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Data Article

Data for simultaneous fermentation of galacturonic acid and five-carbon sugars by engineered *Saccharomyces cerevisiae*



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ABSTRACT

Saccharomyces cerevisiae expressing heterologous pathways for xylose, arabinose, and galacturonic acid metabolism has been constructed by a Cas9-based genome editing technology [1]. The fermentation performance of the final strain (YE9) was tested under various substrate conditions, and the fermentation parameters were calculated. The dataset can be used for designing bioprocesses for pectin-rich biomass.

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1. Data

This dataset contains 1) the construction of engineered *Saccharomyces cerevisiae* strain (YE9) capable of fermenting galacturonic acid, arabinose, and xylose, and 2) its fermentation data with different carbon sources (galacturonic acid, arabinose, xylose, galactose, glucose, and fructose) and their mixtures, all of which present in pectin-rich biomass. In Fig. 1, the fermentation patterns of the YE9 strain with natively fermentable sugars (glucose, fructose, and galactose) as a sole carbon source

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Specifications Table

Subject	Applied Microbiology and Biotechnology
Specific subject area	Yeast metabolic engineering
Type of data	Tables and Figures
How data were acquired	The fermentation data were obtained by HPLC (Agilent Technologies 1260 series).
Data format	Raw and Analysed
Parameters for data collection	Fermentation conditions at 30°C and 130 rpm.
Description of data collection	Time series analysis of fermentation samples.
Data source location	Institution: Kyungpook National University City/Town/Region: Daegu Country: Korea
Data accessibility	With the article
Related research article	Author's name: Deokyeol Jeong, Suji Ye, Heeyoung Park, and Soo Rin Kim Title: Simultaneous fermentation of galacturonic acid and five-carbon sugars by engineered <i>Saccharomyces cerevisiae</i> Journal: Bioresource Technology https://doi.org/10.1016/j.biortech.2019.122259

Value of the Data

- The dataset contains the construction strategy and fermentation data for the engineered strain simultaneously fermenting representative three carbon sources (xylose, arabinose, galacturonic acid) in pectin-rich biomass.
- The fermentation data of the YE9 strain expressing the three pathways can be useful for process design utilizing pectin-rich biomass consisting mainly of galacturonic acid and arabinose.
- Based on the fermentation data of the YE9 strain, feasible options for strain engineering can be broadened for industrial bioprocesses.

are presented. In Table 1, the fermentation profiles of the YE9 strain with xylose, arabinose, and galacturonic acid in comparison to its wild type strain (D452-2). In Fig. 2, the YE9 strain was tested for xylose and galacturonic acid consumption rates in a mixture of 40 g/L xylose and various galacturonic acid concentrations. In Table 2, the fermentation parameters of the YE9 strain with a mixture of galacturonic acid and co-substrates.

2. Experimental design, materials, and methods

2.1. Strain construction by Cas9-based genome editing

To construct the YE9 strain, four consecutive transformations were performed as summarized in Fig. 3 using strains listed in Table 3. Briefly, the strain construction includes three parts: 1) guide RNA (gRNA) plasmid construction, 2) donor DNA preparation, and 3) yeast transformation.

1) Guide RNA (gRNA) plasmid construction

gRNA sequences are designed to be target cut site-specific and 20-bp long, as listed in Table 4. The plasmids expressing each gRNA sequence were constructed by the fast cloning method [2], which is a PCR-based protocol for plasmid mutagenesis. To construct the pRS42H-ALD6.1 plasmid, for example, the pRS42H-GND1.1 plasmid (a template plasmid) [3] was amplified with the primers Kim044/Kim045 (Table 5). The PCR products were treated with *DpnI* and used to transform *E. coli* TOP10 (Invitrogen, Carlsbad, CA, USA). The transformants were selected on an LBA (5 g/L yeast extract, 10 g/L tryptone, 10 g/L NaCl, and 100 µg/mL ampicillin) agar plate. The gRNA sequence of the resulting plasmid was confirmed by Sanger sequencing using a universal primer for the T3 promoter. All other gRNA plasmids were constructed using the same procedure but different primers, as listed in Table 5.

