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ORIGINAL ARTICLE

Cloning, Prokaryotic Expression, and Enzyme Activity of a UDP-Glucose Flavonoid 3-O-Glycosyltransferase from Mulberry (*Morus alba* L.) Leaves

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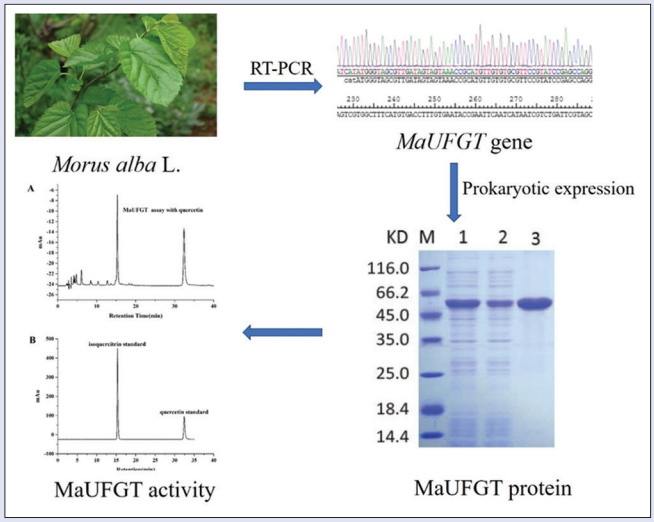
ABSTRACT

Background: Mulberry leaves are traditional Chinese medicines, which have pharmacological activities such as anti-inflammation, anti-oxidation, antitumor, and hypoglycemic. Moreover, flavonol glycosides are one of the main functional ingredients. However, the biosynthetic pathway involved in mulberry flavonol glycosides has not been clear. UDP-glucose flavonoid 3-*O*-glycosyltransferase (UFGT) is a key enzyme in the biosynthesis pathway of flavonol glycosides, which can glycosylate unstable flavonols to form stable flavonol glycosides. **Objectives:** In this article, *MaUFGT*, a cDNA encoding the UFGT from mulberry leaves, was cloned, codon optimized, and expressed in *Escherichia coli* to study its effect *in vitro*. **Materials and Methods:** Mulberry UDP-Glucose flavonoid-3-O-glucanotransferase (*MaUFGT*) gene was reverse transcription-polymerase chain reaction amplified using the cDNA obtained from young leaves of mulberry, and the full-length *MaUFGT* gene was synthesized by codon-optimized whole-gene synthetic method. Then, the plasmid pCzn1/MaUFGT was constructed and heterologously expressed in *E. coli*. After denatured, renatured, and purified, the recombinant protein was used to evaluate its function *in vitro* by determining the final product by high-performance liquid chromatography. **Results:** The target protein was in the range of 45–66 KD and mainly present in the form of inclusion bodies. The obtained protein was found to transfer UDP-glucose glycosyl moieties to the 3-hydroxyl group of quercetin or kaempferol to form the corresponding products *in vitro*. **Conclusion:** The MaUFGT was preliminarily proved to be involved in flavonoid 3-*O*-glucoside biosynthesis.

Key words: Enzyme activity analysis, gene cloning, Mulberry (*Morus alba* L.) leaves, prokaryotic expression, UDP-glucose flavonoid 3-O-glycosyltransferase

SUMMARY

- MaUFGT, a cDNA encoding the UDP-glucose flavonoid 3-*O*-glycosyltransferase (UFGT) from mulberry leaves, was cloned, codon optimized, and expressed in *Escherichia coli*. The obtained target protein was found to catalyze the transfer of UDP-glucose to quercetin or kaempferol to form isoquercitrin or astragalin *in vitro*. The MaUFGT was preliminarily proved to be involved in flavonoid 3-*O*-glucoside biosynthesis.



Abbreviations used: UFGT: UDP-glucose flavonoid 3-*O*-glucosyltransferase; UDPG: Uridine 5′-diphospho-α-D-glucose; RT-PCR: Reverse transcription-polymerase chain reaction; IPTG: Isopropyl-β-D-thiogalactoside; PSPG: Plant secondary product glycosyltransferases; HPLC: High-performance liquid chromatography; ORF: Open reading frame; DHK: Dihydrokaempferol; DHQ: Dihydroquercetin; PAL: Phenylalanine ammonia lyase; C4H: Cinnamic acid-4-hydroxylase; 4CL: 4-coumarate-CoA ligase; CHS: Chalcone synthase; CHI: Chalcone isomerase; F3H: Flavanone 3-hydroxylase; FLS: Flavonol synthase; F3′H: Flavonoid 3′-hydroxylase; PAS: PCR-based Accurate Synthesis.

