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

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, dihydrogenestein, _Lactobacillus rhamnosus_, chemical analysis, microbiology

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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-(4′-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 microbial 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 microbacteria 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-hydrazyl-hydrate (DPPH) free radical assay. In addition, the improved anti-melanogenic effects of the fermented EPL (FEPL) compared with EPL were assessed through measurement of melanin production and anti-tyrosinase activity in α-melanocyte-stimulating hormone (α-MSH)–stimulated B16-F10 cells.

**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-aminopropene) 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), 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.