การโคลนและจำลองโครงสร้างของไกลโคซิลทรานเฟอเรส Os02g11110 และ Os11g38650 จากข้าว (*Oryza sativa* L.)

Molecular Cloning and Docking of Two Glycosyltransferases, Os02g11110 and Os11g38650, from *Oryza sativa* L.

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บทคัดย่อ

ไกลโคซิลทรานเฟอเรส แฟมิลี่ที่1 (GT1) หรือ UGT ในอาณาจักรพืชเป็นกลุ่มเอนไซม์ขนาดใหญ่ เกี่ยวข้องกับการย้ายหมู่น้ำตาลหรือไกลโคซิเลชั่นจากน้ำตาลนิวคลีโอไทด์ (UDP-sugar) ไปยังหมู่ตัวรับที่มีความจำเพาะกับฟังก์ชันของเอนไซม์สังเคราะห์สารไกลโคไซด์ เพื่อปรับเปลี่ยนคุณสมบัติการละลาย เพิ่มความเสถียร และเปลี่ยนสมบัติทางชีวภาพ อีกทั้งไกลโคไซด์ใช้เป็นยาต้านมะเร็ง ยาโรคหัวใจ และต้านแบคทีเรีย ในปัจจุบันการศึกษาเกี่ยวกับเอนไซม์ในกลุ่มนี้ยังมีน้อย โดยประมาณ 220 ยีนในข้าวยังไม่มีการศึกษา ในงานวิจัยนี้จึงสนใจไกลโคซิลทรานเฟอเรสจากข้าว คือ OsUGT02 จากยีน *Os02g11110* และ OsUGT11 จากยีน *Os11g38650* โดยทำการโคลนลงใน *E.coli* BL21 Star™ (DE3) ผลิตเอนไซม์และทำให้บริสุทธิ์ด้วย His-Tag คอลัมน์ ได้เอนไซม์ในรูปแบบที่ละลาย จากนั้นทำนายโครงสร้างโปรตีนสามมิติด้วยโปรแกรม I-TASSER พบว่า OsUGT02 มีโครงสร้างตรงกับ LOC_Os04g12970.1 (PDB ID: 5tmb)จาก O. sativa ที่ 30% และ OsUGT11 มีโครงสร้างตรงกับ UGT72B1 (PDB ID: 2vg8)จาก *Arabidopsis thaliana* ที่ 50% ใช้โปรแกรม SwissDock ทำนายการจับกับน้ำตาลนิวคลีโอไทด์ด้วย พบว่า UDP-glucose จับที่บริเวณ PSPG motif งานวิจัยนี้ใช้ไปความรู้เบื้องต้นในการนำไปสังเคราะห์ไกลโคไซด์

คำสำคัญ : ไกลโคซิลทรานเฟอเรส แฟมิลี่ที่ 1 น้ำตาลนิวคลีโอไทด์ ไกลโคไซด์ ข้าว

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Abstract

Glycosyltransferase family 1 (GT1) or UGTs are the largest group of enzymes in plant kingdom. UGT transfers sugar moieties from UDP-sugar to specific acceptors for glycoside synthesis via glycosylation to enhance their solubility and stability, and modify biological activities. Glycosides have been used as antitumor drugs, cardiac-related drugs, and antifungal and antibacterial agents. Up to now, an understanding of enzyme characteristics has been limited since a few specific acceptors for UGTs were reported. For example, 220 putative genes of UGT in rice have not been characterized. In this study, two rice (*Oryza sativa* L.) UGTs, namely OSUGT02 (*Os02g11110*) and OsUGT11 (*Os11g38650*), were cloned into *E.coli* BL21 Star™ (DE3), expressed and purified using His-tag column. Protein structure was predicted by I-TASSER. OsUGT02 showed 30% identity with LOC_Os04g12970.1 (PDB ID: 5tmb) from *O. sativa*. OsUGT11 showed 50% identity with UGT72B1 (PDB ID: 2vg8) from *Arabidopsis thaliana*. Molecular docking, which was performed by SwissDock to predict ligand-enzyme binding, showed that UDP-glucose and enzyme complex was formed through PSPG motif.

