Biotransformation of *Pueraria lobata* Extract with *Lactobacillus rhamnosus* vitaP1 Enhances Anti-Melanogenic Activity

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Isoflavone itself is less available in the body without the aid of intestinal bacteria. In this study, we searched for isoflavone-transforming bacteria from human fecal specimens (n = 14) using differential selection media. Isoflavone-transforming activity as the production of dihydrogenistein and dihydrodaidzein was assessed by high-performance liquid chromatography and we found *Lactobacillus rhamnosus*, named *L. rhamnosus* vitaP1, through 16S rDNA sequence analysis. Extract from *Pueraria lobata* (EPL) and soy hypocotyl extract were fermented with *L. rhamnosus* vitaP1 for 24 and 48 h at 37°C. Fermented EPL (FEPL) showed enhanced anti-tyrosinase activity and antioxidant capacities, important suppressors of the pigmentation process, compared with that of EPL (p < 0.05). At up to 500 μg/ml of FEPL, there were no significant cell cytotoxicity and proliferation on B16-F10 melanoma cells. FEPL (100 μg/ml) could highly suppress the content of melanin and melanosome formation in B16-F10 cells. In summary, *Lactobacillus rhamnosus* vitaP1 was found to be able to biotransform isoflavones in EPL. FEPL showed augmented anti-melanogenic potential.

**Keywords:** *Pueraria lobata*, dihydrodaidzein, dihydrogenistein, *Lactobacillus rhamnosus*, chemical analysis, microbiology

Introduction

*Pueraria lobata* is a species of climbing plant belonging to the Leguminosae family that contains various phenolic compounds and isoflavonoids, including daidzin, daidzein, genistin, and puerarin, which are commonly used to alleviate liver damage and bone loss [1, 2]. Extract of *P. lobata* (EPL) has been used as an alternative herbal remedy for alleviating postmenopausal symptoms [3]. Recently, the aerial part of *Pueraria thunbergiana* was found to have an anti-melanogenetic effect in vitro and in vivo [4]. Pigmentation of skin is one aspect of skin aging that is intrinsically or extrinsically associated with reactive oxygen species formation, effects of declining hormone levels, and ultraviolet radiation [5, 6]. In this regard, cosmeceuticals are used to improve skin appearance, and many compounds such as retinoids and botanicals such as soy isoflavones have been used to develop safe cosmeceuticals [5]. Corticosteroids, tretinoin, and hydroquinone are commercially used to treat problems of skin color and the effects of aging [6]. However, commercial use of these agents is associated with a variety of side effects [6]. Extract
of the aerial part of *P. lobata* contains a lot of isoflavones, and topical administration of this extract suppressed the pigmentation response in an in vivo study [4]. This suggested that isoflavones might be an active compound with anti-melanogenic activity. However, there is no evidence that isoflavones directly exert an anti-melanogenic effect.

Isoflavones are a subclass of flavonoids that includes over 6,000 identified family members and are abundant phenolic compounds in soy beans, soy foods, legumes, and fungus [7, 8]. In addition to their antioxidant, antimicrobial, and anti-inflammatory activities, isoflavones possess weak estrogenic (agonistic) activity through binding to the estrogen receptor [7]. The physiological effects of ingested flavonoids in individuals are dependent on their bioavailability in the intestine [9–11]. After ingestion, isoflavone glucosides (e.g., daidzin, genistin, and glycitin) are hydrolyzed to aglycones (e.g., daidzein, genistein, and glycitein) by glucosidases in the small intestine [10, 12] and further metabolized in the liver [13]. Among isoflavone metabolites produced by intestinal bacteria, equol [7-hydroxy-3-(49-hydroxyphenyl)chroman] belongs to the family of nonsteroidal estrogen compounds [14] and is known to have higher estrogenic and antioxidant activities than daidzin and genistein [12, 15]. Interestingly, intervention studies have shown that risk reduction of hormone-dependent diseases for the same dose of isoflavone supplementation was highly affected by the intestinal microflora flora in some individuals [15, 16]. These findings suggest that individuals have different intestinal microflora due to differences in environmental factors, such as antibiotic use, diet, and hormones. Therefore, identification of intestinal bacteria involved in isoflavone biotransformation will be important for increasing the health benefits of isoflavones [17] and will help provide a benefit of isoflavones in individuals with insufficient intestinal bacteria involved in the biotransformation of isoflavones. In topical application of isoflavones for skin whitening, transformation of isoflavones similar to the metabolism by microbiota in intestine will be beneficial since isoflavone itself has low potential bioavailability and estrogenic effects.

