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Enhancement of the Tyrosinase Inhibitory Activity of Mori Cortex Radicis Extract by Biotransformation Using *Leuconostoc parameseteroides* PR

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Mori Cortex Radicis (MCR), the root bark of *Morus alba* L., consists of various phytochemicals and exhibits a strong inhibitory effect on tyrosinase. To enhance the tyrosinase inhibitory activity of MCR extract without further purification of bioactive compounds, whole MCR extract was biotransformed with crude enzyme extract from a selected lactic acid bacterium, *Leuconostoc parameseteroides* PR (LP). Mulberroside A (MA), a major stilbene glucoside of MCR, contains two β-glucosyl residues at the C3 and C4′ positions of oxyresveratrol (OXY). The crude enzyme of LP hydrolyzed the two glycosidic bonds of MA effectively, and 97.1% of MA was biotransformed into OXY within 2 h. Commercial almond β-glucosidase hydrolyzed only one site of the two glycosidic bonds of MA, and 68.7% of MA was biotransformed to OXY-glucoside. The tyrosinase inhibitory activity of the crude extract of MCR was increased approximately 6.5-fold by biotransformation using LP, and the IC_{50} value of the transformed MCR was 3.7 μg/mL.

Key words: biotransformation; tyrosinase inhibition; Mori Cortex Radicis; *Leuconostoc parameseteroides* PR; oxyresveratrol

Tyrosinase (EC 1.14.18.1), also known as polyphenol oxidase, is widely distributed in microorganisms, animals, and plants.[1)] It plays a significant role in the modulation of melanin production, which is involved in the formation of melanins, by catalyzing two distinct reactions: the hydroxylation of L-tyrosine, and the oxidation of L-DOPA (3,4-dihydroxyphenylalanine) to DOPA-quinone.[2)] Hydroxylation by tyrosinase is the first step, and a rate-limiting step of melanogenesis. Many studies have focused on identifying an effective tyrosinase inhibitor as a way to control tyrosinase activity and consequent melanogenesis.[3)] Most reported tyrosinase inhibitors are phenol/catechol derivatives that have structures similar to substrates of tyrosinase, such as tyrosine and DOPA.

Among reported tyrosinase inhibitors, hydroquinone, arbutin, and kojic acid are known for their effectiveness and have been used for many years in the cosmetics industry as whitening agents, but safety concerns have arisen because of the side effects of these compounds. In this regard, various naturally occurring herbal extracts and their active phytochemicals, such as flavonoid-like agents and other derivatives, have gained attention as potentially more desirable putative hypopigmenting agents.[4–6)]

Botanical structures of the mulberry tree (*Morus alba* L.), including the fruit, leaves, and root bark, have been used widely for many years in traditional Oriental medicine to treat fever, protect the liver, remove sputum, improve eyesight, strengthen joints, facilitate the discharge of urine, and lower blood pressure.[7,8)] The root bark of *Morus alba* L., Mori Cortex Radicis (MCR), is rich in phytochemicals such as 1-deoxynojirimycin, γ-aminobutyric acid, benzofuran derivatives, coumarins, and various flavonoids including hydroxystilbene derivatives.[9,10)] Stilbene glucosides such as mulberroside A (MA), oxyresveratrol-2-*O*-β-D-glucopyranoside, and oxyresveratrol-3′-*O*-β-D-glucopyranoside comprise the main components of MCR aqueous extract[10,11)] and are not found in *Morus alba* L. leaves or fruits, suggesting that there are significant differences in the components of the root bark, leaves, and fruits.[12)]

Currently, many studies are being conducted to investigate the health benefits of MCR, including antioxidative, antiviral, antiasthmatic, hypolipidemic, antihyperglycemic, and antiatherogenic effects.[13–18)] MA and its aglycone, oxyresveratrol (OXY), exhibit antioxidant activities, and show an inhibitory effect against $FeSO_4/H_2O_2$-induced lipid peroxidation in rat microsomes, and a scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals.[19,20)] Oxidative stress caused by free radicals has been proposed as a contributing factor in hyperpigmentation, suggesting that the antioxidant activity of MCR can serve as a depigmenting agent. In addition, Lee *et al.* reported that MCR extract exhibited the greatest tyrosinase inhibitory effect among 285 oriental herb extracts and showed no toxicity in eye irritation and skin sensitization tests on animals, or in human skin irritation tests.[21)]

