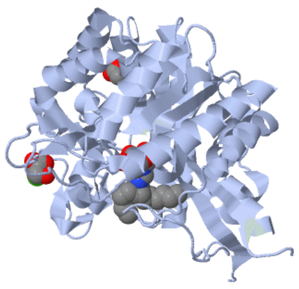
ENZYMATIC SYNTHESIS OF GLYCOSYLATED COMPOUNDS BY RICE Os9BGlu31 TRANSGLUCOSIDASE AND ITS MUTANTS

Eko Suyanto[1,2,3], Sunaree Choknud[1,2], Jaggaiah Naidu Gorantla[1,2], and James R. Ketudat-Cairns[1,2,4*]

[1] School of Chemistry, Institute of Sciences, Suranaree University of Technology, Nakhon Ratchasima, Thailand
[2] Center for Biomolecular Structure, Function and Application, Suranaree University of Technology, Nakhon Ratchasima, Thailand
[3] Biology Department, Faculty of Mathematic and Sciences, Brawijaya University, Malang, Indonesia
[4] Laboratory of Biochemistry, Chulabhorn Research Institute, Bangkok, Thailand
* Email: cairns@sut.ac.th

INTRODUCTION



In our body, glycoconjugate are important compounds and have a crucial role in fundamental biology, with many applications for therapeutics of human health, such as anti-microbial vaccine, anti-cancer agents, antibiotics, antifungal, anti-parasitic agent, anti-inflammatory agent food ingredients and other many functions. Glycoconjugate can be formed by chemical or enzyme-catalyzed glycosylation reaction. Enzyme-catalyzed glycosylation has been recognized as a feasible tool to synthesis glycosylated products. Rice Os9BGlu31 (EC 3.2.1.21) is one of enzyme in glycoside hydrolase family 1 (Gh1), a family that mostly catalyzes hydrolysis reaction. Os9BGlu31, however, mainly has transglycosylation activity that can transfer a glucosyl moiety to another aglycon moiety to form new glycosylated compounds through a retaining mechanism. This reaction may improve the bioactivity, stability, solubility , and physicochemical and physiological properties of the compounds, such as promising functional compounds and pharmaceuticals. In this study, we investigated the ability of rice Os9BGlu31 transglucosidase for glycosylation of phytosterols and phenolic acids to synthesis glycoside or glucosyl esters.

RESULTS

Protein expression and purification

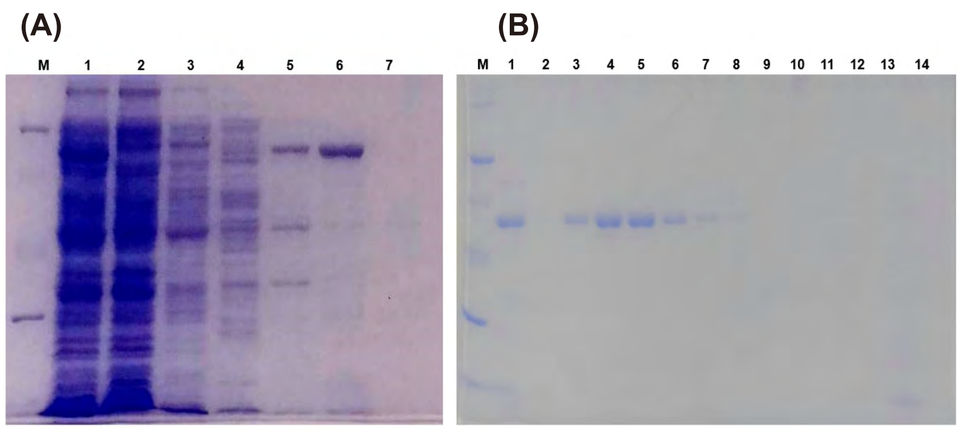


Figure 1. SDS-PAGE result of Os9BGlu31 after purification by IMAC. (A) 1st IMAC, (B) 2nd IMAC.