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INTRODUCTION

Mulberry (*Morus alba* L.) leaves are used as a traditional Chinese medicine. Now, studies have shown that mulberry leaves mainly contain flavonoids,[1-3] alkaloids,[4-7] and phenylpropanoids.[8,9] Flavonoids are one of the main active constituents of mulberry leaves, including rutin, isoquercitrin, astragalin, quercetin-3-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside, and kaempferol-3-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside.[10,11] These flavonol glycosides with quercetin or kaempferol as aglycon have anti-inflammatory, antioxidative, antitumor, hypoglycemic, and other pharmacological activities.[1,12-14]

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The biosynthesis of the flavonol glycosides has been extensively studied,[15-17] and our group had obtained the transcriptome data of *M. alba* L.[18] Based on the results, we proposed the glavonol glycoside biosynthesis pathway in mulberry leaves, which is shown in Figure 1. The biosynthesis of flavonol glycosides begins with phenylalanine, which produces cinnamic acid under the action of phenylalanine ammonia lyase.[15] Cinnamic acid is catalyzed by cinnamic acid-4-hydroxylase and 4-coumarate-CoA ligase to form *p*-coumaroyl-CoA. Subsequently, chalcone synthase catalyzes the condensation of *p*-coumaroyl-CoA and three molecules of malonyl-CoA to produce naringenin chalcone, which is eventually converted into naringenin flavanone with the participation of chalcone isomerase.[19] With the action of flavanone 3-hydroxylas, dihydrokaempferol (DHK) is generated. DHK can also be further hydroxylated by flavonoid 3´-hydroxylase to produce dihydroquercetin (DHQ). DHK and DHQ are catalyzed by flavonol synthase to form kaempferol and quercetin, respectively.[20] Finally, the formation of flavonol glycosides from kaempferol or quercetin is catalyzed by UDP-glucose flavonoid 3-*O*-glucosyltransferase (UFGT). In detail, the kaempferol can be changed into astragalin and kaempferol-3-*O*-(6”-*O*-acetyl)-β-D-glucopyranoside, while quercetin can be glycosylated into isoquercitrin, rutin, and quercetin-3-*O*-(6”-*O*-acetyl)-β-D-glucopyranoside.[21]

UFGT is a very important modification enzyme in plants, which catalyzes the transfer of glucosyl moiety to form stable flavonol glycosides from unstable flavonols.[22-23]

Moreover, UFGT is closely correlated with the biosynthesis and transformation of flavonoids and anthocyanin.[24] As reported by Wei *et al*.,[25] overexpression of UFGT in *Solanum tuberosum* L. significantly increased the color and anthocyanin content of potato tubers. Aza-González *et al*.[26] reported that there was a positive correlation between the accumulation of anthocyanins and the expression of UFGT in pepper fruit during maturation. Our group found that the expression level of UFGT gene in mulberry leaves in different growth seasons was significantly and positively correlated with the content of flavonol glycosides.[27] In summary, UFGT plays an important role in the biosynthesis of flavonoid glycosides. However, the clone and function of the UFGT gene in *M. alba* L. has not been reported in literature. Therefore, it is important to clarify the function of the UFGT gene in mulberry leaves to help in the elucidation of the flavonoid biosynthetic pathway.

In this article, an UFGT gene (*MaUFGT*) was isolated from mulberry leaves by reverse transcription-polymerase chain reaction (RT-PCR) method. We investigated the gene structure and deduced the amino acid composition. Next, the gene was optimized according to the codon preference of *Escherichia coli* for heterologous expression. The recombinant protein synthesis was induced by isopropyl-β-D-thiogalactoside (IPTG) and purified using affinity chromatography. Finally, we established an enzymatic reaction system *in vitro* and identified the reaction products through high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Plant materials

Mulberry leaves were collected from mulberry plantations located in N32°12´13.50´´and E119°30´45.26´´. The top young leaves were surface sterilized with 70% ethanol and rinsed several times by sterile water. Then, these leaves were immediately frozen in liquid nitrogen and stored at −80°C for future use.

