BIO Integration

Advances in the Production of Minor Ginsenosides Using Microorganisms and Their Enzymes

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Abstract

Minor ginsenodes are of great interest due to their diverse pharmacological activities such as their anti-cancer, anti-diabetic, neuroprotective, immunomodulator, and anti-inflammatory effects. The miniscule amount of minor ginsenosides in ginseng plants has driven the development of their mass production methods. Among the various production methods for minor ginsenosides, the utilization of microorganisms and their enzymes are considered as highly specific, safe, and environmentally friendly. In this review, various minor ginsenosides production strategies, namely utilizing microorganisms and recombinant microbial enzymes, for biotransforming major ginsenosides into minor ginsenoside, as well as constructing synthetic minor ginsenosides production pathways in yeast cell factories, are described and discussed. Furthermore, the present challenges and future research direction for producing minor ginsenosides using those approaches are discussed.

Keywords

biotransformation, biosynthesis, β-glucosidase, Ginsenosides, minor ginsenosides.

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Introduction

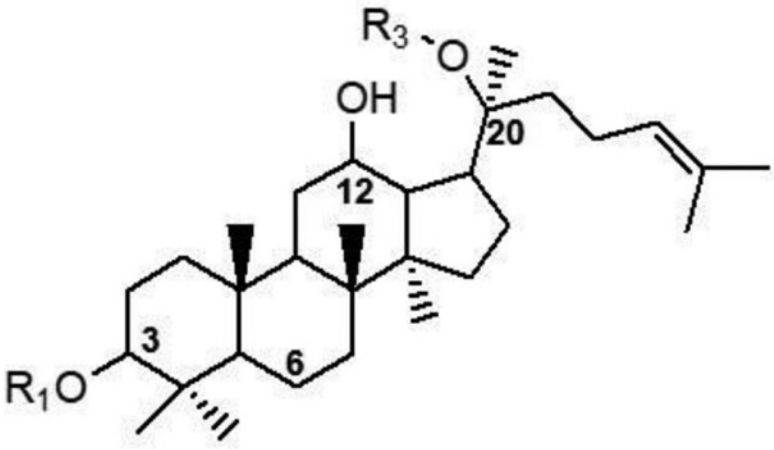
Ginseng, a plant belonging to the Araliaceae family and the genus *Panax*, has been used to treat different kinds of ailments and disease in East Asian countries for millennia [1, 2]. Recently, the popularity of ginseng as a nutraceutical and alternative medicine has been increasing worldwide [3, 4]. The global ginseng market has been reported to be valued at over two billion U.S. dollars and is expected to grow exponentially [5].

The beneficial health effects of ginseng are mainly attributed to ginsenosides, the major bioactive compound of ginseng [6, 7]. Various *in vitro* and *in vivo* studies have demonstrated the diverse pharmacological activities of ginsenosides such as anti-microbial, antioxidant, anti-inflammatory, skin-protective, neuroprotective, anti-cancer, and anti-diabetic effects **(Table 1)**. Most of ginsenosides are classified as protopanaxadiol (PPD), and protopanaxatriol (PPT) types [8]. Both types consist of an aglycon (a non-sugar component) of a dammarane skeleton (PPD or PPT) as an aglycon and one to four molecules of sugar moieties at C-3 and C-20 positions in the case of PPD-type ginsenosides or at C-6 and C-20 positions in the case of PPT-type ginsenosides [9, 10].

Meanwhile, based on their abundance in ginseng, ginsenosides can be classified as major ginsenoside and minor ginsenosides. Major ginsenosides [i.e., Rd, Rc, Rb_2, and Rb_1 (PPD-type); Rg_1, Rf, and Re (PPT-type)] constitute more than 90% of the total ginsenosides, while minor ginsenosides [i.e. F_2, Rg_3, Rh_2, and Compound K (PPD-type); Rg_2, Rh_1, and F_1 (PPT-type)] are only present in small quantities in ginseng [50, 51]. Despite showing some therapeutic effects such as, anti-inflammatory, anti-diabetic, and neuroprotective effects, major ginsenosides are not easily absorbed by the human body [52]. On the other hand, while they are only present in very low amounts in ginseng, minor ginsenosides are considered as being more pharmacologically active than major ginsenosides due to their smaller molecule size, better permeability across the cell membrane, and thus their higher bioavailability [53, 54].

