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Biosynthesis of Pinocembrin from Glucose Using Engineered *Escherichia coli*

Bong Gyu Kim[1†], Hyejin Lee[2†], and Joong-Hoon Ahn[2*]

[1]*Department of Forest Resources, Gyeongnam National University of Science and Technology, Jinju-si 660-758, Republic of Korea*
[2]*Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Republic of Korea*

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*Corresponding author
Phone: +82-2-450-3764;
Fax: +82-2-3437-6106;
E-mail: jhahn@konkuk.ac.kr

†These authors contributed equally to this work.

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Pinocembrin is a flavonoid that exhibits diverse biological properties. Although the major source of pinocembrin is propolis, it can be synthesized biologically using microorganisms such as *Escherichia coli*, which has been used to synthesize diverse natural compounds. Pinocembrin is synthesized from phenylalanine by the action of three enzymes; phenylalanine ammonia lyase (PAL), 4-coumarate:CoA ligase (4CL), and chalcone synthase (CHS). In order to synthesize pinocembrin from glucose in *Escherichia coli*, the *PAL*, *4CL*, and *CHS* genes from three different plants were introduced into an *E. coli* strain. Next, we tested the different constructs containing 4CL and CHS. In addition, the malonyl-CoA level was increased by overexpressing acetyl-CoA carboxylase. Through these strategies, a high production yield (97 mg/l) of pinocembrin was achieved.

Keywords: Flavonoid biosynthesis, metabolic engineering, pinocembrin

The amino acids tyrosine and phenylalanine are primary metabolites that serve as entry points for the synthesis of flavonoids in plants. In plants, phenylalanine undergoes deamination by phenylalanine ammonia lyase (PAL) that leads to the synthesis of cinnamic acid. Sequential hydroxylation and *O*-methylation result in the production of hydroxycinnamic acids, including *p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid [4]. *p*-Coumaric acid can be synthesized from phenylalanine by deamination and hydroxylation, or from tyrosine by deamination. PALs from monocotyledons recognize not only phenylalanine but also tyrosine [8, 23], whereas PALs from dicotyledonous plants recognize only phenylalanine [3].

Hydroxycinnamic acids serve as substrates for various compounds, including flavonoids, isoflavonoids, stilbenes, aurones, catechins, proanthocyanidins, lignans, lignin, and phenylpropenes [25]. The first committed step for the synthesis of these compounds is the attachment of coenzyme A to hydroxycinnamic acids. Coenzyme A is attached to cinnamic acid or *p*-coumaric acid by 4-coumarate:CoA ligase (4CL) to form cinnamoyl-CoA or *p*-coumaroyl-CoA [5]. Flavanones (naringenin and pinocembrin) are synthesized from the condensation of one molecule of hydroxycinnamoyl-CoA and three molecules of malonyl-CoA. Condensation of one molecule of cinnamoyl-CoA with three molecules of malonyl-CoA leads to the synthesis of pinocembrin, whereas *p*-coumaroyl-CoA and malonyl-CoA are required for the synthesis of naringenin [2]. These two flavanones are the most essential components required in the synthesis of diverse flavonoids, including flavones, flavonols, isoflavones, and anthocyanins [27].

Pinocembrin, one of the major ingredients of propolis [6], exerts diverse activities, including antibacterial activity [26], antifungal activity [21], inhibition of atherosclerosis [30], and protection of neurons in Alzheimer's disease [16]. Pinocembrin has been extracted from propolis [22], and the biological synthesis of pinocembrin has been performed using microorganisms introduced with an artificial gene cluster. Mostly, cinnamic acid is used as the starting material for the synthesis of pinocembrin [14, 15, 20, 29]. Biosynthesis of pinocembrin from cinnamic acid does not require PAL, which converts phenylalanine to cinnamic acid. Glucose has also been used as the starting material to synthesize pinocembrin using *Escherichia coli* [19, 28]. Another flavanone, naringenin, was also synthesized from glucose in *E. coli* by modulating its tyrosine biosynthesis