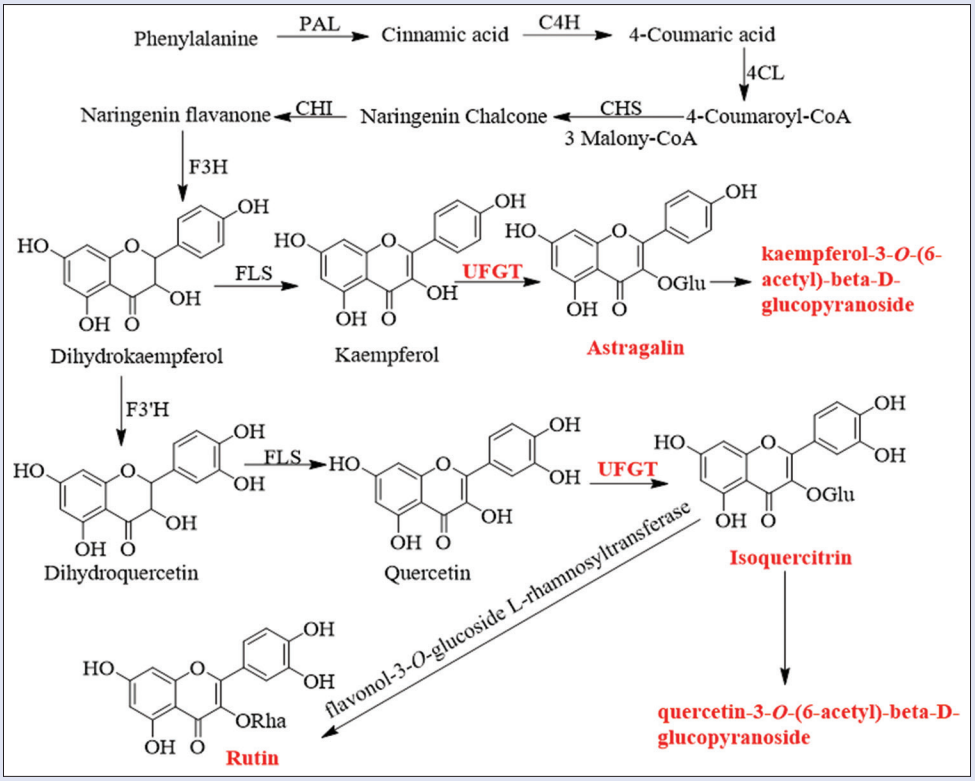

Figure 1: Proposed flavonol glycoside biosynthesis pathway in mulberry (*Morus alba* L.) leaves.[19] PAL: Phenylalanine ammonia lyase; C4H: Cinnamic acid-4-hydroxylase; 4CL: 4-coumarate-CoA ligase; CHS: Chalcone synthase; CHI: Chalcone isomerase; F3H: Flavanone 3-hydroxylase; FLS: Flavonol synthase; F3´: Flavonoid 3´3avonoidaseo; UFGT: UDP-glucose flavonoid 3-*O*-glucosyltransferase

Total RNA extraction and cDNA synthesis

Mulberry leaves were quickly ground into powder using liquid nitrogen. The Trizol reagent (Sangon Biotech, Shanghai, China) was used to extract total RNA. The purity and concentration of total RNA were detected by a nucleic acid protein detector (Biospec-mini230, Shimadzu Corporation, Japan), and total RNA integrity was evaluated with 1% agarose gel electrophoresis. The first strand of cDNA was synthesized using 1 μg of total RNA of mulberry leaves as a template according to the instructions of the RT kit (Thermo Scientific, Lithuania).

MaUFGT cloning

Primer Premier 5.0 software (Premier Biosoft Inc., Canada) was used to design primers UFGT-F and UFGT-R according to mRNA sequence of *Morus notabilis* (accession number XM_010089319) and *M. alba* (accession number KJ616402.1) for the RT-PCR amplification. RT-PCR was carried out using the primers UFGT-F (5'-ATGGGTTCAGTTGATTCAAGCAAAC-3') and UFGT-R (5'-TTAGCATTTATCACCAGACAAGAGAG-3'). The PCR reaction system (TaKaRa Bio, Dalian, China) was as follows (50 μl total volume): 35.5 μL H_2O, 5 μL Green Buffer, 0.5 μL of 10 mmol/L dNTPs, 1.0 μL Tag DNA polymerase, 2 μl of each primer (10 μM), and 4 μL cDNA. PCR procedure: predenaturation at 94°C for 3 min; 94°C for 45 s, 57°C for 45 s, 72°C for 90 s, 35 cycles; 72°C extension for 7 min, and storage at 4°C. The PCR products were analyzed by 1% agarose gel electrophoresis. Then, the target band was recovered using the Axygen's DNA gel recovery kit (TaKaRa Bio, Dalian, China) and ligated to the pMD18-T vector (TaKaRa Bio, Dalian, China) at 4°C overnight. The next day, the ligation product was transformed into *E. coli* DH5α competent cells (Tiangen Biotech Co., Ltd., Beijing, China) to obtain positive colonies using ampicillin resistance and blue-white screening. Positive colonies were randomly selected and verified by PCR, followed by validation via DNA sequencing by Sangon Biotech (Shanghai, China).

