Supplementary Infromation

Construction and optimization of efficient glucose-xylose co-fermenting yeast *Yarrowia lipolytica* for green and sustainable succinic acid production from lignocellulosic biomass

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# These authors contributed equally to this work.

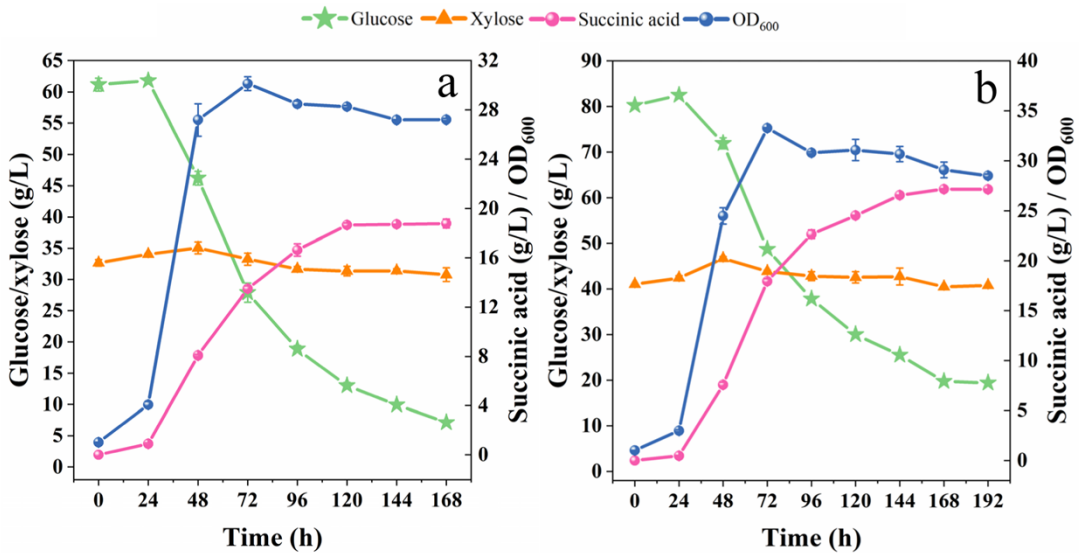

Fig. S1 Time-course profiles of sugar consumption, cell growth (OD_{600}), SA production during shake flask culture of *Y. lipolytica* BAS4332 on YPD60X30 (a) and YPD80X40 (b) medium.