The aim of this study was to identify isoflavone-transforming bacteria from human gut microbiota and apply them to the biotransformation of isoflavones from *P. lobata* using an in vitro fermentation system. The selected bacterium was identified as *Lactobacillus rhamnosus* through DNA sequence analysis and was named *L. rhamnosus* vitaP1. Enhanced antioxidant capacity following biotransformation of EPL with *L. rhamnosus* vitaP1 was determined by oxygen radical absorbance capacity (ORAC) and 2,2-diphenyl-1-picryl-

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**Materials and methods**

**Materials**

Dihydrodaidzein (DHD), dihydrogenistein (DHG), and isoflavone (daidzein and daidzin) were purchased from Santa Cruz Biotechnology (USA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2’-azobis (2-aminopropane) dihydrochloride (AAPH), DPPH, β-phycoerythrin, mushroom tyrosinase, and α-MSH were purchased from Sigma (USA). Unless indicated otherwise, all other chemicals were obtained from Sigma. Trypticase soy agar (TSA), ethylene bromide agar (EMA), reinforced clostridial medium (RCM), brain heart infusion (BHI) medium, and lactobacilli deMan-Rogosa-Sharpe medium (MRS) were purchased from Becton & Dickinson (USA). Gifu anaerobic medium (GAM) was obtained from Nissui Pharmaceutical (Japan).

**Extraction of Plant Material**

*Pueraria lobata* (Willd.) Ohwi roots were purchased from Daegu Oriental Medicine Market and identified by Dr. T. H. Park of Daegu University (Korea). Roots of *P. lobata* were chopped into small pieces and extracted three times in 20% ethanol for 24 h each time at room temperature. Various ethanol concentrations were tested, and 20% gave the best yield. The extract was subsequently filtered to remove any particulates and concentrated under vacuum at 50°C. The concentrated crude extract of *P. lobata* (EPL) was lyophilized to obtain a powder and stored at –20°C for further experiments (20.5% yield). Soy hypocotyl extract was prepared as follows. Briefly, 100 g of soybean germ obtained from Dr. Chung’s Food Co., Ltd (Korea) was mixed with 1 L of 30% ethanol and extracted at 80°C for 5 h. The mixture was subsequently filtered to remove any particulate materials and vacuum concentrated at 50°C. The concentrated crude extract was lyophilized to obtain a powder that was stored at –70°C for further experiments.

**Collection of Human Fecal Samples**

To obtain bacteria capable of biotransforming isoflavones to their more bioavailable metabolites, feces were collected from healthy volunteers (6 females and 8 males, 25–41 years old) who had consumed EPL for 2 weeks. The volunteers were fully informed of the aims of the study and gave their written consent. All procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, as well as the 1964 Helsinki declaration and its later amendments or comparable ethical standards.
Isolation and Identification of Isoflavone-Biotransforming Bacteria

Fresh fecal specimens from the subjects were suspended in 1 ml of sterile phosphate-buffered saline (PBS), and 0.1 ml of fecal suspension was spread on GAM, TSA, EMA, RCM, BHI, and MRS media and cultured for 24 h at 37°C. Individual colonies were selected on the basis of morphology and color and further cultured in the presence of isoflavone. Production of isoflavone metabolites by each bacterium was determined by high-performance liquid chromatography (HPLC).

High-Performance Liquid Chromatography Analysis

An LC-20AD (Shimadzu, Japan) equipped with PDA detector (280 nm) and a C18 column (Skypack, Korea; 4.6 x 250 mm, 5 μm) and column oven (40°C) with 10-μl injection volume was used for HPLC analysis. The mobile phase consisted of solvent system A (water/acetic acid = 100:1) and solvent system B (water:acetoni trile:acetic acid = 50:50:1) in gradient modes. Data were analyzed using the instrument-embedded software.

