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연구논문

균사체 생물전환기술을 이용한 리퀘리티게닌 생산과 항노화 활성

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Production of Liquiritigenin with Cell-based Biotransformation and Its Anti-Aging Activity

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Abstract: In this study, an efficient whole cell-based biotransformation for the production of liquiritigenin was developed using *Laetiporus sulphureus* CS0218 as biocatalyst and aqueous extracts of *Glycyrrhiza uralensis* as co-substrate, respectively. In order to determine the efficacy of this method, the optimal bioconversion conditions including mycelial growth, three important enzyme activities (β-glucosidase, α-rhamnosidase and β-xylosidase), and apparent viscosity of culture broth were monitored. After optimization, aqueous extracts of *G. uralensis* were added to the culture medium to directly produce algycone liquiritigenin. By applying this strategy, 67.5% of liquiritin was converted to liquiritigenin at pH 3.0 after 9 days of incubation and finally liquiritigenin was purified from the reaction mixture. And then, their biological activities including anti-oxidant and superoxide dismutase were observed. In fact, purified liquiritigenin was capable of bi-directional functions (i.e., either up-regulation or down-regulation of SIRT1 which is associated with aging). The results indicate that this strategy would be beneficial to produce biologically active liquiritigenin and could be used in pharmaceutical, cosmetic and food applications.

Keywords: Liquiritigenin, Whole cell based biocatalyst, *Glycyrrhiza uralensis*, *Laetiporus sulphureus* CS0218, Anti-aging

1. INTRODUCTION

Glycyrrhiza uralensis has been used as traditional and alternative medicine in some countries [1-3] because various flavonoids including liquiritin, liquiritigenin, iso-liquiritigenin and other bioactive derivatives have tremendous pharmacological functions. It was found that liquiritin exist mainly as glucoside form of bioactive compound [4,5], while liquiritigenin, an aglycone of liquiritin is present in small amounts in *G. uralensis* [1]. There are several studies on their beneficial effects including prevention of carcinogenesis [6,7], hepatoprotective effects [1], and anti-inflammatory effects [4]. Recent studies also demonstrated that aglycones are digested much faster and enhanced their functions than their corresponding glucosides in humans [8,9]. In particular, the glucosides do not easily digest and absorb completely in intestinal epithelial cells in humans. Therefore, the hydrolysis of the isoflavone glucosides is necessary to increase their absorption in the intestine and promote health related beneficial effects [9,10]. So, much effort has been devoted to develop an efficient and simple enzymatic bioconversion method instead of the harsh chemical treatment [9,11-13].

The basidiomycetes *Laetiporus sulphureus* has been widely cultivated and studied for medicinal purposes [14,15]. For instance, the fruiting body and exopolysaccharides produced in submerged culture of this fungus have attracted considerable

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attention due to their biological properties including anti-tumor, anti-oxidant, immunostimulatory and cell proliferation effects [15-20]. Thus, some investigators have been focused on optimization of culture conditions in flask culture and bioreactors [21-23]. The submerged culture of mushrooms has many advantages: 1) it allows for higher mycelial concentration and 2) it can be produced a series of targeted metabolites such as exopolysaccharides and thermostable enzymes as well as other functional molecules [22,24,25]. Some hydrolyzing enzymes including glucanase and xylanase have been produced from various mushrooms and characterized [14,26,27]. Among them, β-glucosidase can be efficiently used for the hydrolysis of glycosidic bonds, acetylglycoside or malonylglycoside to increase the concentration of bioactive aglycones [9,11,28,29].

In the present study, an efficient whole cell-based bioconversion process for the production of aglycone liquiritigenin was developed using *L. sulphureus* CS0218 as whole cell biocatalyst and aqueous extracts of *G. uralensis* as co-substrate. To the best of our knowledge, this is the first example of development and characterization of purified liquiritigenin. We believe that this method enable production of biologically active liquiritigenin and can be used in industrial applications.