Keywords: GT1, UGT, glycoside, UDP-glucose, *Oryza sativa*

Introduction

Glycosyltransferase (GT) transfers sugar moieties from activated sugar donor to a specific acceptor for glycoside synthesis via glycosylation, which leads to the structural diversity of secondary metabolites (Schwab, 2003) found in viruses, bacteria and eukaryotes (Breton et al., 2006). GTs have been classified into 101 families primarily based on amino acid sequence similarity (http://www.cazy.org/) (Cantarel *et al*., 2009). Glycosyltransferase family 1 (GT1) is the largest group in the plant kingdom often referred to as UDP glycosyltransferases (UGTs). UGTs have the GT-B fold structure consisting of two β/α/β Rossmann-like domains (Lairson *et al*., 2008). Catalytic site located between N-terminal domain interacts with the substrate whereas C-terminal domain interacts with the sugar donor (Breton *et al*., 2006). UGTs transfer sugar moieties from UDP sugar to acceptor for glycoside synthesis (Fig. 1). Sugar acceptors in plants including all major classes of secondary metabolites are terpenoids, phenylpropanoids, flavonoids and alkaloids (Vogt & Jones, 2000). UGT plays an important role in enhanced water solubility, increased stability, detoxification of xenobiotic and regulation of plants hormone (Yonekura-Sakakibara & Hanada, 2011). Glycosides are very important in therapeutics such as increased apoptosis of cancer (Rascon-Valenzuela *et al*., 2016), antibiotic (Zhou *et al*., 2016), anti-inflammation (Francisco *et al*., 2014).

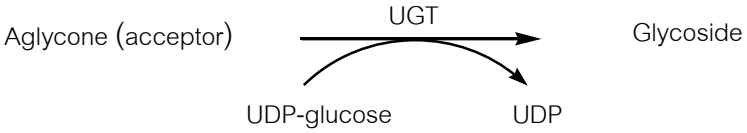


Figure 1 UGT transfers glucose to aglycone for glycoside synthesis

According to CAZY database, GT1 has a total of 4578 UGT genes in eukaryote. Most of the enzymes have not been characterized such as glycosyltransferase in *Brassica napus*, *Cinchona calisaya, Citrullus lanatus, Daucus carota subsp. Sativus, Solanum lycopersicum, Vitis vinifera* and *O sativa.*

O. sativa or rice is one of the world's most biodiversed crop (Ko *et al*., 2008). The characterization of rice GT1 shows that it transfers sugar moieties to flavonoid acceptors such as luteolin, apigenin and kaempferol (Ko *et al*., 2006). Rice contains flavonoid-O Diglucosyltransferase (Kim *et al*., 2009) and flavonoid glucosyltransferase (Hong *et al*., 2007). A total of 280 rice glycosyltransferase genes belongs to family 1 but 220 genes of rice GT1 have not been functionally characterized (http://www.cazy.org/) (Cantarel *et al*., 2009). This study represented recombinant OsUGT11 and OsUGT02 in soluble protein. Predicted interaction between UGTs and UDP-sugar using homology modeling and molecular docking.

Methods

1. Bioinformatic tools

The UGT coding sequences (CDS) were obtained from Rice Phylogenomic database (http://ricephylogenomics.ucdavis.edu/) (Cao *et al*., 2008). *Os02g11110* and *Os11g38650* which were expressed in leaves were selected. The neighbor-joining phylogenetic tree of UGTs from rice and *A. thaliana* was constructed with 1000 bootstrap replicates using MEGA 7.0 software (Kumar *et al*., 2016).

2. Cloning of *Os02g11110* and *Os11g38650*

Leave of one month old *O. sativa* L. 'Khao Dawk Mali 105' were harvested. Total RNA from leaves was isolated by using EZNA® Plant RNA Kit (Omega Bio-tek, USA). The cDNA was synthesized by ReverTra Ace® qPCR RT Kit (Toyobo, Japan). The coding region of glycosyltransferase gene was amplified by PCR using cDNA template at the final concentration of 2 ng/µl. The forward primer and reverse primer were as follow: *Os02g11110* forward primer 5'-CATATGGCAGCTGAGTCCACAGCACAGGC -3' (*Nde*I site underline) *Os02g11110* reverse primer 5'- GAATTCCTATTCCACTCCAGTTTGCGTGAACCTCG -3' (*EcoR*I site underline). *Os11g38650* forward primer 5'-CATATGGAGAACGGCAAGTGCAACG-3' (*Nde*I site underline). *Os11g38650* reverse primer 5'-GAATTCCTA CTTCTCCCCTCCTGTCCACTTG-3' (*EcoR*I site underline). The final