Amplification of 16S rRNA and Bacteria Identification

To identify the bacterial strain, we performed sequence-based analysis of 16S rRNA in particular, the internal transcribed spacer (ITS) [18]. Genomic DNA was isolated from candidate bacterial colonies using a genomic DNA prep kit (Solgent Co., Korea). The polymerase chain reaction (PCR) was performed on 40 ng genomic DNA with 10 pmol 16S rRNA-specific primer (forward primer 27F: 5'-agagtttgatcMtggctcag-3'; reverse primer 1492R: 5'ggYtaccttgttacgactt-3') using TaqPCR premix (Qiagen, Germany). After electrophoresis on a 0.8% agarose gel, the PCR product was purified with a QIAquick Gel Extraction kit. DNA sequence analysis was performed by a commercial company (Solgent Co., Korea), and identification of bacterial strains was based on sequence homology with data from the National Center for Biotechnology Information.

Fermentation of EPL with \( \text{L. rhamnosus vitaP1} \)

EPL and soy hypocotyl extract (2% solutions) were fermented with 1% \( \text{L. rhamnosus vitaP1} \) for 48 h at 37°C with shaking (250 rpm). The FEPL and fermented soy hypocotyl extract (FSHE) were re-extracted with ethyl acetate hexane (EtOAc). Concentrated (250rpm). The FEPL and fermented soy hypocotyl extract (FSHE) were re-extracted with ethyl acetate hexane (EtOAc). Concentrated (250rpm). The FEPL and fermented soy hypocotyl extract (FSHE) were re-extracted with ethyl acetate hexane (EtOAc). Concentrated (250rpm). The FEPL and fermented soy hypocotyl extract (FSHE) were re-extracted with ethyl acetate hexane (EtOAc).

Oxygen Radical Absorbance Capacity Assay

Samples (0–100 μg/ml) were reacted with 75 mM phosphate buffer and β-phycoerythrin (Sigma). After addition of AAPH, changes in fluorescence were measured using a fluorometer (Victor²; Perkin Elmer, USA) with excitation at 535 nm and emission at 590 nm at 2-min intervals for 1 h [19]. Trolox (6.25 μg/ml) was used as a positive control, and results are expressed as an index of the control.

DPHH Radical Scavenging Activity

The purple color of DPPH solution fades rapidly after interaction with proton-radical scavengers. The radical scavenging activity of FEPL was determined according to a previous report. Different concentrations of FEPL (0.2 ml, 0–1,000 μg/ml) were mixed with 100 mM Tris-HCl buffer (pH 7.4) and then added to 1 ml of 500 μM DPPH in ethanol (final concentration 250 μM). After vigorous shaking, the mixture was left in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. DPPH radical scavenging activity was expressed as percentage of the control (0 μg/ml FEPL).

Determination of Tyrosinase Activity

Tyrosinase is a binuclear copper-containing metalloprotein that catalyzes a two-step oxidation of monophenols [20]. Tyrosinase activity was measured by a colorimetric method [19]. Briefly, 20 μl of mushroom tyrosinase (1,000 units/ml) was added to 170 μl of reaction mixture (1 mM L-tyrosine, 50 mM phosphate buffer (pH 6.5), DW; 10:10:9) in the presence or absence of sample. The reaction was conducted at 25°C for 20 min and the absorbance was measured at 490 nm. Tyrosinase activity (±) was calculated using the following equation: \( \frac{[(\text{Sample} + \text{tyrosinase}) – (\text{sample alone})]}{(\text{tyrosinase})} \times 100 \). Dose-response curves of activity were obtained, and the value corresponding to 50% reduction of the tyrosinase activity (IC50) was calculated.

Cell Culture

B16-F10 (\( \text{Mus musculus} \) skin melanoma) cells were obtained from the American Type Culture Collection (ATCC, USA). The cells were cultured in Dulbecco’s Modified Eagle’s Medium (GIBCO, USA) supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal bovine serum (GIBCO). The cells were grown at 37°C in fully humidified air with 5% CO2 and subcultured twice weekly.

Cell Cytotoxicity Test

Cell cytotoxicity was measured by quantitative colorimetric assay with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], which measures the mitochondrial activity of living cells [20]. Exponentially growing B16-F10 cells were seeded at \( 2 \times 10^4 \) cells/well in 96-well tissue culture plates and treated with different concentrations of FEPL (0-2,000 μg/ml) for 24 and 48 h. After addition of MTT, the formation of formazan within the cells was quantified by measuring the optical density at 550 nm using a microplate reader (Molecular Devices, USA). Cytotoxicity was expressed as the percentage of untreated control cells.