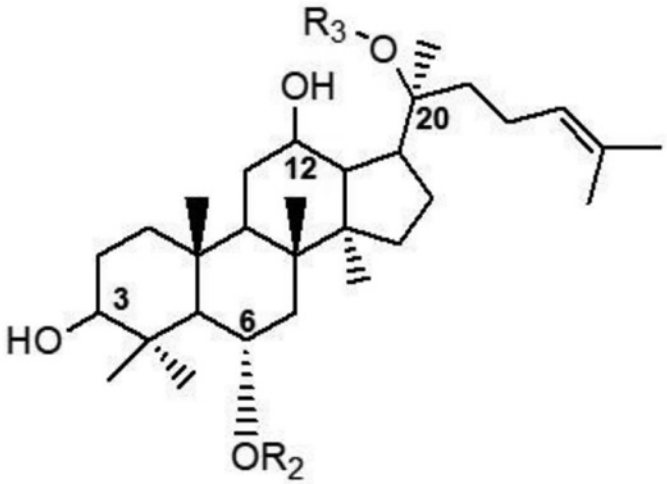
Minor ginsenosides are of commercial interest due to their diverse biological activities and high pharmacological activities. However, the miniscule amount of minor ginsenosides extracted from ginseng cannot satisfy the needs of scientific and clinical studies, as well as commercial purposes

Table 1 Example of PPD- and PPT-type Ginsenosides with Their Health Benefits



20(S)-Protopanaxadiol-type ginsenoside

Name		R1 (C3)	R3 (C20)	Health Benefits	References
Rb_1	Major ginsenosides	Glc^1–^2Glc	Glc^1–^6Glc	Neuroprotective and anti-diabetic effects	[11, 12]
Rb_2		Glc^1–^2Glc	$Ara(p)^1$–^6Glc	Neuroprotective and anti-inflammatory effects	[13, 14]
Rc		Glc^1–^2Glc	$Ara(f)^1$–^6Glc	Anti-inflammatory and skin-protective effects	[15, 16]
Rd		Glc^1–^2Glc	Glc–	Anti-cancer and neuroprotective effects	[17, 18]
F_2	Minor ginsenosides	Glc–	Glc–	Anti-oxidant, anti-cancer, and skin-protective effects	[19–21]
Rg_3		Glc^1–^2Glc	H	Anti-depressant, anti-virus, and anti-cancer effects	[22–24]
Gypenoside XVII (GypXVII)		Glc–	Glc^1–^6Glc	Cardioprotective and neuroprotective effects	[25, 26]
Gypenoside LXXV (GypLXXV)		H–	Glc^1–^6Glc	Skin-protective and anti-cancer effects	[27, 28]
Rh_2		Glc–	H–	Skin protective, immunomodulator, anti-virus, and anti-cancer effects	[29–32]
Compound K (CK)		H	Glc–	Anti-epileptic, anti-cancer, and skin-protective effects	[33–35]



20(S)-Protopanaxatriol-type ginsenoside

Name		R2 (C6)	R3 (C20)	Health Benefit	References
Re	Major ginsenosides	Rha^1–^2Glc	Glc–	Anti-diabetic, kidney-, and heart-protective effects	[36–38]
Rf		Glc^1–^2Glc	H	Neuroprotective and anti-inflammatory effects	[39, 40]
Rg_1		Glc–	Glc–	Neuroprotective and hepatoprotective effects	[41, 42]
Rg_2	Minor ginsenosides	Rha^1–^2Glc	H-	Skin-protective, antidepressant, and anti-inflammatory effects	[43–45]
Rh_1		Glc-	H	Anti-oxidant, anti-inflammatory, immunomodulator, and anti-cancer	[46, 47]
F_1		H	Glc-	Anti-aging, anti-oxidant, skin-protective, immunomodulator	[48, 49]

Ara(f): α-l-arabinofuranosyl; Ara(p): α-l-arabinopyranosyl; Glc: α-d-glucopyranosyl; Rha: α-l-ahamnopyranosyl.

[55, 56]. Therefore, it is very important to develop useful methods for the mass production of minor ginsenosides.