2. MATERIALS AND METHODS

2.1. Microorganisms, culture media, and chemicals

L. sulphureus CS0218, which was originally isolated from a mountainous district in Jeonnam Province, Korea, was obtained from the culture collection of CoSeedBiopharm (Cheongju, South Korea). The stock of this microorganism was maintained on a potato-dextrose agar (PDA) slant. To be precise, the slants were inoculated and cultivated at 25°C for seven days and then stored at 4°C for further experiments. The seed culture was carried out in 100 mL of the medium containing 10 g/L glucose, 3 g/L yeast extract, and 3 g/L polypeptone on a rotary shaker-incubator at 140-150 rpm for five days at 25°C. The basal medium for the submerged culture of *L. sulphureus* CS0218 was a modified mushroom culture medium (MCM) consisting of 20 g/L glucose, 2 g/L yeast extract, 2 g/L polypeptone, 2 g/L $MgSO_4 7H_2O$, 0.5 g/L of KH_2PO_4, and 0.5 g/L K_2HPO_4 in distilled water. The initial pH of the culture was adjusted to 6.0 before sterilization. The standards of liquiritin and liquiritigenin were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Culture conditions

L. sulphureus CS0218 was cultivated on PDA broth and transferred to the seed culture medium by punching out 4-5 mm of the agar plate with a homemade cutter. The seed culture was continued for five days in a 250-mL flask containing 100 mL of the basal medium at 25°C. The aqueous extract of *G. uralensis* (18%, w/v) was used as an additional source of carbon to convert liquiritin into liquiritigenin by fermentation with *L. sulphureus* CS0218. The bioconversion process was conducted in a 250-mL flask containing 100 mL of aqueous *G. uralensis* extract containing 2 g/L yeast extract, 1 g/L polypeptone, 2 g/L $MgSO_4 7H_2O$, 0.5 g/L $KHPO_4$, and 0.5 g/L K_2HPO_4 after inoculation with 2% (v/v) seed culture broth.

2.3. Extraction of *G. uralensis*

G. uralensis was obtained from the Korea Cosmeceutical Material Bank (Daegu Haany University, Korea), sliced into small pieces, and homogenized. The homogenate (~200 g) was mixed with 1.5 L of distilled water and autoclaved, with subsequent cooling to room temperature before centrifuging at 7000 *g* for 20 min. The supernatant was filtered through a membrane filter (pore size 0.45 μm) and stored at 20°C until use in the microbial bioconversion reaction. Total reducing sugar (52.46%) in the extract of *G. uralensis* was quantified using the phenol-sulfuric acid method.

2.4. Quantification of mycelial biomass and exopolysaccharides

To measure the dry cell weight of the mycelium, we centrifuged each sample from the culture broth (at various fermentation intervals) at 9000 *g* for 20 min, washed three times with distilled water, and dried in an oven at 85-90°C overnight. To measure the exopolysaccharide concentration, we filtered the culture broth through a Whatman filter paper and mixed the filtrate with four volumes of absolute ethanol; the mixture was stirred overnight at 4°C, as described previously [21,22]. The precipitated exopolysaccharide was centrifuged at 9000 *g* for 15 min, and the supernatant was removed. The crude exopolysaccharides were lyophilized. Unless otherwise stated, all experiments were carried out at least in duplicate to ensure reproducibility.

2.5. Enzyme activity assay

The enzyme activity during the submerged culture of *L. sulphureus* CS0218 with aqueous extract of *G. uralensis* was measured as described in a previous reported [11]. After removal of the mycelium from the culture broth at various intervals, the supernatant fraction was concentrated using a Amicon Ultra 0.5 mL centrifugal filter (EMD Millipore Corporation, Billerica, MA, USA) and resuspended in acetate buffer (pH 5.0). Then, 5 mM *p*-nitrophenyl-conjugated substrates that were dissolved in the same buffer were added to the concentrated solution, and the mixture was incubated for 30 min at 37°C. α-L-rhamnosidase, β-D-glucosidase, and β-D-xylosidase activities were assayed

using *p*-nitrophenyl-pyranoside substrates (Sigma-Aldrich): *p*-nitrophenyl α-L-rhamnopyranoside, *p*-nitrophenyl β-D-glucopyranoside, and *p*-nitrophenyl β-D-xylopyranoside, respectively. One unit of the enzymatic activity was defined as the amount of enzyme releasing 1 μmol of *p*-nitrophenol per minute in 50 mM citrate phosphate buffer at pH 6.0 and 37°C. After incubation of the reaction mixture, 2 mL of 1 M Na_2CO_3 was added to stop the reaction. Finally, the released *p*-nitrophenol was quantified using a spectrophotometer at 420 nm. All enzymatic assays were performed at least in duplicate.

2.6. Analytical methods

The products of the microbial bioconversion were analyzed using a high performance liquid chromatography system (HPLC) (Waters Corporation, Milford, MA, USA) equipped with the RP18 column (250 × 4.6 mm i.d. [internal diameter]) and a diode array detector. The column temperature was maintained at 25°C, and a mixture of acetic acid and methanol was used as the mobile phase at the flow rate of 0.6 mL/min. The eluate was examined by absorbance at 280 nm.