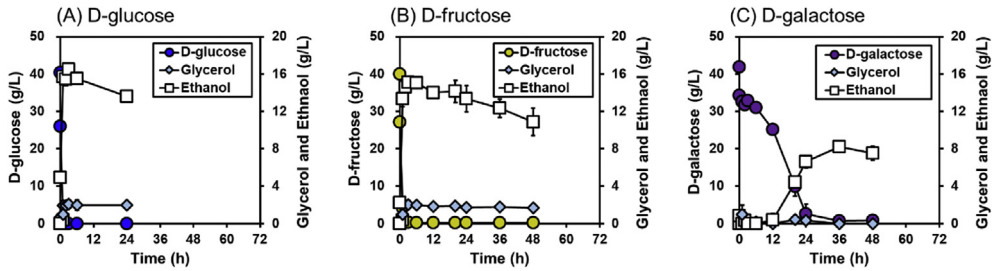


Fig. 1. Fermentation profiles of the YE9 strain in a complex medium containing (A) 40 g/L D-glucose, (B) 40 g/L D-fructose, and (C) 40 g/L D-galactose as the sole carbon sources. Fermentations were performed under oxygen-limited conditions (130 rpm) with a starting cell density of 25 g/L. All experiments were performed in biological triplicate, and the error bars indicate the standard deviations.

2) Donor DNA preparation

Donor DNA fragments were prepared by PCR using the primers listed in Table 6. Each of the fragments was flanked by 40–50 bp to allow *in vivo* assembly and genome integration through homologous recombination. Each assembly was an expression cassette of a heterologous gene as described in Fig. 3A. The donor DNAs for the xylose expression cassettes were designed to achieve complete removal of a target gene when genome integrated. On the other hand, the expression cassettes of the arabinose pathway and galacturonic acid pathway were integrated into an intergenic region without interfering neighboring genes.

3) Yeast transformation

For yeast transformation, a gRNA plasmid (4 μ g) and donor DNA fragments (4 μ g each) were used to transform a designated strain harboring pRS41N-Cas9 [3]. The resulting transformants were selected on a YPD agar plate supplemented with 100 μ g/mL nourseothricin sulfate (Gold Biotechnology, St. Louis, MO, USA) and 300 μ g/mL hygromycin B (Invitrogen, Carlsbad, CA, USA). Selected transformants were serially sub-cultured in YPD medium supplemented with 100 μ g/mL nourseothricin sulfate to only remove the existing gRNA plasmids. Correct assembly and integration was then confirmed by yeast colony PCR with the primers listed in Table 7. Through four consecutive transformations, as described in Fig. 3, the YE9 strain was finally constructed.

Table 1

Fermentation profiles of the native *S. cerevisiae* strain (D452-2) and engineered strain (YE9) expressing heterologous pathways for metabolizing D-xylose, L-arabinose, and D-galacturonic acid (galUA).

Strain	Substrate	Substrate consumed (g/L)	Substrate consumption rate (g/L/h)	Products (g/L)		Parameters ^{b)}		
				Glycerol	Ethanol	Y_{Glycerol}	Y_{Ethanol}	P_{Ethanol}^*
D452-2	D-xylose ^{a)}	5.9 ± 0.2	0.19 ± 0.01	0.3 ± 0.0	<i>n. d.</i>	0.07 ± 0.02	<i>n. d.</i>	<i>n. d.</i>
	L-arabinose	1.3 ± 0.6	0.08 ± 0.03	<i>n. d.</i>	<i>n. d.</i>	<i>n. d.</i>	<i>n. d.</i>	<i>n. d.</i>
	galUA	< 0.0	< 0.00	<i>n. d.</i>	<i>n. d.</i>	<i>n. d.</i>	<i>n. d.</i>	<i>n. d.</i>
YE9	D-xylose	33.7 ± 0.5	1.41 ± 0.02	0.6 ± 0.1	11.3 ± 0.1	0.02 ± 0.00	0.34 ± 0.01	0.05 ± 0.00
	L-arabinose	30.2 ± 0.1	0.63 ± 0.07	<i>n. d.</i>	1.9 ± 0.1	<i>n. d.</i>	0.07 ± 0.00	< 0.00
	galUA	6.7 ± 0.7	0.27 ± 0.01	0.3 ± 0.1	0.3 ± 0.0	0.04 ± 0.01	0.08 ± 0.02	< 0.00

^{a)} Fermentations were performed in a complex medium containing 40 g/L D-xylose, 40 g/L L-arabinose, or 20 g/L D-galacturonic acid under oxygen-limited conditions (130 rpm) with a starting cell density of 25 g/L. Substrate consumption rate was calculated for 24 h and the others were calculated for 72 h.