Minor ginsenosides production methods

The most common approach to producing minor ginsenosides is by hydrolyzing the sugar moieties of major ginsenosides [57]. The hydrolysis can be conducted through physical (heat and microwave transformation), chemical (acid and alkali hydrolysis), and biological (biotransformation) methods. Heat transformation *via* baking and steaming transforms PPD-type major ginsenosides Rb_1, Rb_2, and Rc2 into Rd, and finally into minor ginsenosides Rg_3, F_2, compound K, and Rh_2, as well as PPT-type major ginsenosides Re and Rf into minor ginsenosides Rh_1 and F_1, with by-products of acetyl-ginsenosides [58, 59]. Meanwhile, acid hydrolysis using hydrochloric acid transformed Rb_1 into Rg_3 [60]. The physical and chemical methods are considered to be fast and simple; however, the use of those methods often results in the formation of undesirable by-products due to the low specificity. Moreover, there are safety and environmental issues, for example, due to the use of high temperature in the heat transformation approach, or strong acid/base in the acid/alkali hydrolysis methods. On the other hand, biological methods which involve enzymes offer higher reaction specificity, can be conducted in mild conditions minimizing safety risks, and are more environmentally friendly [61]. Thus, there is growing interest in using biological methods for the mass production of minor ginsenosides.

In general, the biological methods for producing minor ginsenosides can be categorized as; the use of microbial cells and enzymes for hydrolyzing sugar moieties of major ginsenosides, and the emerging biosynthesis methods, where the minor ginsenoside biosynthesis pathway is introduced into microorganism hosts. The present paper reviews the advances on using those biological methods for producing minor ginsenosides.

Biotransformation of major ginsenosides into minor ginsenosides using microorganisms

A wide variety of microorganisms have been used in the biotransformation of major ginsenosides into minor ginsenosides **(Table 2)**. The biotransformation activities are mainly attributed to β-glucosidases (β-d-glucopyranoside glucohydrolase) [E.C.3.2.1.21] which hydrolyze the glycosidic bonds of the sugar moieties of the major ginsenosides at the C-3, C-6, and C-20 positions [62]. However, due to specific structures of the aglycon (dammarane skeleton), only specific β-glucosidases, thus specific microorganisms are able to hydrolyze ginsenoside-β-glucoside linkages [63].

Ginseng plantation fields are one of the main sources of microorganisms with major ginsenoside-biotransforming activities. Endophytic microorganisms, spend all or part of their life cycle inside ginseng plants without damaging the plant tissues or inducing defense responses, for example, *Burkholderia* sp. GE 17-7 and *Flavobacterium* sp. GE 32, as well as fungi *Arthrinium* sp. GE 17-18 [64]. Those microorganisms exhibit hydrolysis activities on PPD-type ginsenosides. *Flavobacterium* sp. GE 32 has been reported to hydrolyze the outer glycosidic linkage of Rb_1 at C-3 to produce Gypenoside XVII and the C-20 position to produce Rd. The bacteria also showed hydrolysis activities on the glycosidic linkage at the C-20 position of Rd to generate Rg_3 [65]. Similar hydrolysis activities on the terminal and inner glucopyranosyl moieties at the C-20 position of Rb_1 to produce Rg_3 were also shown by *Burkholderia* sp. GE 17-7 [66]. Meanwhile *Arthrinium* sp. GE 17-18 was shown to have hydrolysis activity on terminal and inner glucopyranosyl moieties at the C-3 position and terminal glucopyranosyl moieties at the C-20 position of Rb_1 to generate CK [67]. Finally, *Cellulosimicrobium* sp. TH-20 isolated from rhizosphere soil of ginseng showed biotransformation activities on PPT-type Re to Rg_2 by hydrolyzing sugar moieties at the C-20 position [68].

