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Glycoside hydrolase family 39 β-xylosidases exhibit β-1,2-xylosidase activity for transformation of notoginsenosides: a new EC subsubclass

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Running title: GH 39 β-1,2-xylosidases

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ABSTRACT: β-1,2-Xylosidase activity has not been recorded as an EC subsubclass. In this study, phylogenetic analysis and multiple sequence alignments revealed that characterized β-xylosidases of glycoside hydrolase family (GH) 39 were classified into the same subgroup with conserved amino acid residue positions participating in substrate recognition. Protein-ligand docking revealed that seven of these positions were probably essential to bind xylose-glucose, which is linked by a β-1,2-glycosidic bond. Amino acid residues in five of the seven positions are invariant, while those in two of the seven positions are variable with low frequency. Both the wild-type β-xylosidase rJB13GH39 and its mutants with mutation at the two positions exhibited β-1,2-xylosidase activity as they hydrolyzed *o*-nitrophenyl-β-D-xylopyranoside and transformed notoginsenosides R_1 and R_2 to ginsenosides Rg_1 and Rh_1, respectively. The results suggest that all these characterized GH 39 β-xylosidases probably show β-1,2-xylosidase activity, which should be assigned an EC number with these β-xylosidases as representatives.

KEYWORDS: *β-xylosidase*; *β-1,2-glycosidic bond*; *enzyme commission*; *notoginsenosides*; *glycoside hydrolase family 39*

INTRODUCTION

Xylans, abundant in many agricultural biomasses, algae, and industrial wastes, are polymerized mainly by xylose with β-1,4-glycosidic or β-1,3-glycosidic linkages.[1–3] The role of β-1,4-xylosidases and β-1,3-xylosidases is to produce xylose from xylans after degradation by endo-xylanases. Engineered *Saccharomyces cerevisiae* can utilize xylose to produce ethanol, xylitol, lactic acid, and other chemicals.[4] Therefore, β-xylosidases are of great importance in the food, beverage, bioenergy, feed, and pharmaceutical industries.[5]

In addition to xylans, some saponins contain xylosyl moieties at their terminals. *Panax notoginseng*, well known as "Sanqi" in Chinese, is recorded in the Compendium of Materia Medica (Bencao Gangmu) and traditionally used for hemostasis. In China, the plant is primarily cultivated in Yunnan and Guangxi provinces. Both notoginsenosides R_1 and R_2 contain a xylosyl group linked by a β-1,2-glycosidic bond with a glucosyl group (Figure 1).

Removal of the xylosyl group from notoginsenosides R_1 and R_2 yields ginsenosides Rg_1 and Rh_1, respectively, which possess anticancer, antioxidant, and anti-inflammatory activities. Recently, Shin et al.[6] reported that the β-xylosidase (TtGH39) from *Thermoanaerobacterium thermosaccharolyticum* was the first β-xylosidase exhibiting β-1,4-xylosidase activity as well as the ability to remove the xylosyl group from notoginsenosides R_1 and R_2. Li et al.[7] also reported that the β-xylosidase (Xln-DT) from *Dictyoglomus thermophilum* could efficiently degrade xylobiose and remove the xylosyl group from notoginsenoside R_1. Both TtGH39 and

Xln-DT are members of the glycoside hydrolase family (GH) 39. Shin et al.[6] and Li et al.[7] focused on the bioconversion of saponins, but they did not stress the key point that the two β-xylosidases had the ability to hydrolyze β-1,2-glycosidic bonds.

To date, β-1,2-xylosidase or notoginsenoside xylohydrolase activity has not been assigned an EC number (http://www.enzyme-database.org). Most β-xylosidases are classified as EC 3.2.1.37 as they catalyze the hydrolysis of β-1,4-linked xylosyl moieties from xylo-oligosaccharides. β-1,3-Xylosidases are classified as EC 3.2.1.72, but seldom reported.[2,3] To determine β-1,2-xylosidase activity, both notoginsenosides and *o*-nitrophenyl-β-D-xylopyranoside are suitable substrates. For example, EC 3.2.1.191–195 show five types of ginsenoside glucohydrolases using various ginsenosides as substrates.

This study aimed to determine whether other GH 39 β-xylosidases, like TtGH39 and Xln-DT, had the ability to hydrolyze β-1,2-glycosidic bonds for transformation of notoginsenosides and further proposed that GH 39 β-xylosidases could be representatives of the new EC subsubclass, β-1,2-xylosidases or notoginsenoside xylohydrolases.

MATERIALS AND METHODS

Sequence, Strains, and Materials. Previously, we obtained a GH 39 β-xylosidase (JB13GH39) from *Sphingomonas* sp. JB13.[8] The sequence of JB13GH39 can be retrieved from the GenBank database (accession No. MG838204). *Escherichia coli* BL21 (DE3) was purchased from TransGen (Beijing, China). *p*-Nitrophenol (*p*NP)

and *p*-nitrophenyl-β-D-xylopyranoside (*p*NPXyl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Notoginsenosides R_1 and R_2, ginsenosides Rg_1 and Rh_1, and *o*-nitrophenyl-β-D-xylopyranoside (*o*NPXyl) were purchased from YuanYe Bio-Technology (Shanghai, China). Mut Express® II Fast Mutagenesis Kit V2 was purchased from Vazyme Biotech (Nanjing, China).

Enzyme Activity toward *o*NPXyl. Recombinant JB13GH39 (rJB13GH39) was expressed in *E. coli* BL21 (DE3) cells using the *pEASY*-E2 vector and purified by Ni^{2+}-NTA affinity chromatography. The details of enzyme preparation were described in our previous study.[8]

o-Nitrophenol is not available to us because of its danger. Therefore, the *p*NP method was used as described previously[8] to evaluate the activity of rJB13GH39 toward *o*NPXyl. One unit of β-1,2-xylosidase activity was defined as the amount of enzyme that releases 1 μmol of *p*NP per minute at pH 4.5 and 50 °C.

