Identification, Isolation and Characterization of a Novel Glucosyltransferase from *Beauveria bassiana*

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1. Introduction

Glycosylation is a widely occurring reaction in nature and one of the important reactions for the formation of various natural glucosides with different applications. Glycosylation is catalyzed by glycosyltransferases (GTs) in living organisms. GTs transfer sugar moieties from activated donor molecules to various acceptors.

Family 1 glycosyltransferase (GT1) includes the uridine 5'-diphosphate (UDP)-glycosyltransferases that are involved in the glycosylation of flavonoids. Thereinto, UDP-glucose is the most typical donor molecule for GT1 and the corresponding GTs are called UDP-glucosyltransferases (UGTs) (Fig. 1).

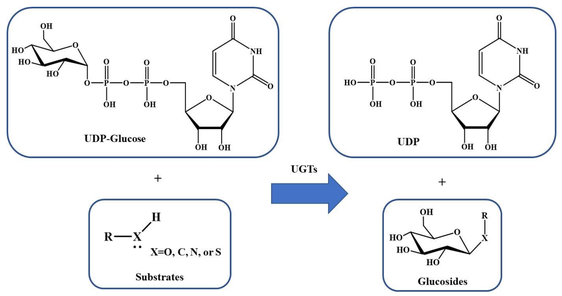

Fig.1 UGTs-mediated glucosylation process.

Flavonoids are polyphenolic natural products with diverse bioactivities, such as antibacterial, antimicrobial, antidiabetes, antioxidant, antitumor, anti-inflammatory, antiallergic, antihypertensive, and antiviral properties.

The most representative antioxidant flavonoid is quercetin, but its low aqueous solubility and poor bioavailability hinder further clinical applications. Here, we report a novel GT from *Beauveria bassiana* that can be used to prepare quercetin-3-O-β-D-glucoside and quercetin-7-O-β-D-glucoside (Fig. 2).

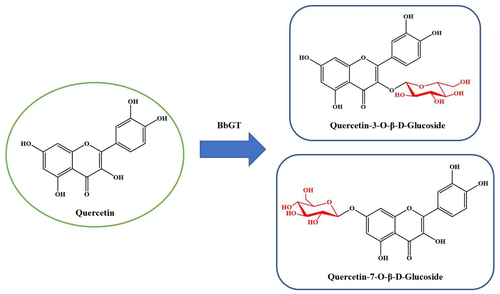

Fig.2 Enzymatic production of glucosylated quercetin

2. Hypothesis

Fungi are well-known for the production of various bioactive compounds. My hypothesis is that these strains are rich in natural product biosynthetic enzymes, some of which may be used for preparation of glycosides. Therefore, our research team is interested in discovering useful glycosyltransferases from microbes.

3. Results and Discussion

3.1 Expression and purification of BbGT

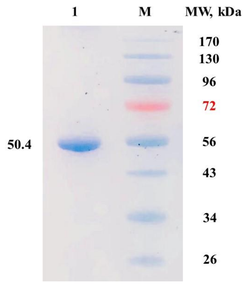

Fig. 3 SDS-PAGE analysis of the purified recombinant BbGT from *E.coli* BL21 (DE3). Lane 1: Purified recombinant BbGT; Lane 2: Standard protein ladder.

The gene was amplified from the genomic DNA of *B. bassiana*, and it was ligated to pET28a to yield the corresponding expression plasmids, namely pET28a-*Bbgt*. The plasmid was expressed in *E. coli* BL21(DE3) with IPTG induction. The protein band of purified enzyme is ~50.4 kDa in the lysate of *E. coli* BL21(DE3)/pET28a-*Bbgt* (lane 1). The yield of BbGT was 13.635 mg/L (Fig. 3).