Bioinformatics analysis of MaUFGT

The amino acid sequence encoded by *MaUFGT* gene was analyzed by Discovery Studio software (Accelrys, San Diego, California, USA). ExPAS ProtParam tool (http://web.expasy.org/protparam/) predicted protein-relative molecular mass and theoretical isoelectric point. MEGA 6.0 software (Arizona State University, USA) were used to generate a multiple sequence alignment. Eight UDP-glucosyltransferase sequences from different plants were aligned by Clustal W (Stanford University, USA). A phylogenetic tree was performed using the neighbor-joining method with MEGA 6.0 software with 1000 bootstrap replicates.[28]

Codon optimization and synthesis of MaUFGT

Codon_bias tool was used to analyze the codon preference of *MaUFGT*. Under the premise of ensuring the amino acid sequence unchanged, the *MaUFGT* was optimized according to *E. coli* codon preference and synthesized based on the method of PCR-based Accurate Synthesis by Zoonbio (Nanjing, China). The full-length splicing primers were designed, and Nde I and XbaI cleavage sites were designed at both ends of the primers. The primer sequences were as follows:

F: 5'-CAAAGTGCATCATCATCATCATATGGGTAGCGTTGATAGTAGTAAACCGCATGTT-3'

R: 5'-GTGCTTTTAAGCAGAGATTACCTATCTAGATTAACATTTGTCACCACTCAGCAGGGCC-3'.

Heterologous expression in *Escherichia coli*

The PCR amplification product was inserted into the pCzn1 vector (Zoonbio, Nanjing, China) by Nde I and Xba I digestion, and the prokaryotic expression plasmid of pCzn1/MaUFGT was obtained and sequenced. The recombinant plasmid was transformed into *E. coli* Arctic Express™ (DE3) competent cells (Zoonbio, Nanjing, China). Monoclonal colonies on solid LB medium were inoculated into 3 ml of LB liquid medium (containing 50 μg/ml ampicillin) and cultured overnight at 37°C. The next day, the culture was expanded at a ratio of 1:100. A volume of 300 μl of the bacterial solution was inoculated into 30 ml of LB liquid medium containing 50 μg/ml ampicillin until the absorbance value of A_{600} reached 0.6-0.8 at 37°C. IPTG (0.5 mM) (Sigma) was added to induce the expression of *MaUFGT*. After induction at 11°C for 12 h at 220 rpm, the cells were treated and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The recombinant protein existed in the form of inclusions. The cells were resuspended with 20 ml of cell lysis buffer (20 mM Tris-HCl containing 1 mM phenylmethylsulfonyl fluoride and bacterial protease inhibitor, pH 8.0) and then sonicated (400 W, for 5 s, and stop for 8 s, total 20 min), followed by centrifugation at 4°C for 20 min at 10,000 g. Then, the precipitate was washed three times by the mixture consisting of 20 mM Tris, 1 mM ethylenediaminetetraacetic acid, 2 M urea, 1% Triton X-100, and 1M NaCl (pH 8.0) and then dissolved in appropriate amount of lysis buffer (20 mM Tris, 5 mM DTT, 8M urea; pH 8.0) at 4°C overnight. The next day, buffer (20 mM Tris-HCl, 100 mM NaCl; pH 8.0) was added into the above supernatant and the protein solution was loaded into a dialysis bag and dialyzed overnight in a solution of 20 mM Tris-HCl and 100 mM NaCl (pH 8.0).

Purification of the refolded protein was performed using Ni^{2+}-IDA affinity chromatography gel (Novagen, USA) according to the manufacturer's instructions. First, the protein solution was loaded onto the Ni-IDA-Sepharose CL-6B affinity column at a flow rate of 0.5 mL/min. Then, the affinity column was washed in turn with Ni-IDA binding buffer at a flow rate of 0.5 ml/min and Ni-IDA washing buffer (20 mM Tris-HCl, 20 mM imidazole, 0.15 M NaCl, pH 8.0) at a flow rate of 1 ml/min until the OD280 value of the effluent reached the baseline. Next, the target protein was eluted with Ni-IDA elution buffer (20 mM Tris-HCl, 250 mM imidazole, 0.15 M NaCl, pH 8.0) at a flow rate of 1 ml/min. Finally, the effluent was collected for SDS analysis.

Enzymatic assay of *MaUFGT in vitro*

The UFGT activity was evaluated referring to the method of Lister *et al.* [29] and Liang *et al.*[30] with slight adjustment. The assay mixtures comprised 100 μl of 4 mg/ml protein, 200 μl of 50 mM glycine buffer (pH 8.6), 30 μl of 2 mg/ml quercetin, and 30 μl of 15 mg/ml uridine 5'-diphospho-α-D-glucose (UDPG, Toronto Research Chemicals Inc., Canada) in 1.5 ml Eppendorf tubes. After the tubes were incubated at 30°C for 30 min in a water bath, 200 μl of 20% trichloroacetic acid in methanol was added to terminate the reaction. The tubes were centrifuged for 5 min at 10,000 ×*g*, and the supernatant was stored at −80°C until other analysis. HPLC analysis was performed with a Shimadzu HPLC system, equipped with a Kromasil C_{18} column (250 mm × 4.6 mm, 5 μm). The mobile phase was acetonitrile (A)-0.5% formic acid aqueous solution at a flow rate of 1 ml/min with a column temperature of 26°C. The gradient flow was set as follows: 0–10 min, 15%–20% A; 10–30 min, 20%–30% A; and 30–40 min, 30%–50% A. The products were detected at 350 nm, and the standards were purchased from the National Institutes for Food and Drug Control.