Determination of Melanin Production in B16-F10 Cells

B16-F10 cells were treated with α-MSH (100 nm) in the presence or absence of FEPL (0, 10, and 100 μg/ml) for 48 h. The cells were washed with PBS and lysed with lysis buffer containing 50 mM sodium phosphate (pH 6.8) buffer, 1% Triton X-100, and 0.1 mM PMSF. After collecting the cell supernatant by centrifugation, the pellets were dissolved in 1 N NaOH for 1 h at 60°C. The
absorbance was measured at 405 nm with a microplate reader.

Statistical Analyses
Each experiment was repeated three or four times, and the results of a representative experiment are shown. The results are expressed as the mean ± SEM and were analyzed using one-way ANOVA followed by Turkey’s method (Systat Software Inc., USA). A statistical probability of \( p < 0.05 \) was considered significant.

Results
Daidzein and genistein are abundant in plants, but DHD and DHG are only obtained through biotransformation with specific microorganisms [21]. Isoflavone derivatives, such as equol, DHD, and DHG, are more potent estrogenic isoflavones than the precursor, but bioconversion is dependent on the presence of intestinal bacteria capable of carrying out the specific metabolism [10]. Importantly, isoflavone-transforming bacteria isolated from human sources must be safe in future applications. Putative bacteria from human feces were selected on the basis of morphology and color of colonies using the differential culture media GAM, TSA, EMA, RCM, BHI, and MRS. After selection of candidate colonies, transformation of isoflavone (daidzein and daidzin) was tested by TLC and HPLC methods. In this study, one bacterial strain isolated from human feces could efficiently transform isoflavone to isoflavone metabolites.

Identification of Isoflavone-Transforming Bacterium
Next, we identified the bacterial strain that could biotransform the isoflavones through an ITS sequence analysis method. Based on the DNA sequences (Fig. S1), the putative isoflavone-transforming bacterium was identified as a *Lactobacillus rhamnosus* and named *L. rhamnosus* vitaP1. This is the first demonstration that *L. rhamnosus* can be involved in the intestinal fermentation of isoflavones.

Production of Isoflavone Metabolites from EPL and Soy Hypocotyl Extract after Fermentation with *L. rhamnosus* vitaP1
To further test the isoflavone-transforming potential of *L. rhamnosus* vitaP1, EPL (2%) and soy hypocotyl extract (2%) were fermented with *L. rhamnosus* vitaP1 for 48 h. As shown in Fig. 1, isoflavone fractions were highly different between EPL and soy hypocotyl extract, reflecting the fact that soy and *P. lobata* extract have different compositions of isoflavones. After fermentation by *L. rhamnosus* vitaP1, the fraction of DHG was highly increased in soy hypocotyl extract.

![Fig. 1. Dihydrogenistein and dihydrodaidzein production after fermentation with *L. rhamnosus* vitaP1.](image-url)

Extract of *Pueraria lobata* (EPL, 2%) and soy hypocotyl extract (2%) were fermented with 1% *L. rhamnosus* vitaP1 for 48 h at 37°C. Fermented EPL (FEPL) and soy hypocotyl extract were re-extracted with ethyl acetate hexane. Production of isoflavone metabolites was analyzed with HPLC. (A) EPL; (B) soy hypocotyl extract; (C) FEPL; (D) fermented soy hypocotyl extract.
extract (Fig. 1D), whereas the fraction of DHD was greatly increased in EPL (Fig. 1C) compared with nonfermented extracts (Figs. 1A and 1B). This result indicates that the L. rhamnosus isolated in this study is able to transform isoflavones into their bioavailable metabolites such as DHD and DHG.

Enhanced Antioxidant Capacity of EPL after Fermentation with L. rhamnosus vitaP1

Biotransformation of isoflavones will result in increased content of DHD, DHG, or equol, which have more potent antioxidant capacity than the precursor. We examined changes in the antioxidant capacity of EPL and FEPL by ORAC assay. As shown in Table 1, FEPL showed more potent antioxidant capacity than unfermented EPL. When antioxidant capacity was determined on the basis of scavenging of DPPH (Fig. 2), FEPL showed a significant increase in antioxidant capacity at concentrations of 100 μg/ml and higher. This result implies that L. rhamnosus vitaP1 can be applied to the biotransformation of isoflavones into their active metabolites.

Table 1. Antioxidant capacity of EPL and FEPL.