3.2 *In vivo* functional characterization of BbGT in *E. coli* BL21(DE3)

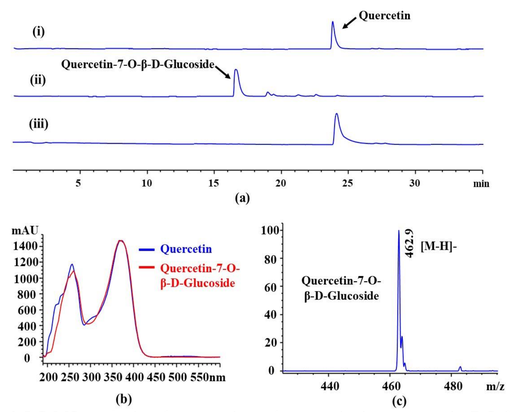

Fig. 4 LC-MS analysis of conversion of quercetin into quercetin-7-O-β-D-glucose by BbGT in *E. coli* BL21(DE3) at 350 nm. **a** HPLC analysis of the glycosylation of quercetin by BbGT. (i) Standard of quercetin; (ii) Quercetin+*E. coli* BL21(DE3)/pET28a-*Bbgt*; (iii) Quercetin+*E. coli* BL21(DE3)/pET28a. **b** UV spectra comparison of the substrate and bioconversion product at 16.5 min. **c** ESI-MS (−) spectrum of the bioconversion product at 16.5 min.

HPLC analysis showed that the vector control did not convert the substrate into any detectable product (Fig. 4, a, trace iii); while the engineered strain yielded a more polar product at 16.5 min (Fig. 4, a, trace ii). Furthermore, the product showed the similar UV spectrum (Fig. 4, b) as the substrate quercetin. Finally, the ESI-MS spectrum (Fig. 4, c) of this compound showed a $[M-H]^-$ ion peak at *m/z* 462.9, indicating that its molecular weight is 464 Da, which is consistent with the addition of a glucose moiety with 162 Da. Therefore, this product was characterized as glucosylated quercetin (Fig. 4).

3.3 Glucosylation of quercetin by BbGT *in* different heterologous expression systems and BbGT *in vitro* functional characterization

Engineered microorganisms such as *Saccharomyces cerevisiae* BJ5464, *Pseudomonas putida* KT2440 and *Pichia pastoris* GS115 are also used in the biotechnological production of medicinally important natural products. Therefore, we tested different heterologous expression systems for recombinant *Bbgt*. Surprisingly, we got different products from different hosts. The purified BbGT enzyme was also reacted with quercetin *in vitro* and showed the same glycosylated products of peak 3 and 4, further confirming that this enzyme is glucosyltransferase (Fig. 5).

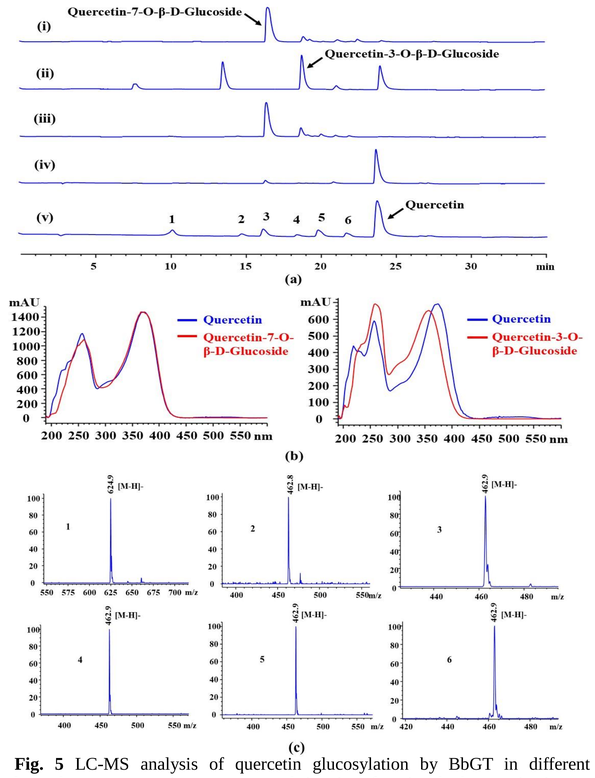

Fig. 5 LC-MS analysis of quercetin glucosylation by BbGT in different heterologous expression systems and *in vitro* functional characterization. **a** HPLC of the *in vivo* and *in vitro* reactions of BbGT with quercetin. (i) Quecetin+*E. coli* BL21(DE3)/pET28a-*Bbgt*; (ii) Quecetin+*S. cerevisiae* BJ5464/pXW55-*Bbgt*; (iii) Quecetin+*P. putida* KT2440/pMIS1-*Bbgt*; (iv) Quecetin+*P. pastoris* GS115/pPICZB-*Bbgt*; (v) *In vitro* functional characterization of *Bbgt*. **b** UV spectra comparison of the substrate and bioconversion products at 16.5 and 19.0 min respectively. **c** ESI-MS (−) spectra of the *in vitro* bioconversion products of *Bbgt*.

