



# Directed evolution of a *Bacillus*-derived glycosyltransferase for enhanced glycosylation efficiency

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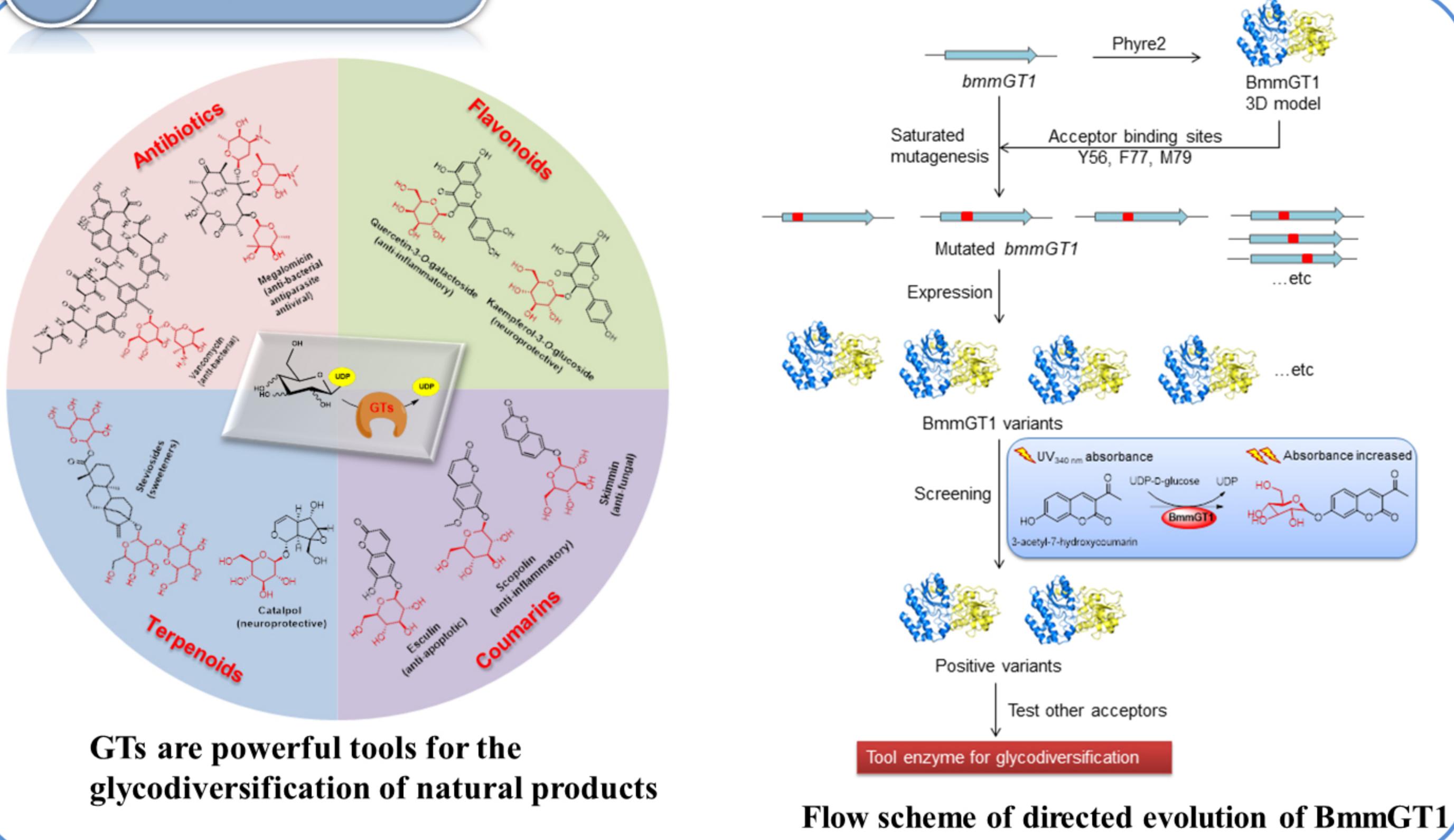
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## Abstract

The *Bacillus*-derived glycosyltransferase (GT) BmmGT1 exhibited broad substrate flexibility, especially towards sugar acceptors, showing a great potential in natural products diversification. However, compared to the natural sugar acceptor macro lactin A (MLN A), the conversion rates of BmmGT1 towards other acceptors are much lower. Herein, the catalytic activities of BmmGT1 towards sugar acceptors was enhanced via directed evolution.