The content of MA in the MCR extract was 12-fold higher than the content of OXY, but the biological activities of OXY were much greater than those of MA. In measurements of antioxidative activity against lipid

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Abbreviations: LP, *Leuconostoc parameseteroides* PR; MA, mulberroside A; MCR, Mori Cortex Radicis; OXY, oxyresveratrol

peroxidation, the IC_{50} values of MA and OXY were 78.4 μM and 3.6 μM, respectively, and the anti-inflammatory effect of OXY was also much greater than that of MA.[19] Moreover, OXY showed approximately 110-fold higher tyrosinase inhibitory activity than did MA against L-tyrosine substrate.[22] Flavonoids generally exist in glycosylated form in plant and food sources, and upon ingestion are absorbed after conversion into deglycosylated aglycones by intestinal enzymes and/or gut microbial enzymes. Flavonoid aglycones exhibit improved bioavailability and greater bioactivity than the respective glycosidic forms.[23–25] When orally administered to rats, MA was metabolized to OXY prior to absorption into the body,[26] and was then transported into the circulating blood and tissues at high rates.[27]

The present study aimed to enhance the tyrosinase inhibitory activity of MA, found in high concentrations in MCR water extract, by bioconversion to a more active oxyresveratrol aglycone using crude enzyme extracts of lactic acid bacteria. To establish a one-step bioconversion process that yields a product useful without further purification with chemical compounds or enzymes, various generally recognized as safe (GRAS) bacteria were screened to identify those with the highest β-glucosidase activity. The MCR extract biotransformed by crude enzyme extract from *Leuconostoc paramesenteroides* PR (LP) exhibited more potent tyrosinase inhibitory activity than did untransformed MCR extract or biotransformed MCR extract prepared using commercially available β-glucosidase purified from almond.

Materials and Methods

Chemicals and reagents. Mushroom tyrosinase, L-tyrosine, *p*-nitrophenyl-β-D-glucopyranoside (PNPG), kojic acid, phosphoric acid, and β-glucosidase from almond were purchased from Sigma-Aldrich (St. Louis, MO), and OXY (purity ≥ 95%) was from Sabinsa (East Windsor, NJ). Methanol (HPLC grade) was from Duksan Pure Chemicals (Gyeonggi, Korea). The water used in this study was purified with the Milli-Q system (Millipore, Bedford, MA).

Extraction of Mori Cortex Radicis. MCR was purchased from the Kyung-dong Market in Seoul, Korea. Fifty g of MCR was sliced thinly with scissors and extracted with distilled water (500 mL) at 50 °C for 3 h. The extract was then filtered through Whatman no. 1 paper to remove any residue, and the filtrate was dried using a freeze dryer (TFD5503; Ilshin Bio Base, Gyeonggi, Korea) to obtain dried powder.

Preparation of crude enzyme extracts from lactic acid bacteria. The bacterial strains used here, which included the species *Bifidobacterium, Lactobacillus*, and *Leuconostoc*, were cultured in MRS medium containing 0.05% (w/v) L-cysteine·HCl at 37 °C anaerobically. *Leuconostoc paramesenteroides* PR (LP) was isolated from Puerariae radix as previously reported.[28] After 18 h of incubation, the bacterial cells were collected by centrifugation (2,500 × *g* for 20 min at 4 °C) and the harvested pellet was washed twice with phosphate buffered saline. The pellets were re-suspended in 100 mM phosphate buffer (pH 6.0). All of the re-suspended solutions were concentrated at a level 10 times the original cultured volume and then sonicated (VCX 400; Sonics & Materials, Newtown, CT) with pulsing (2.0 s, 1.0 s) for 30 min in order to extract the enzymes. The disrupted bacterial solutions were centrifuged at 12,000 × *g* for 3 min, and the supernatants were used as crude enzyme extracts from the microorganisms.

β-Glucosidase activity assay. β-Glucosidase activity was measured based on the rate of release of *p*-nitrophenol from PNPG. Crude enzyme extracts (90 mL) was pre-warmed to 37 °C for 15 min, and 10 μL of 5.0 mM PNPG was added as substrate. Commercial β-glucosidase from almond was used as positive control. After incubation at 37 °C for 30 min, the reaction was stopped by adding 100 μL of 1.0 M Na_2CO_3, and then the optical density (OD) was measured at 405 nm (Model 680 Microplate Reader; Bio-Rad Laboratories, Hercules, CA). At the same time, the protein concentration of each enzyme extract was measured by the Bradford method. One unit of activity defines as the amount of enzyme that releases 1 μmol of *p*-nitrophenol per min. Values are means of at least three measurements. Relative activity against commercial β-glucosidase was calculated by a comparison of enzyme activities after adjusting the amount of purified/crude enzymes so that the final OD value did not exceed 1.0 at 30 min of incubation.

