SUPPLEMENTARY MATERIALS

TO

Identification of an inducible glucosyltransferase from *Phytolacca americana* L. cells that are capable of glucosylating capsaicin

BY

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Supplementary Table IS. *Glucosyl-acceptor specificity of PaGT 1 and PaGT2*

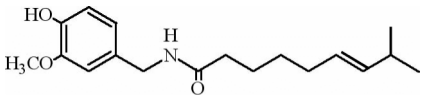
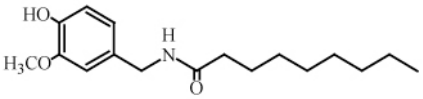
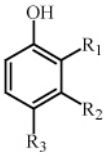
Substrate	Relative activity[a] (%)	
	PaGT1	PaGT2
 Capsaicin	nd	nd
 8-Nordihydrocapsaicin	nd	nd
		
Salicylic acid (R_1 = -COOH, R_2 = -H, R_3 = -H)	nd	nd
m-Hydroxy benzoic acid (R_1 = -H, R_2 = -COOH, R_3 = -H)	nd	nd
p-Hydroxy benzoic acid (R_1 = -H, R_2 = -H, R_3 = -COOH)	nd	7.3
Salicyl alchol (R_1 = -CH_2OH, R_2 = -H, R_3 = -H)	1.7	2.4
Hydroquinone (R_1 = -H, R_2 = -H, R_3 = -OH)	nd	13.2

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Table IS. (*continued*)

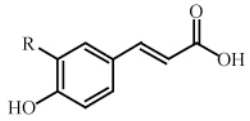
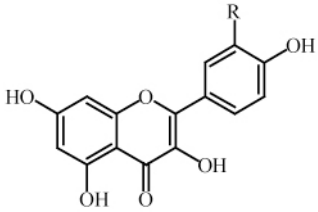
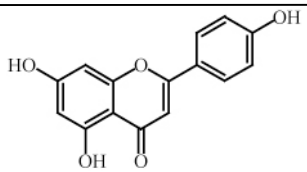
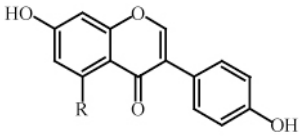
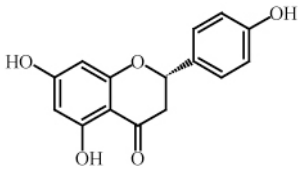
		
trans-p-Coumaric acid (R = -H)	nd	nd
Caffeic acid (R = -OH)	6.7	nd
		
Kaempferol (R = -H)	36	70 (2)
Quercetin (R = -OH)[b]	100	100 (3)
		
Apigenin	41	nd
		
Daidzein (R = -H)	20	10.7
Genistein (R = -OH)	69	5.4
		
Naringenin	77	nd

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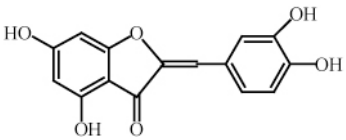
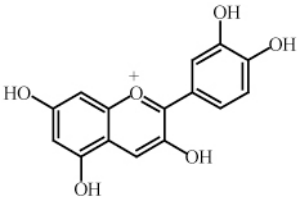
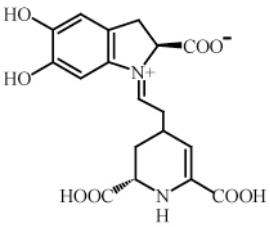
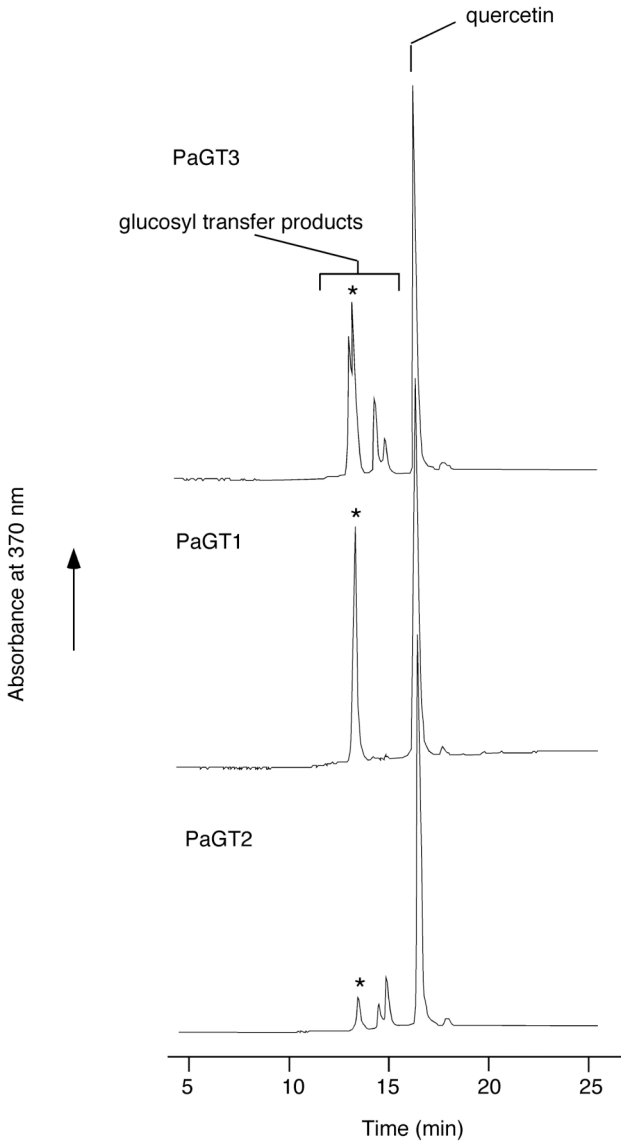
Table IS. (*continued*)

