Kang *et al. Applied Biological Chemistry* (2022) 65:59
https://doi.org/10.1186/s13765-022-00727-5

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE Open Access

Production of isoquercitrin from quercetin by biotransformation using *Bascillus* sp. CSQ10 isolated from *Camellia sinensis* cultivation soils

Ju-Yeong Kang[1†], Won-Jung Park[1†], Youngdae Yoon[2*] and Bong-Gyu Kim[1*]

Abstract

Microorganisms are widely used to produce biologically active substances owing to their versatile ability to convert inexpensive compounds into physiologically active compounds. In this study, we isolated a microorganism capable of converting quercetin to isoquercitrin, a substance with various biological functions, from tea cultivation soils. A *Bacillus* strain was isolated and verified as *Bacillus* sp. CSQ 10 using 16sRNA gene analysis. When quercetin was fed as a substrate for *Bacillus* sp. CSQ10, isoquercitrin was produced through biotransformation. Furthermore, *Bacillus* sp. CSQ10 was able to biotransform isoquercitrin to quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside when the medium for biotransformation was replaced with yeast extract–peptone–dextrose (YPD) medium. Based on these findings, the biotransformation performance of *Bacillus* sp. CSQ10 was verified by optimizing the experimental conditions for the culture system at the laboratory scale in terms of temperature, cell density, biotransformation medium, and substrate concentration. The best biotransformation yields were achieved at 37 °C, 6.0 OD_{600}, with YPD, and 181.0 mg/L of quercetin supply. Conclusively, 193.3 mg/L and 198.8 mg/L of isoquercitrin and quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside, respectively, were produced by *Bacillus* sp. CSQ 10 under these optimized experimental conditions.

Keywords: Biotransformation, *Bacillus* sp., *Camellia sinensis*, Flavonoid

Introduction

Forests are one of the largest ecosystems on Earth, and a large amount of organic matter, which is an important carbon source in forest ecosystems, enters the forest soil each year [1]. Organic matter is used as a carbon source by various microorganisms that inhabit forest soils. It had been reported that the growth of microorganisms can be inhibited by polyphenols contained in organic matters, such as flavonoids, stilbenes, anthocyanins, coumarins, lignans, quinones, phenolic acids, and tannins [2]. To overcome the adverse effects of these compounds, microorganisms possess various enzymes, including cytochrome P450 oxidases [3], UDP-glucuronosyltransferases [4], glutathione *S*-transferases [5], and methyltransferases [6], which detoxify or decompose substances that inhibit their growth. In particular, microorganisms inhabiting forest soils have various genes encoding enzymes involved in detoxification mechanisms for their own growth inhibitory substances [7]. Among these detoxification mechanisms, glycosylation of phenolic compounds catalyzed by uridine diphosphate-dependent UDP-glycosyltransferase is a well-known detoxification mechanism [8]. In addition, the glycosylation of phenolic compounds results in a variety of biological activities for human health, depending on the glycosylation positions [9]. In this respect, forest soils have been considered a useful reservoir for identifying valuable microorganisms as well as various metabolic enzymes, including glycosyltransferases.

†Ju-Yeong Kang and Won-Jung Park contributed equally to this work

*Correspondence: yyoon21@gamil.com; dkimbk@gnu.ac.kr

[1] Division of Environmental and Forest Science, Gyeongsang National University, Jinju 52725, Republic of Korea
[2] Department of Environmental Health Science, Konkuk University, Seoul 05029, Republic of Korea

Glycosylation of flavonoids is a reaction that occurs at the final stage of plant biosynthesis, catalyzed by glycosyltransferase, and plays a role in increasing the solubility and stability of flavonoids in plants [10]. Glycosylation may lead to changes in the pharmacological properties of flavonoids by increasing bioavailability or reducing the risk of substances causing acute toxicities or adverse effects [9]. Therefore, the enzymes responsible for glycosylation and the biological activities of diverse glycosylated flavonoids have been actively investigated.

Isoquercitrin (quercetin-3-*O*-β-D-glucopyranoside) is a glycoside of quercetin and a major glycoside of flavonols along with rutin (quercetin-3-*O*-rutinoside). As reported in previous studies, isoquercitrin was found in various plant species including *Rosa soulieana* Crepin [11], *Eucommia ulmoides* Oliv [12], *Crataegus pinnatifida* Bge [13], *Crataegus azarolus* L [14], *Arbutus unedo* L. [15], various onion species [16, 17], and amaranth [18]. It had been reported isoquercitrin exhibits biological activity in kidney-related diseases [19, 20]. Although isoquercitrin is present in many plants, its content is too low for sufficient amounts to be obtained for the diet and pharmaceutical industries [21]. To our knowledge, selectively removing rhamnose from rutin using rhamnosidase and then purifying isoquercitrin was the only method to produce isoquercitrin [21]. In addition, it was possible to obtain isoquercitrin by chemical hydrolysis of rutin present in buckwheat or red bean in large amounts. However, it was not a suitable method because chemical hydrolysis preferred to produce quercetin rather than isoquercitrin. Therefore, it was inferred that the conventional method was insufficient and inefficient for producing isoquercitrin [22].

There is a need to develop efficient methods to produce biologically active compounds, including various glycosylated polyphenolic compounds. In this regard, the biotransformation has been highlighted as an alternative method to produce biological active compounds, and many researchers have contributed their efforts [23]. Biotransformation is the process of converting substrates to products using biological systems. A supply of various cofactors and donors is required when physiologically active substances are produced by enzymatic methods. However, there is no need to supply these factors for the biotransformation by microorganisms. Compared to chemical synthesis, biotransformation has advantages in that the reaction conditions are simple, safe, and generate less contaminants [23–25]. For these reasons, biotransformation has been successfully applied in the large-scale industrial production of biologically active materials for new drugs and cosmetics [26, 27]. Similarly, it would be invaluable to produce isoquercitrin through biotransformation because of the advantages of simple processes and high production yields over conventional methods.

To obtain the microorganisms capable of producing isoquercitrin, the microorganisms from the tea plantation area were screened. Since the contents of flavonoids were relatively high in tea plants, the chance to isolate the microorganisms capable of metabolizing flavonoid-like natural compounds would be higher from tea plant area. In this study, the microorganisms were isolated from the tea plantation soils, the biotransformation efficiency was characterized. Among them, a *Bacillus* strain with good biotransformation efficiency was isolated and identified, and its biotransformation performance was elucidated.

Materials and methods

Isoquercitrin-producing bacteria isolation and species identification

The soils were collected from tea plantations under the cedar (*Cryptomeria japonica*) and cypress (*Chamaecyparis pisifera*) in the forest around Jinju-si. Using a soil auger (EP 1055, USA), approximately 200*g* of soil was collected 10 cm beneath the topsoil and stored in a − 20 °C freezer until used for the experiment. The soil samples were passed through a 2-mm sieve to remove coarse-grained soils from the soil samples. One gram of soil was suspended in 9 mL of sterilized water and serially diluted 10^{-5} times. The diluted soil samples were spread evenly on LB agar plates on a clean bench and incubated at 30 °C for 48 h. The bacterial colonies were inoculated LB media and incubated overnight. Then, each cell was adjusted to 1.0 of OD_{600} in 2 mL of M9 media containing 2% glucose and fed quercetin with 200 μM of final concentration. The cells were incubated at 30 °C for 12 h and the samples prepared by ethyl acetate extraction for HPLC analysis. The colonies showing new peaks with the different retention time of quercetin were selected for further investigations.

Bacteria identification

Bacterial species were identified using 16S rRNA sequence analysis. The primers used for gene amplification were as follows: forward primer 877F: 5′-CGGAGA GTTTGATCCTGG-3′, and reverse primer 878R: 5′-TAC GGCTACCTTGTTAGCGAC-3′. Polymerase chain reaction (PCR) was used to amplify the 16S rRNA gene from the genomic DNA of bacteria using the Qiagen DNeasy kit (Hilden, Germany). The PCR reaction solutions were prepared by mixing 0.5 μL of genomic DNA as templates, 2 μL of 10X reaction buffer, 2 μL of Q buffer, 2 μL of 2.5 mM dNTP, 0.5 μL of Qiagen Hot Taq polymerase (Hilden, Germany), 0.2 μL of forward primer (100 pmol/

μL), 0.2 μL reverse primer (100 pmol/μL), and 12.6 μL sterilized water. PCR was performed at 94 °C for 15 min to activate hot Taq polymerase, followed by 1 min at 94 °C, 1 min at 55 °C, and 1 min and 30 s at 72 °C for a total of 35 cycles. To fill this gap, the mixture was incubated at 72 °C for 7 min.