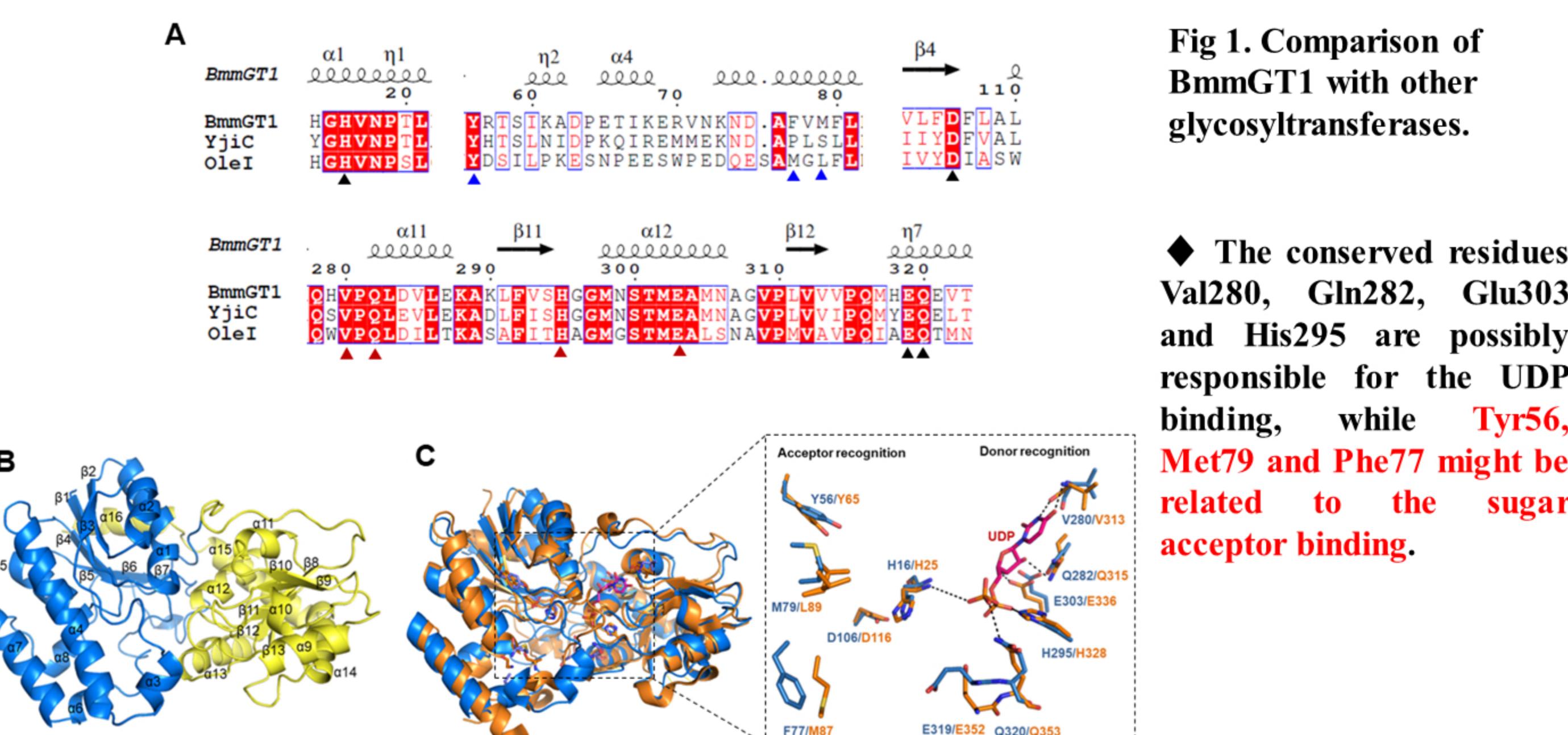
- ◆ A small library of variant BmmGT1 with mutated binding sites mutants were generated and screened using a 3-acetyl-7-hydroxycoumarin-based ultraviolet spectrophotometry screening method.
- ◆ Two mutants (M79F and M79W) were obtained, showing ~2.1-fold higher glucosylation activities towards 3-acetyl-7-hydroxycoumarin than the wild-type BmmGT1.
- ◆ Their catalytic activities towards other sugar acceptors were further tested, and the glucosylation activities of M79W was identified with ~3.6-fold and ~1.2-fold increase towards MLN A and piericidin A, respectively.

