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Biotransformation of isoflavones daidzein and genistein by recombinant *Pichia pastoris* expressing membrane-anchoring and reductase fusion chimeric CYP105D7

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ABSTRACT

The biotransformation of soy isoflavones into *ortho*-hydroxyisoflavones by CYP105D7 from *Streptomyces avermitilis* MA4680 is investigated through chimeric expression in *Pichia pastoris*. Using N-terminal fusion with the transmembrane domain of CYP57B3 from *Aspergillus oryzae* and C-terminal fusion with a P450 reductase from *Saccharomyces cerevisiae*, CYP105D7 was expressed in the form of reductase fusion and membrane anchoring in *P. pastoris*. Recombinant *P. pastoris* expressing the chimera catalyzed the biotransformation of both daidzein and genistein. This is the first study to show the catalyzing activity of CYP105D7 towards genistein. The major product from daidzein was identified as 6-hydroxydaidzein by comparing the results of the ultra-performance liquid chromatography analysis with the authentic standard. The major product from genistein was purified using preparative high-performance liquid chromatography and identified as 3′-hydroxygenistein based on nuclear magnetic resonance and mass data. The recombinant *P. pastoris* produced 6-hydroxydaidzein and 3′-hydroxygenistein in a 5-l fermenter, with maximal yields of 7.5 and 15.0 mg/l, respectively. The production of 3′-hydroxygenistein was higher than any previously reported in the literature.

1. Introduction

Cytochrome P450 monooxygenases (CYP) are a super-family of heme-containing enzymes that catalyze a variety of chemical reactions with a broad range of substrates [1]. The typical CYP-catalyzed reaction is $RH + O_2 + NAD(P)H + H^+ \rightarrow ROH + H_2O + NAD(P)^+$. CYPs require a tight interaction with auxiliary proteins, electron transferring proteins from a cofactor. They can be classified into several groups based on the nature of the electron transfer proteins [2]. The first of these, type I, is the mitochondrial/bacterial cytochrome P450 system, in which the electrons required for CYP reaction are delivered from NAD (P) H to CYP *via* ferredoxin reductase (Fpr) and ferredoxin (Fdx); notably, these proteins are in soluble form in cytoplasm. In contrast, the type II cytochrome P450 system is a microsomal system, in which electrons are transferred from NADPH *via* cytochrome P450 reductase (CPR). In this system, both CYP and CPR are usually membrane-anchored in endoplasmic reticulum (ER). In addition, some P450s are termed self-sufficient P450s, and these usually exhibit great catalytic activity because of the high efficiency of electron transfer from CPR to its fused P450 partner [3]. Some recent studies have mimicked naturally self-sufficient P450s, and artificial self-sufficient P450s have been developed by the genetic fusion of non-self-sufficient P450 with CPR, which greatly improved the catalytic activities of artificial self-sufficient P450s [4].

Isoflavones are naturally occurring dietary phytoestrogens that are distributed in some plants [5]. The two major isoflavone aglycons found in soybeans are daidzein and genistein. These have been intensively investigated because of their possible role in preventing certain hormone-dependent diseases as well as others, including breast and prostate cancers, osteoporosis, and cardiovascular diseases [6]. In structure-activity relationships, the number and positions of functional groups in the chemical structures of isoflavones dramatically affect the functions of the latter [7]. Among the various soy isoflavone derivatives, *ortho*-hydroxydaidzein and *ortho*-hydroxygenistein are important because of the *ortho*-dihydroxyl groups in their structures, which may enhance bioactivity compared with those of their precursors, daidzein and genistein. However, despite possessing multiple bioactivities, *ortho*-hydroxydaidzein and *ortho*-hydroxygenistein rarely exist in nature [8]. In recent years, scientists have successfully used genetically recombinant microorganisms harboring CYPs to produce *ortho*-hydroxydaidzein and *ortho*-hydroxygenistein to overcome the problem of scarcity [8].

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Some CYPs with the capacity to catalyze the *ortho*-hydroxylation of soy isoflavones have recently been identified [9,10], among which CYP105D7 from *Streptomyces avermitilis* MA-4680 has been the most comprehensively studied. This CYP catalyzed 3′-hydroxylation of daidzein, but was not active against genistein [10]. The kinetics of the enzyme was studied in recombinant *Escherichia coli* [11] and *S. avermitilis* [12]. The genetic fusion of CYP105D7 with a redox partner was found to improve its catalytic activity [13]. Scientists have recently identified natural electron transfer proteins for CYP105D7 in *S. avermitilis* [14], finding that the gene *cyp28* encoding CYP105D7 in *S. avermitilis* was up regulated by daidzein feeding [15]. However, despite the previous in-depth studies on CYP105D7, it has never been expressed in eukaryotic cells [10–15].