The PCR reaction product was separated using electrophoresis on 1.5% agarose gel and the PCR product was purified using a DNA purification kit (Bioneer, Daejeon, Korea). The purified 16S rRNA gene was cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA) and the sequences were analyzed. The similarity and multiple sequence alignment of the 16S rRNA gene sequences were analyzed using the blastn and CLUSTAL W programs provided by the National Center for Biotechnology Information (NCBI). Phylogenetic analysis of *Bacillus* strains was performed using the neighbor-joining method built into the Molecular Evolutionary Genetics Analysis 6.0 (MEGA 6.0) program [28]. To evaluate the reliability of the branching pattern, bootstrapping generated by 1000 sampling replicates was performed.

Biotransformation for isoquercitrin production

Single colonies obtained from the soils were inoculated in 2 mL of Luria Broth (LB) medium and cultured overnight at 30 °C in an incubator at 200 rpm. The precultured cells were inoculated into 25 mL of fresh LB in 250 mL flasks to reach an 0.1 of optical density at 600 nm (OD_{600}), and then incubated for 3 h in a shaking incubator at 30 °C and 200 rpm. The cell cultures were chilled on ice for 10 min, and the cells were harvested by centrifugation (Hannil UNION32R, Korea) at 4 °C, 4000 rpm, and 15 min. Cells were washed twice with M9 minimal medium (M9 salt, glucose 20 g/L) and then suspended in the appropriate media to an 0.6 of OD_{600} value. The biotransformation processes were performed in a shaker incubator at 30 °C, followed by feeding of quercetin as a substrate. 50–500 μM ranges of quercetin was tested as a substrate for screening, and then fixed the concentration as 200 μM for further experiments to obtain maximum biotransformation efficiency. To verify the biotransformation efficiency, 500 μL of the culture solution was collected after 12 h of incubation and the reactants were extracted by mixing with the same amount of ethyl acetate. In addition, cell cultures were collected after 1, 2, and 3 days of incubation to track the biotransformation performance of bacterial cells. The supernatant was recovered by centrifugation for 1 min at 13,500 rpm using a desktop centrifuge (Eppendorf 5424R, Germany), and the extraction process was repeated [23]. The supernatants were completely dried under the vacuum dryer and dissolved in dimethyl sulfoxide (DMSO) before subjected to the high-performance liquid chromatography (HPLC) [24, 25].

HPLC analysis

The extracts of bacterial cell culture were analyzed using a Shimadzu HPLC system (Shimadzu, Japan) equipped with a Polaris 5 C18-A column (250 mm × 4.6 mm, Agilent Technologies) and a photodiode array (PDA). For HPLC analysis, tertiary distilled water added with formic acid to a final concentration of 0.1% was used as buffer A and acetonitrile containing formic acid added to a final concentration of 0.1% was used as buffer B. The HPLC analysis was performed under the following experimental conditions: 10% B buffer at 0 min, 45% B buffer at 8 min, 70% B buffer at 15 min, 90% B buffer at 17 min, 20 min, 12 B buffer for 20.1 min, and 10% B buffer for 25 min.

Results and discussion

Isolation of quercetin metabolizing bacteria

The soils from the tea planting area were diluted, plated on LB agar plates, and incubated for 48 h at 30 °C. As a result, 2.46×10^7 colony-forming units (CFU)/g soil of the total number of culturable bacteria were obtained on LB agar plates. This was slightly higher than the results from the soils collected from the city park reported as 1.96×10^7 CFU/g [29]. However, it was similar to 2.5×10^8 CFU/g of culturable bacteria reported from the investigation of the soils in Chang Qing Graden (CQG), which consisted of hardwood trees aged more than 20 years [30]. Although the bacterial species were not compared, it was inferred that soils with large amounts of organic matter were favored by microorganisms.

To elucidate the biotransformation performance of the isolated bacteria, quercetin was fed into the cells as a substrate. After the biotransformation processes, the samples prepared by ethyl acetate extraction were subjected to HPLC analysis [23]. Approximately 200 colonies were tested, of which 11 colonies showed new peaks at different retention times of quercetin, therefore, 5.5% of bacteria isolated from tea planting soils were able to convert quercetin (Additional file 1). The biotransformation ability of the bacteria was isolated through subculture processes, and their biotransformation ability to generate products from quercetin was confirmed by repeated experiments. Among the bacteria possessing biotransformation activity, the most reactive strain was selected and investigated in the subsequent experiments.

Identification of quercetin metabolizing bacteria

16S rRNA sequencing was performed to identify isoquercitrin-producing bacteria isolated from tea-planting soils.

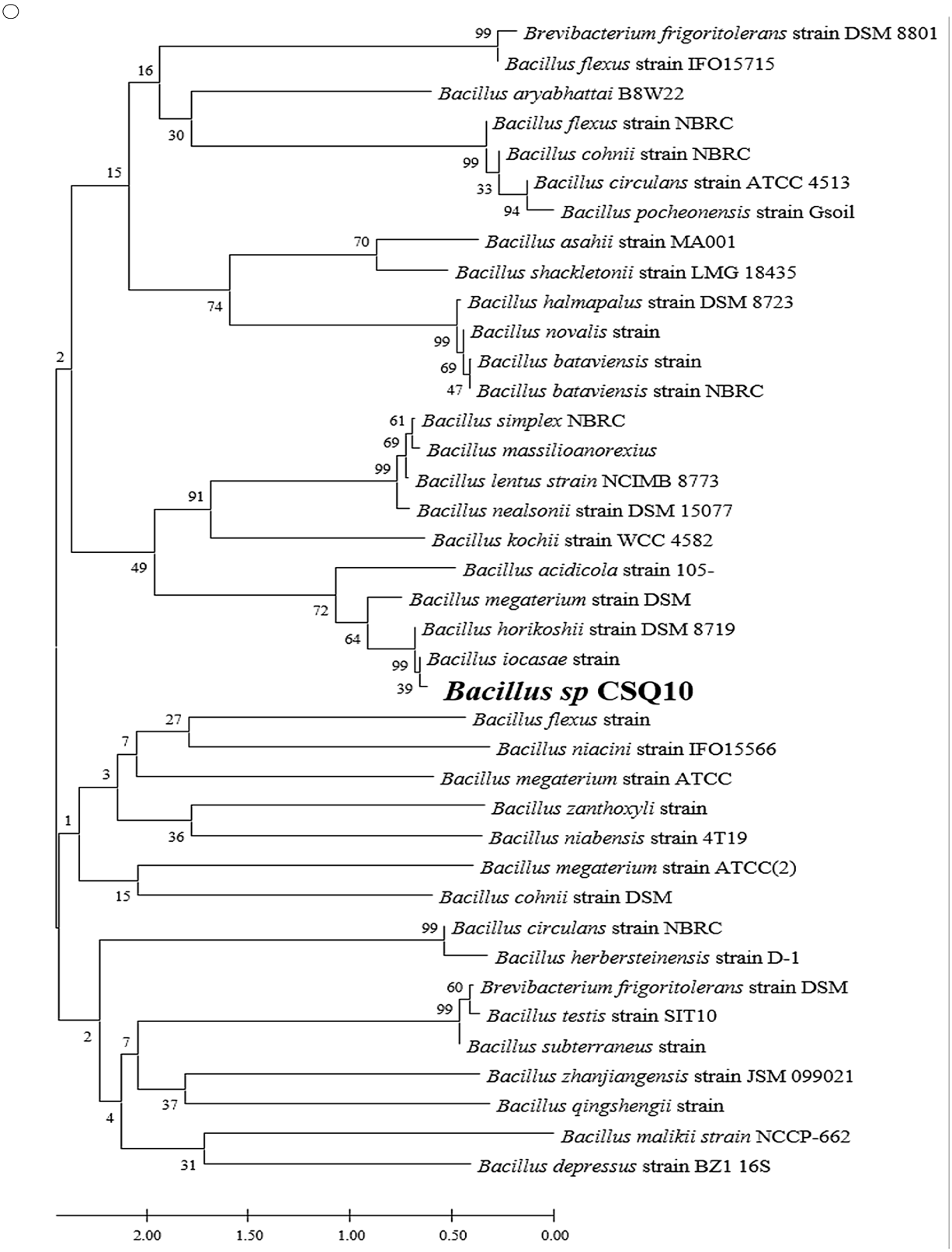

Fig. 1 A neighbor-joining phylogenetic tree based on the 16S rRNA gene of *Bacillus* sp. CSQ10 with 16S rRNA gene sequences from other bacteria. The Kimura two-parameter model was used to determine the distance matrix. Bootstrap values were calculated from 1000 replicates regenerated using a random method