Biotransformation of Mori Cortex Radicis extract by the crude enzyme extract of LP. One mL of 20 mg/mL MCR extract in phosphate buffer (pH 6.0) and 1 mL of crude enzyme extract of LP, as selected in the β-glucosidase activity screening, were mixed and the mixture was incubated at 37 °C for 2 h. Commercial β-glucosidase from almond was used as positive control. The activity of the enzyme used was identical to that of the crude enzyme of LP. After incubation, 1 mL of the mixture was extracted 3 times with 600 μL of *n*-butanol (BuOH). The BuOH layer was dried with a speed vacuum evaporator (Modulspin 4080C; BioTron, Bucheon, Korea). The average weight of the residues was 1.3 mg. The residues were used in an assay for tyrosinase inhibitory activity and in HPLC analysis.

HPLC and LC/MS analysis. HPLC analysis was performed with a Dionex P680 HPLC pump and a Dionex UVD170U detector (Dionex Softron, Germering, Germany). The samples were separated on an Alltima HP C18 HL column (250 × 4.6 mm, 5 μm) from Alltech Associates (Deerfield, IL) and in a Dionex TCC-100 column oven (Dionex Softron) at 40 °C. The mobile phase consisted of 0.02% phosphoric acid water (mobile phase A) and MeOH (mobile phase B), and was run at a flow rate of 1 mL/min on a gradient program, as follows: 10–20% B (0–10 min), 20–23% B (10–15 min), 23–28% B (15–25 min), 28% B (25–35 min), and 28–50% B (35–70 min). After completion of elution, a 15-min reconditioning time was imposed before the next injection. The samples were dissolved in MeOH, and a 10-μL aliquot of the solution was injected. MA and OXY were detected at 300 nm.

Qualitative analysis for MA and OXY by LC/MS was performed with an HP 1100 High-Performance Liquid Chromatography (Hewlett-Packard, Palo Alto, CA) equipped with a QUATTRO LC Triple Quadrupole Tandem Mass Spectrometer (Micromass, Beverly, MA).

Preparation of mulberroside A-enriched fraction (MEF) by preparative HPLC. The peak at 10.1 min in LC/MS analysis was assumed to be MA based on the ion peaks at m/z 569 ($[M + H]^+$). It was enriched by preparative HPLC, and the content of the MA in this MA-enriched fraction (MEF) was verified to be 87.7% (w/w) by analytical HPLC. The solution of MCR extract was fractionated by BuOH, and the BuOH layer was evaporated using a speed vacuum evaporator. The residue was dissolved in MeOH and filtered with a syringe filter (Millex SLLHR04NL, 0.45 μm PTFE, 4-mm LH; Millipore) to conduct preparative HPLC. An HPLC pump (model ACME 9000; Younglin Instrument, Gyeonggi, Korea), a reversed-phase column (21.2 mm ϕ × 250 mm, HiQSil C18 HS-10; Kya tech, Tokyo, Japan), UV detector (UV VIS detector, Younglin Instrument, Gyeonggi, Korea), and Autochro 3000 software (Gilson, Middleton, WI) were used to isolate the MA fraction. The mixture of solvent (A) 0.1% TFA water and solvent (B) MeOH flowed at a rate of 20 mL/min for 70 min with a linear gradient profile, as described in the HPLC and LC/MS analysis section above.

Tyrosinase inhibition assay. Tyrosinase activity using L-tyrosine as substrate was determined spectrophotometrically by a previously described method, with slight modifications.[29] Nine units of mushroom tyrosinase in 20 μL of phosphate buffer (20 mM, pH 6.8) was added to an assay mixture containing 160 μL of 1 mM L-tyrosine solution and 20 μL of a test sample solution. The L-tyrosine and test samples were dissolved in 20 mM phosphate buffer (pH 6.8). After incubation of the reaction mixture at 37 °C for 40 min, the absorbance of the mixture was measured at 490 nm using a microplate reader. The

