

Table S1 The primers used in this study.

Primers	Sequence (5'-3')	Function
XDH F	cggttgagcatccgttgattccgaaccatatgagagaccgggtggcggcgattttgt	ssXDH expression cassette for pUC-X
XDH R	aaatacgccgcaaccggctctctgtttaacaaacccaaaaggccgaaggctgg	ssXR expression cassette for pUC-X
XR F	cagccttcggccctttgggtttatttaaatagagaccgggtggcggcgatttt	ylXX expression cassette for pUC-X
XR R	gcaggacatcctactcgtcgcggccgcgcaaattaaagccttcgagcgtcc	ylXX expression cassette for pUC-X
XK F	ggacgctcgaaggcttaatttgcgcccgcgtttaacagagaccgggtggcggcg	expression cassette for pUC-X
XK R	cgtagggacagccatggaggtacccccagctttttctccaggcaggcgttttcg	expression cassette for pUC-X
TEF F	tagcatacattatacgaagttatacgcgtagagaccgggtggcggcgattttgt	ICL expression cassette for pUC-I
ICL-TEF R	cttggttaggtagcagcaccatctcgggttagtactgcaaaaagtgtg	ICL expression cassette for pUC-I
ICL F	cagcactttttgcagtactaacccagatgccgaacagcagcgattcaaca	AMPD expression cassette for pUC-A
ICL R	ggcaacgtggggacagccatggaggtaccttaaagcttgacttgaactgttc	AMPD expression cassette for pUC-A
AMPD-TEF R	tgatatcattgcttctcggcatctcgggttagtactgcaaaaagtgtg	YHMP expression cassette for pUC-Y
AMPD F	cagcactttttgcagtactaacccagatgccgcagcaagcaatggatatca	YHMP expression cassette for pUC-Y
AMPD R	ggcaacgtggggacagccatggaggtaccttaaagcttgacttgaactgttc	YHMP expression cassette for pUC-Y
YHMP-TEF R	cttggttaggtagcagcaccatctcgggttagtactgcaaaaagtgtg	Cit1 expression cassette for pUC-C1
YHMP F	tgagcgacctgttgtaagcactagcactggccggtcgataatttaactg	Cit2 expression cassette for pUC-C2
YHMP R	ccgtttaccgcgcgcttggttgaatttgaattcaaacccaaaaggccgaaggctggg	<i>DGA1</i> gene knockout cassette for pUC-ΔDGA1-
Cit1-TEF R	cacgggcggttcgaaggggatcatctcgggttagtactgcaaaaagtgtg	<i>DGA1</i> gene knockout cassette for pUC-ΔDGA1-
Cit1 F	cagcactttttgcagtactaacccagatgatccctctcgaaccgcccgtg	HUH
CIT R	gcaacgtggggacagccatggaggtaccttatttggcgaccttaata	HUH
Cit2-TEF R	acggcgggacgaatagcagaatcatttgaatgattcttatactcagaaggaaatg	HUH
Cit2 F	tgagtataagaatcattcaaaatgattctgctattctcccgcggttcgatctt	<i>DGA2</i> gene knockout cassette for pUC-ΔDGA2-
Cit2 R	agcgtgacataactaattacatgacctaggttagagcttgaggccaacgagctca	<i>DGA2</i> gene knockout cassette for pUC-ΔDGA2-
1-AMP F	tgtagcttaaacagagagtgatgtgtatcgaatttaaatagcttggcgtaatcat	HUH
1-AMP R	acgcctccatctatagattccccctgtggccctgtatttaataattcactggccgctg	HUH
DGA1UP F	acgttgtaaaacgacggccagtgaaattttaataacagggccacagggggaatctata	HUH
DGA1UP R	atgcaccactggaagatccgggaattcgtttaacagcttttgtttgtgtgacttgc	HUH
DGA1DN F	gaacagcttaattaaggtaccaagcttgcggccgcggaaaactgcctgggttaggcaaa	HUH
DGA1DN R	cagctatgacctgattacgccaagctatttaaatcgatacacatctcactctcgttg	HUH
2-AMP F	actccgtttccaatcagcaccgaggccaactcgtaatttaaatagcttggcgtaatcat	HUH
2-AMP R	tcgtagagcagttcagctcgtactcgcacgatccatttaataattcactggccgctg	HUH
DGA2UP F	gacgttgtaaaacgacggccagtgaaattttaaatggatcgtcaggtacgagctgaa	HUH
DGA2UP R	atgcaccactggaagatccgggaattcgtttaactttgcggcggttacgggtacagcg	HUH
DGA2DN F	gaacagcttaattaaggtaccaagcttgcggccgcataacactcatcagtagccttta	HUH
DGA2DN R	cagctatgacctgattacgccaagctatttaaatcaggttggcctcgggtgctgatt	HUH