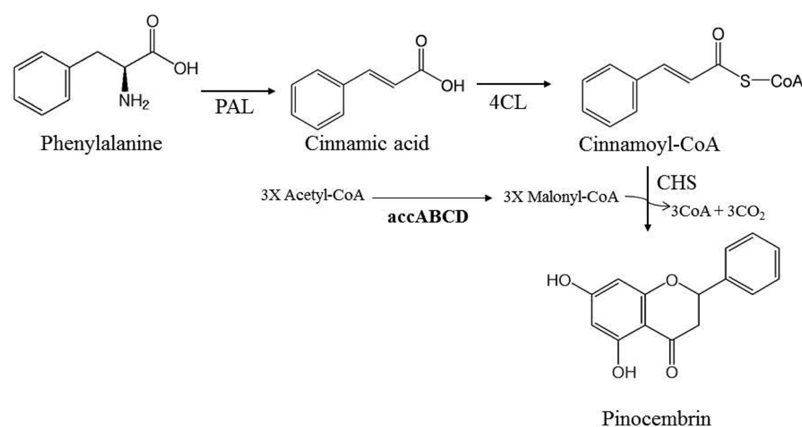

Fig. 1. Biosynthesis pathway for pinocembrin.
PAL, phenylalanine ammonium lyase; 4CL, 4-coumarate CoA ligase; CHS, chalcone synthase; accABCD, acetyl-CoA carboxylase.

pathway [24]. Compared with the synthesis of flavanone from glucose, the synthesis of flavanone from hydroxycinnamic acids such as cinnamic acid and *p*-coumaric acid leads to enhanced production of pinocembrin or naringenin. However, the synthesis of pinocembein from glucose has an economical advantage, because glucose costs much less than cinnamic acid.

Three enzymes are required to synthesize pinocembrin from glucose (Fig. 1). PAL converts phenylalanine into cinnamic acid, which is then converted into cinnamoyl-CoA through 4CL. CHS condensates cinnamoyl-CoA and three molecules of malonyl-CoA to make pinocembrin. The PAL used in this study had approximately 1,280-fold higher catalytic efficiency for phenyalanine than for tyrosine [3]. The *PAL* gene from *Arabidopsis thaliana* (GenBank XM_002877863) was cloned by reverse-transcription polymerase chain reaction as described previously [12]. The *4CL* (*Os4CL*) and *CHS* (*PeCHS*) genes, which were cloned as previously described [13, 10], were regulated by independent *T7* promoters. *Os4CL* was cloned into the *Eco*RI/*Not*I site of the pCDFDuet vector. *PeCHS* was subcloned into the second cloning site, *Nde*I/*Kpn*I, of pCDFDuet harboring *Os4CL*. *E. coli* BL21 (DE3) were transformed with three genes (pA-AtPAL and pC-pOs4CL-pCHS in Table 1). *E. coli* transformants (B-Pin1) were grown in Luria-Bertani (LB) broth containing 50 μg/ml of chloramphenicol and 50 μg/ml of spectinomycin. The culture was inoculated into fresh LB medium containing 50 μg/ml chloramphenicol and 50 μg/ml of spectinomycin, and the culture was incubated at 37°C with shaking until an OD_{600} of 1.0 was attained. The cells were harvested and resuspended with M9 medium containing 2% glucose, 0.2% yeast, 50 μg/ml of chloramphenicol, 50 μg/ml spectinomycin, and 0.1 mM IPTG. The resulting culture was incubated at 30°C for 24 h with shaking at 180 rpm, and then 300 μl of the culture was extracted with 600 μl of ethylacetate. The supernatant was collected, dried, and dissolved in 100 μl of dimethyl sulfoxide; 10 μl of the resultant solution was analyzed by high-performance liquid chromatography (HPLC), using an Ultimate 3000 HPLC (Thermo Scientific, USA) equipped with a photodiode array (PDA) detector and a Varian C18 reverse-phase column (Varian, 4.60 × 250 mm, 3.5 μm particle size). The separation condition for HPLC was as described by Kim *et al*. [12]. HPLC analysis of the culture showed a new peak (Fig. 2); the molecular mass of the corresponding product of this peak was 256 Da, which corresponded with the molecular mass of pinocembrin. Empty vectors (pACYCDuet and pCDFDuet) were introduced into BL21 as controls, and the resulting transformant did not synthesize any product (data not shown). In order to determine the structure of this reaction product from B-Pin1, nuclear magnetic resonance (NMR) analysis [11] was carried out and the following data were obtained: ^{1}H NMR (400 MHz, MeOD) δ 7.47–7.49 (m, 2H), 7.35–7.43 (m, 3H), 5.93 (d, *J* = 2.2 Hz, 1H), 5.89 (d, *J* = 2.1 Hz, 1H), 5.44 (dd, *J* = 12.8, 3.0 Hz, 1H), 3.09 (dd, *J* = 17.1, 12.8 Hz, 1H), and 2.76 (dd, *J* = 17.1, 3.1 Hz, 1H). These data are in concordance with those reported previously [1, 30]. Therefore, the structure of product was confirmed to be pinocembrin.