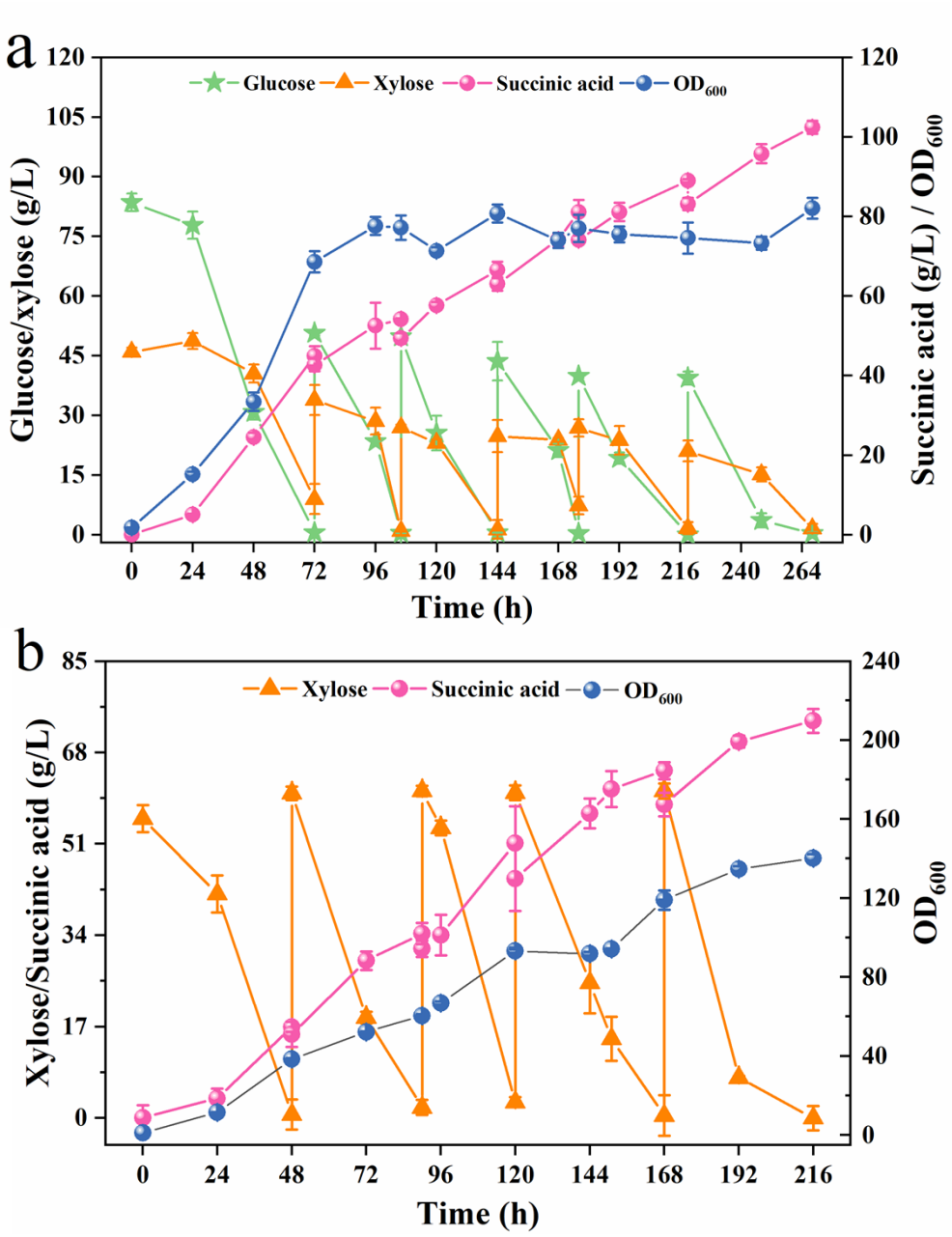

Fig. S2 Fed-batch fermentation profile of engineered *Y. lipolytica* strain BDic5 using glucose-xylose mixture (a) or pure xylose (b) as carbon resources in shake flasks.