R		
A-AMP F	agctccaggctctcgaatagactccatttaaatagcttggcgtaatcatggtcatagct	
A-AMP R	agactctctggtgcataccatctgcaatttaaatattcactggccgctcgttttac	<i>ACL</i> gene
ACLUP F	cgttgtaaacgacggccagtgaaatttttaattgcagatggtatgcaccagagagtc	knockout
ACLUP R	caccactggaagatccgggaattcgtttaaacgtctgatgaggtgagttaaggggga	cassette
ACLDN F	gaacagcttaattaaggtaccaagcttgcggccgatctaggtgatagaatatatag	for pUC-
ACLDN R	ctatgacctgattacgccaagctatttaaatggagtctatcgagagcctggagct	Δ ACL-
S-AMP F	actcctgttccaatcagcaccgaggccaactgtaatttaaatagcttggcgtaatcat	HUH
S-AMP R	tcgtagagcagtttcagctcgtactcgacgatccATTTAAATAattcactggccgct	
SCFUP F	ttgtaaacgacggccagtgaaatttttaaatgacactggtgtccatgcagcatga	<i>y/SCF</i> gene
SCFUP R	cactggaagatccgggaattcgtttaaaccttggagagtgtatgagttgtgag	knockout
SCFDN F	gcttaattaaggtaccaagcttgcggccgctcgatcccatccgattttggc	cassette
SCFDN R	ctatgacctgattacgccaagctatttaaatagatcgtagcctcgtcctccc	for pUC-
A2-TEF F	cttaattaaggtaccaagcttgcagagaccgggttggcggcgt	<i>y/SCF</i> -
Yht1-TEF	gttgatgatgtagcagtgccatctgcggtagtagtactgcaaaa	HUH
R		
Yht1 F	gcagtactaaccgagatgggactcgtaacatcatcaaccgtg	Yht1
Yht1 R	ggcaacgtggggacaggccatggactagacagactcaatgtaga	expression
Yht-XPR	ttgagtctgtctagtcctatggcctgtccccacgttcccgtctt	cassette for
F		pUC-Y1
A2-XPR	ttacgcagccgatcttaattaaggacacgggcatctcacttgcg	
R		
Yht2-TEF	aaatacagccacaafaatggccatctgcggtagtagtactgcaaaa	
R		
Yht2 F	ttgcagtactaaccgagatggccattatgtggctgtatttggcttttggga	Yht2
Yht2 R	gcaagaccggcaacgtggggacaggccatggactaatccgaatcaaatccagaat	expression
Yh2-XPR	ttgattcggattagtcctatggcctgtccccacgttcccgtctt	cassette for
F		pUC-Y2
Yht3-TEF	gtcggcatagcactagtgacatctgcggtagtagtactgcaaaa	
R		
Yht3 F	gcagtactaaccgagatgtccactagtgctatgaccgacgatt	Yht3
Yht3 R	caacgtggggacaggccatggactaagaggactcggagaagt	expression
Yh3-XPR	ccgagtctcttagtcctatggcctgtccccacgttcccgtctt	cassette for
F		pUC-Y3
Yht4-TEF	ttgagaaagacaaagcctcgcctatctgcggtagtagtactgcaaaa	
R		
Yht4 F	gcagtactaaccgagatggcgaggcttcttcttcaaacgg	Yht4
Yht4 R	ggggacaggccatggattaaacagtctcgggtactgaggatg	expression
Yh4-XPR	tcagtacaccgagactgttaatccatggcctgtccccacgttgc	cassette for
F		pUC-Y4
Yht5-TEF	ggtaggggttatggacctgtacatctgcggtagtagtactgcaaaaa	
R		
Yht5 F	gcagtactaaccgagatgtacaaggctcacaaccctacctca	Yht5
Yht5 R	tggggacaggccatggattagacatgctcagtccaggatactg	expression
Yh5-XPR	tcttgaactgagcatgtctaattccatggcctgtccccacgttgc	cassette for
F		pUC-Y5
Yht6-TEF	ggtaatttgagcgttccaatcatctgcggtagtagtactgcaaaaa	

