

Supplementary Materials

Table S1. List of Lsc3 mutants and mutagenic oligonucleotides used in the study. Mutated codon is indicated on grey background and mutated nucleotides are underlined. Publications addressing the mutants are indicated. Some of mutants were designed to mimic respective mutants of *Zymomonas mobilis* levansucrase [1]. Mutagenesis was performed as shown in section 3.1 of the main text and in Ref [2].

Mutation in Lsc3	Mutagenic Primer	Primer Sequence (5'→3')	Origin
Asp31Asn (D31N)	D31NRev	ACCTTCAGCGCAT <u>T</u> GGCG	This work
Trp61Ala (W61A)	W61ARev	ATGGTGTCC <u>G</u> GATGAATACCG	[3]
Trp61Asn (W61N)	W61NRev	ATGGTGT <u>C</u> GTTGATGAATACCG	[3]
Asp62Ala (D62A)	D62ARev	CATGGTGG <u>C</u> CCAGATGAAT	[3]
Thr63Ala (T63A)	T63ARev	AGCGGCATGG <u>C</u> GTCCAGATG	[3]
Leu66Ala (L66A)	L66ARev	TCTCGC <u>G</u> CCGGCATGGTG	[3]
Trp109Ala (W109A)	W109ARev	TGTCGATCTTCC <u>G</u> CGTCACGC	This work
Trp109Phe (W109F)	W109FRev	TGTCGATCTT <u>C</u> AAAGTCACGC	This work
Trp109Arg (W109R)	W109RRev	TGTCGATCTTCC <u>C</u> GGTCACGC	[1]; this work
Glu110Asp (E110D)	E110DRev	ATGTCGATC <u>G</u> TCCCAGTCACGC	This work
His113Ala (H113A)	H113ARev	CGCGGCCA <u>G</u> CTCGATCTTCC	This work
His113Gln (H113Q)	H113QRev	GCGTGC <u>G</u> CGGCC <u>T</u> TGTCG	This work
Glu146Gln (E146Q)	E146QRev	GGTGTGCCCCGCCA <u>C</u> TGTCGAGTGGTGG	[1]; this work
Asp219Ala (D219A)	D219ARev	ACTGGGG <u>G</u> CGCGAAAGTTC	[3]
Pro220Ala (P220A)	P220ARev	ATGAACGGACTGG <u>C</u> GTGCGG	[3]
Asp225Ala (D225A)	D225ARev	CATCATTAGGG <u>G</u> CAATGAACGGAC	[3]
Asp225Asn (D225N)	D225NRev	CATCATTAGGAT <u>A</u> AATGAACGGAC	[3]
Glu236Gln (E236Q)	E236QRev	TCACCGGCGACATTG <u>C</u> CTGAAACACCA	[1]; this work
Val248Ala (V248A)	V248AFw	TTCGCACACAG <u>C</u> AGGGGTTGC	[1]; this work
Asp300Ala (D300A)	D300AFw	GGCGTGAATG <u>C</u> GACACCGAG	This work
Asp300Asn (D300N)	D300NFw	TGAAT <u>A</u> ATCAGACCGAGCGCC	[2]
Gln301Ala (Q301A)	Q301AFw	TGAATGATG <u>C</u> GACCGAGCGC	[3]
Gln301Glu (Q301E)	Q301EFw	TGAATGATGAGACCGAGCGC	[3]
Thr302Met (T302M)	T302MFw	AATGATCAGATG <u>G</u> GAGCGCCC	[3]
Thr302Pro (T302P)	T302PFw	AATGATCAG <u>C</u> CCGAGCGCCC	[2]
Glu303Ala (E303A)	E303AFw	TGATCAGACCG <u>C</u> GCGCC	[3]
Glu303Gln (E303Q)	E303QFw	TGATCAGAC <u>C</u> CAGCGCCCCG	This work
Arg304Ala (R304A)	R304AFw	TCAGACCGAGG <u>C</u> CCCCGCACTATG	[3]
Arg304Cys (R304C)	R304CFw	TCAGACCGAGT <u>G</u> CCCCGCACTATG	[3]
His306Ala (H306A)	H306AFw	AGCGCCC <u>G</u> CCATGTTTTTCAAG	[3]
His321Leu (H321L)	H321LFw	CACCATCAGT <u>C</u> TGAAGTTCACGTATGCC	[2]
His321Lys (H321K)	H321KFw	CACCATCAGT <u>A</u> AAAAGTTCACGTATGCC	[2]
His321Arg (H321R)	H321RFw	CACCATCAGT <u>C</u> GCAAGTTCACGTATGCC	[2]
His321Ser (H321S)	H321SFw	CACCATCAGT <u>A</u> GCAAGTTCACGTATGCC	[2]
Asp333Ala (D333A)	D333AFw	GGGCCAG <u>C</u> GGGGTGTACGG	This work
Asp333Asn (D333N)	D333NFw	GGGCCA <u>A</u> ACGGGGTGTACGG	[1]; this work