Table 2 Production of Minor Ginsenosides in Different Microorganism

Microorganism	Source of Microorganism	Transformation Pathway	Remarks
***Flavobacterium* sp. GE 32** [65]	Root of *Panax ginseng* (Jilin, China)	$Rb_1 \rightarrow$ GypXVII $Rb_1 \rightarrow Rd \rightarrow Rg_3$	–
***Burkholderia* sp. GE 17–7** [66]	*P. ginseng* (Jilin, China)	$Rb_1 \rightarrow Rd \rightarrow Rg_3$	Rg_3 was produced with conversion rate of 98% after 15 h
***Arthrinium* sp. GE 17–18** [67]	Root of *P. ginseng* (Jilin, China)	$Rb_1 \rightarrow Rd \rightarrow F_2 \rightarrow CK$	Endophytic fungi
***Cellulosimicrobium* sp. TH-20** [68]	Soil of ginseng field (Fusong, China)	$Re \rightarrow Rg_2$	Re (30 mg) was transformed into Rg_2 (24 mg) with a yield of 96%
***C. allociferrii* JNO301** [70]	Meju (dried fermented soybeans) from South Korea	$Rb_1 \rightarrow Rd \rightarrow F_2$ $Rf \rightarrow Rh_1$	Yeast
***Leuconostoc mesenteroides* WIKim19** [71]	Kimchi (fermented vegetable) from South Korea	$Rb_1 \rightarrow Rd \rightarrow Rg_3$	–
***Lactobacillus rhamnosus* GG** [72]	Culture collection	$Rb_1 \rightarrow Rd$	Probiotic microorganism
Schizophyllum commune [73]	Culture collection	$Rb_1 \rightarrow Rd \rightarrow F_2 \rightarrow CK$	Edible and medicinal fungi

Generally recognized as safe (GRAS) microorganisms (i.e., probiotics, microorganisms from fermented foods, etc.), which are non-pathogenic and considered as safe to be used for nutraceutical and pharmaceutical purposes, were also reported to have major ginsenoside-biotransforming activities [69]. *Candida allociferrii* JNO301 yeast isolated from Korean fermented soybean showed hydrolysis activity on outer glucopyranosyl moieties at the C-3 and C-20 positions of Rb1 to produce F_2. Interestingly the yeast also exhibited biotransformation activity on PPT-type ginsenoside Rf into Rh_1, by hydrolyzing outer sugar moieties at the C-6 position [70].

Biotransformation of major ginsenosides into minor ginsenosides using β-glucosidase from microorganisms

Purified recombinant enzymes are considered superior to the enzymes isolated and purified from cultured microorganisms due to their higher selectivity and activity [74–76]. Gram-scale quantities of minor ginsenosides were produced using enzymes from *Microbacterium* sp. Gsoil 167 isolated from ginseng plantation and *Lactobacillus ginsenosidimutans* EMML 3041 from kimchi (fermented vegetable) [27, 77] **(Table 3)**. Thermostable β-glucosidase were also reported to exhibit ginsenoside-biotransformation activities which resulted in the production of Gram-scale quantities of minor ginsenosides. Those thermostable enzymes are of industrial interest as the enzymes can be used in combination with heat and acid hydrolysis to further accelerate and increase the yield of major ginsenoside biotransformation. While most of ginsenoside-biotransforming microorganisms and enzymes are isolated from the East Asia region, interestingly one bacterial isolate from Indonesia has β-glucosidase that can hydrolyze the outer sugar moieties at C-3 and C-20 of Rb_1 to produce F_2 [62].

Based on the data in **Table 3**, *Escherichia coli* is the most common host for the production of recombinant β-glucosidase. This is due to the simplicity of *E. coli* genetic modification and its rapid growth in relatively inexpensive media [78, 79]. However, *E. coli* is not preferable for application in food and pharmaceutical industries due to its non-GRAS status [80]. Efforts to produce ginsenoside transforming-β-glucosidase in GRAS microorganisms, such as *Corynebacterium glutamicum* and *Lactococcus lactis* have been attempted. *Microbacterium testaceum* β-glucosidase was successfully expressed in *C. glutamicum* and exhibit biotransformation activity for both PPD- and PPT-type ginsenosides, and resulted in the production of Gram-scale quantities of CK and F_1 [56]. However, in general, the quantity and activity of ginsenoside biotransforming-β-glucosidases produced in GRAS hosts are not as high as recombinant β-glucosidases produced in *E. coli* [81, 82].

Biosynthesis of minor ginsenoside in microorganisms

Despite the high efficiency, biotransformation methods for producing minor ginsenosides require ginseng extracts as the raw material which are produced through time-consuming (typically, 5–6 years are required to produce marketable ginseng), labor-intensive, energy-consuming, and high-cost processes and can be affected by many factors such as soil quality, climate, pathogens, and pests [92, 93]. The discovery of genes encoding enzymes involved in the minor ginsenosides synthesis pathway coupled with the advances in synthetic biology tools, has allowed the construction of microbial cell factories, which can provide more sustainable and cost-effective alternative to mass-produced minor ginsenosides from renewable resources [94, 95].

