J. Microbiol. Biotechnol. (2016), 26(3), 493–497
http://dx.doi.org/10.4014/jmb.1512.12085

jmb

Glucosyl Rubusosides by Dextransucrases Improve the Quality of Taste and Sweetness

Jin-A Ko[1†], Young Bae Ryu[1†], Ji-Young Park[1], Cha Young Kim[1], Joong Su Kim[1], Seung-Hee Nam[2], Woo Song Lee[1*], and Young-Min Kim[2*]

[1]*Eco-Friendly Bio-material Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup 56212, Republic of Korea*
[2]*Department of Food Science and Technology and BK21 Plus Program, Graduate School of Chonnam National University and Bioenergy Research Center, Gwangju 61186, Republic of Korea*

Received: January 4, 2016
Revised: January 18, 2016
Accepted: January 25, 2016

First published online
January 26, 2016

*Corresponding authors
Y.-M.K.
Phone: +82-62-530-2142;
Fax: +82-62-530-2149;
E-mail: u9897854@jnu.ac.kr
W.S.L.
Phone: +82-63-570-5001;
Fax: +82-63-570-5019;
E-mail: wslee@kribb.re.kr

†These authors contributed equally to this work.

pISSN 1017-7825, eISSN 1738-8872

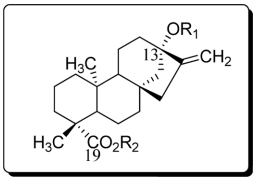
Glucosyl rubusosides were synthesized by two dextransucrases. LcDexT was obtained from *Leuconosotoc citreum*, that LlDexT was obtained from *Leuconostoc lactis*. LcDexT and LlDexT regioselectively transferred a glucosyl residue to the 13-*O*-glucosyl moiety of rubusoside with high yield of 59–66% as analyzed by TLC and HPLC. Evaluation of the sweetness of these glucosyl rubusosides showed that their quality of taste, in particular, was superior to that of rubusoside. These results indicate that transglucosylation at the 13-*O*-glucosyl moiety of rubusoside by different regioselective dextransucrases can be applicable for increasing its sweetness and quality of taste.

Keywords: Acceptor reaction, rubusoside, dextransucrase, *Leuconostoc lactis*, *Leuconostoc citreum*

Rubusoside, the β-D-glucosyl ester of 13-*O*-β-D-glucosyl-steviol, has been isolated as the major sweet principle from the leaves of *Rubus suavissimus* S. Lee (Rosaceae) grown in Southern China at yields greater than 5% [15]. Rubusoside is used to produce tiancha (Chinese sweet tea). Although rubusoside exhibits 114-fold greater natural sweetness than sucrose, its disadvantage is a slightly bitter taste and aftertaste. Many researchers have attempted to improve its sweetness level by adding transglycosyl or -fructosyl residues using cyclomaltodextrin glucanotransferase (CGTase) [1], α-galactosidase [5], and β-fructofuranosidase [3], respectively (Table 1). In spite of extensive research, the association between the structure and the sweetness/quality of taste of the derivatives is yet to be significantly elucidated. Rubusoside also is an effective solubilizing agent [17], such as liquiritin, teniposide [10], etoposide, and astragalin [2] in the pharmaceutical industry.

Dextransucrase (DexT; E.C. 2.4.1.5) is a type of glucansucrase that catalyzes the polymerization reaction from sucrose to dextran through a transglucosylation reaction. The transglucosylation activity of DexT has been exploited to enhance the functionality of several materials, including the anticoagulant properties of salicin [12], the development of acarbose as a novel inhibitor of α-amylase [16], and improving the solubility of catechin [9] for use as a pharmaceutical material. Recently, we found that two DexTs (LlDexT and LcDexT) from *Leuconostoc lactis* EG001 [4] and *L. citreum* KM20 [6] could transform steviol glucoside at different regioselectivity, such as alpha-1,3 or alpha-1.6 transglucosylation activity for LlDexT [4] or LcDexT [6]. Rubusoside was transformed into transfer products (glucosyl rubusoside 1 and glucosyl rubusoside 2)

Table 1. Regiospecific transfer products of rubusoside by various enzymes and their yields.