After the amplified 16S rRNA from the genomic DNA of bacteria was introduced into a pGEMT-easy vector (Promega, USA), 16s RNA gene sequences were determined by nucleotide sequence analysis (Additional file 2). The nucleotide sequences were compared and analyzed using the 16S ribosomal RNA gene sequencing database with the blastn program of the Basic Local Alignment Search Tool (BLAST). The 16S rRNA sequence of bacteria showed high homology with *Bacillus aryabhattai* B8W22 (99.8%), *Bacillus megaterium* strain ATCC 14581 (98.67%), *Bacillus flexus* strain IFO15715 (98.67%), *Bacillus zanthoxyli* strain 1433 (99.65%), *Bacillus iocasae* strain S36 (96.72%), and *Bacillus qingshengii* strain G19 (97.97%). As shown in Fig. 1, the isolated *Bacillus* strain was closest to *Bacillus iocasae* and *Bacillus horikoshii* DSM8719 and belonged to the same group as *Bacillus megaterium* and *Bacillus acidicola* based on phylogenetic analysis. The isolated strain possessing the biotransformation activity of quercetin was named *Bacillus* sp. CSQ10.

Characterization of biotransformation performance of *Bacillus* sp. CSQ10

Since *Bacillus* sp. CSQ10 isolated from tea planting soils showed the ability to metabolize quercetin, and its biotransformation performance was investigated. The quercetin was added as substrate to cells resuspended in M9 medium supplemented with 2% glucose. After 12 h of incubation, 2 mL of cell culture was extracted with ethyl acetate to prepare the samples for HPLC analysis. As shown in Fig. 2, a new substance (P1) was observed at 11.09 min (Fig. 2C), except at 14.39 min (Fig. 2A), which is the retention time for quercetin. Although the UV absorbance of P1 was similar to that of quercetin, the maximum absorbance of band I shifted from 371 to 354 nm (boxes in Fig. 2A, C). It is known that the shift of band I maximum absorbance in flavonols is caused by a hypsochromic shift induced by structural changes at position 3 [31]. Thus, it was assumed that P1 would undergo a conformational change at position 3 of quercetin. To verify this assumption, P1 was used for mass spectrometry (MS) to estimate its molecular weight. The results of MS analysis of P1 are shown in Fig. 2D. The ion peak at 487.1 $[M+H]^+$ was estimated to be quercetin-hexose-Na^+, 465.2 $[M+H]^+$ was quercetin-hexose, and 302.8 $[M+H]^+$ was quercetin. In addition, major ions such as 463.4 $[M-H]^-$, 300.2 $[M-H]^-$, 271.2 $[M-H]^-$, 255 $[M-H]^-$, 179.1 $[M-H]^-$, and151.0 $[M-H]^-$ were observed in the ion negative MS analysis (Fig. 2E). The ionization pattern determined by MS analysis was the same as the MS fragmentation pattern of quercetin-3-*O*-glucoside in a previous study [32]. This was further confirmed by analyzing commercial quercetin 3-*O*-glucoside. As shown in Fig. 2B, the retention time and UV absorbance of quercetin 3-*O*-glucoside are indistinguishable from those of P1. Therefore, it can be concluded that *Bacillus* sp. CSQ10 exhibits biotransformation activity to produce isoquercitrin through biotransformation using quercetin as a substrate.

Isoquercitrin had been first isolated from the seed pods of *Cercis canadensis* L. (eastern redbud) [33] and then from various plants [11–18, 34, 35]. Isoquercitrin is a biologically active substance of St. John's wort (*Hypericum perforatum* L.) [36], a medicinal plant found in many plant-derived beverages such as fruits, vegetables, tea, and wine [22]. Additionally, it is known that isoquercitrin exhibits various physiological activities such as anti-inflammatory and anti-obesity [37], antihyperglycemic [38], colon inflammation relief [39], skin cancer growth inhibition [40], and liver cancer growth inhibition [41]. Despite these various physiological activities, it is challenging to supply isoquercitrin commercially because the isoquercitrin content in plants is insufficient. In this regard, it would be invaluable to isolate new bacterial strain to produce isoquercitrin by one-step of biotransformation.

Effects of media on biotransformation activity of *Bacillus* sp. CSQ10

The biotransformation efficiency of microorganisms has been reported to be modulated by the components of the media [24, 25]. The biotransformation activity of *Bacillus* sp. CSQ10 was investigated under different biotransformation media, including Luria Broth (LB) medium (BD-Difco, USA), Terrific Broth (TB) medium (BD-Difco, USA), yeast extract –peptone–dextrose (YPD) medium (BD-Difco, USA), Andrw's Magic Media (AMM)-Glu medium [42], AMM-Gly medium, YM9 (10 g yeast extract and M9 salt)-Glu medium, and M9-Glu medium (Fig. 3). Following the same procedures

(See figure on next page.)

Fig. 2 HPLC analysis of reaction product by biotransformation of *Bacillus* sp. CSQ10 with quercetin as a substrate. **A** HPLC chromatogram of authentic quercetin; **B** HPLC chromatogram of authentic isoquercitrin; **C** HPLC chromatogram of reaction products obtained through biotransformation of *Bacillus* sp. CSQ10; **D** MS spectra of P1 in the positive ion mode; **E** negative ion mode of P1. S1, quercetin standard; S2, isoquercitrin standard; P1, reaction product 1