The ginsenoside biosynthesis pathway has mainly been introduced for three species of yeasts (*Saccharomyces cerevisiae*, *Pichia pastoris*, and *Yarrowia lipolytica*) which are on the GRAS list of microorganisms, and are compatible for the expression of plant-derived heterologous enzymes [95]. In general, there are three general strategies to produce minor ginsenosides in yeast cell factories: (1) improving the yield of the yeast native mevalonic acid (MVA) pathway; (2) introducing genes for the synthesis of ginsenosides aglycons; and (3) introducing uridine diphosphate (UDP)-glycosyltransferase genes for the addition of sugar moieties to the aglycons **(Figure 1)**.

The MVA pathway produces isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) which are important precursors for the synthesis of ginsenosides, from acetyl coenzyme A (acetyl-CoA). The native MVA pathway of yeasts is improved by overexpressing 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) which catalyzes the production of mevalonate from 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA). Furthermore, to eliminate the post-transcriptional feedback inhibition, *hmgr* gene with a truncated N-terminal (*thmgr*), which lacks its N-terminal transmembrane sequence coding for membrane-binding activity, is used [96]. Additionally, the acetyl-CoA supply to the pathway can be improved, for example, *via* the overexpression of the *ALD6* gene (encoding NADP-dependent aldehyde dehydrogenase) along with the introduction of a synthetic codon-optimized acetyl-CoA synthase mutant from *Salmonella enterica* [97].

IPP and DMAPP are then converted to 2,3 oxidosqualene, the precursor of ginsenoside aglycons. The yield of 2,3 oxidosqualene is improved by the overexpression of squalene synthase (SQS)- and squalene epoxidase (SQE)-encoding genes. To minimize the utilization of 2,3 oxidosqualene by competing yeasts in the native ergosterol pathway, downregulation of the Erg7/LS (lanosterol synthase)-encoding gene is conducted. The subsequent conversion of 2,3 oxidosqualene into dammarenediol and protopanaxadiol/protopanaxatriol was facilitated by the introduction of dammarenediol II synthase and cytochrome P450 (*CYP*) [protopanaxadiol synthase (*CYP716A47*) and protopanaxatriol synthase (*CYP716A53v2*)] genes from *P. ginseng* and NADPH-cytochrome P450 reductase (*CPR*) from *Arabidopsis thaliana* or *P. ginseng* [92, 98, 99].

Finally, the addition of sugar moieties to PPD and PPT aglycons was facilitated by the introduction of glycosyltransferases-encoding genes. UDP-glycosyltransferases from *P. ginseng*, PgUGT45 and PgUGT74AE2, catalyze the transfer of a glucose moiety from UDP-glucose (UDP-Glc) to the C3 hydroxyl groups of PPD to form Rh_2, whereas PgUGT29

Table 3 Production of Minor Ginsenosides Using Different Recombinant β-Glucosidase