We recently expressed CYP57B3 from *Aspergillus oryzae* by fusion with a reductase domain of a self-sufficient CYP102A1 from *Bacillus megaterium* to form an artificial, self-sufficient P450 in *Pichia pastoris*, and we demonstrated that the recombinant strain efficiently catalyzed the *ortho*-hydroxylation of daidzein [16]. The production of 6-hydroxydaidzein by this strain was higher than any previously reported in the literature. On this basis, we proposed that using the eukaryotic yeast to express CYP, and then catalyzing *ortho*-hydroxylation of flavonoids, would be more beneficial compared to the expression in *E. coli*. Hence, in the present study, we attempted to express the previously well-studied CYP105D7 from *S. avermitilis* MA4680 in the membrane-anchoring and reductase fusion form in *P. pastoris*, and we investigated the production of *ortho*-hydroxyisoflavones by a recombinant strain expressing the chimeric enzyme.

2. Materials and methods

2.1. Microorganisms and chemicals

S. avermitilis MA4680 (BCRC12382) and *S. cerevisiae* (BCRC57896) were obtained from the Bioresources Collection and Research Center (BCRC) (Food Industry Research and Development Institute, Hsinchu, Taiwan) and cultivated according to the BCRC protocol. The EazySelect™ Pichia expression kit, containing *P. pastoris* X-33, plasmid vector pGAPZA™, yeast nitrogen base (YNB) medium without amino acid, and the antibiotic zeocin™ were obtained from the Invitrogen Company (Carlsbad, CA). Daidzein, genistein, and δ-aminolevulinic acid were purchased from Sigma-Aldrich (St. Louis, MO). The other reagents and solvents used in the study were of analytical grade and commercially available.

2.2. Construction and expression of mCYP105D7-sCPR fusion P450 *in* P. pastoris

The construction process is shown in Fig. 1(A). The primers used for the polymerase chain reaction (PCR) are shown in Table 1. PCR was used to amplify the transmembrane domain (TMD, the first 45 amino acids in the sequence) of the CYP57B3 gene [Fig. 1(B)] from pGAP-CYPBM3R, which was constructed in our previous study [16].

Table 1
Primer sequences used in the present study. Underlined sequences are restriction enzyme sites. Highlighted sequences are the Kozak sequence, followed by initiation codon in TMD-f; silent mutations in TMD-r; and extra sequences in sCRP-f and sCPR-r.

Primer name	DNA sequence (5′–3′)
TMD-f	CCGAATTCAAAATGATAGGGACGGTCTTGGACACA
TMD-r	GAGATCTACGTTTCGAAGGGGAGAGAAGAAAAGGT
CYP105D7-f	AATTCGAAACGTACCCGGTACGTCCGTGTCAGC
CYP105D7-r	AAGGGAGATCTCCAGGTCACGGGGAGTTCCAGC
sCPR-f	TTTAGATCTCCTGCTGTCAGCTCGGGCAACAGAGA
sCPR-r	AACCTCGAGGCCCAGACATCTTCTTGGTATCTACCTG