Next, we tested the effect of the promoter on the production of pinocembrin. Two constructs were built with *Os4CL* and *PeCHS* (Table 1); the first construct (pseudo-operon type) has two *T7* promoters (pC-pOs4CL-pCHS), each of which controls the expression of Os4CL and PeCHS, respectively. The second construct (operon type) has *Os4CL* and *PeCHS* genes under the control of one *T7*

Table 1. Plasmids and strains used in the present study.

Plasmids / *E. coli* strains	Relevant properties or genetic marker	Source or reference
Plasmids		
pACYCDuet	P15A ori, Cm^r	Novagen
pCDFDuet	CloDE13 ori, Str^r	Novagen
pETDuet	f1 ori, Amp^r	Novagen
pA-AtPAL	pACYCDuet harboring *PAL* from *Arabidopsis thaliana*	This study
pC-pOs4CL-pPeCHS	pCDFDuet harboring *4CL* from *Oryza sativa* and *CHS* from *Populus euramericana*. Each gene is controlled by an independent *T7* promoter.	This study
pC-pPeCHS Os4CL	pCDFDuet harboring *CHS* from *Populus euramericana* and *4CL* from *Oryza sativa*. Both genes are controlled by one *T7* promoter.	This study
pC-pAtPAL- pPeCHS- Os4CL	pCDFDuet harboring AtPAL, *4CL* from *Oryza sativa*, and *CHS* from *Populus euramericana*.	This study
pE-pPeCHS- Os4CL	pETDuet harboring *4CL* from *Oryza sativa* and *CHS* from *Populus euramericana*. Both genes are controlled by one *T7* promoter.	This study
pA-accABCD	pACYCDuet harboring *accA*, *accB*, *accC*, and *accD* from *Photorhabdus luminescens*	This study
Strains		
BL21 (DE3)	F^- *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm lon* (DE3)	Novagen
B-Pin1	BL21 harboring pA-AtPAL and pC-pOs4CL-pPeCHS	This study
B-Pin2	BL21 harboring pA-AtPAL and pC-pPeCHS Os4CL	This study
B-Pin3	BL21 harboring pA-accABCD and pC-AtPAL-pOs4CL-PeCHS	This study
B-Pin4	BL21 harboring pA-accABCD, pC-AtPAL-pOs4CL-PeCHS, and pE-Os4CL-PeCHS	This study