Enzymatic Transformation of Notoginsenosides R_1 and R_2. The 400 μL (pH 4.5) reaction included 10 μg of rJB13GH39 and 4 mM substrate. The mixture was incubated in a water bath at 30 °C for 24 h. The products were qualitatively detected by electrospray ionization mass spectrometry (ESI-MS) before quantitative analysis. ESI-MS was carried out in positive mode on a quadrupole time-of-flight high-resolution mass spectrometer (6540 series, Agilent Technologies, Santa Clara, CA, USA) with the following parameters: fragmentor, 135 V; capillary, 3500 V;

skimmer, 65 V; gas (nitrogen) temperature, 350 °C; drying gas, 8 L/min; and nebulizing gas, 30 psi. For quantitative analysis, the products from notoginsenosides R_1 and R_2 were prepared as previously described[6] and analyzed by ultra-high-performance liquid chromatography (UHPLC) at 203 nm with a Hypersil ODS column (4.0 mm × 250 mm, 5 μm; Agilent Technologies). The column was eluted at room temperature with 20:80 (v/v) acetonitrile/water at 1.0 mL/min for R_1 and Rg_1 and with 20:80 to 55:45 (v/v) acetonitrile/water for 60 min at 1.5 mL/min for R_2 and Rh_1.

Phylogenetic Analysis. Phylogenetic analysis of GH 39 members was performed by MEGA using the built-in Jones–Taylor–Thornton model for distance matrices calculation and neighbor-joining algorithm for phylogenetic tree construction with 1000 bootstrap replications.[9] A total of 14 identified GH 39 β-xylosidases were selected for phylogenetic analysis: TtGH39 and Xln-DT showing both β-1,4-xylosidase and β-1,2-xylosidase activities for transformation of notoginsenosides;[6,7] XynB_thesa (accession no. AAA27369 or 1UHV) from *Thermoanaerobacterium saccharolyticum* showing both β-1,4-xylosidase and β-1,2-xylosidase activities using *p*NPXyl and *o*NPXyl as substrates;[10,11] XynA (AAB87373) and XynB (AAA23063) from *Caldicellulosiruptor saccharolyticus* as well as BxyH (AEE47384) from *Cellulomonas fimi* showing β-1,2-xylosidase activity using *o*NPXyl as substrate;[12,13] XynB1 (ABI49941 or 2BS9) from *Geobacillus stearothermophilus* having solved crystal structure with molecule

2,5-dinitrophenyl-β-xyloside as ligand;[14] JB13GH39,[8] SlpA (AFK86459) from *T. saccharolyticum*,[15] XylBH39 (BAB04787) from *Bacillus halodurans*,[16] WSUCF1 (EPR27580) from *Geobacillus* sp.,[17] Bxl39A (CAD48308) from *Clostridium stercorarium*,[18] Coxyl A (ADQ03734) from *Caldicellulosiruptor owensensis*,[19] and CcXynB2 (AAK24328 or 4EKJ) from *Caulobacter crescentus*[20,21] showing β-1,4-xylosidase activity.

Sequence Analysis. To illuminate patterns of amino acid conservation, the multiple sequence alignment of the 14 identified GH 39 β-xylosidases were uploaded to the Weblogo server[22] to generate sequence logos.

Protein Structure Modeling. The protein homology model of JB13GH39 was built using SwissModel (http://swissmodel.expasy.org/), followed by quality estimation with GMQE, QMEAN Z-score, and a local quality plot.

Protein-ligand Docking. The ligand, designated as xylose-glucose, was a compound linked by a β-1,2-glycosidic bond. Xylose-glucose was retrieved from the PubChem database with the accessing number CID 3083150. Docking sites of JB13GH39 were predicted using the Dock Ligands (CDOCKER) protocol by Discovery Studio v2.5 software (Accelrys, San Diego, CA, USA).

Site-directed Mutagenesis. Site-directed mutagenesis was used to substitute

Tyr257 and Tyr311 of JB13GH39 with Thr and Pro, designated as mutants Y257(Ps266)T and Y311(Ps322)P, respectively. The mutation was introduced using Mut Express® II Fast Mutagenesis Kit V2 according to the manufacturer's instructions. Two primer sets were designed: ACAGCaccGGCGTCGATGGCGGCTTTCTCGAC and ATCGACGCCggtGCTGTGCGTGGTGACGAAAT for mutant Y257(Ps266)T and ATGGAGCACCAGCccaACGCCGCGCGATGCCGTG and TtggGCTGGTGCTCCATTCGGTGAAATAGAGT for mutant Y311(Ps322)P. Mutated nucleic acids were confirmed by DNA sequencing (Tsingke, Beijing, China). Expression and purification of the mutated enzymes were performed as those of rJB13GH39.

Activity of Mutants. The *p*NP method was used as described previously[8] to determine the activity of the mutants toward *p*NPXyl. One unit of β-1,4-xylosidase activity was defined as the amount of enzyme that releases 1 μmol of *p*NP per minute at pH 4.5 and 50 °C. β-1,2-Xylosidase activity of the mutants toward *o*NPXyl and notoginsenosides was detected by *p*NP method and UHPLC, respectively, as that of rJB13GH39.

RESULTS AND DISCUSSION

β-1,2-Xylosidase Activity of rJB13GH39. rJB13GH39 was successfully expressed and purified as previously described.[8] The specific activity of rJB13GH39

toward *o*NPXyl was 31.7 ± 1.2 U/mg. The ESI-MS spectra indicated that notoginsenosides R_1 and R_2 were converted by the activity of purified rJB13GH39 to ginsenosides Rg_1 and Rh_1, respectively (Figure S1). Xylose was also observed in those products (Figure S1). As shown in the UHPLC chromatograms, 100.0 % of notoginsenoside R_1 and 90.3 % of notoginsenoside R_2 were individually hydrolyzed (Figures 2A, 3A).

β-Xylosidases have been found in 11 families, including GH 1, 3, 5, 30, 39, 43, 51, 52, 54, 116, and 120.[5,23] Why are β-xylosidases so diverse? What are the differences between these β-xylosidases? One explanation may be that β-xylosidases from each glycoside hydrolase family have specific activity toward certain xylose-containing substances. Previous studies have shown that the xylosyl moiety of notoginsenosides R_1 and R_2 can be removed by GH 39 β-xylosidases TtGH39 and Xln-DT.[6,7] This study confirmed that GH 39 β-xylosidase rJB13GH39 had the ability to degrade notoginsenosides R_1 and R_2. Furthermore, Li et al.[7] reported that three GH 3 β-xylosidases, Tth XyB3, Tpe Xln3, and XlnD, as well as one GH 120 β-xylosidase, Tth Xyl, were unable to degrade notoginsenoside R_1. Additionally, we found that the GH 43 β-xylosidase HJ14GH43 did not function as a β-1,2-xylosidase (data not shown). Therefore, the functional signature of GH 39 β-xylosidases may be their ability to cleave the β-xylose-(1→2)-β-glucose bond. However, the function is not exclusive to GH 39 β-xylosidases, as the GH 1 β-glucosidase from *Pyrococcus furiosus* has β-xylosidase activity and can also convert notoginsenosides R_1 and R_2 to ginsenosides Rg_1 and Rh_1, respectively.[24]