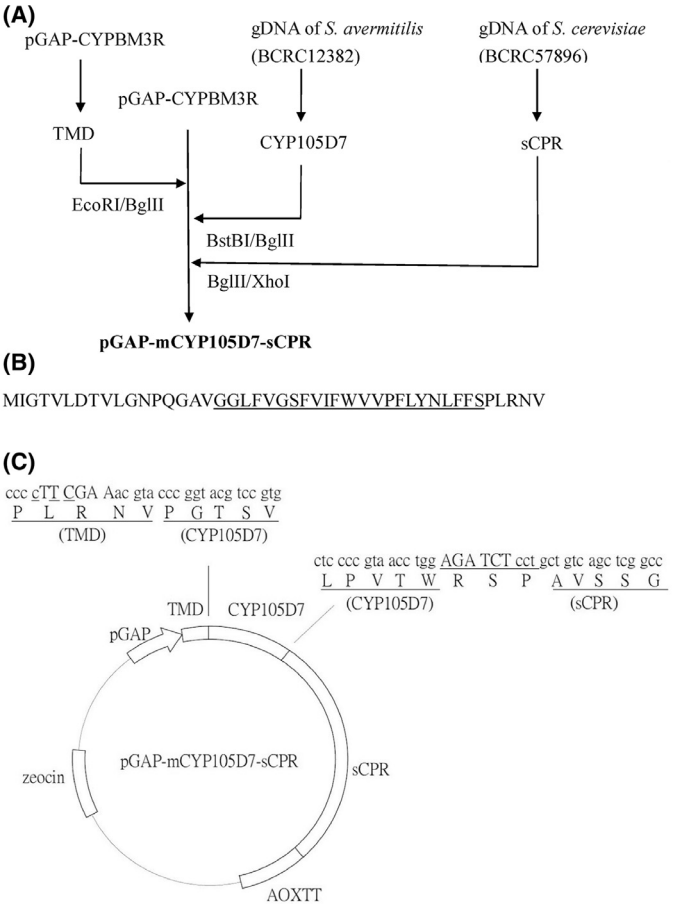

Fig. 1. Construction of the expression vector pGAP-mCYP105D7-sCPR. (A) The construction process. (B) Amino acid sequences of TMD from CYP57B3, where the sequences forming transmembrane α-helix structure are underlined. (C) The map of pGAP-mCYP105D7-CPR, where the silent mutations in TMD and the linker sequences joining both CYP105D7 and sCPR are underlined. Restriction enzyme sites are marked by capital.

To create a restriction site BstBI at 3′ end of the TMD, three silent point mutations were generated in the reverse primer of the TMD, as shown in Table 1 and Fig. 1(C). The TMD was sub-cloned into the EcoRI–BglII sites of pGAP-CYPBM3R, which was followed by sub-cloning the PCR-amplified CYP105D7 gene on the BstBI–BglII sites. For the N-terminal fusion with TMD of CYP57B3 and the C-terminal fusion with a cytochrome P450 reductase from *S. cerevisiae* (sCPR), the amplified CYP105D7 was begun from the third amino acid, and the stop codon at the end was deleted. Similarly, the first 41 amino acids and the stop codon of the sCPR sequence were deleted by PCR method. Finally, the PCR-amplified sCPR was sub-cloned into the BglII–XhoI sites to form pGAP-mCYP105D7-sCPR, in which the C-terminal of the coding chimera containing 6X-His was detected by western blot. The map of pGAP-mCYP105D7-sCPR, with the amino acid sequences of joint regions between TMD and CYP105D7 or between CYP105D7 and sCPR, is shown in Fig. 1(C).

The resulting vector was then linearized with the BsmI restriction enzyme and transformed into *P. pastoris* X-33 using the electroporation method provided in the expression kit manual. The recombinants were selected with zeocin, and the insertion of the fusion gene into the genomic DNA of the recombinants was confirmed using the PCR method described above.

2.3. Preparation of membrane-anchoring proteins

Recombinant *P. pastoris* was cultivated in a 20-ml YNB medium (Invitrogen), which was supplemented by 2% dextrose, 100 μg/ml

zeocin, and 250 μM δ-aminolevulinic acid. The cultivation was carried out by shaking at 240 rpm for 48 h at 28 °C. The recombinant cells were harvested and washed three times in ice-cold phosphate-buffered saline (PBS). The washed cells were suspended in a cold lysis buffer containing 20 mM sodium phosphate (pH 6.8), 1% Triton X-100, 1 mM phenylmethanesulfonyl fluoride (PMSF), and 1 mM ethylenediaminetetraacetic acid (EDTA). Then 5000 U/ml of lyticase (Sigma) were added to disrupt the cells, followed by incubation at 28 °C for 30 min. The undisrupted cells and cell debris were removed by centrifugation at 5000*g* for 5 min at 4 °C. The supernatant containing all crude proteins was separated in advance by centrifugation at 100,000*g* for 30 min at 4 °C. The pellet containing the membrane-anchoring proteins was suspended in a cold lysis buffer, and the supernatant contained cytosolic proteins. The protein concentrations of all crude proteins, membrane-anchoring proteins, and cytosolic proteins were determined by a Bradford assay using bovine serum albumin as the standard [17].

2.4. Western blotting

The analyzed proteins (10 μg) were separated using 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and then blotted onto polyvinyl difluoride membranes (MP Biomedicals Co., Irvine, CA). The membranes were blocked with 5%-nonfat skimmed milk in a tris buffered saline-tween buffer. The expressed CYP fusion protein with His-tag was detected using rabbit polyclonal antibodies and mouse monoclonal anti-His-tag antibodies, respectively. The membranes were further incubated with horseradish peroxidase-conjugated secondary antibody. All bound antibodies were then detected using an Amersham ECL system (Amersham Pharmacia Biotech, Piscataway, NJ).