β-Glucosidase	Source of Microorganism	Transformation Pathway	Yields
Recombinant β-glucosidase from *Microbacterium* sp. Gsoil 167 expressed in *E. coli* BL21(DE3) [27]	Soil of ginseng field (Pocheon, South Korea)	• GypXVII → GypLXXV	• GypXVII (10 g) was transformed into GypLXXV (5.7 g; 69.6% recovery; and 97.8 chromatography purity)
Recombinant β-glucosidase from *Arachidicoccus ginsenosidimutans* sp. nov. expressed in *E. coli* BL21(DE3) [83]	Ginseng compost (South Korea)	• Rb_1 → GypXVII → F_2 → CK • Rd → F_2	–
Recombinant β-glucosidase from *Microbacterium esteraromaticum* expressed in *E. coli* BL21(DE3) [84]	Soil of ginseng field (South Korea)	• Re → Rg_2 • Rg_1 → Rh_1	• Re (1 mg) was transformed into Rg_2 (0.83 mg; 100% molar conversion yield within 150 min) • Rg_1 (1 mg) was transformed into Rh_1 (0.6 mg; 78% molar conversion yield within 15 min)
Recombinant β-glucosidase from *M. esteraromaticum* expressed in *E. coli* BL21(DE3) [85]	Soil of ginseng field (South Korea)	• Rb_1 → Rd → CK	• Rb_1 (1 mg/ml) was transformed into CK (0.46 mg/ml; 77% molar conversion yield within 60 min)
Recombinant β-glucosidase from *Pseudonocardia* sp. *Gsoil 1536* expressed in *E. coli* BL21(DE3) [51]	Soil of ginseng field (Pocheon, South Korea)	• Re → Rg_2	• Re (150 g) was transformed into Rg_2 (150 g; 84.0±1.1% chromatographic purity)
Recombinant β-glucosidase from *Lactobacillus brevis* expressed in *E. coli* BL21(DE3) [86]	Kimchi (fermented vegetable) from South Korea	• GypXVII → CK	• GypXVII was transformed into CK (89% molar conversion yield within 6 h)
Recombinant β-glucosidase from *L. ginsenosidimutans* EMML 3041 expressed in *E. coli* BL21(DE3) [77]	Kimchi (fermented vegetable) from South Korea	• Rb_1 → Rd → Rg_3	• Rb_1 (50 g) was transformed into Rg_3 (30 g; 74.3% chromatographic purity)
Recombinant β-glucosidase from *Thermotoga thermarum* DSM 5069 expressed in *E. coli* JM109 (DE3) [87]	Culture Collection	• Re → Rg_2 • Rg_1 → Rh_1	• Re (10 g/L) was transformed into Rg_2 (8.02 g/L) within 60 min at 85 °C and PH 5.5. • Rg_1 (2 g/L) was transformed into Rh_1 (1.56 g/L) within 60 min at 85 °C and PH 5.5
Recombinant β-glucosidase from *Caldicellulosiruptor bescii* in *E. coli* [88]	Culture Collection	• Rb_1 → Rd → F_2 → CK • Rc → Rd → F_2 → CK	• CK was produced from Rb_1 with the productivity of 1000 (μM/h) within 80 min at 80 °C and PH 5.5 • CK was produced from Rc with the productivity of 400 (μM/h) within 180 min at 80 °C and and PH 5.5
Recombinant β-glucosidase from *Thermotoga neapolitana* DSM 4359 in *E. coli* [89]	Culture Collection	• Re → Rg_2	• Re (2 mg/ml) was transformed into Rg_2 (1.66 mg/ml; 100% molar conversion yield) within 3 h at 85 °C and PH 5.5
Recombinant β-glucosidase from *Serratia marcescens* LII61 expressed in *E. coli* BL21(DE3) [62]	Slaughterhouse waste (Indonesia)	• Rb_1 → Rd → F_2 • Rb_1 → GypXVII → F_2	–
Recombinant β-glucosidase from *M. testaceum* ATCC 15829 expressed in *C. glutamicum* ATCC 13032 [56]	Culture Collection	• Rb_1 → GypXVII → GypLXXV → F_2 → CK • Rd → F_2 → CK • Re → F_1 • Rg → F_1	• CK (7.59 g/L) was produced from PPD-type ginsenoside mixtures in 24 h • F_1 (9.42 g/L) was produced from PPT-type ginsenoside mixtures in 24 h
Recombinant β-glucosidase from *Paenibacillus mucilaginosus* KCTC 3870 expressed in *C. glutamicum* ATCC 13032 [81]	Culture Collection	• Rg_3 → Rh_2	–
Recombinant β-glucosidase from *Flavobacterium johnsoniae* expressed in *L. lactis* subsp. *cremoris* NZ9000 [90]	Culture Collection	• Rb_1 → Rd → F_2 → CK • Rb_1 → Rd → Rg_3	–
Recombinant β-glucosidase from *P. mucilaginosus* expressed in *L. lactis* subsp. *cremoris* NZ9000 [91]	Culture Collection	• Rb_1 → Rd → F_2	• F_2 was produced from PPD-type ginsenoside mixtures with 74% conversion yield