R		
Yht6 F	gcagtactaaccgcagatgattggaacgctcaaattaaccagg	Yht6
Yht6 R	gtggggacaggccatggattacaattgagagggagggcgctc	expression
Yh6-XPR	ccccctcctcaattgtaatccatggcctgtccccacgttgcc	cassette for
F		pUC-Y6
Hxk1-TEF	tttcggggaccaagatgaaccatctgcggttagtactgcaaaaa	
R		
Hxk1 F	gcagtactaaccgcagatggttcatcttgggtccccgaaaacccc	Hxk1
Hxk1 R	ggggacaggccatggactaaatcgtacttgacaccgggctt	expression
Hxk1- XPR F	cggtgtcaagtacgatatttagtccatggcctgtccccacgttg	cassette for
E3-FBA F	caccactgcactaccactacacccatattggtacgtagcaacaacagtgtac	pUC-Y6
pfk-FBA	gaacaggtgcctgaggtggagccattgtgtgatgttagttaga	
R		
pfk F	agattcgaatctaaactacacatcacacaatggctccacctcaggcacctgttc	PFK1
pfk R	aagcgtgacataactaattacatgagtcgtagtgcggcacagacctgga	expression
pfk-cyc F	ctttccaggtctgtgccgacactacgactcatgtaattagttatgtca	cassette for
cyc R	gttcatgcaccactggaagatccgggaattcgaatgaaagccttcgagcgtccc	pUC-PFK

Table S2 Sugar and typical inhibitors contents in DLCA(sa) corn stover hydrolysates at different solid loading.

Solid loading	Sugar concentrations (g/L)		Inhibitors concentrations (g/L)					
	Glucose	Xylose	Formic acid	Levulinic acid	Furfural	HMF ²	Acetic acid	Phenols
25%	88.1±1.2	54.9±0.7	0.47±0.01	1.54±0.02	N.D ¹	0.72±0.03	2.83±0.06	0.92±0.01
30%	100.4±0.4	60.0±1.1	0.57±0.05	1.83±0.02	N.D ¹	0.91±0.05	3.40±0.02	1.15±0.06
35%	121.1±1.3	74.8±0.02	0.64±0.03	1.91±0.06	N.D ¹	1.10±0.08	3.86±0.07	1.38±0.04

1. N. D data not detected

2. Hydroxymethylfurfural

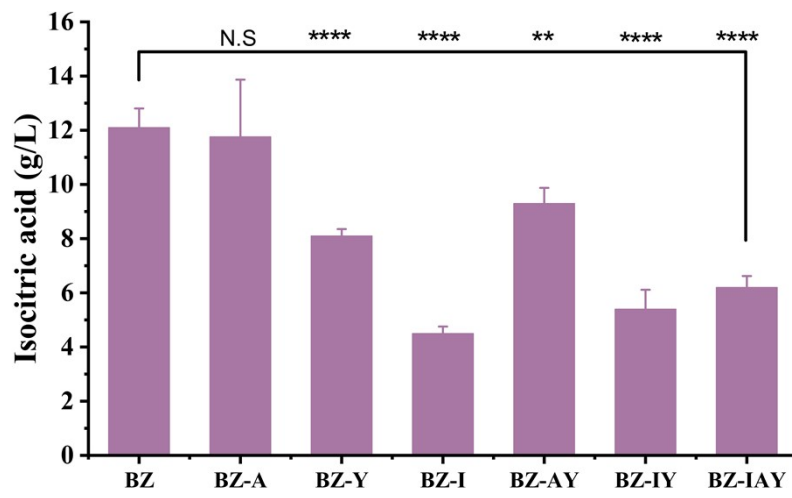


Fig. S1 Isocitric acid production of the engineered strain.