Enzyme	Substrate	Major transfer products		Yield (%)	References
		R_1	R_2		
α-Galactosidases	Raffinose	β-Glc(6 → 1)α-Gal	β-Glc	12.0	[5]
		β-Glc	β-Glc(6 → 1)α-Gal	8.6	
		β-Glc(6 → 1)α-Gal(6 → 1)α-Gal	β-Glc	2.7	
Arthrobacter sp. K-1 β-fructofuranosidase	Sucrose	β-Glc	β-Glc(2 → 6)β-Fru	13.3	[3]
Bacillus circulans CGTase	Soluble starch	β-Glc[(1 → 4)β-Glc]$_n$	β-Glc[(1 → 4)β-Glc]$_n$	-	[11]
L. citreum KM20 dextransucrase	Sucrose	Expected β-Glc(6 → 1)α-Glc	β-Glc	59	This study
L. lactis EG001 dextransucrase	Sucrose	Expected β-Glc(3 → 1)α-Glc	β-Glc	66	This study

-, not observed.

with enhanced sweetness and significantly improved quality of taste. In this study, we demonstrated the synthesis of different regiospecific monoglucosyl glucosyl rubusosides using these two DexTs and elucidated their structure-sweetness association (Fig. 1).

The two recombinant DexTs were expressed in *Escherichia coli* BL21 (DE3), harboring the two dextransucrase genes, at 20°C for 12 h as previously described [4, 6]. Approximately 16 mg (98 U/mg) and 3.5 mg (126 U/mg) of final purified LcDexT (170 kDa) and LlDexT (165 kDa), respectively, were obtained from 1 L of culture broth. DexT activity was determined by quantifying the level of fructose liberated from sucrose by the addition of dinitrosalicylic acid [8]; one unit of DexT activity was defined as the quantity of

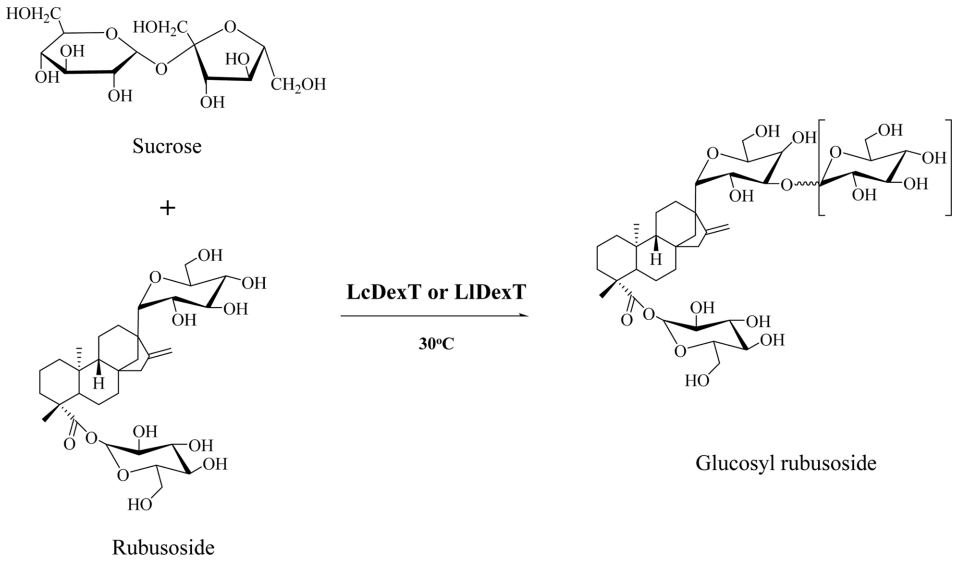


Fig. 1. Schematic representation for the glucosyl rubusoside enzymatically synthesized by LcDexT or LlDexT.

