

Supplementary material for:

Engineering natural *S. cerevisiae* isolates for enhanced one-step cellulosic ethanol production

Letitia Sabina Minnaar ^a, Kentaro Inokuma ^b, Tomohisa Hasunuma ^{b, c}, and Riaan den Haan* ^{a, d}

^a Institute for Microbial Biotechnology and Metagenomics, Department of Biotechnology, University of the Western Cape, Bellville, 7530, South Africa

^b Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan

^c Engineering Biology Research Center, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan

^d Department of Microbiology, Stellenbosch University, Stellenbosch, 7600, South Africa

*Corresponding author

Table S1 Plasmids used in this study

Plasmid	Description and application	Reference
pRDH180	Contains <i>ENO1_P</i> and <i>ENO1_T</i> , and <i>T.reg2</i> gene cassette (used to produce homology repair template DNA for <i>T.reg2</i> integration to be expressed as secreted free enzyme)	Brevnova et al. (2011)
pMI529	Contains <i>ENO1_P</i> and <i>ENO1_T</i> , and <i>T.e.cbh1</i> gene cassette (used to produce homology repair template DNA for <i>T.e.cbh1</i> integration to be expressed as secreted free enzyme)	Ilmén et al. (2011)
pMU784	Contains <i>PGK1_P</i> and <i>PGK1_T</i> , and <i>C.l.cbh2</i> gene cassette (used to produce homology repair template DNA for <i>C.l.cbh2</i> integration to be expressed as secreted free enzyme)	Ilmén et al. (2011)
pIBG-SSAD	Contains <i>SED1_P</i> and <i>DIT1_T</i> , and <i>A.a.bgl1</i> gene cassette (used to produce homology repair template DNA for <i>A.a.bgl1</i> integration to be expressed as cell-tethered enzyme)	Inokuma et al. (2016)
pIAUR-CICBH2b-SSSD	Contains <i>SED1_P</i> and <i>DIT1_T</i> , and <i>C.l.cbh2</i> gene cassette (used to produce homology repair template DNA for <i>C.l.cbh2</i> integration to be expressed as cell-tethered enzyme)	Inokuma et al. (2021)
pIL2EG-SSSD	Contains <i>SED1_P</i> and <i>DIT1_T</i> , and <i>T.reg2</i> gene cassette (used to produce homology repair template DNA for <i>T.reg2</i> integration to be expressed as cell-tethered enzyme)	Inokuma et al. (2016)
pIU5-TeCBH1c-SSSD	Contains <i>SED1_P</i> and <i>DIT1_T</i> , and <i>T.e.cbh1</i> gene cassette (used to produce homology repair template DNA for <i>T.e.cbh1</i> integration to be expressed as cell-tethered enzyme)	Inokuma et al. (2021)
pCas9-NAT	Yeast episomal plasmid with <i>cas9</i> expression cassette	ADDGENE
pRS42-G-ChX	sgRNA scaffold plasmid that targets an intergenic region on Chromosome 10	Jacob et al. (2022)
pRS42-G-ChXI	sgRNA scaffold plasmid that targets an intergenic region on Chromosome 11	Kruger and Den Haan (2022)
pRS42-G-ChXII	sgRNA scaffold plasmid that targets an intergenic region on Chromosome 12	Jacob et al. (2022)
pRS42-G-Δ	sgRNA scaffold plasmid that targets delta sequences within the yeast genome	Jacob et al. (2022)
pRS42-G-CAN1	sgRNA scaffold plasmid that targets intergenic regions at <i>CAN1</i> sites	This laboratory

