

Supporting Information

Characterization of the first α -(1→3) branching sucrases of GH70 family

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Table S1: Ratios and amounts of sucrose and dextran used to synthesize α -(1→3) branched dextrans

Ratio	5.6	3	1	0.5	0.25	0.1	0.05
Sucrose (g L ⁻¹)	170	150	100	50	25	10	5
Dextran (g L ⁻¹)	30	50	100	100	100	100	100

Table S2: Main sequence features of the putative branching sucrases

Enzyme	Organism	Size ^a	SP ^b	BRS-A query			BRS-B query		
				% id ^c	% si ^d	% QC ^e	% id	% si	% QC
BRS-C	<i>Ln. fallax</i> KCTC3537	1774 aa 197.1 kDa	Yes	49%	64%	99%	54%	69%	99%
BRS-D	<i>Lb. kunkei</i> EFB6	1463 aa 163.6 kDa	No	56%	70%	69%	50%	63%	69%

^a The size is given in amino acids (aa) and in kDa

^b SP : Signal peptide

^c %id : percentage of identity
^d %si : percentage of similarity
^e QC : Query coverage

Table S3. Expression system (vector and expression host) used to produce recombinant branching sucrose (BRS-C and BRS-D) and their production level in *E. coli* cells

	Expression system		Production level (U L ⁻¹ of culture)
	Vector	Host	
BRS-C	pET-55-DEST	<i>E. coli</i> BL21 star DE3	2000
BRS-D	pET28b	<i>E. coli</i> BL21 star DE3	150

Table S4. Reaction on sucrose (292 mM) using BRS-C and BRS-D branching enzymes. Comparison of the production yields.

	Leucrose yield	Glucose yield	Small oligosaccharide yield
BRS-C	49%	44%	7%
BRS-D	6%	76%	18%