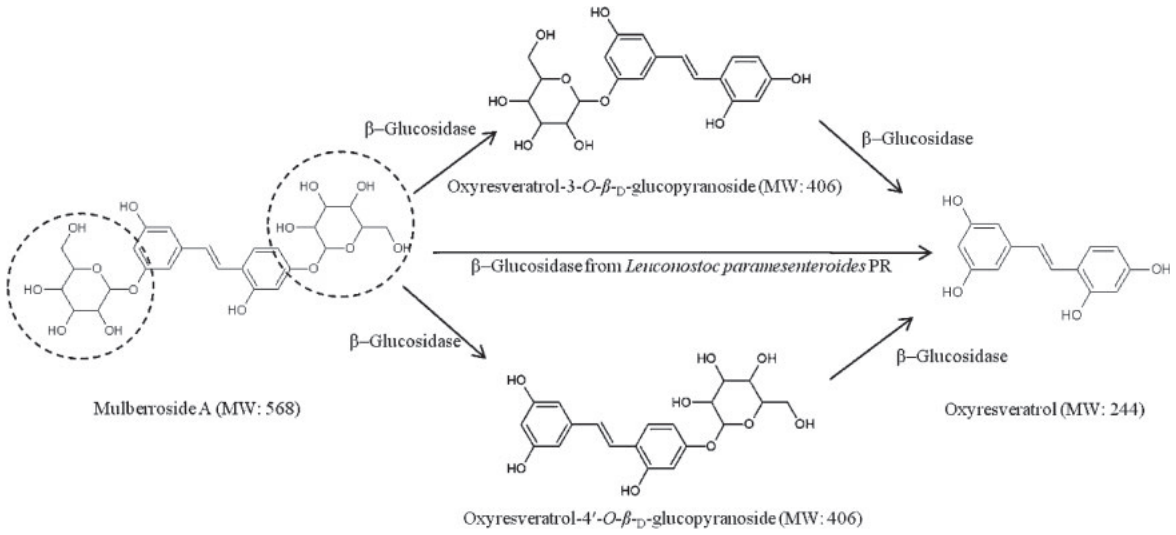

Fig. 1. Biotransformational Pathway of Mulberroside A (MA) to Oxyresveratrol (OXY).

inhibitory effects of the samples were represented as % inhibition = (A–B)/A × 100, where A = net OD_{490} without a test sample and B = net OD_{490} with a test sample. The net OD_{490} value was the difference between OD_{490} after 40 min of reaction and OD_{490} before the reaction.

Results and Discussion

Screening of bacterial strains suitable for the biotransformation of Mori Cortex Radicis

MA, a stilbene glucoside and a major component of MCR, contains two β-glucosyl residues, at the C3 and C4′ positions of OXY (Fig. 1). To improve the tyrosinase inhibitory activity of MCR extract by bioconversion of MA to OXY, the β-glucosidase activities of various crude enzyme extracts from GRAS microorganisms, especially lactic acid bacteria, were screened by pNP releasing assay (Table 1). Commercially available β-glucosidase from almond was used as positive control. Among the lactic acid bacteria tested, *Leuconostoc paramesenteroides* PR, *Bifidobacterium longum* subsp. *infantis* ATCC 15697, *B. pseudocatenulatum* SJ32, and *B. adolescentis* Int57 showed greater β-glucosidase activity than the commercial almond β-glucosidase enzyme. Among these, *L. paramesenteroides* PR (LP), isolated from Puerariae radix as previously reported,[28] showed the greatest β-glucosidase activity, 2-fold higher than that of the positive control. Based on this result, LP was chosen as a suitable strain for biotransformation of the MCR extract.

Differences in the bioconversion patterns of MA under treatment by LP crude enzyme extract and commercial β-glucosidase

Biotransformation methods using crude enzyme extracts from GRAS microorganisms have been applied in various fields, including the food, pharmaceutical, and cosmetic industries.[23,24,30–34] Because the deglucosylated form of hydroxystilbene has more potent tyrosinase inhibitory activity than its glucosylated form,[22,35] the biotransformation of stilbene glycoside to an aglycone has been performed using commercial deglycosylation enzyme products such as cellulase and Pectinex®.[22,36] Although these products are used widely as hydrolyzing