promoter (pC-pPeCHS- pOs4CL). In order to make this construct, *PeCHS* was amplified with the following primers: 5′-ATGATTCGATGGCACCGTCGATTGAGGA-3′ (forward, *Eco*RI site is underlined) and 5′-ATTCTAGATCATGAG TAAATTGTTTGTTCTACTG-3′ (reverse, *Xba*I site is underlined). *Os4CL* was amplified with the following primers: 5′-ATACTAGTaggaggattacaaaATGGATCCGA-3′ (forward, lower case indicates ribosome-binding site and *Spe*I site is underlined) and 5′-ATGCGGCCGCTTAGCTGCTTTTGG GCGC-3′; reverse, *Not*I site is underlined.). After the *PeCHS* PCR product was digested with *Xba*I and the *Os4CL* PCR product was digested with *Spe*I, the resulting fragments were ligated. PCR was performed with *Os4CL* forward and *PeCHS* reverse primers. The resulting PCR product was digested with *Eco*RI/*Not*I and subcloned into the corresponding site of pCDFDuet. Strain B-Pin2, which contained the operon type construct produced more pinocembrin (approximately 58 mg/l) than strain B-Pin1 (approximately 46 mg/l) (Fig. 3). These results suggested that the coordinated expression control of *4CL* and *CHS* by one *T7* promoter is better than the independent expression of *4CL* and *CHS* by two *T7* promoters. AtPAL was also subcloned into the second cloning site of pC-p PeCHS-Os4CL. A previous study [7] showed that the pinocembrin production was better when all three *PAL*, *4CL*, and *CHS* genes were controlled by three independent *T7* promoters. It was claimed that more proteins were expressed when the three genes were independently controlled by *T7* promoter

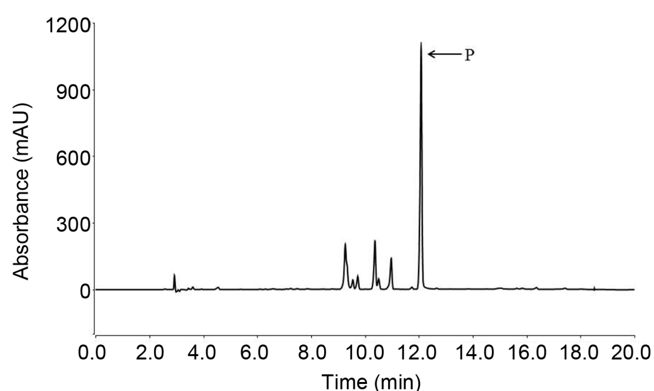

Fig. 2. HPLC analysis of pinocembrin produced by *Escherichia coli* strain B-Pin1.

P is pincembrin, whose structure was determined by NMR.

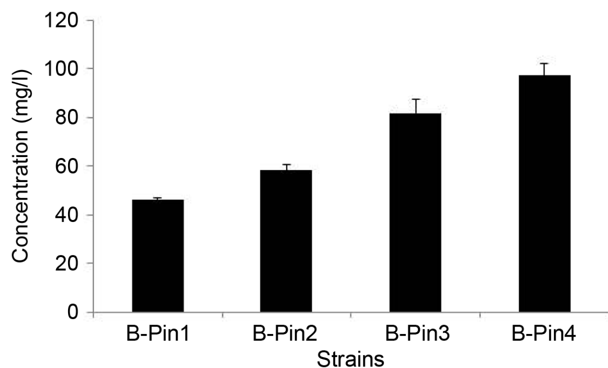

Fig. 3. Production of pinocembrin by different *Escherichia coli* strains.

than when all three genes were controlled by one promoter. However, this contradicted our observation in the present study. We believe that the order of genes is one of the critical factors that influences the yield of the final product. The gene order in the previous study [7] was *PAL*, *4CL*, and *CHS*. With such an order, the upstream products such as cinnamic acid and cinnamoyl-CoA would accumulate because expression of PAL and/or 4CL is greater than that of CHS. However, CHS was located in front of 4CL in our pseudo-operon construct. Therefore, less reaction intermediates were likely to be accumulated and, as a result, this construct had better productivity. The importance of gene order for the final yield was also observed in resveratrol biosynthesis [16]. The operon construct with proper order of genes resulted in an enhanced final yield of resveratrol [16].