188 Transformation of notoginsenosides R_1 and R_2 by GH 39 β-xylosidases seems to
189 be highly efficient. Notoginsenoside R_1 is converted into ginsenoside Rg_1 by GH 39
190 β-xylosidase Xln-DT after 30 min with a corresponding molar conversion yield of 100
191 % in total.[7] GH 39 β-xylosidase TtGH39 completely converts notoginsenosides R_1
192 and R_2 to ginsenosides Rg_1 and Rh_1 after 4 and 18 h, respectively.[6] Greater than 90 %
193 of notoginsenosides R_1 and R_2 were hydrolyzed when the reactions occurred at 30 °C
194 for 24 h using rJB13GH39.
195
196 **Sequence and Phylogenetic Analyses.** Previously, GH 39 enzymes were
197 regarded as α-L-iduronidases or β-xylosidases. According to the previous
198 phylogenetic analysis performed by Ali-Ahmad et al.,[25] the GH 39 family has four
199 subgroups. In this study, GH 39 enzymes were also classified into 4 subgroups
200 (Figure 4). The results suggest that GH 39 enzymes should show at least 4 types of
201 activity. As shown in Figure 4, subgroup I includes two α-L-iduronidases from *Homo*
202 *sapiens*[26] and *Mus musculus*.[27] NF2152, in subgroup III from the rumen anaerobic
203 fungus *Neocallimastix frontalis*, was reported to release β-1,2-arabinobiose from
204 sugar beet arabinan and both β-1,2-arabinobiose and α-1,2-galactoarabinose from rye
205 arabinoxylan.[28] The GH 39 member in subgroup IV, PslG from *Pseudomonas*
206 *aeruginosa* found in chronic leg ulcers, was found to disrupt biofilm matrix
207 exopolysaccharide in bacteria.[29] GH39wh2 from the human gut bacteria *Bacteroides*
208 *cellulosilyticus* was classified in subgroup IV and predicted to harbor

endo-glycosidase activity.[25] TtGH39, Xln-DT, and JB13GH39, as well as other characterized β-xylosidases, were classified into subgroup II.

On the basis of the multiple sequence alignment of the 14 identified GH 39 β-xylosidases in subgroup II, conserved amino acid residue positions are shown in Figure 5. According to the alignment and the three GH 39 β-xylosidases XynB_thesa,[10,11] XynB1,[14] and CcXynB2[20,21] that have crystal structure data, Glu in position (Ps) 317 corresponds to the catalytic nucleophile, Glu in Ps195 corresponds to the general acid/base residue, and His in Ps88, Phe in Ps150, Asn in Ps194, Phe in Ps201, His in Ps264, Tyr in Ps266, Tyr in Ps322, Trp in Ps356, Phe in Ps362, Glu in Ps364, and Phe in Ps376 correspond to substrate recognition.

Protein-ligand Docking. To uncover which amino acid residue positions are essential for β-1,2-xylosidase activity, protein-ligand docking was performed.

The homology model of JB13GH39 was built previously with CcXynB2 as a template.[8] The quality of the model was high, taking the following values into consideration: 66.5 % sequence identity, 0.78 GMQE score, -1.49 QMEAN Z-score, and fewer than 4 residues (0.8 % of the total residues) showing a score below 0.6 in the local quality plot.[8]

The xylose-glucose ligand successfully docked with the catalytic pocket of JB13GH39 with 91 poses (Figure 6). Each pose was analyzed to show the hydrogen bond interactions of amino acid residues with the ligand (Figure 7). The results revealed that His88, Glu189, Tyr257, Glu306, Tyr311, Typ344, and Glu352 of

JB13GH39 bound xylose-glucose with high frequency (Figure 7). These results revealed that the seven conserved amino acid residue positions were probably essential for β-1,2-xylosidase activity.

As shown in Figure 5, the positions of His88 (Ps88), Glu189 (Ps195), Glu306 (Ps317), Trp344 (Ps356), and Glu352 (Ps364) are not substituted with other amino acid residues, while the positions of Tyr257 (Ps266) and Tyr311 (Ps322) can be substituted with Thr and Pro with low frequency, respectively. As such, it is important to figure out whether β-1,2-xylosidase activity is lost when the positions of Tyr257 (Ps266) and Tyr311 (Ps322) are substituted with Thr and Pro, respectively.

Expression and Purification of Mutants. The JB13GH39 residues Tyr257 and Tyr311 were successfully substituted with Thr and Pro, respectively, and confirmed by DNA sequencing (Figure S2). Under the same conditions as those of the expression and purification of rJB13GH39, mutants Y257(Ps266)T and Y311(Ps322)P were expressed in cells and purified to an electrophoretically pure state (Figure S3).

Activity of Mutants. Both Y257(Ps266)T and Y311(Ps322)P showed β-1,4-xylosidase activity, with specific activities of 0.106 ± 0.002 and 28.641 ± 1.380 U/mg toward *p*NPXyl, respectively. The specific activities of Y257(Ps266)T and Y311(Ps322)P were reduced by 99.7 % and 24.2 %, respectively, compared with that of rJB13GH39 (37.8 ± 0.9 U/mg).[8] The decrease in the activity of Y257(Ps266)T

253 may be attributed to the function of Y257 (Ps266): the tyrosine enables the catalytic
254 nucleophile to attack the anomeric center correctly.[10]

255 Both Y257(Ps266)T and Y311(Ps322)P showed β-1,2-xylosidase activity. The
256 specific activities of Y257(Ps266)T and Y311(Ps322)P toward *o*NPXyl were 0.073 ±
257 0.030 and 7.619 ± 0.429 U/mg, respectively. More than 90 % of notoginsenosides R_1
258 and R_2 were converted by purified Y311(Ps322)P to ginsenosides Rg_1 and Rh_1,
259 respectively (Figures 2B, 3B). The rates at which Y311(Ps322)P hydrolyzes
260 notoginsenosides R_1 and R_2 are almost the same as those of rJB13GH39. However,
261 just as there was a substantial reduction in β-1,4-xylosidase activity, a substantial
262 reduction in Y257(Ps266)T β-1,2-xylosidase activity was observed: 57.0 % of
263 notoginsenoside R_1 and 7.5 % of notoginsenoside R_2 were individually hydrolyzed by
264 purified Y257(Ps266)T (Figures 2C, 3C).