2.5. Fermentation and ultra-performance liquid chromatography analysis

A seed culture of recombinant *P. pastoris* was cultivated in 100 ml of an YPD medium, containing 100 μg/ml zeocin and then agitated at 200 rpm for 48 h at 28 °C. The seed culture was inoculated into a 5-l jar fermenter containing 2.5 l YPD medium supplemented with 2% dextrose, 250 μM δ-aminolevulinic acid, and 100 μM isoflavones, followed by cultivation with aeration (0.5, v/v/m) and agitation at 280 rpm for 72 h at 28 °C. A 10-ml aliquot of the culture was collected at several different time intervals, and the amounts of isoflavones and products were measured by ultra-performance liquid chromatography (UPLC) analysis. The operational conditions for the UPLC analysis by an analytic C18 reversed-phase column (Acquity UPLC BEH C18, 1.7 μm, 2.1 i.d. ×100 mm, Waters, USA) included the following: a gradient elution using water (A) containing 1% (v/v) acetic acid and methanol (B) with a linear gradient for 5 min with 15–35% B at a flow rate of 0.3 ml/min; injection volume of 0.2 μl; and detection of the absorbance at 260 nm.

2.6. Purification and Identification of ortho-hydroxygenistein product

A seed culture of recombinant *P. pastoris* was cultivated in a 100-ml YNB medium containing 2% dextrose and 100 μg/ml zeocin and the agitated at 200 rpm for 48 h at 28 °C. The seed culture was inoculated into a 5-l jar fermenter containing a 2.5 l YNB medium supplemented with 2% dextrose, 250 μM δ-aminolevulinic acid, and 100 μM genistein, which was followed by cultivation with aeration (0.5, v/v/m) and agitation at 280 rpm for 72 h at 28 °C. Two batches of 2.5 l fermentation were created for the purification of the biotransformation product. Following fermentation, the amount of broth was twice extracted with an equal volume of ethyl acetate; the extracts were then combined and condensed under a vacuum. The residue was then suspended in 100 ml of 50% methanol. After filtration with a 2.2 μm nylon membrane under a vacuum, the metabolite was purified by preparative high performance liquid chromatography (HPLC). The operational conditions for the preparative HPLC analysis by a preparative C18 reversed-phase column (Inertsil, 10 μm, 20.0 i.d. ×250 mm, ODS 3, GL Sciences, Japan) included a gradient elution using water (A) and 25–50% methanol (B) in a linear gradient for 25 min at a flow rate of 15 ml/min; absorbance was detected at 260 nm. The injection volume was 10 ml. The elution corresponding to the peak of the metabolite in the UPLC analysis was collected, condensed under a vacuum, and then crystallized by freeze drying it. Finally, 5.3 mg crystal compound were obtained, and the structure of the compound was confirmed by nuclear magnetic resonance and mass spectra analysis.

3′-hydroxygenistein: ESI/MS *m*/*z*: 295 $[M-H]^+$; ^{1}H NMR (DMSO-*d*6, 500 MHz) δ: 6.20 (1H, d, *J* = 1.8 Hz, H-6), 6.36 (1H, d, *J* = 1.8 Hz, H-8), 6.76 (1H, d, *J* = 8.0 Hz, H-5′), 6.78 (1H, dd, *J* = 8.0, 1.8 Hz, H-6′), 6.96 (1H, d, *J* = 1.8 Hz, H-2′), 8.22 (1H, s, H-2); ^{13}C NMR (DMSO-*d*6, 125 MHz) δ:180.6 (C-4, C = O), 164.7 (C-7), 162.3 (C-5), 157.9 (C-9), 154.3 (C-2), 145.8 (C-3′), 145.2 (C-4′), 122.8 (C-1′), 122.0 (C-3), 120.4 (C-6′), 116.8 (C-5′), 115.8(C-2′), 104.8 (C-10), 99.4 (C-6), 94.1 (C-8). Based on the data and comparing them with the values in the literature, the compound was identified as 3′-hydroxygenistein by [18].