 Aureusidin	102	42
 Cyanidin	49	nd
 Betanidin	17	nd

[a] Assay conditions are described in Materials and methods. When multiple transfer products could be separated under the analytical HPLC conditions employed, the number of the separated peaks is shown in parenthesis. The relative activities were determined from the sum of product peak integrals, assuming that the extinction coefficient of the reaction product(s) was the same as that of the substrate. The activity for quercetin was taken to be 100%. nd, Activity not detected.

[b] Apparent K_m values of PaGT1 and PaGT2 for quercetin were 84±19 μM and 34±5 μM, respectively, with 100 μM UDP-glucose used as a glucosyl donor. Specific activities of enzyme preparations of PaGT1 (purity, 12%) and PaGT2 (purity 27%) used in these kinetic assays were 9.4±0.9 nkatal/mg and 6.2±0.4 nkatal/mg, respectively.



Supplementary Figure 1S. HPLC analysis of transglucosylation from UDP-glucose to quercetin catalyzed by PaGT3 (*top*), PaGT1 (*middle*), and PaGT2 (*bottom*).

The reaction mixture (100 μl) consisted of 100 μM quercetin, 100 μM UDP-glucose, 20 mM potassium phosphate (pH 6.5 for PaGT3, pH 7.0 for PaGT1, and pH 8.0 for PaGT2), and enzyme. After a 10-minute pre-incubation of the mixture without quercetin at 30°C, the reaction was started by addition of quercetin. After incubation at 30°C for 60 min, the reaction was stopped by the addition of 100 μl of 1% (v/v) trifluoroacetic acid (TFA) in 40% (v/v) acetonitrile. The substrates and glucosylated products were separated by reversed-phase HPLC on a COSMOSIL $5C^{18}$-MS-II column (4.6 x 150 mm, Nacalai tesque, Kyoto, Japan) using a linear gradient of 10 to 80% (v/v) CH_3CN containing 0.1% (v/v) TFA in 15 min at a flow rate of 0.5 ml/min. The compounds were detected at 370 nm using a SPD-10A *VP* UV-visible detector (Shimadzu, Kyoto, Japan). *Asterisks* indicate peaks of quercetin 3-*O*-glucoside (isoquercitrin). Note that reversed-phase HPLC analyses with C_{18} columns using acetonitrile/water/TFA systems (Kramer CM et al 2003, Cartwright AM et al 2008) suggest that quercetin

monoglucosides are generally eluted, under such conditions, in the following order: 7-*O*-glucoside, 3-*O*-glucoside, 4'-*O*-glucoside, and then, 3'-*O*-glucoside.

References

Kramer CM, Prata RTN, Willits MG, Luca VD, Steffens JC, Graser G (2003) Cloning and regiospecificity studies of two flavonoid glucosyltransferases from *Allium cepa*. *Phytochemistry* 64: 1069-1076

Cartwright AM, Lim E-K, Kleanthous C, Bowles DJ (2008) Kinetic analysis of regiospecific glucosylation by two glycosyltransferases of *Arabidopsis thaliana*: Domain swapping to introduce new activities. *J Biol Chem* 283:15724-15731