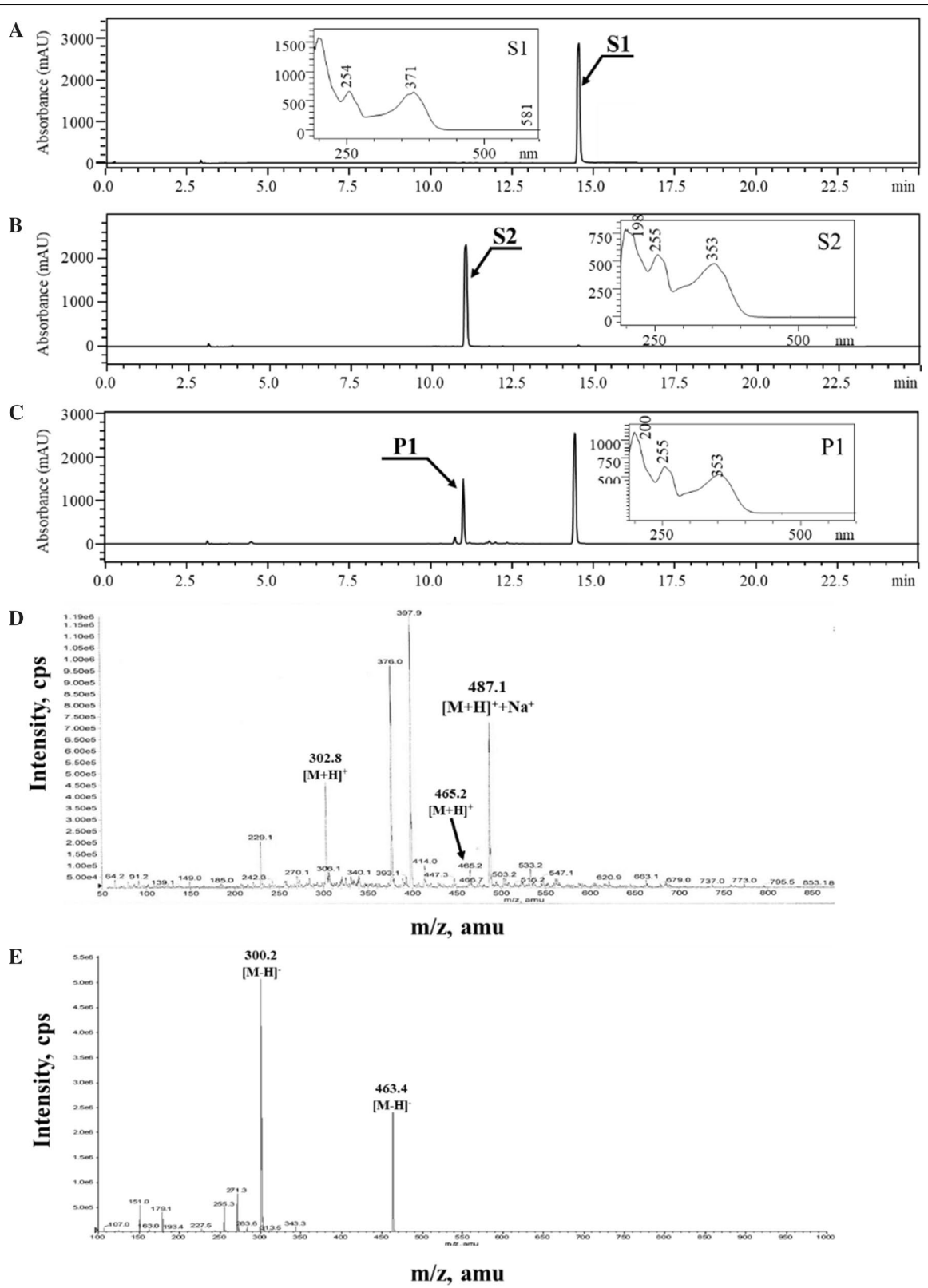

Fig. 2 (See legend on previous page.)

described previously, *Bacillus* sp. CSQ10 cells adjusted to 0.6 of OD_{600} were fed 200 μM quercetin as a final concentration and incubated for 12 h at 30 °C. As a result, the highest amount of isoquercitrin was produced in YPD medium as 56.2 mg/L, followed by M9-glucose medium (36.3 mg/L), AMM-glucose medium (38.6 mg/L), and YM9-glucose medium (20.9 mg/L) (Fig. 3A). From the HPLC analysis, only one peak (11.09 min) was produced in the biotransformation medium of LB, TB, AMM-Glu, AMM-Gly, and M9-Glu, while two peaks were observed at 11.09 min and 12.07 min in the biotransformation medium of YPD and YM9-glucose (Fig. 3D). As verified above, the peak indicated as P1 was identified as isoquercitrin by comparing the elution time and UV absorbance with those of authentic isoquercitrin. Although the peak indicated as P2 had a different elution time, the UV absorbance spectrum was indistinguishable from that of isoquercitrin (Fig. 3D). Thus, it can be assumed to be a derivative of P1, isoquercitrin.

To characterize the new product of biotransformation, P2 was used for MS analysis. As shown in Fig. 3E, major ion peaks such as 528.7 $[M+H]^+$, 506.7 $[M+H]^+$, and 302.8 $[M+H]^+$ were detected. It could be interpreted as that the molecular weight of 528.7 $[M+H]^+$ was Na^+ attached to quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside, 506.7 $[M+H]^+$ was quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside, and 302.8 $[M+H]^+$ was quercetin aglycone. In addition, major ion peaks, such as 301.5 $[M-H]^-$, 463.5 $[M-H]^-$, and 505.6 $[M-H]^-$ were observed in the ion-negative MS analysis (Fig. 3F). The molecular weights of 301.5 $[M+H]^-$, 463.5 $[M+H]^-$, and 505.6 $[M+H]^-$ were estimated to be quercetin, quercetin-3-*O*-glucoside, and quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside, respectively. Therefore, it was necessary to conduct nuclear magnetic resonance (NMR) analysis to verify exact structure of P2. For the preparation of NMR samples, 500 L of cells was reacted with quercetin and extracted with ethyl acetate. The extracts were then subjected to HPLC to purify P2 and 6 mg of P2 was obtained. The samples were subjected to ^{1}H-NMR analysis following the procedures described by Kim et al. [24]. The NMR data was analyzed as follows: ^{1}H-NMR (400 MHz, Acetone-d_6); δ 6.21 (H, d, J = 2.0 Hz), δ 6.41(H, d, J = 2.0 Hz), δ 7.59 (1H, d, J = 2.0 Hz), δ 6.84 (H, d, J = 2.0 Hz), δ 7.60 (H, dd, J = 2.0, 8.6 Hz), δ 5.11 (H, d, J = 7.7 Hz), δ 4.6 (H, m), and δ 4.17(H, m). The structure of P2 was determined by comparison with ^{1}H-NMR spectrum published by Jeon et al. [43]. Conclusively, the new product indicated as P2 was identified as quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside. The results revealed that the *Bacillus* sp. CSQ10 isolated from soil can convert quercetin to isoquercitrin and quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside. Moreover, it would be inferred that *Bacillus* sp. CSQ10 has genes that catalyze the biotransformation of quercetin, and the gene encoding an enzyme that attaches an acetyl group on the glucoside of isoquercitrin would be induced by components only in YPD and YM9-glucose.

Optimization of isoquercitrin production

To maximize the yield of isoquercitrin, biotransformation of quercetin by *Bacillus* sp. CSQ10 was examined under various experimental conditions, including temperature, cell density, and substrate concentration. First, the amount of isoquercitrin was determined at different temperatures, including 25, 30, and 37 °C. As shown in Fig. 4A, the biotransformation efficiency of isoquercitrin at 30 °C and 37 °C was similar (59 mg/L), whereas the efficiency decreased about threefold at 25 °C (20 mg/L). This is because the growth rate of *Bacillus* sp. CSQ10 was reduced at 25 °C compared to other culture temperatures, so the supply of UDP-glucose, a sugar donor, was limited; therefore, the biotransformation efficiency was lowered. To verify the effects of cell density on the biotransformation efficiency, the initial cell densities for feeding quercetin were adjusted to 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 of OD_{600}. As a result, the biosynthesis of isoquercitrin increased as the cell density increased, and the highest biosynthesis (128 mg/L) was observed at a cell density of 3.0 of OD_{600} (Fig. 4B). However, no further increase in isoquercitrin biosynthesis was observed even when the cell density was increased to over 3.0 OD_{600} (Fig. 4B). Finally, the effects of quercetin concentration on isoquercitrin biosynthesis were investigated. The cell density of *Bacillus* sp. CSQ10 in M9-glucose medium was adjusted to 3.0 of OD_{600} and 10 different concentrations (100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 μM) were tested (Fig. 4C). The highest production of isoquercitrin was 128 mg/L with 600 μM of quercetin supply, and approximately 10% less biotransformation efficiency (approximately 118 mg/L) was observed with 400,

(See figure on next page.)