<table>
<thead>
<tr>
<th>Concentration of extract (μg/ml)</th>
<th>Relative ORAC value</th>
<th>EPL</th>
<th>FEPL</th>
</tr>
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<tbody>
<tr>
<td>100</td>
<td>0.80 ± 0.025</td>
<td>0.84 ± 0.018</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.70 ± 0.030</td>
<td>0.84 ± 0.073*</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.56 ± 0.040</td>
<td>0.85 ± 0.089*</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>0.37 ± 0.057</td>
<td>0.65 ± 0.025*</td>
<td></td>
</tr>
<tr>
<td>6.25</td>
<td>0.21 ± 0.043</td>
<td>0.46 ± 0.044*</td>
<td></td>
</tr>
</tbody>
</table>

Antioxidant capacity was determined by oxygen radical absorbance capacity (ORAC) assay. Trolox (6.25 μg/ml) was used as a positive control, and its activity was set at 1.0. Data are the mean ± SEM (n = 9) expressed relative to Trolox. *p < 0.05 versus EPL.

Table 2. Anti-tyrosinase activity of EPL and FEPL.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (μg/ml)</th>
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<tbody>
<tr>
<td>EPL</td>
<td>&gt;500</td>
</tr>
<tr>
<td>FEPL</td>
<td>423.21 ± 30.28</td>
</tr>
<tr>
<td>Daizin</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Daidzein</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Dihydrodaidzein</td>
<td>150.50 ± 4.55</td>
</tr>
<tr>
<td>Arbutin</td>
<td>417.75 ± 33.53</td>
</tr>
</tbody>
</table>

Anti-tyrosinase activity was determined by a colorimetric method using purified tyrosinase and L-tyrosine. Dose-response curves of activity were obtained, and the IC_{50} values were calculated. Data are the mean ± SEM (n = 10).
Inhibitory Effect of Fermented EPL on Formation of Melanosomes in B16-F10 Cells

α-MSH activates the melanocortin-1 receptor on melanocytes and thereby increases tyrosinase activity and eumelanin production [23]. Melanosome formation is an important process in the progression of melanogenesis. Therefore, the inhibitory activity of FEPL against α-MSH-stimulated melanosome production was determined in B16-F10 cells (Fig. 4). FEPL highly suppressed the melanosome formation that was stimulated by α-MSH (p < 0.05). In addition to the direct inhibitory action of tyrosinase (Table 2), this inhibitory effect of FEPL on melanosomes could be another beneficial activity in the treatment of excess melanogenesis.

Inhibitory Effect of FEPL on Production of Melanin

FEPL could suppress the α-MSH-induced formation of melanosomes, which is regulated by an intracellular enzymatic cascade. Next, we determined the inhibitory effect of FEPL on production of melanin in B16-F10 cells. FEPL alone did not increase the production of melanin in B16-F10 cells at 24 h (Fig. 5A) or 48 h (Fig. 5C). Exposure to α-MSH (100 nM) significantly increased the production of melanin, but this increase in melanin production was highly suppressed in the presence of FEPL (10 and 100 μg/ml) at both 24 h (Fig. 5B) and 48 h (Fig. 5D). As FEPL showed in vitro anti-tyrosinase activity (Table 2), anti-tyrosinase activity was measured in α-MSH-stimulated B16-F10 cells. As shown in Fig. 6, FEPL at a concentration of 100 μg/ml highly suppressed the tyrosinase activity. In accordance with the results from Fig. 5 and Fig. 6, FEPL has an inhibitory effect on the production of melanin, and this effect might be associated with the high contents of isoflavone metabolites such as DHD and DHG.

Discussion

In this study, we found that a L. rhamnosus strain could biotransform the isoflavones of EPL into the more bioavailable DHD and DHG (Fig. 1). FEPL suppressed production of melanin through increased antioxidant capacity (Table 1 and Fig. 2), suppression of melanin production (Figs. 4 and 5), and direct inhibition of tyrosinase activity (Table 2 and Fig. 6). Therefore, EPL would be a good source
of isoflavones, and its biotransformation by *L. rhamnosus* vitaP1 strain will be applicable in the development of bioactive skin-whitening compounds and future applications of isoflavone metabolites.

Numerous studies based on natural product chemistry have been performed to find novel medicinal compounds through traditional and dereplication-driven approaches based on bioguided isolation, microfractionation, and...
Cells were treated with α-MSH in the presence or absence of FEPL and tyrosinase activity was determined by a colorimetric method. Data are the mean ± SEM expressed as a percentage of the untreated activity in B16-F10 cells.