3.4 Characterization of two major glycosylated products in different microorganism hosts as quercetin-7-O-β-D-glucoside (3) and quercetin-3-O-β-D-glucoside (4)

Compound 3 was glycosylated at C-7, which was confirmed by the HMBC correlation of H-1" (δ 5.07, d, J=7.5 Hz) to C-7 at δ 162.7. Meanwhile, compound 4 was glycosylated at C-3, which was confirmed by the HMBC correlation of H-1" (δ 5.46, d, J=7.5 Hz) to C-3 at δ 133.3 (Fig. 6)

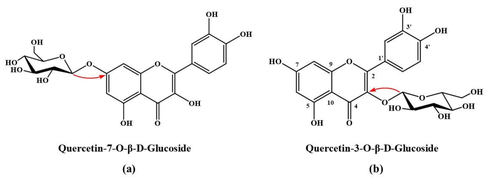

Fig. 6 Chemical structures of glucosylated quercetin. **a** Chemical structure of compound **3** in Quecetin+*E. coli BL21*(DE3)/pET28a-*Bbgt*; **b** Chemical structure of compound **4** in Quecetin+*S. cerevisiae* BJ5464/pET28a-*Bbgt*.

3.5 Determination of the optimal reaction conditions of BbGT

The optimal catalyzing temperature and pH conditions are 35℃ and 8.0 respectively. And the BbGT activity was stimulated by Ca^{2+}, Mg^{2+}, and Mn^{2+}, and when Zn^{2+} was added to the reaction system, we found that it showed strong inhibition to this enzyme (Fig. 7) .

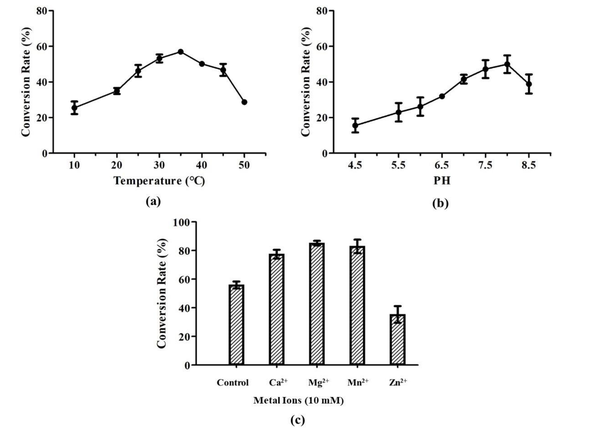

Fig. 7 Determination of the optimum reaction conditions of BbGT. **a** Effect of reaction temperature on the BbGT glucosylation activity. **b** Effect of reaction pH on the BbGT glucosylation activity. **c** Effect of reaction various metal ions on the BbGT glucosylation activity. Data are presented as the mean ± SD from three independent experiments.

Future Work

- Optimize the *in vivo* production conditions of quercetin glycosides using different hosts expressing BbGT.
- Production of quercetin glycosides by repeated uses of *E. coli* BL21(DE3)/pET28a-BbGT in 1L batch reaction system.

Conclusion

This research was focused on the discovery of the novel glucosyltransferase from *Beauveria bassiana* ATCC 7159, which provides a novel tool for the preparation of water-soluble derivatives of medicinally important natural product.

This enzyme can be used to prepare different glucosides with different expression hosts. Meanwhile, different sugar-acceptors can be glucosylated at different chemical structure positions with different sugar moieties. Therefore, the BbGT enzyme is expected to be useful molecular tool for increasing the bioavailability of various health-beneficial natural products, so that the bioactive components with low bioavailability can be better utilized by the human body.