Fig. 3 Effect of biotransformation medium on isoquercitrin production by *Bacillus* sp. CSQ10. **A** Isoquercitrin production titers according to biotransformation medium; **B** standard quercetin; **C** standard isoquercitrin; **D** HPLC analysis of reaction products of YPD medium biotransformation; **E** MS spectra of P2 in positive ion mode; **F** MS spectra negative ion mode of P2. *Bacillus* sp. CSQ10 cell density was adjusted to $OD_{600} = 0.6$, with 2 mL of each medium and 200 μM quercetin. The resulting culture was biotransformed at 30 °C for 12 h, with shaking at 200 rpm. The reaction products were extracted with two volumes of ethyl acetate and analyzed using HPLC. Error bars indicate mean values ± from three independent experiments

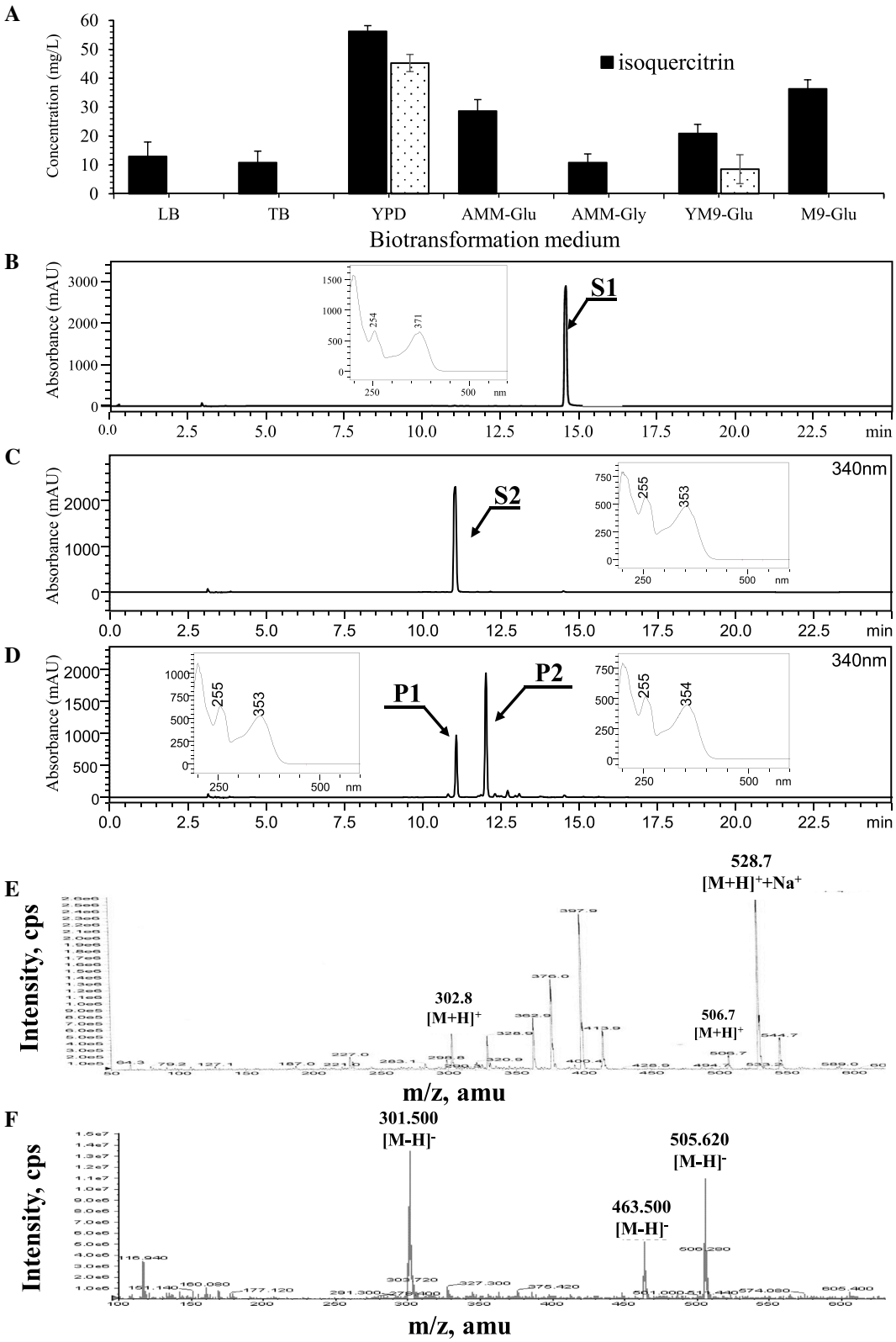

Fig. 3 (See legend on previous page.)

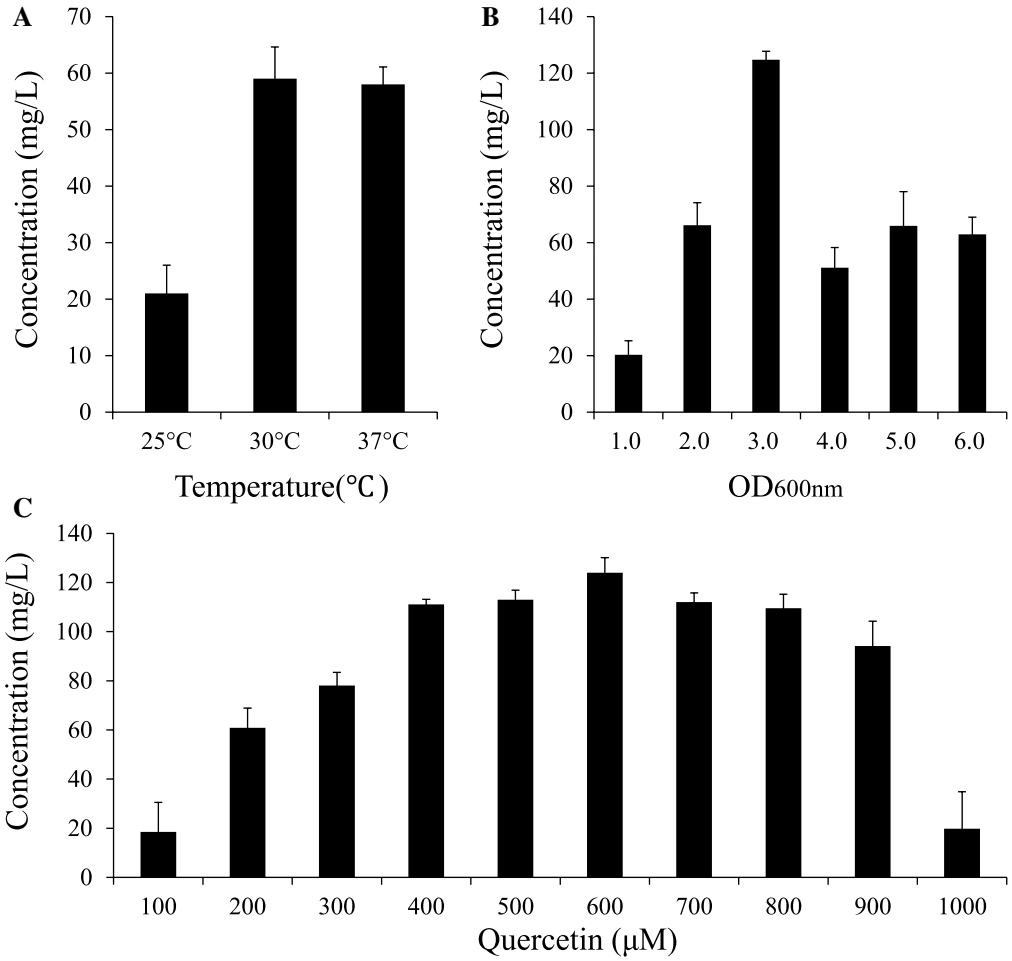

Fig. 4 Determination of optimum temperature (**A**), cell density (**B**), quercetin feed concentration (**C**) for isoquercitrin production. Biotransformation was performed for 12 h at 30 °C after *Bacillus* sp. CSQ10 cell densities were adjusted to an OD600 of 0.6. The reaction products were extracted with two volumes of ethyl acetate and analyzed using HPLC. Error bars indicate mean values ± from three independent experiments

500, 700, and 800 μM of quercetin supply. Additionally, it was observed that the isoquercitrin production was slightly decreased at 900 μM of quercetin and rapidly decreased at 1000 μM of quercetin supply (Fig. 4C). This result seems to be caused by the antibacterial effect of quercetin on *Bacillus* sp. CSQ10, as well as by the metabolic load for isoquercitrin biosynthesis Based on these results, it can be concluded that the optimum conditions for biotransformation to produce isoquercitrin from quercetin are M9-glucose medium, 30 °C, and 600 μM quercetin.