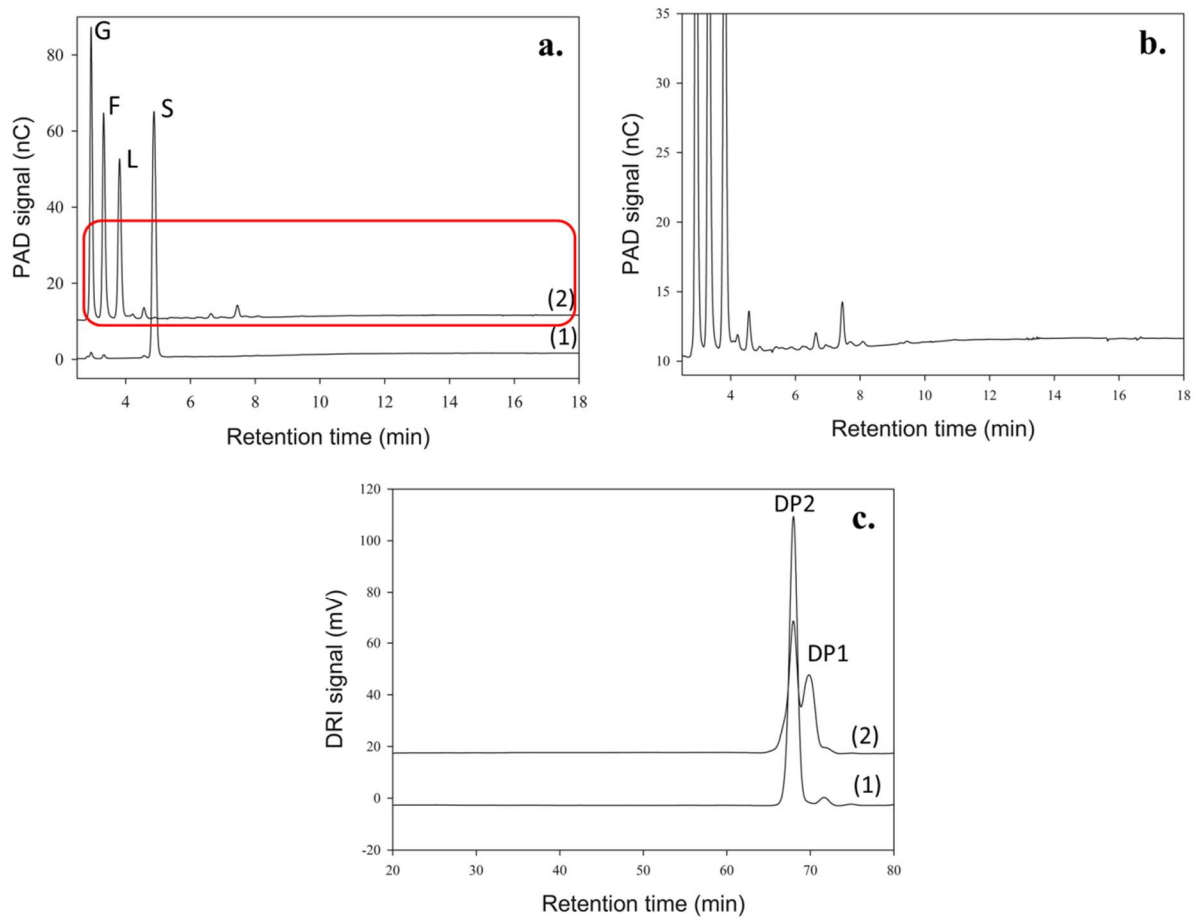


FIGURE S1. Analysis of the products synthesized using BRS-B enzyme from sucrose (292 mM). (a) HPAEC-PAD chromatograms of (1) Enzymatic reaction stopped after 1 minute and (2) Enzymatic reaction stopped after 8 hours. G indicates peak corresponding to glucose, F, fructose, L, leucrose and S, sucrose. (b) Enlargement of HPAEC-PAD chromatograms, corresponding to the red frame in the chromatogram (a). (c) HPSEC-RI profile of (1) Enzymatic reaction stopped after 1 minute and (2) Enzymatic reaction stopped after 8 hours. DP1 indicates peak corresponding to monosaccharides (glucose and fructose) and DP2 indicates peak corresponding to disaccharides (sucrose and leucrose).

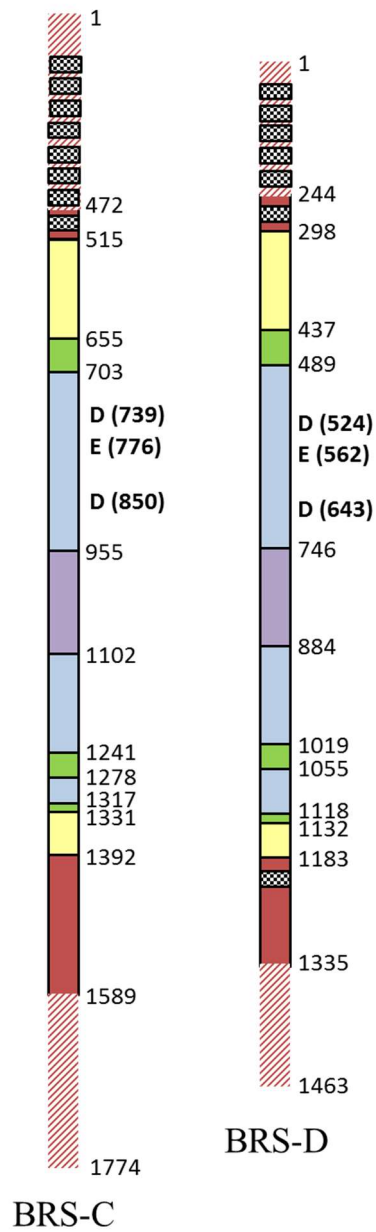


FIGURE S2. Schematic organization of the three putative branching sucrases (BRS-C and BRS-D) based on protein sequence alignment with GTF-180-ΔN. Domain V is represented in red, domain IV in yellow, domain B in green, domain A in blue and domain C in purple. Catalytic residues are highlighted in bold and YG repeats are indicated with chequered motifs. Hatched red lines represent zones where no 3D structure information is available for GTF-180-ΔN

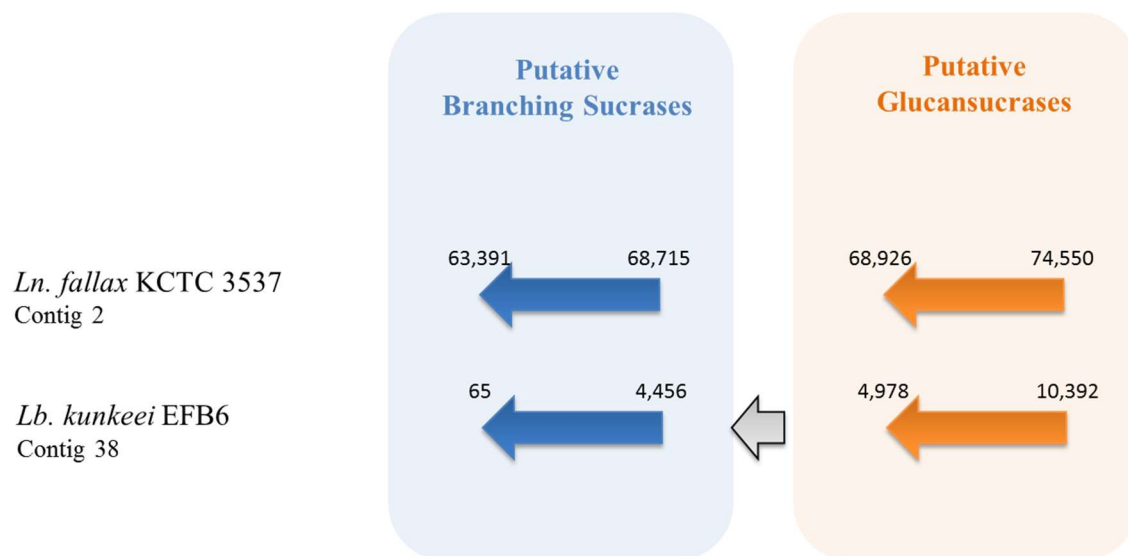


FIGURE S3. Representative tandem organization of the three putative branching sucrases (BRS-C and BRS-D) with other putative glucansucrases.