Figure S1. Typical high-performance liquid chromatography (HPLC) analysis of reaction products of Lsc3 wild-type (Lsc3 wt; upper panel) and a mutant with reduced catalytic activity (e.g. Leu66Ala; lower panel) conducted using CTAB-permeabilized cells (violet line) or purified protein (red line). Peaks corresponding to fructose (F), glucose (G), sucrose (S) and FOS with degree of polymerization (DP) 3 to 6 are designated. Detection sensitivity was reduced to fully accommodate the peaks of glucose and sucrose. Due to that, FOS with DP > 5 cannot be seen on the chromatogram. Lsc3 wt and Leu66Ala mutant both synthesize FOS with DP up to 7 (see Table 1 of the main text and Ref [3]). Reactions were performed as shown in sections 3.5 and 3.6 of the main text and products were analyzed by HPLC as in section 3.9 and Ref [3]. LSU – light scattering unit determined on evaporative light scattering (ELS) detector.

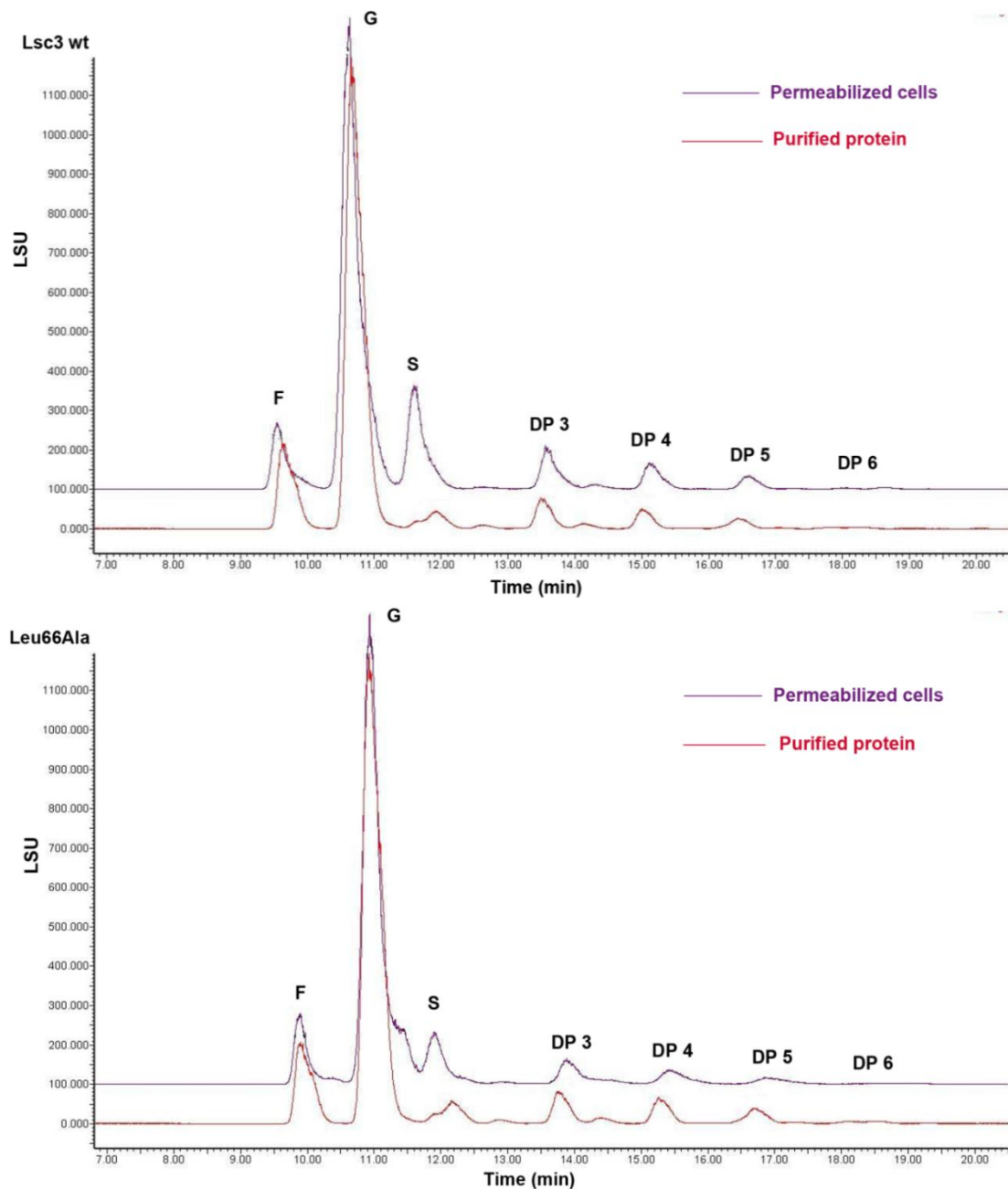


Figure S2. Alignment of protein sequences of 22 levansucrases. Fragment containing the position corresponding to Asp31 of *Pseudomonas syringae* pv. tomato levansucrase Lsc3 is shown and Asp31 residue is indicated as # above the MUSCLE [4] alignment. Sequences were extracted from UniProt [5] database. Levansucrase protein sequences of following bacteria are shown: *Pseudomonas syringae* pv. phaseolicola, *Serratia odorifera*, *Pseudomonas fluorescens*, *Rahnella aquatilis*, *Erwinia amylovora*, *Pseudomonas chlororaphis* subsp. *aurantiaca*, *Zymomonas mobilis*, *Gluconobacter oxydans*, *Gluconacetobacter diazotrophicus*, *Beijerinckia indica*, *Burkholderia phymatum*, *Burkholderia pseudomallei*, *Clostridium acetobutylicum*, *Streptomyces viridochromogenes*, *Cellulomonas flavigena*, *Actinomyces viscosus*, *Halorubrum lacusprofundi*, *Halalkalicoccus jeotgali*, *Halomicrobium mukohataei*, *Haloarcula marismortui*, *Bacillus subtilis*.

		Asp31N											
		#											
Q88BN6	Lsc3 <i>P. s.pv.tomato</i>	IKYPP	TV---	WSRAD	ALK	VNEND	PTT--	-----	TPPLV	SAD	FFP	52	