^{b)} Y_{Glycerol} , glycerol yield (g glycerol/g substrate); Y_{Ethanol} , ethanol yield (g ethanol/g substrate); P_{Ethanol}^* , specific ethanol productivity (g ethanol/g cell/h); *n. d.*, not detected.

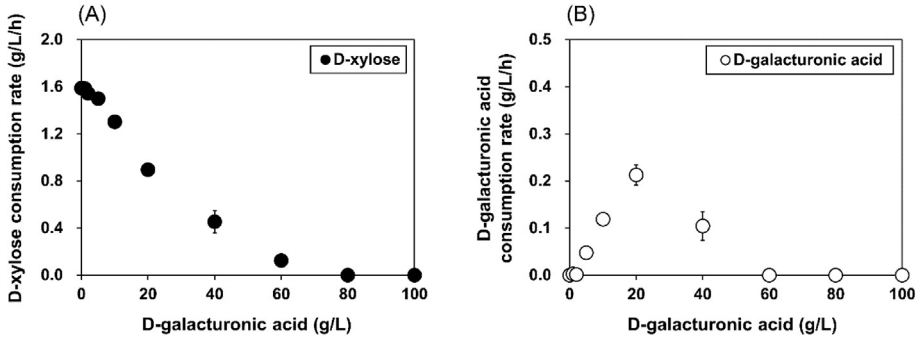


Fig. 2. Effect of D-galacturonic acid on the rate of D-xylose consumption in the YE9 strain. Consumption rate of D-xylose (A) and D-galacturonic acid (B) was evaluated under 40 g/L D-xylose and different D-galacturonic acid concentrations (0–100 g/L). All experiments were performed in biological triplicate, and error bars indicate standard deviations and were not visible when smaller than the symbol size.

2.2. Fermentation

For fermentation of the YE9 strain, one colony was pre-cultured in YP medium (10 g/L yeast extract and 20 g/L peptone) supplemented with 20 g/L of glucose for 36 h at 30°C and 250 rpm. Cells were centrifuged, washed twice, and re-suspended in YP medium supplemented with desired carbon sources. The initial cell density of fermentation was 25 g/L dry weight, which corresponds to approximately 125 g/L wet weight, and this conversion factor was obtained from a prior study [4]. In the industrial bioethanol processes, >90% cells are recycled in repeated batch-type fermentation;

Table 2

Fermentation profiles of mixed culture by engineered *S. cerevisiae* YE9 strain expressing heterologous pathways metabolizing D-xylose, L-arabinose, and D-galacturonic acid (galUA).

Medium ^{a)}	Substrate consumed (g/L)		galUA consumption rate (g/L/h)	Products (g/L)		Parameters ^{b)}			
	galUA	Sugars		Glycerol	Ethanol	Y_{Glycerol}	Y_{Ethanol}	P_{Glycerol}^*	P_{Ethanol}^*
galUA	6.7 ± 0.7	–	0.27 ± 0.01	0.3 ± 0.1	0.3 ± 0.0	0.04 ± 0.01	0.08 ± 0.02	< 0.00	< 0.00
galUA + Glucose	3.3 ± 0.2	36.7 ± 0.1	0.14 ± 0.01	2.4 ± 0.3	16.9 ± 0.2	0.06 ± 0.01	0.40 ± 0.01	0.06 ± 0.00	0.66 ± 0.01
galUA + Fructose	4.5 ± 0.3	36.1 ± 0.8	0.18 ± 0.02	2.9 ± 0.1	16.9 ± 0.5	0.07 ± 0.00	0.36 ± 0.01	< 0.00	0.65 ± 0.03
galUA + Galactose	4.6 ± 1.2	25.4 ± 7.3	0.17 ± 0.03	1.6 ± 0.7	2.4 ± 1.1	0.04 ± 0.01	0.05 ± 0.02	< 0.00	< 0.00
galUA+ Xylose	13.1 ± 0.4	33.3 ± 0.5	0.49 ± 0.02	4.5 ± 0.1	12.8 ± 0.3	0.08 ± 0.00	0.23 ± 0.01	0.01 ± 0.00	0.04 ± 0.00
galUA+ Arabinose	11.9 ± 0.7	28.4 ± 0.1	0.32 ± 0.03	4.2 ± 0.2	4.1 ± 0.5	0.11 ± 0.01	0.11 ± 0.02	< 0.00	< 0.00
galUA +Xylose (X) +Arabinose (A)	15.3 ± 0.6	33.7 ± 0.1 (X) 25.9 ± 4.4 (A)	0.49 ± 0.04	5.3 ± 0.6	16.5 ± 1.2	0.07 ± 0.00	0.22 ± 0.01	< 0.00	0.02 ± 0.00

