

Supporting information

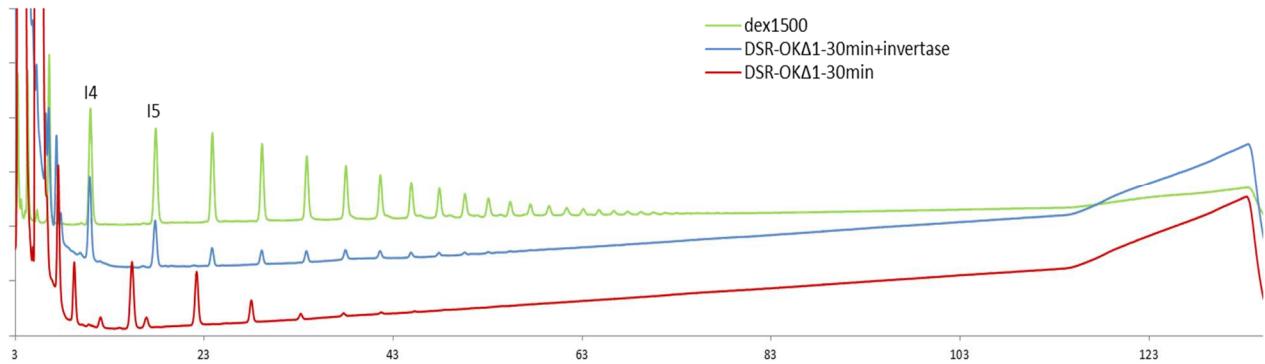


Figure S1: Invertase digestion. HPAEC-PAD chromatogram of products formed after 30 min of reaction on 292 mM sucrose by DSR-OKΔ1, before (red) and after (blue) digestion with invertase. Commercial dextran 1500 is shown as standard (green).

N-terminal

		Helix		
DSR- Δ 2	NNEFIYFGLD-GVGQSAIEYQFEKGLTS	ONSVATSENAAKSYDTKSFTNVGFLTANSWY	451	
3KLK	GNDWIYFKDGTAGTNAKLQFDKGTTI	ADEOYRRGNEAYSYDDKSIEVNGLYTADTWY	824	
4AMC	GNNWIYFDSDTGVGTNALELQFAKGTV	SSNEQYRNGNAAYSYDDKSIEVNGLYTADTWY	823	
3AIE		SEAOVQIVSTPDAEHDVHLYTAESIWY	722	

DSR-MA2	RPTDILRNGTKWE PSTE TD FRL LMT WWP DKEV QANY LN YM S AL GLG --- DQ KI YT GASS	509
3KLK	RP KI QILK DGT TWT DSK ETD MPR I L M VWW PNT V T QAY YL N YM K Q YG N LL P AS L P S F ST DAD	884
4AMC	RP KI QILK DGT TWT DSK ETD MPR I L M VWW PNT L T QAY YL N YM K Q H G N LL P AS L P F FN A DAD	883
3AIE	RP KI YL KDG K TWT Q S TE KDF RPL L M T WWP D Q E T Q R Q YV N YM N A Q L G I --- H Q T Y N T A T S	328
DSR-OKA1	RP A D I L E N G T D W R A S R A D E F R I L T T W W P D K Q T E V N Y L N Y M K T Q G F I T N --- D Q D F K L S D D	316

DSR-ΔM2	QLDLNNAALIVQEAIKKISLEKSTKWLDDSIKSFIKSKRKDIQGNLVDTNPQWTIDSET	569
3KLK	SAELNHYSELVQQNIEKRISETGSTDWLRTLMEFV-----TKNSMWNKDSEN	932
4AMC	PAELNHYSEIVQQNIEKRISETGNTDWLRTLMLHDVF-----TNNPMWNKDSEN	931
3AIE	PLQLNLAAQTIQTKEEKKITAEEKNTNWLRQITISAFTV-----KTQSAPWNSDCEK	376
DSR-OKΔ1	QLLLNHAQSVQGEIJKKISQGQSTDWLTLTLLQTFI-----NQQPSWNGESED	364

