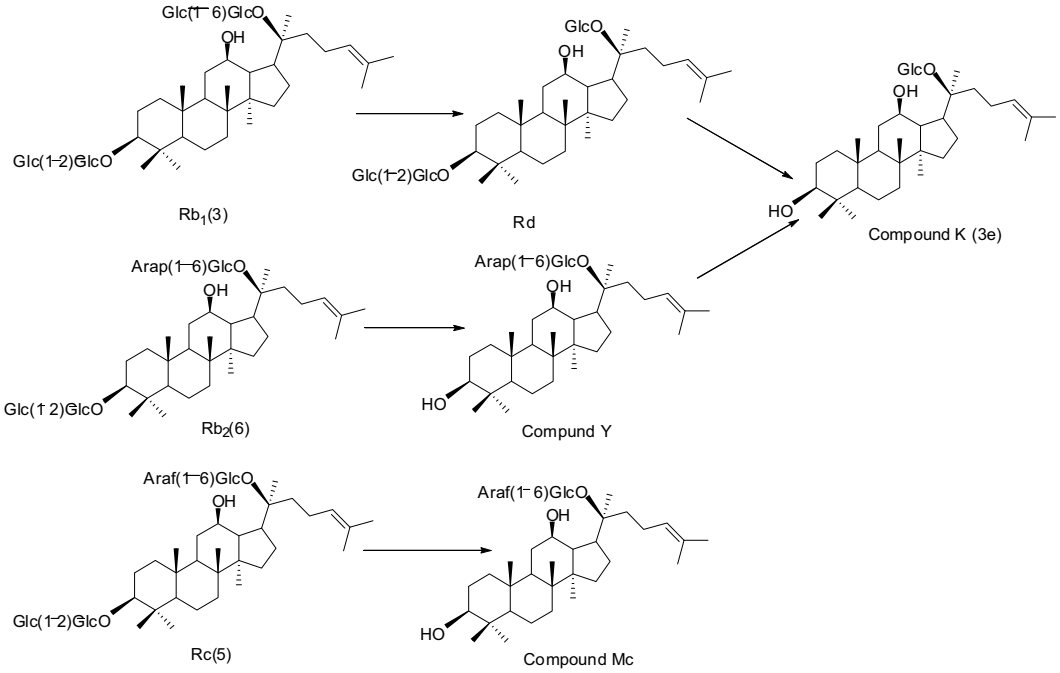


Fig. 11 Transformation pathways from ginsenoside Rb_1, Rb_2, and Rc to Compound K, Compound Y, and Compound Mc by *β*-glycosidase from *Sulfolobus acidocaldarius*, respectively.

β-galactosidase, and *β*-xylosidase *et al*. The selection of a ginsenoside hydrolyzing enzyme largely relies on the type of sugar moiety of the ginsenoside or the type of the aglycone (PPD and PPT), because this determines its substrate specificity. The enzyme (for example *β*-glucosidase) from different sources may transform the same substrate into different metabolites or into the same product through a different pathway.

Biotransformation of PPD-type ginsenosides

A thermostable *β*-glycosidase from *Sulfolobus acidocaldarius* has concomitant *β*-glucosidase, *β*-galactosidase, and *β*-xylosidase activities due to its broad specificity. The rare ginsenosides Compound K (3e), Compound Y and Compound Mc were produced from the major ginsenosides Rb_1 (3), Rb_2 (6), Rc (5), and Rd (3a) by this enzyme via three pathways: Rb_1→Rd→Compound K, Rb_2→Compound Y→ Compound K, and Rc→Compound Mc with a mole yield of 99% after 24 h. Each of the ginsenosides was identified by HPLC-MS (Noh *et al*. 2009; Noh and Oh 2009) (**Fig. 11; Table 3**).

