Supporting information

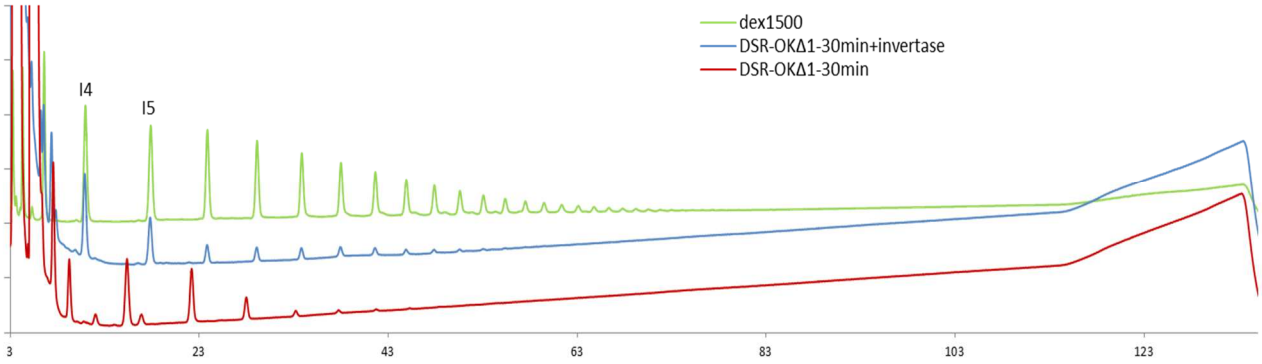
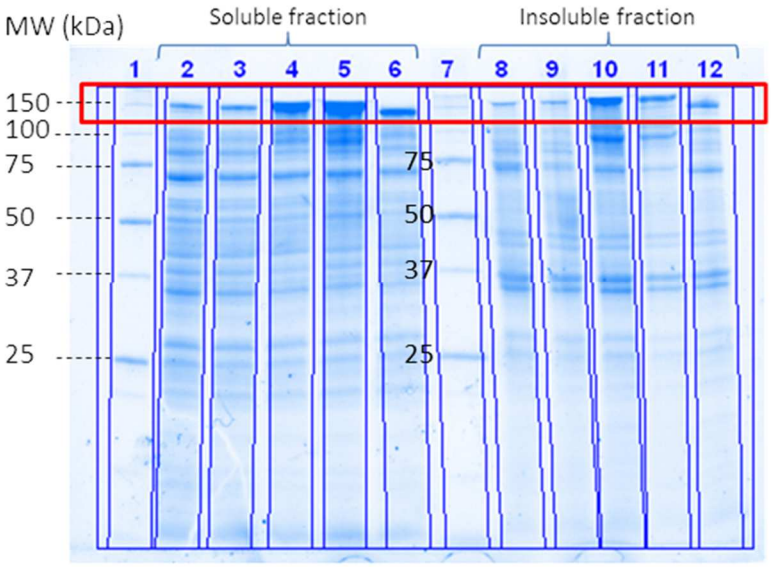


Figure S1: Invertase digestion. HPAEC-PAD chromatogram of products formed after 30 min of reaction on 292 mM sucrose by DSR-OKΔ1, before (red) and after (blue) digestion with invertase. Commercial dextran 1500 is shown as standard (green).

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.



Enzyme	$U/L_{culture}$
Chimera1	394
Chimera2	325
Chimera3	16175
Chimera4	11002
DSR-MΔ2	13250

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	GGAGATATACCATGGCACATGGCGAGTTTGTTAAGG	Amplification of *dsr*okΔ1 gene
DSR-OKΔ1rev	CCTTAACAAACTCGCCATGTGCCATGGTATATCTCC	
DSR-OKΔVfor	CACCCAAAATGATGATTTCACAG	Deletion of entire domain V
DSR-OKΔVrev	GCCGGGCTGATTAGTCAGC	
DSR-OKΔ4for	CACCATGGCACATGGCGAGTTTG	Deletion of domain V at Cterm end
DSR-OKΔ4rev	GCCGGGCTGATTAGTCAG	
DSR-OKΔ3for	GCCGCCCCCTTCACCATGGCACATGGCGAGTTTGTTAAGG	Deletion of pockets D, E, F and G
DSR-OKΔ3rev	GGCGCGCCCACCCTTATTAGCAACCTGCCGGCCTCTTTGATC	
DSR-OKΔ2for	GCCGCCCCCTTCACCATGGCACATGGCGAGTTTGTTAAGG	Deletion of pockets F and G
DSR-OKΔ2rev	GGCGCGCCCACCCTTTGTTTGAAAACCGGTCACAATCTGCC	
Y153Arev	GCGGCGTTATTAACTGTTTC	Mutation of Y153 in pocket B
Y153Afor	GTTAATAACGCCGCCACAG	
F1098Arev	CTTGGACAGCAGCGTTTTTGG	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	CGCTGCCCTTGGCTTCG	
F1354Afor	TGATCACAAATCGTGCCATGCGC	Mutation of F1354 in pocket F
F1354Arev	CCGGCGCATGGCACGATTTG	
Y1420Afor	GACGCCTTCTCCCCAGAC	Mutation of Y1420 in pocket G
Y1420Arev	GGAGAAGGCGTCATTTGTGAC	

Table S3: Primers used for chimera constructions

		Primer name	Primer sequence	template	size of the fragment (bp)
Chimera 1	PCR 1	DSROK-IV Fwd	GTGTTGCTACAAGTCATAATGCCGTTTATTCGACC	pET55-*dsrok*	2538
		DSROK-IV Rev	TATTTGACTCTTGATTCATCAGCTGTTTAGGCAGAAATG		
	PCR 2	pET-55-DSRM-V Fwd	ATGAATCAAGAGTCAAATACTG	pET55-*dsrmΔ1*	6740
		pET-55-DSRM-V Rev	ATGACTTGTAGCAACACTG		
Chimera 2	PCR 1	DSROK Fwd	GACACCAACCCAGGGTGGAATGGGGAAAGTGAAGATCC	pET55-*dsrok*	2079
		DSROK Rev	GTCTTTTAAGACGTAGTTGATCCCACGGCCCTGGATGTT		
	PCR 2	pET55-DSRM-IV-V Fwd	ATCAACTACGTCTTAAAAGACTGG	pET55-*dsrmΔ1*	7231
		pET55-DSRM-IV-V Rev	CCCTGGGTTGGTGTCTAC		
Chimera 3	PCR 1	DSRM-IV Fwd	GATGATTTCACAGCTCATAATGCTGCAAAGTCTTATGATACCAAAAG	pET55-*dsrmΔ1*	2685
		DSRM-IV Rev	GCCGGGCTGATTAGTAAGCTGCTTGGGCAGACG		
	PCR 2	pET55-DSROK-V Fwd	ACTAATCAGCCCGGCGAA	pET55-*dsrok*	7166
		pET55-DSROK-V Rev	ATGAGCTGTGAAATCATCATTTTG		
Chimera 4	PCR 1	DSRM Fwd	AACCAGCAGCCCTCTTGGACGATTGATAGTGAAAC	pET55-*dsrmΔ1*	2194
		DSRM Rev	CGCGCAGCACATAATAAGCTCCTTTTCCCTGAATGTTAG		
	PCR 2	pET55-DSROK-IV-V Fwd	GCTTATTATGTGCTGCGC	pET55-*dsrok*	7625
		pET55-DSROK-IV-V Rev	AGAGGGCTGCTGGTTGAT		