## Introduction

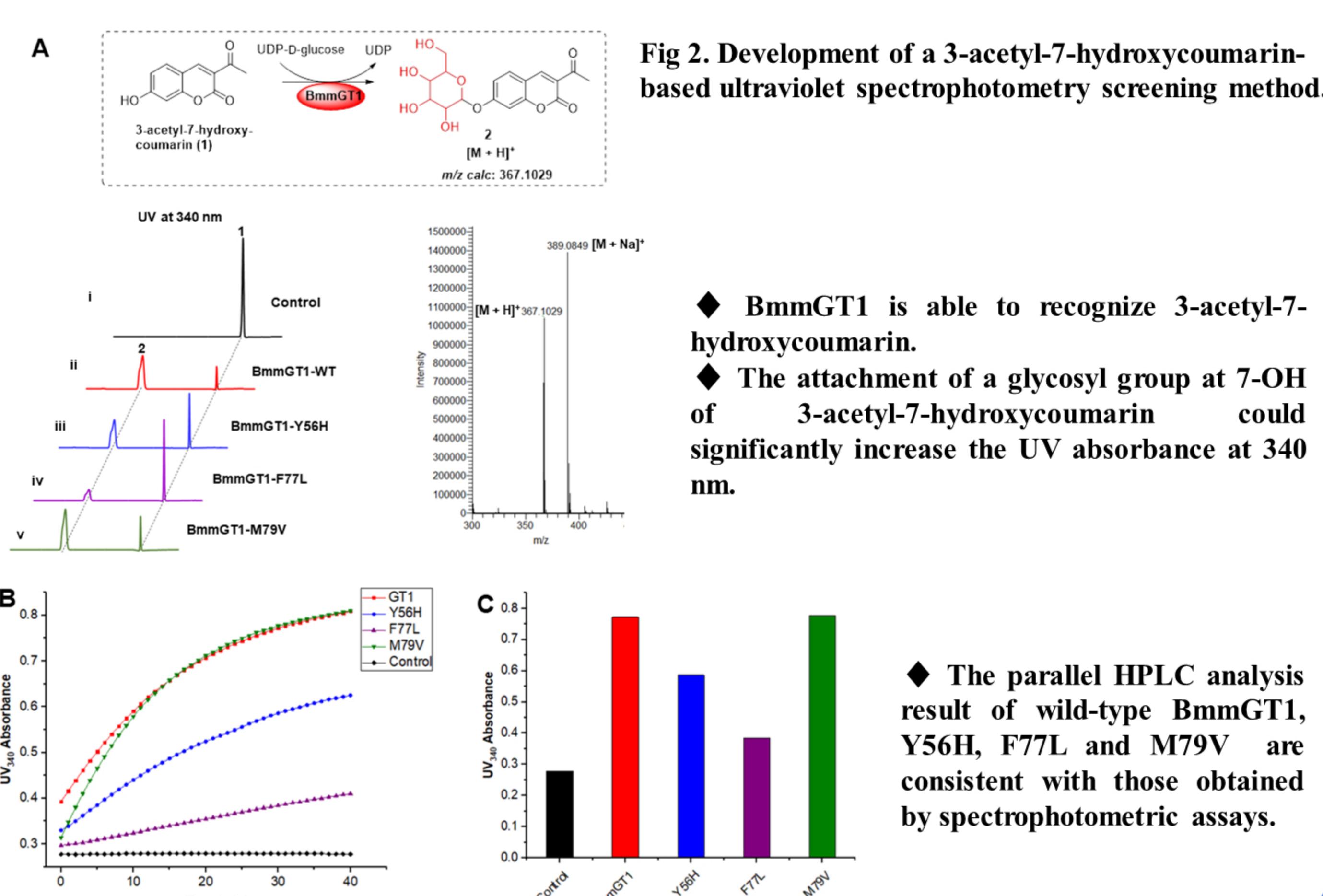


## Results

### Identifying the candidate residues of BmmGT1 for directed evolution