265 Phylogenetic analysis revealed that characterized β-1,4-xylosidases and
266 β-1,2-xylosidases of GH 39 were classified into the same subgroup. Multiple
267 sequence alignments revealed that these GH 39 β-xylosidases have conserved amino
268 acid residue positions that participate in substrate recognition. Among these conserved
269 amino acid residue positions, seven were probably essential to xylose-glucose binding
270 revealed by protein-ligand docking. Amino acid residues in five of the seven positions
271 are invariant, while those in two of the seven positions are variable with low
272 frequency. Mutation at the two positions maintained β-1,4-xylosidase and
273 β-1,2-xylosidase activity at the expense of decreased activity. These results suggest
274 that all GH 39 β-xylosidases (GH 39 subgroup II) probably show β-1,2-xylosidase

activity for transformation of notoginsenosides. As such, we proposed that GH 39 β-xylosidases could be representatives of a new EC subsubclass.

In conclusion, based on molecular analysis, conserved amino acid residue positions of characterized GH 39 β-xylosidases involved in β-1,2-xylosidase activity were revealed. Activity determination showed that both wild-type rJB13GH39 and its mutants exhibited β-1,2-xylosidase activity. These results confirm the cleavage of terminal β-xylose-(1→2)-β-glucose bond by these GH 39 β-xylosidases for transformation of notoginsenosides, and such cleavage activity should be assigned an EC number. These characterized GH 39 β-xylosidases could be representatives of the new EC subsubclass.

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Notes

The authors declare that they have no conflict of interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: .**Figure S1** shows the ESI-MS spectra of the enzymatic transformation of notoginsenosides R_1 and R_2 using rJB13GH39. **Figure S2** shows the DNA sequencing results for mutants Y257(Ps266)T and Y311(Ps322)P using the universal sequencing primers T7 and T7t. **Figure S3** shows SDS-PAGE analysis of rJB13GH39 and its mutants Y257(Ps266)T and Y311(Ps322)P.

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FIGURE CAPTIONS

Figure 1. The diagram of enzymatic transformation of notoginsenosides R_1 and R_2 to ginsenosides Rg_1 and Rh_1, respectively.

Figure 2. UHPLC analysis for the conversion of notoginsenosides R_1 to ginsenosides Rg_1 using rJB13GH39 (A), Y257(Ps266)T (B), and Y311(Ps322)P (C).

Figure 3. UHPLC analysis for the conversion of notoginsenosides R_2 to ginsenosides Rh_1 using rJB13GH39 (A), Y257(Ps266)T (B), and Y311(Ps322)P (C).

Figure 4. The phylogenetic tree constructed on the basis of GH 39 enzymes.
The enzyme name, accession number, and source are given. Bootstrap values (n = 1000 replicates) are reported as percentages. The scale bar represents the number of changes per amino acid position.

Figure 5. Sequence logos generated on the basis of the multiple sequence alignment of the 14 identified GH 39 β-xylosidases in subgroup II shown in Figure 4.
Arrows show the putative catalytic and substrate recognition residues. Asterisks show the conserved docking positions shown in Figure 7.

Figure 6. Xylose-glucose ligand docking with the catalytic pocket of JB13GH39 with 91 poses.

Hydrogen bonds are represented by green-dashed lines.

Figure 7. Heat plot showing the hydrogen bond interactions of amino acid residues with the 91 poses of the xylose-glucose ligand.

Arrows show the conserved docking positions.