2.7. Measurement of viscosity of the culture broth

The measurement of rheological properties was performed on the culture broth collected after each fermentation period using a Brookfield programmable LVD-VII$^+$ digital viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, Massachusetts, USA) fitted with a small sample adapter, as described previously [30].

2.8. Purification of liquiritigenin

The reaction samples were spotted onto a silica TLC plate using a glass capillary tube, and the plate was placed in a chamber (27.0 cm × 7.5 cm × 25.0 cm) that was saturated with the desired solvent system prior to use. Isolation of liquiritigenin was performed using a mobile phase made of the acetone: chloroform mixture (1:1). Each TLC plate was allowed to develop until the solvent front was approximately 1 cm away from the top of the plate. After the development, the plates were removed from the chamber and dried at room temperature. The developed plates were scanned using 254 nm UV absorption to obtain fingerprint profiles of thin layer chromatography scanning (TLCS). The photographic TLC images were acquired using the EPI Chemi3 Darkroom (UVP Biolmaging, Upland, CA, USA).

2.9. Enzyme assay of protein deacetylase sirtuin 1 (SIRT1)

The enzyme activity of sirtuin 1 (SIRT1) was measured using a SIRT1 assay kit, which was obtained from Anaspec Inc. (CA, USA). Iso-liquiritigenin (100 μM) was used as a positive control, and nicotinamide (3 mM) served as a negative control. Aliquots (10 μL) of the partially purified liquiritigenin and control were added into the wells of a 96-well microplate with 40 μL of the enzyme solution. After 10 min incubation at 37°C, 200 μM NAD^+ was added and allowed to react with the 10 μM substrate at 37°C for 30 min. After the incubation, 50 μL of the developer reagent containing 3 mM nicotinamide was added, and the plate was incubated for an additional 10 min at 37°C. The plate was allowed to cool down at room temperature and then was scanned using an Infinite M200 Pro microplate reader (TECAN Devices, CA, USA) at the excitation wavelength of 490 nm and emission wavelength of 520 nm. All experiments were performed in triplicate.

2.10. Anti-oxidant activity assay

The anti-oxidant activity was assessed using the OxiSelect Total Anti-oxidant Capacity (TAC) Assay Kit (Cell Biolabs, Inc., CA, USA). The antioxidant activity was extrapolated using the standard curve for the anti-oxidant activity of uric acid. The uric acid standard stock solutions were made by preparing 10 mg/mL uric acid or a 60 mM solution in 1N NaOH and were stored at -80°C. The stock solution was used to prepare serial dilutions (from 0.0039 to 1.0 mM) of uric acid in deionized water, while water served as the blank sample. The supplied reaction buffer (180 μL) was added to each sample of the isolated liquiritigenin with a uric acid standard (20 μL) and the mixture was vortexed. Then, the reaction was terminated by the addition of 50 μL of the supplied stop solution. The absorbance of the microplate wells was measured at 490 nm (copper reduction). The baseline readings were subtracted from the absorbance values to obtain corrected absorbance values. The anti-oxidant capacity of purified liquiritigenin was expressed as μM of uric acid equivalent using the uric acid standard concentration curve.

2.11. Superoxide dismutase (SOD) activity assay

The SOD activity was assessed using the OxiSelect Superoxide Dismutase Activity Assay (Cell Biolabs, Inc., CA, USA). One unit of SOD activity was defined as the amount of the enzyme that has a 50% inhibitory effect on the coupled system consisting of xanthine and xanthine oxidase at pH 7.8 and 25°C.

2.12. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine statistical significance of differences in the SIRT1 deacetylase activity among all groups tested. In all cases, $p<0.05$ was assumed to denote statistical significance.