Table 1. β-Glucosidase Activities of Various Bacterial Strains

β-Glucosidase sources	Relative activity
β-Glucosidase from almond*	1.000
Leuconostoc paramesenteroides PR	2.063
Bifidobacterium longum subsp. *infantis* ATCC 15697	1.832
B. pseudocatenulatum SJ32	1.770
B. adolescentis Int57	1.589
B. adolescentis ATCC 15703	0.502
B. breve ATCC 15700	0.470
Lactobacillus delbrueckii subsp. *delbrueckii* ATCC 9649	0.175
B. longum RD47	0.088
Bifidobacterium sp. RD54	0
B. longum subsp. *longum* ATCC 15707	0
B. bifidum ATCC 29521	0
B. bifidum BGN4	0

*Positive control
Relative activities of the various compounds were calculated on the basis of specific activity adjusted to the concentration of protein, and are represented as ratios to positive control (1.000).

enzymes, we reasoned that a biotransformation process based on microbial fermentation without a purification process of enzyme sources might represent a more convenient and economical method for the industrial production of bioactive herbal extracts. Therefore, suitable microbial strains were investigated to identify the most efficient organism for the biotransformation of the MA in MCR extract to OXY. It has been found that lactic acid bacteria, which are isolated from food sources and intestinal microbiota, possess β-glucosidase activity and can hydrolyze β-glucosidic bonds in a wide spectrum of substrates in plants. Biotransformation with an enzyme extract from lactic acid bacteria would provide not only a means of converting MA to OXY, but would also yield a substance safe for use as a skin-whitening agent.

In the present study, the MCR extract biotransformed by a crude enzyme extract from LP showed a different HPLC profile from that of the MCR extract biotransformed by commercial almond β-glucosidase (Fig. 2). As shown in Fig. 2B, the content of MA in the MCR extract decreased and the content of OXY increased after 2 h of biotransformation by LP, but the MCR extract biotransformed by almond β-glucosidase showed

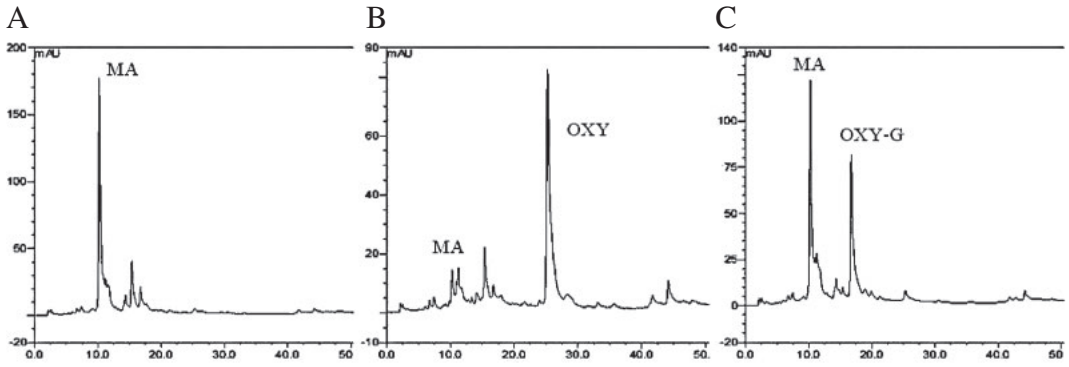

Fig. 2. HPLC Chromatograms of Mori Cortex Radicis (MCR) Extract after Biotransformation.

A, Non-biotransformed MCR extract (control); B, MCR extract biotransformed by crude enzymes from *Leuconostoc paramesenteroides* PR; C, MCR extract biotransformed by commercial β-glucosidase from almond. Elution was monitored with a UV absorption detector at 300 nm. MA, a peak of mulberroside A (t_R, 10.1 min; ESI-MS m/z 569 $[M + H]^+$, 567 $[M - H]^-$); OXY, a peak of oxyresveratrol (t_R, 25.1 min; ESI-MS m/z 243 $[M - H]^-$); OXY-G, a peak of oxyresveratrol-glucoside (t_R, 16.7 min; ESI-MS m/z 405 $[M - H]^-$).

