Supplementary Materials

Mutants with enhanced cellobiose-fermenting ability from thermotolerant *Kluyveromyces marxianus* DMKU 3-1042, which are beneficial for fermentation with cellulosic biomass

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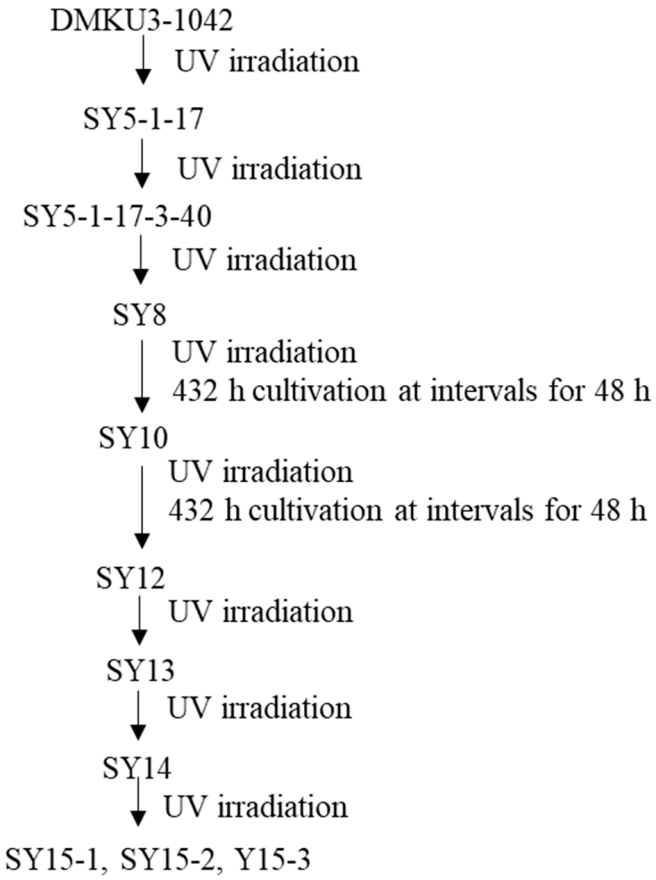

Figure S1. Schematic procedure for development of cellobiose-fermenting mutants from *K. marxianus* DMKU3-1042. The details are described in Materials and Methods.

Table S1. Mutations in protein-coding regions of isolated mutants.

Mutants	Gene	Product	Amino acid change	Function
SY10	KLMA_10393	LrgB superfamily	H → P	Uncharacterized protein
	KLMA_10684	Uncharacterized protein	T → P	Transporter
	YRM1	Zinc finger transcription factor YRR1	I → K	Transcription factor
	CDC55	Protein phosphatase PP2A regulatory subunit B	S → F	Cell cycle
	SLU7	pre-mRNA-splicing factor SLU7	Q insertion	Transcription factor
	RGT1	Glucose transport transcription regulator RGT1	Y → stop codon	
	UGA4	GABA-specific permease	F → L	Transporter
	CDC10	Cell division control protein 10	I → T	Cytoskeleton protein
	MDR1	GTPase-activating protein GYP2	Deletion	Membrane trafficking
	UBP12	Ubiquitin carboxyl-terminal hydrolase 12	E → G	Peptidases and inhibitor
	GAL1	Galactokinase	S → P	Galactose metabolism
	MID1	Stretch-activated cation channel MID1	P → S	Transporter
SY13	AMN1	Antagonist of mitotic exit network protein 1	K → Stop codon	Cell cycle
	SRP101	Signal recognition particle receptor subunit alpha	K → R	Signaling and cellular processes
	FKS3	1,3-beta-glucan synthase component GSC2	L → S	Starch and sucrose metabolism
	CSF1	Protein CSF1	L → F	Uncharacterized protein
	UPC2	Sterol regulatory element-binding protein ECM22	S → F	Uncharacterized protein
	KLMA_50610	Adenylosuccinate synthetase	K → E	Purine metabolism, Alanine,

				aspartate and glutamate metabolism
	SPT7	Transcriptional activator SPT7	L → M	Transcription factor
	KLMA_10393	LrgB superfamily	H → P	Uncharacterized protein
	KLMA_10684	Uncharacterized protein	T → P	Transporter
	YRM1	Zinc finger transcription factor YRR1	I → K	Transcription factor
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	RGT1	Glucose transport transcription regulator RGT1	Y → Stop codon	Transcription factor
	UGA4	GABA-specific permease	F → L	Transporter
	CDC10	Cell division control protein 10	I → T	Cytoskeleton protein
	MDR1	GTPase-activating protein GYP2	Deletion	Membrane trafficking
	UBP12	Ubiquitin carboxyl-terminal hydrolase 12	E → G	Peptidase and inhibitor
	GAL1	Galactokinase	S → P	Galactose metabolism
	MID1	Stretch-activated cation channel MID1	P → S	Transporter
SY14	HGT1	High-affinity glucose transporter	Y → Stop codon	Transporter
	NGL1	RNA exonuclease NGL1	F → S	Transcription
	LAC12	Lactose permease	M → L	Transporter
	KHT2	Hexose transporter 2	S → G	Transporter
	RAG1	Low-affinity glucose transporter	N → D	Transporter
	MEC1	Serine/Threonine-protein kinase MEC1	P → S	Cellular Processes
	KLMA_80374	Uncharacterized protein	P → L	Uncharacterized protein

	AMN1	Antagonist of mitotic exit network protein 1	K → Stop codon	Cell cycle
	SRP101	Signal recognition particle receptor subunit alpha	K → R	Signaling and cellular processes
	FKS3	1,3-beta-glucan synthase component GSC2	L → S	Starch and sucrose metabolism
	CSF1	Protein CSF1	L → F	Uncharacterized protein
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	GAL1	Galactokinase	S → P	Galactose metabolism
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SY15-1	PRP16	Pre-mRNA-splicing factor ATP-dependent	M → I	Peptidase and inhibitor

	RNA helicase PRP16		
RSN1	Uncharacterized protein RSN1	W → Stop codon	Uncharacterized protein
KLMA_30481	Glyoxalase super family protein	S → L	Uncharacterized protein
HKR1	Glycoprotein	T → P	Cell wall
HGT1	High-affinity glucose transporter	Y → Stop codon	Transporter
NGL1	RNA exonuclease NGL1	F → S	Transcription
LAC12	Lactose permease	M → L	Transporter
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	MID1	Stretch-activated cation channel MID1	P → S	Transporter
SY15-2	RCY1	Recyclin-1	N → I	Ubiquitin system
	CCT4	T-complex protein 1 subunit delta	G → N	Signaling and cellular processes
	HGT1	High-affinity glucose transporter	Y → Stop codon	Transporter
	NGL1	RNA exonuclease NGL1	F → S	Transcription
	LAC12	Lactose permease	M → L	Transporter
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SY15-3	ILV2	Acetolactate synthase	R → P	Valine, leucine and isoleucine biosynthesis
	GAR1	H/ACA ribonucleoprotein complex subunit 1	S → C	Translation
	HGT1	High-affinity glucose transporter	Y → Stop codon	Transporter
	NGL1	RNA exonuclease NGL1	F → S	Transcription

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