Under optimized conditions, we monitored isoquercitrin production for 72 h (Fig. 5). The biotransformation rate to produce isoquercitrin was high at the initial stage until 4 h, and 123 mg/L of isoquercitrin was produced. Subsequently, the rate gradually decreases. The highest production of isoquercitrin was 193 mg/L after 24 h of incubation, while 11.3 mg/L of quercetin remained. However, no further increase in isoquercitrin biosynthesis

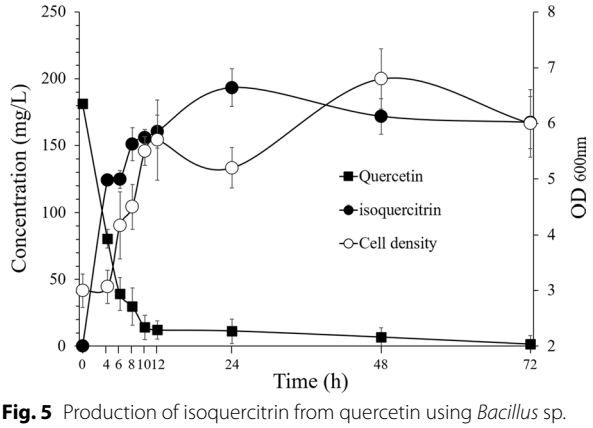

Fig. 5 Production of isoquercitrin from quercetin using *Bacillus* sp. CSQ10. Quercetin (600 μM) was then added. The reaction mixture was collected periodically and the production of isoquercitrin was monitored. Error bars represent mean values ± SD of three independent experiments

was observed, even after 72 h of incubation. During this biotransformation process, the cell density gradually increased to 6.8 of OD_{600} value at 48 h, and then decreased slightly after 48 h.

Optimization of Quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside

Isolated *Bacillus* sp. CSQ10 possesses the ability to biotransform isoquercitrin to quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside, as well as quercetin to isoquercitrin with YM9-glu and YPD as transformation media. The first identification of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside was from *Petasites japonicus* [44] and was recently reported in *Pinus densiflora* needles [43]. It has been reported that quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside has biological effects such as protection of skin cells from UV radiation [45], aldose reductase inhibitory activity [46], and antioxidant activity. Although several types of biological activities have been reported, the process has been hampered to explore their biological activity and supply as a diet because of the difficulty in obtaining sufficient amounts. In this regard, the biotransformation of quercetin by *Bacillus* sp. CSQ10 is valuable for the production of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside.

To achieve the maximum yield, the experimental conditions for quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside production were optimized by testing various temperatures, cell densities, and substrate supply concentrations in YPD medium (Fig. 6). The biosynthesis of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside occurred at 37 °C during the biotransformation process (97 mg/L), and 200 mg/L of isoquercitrin was obtained (Fig. 6A). As the temperature decreased, the production of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside also decreased. This could be attributed to the retarded growth rate of *Bacillus* sp. CSQ10 limits the supply of UDP-sugar donors and acetyl-CoA, thereby resulting in a decrease in biosynthesis. To investigate the effects of the initial cell density on the biotransformation of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside production yield, quercetin was fed to *Bacillus* sp. CSQ10 at 1.0 to 10.0, OD_{600} in the YPD medium. The cells underwent the biotransformation process by incubating at

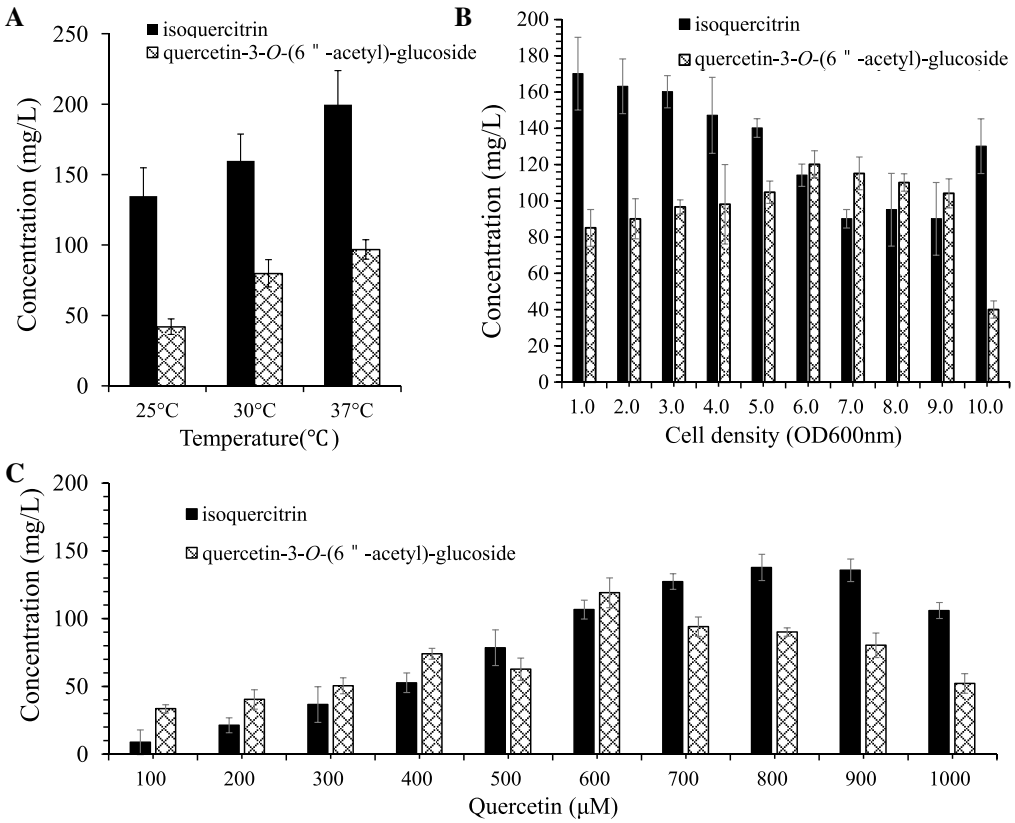

Fig. 6 Determination of optimum temperature (**A**), cell density (**B**), quercetin feed concentration (**C**) for quercetin-3-*O*-(6″-acetyl)-β-D-glucoside production. Biotransformation was performed for 12 h at 30 °C after *Bacillus* sp. CSQ10 cell densities were adjusted to an OD600 of 0.6. The reaction products were extracted with two volumes of ethyl acetate and analyzed using HPLC. Error bars indicate mean values ± from three independent experiments

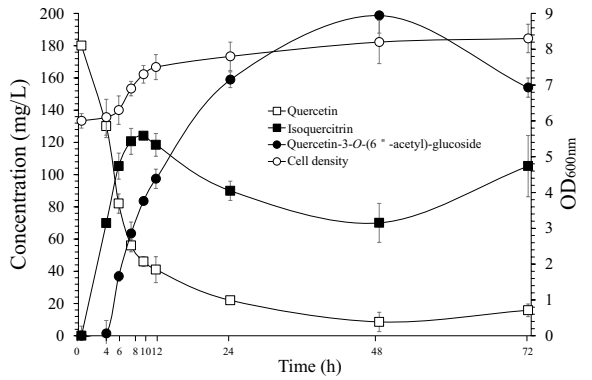

Fig. 7 Production of quercetin-3-*O*-(6″-acetyl)-β-D-glucoside from quercetin using *Bacillus* sp. CSQ10. Quercetin (600 μM) was then added. The reaction mixture was collected periodically and the production of quercetin-3-*O*-(6″-acetyl)-β-D-glucoside was monitored. Error bars represent mean values ± SD of three independent experiments

37 °C for 12 h, and the biotransformation yields were determined by HPLC analysis. As shown in Fig. 6B, the production of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside was increased to the 6.0 of OD_{600} showing highest amount as 120 mg/L, and the biotransformation efficiency was gradually decreased. In contrast, the amount of isoquercitrin biosynthesized from quercetin was inversely proportional to the amount of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside. Therefore, we inferred that *Bacillus* sp. CSQ10 can convert quercetin to isoquercitrin, and isoquercitrin to quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside in YPD media. To verify the effects of quercetin concentration, the range of 100 to 1,000 μM of quercetin was fed to *Bacillus* sp. CSQ10 was adjusted to 6.0 an OD_{600} values of YPD. As shown in Fig. 6C, the highest production of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside as 118.9 mg/L was observed from 600 μM of quercetin supply and it was decreased with higher concentration of quercetin. Additionally, the production efficiency of isoquercitrin increased up to 900 μM in YPD media, while the highest efficiency was observed at 600 μM in M9-glu (Fig. 5C).