Table S2 Primers used for homology template and gene integration confirmations

Primer name	Sequence (5'-3')	Application
Homology repair template amplification		
Ch10.ENO1_p-L	GCAGTTATCTCTGTGTGYCCAGATCCCTT	Amplify homology repair template
Ch10.ENO1_T-R	CTACATGTAATTGTGCGGTGCAGGGAGG	DNA (<i>ENO1_p-T.reg2-ENO1_T</i>) with Chromosome 10 target homology
DELTA-ENO1-L	CTTAAGATGCTCTTCTTATTCTATTAAAAA	Amplify homology repair template
DELTA-ENO1-R	TAGAAAATGACTTCTAGGCGGGTATCTACTG	DNA (<i>ENO1_p-T.e.cbh1-ENO1_T</i>) with delta (Δ) target homology
DELTA-PGK1p-L	CTTAAGATGCTCTTCTTATTCTATTAAAAATAGAAAAT GATCCCTCCTTCTTGAATTG	Amplify homology repair template
DELTA-PGK1t-R	GTTTGTTTGCGAAACCTATGCTCTGTTGTTTCGGATT TGAAACGCAGAATTTTCGAG	DNA (<i>PGK1_p-C.l.cbh2-PGK1_T</i>) with delta (Δ) target homology
Ch11_SEDp-L	TGTA AACAGGTATTGGCTGCTTCATAGTACACCCAA	Amplify homology repair template
Ch11_DITt-R	TTGATTGGATATAGAAAATTAACGTAAGGCAGTATC GCAACTCTGAAATGTCAAACGGTCGTCGTATAAATA AATGTTACTCCGCAACGCTTTTCTG	DNA (<i>SED1_p-SS-A.a.bgl1-AD-DIT1_T</i>) with Chromosome 11 target homology
ChX_SEDp_L	GCAGTTATCTCTGTGTCCAGATCCCTTTGAAGTAAAG	Amplify homology repair template
ChX_DITt_R	TTTATTGGATATAGAAAATTAACGTAAGGCAGTATC CTACAGTAATTGTGCGGTGCAGGGAGGCAATGTTTA GTGCTTACTCCGCAACGCTTTTCTG	DNA (<i>SED1_p-SS-T.reg2-AD-DIT1_T</i>) with chromosome 10 target homology
DELTA_SEDp_L	CTTAAGATGCTCTTCTTATTCTATTAAAAATAGAAAAT GAATTGGATATAGAAAATTAACGTAAGGCAGTATC	Amplify homology repair template
DELTA_DITt_R	GTTTGTTTGCGAAACCTATGCTCTGTTGTTTCGGATT TGATTACTCCGCAACGCTTTTCTG	DNA (<i>SED1_p-SS-T.e.cbh1-AD-DIT1_T</i>) with delta sequence target homology
DELTA_SEDp_L	CTTAAGATGCTCTTCTTATTCTATTAAAAATAGAAAAT GAATTGGATATAGAAAATTAACGTAAGGCAGTATC	Amplify homology repair template
DELTA_DITt_R	GTTTGTTTGCGAAACCTATGCTCTGTTGTTTCGGATT TGATTACTCCGCAACGCTTTTCTG	DNA (<i>SED1_p-SS-C.l.cbh2-AD-DIT1_T</i>) with delta sequence target homology
Ch12_SED1p_L	GCGTCCTACAGCGTGATGAAAATTCGCCTGCTGCA	Amplify homology repair template
Ch12_DIT1t_R	AGATATTGGATATAGAAAATTAACGTAAGGCAGTATC CTGTCAAACCTTCTGAGTTGCCGCTGATGTGACACTGT GACTTACTCCGCAACGCTTTTCTG	DNA (<i>SED1_p-SS-C.l.cbh2-AD-DIT1_T</i>) with chromosome 12 target homology
CAN1_SEDp_L	GGTCAATACCATTGAAAGATGAGAAAAGTAAAGAAT	Amplify homology repair template
CAN1_DITt_R	TGTAATTGGATATAGAAAATTAACGTAAGGCAGTATC GGCAATCATACCAATATGTCTTTGCTTAAGCTCTCTCT TCTTACTCCGCAACGCTTTTCTG	DNA (<i>SED1_p-SS-C.l.cbh2-AD-DIT1_T</i>) with CAN1 site target homology
Confirmation of gene integrations		
ENO1_L	GTAACATCTCTCTTGTAATCCCTTATTCCTTCTAGC	Confirm integration of <i>ENO1_p-T.reg2-ENO1_T</i> in transformants
EGR-Rev	ATCTGGATTAGTAAGTGAAGACAAAGCAG	
ENO1_L	GTAACATCTCTCTTGTAATCCCTTATTCCTTCTAGC	Confirm integration of <i>ENO1_p-T.e.cbh1-ENO1_T</i> in transformants
CBH1R_Rev	TGTTGAGAGAAGTCGTCGGTGTAC	