3. Results and discussion

3.1. Expression of chimeric CYP105D7 in P. pastoris

Based on the results of our previous study [16], we proposed that using eukaryotic yeast to express CYP and then catalyzing *ortho*-hydroxylation of soy isoflavones may be more beneficial compared to their expression in *E. coli*. Based on several previous studies [10–15], we selected CYP105D7 from *S. avermitilis* MA4680 as a test model for the present study. It was proven that the activity of this enzyme could be increased by genetic fusion with P450 reductase [13]. Therefore, at the beginning of the present study, we genetically fused CYP105D7 with a P450 reductase from *S. cerevisiae* (sCPR), expressed the fusion protein in *P. pastoris*, and then determined the biotransformation activity of the recombinant yeast expressing the fusion protein. Unfortunately, no activity in the biotransformation of daidzein or genistein was observed (data not shown). It is known that prokaryotic CYPs are cytosolic soluble forms; in contrast, most eukaryotic CYPs are membrane-anchoring forms. Therefore, we tried to express bacterial CYP105D7 as a membrane-anchoring form in *P. pastoris*. We used the TMD of the CYP57B3 from *A. oryzae*, which was proven to be well expressed in *P. pastoris* [16]. The TMD was fused with CYP105D7, which was followed by fusion with sCPR. The construction process and the map of the expression plasmid (pGAP-mCYP105D7-sCPR) are shown in Fig. 1. The chimeric proteins TMD-CYP105D7-sCPR expressed in recombinant yeast were detected by SDS-PAGE and Western blot analysis using anti-His-tag antibodies. The results showed that the chimeric proteins were present in the membrane-anchoring fractions, with a molecular weight of between 110 and 130 kDa (Fig. 2), which was consistent with the calculated 121 kDa of the molecular weight of the chimera (4.9, 43.8, and 72.3 kDa for TMD, CYP105D7, and sCPR, respectively).

3.2. Biotransformation of soy isoflavones by recombinant P. pastoris expressing chimeric TMD-CYP105D7-sCPR

The catalytic activity on the biotransformation of soy isoflavones by the chimeric CYP105D7 was determined by feeding the isoflavones the fermentation broth of recombinant yeast expressing the chimeric protein and then analyzing the broth by UPLC. Fig. 3 shows the UPLC profiles of fermentation broth at 72 h with daidzein (B) or genistein (C) feeding. With regard to the biotransformation of daidzein, the results showed that one major *ortho*-hydroxydaidzein derivative, 6-hydroxydaidzein, with a UPLC retention time of 3.25 min, was

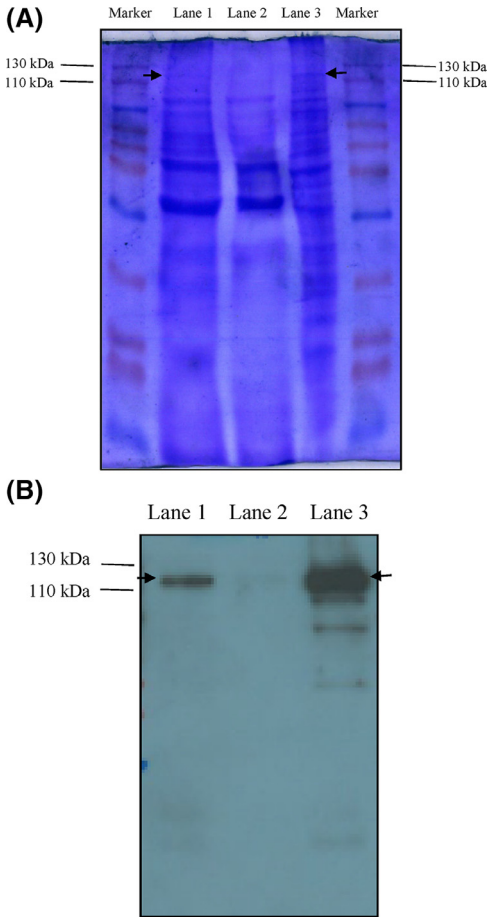

Fig. 2. Identification of expressed mCYP105D7-CPR fusion protein by SDS-PAGE (A) and Western blotting (B). Crude proteins from whole cell lysis (lane 1), soluble fraction (lane 2), and membrane-bound fraction (lane 3) were separated by SDS-PAGE and detected using mouse monoclonal anti-His-tag antibody. Arrows indicate the expressed chimeric TMD-CYP105D7-sCPR protein.

produced during fermentation. In the biotransformation of genistein, a biotransformation product appeared as a new peak at a retention time of 3.6 min in the UPLC profile. Because of the lack of an authentic standard, the produced metabolite was further isolated using a preparative HPLC method and identified using spectrophotometric methods. Based on the nuclear magnetic resonance and mass spectra data and the results of the comparison with the values in the literature, the compound was identified as 3−hydroxygenistein [18]. The biotransformation process of soy isoflavones by the recombinant yeast expressing chimeric TMD-CYP105D7-sCPR is shown in Fig. 4.