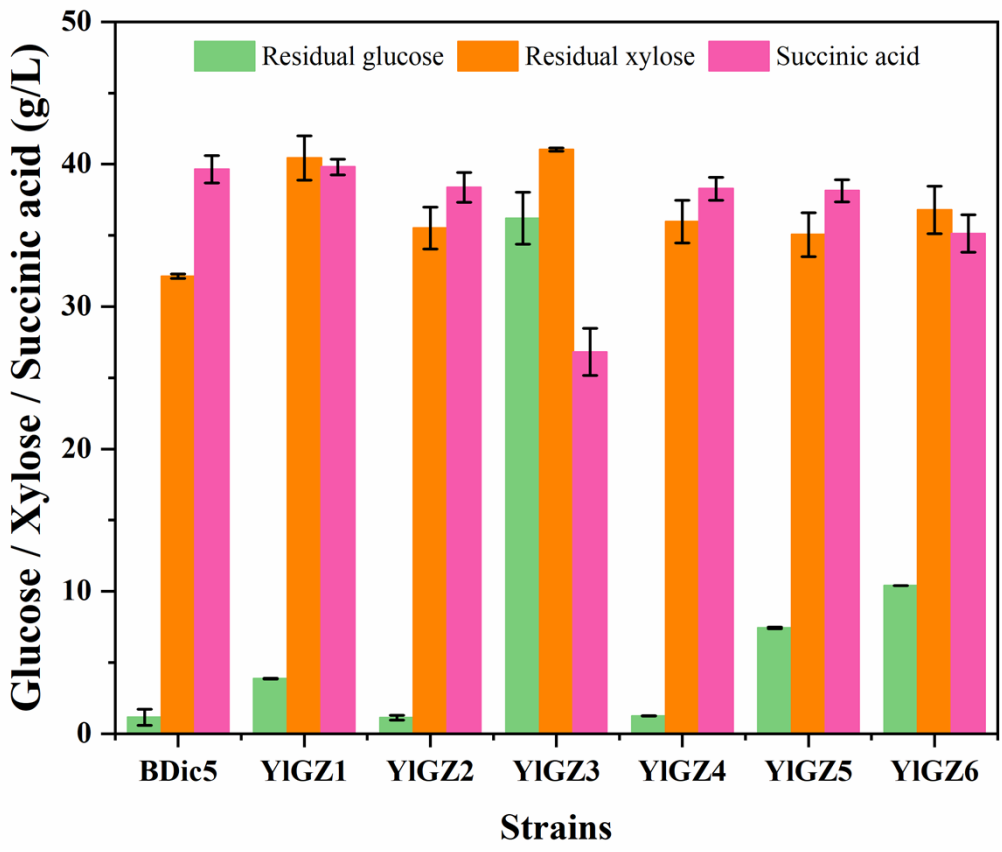

Fig. S3 Sugar consumption and SA production after 72 h of fermentation with different strains. Strains YlGZ1-6 represent overexpressing the *Yht1-6* genes in *Y. lipolytica* BDic5.

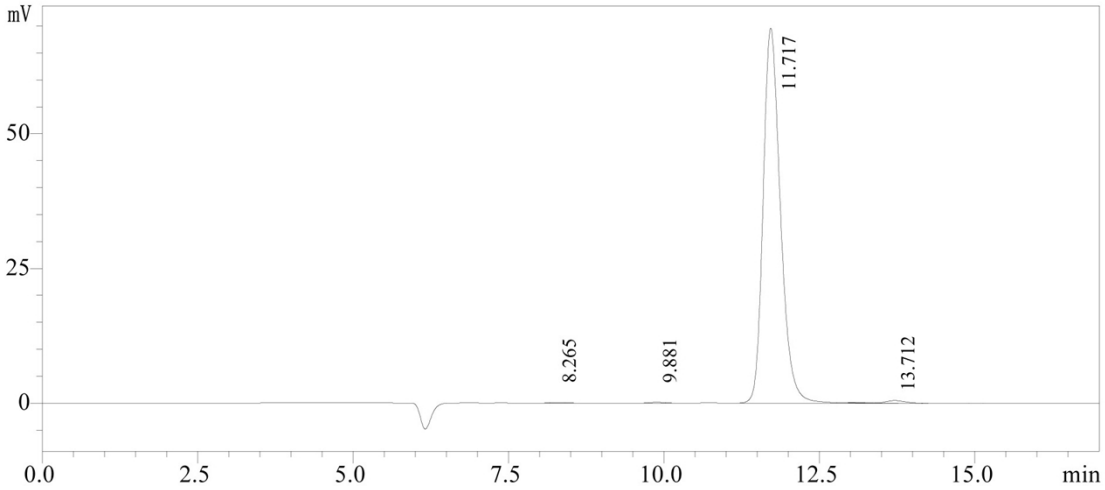

Fig. S4 HPLC chromatogram for recoverd SA from fermentation broth.

Table S1 Sugar concentrations and main inhibitors present in DLCA(sa)-CS hydrolysates with different solid loading.

Solid loading	Glucose (g/L)	Xylose (g/L)	Formic acid (g/L)	Acetic acid (g/L)	Hydroxymethylfurfural (g/L)	Furfural (g/L)	Levulinic acid (g/L)	Phenols (g/L)
20%	70.49 ± 0.12	40.02 ± 0.25	0.39 ± 0.02	1.86 ± 0.05	0.56 ± 0.04	N. A	1.47 ± 0.05	0.84 ± 0.04
25%	84.90 ± 0.24	57.22 ± 0.34	0.46 ± 0.03	2.21 ± 0.06	0.69 ± 0.09	N. A	1.64 ± 0.05	0.99 ± 0.05
30%	103.91 ± 0.39	70.97 ± 0.08	0.55 ± 0.02	2.65 ± 0.08	0.80 ± 0.08	N. A	1.84 ± 0.06	1.18 ± 0.06
35%	124.50 ± 0.29	85.39 ± 0.64	0.66 ± 0.04	3.14 ± 0.08	0.94 ± 0.07	N. A	1.96 ± 0.10	1.41 ± 0.07

N. A: data not available

Table S2 Primers used in this study for gene expression and deletion.