3. Results and Discussion

3.1. Time profiles of mycelial growth and exopolysaccharide production

In this study, we used *L. sulphureus* CS0218 as whole cell biocatalyst for the production of liquiritigenin by adding aqueous extract of *G. uralensis* into the medium (Fig. 1). To determine the optimal culture conditions for this fungus, the concentration of the mycelial biomass and exopolysaccharides were studied. Glucose and yeast extract gave the best results in mycelial growth and highest exopolysaccharide yield (data not shown). The amounts of the mycelial biomass quickly increased after day 3 of fermentation and reached the maximal level of 12.8 g/L after day 8 of the fermentation, whereas the highest exopolysaccharide concentration was 2.9 g/L on day 8, suggesting that the exopolysaccharide production shows a growth-associated trend (data not shown). The submerged culture of the edible fungus rather than the solid-state fermentation (SSF), has received much attention because of the increased production of the mycelial biomass and target metabolites that exhibit potent functional properties that include antitumor, immunostimulatory, and antiobesity effects [15,31,32]. In our previous studies, we found that many edible mushrooms are able to produce bioactive molecules in submerged culture in a flask and a bioreactor [21,22,30]. We mostly wanted to use this fungus as whole-cell-based catalyst for a bioconversion reaction. An interesting feature of this fungus is its ability to grow well under the above-mentioned culture conditions. We assumed that these properties would greatly enhance our proposed model. Therefore, we also analyzed other properties of this fungus in relation to our cell-based catalyst model in the experiments described here.

3.2. Rheological analysis of culture broth

As aforementioned before, culture broth rheology is one of the critical parameters affecting mycelial growth and exopolysaccharide production of various filamentous fungi under submerged-culture conditions [30]. Thus, we observed the changes in viscosity during the whole fermentation period. Apparent viscosity increased gradually when *L. sulphureus* CS0218 grew exponentially and entered a stationary phase that lasted until the end of the fermentation. In fact, the highest viscosity (1050 cP) was detected on days 8 of the fermentation; these findings are indicative of a high concentration of mycelial biomass and strong production of exopolysaccharides. We can conclude that the rheological properties of the broth of *L. sulphureus* CS0218 significantly affect the mycelial growth, morphological changes (*i.e.*, size, shape, and mass), and exopolysaccharide production as well as enzyme production. The highest apparent viscosity was 1050 cP, which is quite different from the results of other fungal fermentation studies [30]. Furthermore, mycelial growth associated with the fungal morphology is strongly dependent on culture conditions [22]. Pellet morphology is another factor affecting the production of exopolysaccharides and mycelial biomass [30].

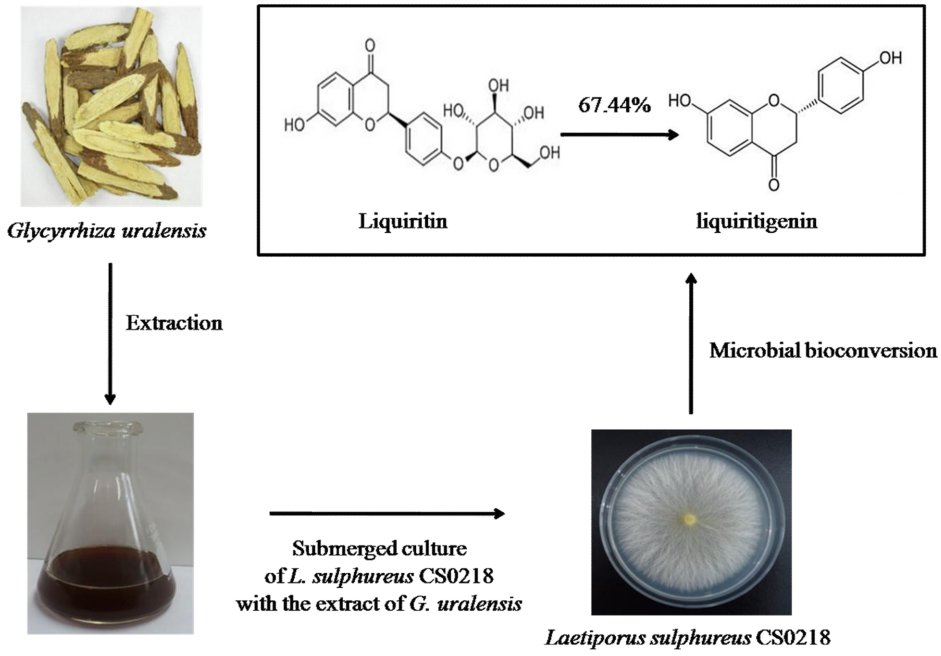

Fig. 1. Schematic description of whole cell-based biocatalyst for the production of liquiritigenin.