Moreover, a *β*-D-glucosidase, designated as GII, from China white jade snail (*Achatina fulica*) can hydrolyze ginsenosides Rb_1, Rb_2, Rb_3 and Rc into their active metabolites, Compound K, Compound Y, Mx and Mc, respectively. It can cleave both *β*-(1→2)-glucosidic linkage at C-3 and *β*-(1→6)-glucosidic linkage at C-20 of ginsenosides as *β*-Glycosidase (Hu *et al*. 2007) (**Fig. 12; Table 3**).

Furthermore, *β*-D-glucosidase extracted from *Fusobacterium* K-60 and *Paecilomyces Bainier* sp. 229 can hydrolyze ginsenoside Rb_1 with a different hydrolytic pathway via a multiple-step: ginsenoside Rb_1→ginsenoside XVII→ ginsenoside F_2→Compound K (Park *et al*. 2001; Yan *et al*. 2010) (**Fig. 13; Table 3**).

The ginsenoside-*β*-glucosidase that hydrolyzes the *β*-(1→2)-glucoside of the ginsenoside Rg_3 sugar moiety to ginsenoside Rh_2 was isolated from the ginseng root. The optimum temperature of the ginsenoside-*β*-glucosidase was 60°C, and the optimum pH was 5.0. The ginsenoside-*β*-glucosidase may be a special *β*-glucosidase that is different from the original exocellulase. The ginsenoside-*β*-glucosidase hydrolyzes over 60% of Rg_3 to Rh_2, but the The *β*-glucosidase from the microorganism strains do not hydrolyze Rh_2. This indicates that the saponin enzyme from the ginseng plant may be a special *β*-glucosidase, *β*-(1→2)-ginsenoside hydrolase (Zhang *et al*. 2001) (**Fig. 14; Table 3**).

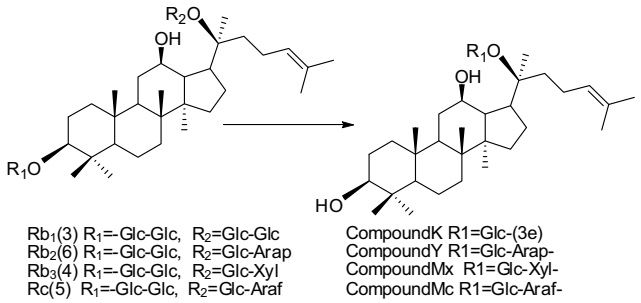


Fig. 12 Transformation pathways from ginsenoside Rb_1, Rb_2, Rb_3 and Rc to Compound K, Compound Y, Compound Mx and Compound Mc by *β*-glucosidase, respectively.

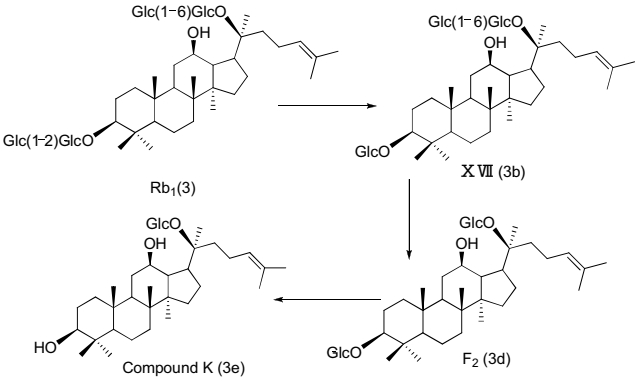


Fig. 13 Transformation pathways from ginsenoside Rb_1 to Compound K by *β*-glucosidase.

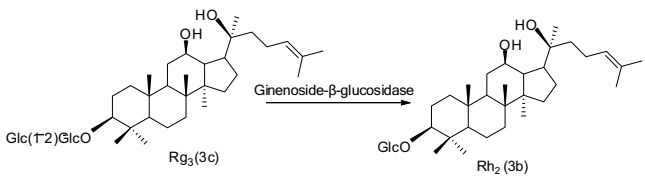


Fig. 14 Biotransformation of Rg_3 by ginsenoside-*β*-glucosidase.