C-terminal

DSR-MA2	AKYFNGNSNIQKGKINYVLDWASNKYFNVSSNDDMSYSLPKQIMNQE---SNTGFIVD-DI	1325
3KLK	AKYFNGTNIHLHGAGYVLRSLNDGKYYNLGTS---TQQLPSQISVQDNEGY-GFVKE-GN	1647
4AMC	AKYFNGTNIHLHGSGYVLKADGGQYYNLGTT---TKQFLPIQTGEKKQGNEGFVKGNQ	1654
3AIE	AKYFNGTNIHLGRGAGYVLDQATNTYFSLVSD---NTFLPKSIIVNP-----	1087
DSR-OKΔ1	AKYFNGNSNIQGFAGAYVLRDSLTDQYFKVTSNDENEALFLPKQITNQP---GETGFSQD-DQ	1082

Figure S2: Sequence alignment of DSR-OKΔ1 and DSR-MΔ2 with GH70 glucansucrases whose structures have been solved (3KLK: GTF180-DN; 4AMC: GTFA-DN; 3AIE: GTF-SI). Blue boxes correspond to the helix at the beginning of domain IV and red lines indicate the swapping areas. In red, domain V; in green, domain B; in yellow, domain IV.

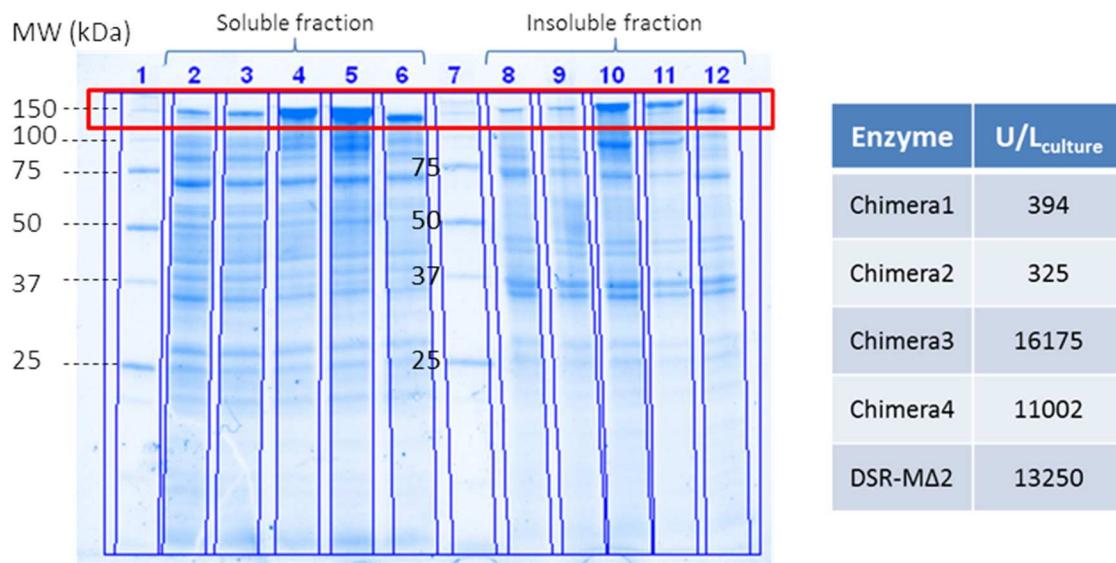


Figure S3: Expression levels of chimeric enzymes in comparison with DSR-MΔ2. Lanes 1 and 7: PrecisionPlus Biorad standard, Lanes 2-6: soluble fractions, Lanes 8-12: insoluble fractions. Lanes 2 and 8: chimera 1, lanes 3 and 9: chimera 2, lanes 4 and 10: chimera 3, lanes 5 and 11: chimera 4, lanes 6 and 12: DSR-MΔ2.

Table S1: Expression levels in the soluble fraction of DSR-OKΔ1 variants

Enzyme	Expression level (U/Lculture)
DSR-OK	18962
DSR-OKΔ1	18709
Y1162A_D	5397
F1228A_E	8392
Y1162A-F1228A_DE	1276
Y1354A_F	13074
Y1420A_G	17750

Table S2: Primers used for the construction of the variants of this study (deletion or mutagenesis)