Q48BC9	<i>P. s.pv.phaseolicola</i>	INYE	PTV---	WSRAD	ALK	VNEND	PTT--	-----	TPPLV	SAD	FFP	52	
D4E4H9	<i>S. odorifera</i>	VNYK	PTI---	WTRAD	ALK	VNEKD	PTT--	-----	TPPLV	SAD	FFP	36	
C3K8D6	<i>P. fluorescens</i>	ANHT	PTR---	WTRAD	ALK	VNEND	PTT--	-----	TPPLV	SPD	FFP	36	
O54435	<i>R. aquatilis</i>	LNYP	PTI---	WTRAD	ALK	VNEND	PTT--	-----	TPPIV	DAD	FFP	36	
Q46654	<i>E. amylovora</i>	YNYK	PTL---	WTRAD	ALK	VHEDD	PTT--	-----	TPPVI	DI	IAFFP	36	
Q93FU9	<i>P. c.subsp.aurantiaca</i>	APHQ	PSL---	WTRAD	ALK	VHADDP	TT--	-----	TPPLV	SAD	FFP	43	
P0DJA3	<i>Z. mobilis</i>	GIAE	PSL---	WTRAD	AMK	VHTDD	PTA--	-----	TMPTI	DYD	FFP	38	
Q5FSK0	<i>G. oxydans</i>	SSTQ	SATQSG	SRWT	ADAMK	IHTDD	PTT--	-----	TMPTI	DY	IAFFP	42	
Q43998	<i>G. diazotrophicus</i>	QAYD	PQSDFT	ARW	TRADALQ	IKAHS	DATVA	AGQNSLPAQL	TPNIN	PAD	FFP	125	
B2IF78	<i>B. indica</i>	QAYD	PQSDFT	AKW	TRANARQ	IKAQSH	VPVS	PDQNSLPLNL	TMPTI	PAD	FFP	90	
B2JVY2	<i>B. phymatum</i>	QAYD	PQSDFT	MRW	TRADIRQ	IKAQSH	AAATA	ADKNSLPLSL	TMPTI	PQD	FFP	100	
Q3JGQ0	<i>B. pseudomallei</i>	QAYD	PQSDFT	MRW	TRADMRQ	IVKQSH	TA-G	ADKNSLPPAL	TMPTI	IAQ	NFFP	95	
Q97I79	<i>C. acetobutylicum</i>	LILR	HNTGYT	SIWS	RQQAQN	FKCT	KEN--	-----	TAPNIN	PNFK		61	
D9X8L9	<i>S. viridochromogenes</i>	RQYT	ADDDHT	AVWS	RADALK	LKQDR	TN---	-----	TAPRV	SPD	FFP	77	
D5UCP7	<i>C. flavigena</i>	AVFS	PRSSYT	ANW	TRADALQ	IRQD	ASN---	-----	TKPRI	PAE	FFP	98	
F2V1I2	<i>A. viscosus</i>	AAHG	V EENYT	AKW	TRADAMQ	IQRV	SNPNAP	SGTNSMPEQL	TMPEI	ISNG	FFP	166	
B9LT89	<i>H. lacusprofundi</i>	GRSR	SR---	WTR	EQAAS	IERR	RGN---	-----	IAPP	AGAP	EL	40	
D8J9C2	<i>H. jeotgali</i>	GRAT	PR---	WTR	EHASK	IERT	DET---	-----	VVPI	IYPP	RE	37	
C7P4M9	<i>H. mukohataei</i>	GTAV	EGHGAR	SGW	SREQASR	IERT	DDT---	-----	TAPI	IYPP	PAT	43	
Q5V249	<i>H. marismortui</i>	GEGR	SGTKGR	AGW	TREQASR	IERT	DDT---	-----	VAPI	IYPP	QPT	43	
P05655	<i>B. subtilis</i>	KPYK	EYTGIS	H-ITR	ADMLO	IPEQ	QKNE--	-----	KY	QVE	EFDS	STI	73
Clustal Consensus				:	:	:	:	:	*				

References

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