concentration of the pair of primer was 0.4 µM. The PCR was performed with MyFi™ DNA Polymerase (Bioline, UK) under the following conditions: Initial denaturation at 95°C for 1 min, denaturation at 95°C 15 secs, annealing at 60°C for 15 secs, extension at 72°C for 1 min, 30 cycle. The PCR products were ligated into RBC cloning vector (RBC Bioscience, Taipei), transformed to *E.coli* XL-1 blue. White colony was selected. The recombinant plasmid was isolated using QIAprep® Spin Miniprep Kit (qiagen, Germany) and subcloned into pET28b with *Nde*I and *EcoR*I site. *Os02g11110* and *Os11g38650* were inserted into pET28b called pET28b-OS02 and pET28b-OS11, respectively and transform to *E.coli* BL21 Star ™ (DE3) for protein expression.

3. Expression and purification of OsUGT02 and OsUGT11

Transformed cells, which contained plasmid pET28b-OS02 and pET28b-OS11 were grown on LB containing 50 µg/ml kanamycin. A single colony was picked and grown in 5 ml LB medium containing 50 µg/ml kanamycin at 37°C overnight. The culture was inoculated into the AIM medium (1% tryptone, 0.5% yeast extract, 0.33% $(NH_4)_2SO_4$, 0.68% KH_2PO_4, 0.71% Na_2HPO_4, 0.05% glucose, 0.2% lactose, 0.015% $MgSO_4$) containing 50 µg/ml kanamycin and grown at 37°C until OD_{600} reached 0.4-0.6. The condition was adjusted to 30°C 16 hour for expressed protein by lactose induction. The cell pellet was harvested by centrifugation at 4,863 x g for 10 min. Cell pellet was lysed by sonication prior to supernatant collection using centrifugation. Recombinant OsUGT02 and OsUGT11 were purified by Histrap FF column (1 ml) (His-Tag column) (GE Healthcare, United States) and analyzed by SDS-PAGE. Protein concentration was determined by NanoDrop™ 2000 UV-Vis Spectrophotometers 280 nm

4. Molecular modeling and docking

Amino acid sequences of OsUGT02 and OsUGT11 were submitted to the I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) (Zhang, 2008) to generate predicted model structures. The interaction with UDP-sugar was docked by SwissDock (http://www.swissdock.ch/) (Grosdidier *et al*., 2011) and analyzed by Chimera and Pymol.

Results and discussion

1. Gene analysis

Os02g11110 and *Os11g38650* gene which were expressed in leaves were selected from the Rice Phylogenomics Database (Cao *et al*., 2008). OsUGT02 has 44% amino acid similarity with OsUGT11. Phylogenetic tree of GT1 were divided into four groups. OsUGT02 and OsUGT11 were classified to group III contain phenylpropanoid glycosyltransferase (UGT71E1, UGT71C1) (Lim *et al*., 2005; Bowles, 2002) and group IV contain flavonoid glycosyltranasferase (LOC_Os01g41430.1, UGT73B2) (Ko *et al*., 2006; Kim *et al*., 2010) respectively (Fig. 2). OsUGT02 was closed to LOC_Os01g41430.1, a flavonoid glycosyltransferase, which

transferred glucose moieties from UDP-glucose to flavonoids acceptor for flavonoids glucoside synthesis (Ko *et al*., 2006). OsUGT11 was closed to LOC_Os02g14680.1.