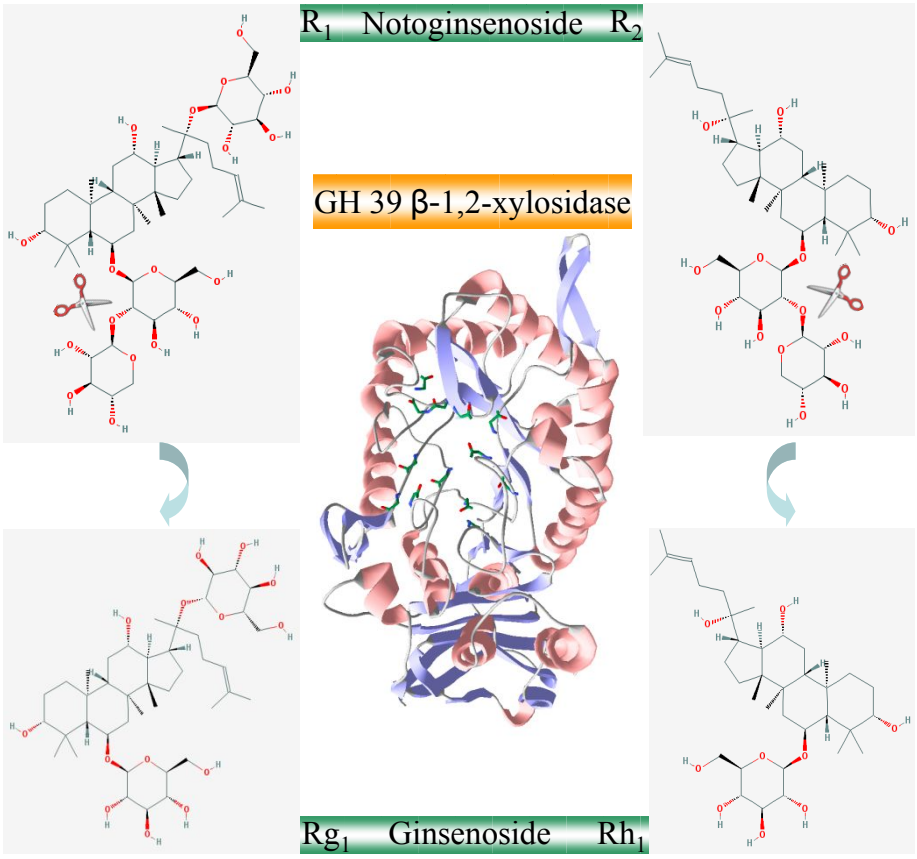
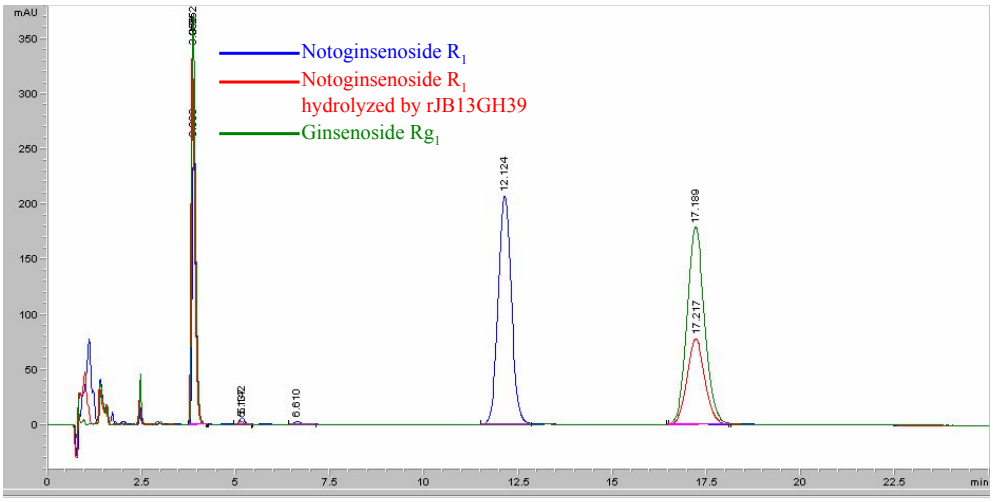
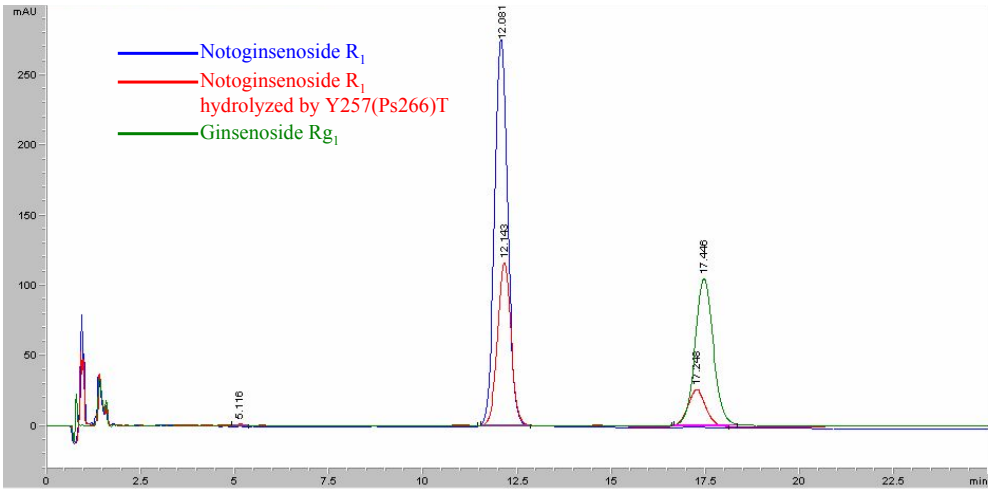

R1 Notoginsenoside R2
GH 39 β-1,2-xylosidase
Rg1 Ginsenoside Rh1

Figure 1.

A


mAU
Notoginsenoside R1
Notoginsenoside R1
hydrolyzed by rJB13GH39
Ginsenoside Rg1
12.124
17.189
17.217
6.610
min

B


mAU
Notoginsenoside R1
Notoginsenoside R1
hydrolyzed by Y257(Ps266)T
Ginsenoside Rg1
12.081
12.143
17.446
17.248
5.116
min

C

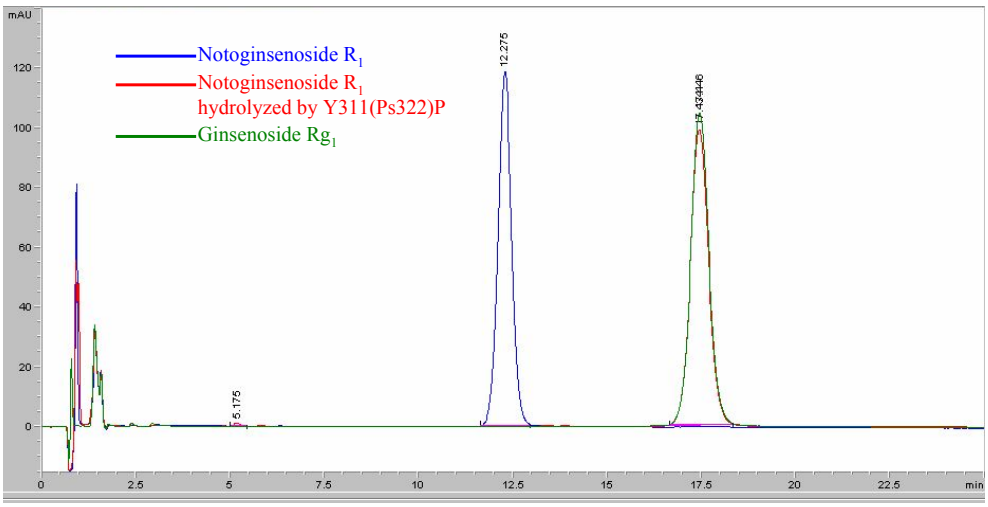
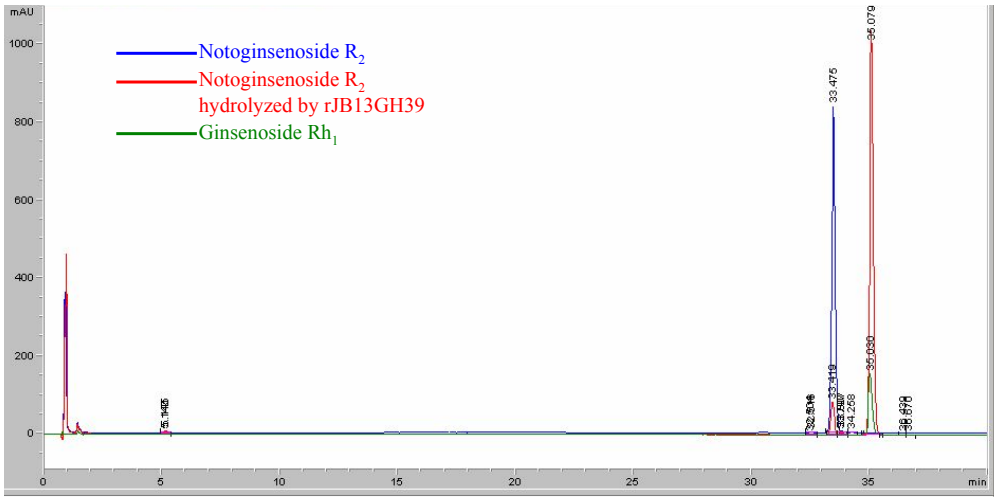
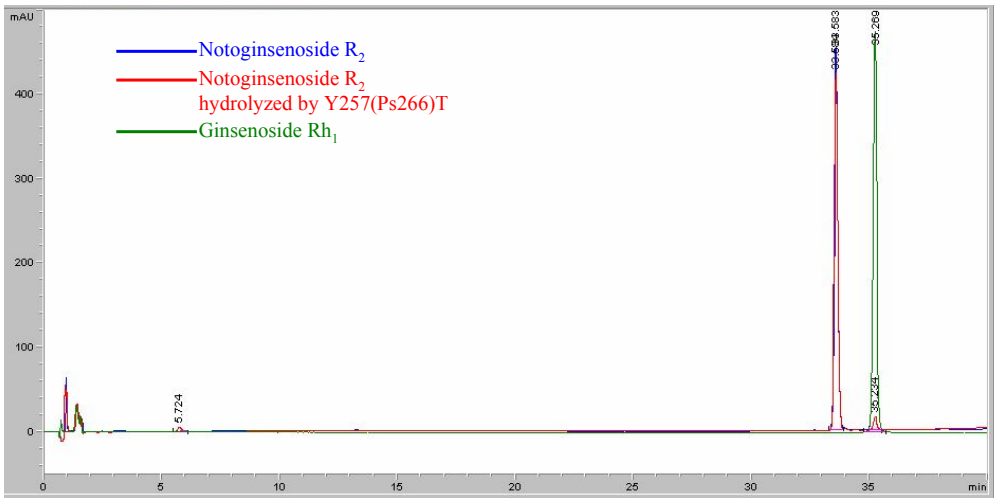