**Fig. 6.** Inhibitory effect of FEPL on α-MSH-induced tyrosinase activity in B16-F10 cells.

Cells were treated with α-MSH in the presence or absence of FEPL and tyrosinase activity was determined by a colorimetric method. Data are the mean ± SEM expressed as a percentage of the untreated activity in B16-F10 cells.

**Table 2.** α-MSH-induced tyrosinase activity and suppression of melanin production were highly increased by fermentation with L. rhamnosus vitaP1. This is a valuable point because more active isoflavone metabolites could be obtained from a relatively small starting quantity. It is speculated that L. rhamnosus vitaP1 will be useful in the biotransformation of EPL and increase the applicability of this bacterium in the fermentation of other extracts containing isoflavones.

Skin depigmentation can be achieved by interference before, during, or after melanin synthesis. Depigmentation is important for medicinal purposes as well as in beauty cosmetics and anti-aging skin products. Melanin is produced in melanosomes, which are special organelles of melanocytes. It is known that the number of melanocytes is the same in all races but the number and distribution of melanosomes in melanocytes determines race-related skin color [28]. Although melanin is a major factor in the determination of skin color and provides protection against ultraviolet-induced skin damage, an abnormal melanin content will cause skin disease or problems, such as albinism, melisma, age spots, moles, and lentigo. In addition to producing an even and light skin tone, skin-whitening agents are also used in anti-aging and skin fairness products. Anti-aging products, including skin-whitening agents, are the most important growing market in the cosmetics industry. Most active ingredients lighten skin tone through interference (e.g., by modulating tyrosinase, melanocortin-1 receptor, or reactive oxygen species production) or change the distribution processes of melanin through inhibition of melanosome transfer or melanin dispersion and acceleration of skin turnover [6]. As found in this research (Table 2), DHD showed potent inhibitory tyrosinase activity and be involved in the hydrolysis reaction of diverse glycosylated phytochemicals [26, 27]. Thus, there is a weaker possibility that using this L. rhamnosus vitaP1 strain will cause safety problems. In addition, this strain may be exploited to enhance the release of aglycones and improve the rate of biotransformation toward bioactive metabolites (Tables 1 and 2). As previously mentioned, the extract of aerial parts of *P. lobata* contains a lot of isoflavones, and topical administration of this extract suppressed the pigmentation response in an in vivo study [4]. The authors suggested that isoflavones might be active compounds showing anti-melanogenic activity. However, isoflavones contained in EPL had less potential anti-tyrosinase activity (Table 2), raising the possibility that the anti-melanogenic effects of isoflavone and/or EPL might be associated with indirect regulation of melanogenesis. Surprisingly, anti-tyrosinase activity and suppression of melanin production were highly increased by fermentation with *L. rhamnosus* vitaP1. This is a valuable point because more active isoflavone metabolites could be obtained from a relatively small starting quantity. It is speculated that *L. rhamnosus* vitaP1 will be useful in the biotransformation of EPL and increase the applicability of this bacterium in the fermentation of other extracts containing isoflavones.

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therefore might be used as a starting compound for development of a skin-whitening agent. When considering EPL for cosmetic application, FEPL is more favorable than EPL itself because it contains higher levels of DHD and shows strong anti-tyrosinase activity (Table 2). In accordance with this result, FEPL (100 μg/ml) not only suppressed tyrosinase activity, but also attenuated both the formation of melanosomes and melanin content in B16-F10 cells (Figs. 4–6).

Collectively, our data show that L. rhamnosus vitaP1 can biotransform isoflavones of soy hypocotyl extract and EPL into more active forms of isoflavones, including DHD and DHG. FEPL can suppress the pigmentation process by inhibiting tyrosinase activity and melanosome formation. In addition to direct inhibitory effects of FEPL and its active compound DHD on melanogenesis by modulation of tyrosinase activity, it is also possible that the anti-melanogenic activity of FEPL might be associated with enhancement of the estrogenic effect, since DHD is known to elicit estrogenic activity and is considered a phytoestrogen (an estrogen-like chemical compound present in plants). This aspect of FEPL should be investigated in future experiments. FEPL may prove to have potential value as a skin-whitening cosmetic additive.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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