Primers	Primer sequences (5'-3')	Functions
UP-Sdh-F	AGTGAATTATTTAAATGCCACAAGTTTGCATCTA TTCTGTACATT	*YlSdh5* deletion
UP-Sdh-R	CACCACTGGAAGATCCGGGAATTCGTTTAAACG CAGGTCGGTTTCGA	*YlSdh5* deletion
DN-Sdh-F	TTAATTAAGGTACCAAGCTTGCGGCCGCTGCTGT CGCGATACGCCAA	*YlSdh5* deletion
DN-Sdh-R	TATTTAAATGCATGCGGCTCTGGCCAGATTGGG GATTAAGGATGAC	*YlSdh5* deletion
UP-Ach-F	ACGACGGCCAGTGAATTATTTAAATGCTATTCTT ACGGTGTACAGTTACG	*YlAch* deletion
UP-Ach-R	ACCACTGGAAGATCCGGGAATTCGTTTAAACTG TGTGAGATGGGGTAGTAC	*YlAch* deletion
DN-Ach-F	ACAGCTTAATTAAGGTACCAAGCTTGCGGCCGC ACACTATATAAGAATGTATTTATTTT	*YlAch* deletion
DN-Ach-R	ACAGCTATGACCATGATTACGCCAAGCTATTTA AATGACGCGAGTAGGTTGACTCATA	*YlAch* deletion
Pck-F	TTTTTGCAGTACTAACCGCAGATGTCCCCTTCTA AAATGAATG	amplification of *ScPck*
Pck-R	GTGACATAACTAATTACATGATTACTCGAATTGA GGACCAGCG	amplification of *ScPck*
Scs-F	TTTTTGCAGTACTAACCGCAGATGTTTTCGCGAA TTGCTGCTCGATCTCGAGCTGTT	amplification of *YlScs*
Scs-R	AGGCCATGGAGGTACCTGGATCCTAAAGCTTTA AAGAGGCAGCTCAAAGGAGACG	amplification of *YlScs*
Dic-F	GCAGTACTAACCGCAGATGTCATCTTTACAAAA ACACTTACCCATGGAGAGCAA	amplification of *YlDic*
Dic-R	GCGTGACATAACTAATTACATGACTAATGTCGC ATTCCAATCTTGTAGAACTTGA	amplification of *YlDic*
Mae-F	TGCAGTACTAACCGCAGATGACCACACCTCAAC CACGAGCAATGA	amplification of *YlMae*
Mae-R	GCGTGACATAACTAATTACATGATTACTGCTCCA AAGGATCAGTTT	amplification of *YlMae*
Yht1-F	GCAGTACTAACCGCAGATGGGACTCGCTAACAT CATCAACCGTG	amplification of *YlYht1*
Yht1-R	GGCAACGTGGGGACAGGCCATGGACTAGACAG ACTCAATGTAGA	amplification of *YlYht1*
Yht2-F	TTTGCAGTACTAACCGCAGATGGCCATTATTGTG	amplification of

	GCTGTATTTGTGGCTTTTGGA	*YlYht2*
Yht2-R	GCAAGACCGGCAACGTGGGGACAGGCCATGGA CTAATCCGAATCAAATCCAGAAT	amplification of *YlYht2*
Yht3-F	GCAGTACTAACCGCAGATGTCCACTAGTGCTAT GACCGACGATT	amplification of *YlYht3*
Yht3-R	CAACGTGGGGACAGGCCATGGACTAAGAGGACT CGGAGAAGT	amplification of *YlYht3*
Yht4-F	GCAGTACTAACCGCAGATGGCGAGGCTTTGTCT TTCTCAAACGG	amplification of *YlYht4*
Yht4-R	GGGGACAGGCCATGGATTAAACAGTCTCGGTGT ACTGAGGATG	amplification of *YlYht4*
Yht5-F	GCAGTACTAACCGCAGATGTACAAGGTCCATAA CCCCTACCTCA	amplification of *YlYht5*
Yht5-R	TGGGGACAGGCCATGGATTAGACATGCTCAGTT CCAGGATACTG	amplification of *YlYht5*
Yht6-F	GCAGTACTAACCGCAGATGATTGGAAACGCTCA AATTAACCAGG	amplification of *YlYht6*
Yht6-R	GTGGGGACAGGCCATGGATTACAATTGAGAGGG AGGGGCGTC	amplification of *YlYht6*
Glk-F	TTTGCAGTACTAACCGCAGATGACAATCACTCTG AGTCAGAAGGT	amplification of *YlGlk*
Glk-R	GACAGGCCATGGACTATGAGTCGTCCGGGTAAG CAGCCAAGG	amplification of *YlGlk*
Hxk-F	GCAGTACTAACCGCAGATGGTTCATCTTGGTCCC CGAAAACCCC	amplification of *YlHxk*
Hxk-R	GGGGACAGGCCATGGACTAAATATCGTACTTGA CACCGGGCTT	amplification of *YlHxk*