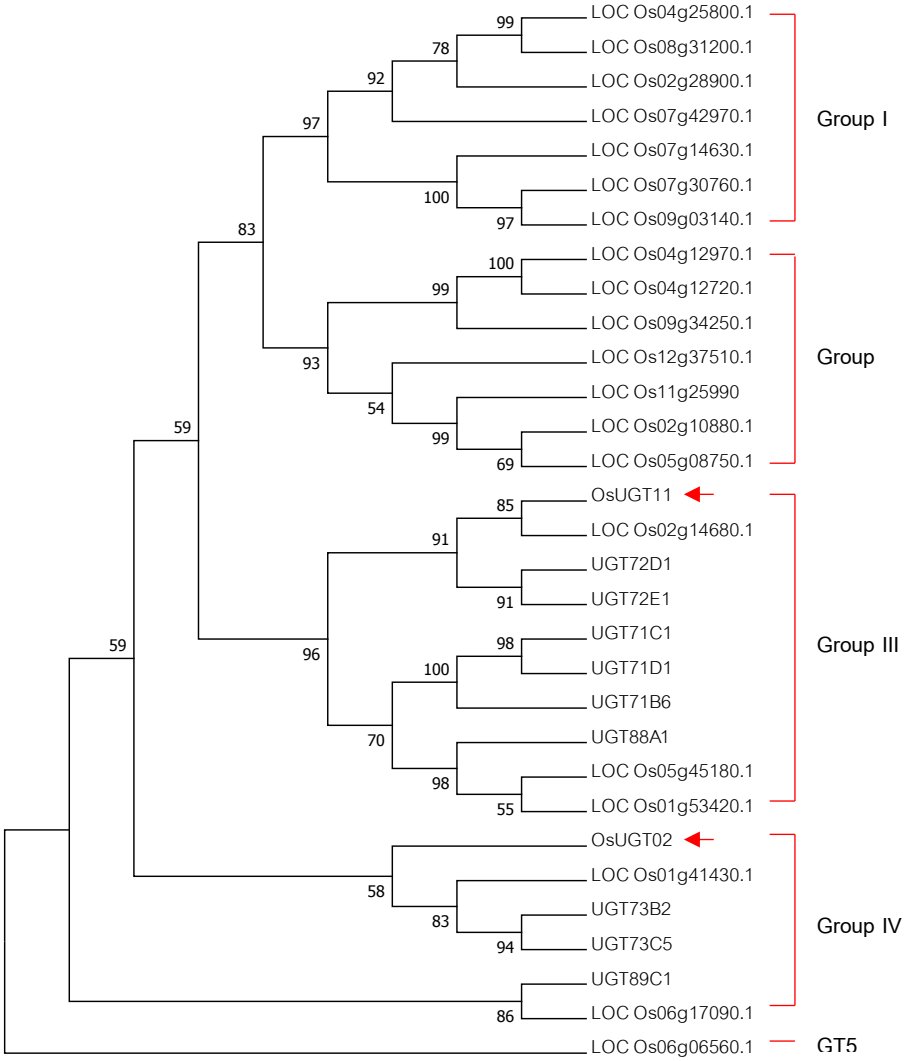


Figure 2 Phylogenetic analysis of UGTs from rice and Arabidopsis constructed by MEGA 7.0. Arrows indicate OsUGT02 and OsUGT11 in group I. The number at the node was indicated the bootstrap values on neighbor joining analysis (GT5, glycosyltransferase family 5)

2. Cloning and expression of OsUGT02 and OsUGT11

Os02g11110 and *Os11g38650* consist of 1476 bp (492 aa) and 1473 bp (491 aa) respectively. Molecular weight of OsUGT02 and OsUGT11 were predicted from deduced amino acid at 53 kDa and 52.5 kDa respectively. Both UGTs were expressed under T7 promoter (pET28b). Six histidine Tag was add to C-terminal for purification process. One step purification was done by eluting His-Tag column with 500 mM imidazole. From SDS-PAGE, first fraction (Fig. 3A and 3B, lane 4) showed the major bands approximately 60 kDa of

OsUGT02 and OsUGT11, (Fig. 3A and 3B). The molecular weight of purified OsUGT02 and OsUGT11 were corresponded to molecular weight values from deduced amino acids. Purified recombinant OsUGT02 and OsUGT11 were collected from His-Tag column at 13 mg and 5 mg per liter of media cultures, respectively.