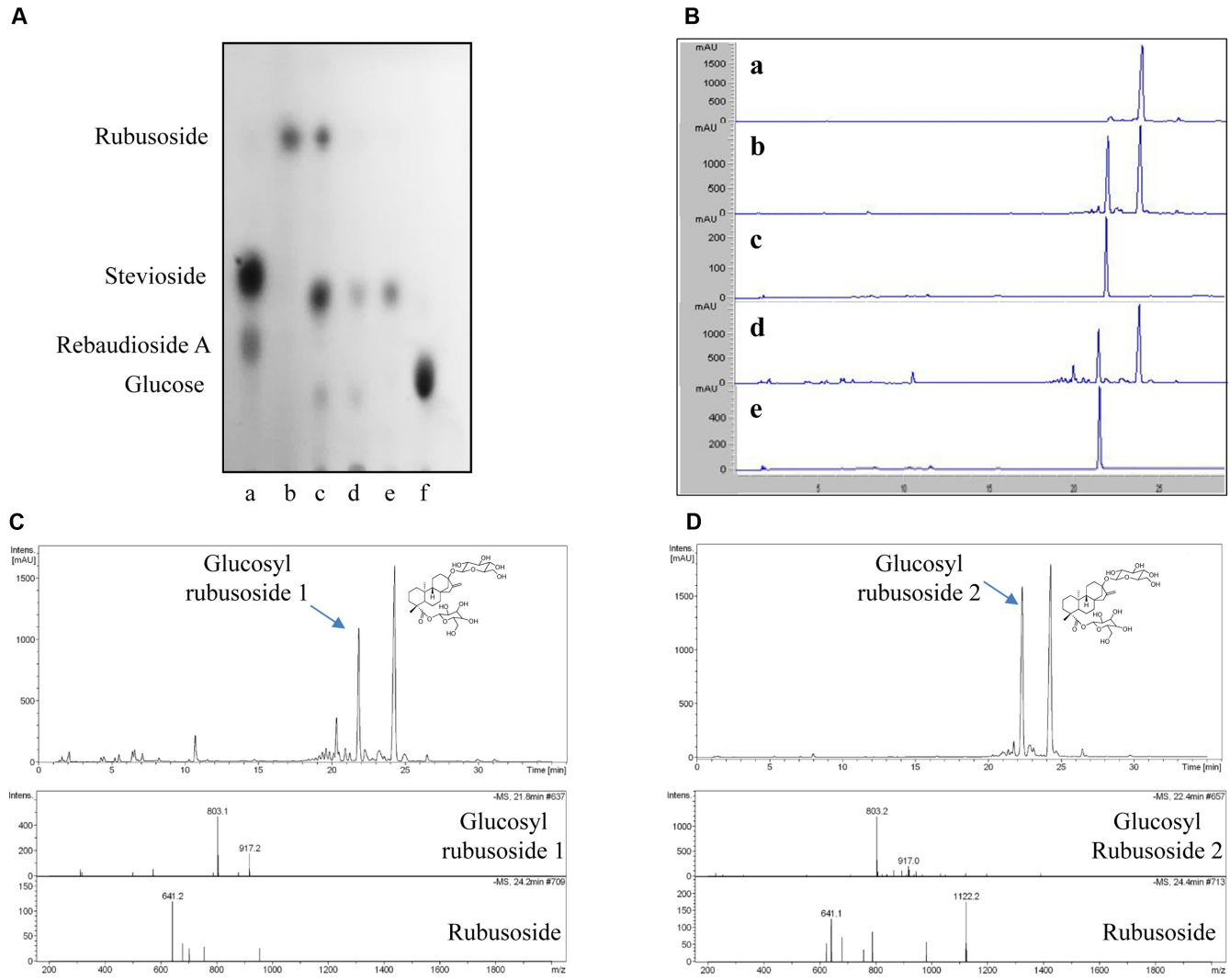


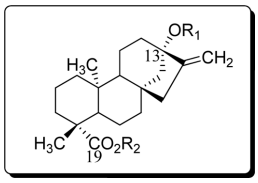
Fig. 2. TLC (**A**), HPLC (**B**), and LC-MS spectra (**C** and **D**) of two glucosyl rubusosides.

(**A**) a, stevioside and rebaudioside A standards; b, rubusoside standard; c, reaction mixture containing the LcDexT with rubusoside; d, purified glucosyl rubusoside 1 by lichroprep RP-18 resin with ethanol; e, finally purified glucosyl rubusoside 1 by prep HPLC. (**B**) a, rubusoside standard; b, reaction mixture of rubusoside and sucrose by LcDexT; c, purified glucosyl rubusoside 1; d, reaction mixture of rubusoside and sucrose by LlDexT; e, purified glucosyl rubusoside 2. (**C**) LC-MS spectra for glucosyl rubusoside 1. (**D**) LC-MS spectra for glucosyl rubusoside 2.

enzyme required to generate 1 μmol of fructose per minute.

The glucosyl rubusoside 1 reaction mixture (10 ml) containing 200 mg sucrose, 200 mg rubusoside, and LcDexT (150 U) was incubated in sodium acetate buffer (pH 5.2) at 30°C for 3 days. The glucosyl rubusoside 2 reaction mixture (7 ml) containing 140 mg sucrose, 200 mg rubusoside, 1 mM $MgCl_2$, and LlDexT (150 U) was incubated in sodium acetate buffer (pH 5.0) at 30°C for 5 days. Following heating at 100°C for 5 min, the reaction aliquots were centrifuged to remove insoluble materials. The supernatants were then analyzed by thin-layer chromatography (TLC), as previously described [13], and high-performance liquid chromatography was carried out in a system (HPLC; Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary HPLC pump, a degasser, an autosampler, and a UV detector (VWD). The reaction mixtures displayed novel single spots in TLC analysis (data not shown) and glucosyl rubusoside 1 and glucosyl rubusoside 2 exhibited retention times of 22.4 min and 21.6 min, respectively, in HPLC analysis, implying that the two DexTs have different regioselectivity for rubusoside (Fig. 2). Liquid chromatography-mass spectrometry (LC-MS) analysis of the glucosyl rubusosides indicated an 803 Da shift, indicating the transfer of one glucosyl moiety to rubusoside. Subsequent to desalting