RESULTS

Isolation and analysis of *MaUFGT*

The total RNA of mulberry leaves was extracted by Trizol method. As shown in supplementary Figure 1, the 28S, 18S and 5S bands

were clear. And the PCR amplification result of *MaUFGT* gene was shown in supplementary Figure 2. The conformed sequence of *MaUFGT* was submitted to NCBI GenBank with the accession number of MH198038. The sequence concluded a complete open reading frame with a size of 1455 bp, which encoded 484 amino acids. This sequence exhibited 39 bp mission and 99% recognition rate compared with the sequence of *M. notabilis* (XM_010089319) and 99% identity to the flavonoid 3-*O*-glucosyltransferase mRNA from *M. alba* (KJ616402.1). The predicted molecular mass of the protein was 54.491 KD (molecular formula: $C_{2439}H_{3751}N_{639}O_{717}S$) and the isoelectric point was 5.3.

Characterization of deduced MaUFGT protein

The MaUFGT protein showed the highest similarity (95.77%) with UDP-glycosyltransferase 85A1 of *M. notabilis* (EXB29476.1) through BLASTP searching. Multiple alignment of amino acid sequence analysis showed that all these sequences contained a plant secondary product glycosyltransferases (PSPG, 44-amino-acid) motif [the blue line region in Figure 2] in C-terminal region. Moreover, the C-terminus contains a highly conserved region of HCGWNS [red box in Figure 2].

A phylogenetic tree was constructed to explore the evolutionary relationships among *MaUFGT* and other plant glycosyltransferases. As depicted in Figure 3, *MaUFGT* is closest to *M. notabilis*, followed by *Trema orientalis*.

The amino acid sequence of MaUFGT was submitted to the online tool SWISS-MODEL, and its predicted model was constructed using UDP-glucuronosyl/UDP-glucosyltransferase (PBD ID: 2pq6.1. A) as a template [Figure 4]. The protein was found to have a similarity to the UDP glucosyltransferase protein of 55.04%, indicating that the protein belongs to the UDP glucose transferase family.

Heterologous expression in *Escherichia coli*

The plasmid pCzn1-MaUFGT was transformed into competent Arctic Express™ (DE3) cells, and their expression was induced by the addition of 0.5 mM IPTG for 3 h. This resulted in the appearance of a new strip with a molecular weight of approximately 54 KD [Figure 5]. However, as shown in Figure 5, the protein almost existed in the form of inclusions. In order to study the protein function, the target protein was denatured with urea and renatured by dialysis method. When the protein was purified on a Ni-NTA column, 250 mM imidazole could wash away the target strip with large amount and high purity [Figure 6], which could be used for subsequent activity analysis.

In vitro enzyme activity assay of the recombinant MaUFGT protein

The purified recombinant protein was subjected to dialysis for enzyme activity assay *in vitro* using quercetin or kaempferol as substrate in the presence of UDPG. Moreover, the products were detected by HPLC. Isoquercitrin and astragalin were verified in the enzyme reaction solution by comparison with the standards [Figures 7 and 8]. The results showed that MaUFGT could catalyze the production of isoquercitrin from quercetin and the production of astragalin from kaempferol. It was confirmed that the recombinant MaUFGT protein had a certain glycosyltransferase activity.