3.3. Time profiles of enzyme production

It is known that glycosidases can catalyze the hydrolysis of glycosidic linkages or glycoside units in predominant isoflavone glucosides or carbohydrate oligomers [9,11,28]. Therefore, we tested whether some of glycosidases produced by *L. sulphureus* CS0218 during fermentation are capable of converting glucosides to aglycones with high conversion activity. To confirm this scenario, three important enzyme activities including β-glucosidase, α-rhamnosidase, and β-xylosidase activities at the end of fermentation were measured (Fig. 2). Notably, the activity of all three enzymes increased until day 9 of fermentation and reached maximal levels of 8.6, 1.28, and 1.12 U/mL for β-glucosidase, α-rhamnosidase, and β-xylosidase, respectively. The activity of β-glycosidase was 6.7-fold and 7.6-fold that of the other two enzymes, respectively.

Some investigators have been reported the microbial bioconversion method using β-glucosidase from several microorganisms including fungi and bacteria [11,13,28]. For example, β-glucosidase produced by *Ganoderma lucidum* and lactic acid bacteria can convert isoflavone glucosides into aglycones in soymilk [9,13]. Indeed, the maximal level of β-glucosidase activity during *L. sulphureus* CS0218 fermentation was approximately 8.6 U/mL. We assumed that β-glucosidase produced by *L. sulphureus* CS0218 has optimal pH in the acidic condition and therefore this may enable the bioconversion of glucosides into aglycones. Because the activity of β-glucosidase was approximately 7-fold that of the other two enzymes, it is likely that β-glucosidase is a major enzyme for conversion to liquiritigenin [11,13]. To see whether β-glucosidase was responsible only for this bioconversion reaction, we also evaluated the activity of the other two hydrolyzing enzymes (rhamnosidase and xylosidase). As a result, the activity of rhamnosidase and xylosidase was not comparable to that of β-glucosidase. There is no doubt that other enzymes can produce and may participate in that bioconversion reaction [9,14,29].

3.4. Bioconversion of liquiritigenin with whole cell-based biocatalyst

As shown in Fig. 2, the activity of β-glucosidase was higher than that of the other two enzymes. We thought that β-glucosidase with a strong activity is probably the great advantage of direct microbial bioconversion of glucoside liquiritin to algycone liquiritigenin. To confirm our hypothesis, *L. sulphureus* CS0218 was grown under optimal medium by adding aqueous extracts of *G. uralensis* as co-substrate for the bioconversion of liquiritin to liquiritigenin. After fermentation for 9 days, the products of the incubation mixture were analyzed by HPLC. Fig. 3 shows the production profiles of liquiritigenin in the medium during incubation with or without whole cell biocatalyst. The most abundant compound in aqueous extracts of *G. uralensis* was liquiritin, which is the glucoside form of liquiritigenin (Fig. 3). Liquiritin was almost completely converted into aglycone liquiritigenin when used *L. sulphureus* CS0218 as biocatalyst.

To evaluate the effects of pH on the bioconversion yield, we measured the concentrations of liquiritin and liquiritigenin at different pH conditions, ranging from 3.0 to 7.0. We found that

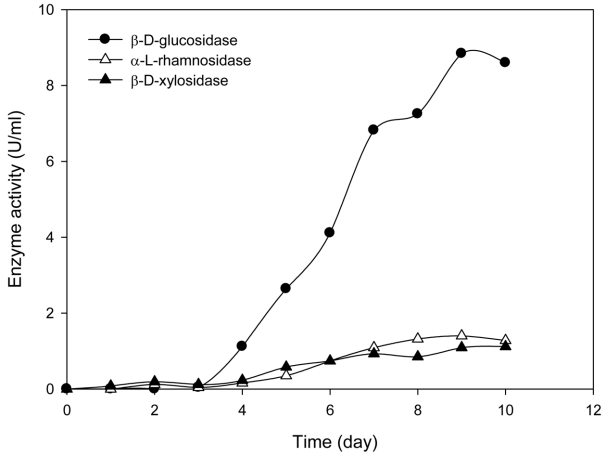

Fig. 2. Time profiles of β-D-glucosidase (●), α-D-rhamnosidase (△), and β-D-xylosidase activity (▲) during submerged culture of *L. sulphureus* CS0218. This fungus was cultured in the mushroom culture medium (MCM) on a rotary shaker-incubator at 150 rpm for 10 days at 25°C. The initial pH of the culture medium was adjusted to 6.0 before sterilization.