Primer Name	Nucleotide Sequence	Main features
DSR-OKΔ1for	GGAGATATAACCATGGCACATGGCGAGTTGTTAAGG	Amplification of <i>dsrokΔ1</i> gene
DSR-OKΔ1rev	CCTAACAAACTGCCATGTGCCATGGTATATCTCC	
DSR-OKΔVfor	CACCCAAAATGATGATTTCACAG	Deletion of entire domain V
DSR-OKΔVrev	GCCGGGCTGATTAGTCAGC	
DSR-OKΔ4for	CACCATGGCACATGGCGAGTTG	Deletion of domain V at Cterm end
DSR-OKΔ4rev	GCCGGGCTGATTAGTCAG	
DSR-OKΔ3for	GCCGCCCTTCACCATGGCACATGGCGAGTTGTTAAGG	Deletion of pockets D, E, F and G
DSR-OKΔ3rev	GGCGCGCCCACCCTTATTAGCAACCTGCCGGCCTTTGATC	
DSR-OKΔ2for	GCCGCCCTTCACCATGGCACATGGCGAGTTGTTAAGG	Deletion of pockets F and G
DSR-OKΔ2rev	GGCGCGCCCACCCCTTGTTGAAAACCGGTACAATCTGCC	
Y153Arev	GCGCGTTATTAACGTGTTTC	Mutation of Y153 in pocket B
Y153Afor	GTTAATAACGCCGCCACAG	
F1098Arev	CTTGGACAGCAGCGTTTG	Mutation of F1098 in pocket C
F1098Afor	CGCTGCTGTCCAAGGCGATG	
Y1162Afor	CTAATCAGGCTGTGACTGATAC	Mutation of Y1662 in pocket D
Y1162Arev	GTTCGTATCAGTCACAGCCTG	
F1228Afor	GTCAACGAAGCCAAGGGC	Mutation of F1228 in pocket E
F1228Arev	CGCTGCCCTGGCTTCG	
F1354Afor	TGATCACAAATCGTGCATGCGC	Mutation of F1354 in pocket F
F1354Arev	CCGGCGCATGGCACGATTG	
Y1420Afor	GACGCCTCTCCCCAGAC	Mutation of Y1420 in pocket G
Y1420Arev	GGAGAAGGCGTCATTGTGAC	

Table S3: Primers used for chimera constructions

		Primer name	Primer sequence	template	size of the fragment (bp)
Chimera 1	PCR 1	DSROK-IV Fwd	GTGTTGCTACAAGTCAT ATGCCGTTATTGACC	pET55- <i>dsrok</i>	2538
		DSROK-IV Rev	TATTTGACTCTTGATTCA CAGCTGTTAGGCAGAAATG		
	PCR 2	pET-55-DSRM-V Fwd	ATGAATCAAGAGTCAAATACTG	pET55- <i>dsrmΔ1</i>	6740
		pET-55-DSRM-V Rev	ATGACTTGTAGCAACACTG		
Chimera 2	PCR 1	DSROK Fwd	GACACCAACCCAGGG TGGAATGGGGAAAGTGAAGATCC	pET55- <i>dsrok</i>	2079
		DSROK Rev	GTCTTTAAGACGTAGTTGAT CCCACGGCCCTGGATGTT		
	PCR 2	pET55-DSRM-IV-V Fwd	ATCAACTACGTCTAAAAGACTGG	pET55- <i>dsrmΔ1</i>	7231
		pET55-DSRM-IV-V Rev	CCCTGGGTTGGTGTCTAC		
Chimera 3	PCR 1	DSRM-IV Fwd	GATGATTTCACAGCTCAT AATGCTGCAAAGTCTTATGATACCAA AAG	pET55- <i>dsrmΔ1</i>	2685
		DSRM-IV Rev	GCCGGGCTGATTAGT AAGCTGCTGGGCAGACG		
	PCR 2	pET55-DSROK-V Fwd	ACTAACAGCCCCGGCGAA	pET55- <i>dsrok</i>	7166
		pET55-DSROK-V Rev	ATGAGCTGTGAAATCATCATTG		
Chimera 4	PCR 1	DSRM Fwd	AACCAGCAGCCCTCT TGGACGATTGATAGTGAAAC	pET55- <i>dsrmΔ1</i>	2194
		DSRM Rev	CGCGCAGCACATAATAAGCT CCTTTCCCTGAATGTTAG		
	PCR 2	pET55-DSROK-IV-V Fwd	GCTTATTATGTGCTGCGC	pET55- <i>dsrok</i>	7625
		pET55-DSROK-IV-V Rev	AGAGGGCTGCTGGTTGAT		