mAU
Notoginsenoside R1
Notoginsenoside R1
hydrolyzed by Y311(Ps322)P
Ginsenoside Rg1
12.275
5.175
min

Figure 2.

A


mAU
Notoginsenoside R2
Notoginsenoside R2
hydrolyzed by rJB13GH39
Ginsenoside Rh1
min

B


mAU
Notoginsenoside R2
Notoginsenoside R2
hydrolyzed by Y257(Ps266)T
Ginsenoside Rh1
min

C

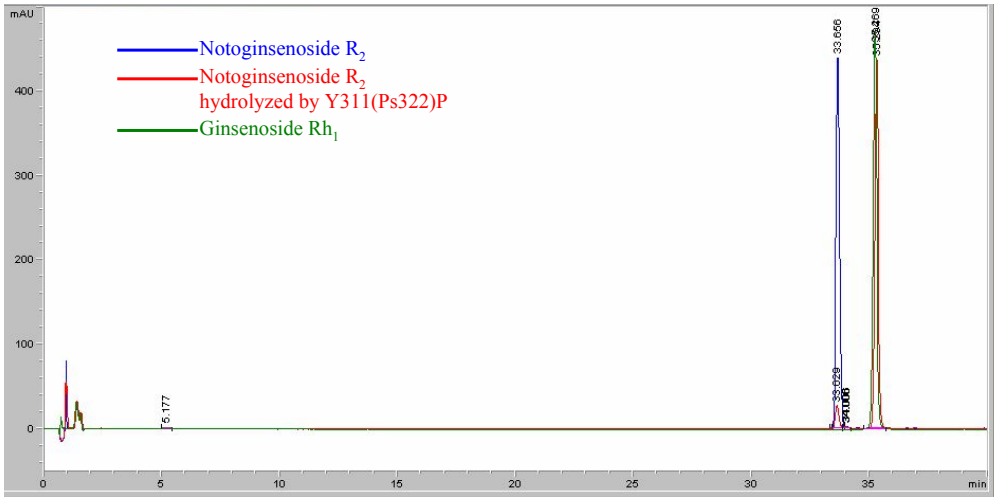

mAU
Notoginsenoside R2
Notoginsenoside R2
hydrolyzed by Y311(Ps322)P
Ginsenoside Rh1
min

Figure 3.