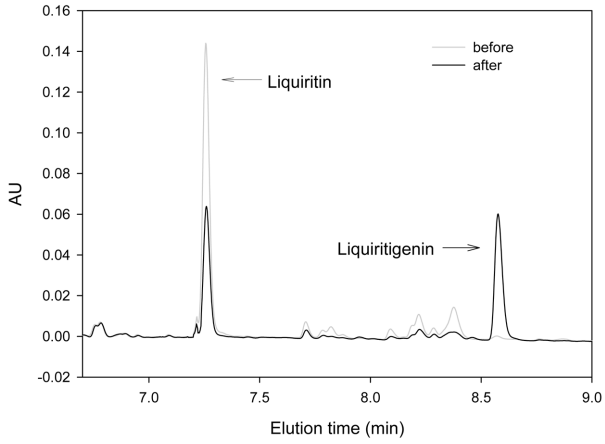

Fig. 3. The changes of liquiritin and liquiritigenin levels in the extract of *G. uralensis* with (after) or without (before) culture of *L. sulphureus* CS0218. This fungus was initially grown in the mushroom culture medium (MCM) and 18% (w/v) of aqueous extracts of *G. uralensis* were added into the culture medium at 150 rpm for 9 days at 25°C. The initial pH of the culture medium was adjusted to 3.0 before sterilization. The contents of reaction mixtures were measured using HPLC.

Table 1. Liquiritigenin contents from incubation mixtures with *L.sulphureus* CS0218 as whole cell based biocatalyst and aqueous extracts of *G. uralensis as* co-substrate at different pH conditions

	Relative conversion ratio (% SD[a])				
	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0
Liquiritigenin (%)	67.44±8.22	57.98±1.85	42.75±1.63	39.37±8.12	24.10±3.45

[a]All measurements were done in triplicate with standard deviations (SD).

the highest conversion yield was approximately 67.44% after 9 days of incubation under acidic pH 3.0 (Table 1). There are few reports on characterization of β-D-glucosidase produced by some microorganisms [26]. According to these studies, β-D-glucosidase is active under acidic conditions. Therefore, we assumed that the β-glucosidase produced by *L. sulphureus* CS0218 mostly acts as a biocatalyst for the production of liquiritigenin. We also tested the possibility of auto-hydrolysis of liquiritin during 9 days incubation at pH 3.0. As a result, there is no hydrolysis of liquiritin under these conditions (data not shown), meaning that bioconversion of liquiritigenin is the result from the action of hydrolyzing enzymes.

To verify the effects of addition of aqueous extracts of *G. uralensis* in a conversion reaction, 18 mL of aqueous extracts (*i.e.*, 5 g/L of total sugar) into the medium on days 0, 3, 6, 9, 12 were added and *L. sulphureus* CS0218 was continuously grown during the whole reaction. It turns out that aqueous extracts of *G. uralensis* can be used as co-substrate for mycelial growth as well as production of liquiritigenin. The changes in the concentrations of liquiritigenin, residual sugar, and mycelial biomass are shown in Fig. 4. The highest production of liquiritigenin was observed in addition of day 3, followed by days 6, 9, and 12 at pH 3.0. Therefore, the change in the residual sugar level was time- dependent manner. This is probably due to well-balanced carbon flux affecting mycelial growth and production

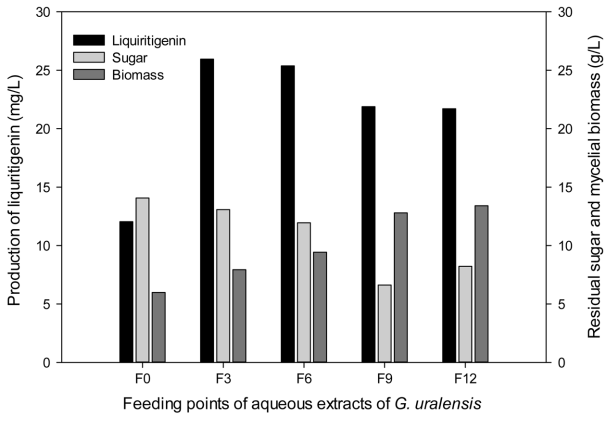

Fig. 4. Time profiles of liquiritigenin, residual sugar and mycelial biomass by adding aqueous extracts of *G. uralensis* to the culture medium at different times (day 0, 3, 6, 9, or 12). The initial pH of the culture medium was adjusted to 3.0 before sterilization.

of hydrolyzing enzymes.

3.5. Activation of SIRT1 expression

To evaluate biological activity of purified liquiritigenin, we tried to purify bioconverted liquiritigenin using preparative TLC (data not shown). The R_f value of converted liquiritigenin was ~0.84. This value is consistent with that of the liquiritigenin standard. Thus, liquiritigenin was detectable as the major compound. However, five unknown minor constituents were also detected (Fig. 5).