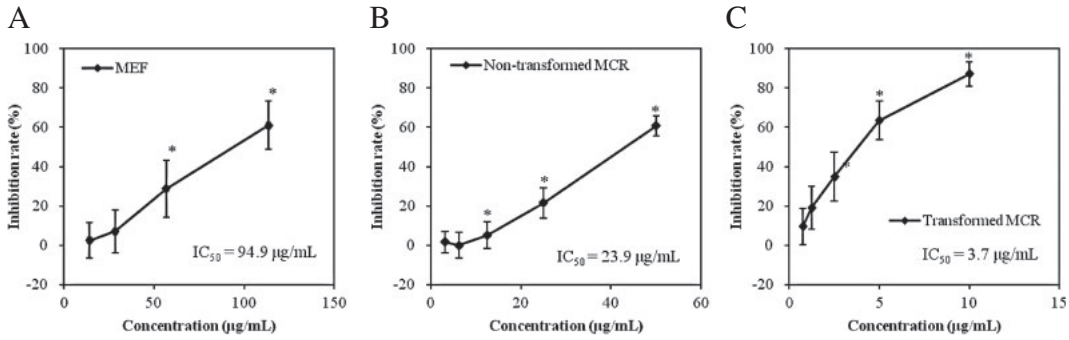

Fig. 3. Tyrosinase Inhibitory Effects of Mori Cortex Radicis (MCR) and Related Compounds against Substrate L-Tyrosine.

A, MEF, mulberroside A-enriched fraction; B, Non-biotransformed MCR extract; C, MCR extract biotransformed by crude enzymes from *Leuconostoc paramesenteroides* PR (LP). Non-biotransformed MCR extract was exposed to the same environment as the biotransformed MCR extract except for the use of phosphate buffer in place of the crude enzyme extract from LP. Asterisk means significant difference ($p < 0.05$) from negative control in tyrosinase inhibition assay (0% inhibition) by Dunnett's multiple range test. IC_{50} values in the tyrosinase inhibition assay are also represented.

no OXY production after 2 h (Fig. 2C). Instead, its HPLC profile showed an increased peak at 16.7 min, which was confirmed by LC/MS analysis (OXY-G, ESI-MS m/z 405 $[M - H]^-$) to be a stilbene glucoside with one glucosyl moiety, like oxyresveratrol-3-*O*-β-D-glucopyranoside or oxyresveratrol-4′-*O*-β-D-glucopyranoside. The crude enzyme extract from LP hydrolyzed both glucosidic bonds of MA effectively. Consequently, most of the MA was biotransformed to OXY within 2 h. The substrate specificity of the commercial almond β-glucosidase was different than that of crude enzyme extract from LP, and its activity cleaved one glycosidic bond of MA at a time. After 2 h, 97.1% of the MA from the MCR extract was biotransformed to OXY by LP, while only 68.7% of the MA was biotransformed to OXY-G, and a small amount of OXY was biotransformed by almond β-glucosidase. Prior to the biotransformation process, no OXY was detected in the MCR extract (Fig. 2A), but biotransformation by LP increased the amount of OXY in the MCR extract to 0.36% (w/w), 17.5 times higher than that in the MCR extract biotransformed by almond β-glucosidase.

Enhanced tyrosinase inhibitory activities of MCR extract biotransformed by LP

Chang *et al.* have reported that an ethanol extract of MCR showed a 0–62% inhibitory effect on mushroom tyrosinase in the range of 0–60 μg/mL.[8] In the present study, the tyrosinase inhibitory activity of the MCR extract was greater than the value reported by Chang *et al.*, with an IC_{50} value of 23.9 μg/mL (Fig. 3B). For comparative purposes, the tyrosinase inhibitory activity of kojic acid was assayed as a positive standard. The IC_{50} value of kojic acid was found to be 13.1 μg/mL in our assay system. The content of MA in the MCR extract was approximately 1.97% (w/w) and no OXY was detected by quantitative analysis in the present study. To investigate the tyrosinase inhibitory activity of MA in MCR extract, a MA-enriched fraction (MEF) of MCR extract was prepared by preparative HPLC. As shown in Fig. 3A and B, the IC_{50} value of MEF was 94.9 μg/mL and the non-biotransformed MCR extract showed tyrosinase inhibitory activity approximately 4-fold higher than that of MEF. The content of MA in the MEF and the non-biotransformed MCR extract were 87.7% (w/w) and 1.97% (w/w) respectively. From these data, it can be inferred that other phytochemicals besides MA in the extract contributed to the greater levels of tyrosinase inhibitory activity. Recently, Zheng *et al.* compared the tyrosinase inhibitory activity of 29 compounds from *Morus nigra* roots, and reported that nine compounds showed greater tyrosinase inhibitory activity levels than kojic acid.[37] Among the compounds isolated, two chalcones, 2,4,2′,4′-tetrahydroxychalcone