Enzymatic synthesis of glycosylated compounds

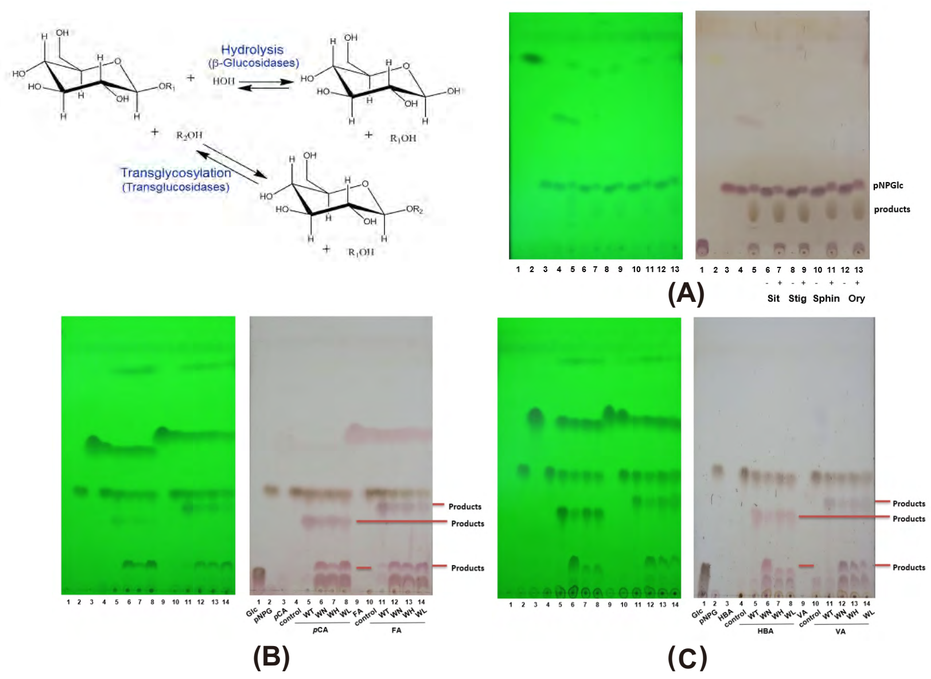


Figure 2. TLC results after transglycosylation reaction by using Os9BGlu31 and its mutants with (A) phytosterols and (B and C) phenolic acids.

Enzyme activity and UHPLC results

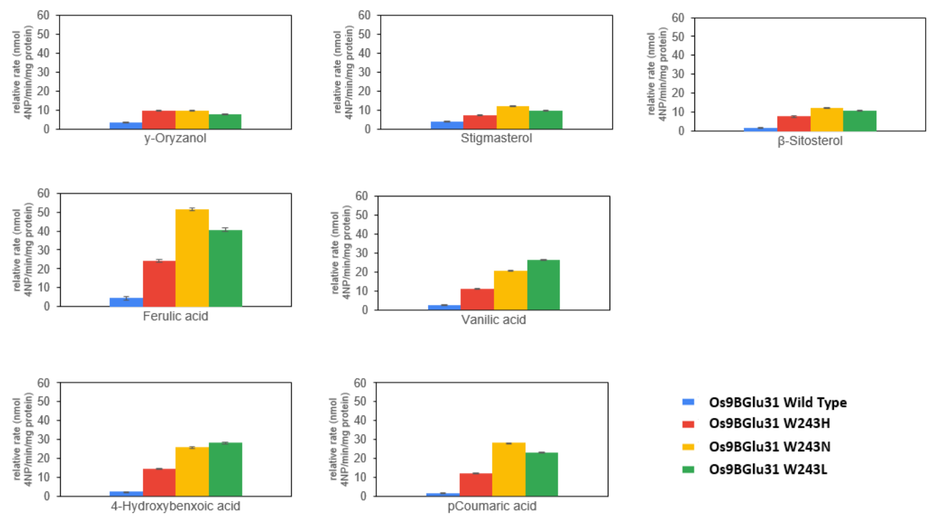


Figure 3. Enzyme activity of Os9BGlu31 and its mutants with several glucosyl acceptors. The mutants had higher activity than wild type (WT) to produce glycosylated compounds. Mutations of Os9BGlu31 are Trp243Asn (W243N), Trp243His (W243H), and Trp243Leu (W243L).