Although there have been many biological studies [2,3,34,35] on various flavonoid components including liquiritin, isoliquiritin, glycyrrhizin, and glycyrrhizic acid, bioconversion of liquiritin (the glucoside form) into liquiritigenin (the aglycone form) by using *L. sulphureus* CS0218 as whole cell biocatalyst has not been described so far. Liquiritigenin has various beneficial properties such as hepatoprotective [1], anti-cancer [6,7] and anti-inflammatory activity [4]. SIRT1 is a NAD^+-dependent protein deacetylase that is involved in the regulation of gene expression, cell metabolism, and differentiation in many organisms including yeast, bacteria, and humans [36]. Recent studies showed that sirtuin is one of the major genes involved in carcinogenesis, DNA repair, inflammation, and aging [36]. It was reported that SIRT1 expression can be up-regulated by some polyphenols such as resveratrol (13-fold), butein (8.5-fold), piceatannol (7.9-fold), iso-liquiritigenin (7.6-fold), fisetin (6.6-fold), and quercetin (4.6-fold) [36]. The effects of liquiritigenin on SIRT1 expression have not been studied yet, although iso-liquiritigenin was shown to be a potent activator of SIRT1 [36, 37]. Therefore, we tested whether purified liquiritigenin could be activated SIRT1 *in vitro*.

For this purpose, purified liquiritigenin was incubated with the SIRT1 enzyme, and the fluorescence intensity was measured on a microplate reader (Fig. 6). The positive control (PC) containing 100 μM of iso-liquiritigenin showed the highest activity. This is consistent with previously result [36]. As shown in Fig. 6, purified liquiritigenin exhibited SIRT1-stimulating activity. Relative to the activity of the control, the activity of SIRT1 deacetylase gradually increased in the range 200 nM to 20 μM. However, as increased liquiritigenin concentration from 40 to 200 μM, the SIRT1 activity became slightly inhibited in a concentration-dependent manner. This is probably that unknown

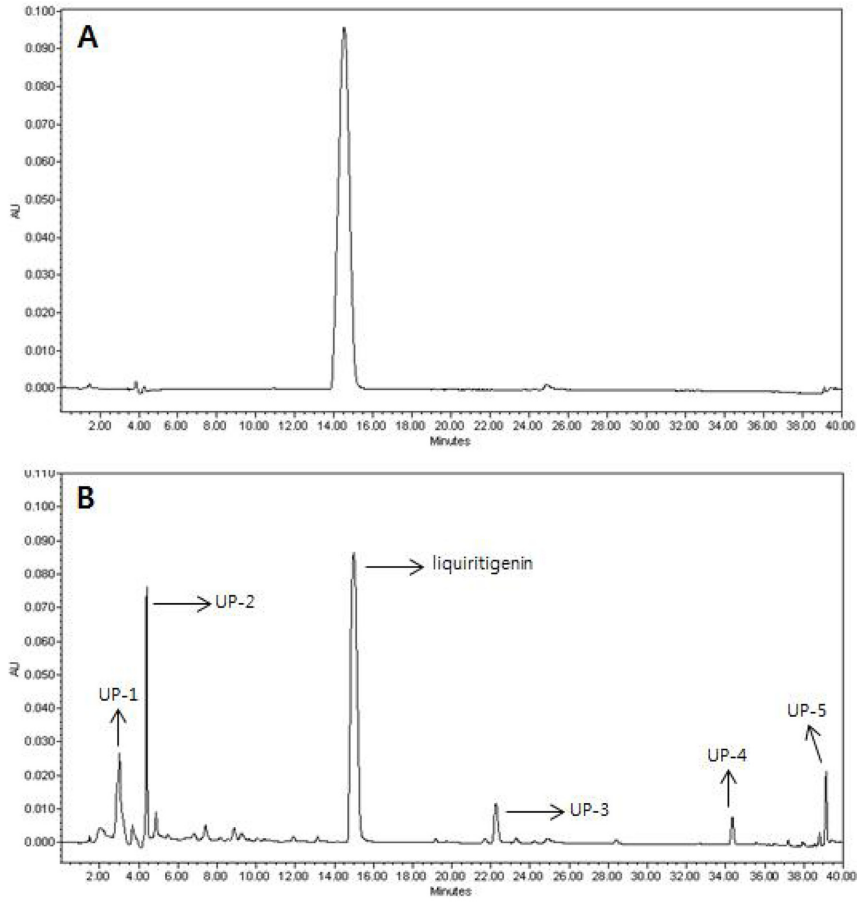

Fig. 5. Amount of purified liquiritigenin using HPLC. (A) standard liquiritigenin as control; (B) incubation mixture after bioconversion reaction. UP1-UP5 indicates unknown fractions.