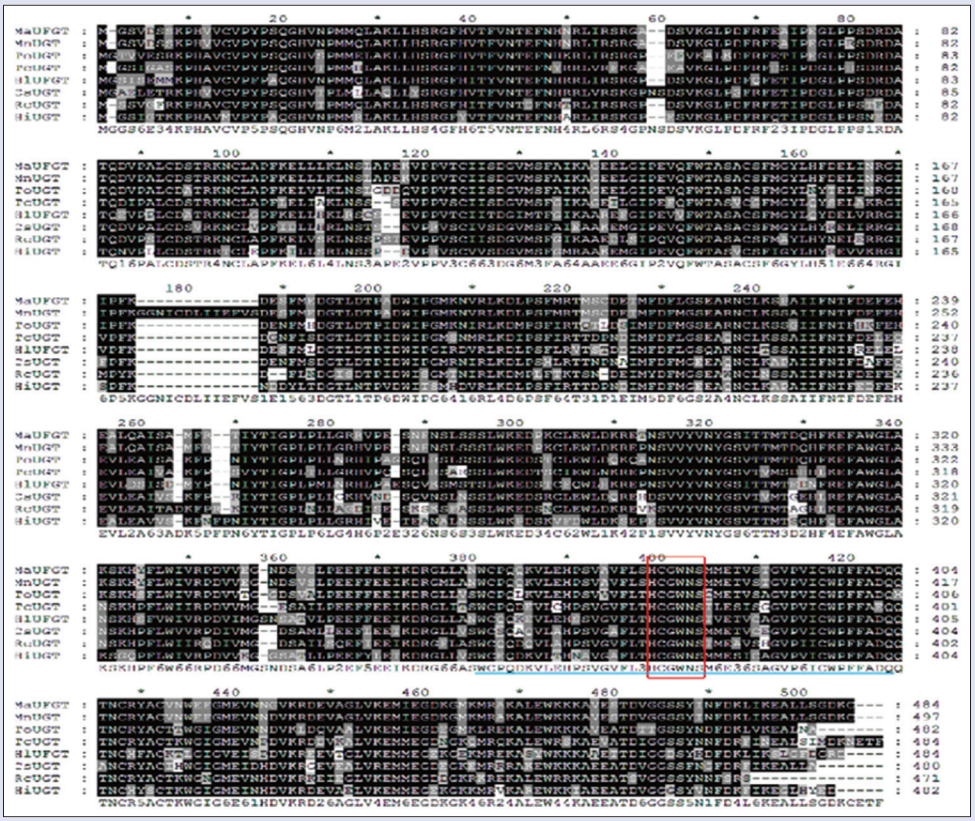

Figure 2: Multiple alignment of amino acid sequences of MaUFGT. The blue line area shows PSPG domain consisting of 44 amino acids and the red frame shows highly conserved residues. The accession numbers are as follows: *Morus alba* (AYO91697.1), *Morus notabilis* (EXB29476.1), *Trema orientalis* (PON90922.1), *Theobroma cacao* (EOX92065.1), *Humulus lupulus* (BAO51840.1), *Camellia sinensis* (BAO51833.1), *Ricinus communis* (EEF29506.1), *Handroanthus impetiginosus* (PIM99761.1)

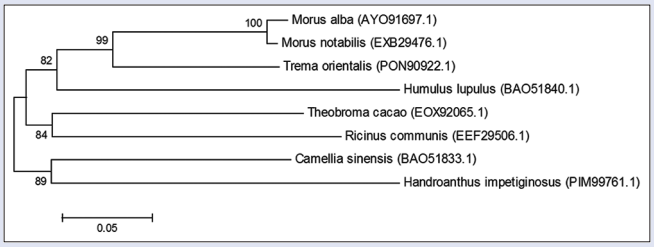

Figure3: Phylogenetic tree of MaUFGT with other plant glycosyltransferases. *Morus alba* (AYO91697.1), *Morus notabilis* (EXB29476.1), *Trema orientalis* (PON90922.1), *Theobroma cacao* (EOX92065.1), *Humulus lupulus* (BAO51840.1), *Camellia sinensis* (BAO51833.1), *Ricinus communis* (EEF29506.1), *Handroanthus impetiginosus* (PIM99761.1)

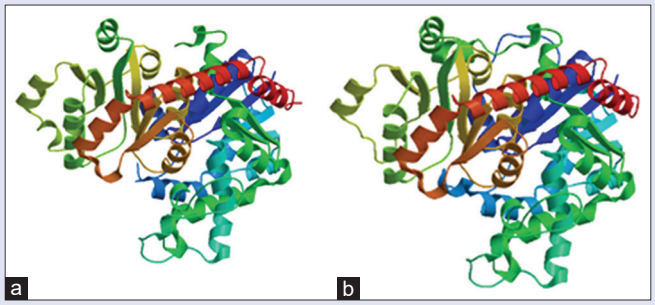

Figure 4: Predicted three-dimensional structure of glycosyltransferase. (a) Three-dimensional structure of UDP-glucuronosyl/UDP-glucosyltransferase (PBD ID: 2pq6.1. A); (b) Three-dimensional structure of MaUFGT

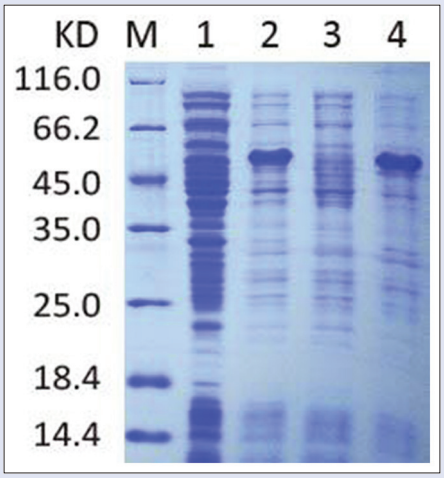

Figure 5: SDS-PAGE of the recombinant MaUFGT protein expression from analysis. M, standard molecular marker; Lane 1, Un-induced pCzn1; Lane 2, Induced pCzn1-MaUFGT; Lane 3, Supernatant of 11°C induction with 0.5 mM IPTG; Lane 4, Precipitate of 11°C induction with 0.5 mM IPTG