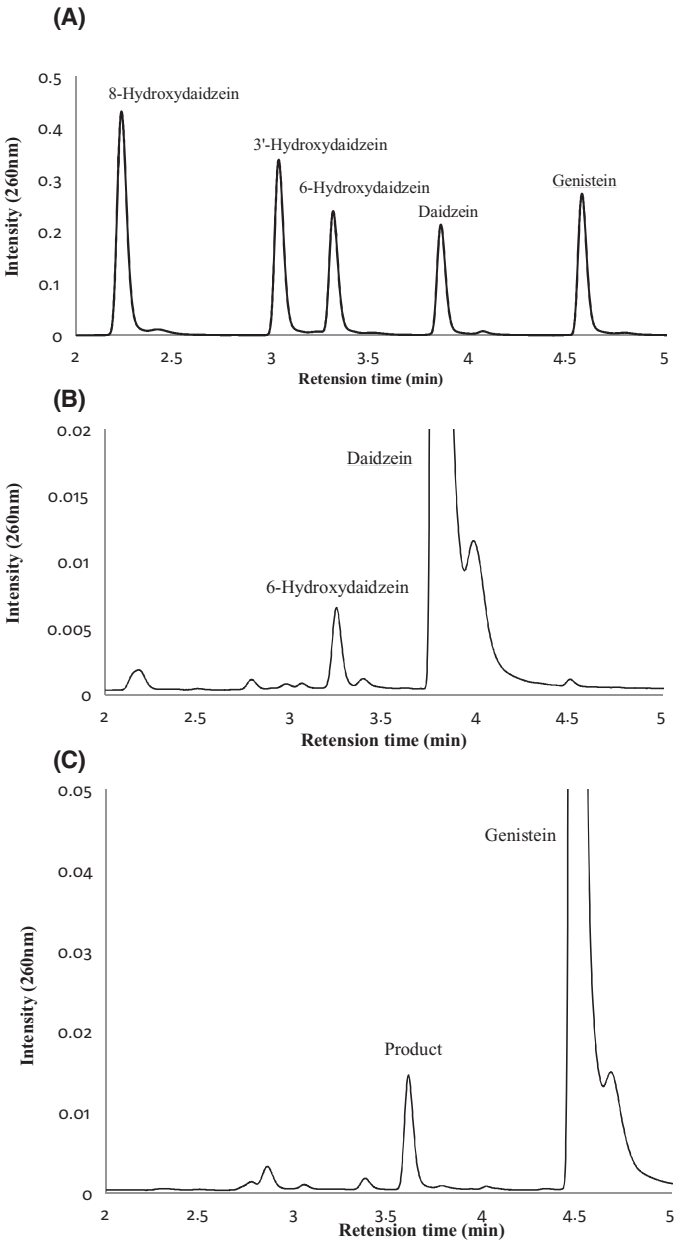

Fig. 3. UPLC profiles of standards (A), fermentation broth feeding with daidzein (B) or genistein (C). The recombinant strain was cultivated in 20 ml YNB medium containing 100 μM isoflavones for 72 h at 28 °C. Samples of the fermentation broth were collected and analyzed by UPLC.

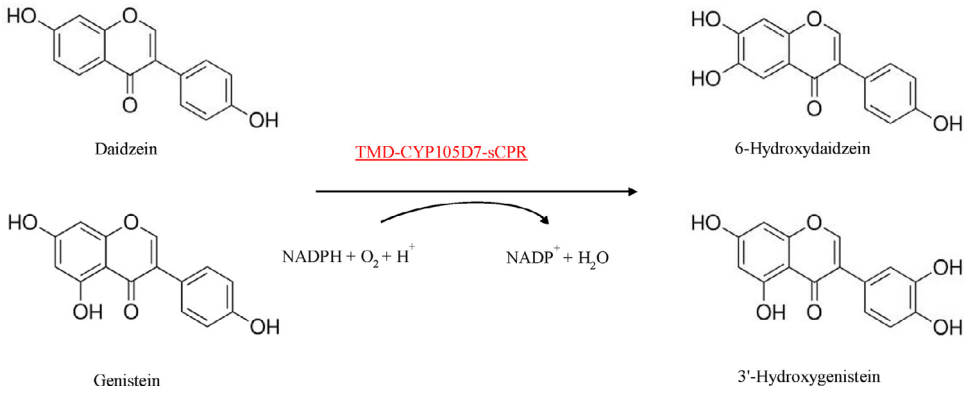

Fig. 4. Biotransformation of isoflavones to *ortho*-hydroxyisoflavones by recombinant *P. pastoris* harboring pGAP-mCYP105D7-CPR.