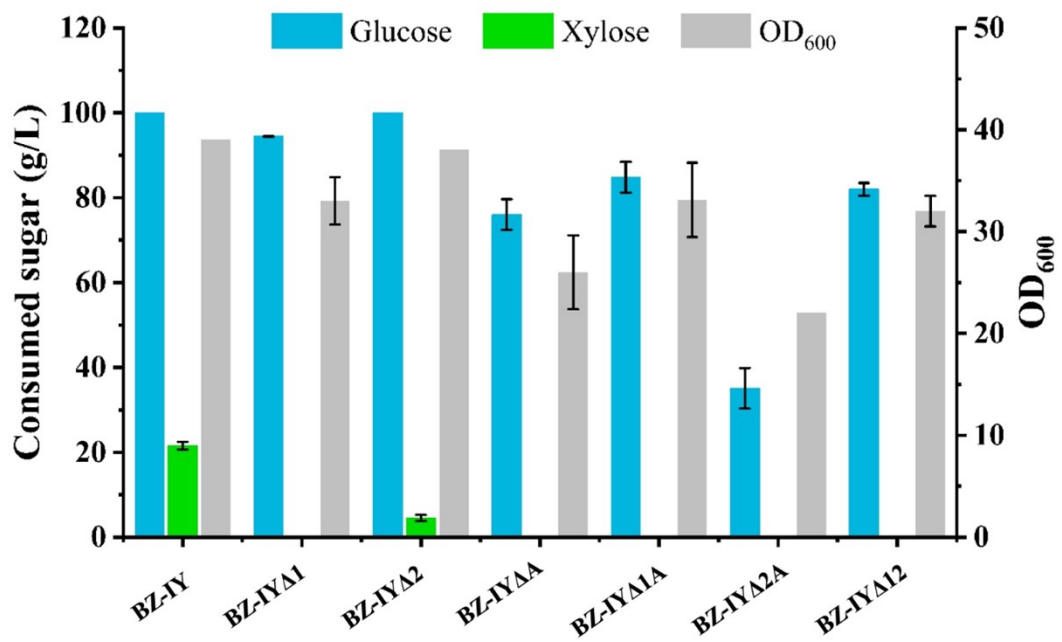


Fig. S2 The effect of deleting *DGA1*, *DGA2*, *ACL* genes on the sugar consumption and OD₆₀₀ of the strain BZ-IY.

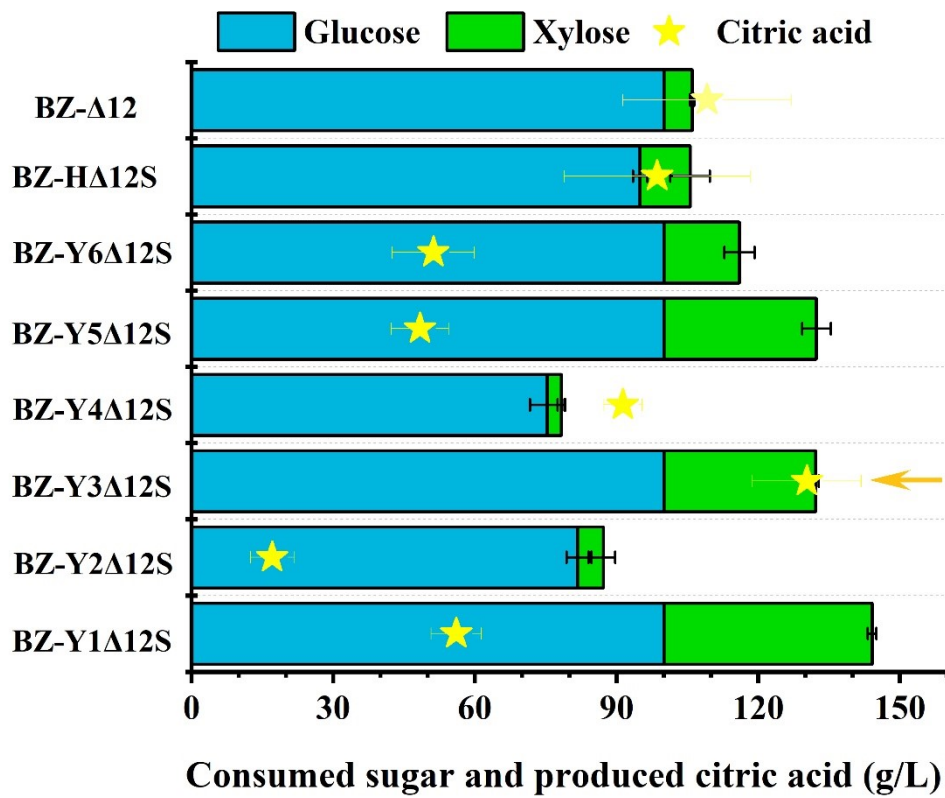


Fig. S3 The effect of overexpression different glucose transporter genes to the sugar consumption rate and citric acid production and in strain BZ-Δ12S.