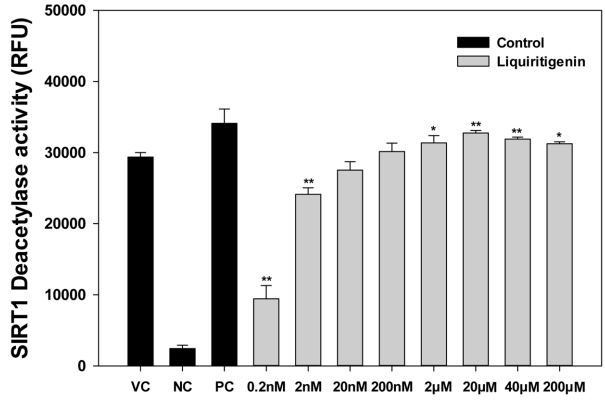

Fig. 6. Measurement of SIRT1 deacetylase activity. Purified liquiritigenin was serially diluted with 10% dimethyl sulfoxide from 0.2 nM to 200 μM. And then, diluted liquiritigenin was incubated with the SIRT1 enzyme containing SIRT1 substrate for 30 min. Then, the fluorescence intensity was measured at excitation/emission 490/520 nm. All measurements were done in triplicate and error bars represent standard deviations, as analyzed by ANOVA (*p<0.05, **p<0.005). VC: vehicle control; NC: negative control (nicotinamide, 3 mM); PC: positive control (100 μM of iso-liquiritigenin).

bioactive compounds or residual ingredients (i.e., impurities) in purified liquiritigenin may inhibit the substrate-binding reaction.

3.6. Measurement of anti-oxidant and SOD activity

Recently, it was reported that some flavonoids derived from plants have anti-oxidant activity in aging, cancer, and other cellular phenomena [38,39]. As shown in Fig. 7A, anti-oxidant capacity slightly increased in a concentration-dependent manner, but the difference was not significant under the conditions tested. In addition, the SOD activity of purified liquiritigenin was close to 62% at 200 μg/mL (Fig. 7B). As increased the concentration of purified liquiritigenin, the inhibition of SOD also gradually increased.

4. Conclusion

In this study, we developed an efficient bioconversion process which consists of *L. sulphureus* CS0218 as whole cell biocat-

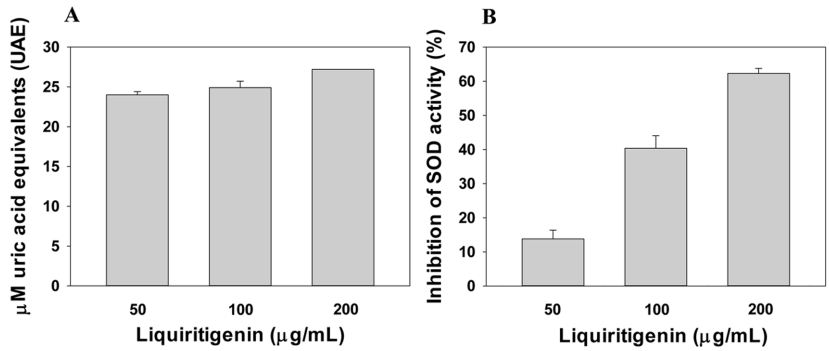

Fig. 7. Anti-oxidant activity (A) and SOD activity (B) of purified liquiritigenin. All measurements were done in triplicate and error bars represent standard deviations.

alyst and aqueous extracts of *G. uralensis* for production of aglycone liquiritigenin. We found that purified liquiritigenin produced by using this strategy is capable of bi-directional functions (i.e., either up-regulation or down-regulation of SIRT1 which is associated with aging in keratinocytes. The results show that it would be beneficial to produce biologically active aglycone liquiritigenin for the use in the pharmaceutical, cosmetic and other applications. Detailed enzymological studies and efficient purification of liquiritigenin are subjects of an upcoming project. In addition, the efficiency of this system needs to be compared with that of commercially available purified enzymes.

Acknowledgements

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