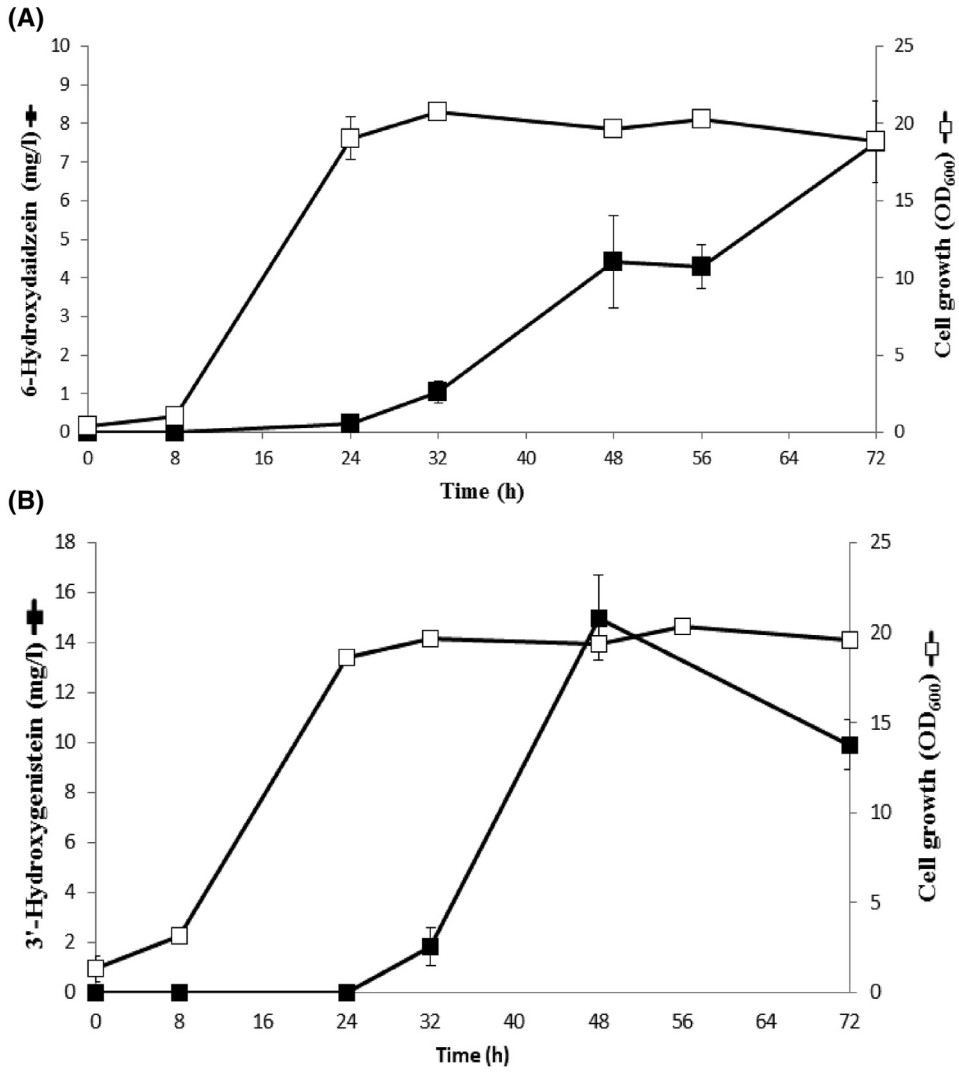

Fig. 5. Cell growth (□) and *ortho*-hydroxyisoflavones production (■) profiles of recombinant *P. pastoris* in a 5 l fermenter feeding with daidzein (A) or genistein (B). The recombinant strain was cultivated with YPD medium containing 100 μM isoflavones for 72 h at 28 °C. Samples of the fermentation were collected and analyzed by UPLC. The average ($n = 2$) is presented, with the S.D. represented by error bars.

Table 2
Production of 6-hydroxydaidzein and 3′-hydroxygenistein by recombinant microorganisms harboring heterogeneous CYPs.

CYP name	CYP sources	Recombinant hosts	Products	Yield (mg/l)	Productivity (mg/l/h)	Ref.
Nfa33880[a]	*N. farcinica*	*E. coli*	6-Hydroxydaidzein	0.56	0.014	[19]
Nfa12130[b]	*N. farcinica*	*S. avermitilis*	6-Hydroxydaidzein	2.3	0.032	[20]
CYP102D1[c]	*S. avermitilis*	*E. coli*	6-Hydroxydaidzein	1.18	0.049	[21]
CYP57B3[b]	*A. oryzae*	*P. pastoris*	6-Hydroxydaidzein	9.1	0.095	[16]
CYP105D7[b]	*S. avermitilis*	*P. pastoris*	6-Hydroxydaidzein	7.5	0.104	Present study
CYP107Y1[a]	*S. avermitilis*	*S. avermitilis*	3′-Hydroxygenistein	6.75	0.094	[10]
CYP57B3[a]	*A. oryzae*	*S. cerevisiae*	3′-Hydroxygenistein	N.D.[d]	N.D.[d]	[22]
CYP105D7[b]	*S. avermitilis*	*P. pastoris*	3′-Hydroxygenistein	15.0	0.313	Present study

[a] Co-expressed with P450 reductase.
[b] Engineered fusion with P450 reductase.
[c] Mutated self-sufficient CYP.
[d] Not determined.