Table 3-1 Biotransformation of ginsenosides by enzymes.

Enzyme kind	Type	Substrate	Product
β-Glycosidase	PPD	Rb_1, Rb_2, Rc and Rd	Compound K, Y and Mc
β-D-glucosidase	PPD	Rb_1, Rb_2, Rb_3 and Rc	Compound K,Y, Mx and Mc
β-D-glucosidase	PPD	Rb_1	Compound K
Ginenoside-*β*-glucosidase	PPD	Rg_3	Rh_2 (3i)
Glucosidase	PPD	20(S, R)-Rg_3	20(S, R)-protopanaxadiol
Ginsenoside-hydrolyzing *β*-glucosidase	PPD	Rb_1	Rg_3
Rb_1-hydrolyzing *β*-glucosidase	PPD	Rb_1	Rd
β-D-Xylosidase	PPD	Ra_1	Rb_2
		Ra_2	Rc
Ginsenosidase	PPD	Rb_1, Rb_2, Rb_3, Rc, Rd	Compound K, F_2, Rh_2
Lactase	PPD	PPD-type saponin mixture	F_2
β-Galactosidase			Compound K
Cellulase			Rd
ginsenoside-*α*-(1→2)-L-rhamnosidase	PPT	20(S,R)-Rg_2	20(S,R)-Rh_1
β-Galactosidase	PPT	PPT-type saponin mixture	Rg_2
β-Galactosidase		PPT-type saponin mixture	Rh_1
Naringinase		PPT-type saponin mixture	F_1 and 20(S)-protopanaxatriol
Hesperidinase		Re	Rg_1
α-Rhamnosidase, *β*-glucosidase	PPT	Re and Rg_1	Rh_1 and F_1
Recombinant *β*-glucosidase	PPT	Rf	Rh_1

Table 3-2 Biotransformation of ginsenosides by enzymes.

Enzyme kind	Hydrolyzing position	Source	Reference
β-Glycosidase	C-3 and C-20	*Sulfolobus acidocaldarius*	Noh *et al*. 2009
			Noh and Oh 2009
β-D-Glucosidase	C-3 and C-20	China white jade snail	Hu *et al*. 2007
β-D-Glucosidase	C-3 and C-20	*Fusobacterium* K-60	Park *et al*. 2001
		Paecilomyces Bainier sp. 229	Yan *et al*. 2010
Ginenoside-*β*-glucosidase	C-3	Ginseng root	Zhang *et al*. 2001
Glucosidase	C-3	*Aspergillus niger*	Liu *et al*. 2010
Ginsenoside-hydrolyzing *β*-glucosidase	C-20	*Paecilomyces Bainier* sp. 229	Yan *et al*. 2008
Rb_1-hydrolyzing *β*-glucosidase	C-20	*Cladosporium fulvum*	Gao *et al*. 2010
β-D-Xylosidase	C-20-O-Xyl	*Bifidobacterium breve* K-110	Shin *et al*. 2003
Ginsenosidase	3-*O*-*β*-Glu, 20-*O*-*β*-Glu, 20-*O*-*β*-Xyl	*Aspergillus* sp. g48p	Yu *et al*. 2007
	20-*O*-*α*-Araf, 20-*O*-*α*-Arap		
Lactase	C-3 and C-20	*Aspergillus oryzae*	Ko *et al.* 2007
β-Galactosidase	C-3 and C-20	*Aspergillus oryzae*	
Cellulase	C-20	*Trichoderma viride*	
Ginsenoside-*α*-(1→2)-L-rhamnosidase	C-6	*Absidia* sp. 39	Yu *et al.* 2002
β-galactosidase	C-20	*Aspergillus oryzae*	Ko *et al.* 2003
β-galactosidase	C-6 and C-20	*Penicillium* sp.	
Naringinase	C-6 and C-20	*Penicillium decumbens*	
Hesperidinase	C-6	*Penicillium* sp.	
α-Rhamnosidase, *β*-glucosidase	C-6 and C-20	*Bacteroides* JY-6	Bae *et al.* 2005
Recombinant *β*-glucosidase	C-6	*Aspergillus niger*	Ruan *et al.* 2009