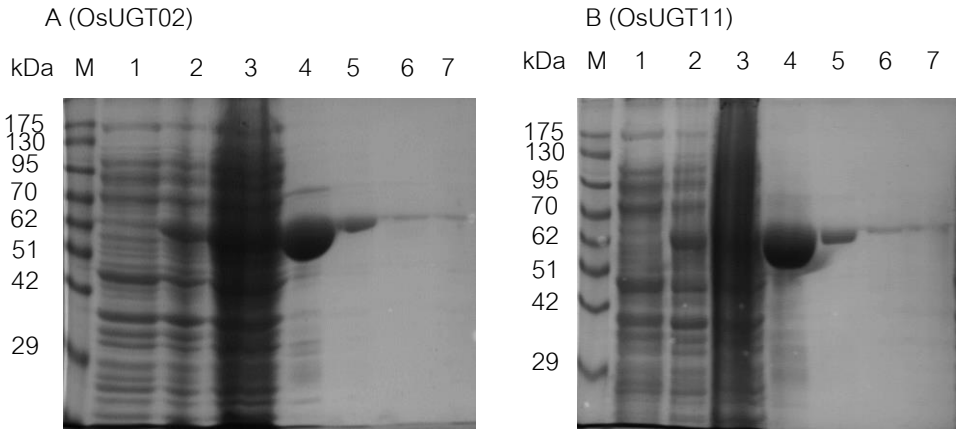
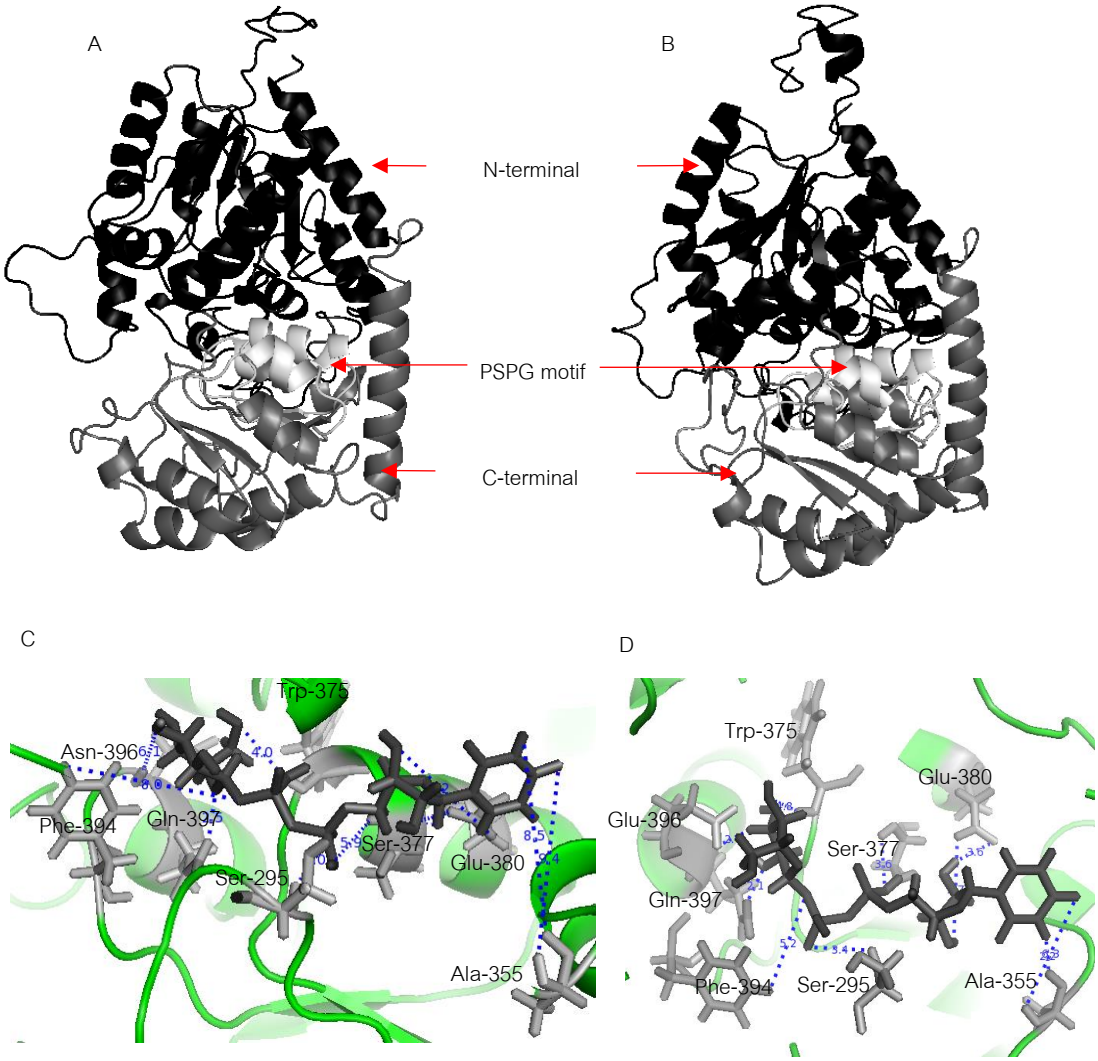