SED1p_check-L	GACAAGCAAAATAAAATACGTTTCGCTC	Confirm integration of <i>SED1p-AaBGL1-DIT1_T</i> in transformants
AaBGL1_check-R	GCTTGGCTGTCGCCACG	
C.l.cbh2_L	AGTCTTAATTAAACAATGGCCAAGAAGTTGTTC	Confirm integration of <i>PGK1p-C.l.cbh2-PGK1_T</i> in transformants
C.l.cbh2_R	AGTCGGCGCGCCTTAGAATGGTG	
SED1p_check-L	GACAAGCAAAATAAAATACGTTTCGCTC	Confirm integration of <i>SED1p-SS-Tr.eg2-AD-DIT1_T</i> in transformants
SED1anchor-R	GGAAGAAGCAGATGTACTGTTGG	
SED1p_check-L	GACAAGCAAAATAAAATACGTTTCGCTC	Confirm integration of <i>SED1p-SS-Te.cbh1-AD-DIT1_T</i> in transformants
CBH1R-Rev	TGTTGAGAGAAGTCGTCGGTGTAC	
SED1p_check-L	GACAAGCAAAATAAAATACGTTTCGCTC	Confirm integration of <i>SED1p-SS-C.l.cbh2-AD-DIT1_T</i> in transformants
J-cbh2-check-R	AATGCATATACGGTTAGCTCATGGTATG	

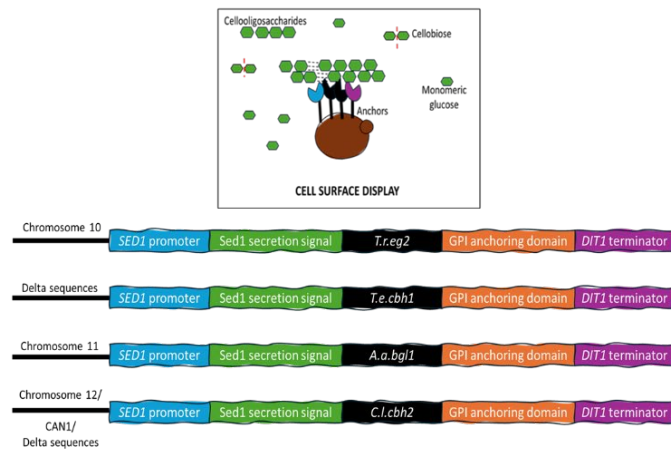
Table S3 Yeast strains constructed in this study with their respective collection numbers at the Biobanks South Africa Yeast Culture Collection at the Department of Microbiology and Biochemistry, University of the Free State.

S/N	Accession Form Nr	UFS Collection Nr	Other Nr (Strain Nr)
<i>S. cerevisiae</i> MH1000 transformed with <i>Tr.EG2</i>, <i>A.a.BGL1</i>, <i>Te.CBH1</i>, & <i>C.l.CBH2</i>	3070	UOFS Y-3424	MH1000_BECC/MH1000_V1
<i>S. cerevisiae</i> YI13 transformed with <i>Tr.EG2</i>, <i>A.a.BGL1</i>, <i>Te.CBH1</i>, & <i>C.l.CBH2</i>	3071	UOFS Y-3425	YI13_BECC/YI13_V1
<i>S. cerevisiae</i> YI59 transformed with <i>Tr.EG2</i>, <i>A.a.BGL1</i>, <i>Te.CBH1</i>, & <i>C.l.CBH2</i>	3072	UOFS Y-3426	YI59_BECC/YI59_V1

Figure S1: Schematic illustration depicting the strain construction strategy and the engineered metabolic pathways implemented in this study.