### Development of a 3-acetyl-7-hydroxycoumarin-based ultraviolet spectrophotometry screening method



### Saturation mutagenesis of sugar acceptor binding residues of BmmGT1

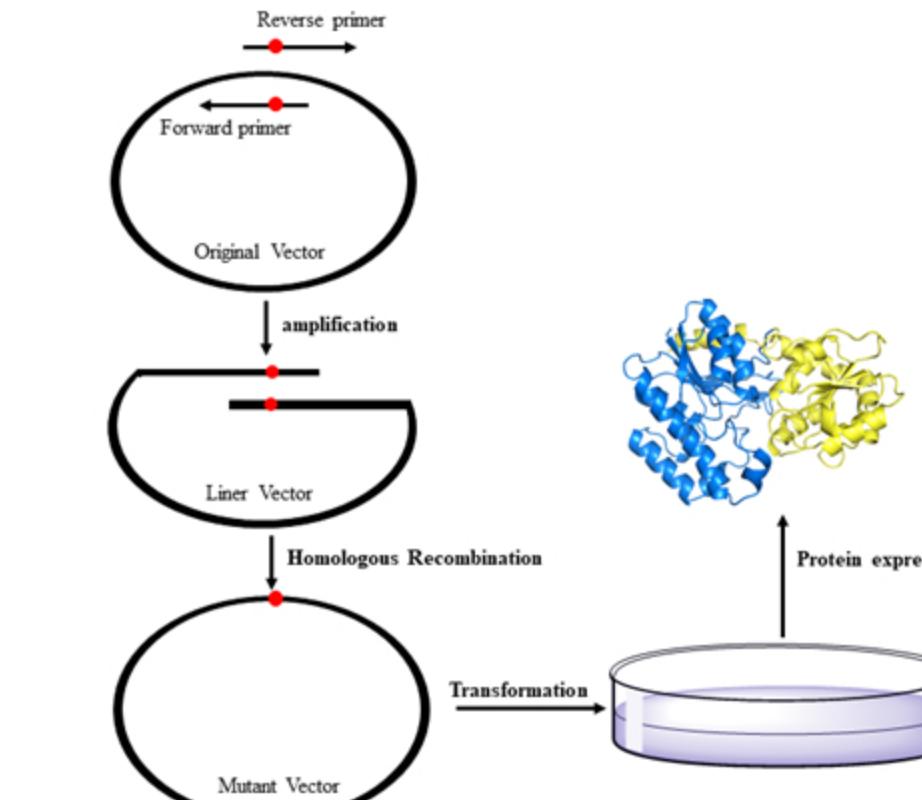
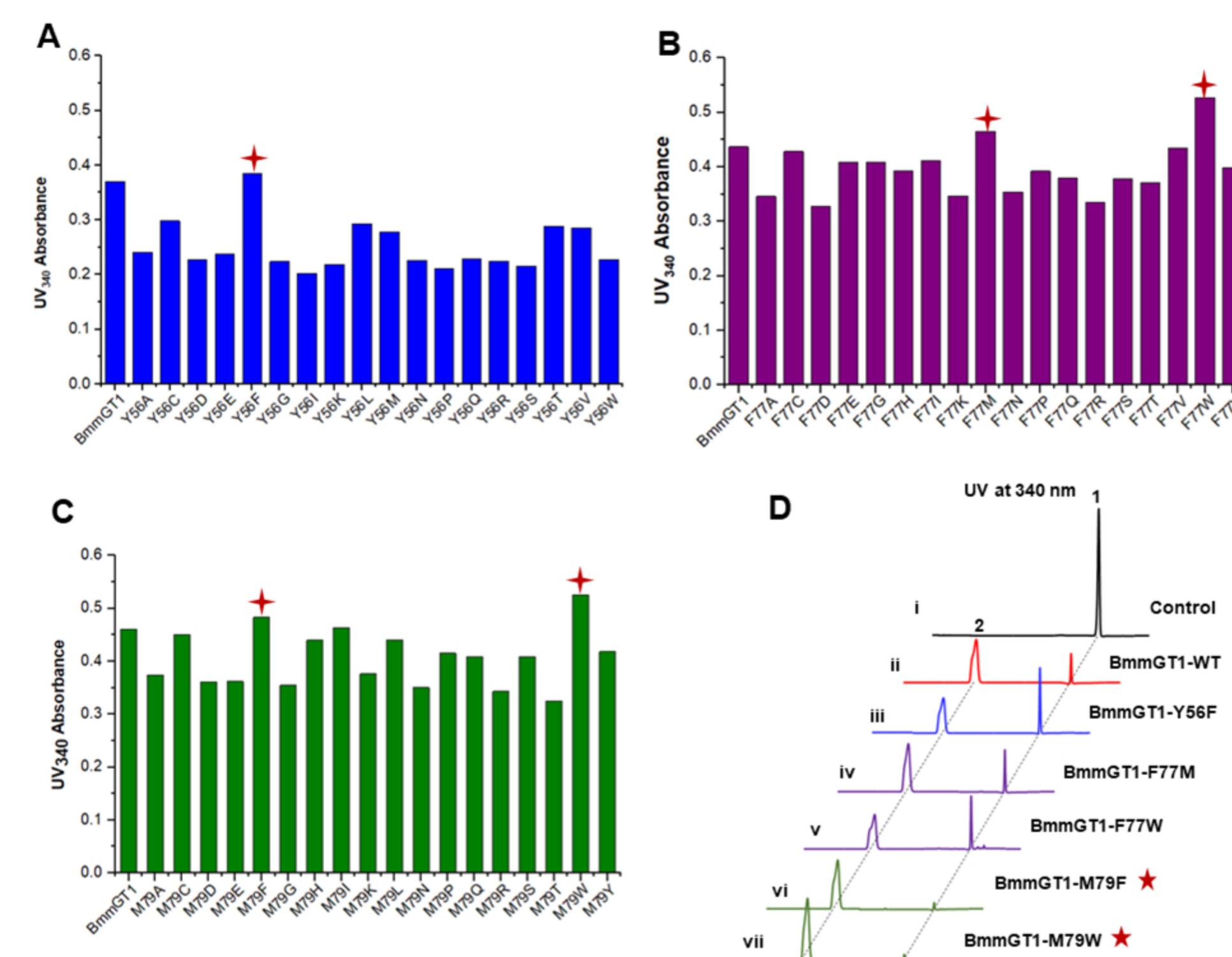


Fig 3. Site-directed mutagenesis procedures of BmmGT1.