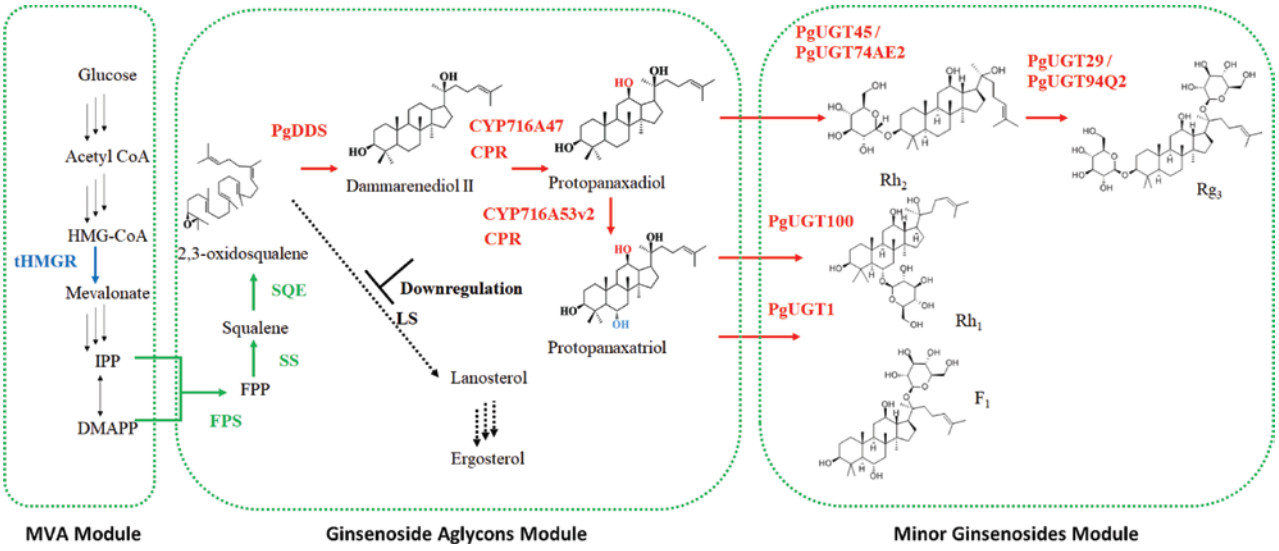


Figure 1 Biosynthetic pathways for minor ginsenosides in metabolically engineered *S. cerevisiae*.
Single arrows represent one-step conversions, while multiple arrows represent multiple steps. Bold, blue arrow represents over-expressed modified yeast endogenous genes (*tHMGR*: truncated 3-hydroxy-3-methylglutaryl-CoA reductase). Bold, green arrows represent over-expressed yeast endogenous genes (FPS: farnesyl pyrophosphate synthase; SS: squalene synthase; SQE: squalene epoxidase). Bold, red arrows represent exogenous plant genes that were introduced into *S. cerevisiae* (*PgDDS*: *P. ginseng* dammarenediol II synthase; *CYP716A47*: *P. ginseng* protopanaxadiol synthase; *CYP716A53v2*: *P. ginseng* protopanaxatriol synthase; CPR: *A. thaliana*/*P. ginseng* NADPH-cytochrome P450 reductase; *PgUGTs*: *P. ginseng* UDP-glycosyltransferases). Dashed arrow represents competing pathway (LS: lanosterol synthase). Intermediates: HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A; IPP: isopentenyl pyrophosphate; DMAPP: dimethylallyl pyrophosphate; FPP: farnesyl diphosphate.