A:

① Multi-gene expression in *S. cerevisiae*



B:

② Substrate hydrolysis and hexose fermentation

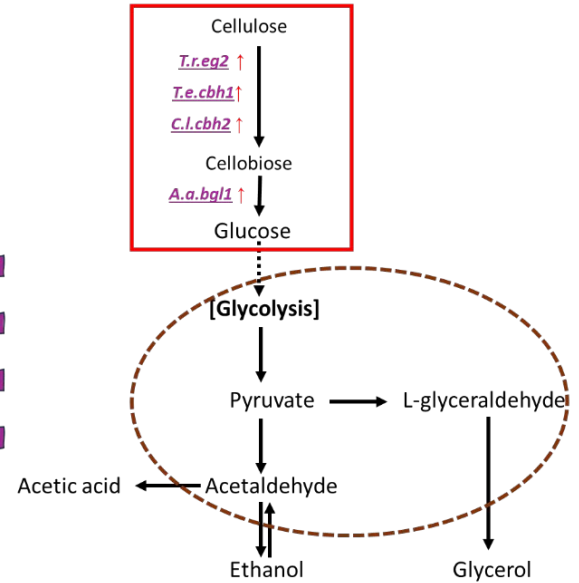


Fig. S1 Schematic illustration depicting the strain construction strategy and the engineered metabolic pathways implemented in this study. (A) the cell surface tethered engineering strategy used, (B) the engineered metabolic module involves heterologous cellulase expression, enabling the hydrolysis of crystalline cellulose into glucose, which can subsequently be assimilated and metabolised by the cell for growth and ethanol production. Please refer to the main text for details of the cellulase genes used.