The intracellular level of malonyl-CoA is critical for the production of pinocembrin, because pinocembrin is synthesized from three molecules of malonyl-CoA and one molecule of cinnamoyl-CoA. To increase the malonyl-CoA levels in *E. coli*, four genes (*accA*, *accB*, *accC*, and *accD*) from *Photorhabus luminescens* (pA-accABCD) were overexpressed. These four genes encode carboxyltransferase α, biotin carboxyl carrier protein, biotin carboxylase, and carboxyltransferase β, respectively, and these subunits comprise acetyl-CoA carboxylase (ACC). A previous study showed that overproduction of these four genes increased the production of flavonoids from phenylpropanoic acids such as cinnamic acid, *p*-coumaric acid, and caffeic acid [13]. The *accA*, *accB*, *accC*, and *accD* genes were cloned using PCR with genomic DNA of *P. luminescens* as a template. *accA*, which was amplified with 5′-ATGGATCCGATGAGTCTGAATTTTCTTGAA-3′ (forward primer, *Bam*HI site underlined) and 5′-CATGTCGACTCAGCAATAACCATATTGCAT-3′ (reverse primer, *Sal*I site underlined), was subcloned into the *Bam*HI/*Sal*I site of pACYC-Duet (Novagen). The resulting plasmid (pA-accA) was digested with *Nde*I/*Xho*I and the *accBC*, which was amplified with 5′-CGCATATGGATATTCGTAAGATAAAAAAAC-3′ (forward primer, *Nde*I site underlined) and 5′-CATCTCGAGTTAAGTTTCCTGTAACCCCAG-3′ (reverse primer, *Xho*I site underlined), was introduced into the *Nde*I/*Xho*I site of pA-accA to obtain the resulting construct pA-accABC. Finally, *accD* was amplified with 5′-ATGGATCCGATGAGCTGGATTGAAAAAATT-3′ (forward primer, *Bam*HI site underlined) and 5′-CATGCGGCCGCTCAGGCATCTTCTTTATTG-3′ (reverse primer, *Not*I site underlined) and cloned into the *Bam*HI/*Not*I site of pACYCDuet (pC-accD). pA-accD was amplified with a primer containing the *Xho*I site and *T7* promoter as a forward primer and the reverse primer of *accD*. The resulting PCR primer was subcloned into the *Sal*I/*Not*I site of pA-accABC to form pA-accABCD. pA-accABCD and pC-pPeCHS-Os4CL-pAtPAL were transformed into *E. coli* BL21 (DE3) (B-Pin3 in Table 1). B-Pin3 produced approximately 82 mg/l pinocembrin (Fig. 3), which was more than that produced by B-Pin2 (58 mg/l), indicating that overproduction of malonyl-CoA increased the production of pinocembrin.

Although B-Pin3 produced more pinocembrin than the other strains, we detected cinnamic acid in the reaction mixture (data not shown). This observation suggests that *Os4CL* and *CHS* encode enzymes for rate-limiting steps in the biosynthesis of pinocembrin from *p*-cinnamic acid. Therefore, introducing more *Os4CL* and *PeCHS* into B-Pin3 would increase the production of pinocembrin. In order to test this hypothesis, additional copies of *Os4CL* and *PeCHS* were introduced into B-Pin3 and the resulting transformant (B-Pin4) was examined for the production of pinocembrin. As expected, B-Pin4 produced approximately 18% more pinocembrin (97 mg/l) than B-Pin3 (82 mg/l).

The production of pinocembrin using B-Pin4 was monitored for 36 h. It was noted that the production of pinocembrin continued to increase until 12 h and then remained stable for the next 24 h. The production of pinocembrin was approximately 97 mg/l at 12 h (Fig. 4). Two studies have reported the production of pinocembrin from glucose [17, 27]. In one study, *acc* from *Corynebacterium glutamicum* was overexpressed along with *PAL*, *4CL*, *CHS*, and chalcone isomerase (*CHI*). After supplement of 3 mM phenylalanine in minimal media, the final yield was 60 mg/l [17]. The other study reported the overexpression of genes involved in phenylalanine biosynthesis (*aroF* and *pheA*) and genes for malonyl-CoA assimilation (*matB*