^{a)} Fermentations were performed in a complex medium containing 20 g/L D-galacturonic acid (galUA) and 40 g/L sugar (D-glucose, D-fructose, D-galactose, D-xylose, L-arabinose, and mixture of D-xylose and L-arabinose) under oxygen-limited conditions (130 rpm) with a starting cell density of 25 g/L. D-galacturonic acid consumption rate was calculated for 24 h and the others were calculated for 72 h.

^{b)} Y_{Glycerol} , glycerol yield (g glycerol/g substrates); Y_{Ethanol} , ethanol yield (g ethanol/g substrates); P_{Glycerol}^* , specific glycerol productivity (g glycerol/g cell/h); P_{Ethanol}^* , specific ethanol productivity (g ethanol/g cell/h).

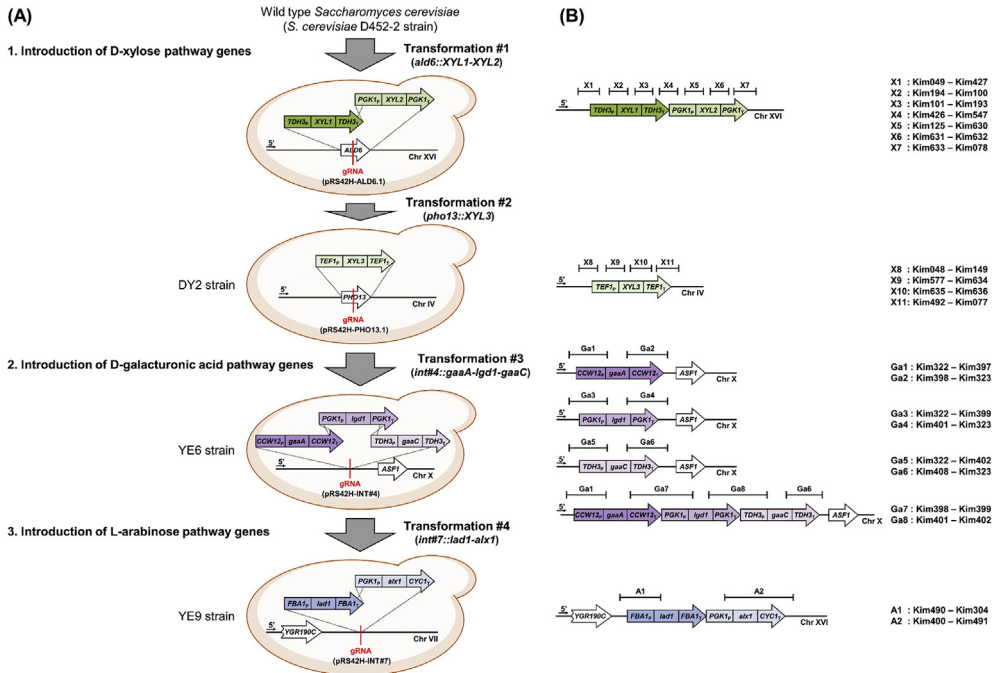


Fig.3. Construction of engineered *S. cerevisiae* YE9 strains expressing heterologous D-xylose, D-galacturonic acid, and L-arabinose pathways. (A) Strain construction using Cas9-based *in vivo* assembly and genome integration strategy. (B) Confirmation primers for correct assembly and integration by yeast colony PCR. The primer sequences are listed in Table S5.

therefore, very high cell density of up to 170 g/L wet weight [5] is often achieved. The concentrations of the carbon sources were selected to reflect the typical chemical composition of pectin-rich biomass (Table 8).

Table 3
Saccharomyces cerevisiae strains used for the construction of YE9.