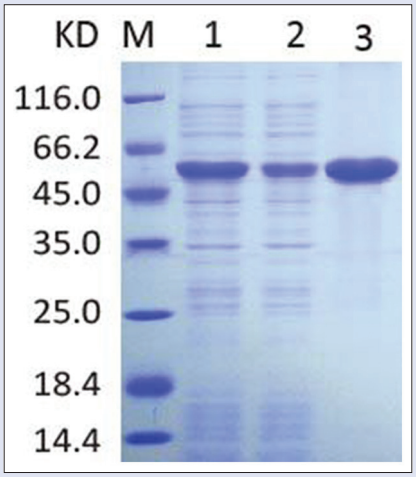

Figure 6: SDS-PAGE analysis of recombinant MaUFGT protein purification. M, standard molecular marker; Lane 1, Un-purified; Lane 2, Flow through; Lane 3, Elution

DISCUSSION

Flavonol glycosides are one of the main functional ingredients in mulberry leaves, which are produced by glycosylation of flavonols. Glycosylation can increase the stability and water solubility of flavonoids,[31] and can also produce a wide variety of flavonol glycosides.[20] UFGT is an enzyme that catalyzes this step of glycosylation in plants. In this study, a UFGT gene was cloned from mulberry leaves. Multiple sequence alignment with other plant glycosyltransferases revealed that the C-terminus of these sequences contained a 44 amino acid PSPG (plant secondary product glycosyltransferases) domain, which was the signature domain of the flavonoid glycosyltransferase and a binding region to a glycosyl donor.[32] The glycosyl donors were UDP-glucose, UDP-galactose, UDP-rhamnose, UDP-xylose, etc.[31] The specificity of the sugar donors was determined by the last amino acid in PSPG domain. If the last amino acid is glutamine (Q or Gln), the glycosyl donor of the enzyme is UDP-glucose. While the last is histidine, the glycosyl donor is UDP-galactose.[31,33] The last amino acid in MaUFGT PSPG domain was Gln, so it was speculated that the glycosyl donor of MaUFGT is UDP-glucose. This can be used to explain why flavonoids in mulberry leaves are present mainly in the form of flavonoid glucosides. Moreover, the C-terminus contains a highly conserved region of HCGWNS [red box in Figure 2], which interacts with UDP-glucose uracil residues.[34] In addition, three-dimensional structure prediction showed that MaUFGT had a 55.04% similarity to the UDP glucosyltransferase. These results indicated that MaUFGT was a member of the UDP glucose transferase family.

Codon optimization can increase the expression level of eukaryotic genes in prokaryotic expression vectors[35,36] and improve the solubility of recombinant proteins.[37-39] In this study, the full-length splicing primers were used to synthesize the *MaUFGT* gene by codon-optimized whole-genome synthesis method and then cloned into the *E. coli* expression vector pCzn1, and the recombinant plasmid pCzn1/MaUFGT was successfully constructed. However, as shown in Figure 5, the recombinant protein was still present in the form of inclusion bodies by SDS-PAGE, which indicated that the codon optimization method in this experiment did not improve the solubility of the recombinant protein. The solubility may be improved by other methods, such as co-expression with molecular chaperones.[40-42] In order to facilitate the determination of enzyme activity, we tried to use different expression vectors, induct at low temperatures, optimize the renaturation conditions, etc., Eventually, we obtained a protein that could be used for the detection of enzyme activity.

The purified recombinant protein was used to evaluate the enzyme activity *in vitro* after dialysis and renaturation.

Because there were all flavonol glycosides with quercetin or kaempferol as aglycon,[11] we selected quercetin or kaempferol as a receptor and