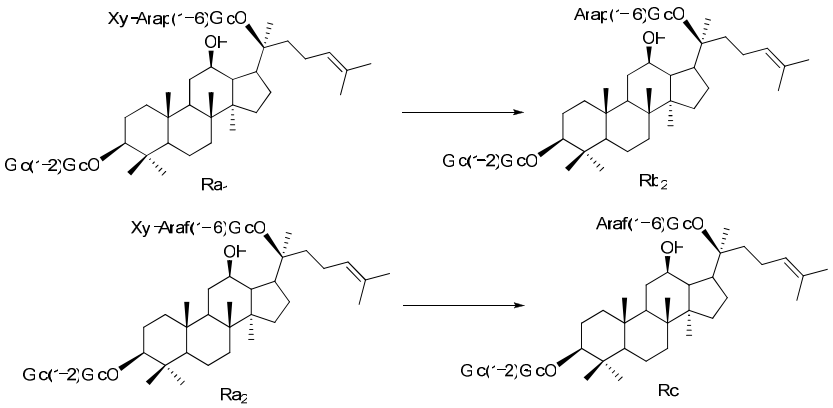


Fig. 15 Proposed metabolic pathway of ginsenosides Ra_1 and Ra_2 by *β*-D-xylosidase.

Furthermore, Liu *et al*. (2010) reported that 20(S, R)-Rg_3 can be transformed into 20(S, R)-protopanaxadiol by the glucosidase from *Aspergillus niger*. The enzymatic reactions were analyzed by reversed-phase HPLC. It was readily found that the glucosidase from *A. niger* was more stable under a slightly acidic environment and unstable as the temperature was above 55°C (Liu *et al*. 2010) (**Table 3**).

A novel ginsenoside-hydrolyzing *β*-Glucosidase from *Paecilomyces Bainier* sp. 229 converted ginsenoside Rb_1 to ginsenoside Rg_3 specifically and efficiently. The hydrolyzing pathway of ginsenoside Rb_1 by the enzyme was Rb_1 →Rd→Rg_3 (Yan *et al*. 2008) (**Table 3**).

Recently, a highly selective ginsenoside Rb_1-hydrolyzing *β*-glucosidase from *Cladosporium fulvum* hydrolyzed

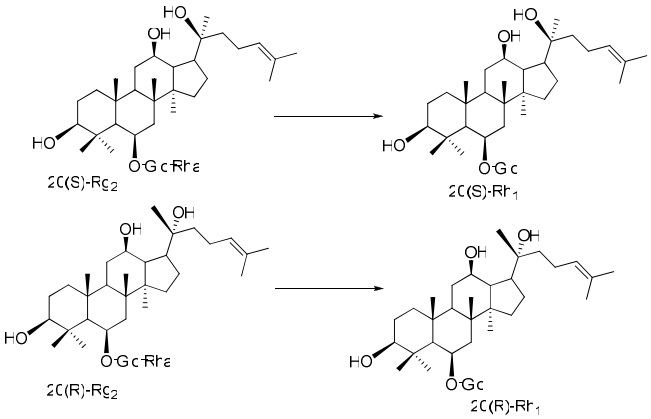


Fig. 16 Biotransformation of ginsenosides 20(S) and 20(R)-Rg_2 by ginsenoside-α-(1→2)-L-rhamnosidase.

β-(1→6)-glucosidic linkage at the C-20 site of ginsenoside Rb_1 to produce ginsenoside Rd, without attack on other β-glucosidic linkages (Gao *et al*. 2010) (**Table 3**).

β-D-Xylosidase has been purified from ginsenoside Ra-metabolizing *Bifidobacterium breve* K-110, which was isolated from human intestinal microflora. β-D-Xylosidase acted to the greatest extent on ginsenoside Ra_1 (11) and ginsenoside Ra_2 (12), but did not act on β-glucopyranoside, β-galactopyranoside or β-D-fucopyranoside (Shin *et al*. 2003) (**Fig. 15; Table 3**).