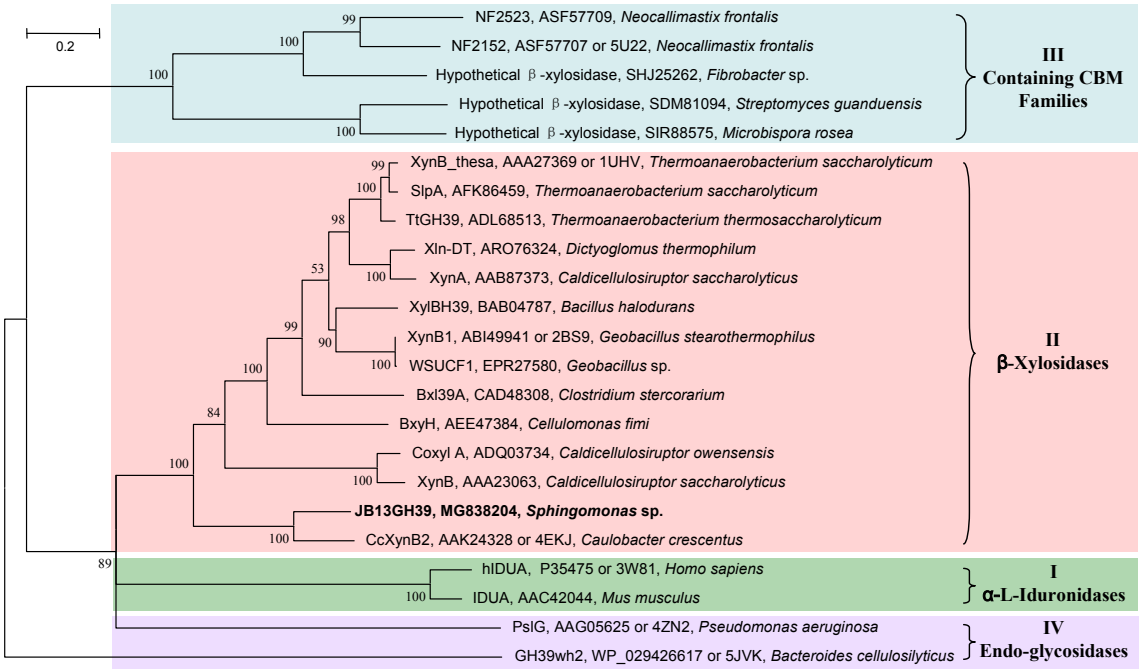

0.2
NF2523, ASF57709, Neocallimastix frontalis
NF2152, ASF57707 or 5U22, Neocallimastix frontalis
Hypothetical β-xylosidase, SHJ25262, Fibrobacter sp.
Hypothetical β-xylosidase, SDM81094, Streptomyces guanduensis
Hypothetical β-xylosidase, SIR88575, Microbispora rosea
III Containing CBM Families
XynB_thesa, AAA27369 or 1UHV, Thermoanaerobacterium saccharolyticum
SlpA, AFK86459, Thermoanaerobacterium saccharolyticum
TtGH39, ADL68513, Thermoanaerobacterium thermosaccharolyticum
Xln-DT, ARO76324, Dictyoglomus thermophilum
XynA, AAB87373, Caldicellulosiruptor saccharolyticus
XylBH39, BAB04787, Bacillus halodurans
XynB1, ABI49941 or 2BS9, Geobacillus stearothermophilus
WSUCF1, EPR27580, Geobacillus sp.
Bxl39A, CAD48308, Clostridium stercorarium
BxyH, AEE47384, Cellulomonas fimi
Coxyl A, ADQ03734, Caldicellulosiruptor owensensis
XynB, AAA23063, Caldicellulosiruptor saccharolyticus
JB13GH39, MG838204, Sphingomonas sp.
CcXynB2, AAK24328 or 4EKJ, Caulobacter crescentus
II β-Xylosidases
hIDUA, P35475 or 3W81, Homo sapiens
IDUA, AAC42044, Mus musculus
I α-L-Iduronidases
PslG, AAG05625 or 4ZN2, Pseudomonas aeruginosa
GH39wh2, WP_029426617 or 5JVK, Bacteroides cellulosilyticus
IV Endo-glycosidases

Figure 4.

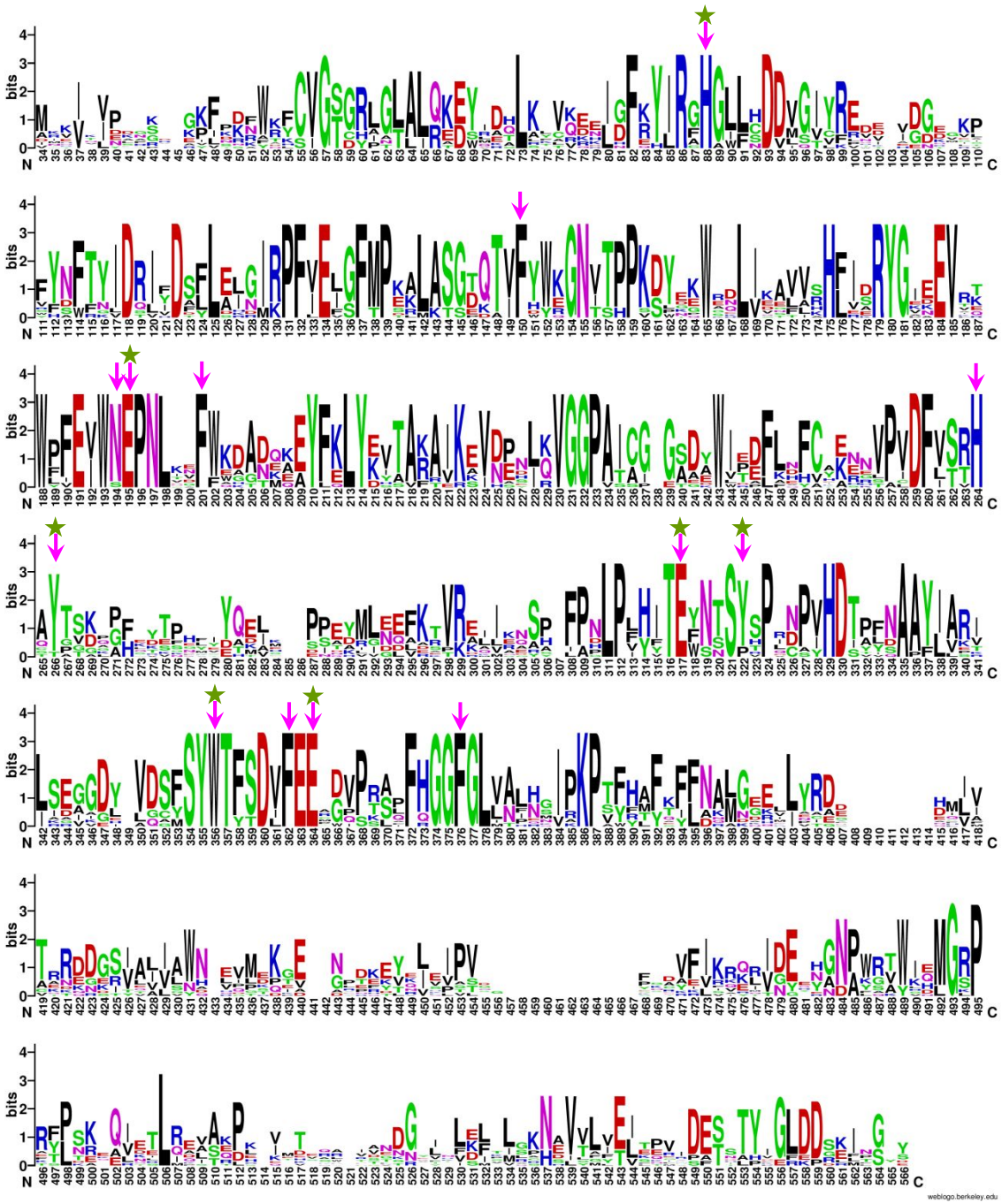


Figure 5.