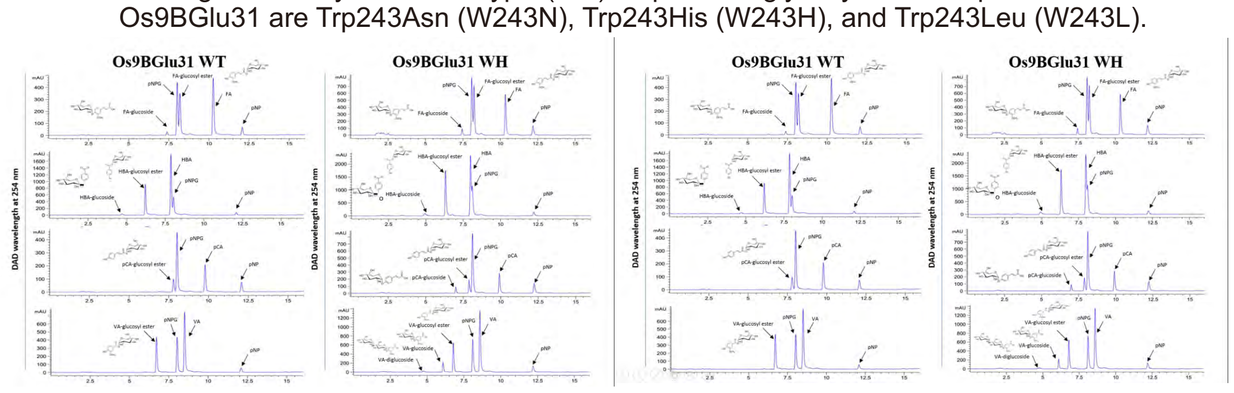


Figure 4. Chromatogram profile of each glycosylated compounds after transglycosylation reaction by using Os9BGlu31 and its mutants. The mutants tended to produce glucosyl esters and glucosides.

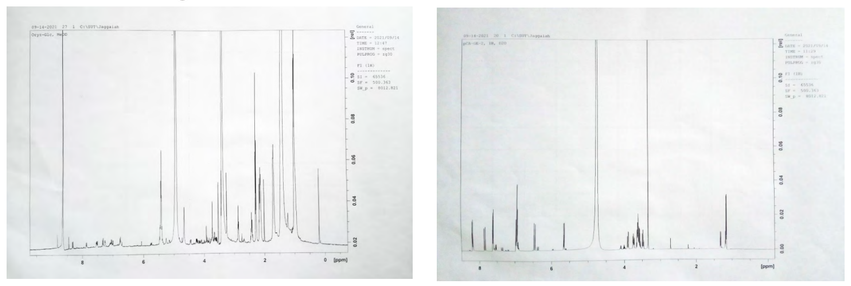
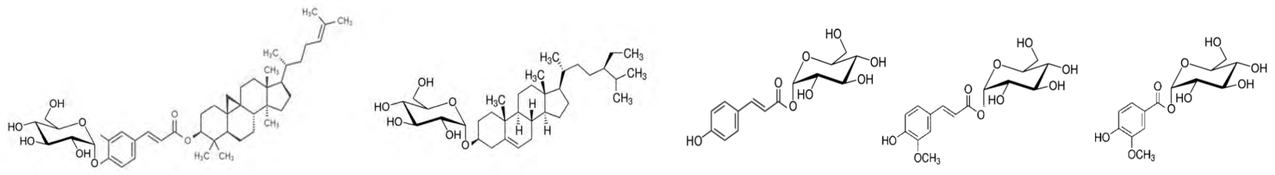


Figure 5. H-NMR spectra of (A) γ-oryzanol glucoside and (B) *p*-Coumaric acid glucosyl ester.

CONCLUSION

Rice Os9BGlu31 transglucosidase and its mutants transferred a glucosyl moiety from *p*-nitrophenol-β-D-glucopyranoside as glucose donor to glucose acceptors through transglycosylation reactions. Its mutants had higher activity than wildtype on phytosterols and phenolic acids to produce glucosides or glucosyl esters. The rice Os9BGlu31 transglucosidase is promising for glycosylation of compounds of interest, which may be improved by engineering the substrate specificity to allow production of a range or novel glycoconjugates.



ACKNOWLEDGEMENT

This research was supported by International Research Network grant IRN62W0004 from the Thailand Research Fund.

REFERENCES

1. Ketudat-Cairns et al. 2015. Plant Sci. 241 : 246-259
2. Khelashvili et al. 2011. Soft Matter 7 : 10299–10312
3. Komvongsa et al. 2015. Biochim. Biophys. Acta 1850 : 1405-1414
4. Nystrom et al. 2012. Eur. J. Lipid Sci. Technol. 114 : 656-669
5. Opassiri et al. 2006. BMC Plant Biology. 29 : 33-45
6. Moradi et al. 2016. Chem. Sci. 7 : 2492-2501
7. Luang et al. 2013. Biol. Chem. 288 (14) : 10111–10123

School of Chemistry,
Institute of Sciences,
Suranaree University of Technology,
Nakhon Ratchasima, Thailand