Figure 3 Expression of recombinant GT1 on 10% SDS-PAGE. M = PiNK Plus Prestained Protein Ladder, 1 = non-induce *E.coli* cell 2 = induced *E.coli* cell, 3 = soluble crude enzyme, 4-7 = purified recombinant enzyme

3. Molecular modeling and molecular docking

Structure of OsUGT02 and OsUGT11 was modeled by I-TASSER. OsUGT02 has 30% structural identity with LOC_Os04g12970.1 from *O. sativa* L. (PDB ID: 5tmb). OsUGT11 has 50% structural identity with UGT72B1 from *A. thaliana* (PDB ID: 2vg8). The enzyme interacts with UDP-sugar through UGT signature PSPG (Plant Secondary Product Glycosyltransferase) motif which is a conserve region located on C-terminal domain (Fig. 4A 4B and 4E) (Zhang *et al*., 2016). The amino acids interacting with UDP-sugar were indicated in the box (Fig. 4E). The interaction between UGT71G1 and UDP-glucose was reported in the previous study (Shao *et al*., 2005). The residues A355, E380, S377, Y394 and S295 interacted with the UDP. The residues W375, N396 Q397 of OsUGT02 and W375, E396 and Q397 of OsUGT11 interacted with the sugar (Table 1) (Shao *et al*., 2005). The interaction of OsUGT02 and OsUGT11 complexes with UDP-glucose were predicted by Swissdock (Fig 4C and 4D). Until now, the crystal structures of OsUGT02 and OsUGT11 have not been reported. We used predicted structure to docking with UDP-glucose, therefore the hydrogen bond distance of rice UGTs more error than X-ray crystal structure. However the amino acids in PSPG motif were predicted to interact with UDP-glucose (Fig.4C 4D and Table 1) is in agreement with the previous report (Shao *et al*., 2005). Gibbs free energy (ΔG) of the docking was favorable to binding model based on cluster formation (Table 2). Base on the results of docking studies, UDP-glucose showed most favorable binding with OsUGT02 and OsUGT11. Therefore, OsUGT02 and OsUGT11 could activate glucoside synthesis by using UDP-glucose as a donor according to molecular docking.



E

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                                                295       305       315       325
OsUGT02       ASTDQRAITAWLDKQATCSVVYVGFGSVLR-KLPKHLSEVGHGLEDSGKPFLWVVKESEA
UGT84A1       ICKSTDKCLEWLDSRPKSSVVYISFGTVAY-LKQEQIEEIAHGVLKSGLSFLWVIRPPPH
UGT71G1       DQAQHDLILKWLDEQPDKSVVFLCFGSMGVSFGPSQIREIALGLKHSGVRFLWSNSAEK-
OsUGT11       PPSPRAACLDWLDRQPDRSVIFVSFGSGGA-LPTEHMRELALGLELSGQRFLWVVRSPSD
UGT72B1       KQSEESECLKWLDNQPLGSVLYVSFGSGGT-LTCKQLNELALGLADSEQRFLWVIRSPSG
                       *** :   **::: **:        .:: *:. *:  *   ***