Table 4 Production of Minor Ginsenosides Using Different Yeast Cell Factories

Host Microorganism	Strategies	Products	Yield
***S. cerevisiae* strain BY4742 (*MAT*α, *his3*Δ*1*, *leu2*Δ*0*, *lys2*Δ*0*, *ura3*Δ*0*)**	• Enhancing MVA pathway of *S. cerevisiae* strain BY4742 • Introducing dammarenediol synthase gene and protopanaxadiol synthase genes and UDP-glycosyltransferase genes from *P. ginseng* (*PgUGT45* and *PgUGT29*) into *S. cerevisiae* strain BY4742	Rh_2, Rg_3	Rg_3 (3.49 ± 0.14 μmol/g dry cell weight) and Rh_2 (1.45 ± 0.27 μmol/g dry cell weight) were produced after 6 days [103]
***S. cerevisiae* strain ZW-PPD-B (PPD-producing yeast strain)** [103]	• Introducing cytochrome P450 *CYP716A53v2*, cytochrome P450 reductase *PgCPR1*, and UDP-glycosyltransferase *PgUGT100* genes into *S. cerevisiae* strain ZW-PPD-B to construct an Rh1-producing yeast strain • Introducing *CYP716A53v2*, *PgCPR1*, and *PgUGT1* genes into *S. cerevisiae* strain ZW-PPD-B to construct an F1-producing yeast strain	Rh_1, F_1	Rh_1 (92.8 ± 12.5 mg/L) and F_1 (42.1 ± 3.2 mg/L) were produced after 6 days [102]
***S. cerevisiae* strain ZD-PPD-016 (PPD-producing yeast strain)** [96]	• Conducting site-directed mutagenesis and iterative saturation mutagenesis to *S. cerevisiae* glycosyltransferase-encoding gene (*UGT51*) • Introducing mutant *UGT51* gene into *S. cerevisiae* strain ZD-PPD-016 to construct an Rh_2-producing yeast strain	Rh_2	Rh_2 (300 mg/L) was produced *via* a 5 L fed-batch fermentation [104]
***S. cerevisiae* strain BY4742 (*MAT*α, *his3*Δ*1*, *leu2*Δ*0*, *lys2*Δ*0*, *ura3*Δ*0*)**	• Modular engineering of the MVA pathway • Optimizing the expression level of cytochrome P450 • Increasing the copy number of UDP-glycosyltransferase-encoding gene (*UGTPg45* from *P. ginseng*) and engineering its promoter to increase the expression level of the enzyme • Conducting *in vivo* directed evolution to the *in vivo* activity of *UGTPg45* in yeast	Rh_2	Rh_2 (2.25 g/L) was produced *via* a 10 L fed-batch fermentation [105]
***Y. lipolytica* ATCC 201249**	• Overexpressing key genes in the MVA pathway • Constructing fusion of cytochrome NADPH-P450 reductase, and P450 monooxygenase (*PPDS*), and introducing the gene into *Y. lipolytica* ATCC 201249 • Introducing UDP-glycosyltransferase genes *UGT1* from *P. ginseng* into *Y. lipolytica* ATCC 201249	CK	CK (161.8 mg/L) was produced *via* 5 L fed-batch fermentation [106]

MVA: Mevalonic acid.

and PgUGT94Q2 catalyze the transfer of a glucose moiety from UDP-Glc to Rh_2 to form Rg_3 [100, 101]. Meanwhile, PgUGT100 and PgUGT1 transfer a glucose moiety from UDP-glucose (UDP-Glc) to the C-6 and C-20 hydroxyl groups of PPT to form Rh_1 and F_1, respectively [102]. The application of the general strategies along with other strategy

such as protein engineering resulted in the production of minor ginsenoside in Gram-scale quantities **(Table 4)**.

Conclusion and future perspectives

Due to their medical importance and high economical value, investigations on the mass-production methods of minor ginsenosides, especially those that involve microorganisms and their enzymes, have garnered great interest. Various microorganisms have been reported to be able to convert major ginsenosides into minor ginsenosides, including GRAS microorganisms which would be suitable for the food and drugs industries. To date the highest yield of minor ginsenosides has been obtained *via* the biotransformation of major ginsenosides using recombinant β-glucosidase expressed in *E. coli* systems. However, *E. coli* is considered as unsafe, and is an inedible bacteria, which would limit its application in the nutraceutical and pharmaceutical industries. Thus, the improvements of recombinant β-glucosidase production in GRAS strains such as *C. glutamicum* and *L. lactis*, for example, through the optimization of growth condition, and media, as well as genetic engineering, are needed.

Finally, the costly, time-consuming, and labor-intensive process of producing ginseng extract as substrates for biotransformation, has driven the development of more sustainable ways to produce minor ginsenosides, especially *via* biosynthesis in yeast cell factories. The introduction of ginsenosides biosynthesis genes from *P. ginseng* coupled with the optimization of yeast native pathways has led to the successful production of minor ginsenosides. Further optimization using protein engineering, synthetic biology, and metabolic engineering approaches, as well as the development of efficient fermentation strategies are critical to unleash the full potential of yeast cell factories to mass produce minor ginsenosides.

Competing interests

The authors declare that they have no competing interests.

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