In a scaled-up experiment, recombinant *P. pastoris* expressing chimeric CYP105D7 was cultivated in a 5-l fermenter. The results are shown in Fig. 5. The maximal concentration and conversion yield of 6-hydroxydaidzein and 3′-hydroxygenistein in the biotransformation were 7.5 mg/l and 30% for 6-hydroxydaidzein [Fig. 5(A)] and 15 mg/l and 60% for 3′-hydroxygenistein [Fig. 5(B)], respectively. The production of both 6-hydroxydaidzein and 3′-hydroxygenistein by genetic microorganisms harboring heterogeneous CYPs found in previous studies and in the present study are summarized in Table 2, which shows that the production of 3′-hydroxygenistein is higher in the present study than that reported in previous studies. 6-Hydroxydaidzein was reported to suppress the cell proliferation of HCT-116 colon cancer *in vitro* and *in vivo* [23], as well as competitively to inhibit tyrosinase activity [24], suppress the adipogenesis of 3T3-L1 preadipocytes [25], promote differentiation of 3T3-L1 preadipocytes [26], and possess antitrypanosomal activity [27].

3′-Hydroxygenistein was reported to possess antiproliferative activity toward T47D tumorigenic breast epithelial cells [28–29], as well as HIV-1 integrase inhibitory activity [30], anti-inflammatory activity [31], and hepatoprotective activity [32]. Based on the multiple bioactivities, the highest productions of the two *ortho*-hydroxyisoflavones derivatives in the present study indicate the potential usage of genetic microorganisms for the industrial mass production of the two *ortho*-hydroxyisoflavones derivatives.

Based on the results of several previous studies [10–15], we selected CYP105D7 from *S. avermitilis* MA4680 as the test model used in the present study. Previously, bacterial hosts expressing soluble CYP105D7 showed no detectable activity in the biotransformation of genistein [10]. The most important finding in the present study is that the recombinant *P. pastoris* expressed the membrane-anchoring and reductase fusion chimeric CYP105D7 catalyzed genistein in 3′-hydroxygenistein. It is well known that the expression of prokaryotic CYPs is better in prokaryotic hosts such as *E. coli*. However, bacterial CYPs are soluble and distributed in cytoplasm, whereas eukaryotic CYPs are membrane-anchored and primarily distributed in ER [2]. In insoluble substrate isoflavones, which are transported *via* the endomembrane system in cells, membrane-anchoring CYPs can access the substrate more easily than the soluble form of CYPs can. Therefore, we hypothesize that using eukaryotic *P. pastoris* to express CYP and then catalyzing the *ortho*-hydroxylation of soy isoflavones may be more beneficial compared to the expression in *E. coli*. According to the results of the present study (Figs. 2, 3, and 5), the recombinant *P. pastoris* expressed the chimeric fusion CYP105D7 catalyzed genistein in 3′-hydroxygenistein with a maximal production of 15 mg/l. Moreover, the production of 3′-hydroxygenistein by the strain was higher than previously reported in the literature (Table 2). Our results showed that the membrane-anchoring and reductase fusion expression of CYP105D7 in *P. pastoris* greatly improved the catalytic activity of the CYP toward genistein whereas the CYP showed no activity toward genistein as expression in *E. coli* before [10–15]. Therefore, we think that the membrane-anchoring and reductase fusion expression of prokaryotic CYPs in *P. pastoris* might be an alternative strategy for improving the catalytic activity of other prokaryotic CYPs.

Both daidzein and genistein were catalyzed by recombinant *P. pastoris* expressed in the membrane anchoring and reductase fusion of chimeric CYP105D7. Both the production and the conversion yields of 3′-hydroxygenistein found in the present study were higher than those observed in previous studies. In addition, the present study is the first to report the catalytic activity of CYP105D7 toward genistein. Based on these findings, we suggest that a strategy involving membrane-anchoring and reductase fusion expression in eukaryotic cells could be also suitable for improving the catalytic activity of other bacterial cytosolic CYPs. Moreover, based on multiple bioactivities, the present study provides evidence of the great potential of using genetic microorganisms in the industrial production of 6-hydroxydaidzein and 3′-hydroxygenistein.

Acknowledgments

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