In summary, the optimal conditions for quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside biosynthesis from quercetin were YPD medium for biotransformation, 37 °C for temperature, and 600 μM of quercetin. As described in the previous section, we monitored the efficiency of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside production for 72 h under the optimized experimental conditions (Fig. 7). Isoquercitrin biosynthesis was first observed during the initial stage, and then the biosynthesis of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside was initiated. Only isoquercitrin (69.9 mg/L) was obtained after 4 h of incubation, whereas no quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside was detected. However, the biosynthesis of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside accelerated from 6 h and reached a maximum biotransformation amount of 198 mg/L after 48 h of incubation. In the case of isoquercitrin, the maximum production was observed after 10 h incubation with 124 mg/L and tended to decrease gradually with increasing incubation time and production of quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside (Fig. 7). The density of cells was also monitored and reached an 8.0 of OD_{600} after 12 h of incubation.

WCBs based on microorganisms have been used to produce high value-added physiological substances from inexpensive substances. Unlike genetically engineered organisms, WCBs for biotransformation have advantages such as being free of antibiotics and inducers, including isopropyl β-D-1-thiogalactopyranoside (IPTG) and arabinose. In addition, there is no need to supply expensive cofactors, as required in enzymatic biosynthesis, because WCBs can utilize their own endogenous cofactors. Moreover, the processes to produce bioactive substances using WCBs were simple to expand to larger quantities. With the advantages of biosynthesis by WCBs, many microorganisms, including Bacillus species, owing to their versatile functions and biological safety, have been implanted in the process of synthesizing bioactive substances. In this study, we isolated microorganisms possessing metabolic mechanisms for isoquercitrin biosynthesis from the soils of tea plantations. Thus, quercetin-metabolizing *Bacillus* sp. CSQ10 was isolated and used as a WCB to produce isoquercitrin from quercetin. Additionally, *Bacillus* sp. CSQ10 can produce quercetin-3-*O*-(6"-*O*-acetyl)-β-D-glucoside when M9-glucose medium supplemented with yeast extract was used as the biotransformation medium. To achieve maximum biotransformation efficiency, the experimental conditions for the biosynthesis of isoquercitrin and quercetin-3-*O*-(6"-*O*-acetyl)-β-d-glucoside from quercetin were optimized. As a result, approximately 193 mg/L of isoquercitrin and 198.7 mg/L of quercetin-3-*O*-(6"-*O*-acetyl)-β-D-glucoside were produced from 181.2 mg/L of quercetin after 48 h of incubation. Conclusively, it would be invaluable to isolated new bacterial strain capable of producing a rare glycosylated flavonoind, isoquercitirn, by one-step biotransformation. Since the biotransformation using *Bacillus* sp. CSQ10 could be an efficient method to produce massive amount isoquercitrin compared to conventional methods, it would be appreciated by industrial fields. Moreover, it is noteworthy that *Bacillus* sp. CSQ10 contains genes encoding enzymes that metabolize quercetin, although they were uncharacterized. Although the genes were not identified at this stage of investigation, it would be next

goals to be achieved. We believed to identify those genes was invaluable not only to enlarge the understanding of flavonoid metabolic pathway in newly isolate strain but also to provide clues for biosynthesizing biological active substances.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-022-00727-5.

Additional file 1: Data S1. HPLC analysis of reaction products of biotransformation of bacteria isolated from tea planting soil using quercetin as a substrate. **Data S2.** The nucleotide sequence of the 16S rRNA gene of Bacillus sp. CSQ10 isolated from Camellia sinensis planting soil.

Acknowledgements
This study was carried out with the support of 'R&D Program for Forest Science Technology (Project No. "FTIS 2020186F10-2222-AA02")' provided by Korea Forest Service (Korea Forestry Promotion Institute).

Author contributions
BGK, JYP, and WJP designed the experiments. WJP, JYP, and BGK performed the experiments and analyzed the data. YY, JYP and BGK composed the manuscript. All authors read and approved the final manuscript.

Funding
Funding was received from the Korea Forest Service ((Korea Forestry Promotion Institute), Support of R&D Program for Forest Science Technology, Project No. 2020186F10-2222-AA02).

Availability of data and materials
All data generated or analyzed in this study are included in this published article.

Declarations

Competing interests
The authors declare that they have no competing interests.