                                335       345       355       365
OsUGT02       SSRPEV------------QEWLDEFMARTATRG-LVVRGWAPQVTILSHHAVGGFLTHCG
UGT84A1       DLKVET------------HVLPQE-LKESSAKGKGMIVDWCPQEQVLSHPSVACFVTHCG
UGT71G1       ------------------KVFPEGFLEWMELEGKGMICGWAPQVEVLAHKAIGGFVSHCG
OsUGT11       EGEVSANYYDAETKKNPFGYLPEGFVERTKEVG-LLVPSWAPQTKVLAHRATGGFLTHCG
UGT72B1       --VANSSYFDSHSQTDPLTFLPPGFLERTKKRG-FVIPFWAPQAQVLAHPSTGGFLTHCG
                                :       *  ::  *.**  :*:* : . *::***

                         375       385       395
OsUGT02       WNSLLEAIARGVPVATWPHFADQFLNERLAVDVLGVGVPIGVTAPVSMLNEEYLTVDRGD
UGT84A1       WNSTMESLSSGVPVVCCPQWGDQVTDAVYLIDVFKTGVRLGRGAT-----E-ERVVPREE
UGT71G1       WNSILESMWFGVPILTWPIYAEQQLNAFRLVKEWGVGLGLRVDYR-----KGSDVVAAEE
OsUGT11       WNSVLESLVHGVPMVAWPLFAEQRQNAVMLTEGAGAAIRVPESK------------GKEK
UGT72B1       WNSTLESVVSGIPLIAWPLYAEQKMNAVLLSEDIRAALRPRAGD--------DGLVRREE
              *** :*::  *:*:   * :.:*  :     .   ..:                    .
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Figure 4 (A) Structure of OsUGT02 (B) Structure of OsUGT11 reveals N-terminal domain, C-terminal domain and PSPG motif. (C) OsUGT02 interacted with UDP-glucose (D) OsUGT11 interacted with UDP-glucose (E) Highlight is indicate PSPG motif and amino acid as interacted with UDP-glucose are indicated in box

Table 1 Interaction between UGT71G1 and Rice UGTs with UDP-glucose

Amino acid	Amino acid atom	UDP-glucose	UDP-glucose atom	Distance (Å)		
				UGT71G1 (Shao et al., 2005)	OsUGT02 (This study)	OsUGT11 (This study)
Ala-355	NH	uracil ring	OH	2.8	9.4	6.3
	OH		NH	2.6	8.5	2.2
Glu-380	O1	ribose	O2	2.7	6.6	5.7
	O2		O3	2.9	8.2	3.6
Ser-377	NH	alpha phosphate	O2	3.1	5.1	3.6
Tyr-394 (UGT71G1) Phe-394 (Rice UGTs)	OH	beta phosphate	O3	3.3	8.0	5.2
Ser-295	NH		O2	3.4	5.4	3.4
Trp-375	NH	sugar	O4	2.9	6.0	4.8
Glu-396 (OsUGT11) Asp-396 (OsUGT02)	H		O3	2.4	6.1	2.7
Gln-397	O1		O2	2.7	2.5	2.1

Table 2 Gibbs free energy (ΔG) of molecular docking

Enzyme	Type of UDP-sugar	ΔG (kcal/mol)
OsUGT02	UDP-glucose	-8.388371
	UDP-galactose	-6.980763
	UDP-xylose	-7.0408783
OsUGT11	UDP-glucose	-11.262657
	UDP-galactose	-5.924764
	UDP-xylose	-5.924764

Conclusions

Glycosyltransferase genes were amplified by PCR using specific primer for *Os02g11110* and *Os11g38650*. Both genes were transformed to *E.coli* BL21 Star™(DE3) and expressed. OsUGT02 and OsUGT11 were purified using His-Tag column. OsUGT02 and OsUGT11 are soluble protein having molecular weight 60 kDa on SDS-PAGE. Structure of OsUGT02 and OsUGT11 were predicted by I-TASSER. OsUGT02 has 30% structural identity with LOC_Os04g12970.1 from *O. sativa* (PDB ID: 5tmb). OsUGT11 has 50% structural identity with UGT72B1 from *A. thaliana* (PDB ID: 2vg8). The amino acids in PSPG motif at C-terminal domain contact with UDP-sugar through hydrogen bond according to the previous report. UDP-glucose is a better sugar donor of OsUGT02 and OsUGT11 than UDP-galactose and UDP-xylose.

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