Table 2. Relative sweetness and quality of taste of glucosyl rubusosides and steviol glucosides.



Compound	R_1	R_2	RS	Quality of taste[a]	References
Rebaudioside A	β-Glc(2 → 1)β-Glc (3 → 1)β-Glc	β-Glc	242	+3	[1]
Stevioside	β-Glc(2 → 1)β-Glc	β-Glc	143	0	[1]
Rebaudioside D	β-Glc(2 → 1)β-Glc (3 → 1)β-Glc	β-Glc(2 → 1)β-Glc	221	+3	[13]
Rebaudioside E	β-Glc(2 → 1)β-Glc	β-Glc(2 → 1)β-Glc	174	+1	[13]
Rubusoside	β-Glc	β-Glc	114	0	[1]
Glucosyl rubusoside1	β-Glc(6 → 1)α-Glc	β-Glc	132 (±8)	+2	This study
Glucosyl rubusoside 2	β-Glc(3 → 1)α-Glc	β-Glc	129 (±9)	+2	This study

RS: relative sweetness to sucrose (on a weight basis).

[a]Quality of taste relative to 3: +3, fairly better; +2, better; +1, slightly better; 0, almost the same.

using AW-90 resin (Samyang Co., Seoul, Korea), the glucosyl rubusosides were isolated by C_{18} resin with an ethanol step gradient. The glucosyl rubusosides were eluted in the 75% (v/v) ethanol fraction and the final purified glucosyl rubusoside 1 and glucosyl rubusoside 2 were obtained at high yields of 59% (147.5 mg) and 66% (164.4 mg), respectively. Previously, Kitahata *et al*. [5] attempted to synthesize rubusoside derivatives using plant or bacterial galactosidases, resulting in yields of 2.7–13.3%.

The relative sweetness and the quality of taste of the synthesized glucosyl rubusosides are listed in Table 2. Improved sweetness intensity was observed for both glucosyl rubusosides; glucosyl rubusoside 1 in particular exhibited 1.16-fold higher sweetness than rubusoside. The quality of taste of the two glucosyl rubusosides (aftertaste and deliciousness) was also greatly improved by the addition of a glucosyl moiety to the free hydroxyl group of the glucosyl moiety at the 13-position of rubusoside. These data indicated that modification of the glucosyl residue at this position may play a critical role in sweetness and quality of taste [5]. Although the sweetness of glucosyl rubusoside 1 and glucosyl rubusoside 2 was greatly enhanced over that of rubusoside, the levels were not as high as that of stevioside and rebaudioside A; however, they were comparable to rebaudioside A in the quality of taste. Elongation of the 13-*O*-glucosyl moiety enhanced the sweetness intensity of the steviol glycosides, and the 13-*O*-glucosyl moiety resulted in an increase in the quality of taste of rubusoside. These characteristics could be utilized to mask the limitations of other sweeteners with modifications (Table 2) at the 13-*O*-glucosyl moiety of rebaudioside A that exhibit high quality of taste [14]. Unfortunately, these compounds are found at less than 0.5% in *S. rebaudiana.* The enzymatically synthesized glucosyl rubusosides presented in this study could be applicable as strong alternatives of rebaudioside A or stevioside.

The two products produced by the DexTs in this study possess three advantages: first, the two DexTs exhibited a high yield, approximately 59–66% of the transglucosylation products of rubusoside (Table 1) compared with 2.7–13.3% yield using α-galactosidase [3] or β-fructofuranosidase [7, 11]; second, one reaction resulted in the synthesis of a single product with different regioselective derivatives at the free 1,6- or 1,3-hydroxyl group of the glucosyl moiety at the 13-position of rubusoside; and third, these regiospecific glucosylations of rubusoside increased the quality of taste and sweetness. Further studies regarding the synthesis of glucosyl stevioside or glucosyl rebaudioside A derivatives using the two DexTs are currently being conducted to confirm the relationship between structure and sweetness/quality of taste.