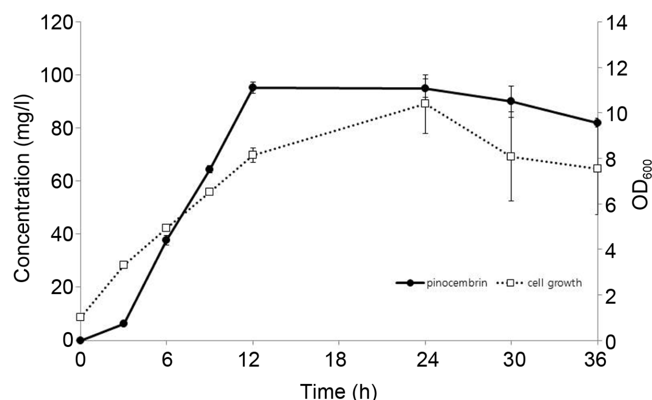

Fig. 4. Production of pinocembrin by *Escherichia coli* strain B-Pin4.

encoding malonyl-CoA synthetase, and *matC* encoding malonate transporter), along with *PAL*, *4CL*, *CHS*, and *CHI*. The final yield was 40 mg/l [27]. The final yield of pinocembrin in both previous studies was much less than that in the current study. Therefore, the proper modulation of the target genes and the supply of the co-substrate are important factors to increase the final yield of pinocembrin.

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References

1. Aboushoer MI, Fathy HM, Abdel-Kader MS, Goetz G, Omara AA. 2010. Terpenes and flavonoids from an Egyptian collection of *Cleome droserifolia*. *Nat. Prod. Res.* **24:** 687-696.
2. Austin MB, Noel JP. 2003. The chalcone synthase superfamily of type III polyketide synthases. *Nat. Prod. Rep.* **20:** 79-110.
3. Cochrane FC, Davin LB, Lewis NG. 2004. The *Arabidopsis* phenylalanine ammonia lyase gene family: kinetic characterization of the four PAL isoform. *Phytochemistry* **65:** 1557-1564.
4. Dixon RA, Paiva NL. 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell* **7:** 1085-1097.
5. Hamberger B, Hahlbrock K. 2004. The 4-coumarate:CoA ligase gene family in *Arabidopsis thaliana* comprises one rare, sinapate-activating and three commonly occurring isoenzymes. *Proc. Natl. Acad. Sci. USA* **101:** 2209-2214.
6. Houghton PJ, Woldemariam TZ, Davey W, Basar A, Lau C. 1995. Quantitation of the pinocembrin content of propolis by densitomety and high performance liquid chromatography. *Phytochem. Anal.* **6:** 207-210.
7. Hwang EI, Kaneko M, Ohnishi Y, Horinouchi S. 2003. Production of plant-specific flavanoes by *Escherichia coli* containing an artificial gene cluster. *Appl. Environ. Microbiol.* **69:** 2699-2707.
8. Jangaard NO. 1974. The characterization of phenylalanine ammonia-lyase from several plant species. *Phytochemistry* **13:** 1765-1768.
9. Jaganath IB, Crozier A. 2010. *Dietary Flavonoids and Phenolic Compound in Plant Phenolics and Human Health.* Fraga CG (ed.). John Wiley & Sons, Hoboken, New Jersey.
10. Kim B-G, Lee E-R, Ahn J-H. 2012. Analysis of flavonoid contents and expression of flavonoid biosynthetic genes in *Populus euramericana* Guinier in response to abiotic stress. *J. Kor. Soc. Appl. Biol. Chem.* **55:** 141-145.
11. Kim BG, Kim HJ, Ahn J-H. 2012. Production of bioactive flavonol rhamnosides by expression of plant genes in *Escherichia coli*. *J. Agric. Food Chem.* **60:** 11143-11148.
12. Kim MJ, Kim B-G, Ahn J-H. 2013. Biosynthesis of bioactive *O*-methylated flavonoids in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **97:** 7195-7204.
13. Lee Y-J, Jeon Y, Lee JS, Kim B-G, Lee CH, Ahn J-H. 2007. Enzymatic synthesis of phenolic CoAs using 4-coumarate: coenzyme A ligase (4CL) from rice. *Bull. Kor. Chem. Soc.* **28:** 365-366.
14. Leonard E, Lim H-K, Saw P-N, Koffas MAG. 2007. Engineering central metabolic pathways for high-level flavonoid production in *Escherichia coli*. *Appl. Environ. Microbiol.* **73:** 3877-3886.
15. Leonard E, Yan Y, Fowler Z, Li Z, Kim C-C, Lim K-H, Koffas MAG. 2008. Strain improvement of recombinant *Escherichia coli* for efficient production of plant flavonoids. *Mol. Pharm.* **5:** 257-265.
16. Lim CF, Fowler ZL, Hueller T, Schaffer S, Koffas MA. 2011. High-yield resveratrol production in engineered *Escherichia coli*. *Appl. Environ. Microbiol*. **77:** 3451-3460.
17. Liu R, Wu C-X, Zhou D, Yang F, Tian S, Zhang L, *et al*. 2012. Pinocembrin protects against β-amyloid-induced toxicity in neurons through inhibiting receptor for advanced glycation end products (RAGE)-independent signaling pathways and regulating mitochondria-mediated apoptosis. *BMC Med.* **10:** 105.
18. Miyahisa I, Funa N, Ohnishi Y, Martens S, Moriguchi T, Horinouchi S. 2006. Combinatorial biosynthesis of flavones and flavonols in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **71:** 53-58.
19. Miyahisa I, Kaneko M, Funa N, Kawasaki H, Kojima H, Ohnishi Y, Horinouchi S. 2005. Efficient production of (2*S*)-flavanones by *Escherichia coli* containing an artificial biosynthetic gene cluster. *Appl. Microbiol. Biotechnol.* **68:** 498-504.
20. Park SR, Ahn MS, Han AR, Park JW, Yoon YJ. 2011. Enhanced flavonoid production in *Streptomyces venezuelae via* metabolic engineering. *J. Microbiol. Biotechnol.* **21:** 1143-1146.