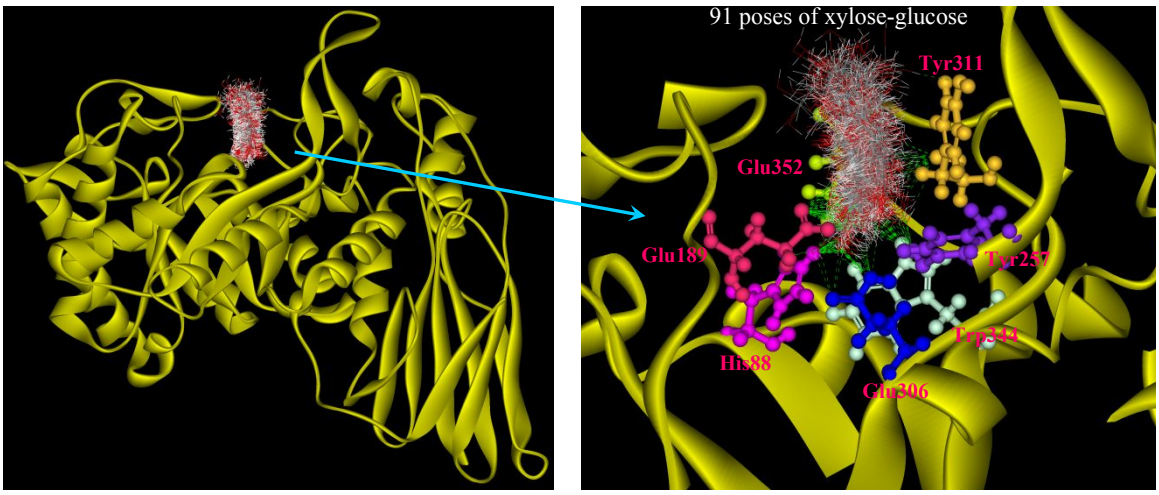

91 poses of xylose-glucose
Tyr311
Glu352
Glu189
Tyr257
His88
Glu306

Figure 6.

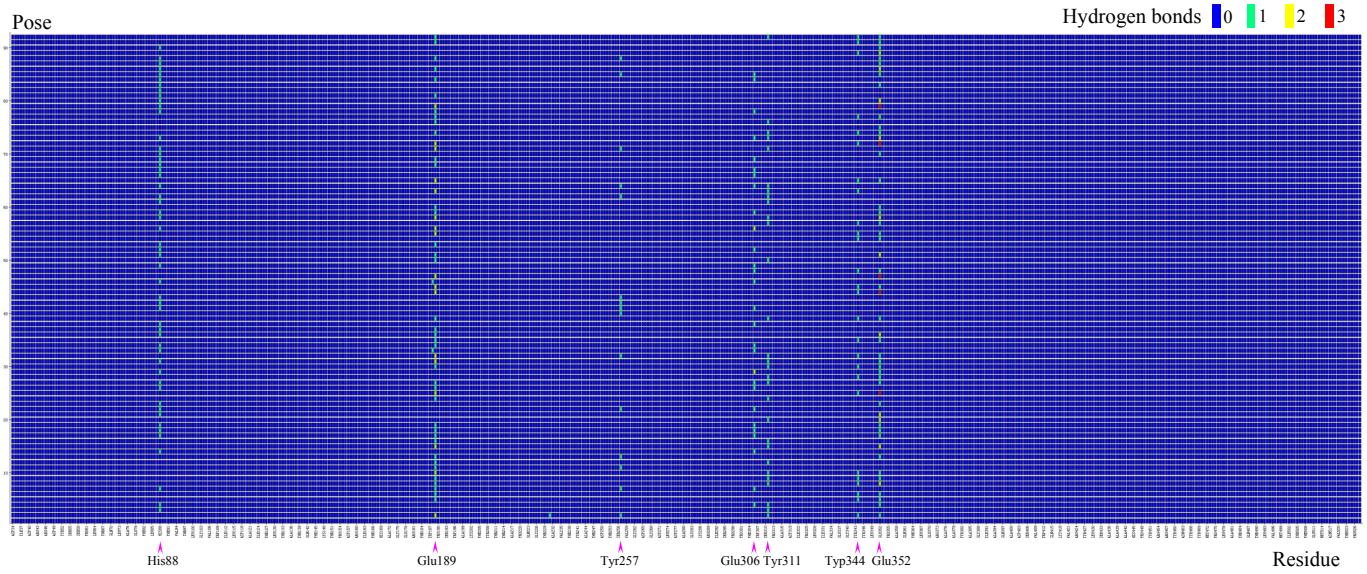
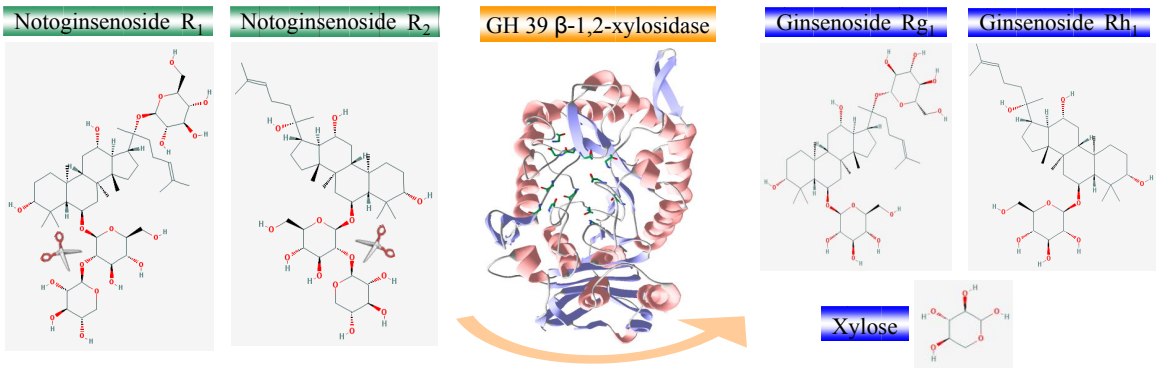

Pose
Hydrogen bonds 0 1 2 3
His88
Glu189
Tyr257
Glu306 Tyr311
Typ344 Glu352
Residue

Figure 7.


Notoginsenoside R1
Notoginsenoside R2
GH 39 β-1,2-xylosidase
Ginsenoside Rg1
Ginsenoside Rh1
Xylose

TOC Graphic