Received: 3 May 2022 Accepted: 16 August 2022
Published online: 26 August 2022

References

1. Robertson AD, Paustian K, Ogle S, Wallenstein MD, Lugato E, Cotrufo MF (2019) Unifying soil organic matter formation and persistence frameworks: the MEMS model. Biogeosciences 16:1225–1248
2. Bouarab-Chibane L, Forquet V, Lanteri P, Clement Y, Leonard-Akkari L, Oulahal N, Degraeve P, Bordes C (2019) Antibacterial properties of polyphenols: characterization and QSAR (Quantitative Structure-Activity Relationship) models. Front Microbiol 10:829
3. Zabel S, Brandt W, Porzel A, Athmer B, Bennewitz S, Schäfer P, Kortbeek R, Bleeker P, Tissier A (2021) A single cytochrome P450 oxidase from *Solanum habrochaites* sequentially oxidizes 7-epi-zingiberene to derivatives toxic to whiteflies and various microorganisms. Plant J 105:1309–1325
4. Mano ECC, Scott AL, Honorio KM (2018) UDP-glucuronosyltransferases: structure, function and drug design studies. Curr Med Chem 25:3247–3255
5. ShylajaNaciyar M, Karthick L, Prakasam PA, Deviram G, Uma L, Prabaharan D, Saha SK (2020) Diversity of glutathione *S*-transferases (GSTs) in Cyanobacteria with reference to their structures, substrate recognition and catalytic functions. Microorganisms 8:712
6. Darsandhari S, Dhakal D, Shrestha B, Parajuli P, Seo JH, Kim TS, Sohng JK (2018) Characterization of regioselective flavonoid *O*-methyltransferase from the *Streptomyces* sp. KCTC 0041BP. Enzyme Microb Technol 113:29–36
7. Gallego A, Fortunato MS, Foglia J, Rossi S, Gemini B, Gomez L, Gomez CE, Higa LE, Koro SE (2003) Biodegradation and detoxification of phenolic compounds by pure and mixed indigenous cultures in aerobic reactors. Int Biodeterior Biodegradation 52:261–267
8. Snoeck S, Pavlidi N, Pipini D, Vontas J, Dermauw W, Van LT (2019) Substrate specificity and promiscuity of horizontally transferred UDP-glycosyltransferases in the generalist herbivore *Tetranychus urticae*. Insect Biochem Mo Biol 109:116–127
9. Yang B, Liu H, Yang J, Kumar V, Jiang Y (2018) New insights on bioactivities and biosynthesis of flavonoid glycosides. Trends Food Sci Technol 79:116–124
10. He B, Bai X, Tan Y, Xie W, Feng Y, Yang GY (2022) Glycosyltransferases: Mining, engineering and applications in biosynthesis of glycosylated plant natural products. Synth Syst Biotechnol 7:602–620
11. Yang C, Li F, Zhang X, Wang L, Zhou Z, Wang M (2013) Phenolic antioxidants from *Rosa soulieana* flowers. Nat Prod Res 27:2055–2058
12. Dai X, Huang Q, Zhou B, Gong Z, Liu Z, Shi S (2013) Preparative isolation and purification of seven main antioxidants from *Eucommia ulmoides* Oliv. (Duzhong) leaves using HSCCC guided by DPPH-HPLC experiment. Food Chem 139:563–570
13. Jurˇikova T, Sochor J, Rop O, Mlcek J, Balla S, Szekeres L, Adam V, Kizek R (2012) Polyphenolic profile and biological activity of Chinese hawthorn (*Crataegus pinnatifida* BUNGE) fruits. Molecules 17:14490–14509
14. Belkhir M, Rebai O, Dhaouadi K, Congiu F, Tuberoso CI, Amri M, Fattouch S (2013) Comparative analysis of tunisian wild *Crataegus azarolus* (yellow azarole) and *Crataegus monogyna* (red azarole) leaf, fruit, and traditionally derived syrup: phenolic profiles and antioxidant and antimicrobial activities of the aqueous-acetone extracts. J Agric Food Chem 61:9594–9601
15. Males Z, Saric D, Bojic M (2013) Quantitative determination of flavonoids and chlorogenic acid in the leaves of Arbutus unedo L using thin layer chromatography. J Anal Methods Chem 18:4057
16. Vlase L, Parvu M, Parvu EA, Toiu A (2013) Phytochemical analysis of *Allium fistulosum* L. and A. ursinum L. Dig J Nanomater Bios 8:457–467
17. Vlase L, Parvu M, Parvu EA, Toiu A (2013) Chemical constituents of three *Allium* species from Romania. Molecules 18:114–127
18. Kraujalis P, Venskutonis PR, Kraujaliene V, Pukalskas A (2013) Antioxidant properties and preliminary evaluation of phytochemical composition of different anatomical parts of amaranth. Plant Foods Hum Nutr 68:322–328
19. Pennesi CM, Neely J, Marks AG Jr, Basak SA (2017) Use of isoquercetin in the treatment of prurigo nodularis. J Drugs Dermatol 11:1156–1158
20. Baell J, Walters MA (2014) Chemistry: Chemical con artists foil drug discovery. Nature 513(7519):481–483
21. Lu Z, Wang J, Lin S, Zhan Y (2013) Degradation of rutin into isoquercitrin by *Bacillus litoralis* strain C44. IOSR J Engin 2:1154–1161
22. Hasumura M, Yasuhara K, Tamura T, Imai T, Mitsumori K, Hirose M (2004) Evaluation of the toxicity of enzymatically decomposed rutin with 13-weeks dietary administration to wistar rats. Food Chem Toxicol 42:439–444
23. Hao Y, Wei Z, Wang Z, Li G, Yao Y, Dun B (2021) Biotransformation of flavonoids improves antimicrobial and anti-breast cancer activities in vitro. Foods 10:2367
24. Kim BG (2019) Biosynthesis of bioactive isokaemferide from naringenin in *Escherichia coli*. J Appl Biol Chem 62:1–6
25. Kim BG (2019) Optimization of bioactive isorhamnetin 3-*O*-glucoside production in *Escherichia coli*. J Appl Biol Chem 62:361–366
26. Venisety RK, Ciddi V (2003) Application of microbial biotransformation for the new drug discovery using natural drugs as substrates. Curr Pharm Biotechnol 4:153–167
27. Tong WY, Dong X (2009) Microbial biotransformation: recent developments on steroid drugs. Recent Pat Biotechnol 3:141–153
28. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729
29. Lee CK, Jang MY, Park HR, Choo GC, Cho HS, Park SB, Oh KC, An JB, Kim BG (2016) Cloning and characterization of xylanase in cellulolytic *Bacillus* sp. strain JMY1 isolated from forest soil. Appl Biol Chem 59:415–423
30. Yang JK, Zhang JJ, Yu HY, Cheng JW, Miao LH (2014) Community composition and cellulase activity of cellulolytic bacteria from forest soils

planted broad-leaved deciduous and evergreen trees. Appl Microbiol Biotechnol 98:1449–1458
31. Kim JH, Kim BG, Park Y, Ko JH, Lim CE, Lim J, Lim Y, Ahn JH (2007) Characterization of flavonoid 7-*O*-glucosyltransferase from *Arabidopsis thaliana*. Biosci Biotechnol Biochem 70:1471–1477
32. Ablajan K, Abliz Z, Shang XY, He JM, Zhang RP, Shi JG (2006) Structural characterization of flavonol 3,7-di-*O*-glycosides and determination of the glycosylation position by using negative ion electrospray ionization tandem mass spectrometry. J Mass Spectrom 41(3):352–360
33. Douglass CD, Wender HWL, SH, (1949) The isolation of isoquercitrin from the seed pods of *Cercis canadensis*. J Am Chem Soc 71:2658–2659
34. Olennikov DN, Tankhaeva LM, Partilkhaev VV (2013) Chemical investigation of *Caragana arborescens* shoots. Nat Prod Commun 8:585–586
35. Fabani MP, Luna L, Baroni MV, Monferran MV, Ighani M, Tapia A, Wunderlin DA, Feresin GE (2013) Pistachio (*Pistacia vera* var Kerman) from Argentinean cultivars. A natural product with potential to improve human health. J Funct Foods 5:1347–1356
36. Paulke A, Schubert-Zsilavecz M, Wurglics M (2006) Determination of St John's wort flavonoid-metabolites in rat brain through high performance liquid chromatography coupled with fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci 832:109113
37. Kim M, Im S, Cho YK, Choi C, Son Y, Kwon D, Jung YS, Lee YH (2020) Anti-obesity effects of soybean embryo extract and enzymatically-modified isoquercitrin. Biomolecules 10(10):1394
38. Ansari P, Flatt PR, Harriott P, Abdel-Wahab YHA (2020) Anti-hyperglycaemic and insulin releasing effects of *Camellia sinensis* leaves and isolation and characterization of active compounds. Br J Nutr 17:1–35
39. Zhang Y, Peng L, Li W, Dai T, Nie L, Xie J, Ai Y, Li L, Tian Y, Sheng J (2020) Polyphenol extract of *Moringa oleifera* leaves alleviates colonic inflammation in dextran sulfate sodium-treated mice. Evid Based Complement Alternat Med 2020:6295402
40. Won YS, Kim JH, Lizardo RCM, Min HJ, Cho HD, Hong SM, Seo KI (2020) The flavonol isoquercitrin promotes mitochondrial-dependent apoptosis in SK-Mel-2 melanoma cell via the PI3K/AKT/mTOR Pathway. Nutrients 12(12):3683
41. Shui L, Wang W, Xie M, Ye B, Li X, Liu Y, Zheng M (2020) Isoquercitrin induces apoptosis and autophagy in hepatocellular carcinoma cells via AMPK/mTOR/p70S6K signaling pathway. Aging (Albany NY) 12(23):24318–24332
42. Jones JA, Vernacchio VR, Lachance DM (2015) ePathOptimize: A combinatorial approach for transcriptional balancing of metabolic pathways. Sci Rep 5:11301
43. Jeon YH, Seo JE, Kim JH, Choi SW (2021) Quantitative changes of flavonol glycosides from Pine needles by cultivar, harvest season, and thermal process. Prev Nutr Food Sci 26:100–108
44. Matsuura H, Amano M, Kawabata J, Mizutani J (2002) Isolation and measurement of quercetin glucosides in flower buds of Japanese butterbur (*Petasites japonicus* subsp. *gigantea* Kitam.). Biosci Biotechnol Biochem 66:1571–1575
45. Kim KM, Im AR, Lee S, Chae S (2017) Dual protective effects of flavonoids from *Petasites japonicus* against UVB-induced apoptosis mediated via HSF-1 activated heat shock proteins and Nrf2-activated heme oxygenase-1 pathways. Biol Pharm Bull 40:765–773
46. Lee DG, Lee KH, Park KW, Han CK, Ryu BY, Cho EJ, Lee S (2015) Isolation and identification of flavonoids with aldose reductase inhibitory activity from *Petasites japonicus*. Asian J Chem 27:991–994
47. Wang JM, Ma YL, Wu XY, Yu L, Xia R, Sun GX, Wu FA (2012) Selective hydrolysis by commercially available hesperidinase for isoquercitrin production. J Mol Catal B: Enzym 81:37–42

Publisher's Note