Figure S2 Enzyme activity for wild-type strains

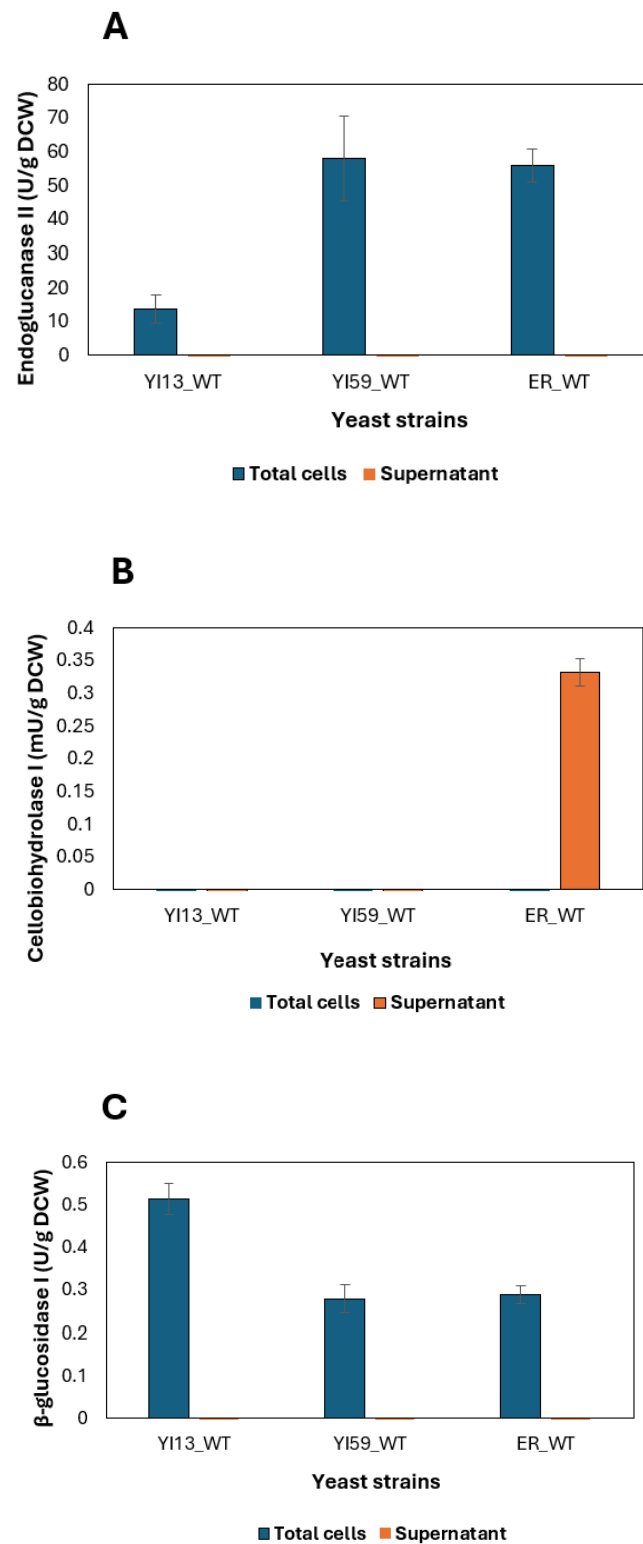


Fig. S2 Background cellulase activities of the parental yeast strains used in this study. (A) endoglucanase activity, (B) cellobiohydrolase activity, (c) β -glucosidase activity. As all of these strains show practically no activity in comparison with transformed strains, this data was omitted from Fig. 1 in the main text.

Figure S2 TEM images

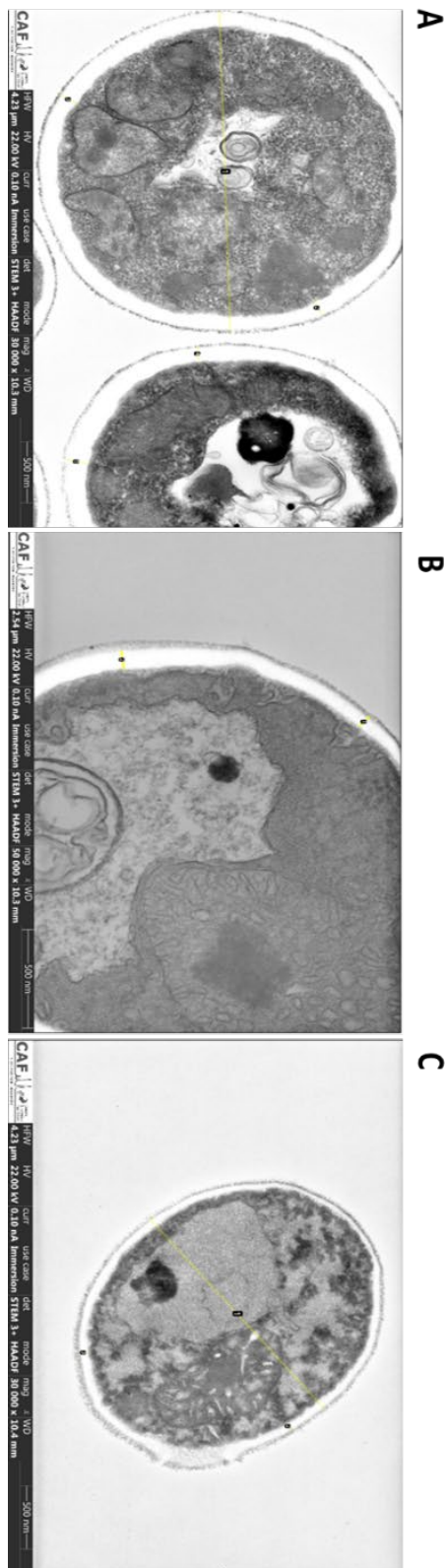


Fig. S3 Cell wall thickness determined with transmission electron microscopy. Yeast strains were cultivated in normal growth conditions until stationary phase. Cells were subjected to microscopic analysis whereby cells were dissected to ultimately determine the thickness of the cell wall. Images show representative individual cells in a population for each of the variant of the YI59 background, namely, (A) YI59_WT, (B) YI59_V1, and (C) YI59_V2

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