21. Peng L, Yang S, Cheng YJ, Chen F, Pan S, Fan G. 2012. Antifungal activity and action mode of pinocembrin from propolis against *Penicillium italicum*. *Food Sci. Biotechnol.* **21:** 1533-1539.
22. Rasul A, Millimouno FM, Eltayb WA, Ali M, Li J, Li X. 2013. Pinocembrin: a novel natural compound with versatile pharmacological and biological activities. *Biomed. Res. Int.* **2013:** 1.
23. Rösler J, Krekel F, Amrhein N, Schmid J. 1997. Maize phenylalanine ammonia-lyase has tyrosine ammonia-lyase activity. *Plant Physiol*. **113:** 175-179.
24. Santos CNS, Koffas M, Stephanopoulos G. 2011. Optimization of a heterologous pathway for the production of flavonoids from glucose. *Metab. Eng.* **13:** 392-400.
25. Vogt T. 2010. Phenylpropanoid biosynthesis. *Mol. Plant* **3:** 2-20.
26. Weston RJ, Mitchella KR, Allen KL. 1999 Antibacterial phenolic components of New Zealand manuka honey. *Food Chem.* **64:** 295-301.
27. Winkel-Shirley B. 2001. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiol*. **126:** 485-493.
28. Wu J, Du G, Zhou J, Chen J. 2013. Metabolic engineering of *Escherichia coli* for (2*S*)-pincocembrin production from glucose by a modular metabolic strategy. *Metab. Eng*. **16:** 48-55.
29. Yan Y, Kohli A, Koffas MAG. 2005. Biosynthesis of natural flavanones in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol*. **71:** 5610-5613.
30. Yang N, Qin S, Wang M, Chen B, Yuan N, Fang Y, *et al*. 2013. Pinocembrin, a major flavonoid in propolis, improves the biological functions of EPCs derived from rat bone marrow through the PI3K-eNOS-NO signaling pathway. *Cytotechnology* **65:** 541-551.
31. Yenjai C, Wanich S, Pitchuanchom S, Sripanidkulchai B. 2009. Structural modification of 5,7-dimethoxyflavone from *Kaempferia parviflora* and biological activities. *Arch. Pharm. Res.* **32:** 1179-1184.