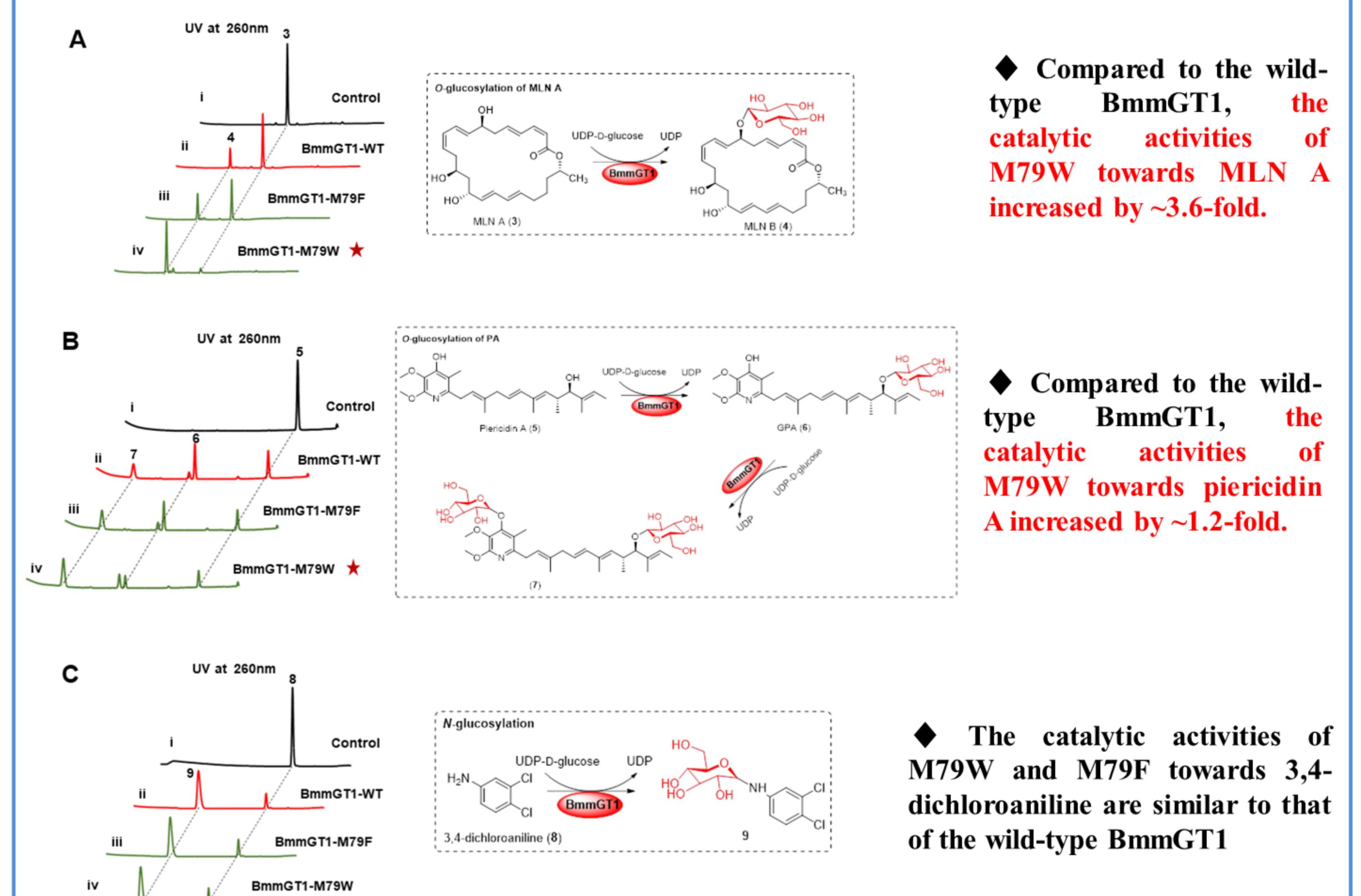
- ◆ A total number of 57 expression vectors harboring site-mutated BmmGT1 at Y56, F77 and M79 were obtained, and introduced into *E. coli* BL21 (DE3).

### Screening of BmmGT1 variants with enhanced glycosylation activities



- ◆ Through directed evolution, the mutants M79F and M79W with ~2.1-fold enhanced glucosylation activity towards 3-acetyl-7-hydroxycoumarin were obtained.

### In vitro characterization of BmmGT1 variants with other sugar acceptors



## Significance

- ◆ An efficient 3-acetyl-7-hydroxycoumarin-based ultraviolet spectrophotometry screening method was developed for quick screening of the variants with enhanced glycosylation activities.
- ◆ Through directed evolution and screening, M79F and M79W, with enhanced catalytic activities towards different sugar acceptors were obtained.
- ◆ Our study would provide guidance for engineering of other natural-product GTs via directed evolution.