and morachalcone A, showed the highest tyrosinase inhibitory activity, with IC_{50} values of 0.062 and 0.14 μM respectively. Because *Morus alba* and *Morus nigra* are of the same genus, it is likely that there are compounds other than MA and OXY in *Morus alba* that exhibit strong tyrosinase inhibitory activity levels in MCR extract. Hence, we reasoned that the combinatorial chemistry of crude MCR extract can impart more benefits by virtue of the combined effects of its various phytochemicals, although MA itself is a good candidate for bioconversion into OXY, which possesses highly potent tyrosinase inhibitory activity. Furthermore, we found that a simple biotransformation process of whole MCR extract sufficiently enhanced its tyrosinase inhibitory activity. The tyrosinase inhibitory activity of the MCR extract was increased by approximately 6.5-fold by biotransformation with a crude enzyme extract from LP (Fig. 3C). The IC_{50} value of the transformed MCR was 3.7 μg/mL, whereas that of the non-transformed MCR was 23.9 μg/mL.

The enhanced tyrosinase inhibitory activity of the biotransformed MCR extract due to LP can be attributed to the increased content of OXY. The strong tyrosinase inhibitory activity of OXY was previously confirmed in other research, which found that the IC_{50} value of OXY was 0.49–1.2 μM depending on the assay conditions.[3,22] In the present study, the IC_{50} value of the OXY standard was 1.1 μM. Among more than 30 stilbene derivatives that occur naturally in various plants, OXY was reported to exert higher potent tyrosinase inhibitory activity than any other stilbene compound. Resveratrol was also found to exhibit tyrosinase inhibitory activity, but its IC_{50} value with a L-tyrosine substrate was 43.5 μM.[36] Resveratrol and its derivatives are abundantly distributed in plants and dietary food sources, including grapes, wine, soybeans, and peanuts. While resveratrol glycosides, methoxides, and polymers represent the most abundant stilbenes in nature, their tyrosinase inhibitory activities were generally found to be negligible in comparison to resveratrol and OXY.[36,38] Kim *et al.*[36] reported that piceid, a glucosidic form of resveratrol, had 8.2-fold less tyrosinase inhibitory activity than resveratrol. A biotransformed extract of *Veratrum patulum* showed an increase of up to 180% in its tyrosinase inhibitory activity because the concentration of resveratrol increased after the hydrolysis of piceid with the use of various commercial enzymes such as cellulase, β-glucosidase, dextranase, and amylase. However, the IC_{50} value of a non-transformed extract of *Veratrum patulum* was 100 μg/mL, and the tyrosinase inhibitory activity of the biotransformed *Veratrum patulum* extract was less than that of a MCR extract biotransformed by LP.

Although OXY exhibited strong tyrosinase inhibitory activity, anti-inflammatory activity, ROS- and RNS-scavenging properties, and neuroprotective effects, the amounts of OXY in food and plant sources are much lower than that of other stilbene derivatives. Chemical synthesis or direct purification from the heartwood of *Artocarpus lakoocha* Roxb. has been used to obtain OXY in large amounts.[39] Recently, Kim *et al.* reported that MA was purified from *Morus alba* roots and biotransformed to OXY to enhance its tyrosinase inhibitory activity.[22] Because the biotransformation in that study was conducted with purified MA and a commercial enzyme, we thought that it might be more helpful to streamline the biotransformation process and to enhance tyrosinase inhibitory activity without purification of OXY. In the present study, we demonstrated the potential enhancement of tyrosinase inhibitory activity by a simple biotransformation process using a crude enzyme extract of LP. After 2 h of biotransformation, 97.1% of MA from the MCR extract was biotransformed to OXY by LP, and the content of OXY in the biotransformed MCR extract increased to 0.36% (w/w). The low IC_{50} value of the transformed MCR extract, 3.7 μg/mL, may have resulted from the increased amount of OXY.

As compared with chemical synthesis and traditional acid/alkali treatment methods, microbial bioconversion represents a simple, efficient means of enhancing the bioactivities of glycones, with benefits including higher conversion efficiency, fewer by-products, better stereospecificity, and the absence of safety concerns. The present study verified that simple biotransformation using a crude enzyme extract of lactic acid bacteria was effective for the production of OXY in a crude extract of MCR. Furthermore, the whole extract of biotransformed MCR can serve as a resource for food-grade biomaterial, offering a sufficiently high level of tyrosinase inhibitory activity due to the combinatorial chemistry of various bioactive phytochemicals.

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