The new type ginsenosidase which hydrolyzing multi-glycosides of ginsenoside, named ginsenoside type I from *Aspergillus* sp. g48p strain was isolated, characterized and generally described. The enzyme can hydrolyze different glycoside of PPD-type ginsenosides as Rb_1, Rb_2, Rb_3, Rc, Rd, but cannot hydrolyze the glycosides of PPT-type ginsenoside such as Re, Rf, Rg_1. So, when the ginsenosidase type I hydrolyzed ginsenosides, the enzyme selected ginsenoside-aglycone type, can hydrolyze different glycosides of PPD type ginsenoside; however no selected glycoside type, can hydrolyze multi-glycosides of PPD type ginsenosides (Yu *et al*. 2007) (**Table 3**).

Ko *et al.* (2007) reported the hydrolysis of protopanaxadiol-type saponin mixture by various glycoside hydrolases was examined. Among these enzymes, crude preparations of lactase from *Aspergillus oryzae*, β-galactosidase from *A. oryzae*, and cellulase from *Trichoderma viride* were found to produce ginsenoside F_2, Compound K and ginsenoside Rd respectively, from protopanaxadiol-type saponin mixture in large quantities (Ko *et al.* 2007) (**Table 3**).

Biotransformation of PPT-type ginsenosides

Yu *et al.* (2002) reported that ginsenoside-α-(1→2)-L-rhamnosidase from *Absidia* sp.39 was purified and characterized. The enzyme hydrolyzed the C-6, α-(1→2)-L-rhamnoside of 20(S) and 20(R)-ginsenoside Rg_2 (13, 14) to produce the 20(S) and 20(R)-ginsenoside Rh_1 (Yu *et al.* 2002) (**Fig. 16; Table 3**).

During investigation of the hydrolysis of a protopanaxatriol-type saponin mixture by various glycoside hydrolases, crude preparations of β-galactosidase from *Aspergillus oryzae* and lactase from *Penicillium* sp. were found to produce two minor saponins, ginsenoside Rg_2 and ginsenoside Rh_1 respectively, in high yields. Moreover, a *naringinase* preparation from *Penicillium decumbens* readily gave an intestinal bacterial metabolite, ginsenoside F_1, as the main product, with a small amount of 20(S)-protopanaxatriol from a protopanaxatriol-type saponin mixture. Also, a hesperidinase from *Penicillium* sp. selectively hydrolyzed ginsenoside Re into ginsenoside Rg_1 (Ko *et al.* 2003) (**Table 3**).

In addition, α-Rhamnosidase and β-glucosidase, partially purified from *Bacteroides* JY-6 can hydrolyzed ginsenoside Re and ginsenoside Rg_1 to ginsenosides Rh_1 and F_1 (Bae *et al.* 2005) (**Table 3**). And recombinant β-glucosidase isolated from *Aspergillus niger* can transformed ginsenoside R_f to Rh_1 (Ruan *et al.* 2009) (**Table 3**).

FUTURE POSSIBILITIES

So far, several documents have been focused on the biotransformation of ginsenosides in order to gain more active compounds or to increase the yield of the pharmaceutical minor active ginsenosides, and remarkable progress already has been made. Continuing attempts are dedicated into utilizing the biotransformation methods for industrialization. Biotransformation by enzymes is more effective because of the high productivity and low cost. Now, the transformations of ginsenosides by some thermo stable enzymes have been applied to industrial exploitation. However, the recombinant enzymes from non-GRAS hosts may cause safety problems when applied to foods. Thus the application of genetic engineering of microorganisms is expected. In a word, biotransformation is a feasible method to modify the structures of ginsenosides, although the possibility of success in exploring the biocatalytic capability of microorganisms and enzymes is enormous.

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