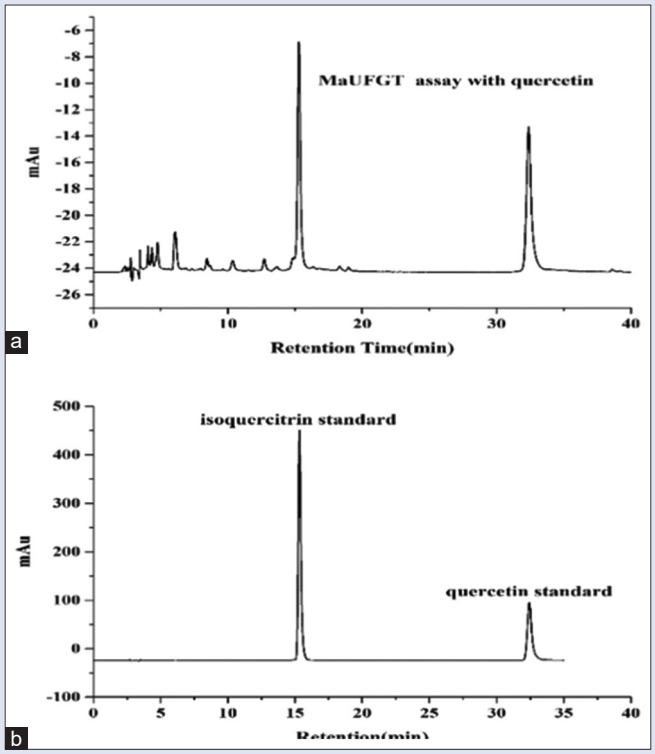

Figure 7: (a) The reaction product of MaUFGT with UDP-Glucose and quercetin. (b) The standards of quercetin and isoquercitin

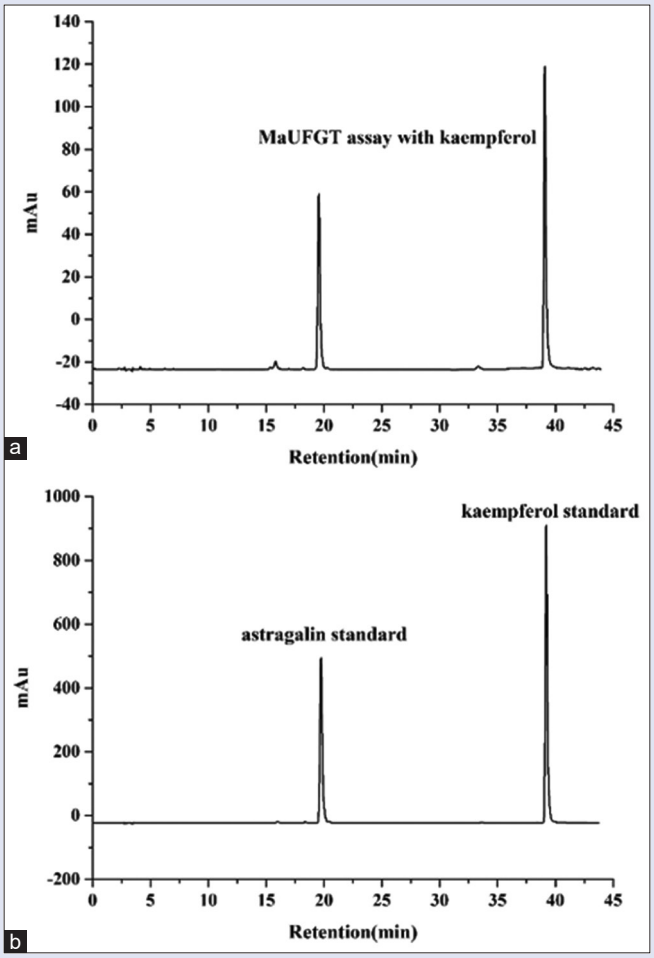

Figure 8: (a) The reaction product of MaUFGT with UDP-Glucose and kaempferol. (b) The standards of kaempferol and astragalin

UDP-glucose as a glycosyl donor to estimate the MaUFGT activity. Isoquercitrin and astragalin were detected in the reaction solution. However, there were no other flavonol glycosides.

It showed that MaUFGT had regioselective, which could only glycosylate the third position of quercetin or kaempferol. This function of the UFGT enzyme has also been confirmed in other plants. For example, Kim *et al.* reported that kaempferol and quercetin were converted into astragalin and isoquercitrin, respectively, catalyzed by the recombinant UFGT from *Oryza sativa*.[43] Liang *et al*. found that UFGT from *M. notabilis* also could catalyze the glycosylation of the third position of quercetin.[30] In addition, some plant UFGTs could also catalyze the glycosylation of anthocyanin.[44] However, whether MaUFGT can catalyze anthocyanins remains to be studied.

CONCLUSION

The full-length splicing primers were designed to synthesize the MaUFGT gene by whole-genome synthesis and then cloned into the expression vector pCzn1. The recombinant plasmid pCzn1/MaUFGT was successfully constructed and expressed in *E. coli*. After the recombinant protein was renatured and purified, the enzyme activity of the recombinant enzyme protein was determined by HPLC. The results showed that the obtained MaUFGT protein can transfer the UDP-glucose glycosyl group to quercetin and kaempferol *in vitro*. The corresponding glycoside was formed on the hydroxyl group, and it was confirmed that MaUFGT was responsible for glycosylation involved in flavonoid biosynthesis of *M. alba* L.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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