Acknowledgments

This research was supported by the KRIBB Research Initiative Program, Fishing Commercialization Technology

Development Program through KIMST funded by the Ministry of Oceans and Fisheries (MOF) and by the Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (Project No. 2010–0020141).

References

1. Darise M, Mizutani K, Kasai R, Tanaka O, Kitahata S, Okada S, *et al*. 1984. Enzymic transglucosylation of rubusoside and the structure-sweetness relationship of steviol-bisglycosides. *Agric. Biol. Chem.* **48:** 2483-2488.
2. George Thompson AM, Lancu CV, Naguyen TT, Kim D, Choe JY. 2015. Inhibition of human GLUT1 and GLUT5 by plant carbohydrate products; insights into transport specificity. *Sci. Rep.* **5:** 12804.
3. Ishikawa H, Kitahata S, Ohtani K, Ikuhara C, Tanaka O. 1990. Production of stevioside and rubusoside derivatives by transfructosylation of β-fructofuranosidase. *Agric. Biol. Chem.* **54:** 3137-3143.
4. Kim YM, Yeon MJ, Choi NS, Chang YH, Jung MY, Song JJ, Kim JS. 2010. Purification and characterization of a novel glucansucrase from *Leuconostoc lactis* EG001. *Microbiol. Res.* **165:** 384-391.
5. Kitahata S, Ishikawa H, Miyata T, Tanaka O. 1989. Production of rubusoside derivatives by transgalactosylation of various α-galactosidases. *Agric. Biol. Chem.* **53:** 2929-2934.
6. Ko JA, Jeong HJ, Ryu YB, Park SJ, Wee YJ, Kim D, *et al*. 2012. Large increase in *Leuconostoc citreum* KM20 dextransucrase activity achieved by changing the strain/inducer combination in an *E. coli* expression system. *J. Microbiol. Biotechnol*. **22:** 510-515.
7. Lee HY, Jung KH. 2014. Enzymatic synthesis of 2-phenoxyethanol galactoside by whole cells of β-galactosidase-containing *Escherichia coli*. *J. Microbiol. Biotechnol*. **24:** 1254-1259.
8. Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*. **31:** 426-428.
9. Moon YH, Kim G, Lee JH, Jin XJ, Kim DW, Kim D. 2006. Enzymatic synthesis and characterization of novel epigallocatechin gallate glucosides. *J. Mol. Catal. B Enzym*. **40:** 1-7.
10. Nguyen TT, Jung SJ, Kim HK, Kim YM, Moon YH, Kim H, Kim D. 2014. Production of rubusoside from stevioside by using a thermostable lactase from *Thermus thermophiles* and solubility enhancement of liquiritin and teniposide. *Enzyme Microb. Technol*. **64-65:** 38-43.
11. Ohtani K, Aikawa Y, Ishikawa H, Kasai R, Kitahata S, Mizutani K, *et al*. 1991. Further study on the 1,4-alpha-transglucosylation of rubusoside, a sweet steviol-biglucoside from *Rubus suavissimus*. *Agric. Biol. Chem.* **55:** 449-453.
12. Seo ES, Kim D, Robyt JF, Day DF, Kim DW, Park HJ, Park HJ. 2004. Modified oligosaccharides as potential dental plaque control materials. *Biotechnol. Prog*. **20:** 1550-1554.
13. Su D, Robyt JF. 1993. Control of the synthesis of dextan and acceptor-products by *Leuconosotc mesenteroides* B-512FM dextransucrase. *Carbohydr. Res*. **248:** 471-476.
14. Tanaka O. 1997. Improvement of taste of natural sweeteners. *Pure Appl. Chem*. **69:** 675-683.
15. Tanaka T, Kohda H, Tanaka O, Chen FH, Chou WH, Leu JL. 1981. Rubusoside (β-D-glucosyl ester of 13-*O*-β-D-glucosyl-steviol), a sweet principle of *Rubus chingii* Hu (Rosaceae). *Agric. Biol. Chem.* **45:** 2165-2166.
16. Yoon SH, Robyt JF. 2002. Synthesis of acarbose analogues by transglycosylation reactions of *Leuconostoc mesenterioides* B-512FMC and B-742CB dextransucrase. *Carbohydr. Res*. **24:** 2427-2435.
17. Zhang F, Koh GY, Hollingsworth J, Russo PS, Stout RW, Liu Z. 2012. Reformulation of etoposide with solubility-enhancing rubusoside. *Int. J. Pharm*. **434:** 453-459.