Strains	Description/relevant genotype ^a	Ref.
D452-2	Wild type; <i>Mata leu2 his3 ura3</i>	[7]
DY02	Expressing the heterologous D-xylose pathway; <i>D452-2 ald6::TDH3_p-XYL1-TDH3_T-PGK1_p-XYL2-PGK1_T pho13::TEF1_p-XYL3-TEF1_T</i>	
YE3	<i>DY02 int#4::CCW12_p-gaaA-CCW12_T</i>	
YE4	<i>DY02 int#4::PGK1_p-igd1-PGK1_T</i>	
YE5	<i>DY02 int#4::TDH3_p-gaaC-TDH3_T</i>	
YE6	Expressing the heterologous D-xylose and D-galacturonic acid pathway; <i>DY02 int#4::CCW12_p-gaaA-CCW12_T-PGK1_p-igd1-PGK1_T-TDH3_p-gaaC-TDH3_T</i>	
YE6 YPR1	<i>YE6 CCW12_p-YPR1</i>	
YE6 gaaD	<i>YE6 int#6::CCW12_p-gaaD-CCW12_T</i>	
YE01	Expressing the heterologous D-xylose, and L-arabinose pathway; <i>D452-2 ald6::TDH3_p-XYL1-TDH3_T-PGK1_p-XYL2-PGK1_T int#1::TEF1_p-XYL3-TEF1_T sor1::FBA1_p-LAD1-FBA1_T-PGK1_p-ALX1-CYC1_T</i>	[8]
YE9	Expressing the heterologous D-xylose, L-arabinose, and D-galacturonic acid pathway; <i>YE6 int#7::FBA1_p-lad1-FBA1_T-PGK1_p-alk1-CYC1_T</i>	

^a *XYL1*, *XYL2*, and *XYL3* are derived from *Pichia stipitis*; *gaaA*, *gaaC*, and *gaaD* are derived from *Aspergillus niger*; *igd1* and *lad1* are derived from *Trichoderma reesei*; *alk1* is derived from *Ambrosiozyma monospora*.

Table 4

Guide RNA (gRNA) plasmids.

gRNA	Target cut site	gRNA and PAM sequences (5'-)	Plasmid name
ALD6.1	<i>ALD6</i>	GTCAGATCACACTTCCAAA TGG	pRS42H-ALD6.1
PHO13.1	<i>PHO13</i>	TCCTTATCTATTAACCTTC CGG	pRS42H-PHO13.1
YPR1.1	<i>YPR1</i>	CATGGTAGATTATTTCTGT GGG	pRS42H-YPR1.1
INT#4	Intergenic region upstream <i>ASF1</i>	CTCTCGAAGTGGTCACGTGC GGG	pRS42H-INT#4
INT#6	Intergenic region upstream <i>ATG33</i>	TTGTCACAGTGCACATCAG CGG	pRS42H-INT#6
INT#7	Intergenic region downstream <i>YGR190C</i>	GATACTTATCATTAAAGAAA TGG	pRS42H-INT#7

Table 5

Primers used for construction of guide RNA plasmids.

Plasmid name	Primers	Sequences (5'-)
pRS42H-ALD6.1	Kim044	<u>AAGATCACACTTCCAAA</u> GGTTTTAGAGCTAGAAATAGCAAG
	Kim045	TTGGAAGTGTGATCTTGACGATCATTATCTTTCACCTGCG
pRS42H-PHO13.1	Kim624	<u>CTTATCTATTAACCTTC</u> GGTTTTAGAGCTAGAAATAGCAAG
	Kim625	AAAGTTAATAGATAAGGAGATCATTATCTTTCACCTGCG
pRS42H-YPR1.1	Kim535	<u>GGTAGATTATTTCTGT</u> GGTTTTAGAGCTAGAAATAGCAAG
	Kim536	CAGATAATAATCTACCATGGATCATTATCTTTCACCTGCG
pRS42H-INT#4	Kim310	<u>TCCAAGTGGTCACGTGC</u> GGTTTTAGAGCTAGAAATAGCAAG
	Kim311	CACGTGACCACCTCGAGAGGATCATTATCTTTCACCTGCG
pRS42H-INT#6	Kim314	<u>TCACAGTGCACATCAGG</u> TTTAGAGCTAGAAATAGCAAG
	Kim315	TGATGTGACACTGTGACAAGATCATTATCTTTCACCTGCG
pRS42H-INT#7	Kim486	<u>AGGAATTAGTTCGCCCG</u> TTTAGAGCTAGAAATAGCAAG
	Kim487	GGCGAACATAATCTTACGATCATTATCTTTCACCTGCG

Table 6

Primers used for construction of donor DNA fragments.

Template genomic DNA ^a	Donor DNA fragments	Primers	Sequences (5'-)
<i>XYL1</i> and <i>XYL2</i> expression cassettes for deleting <i>ALD6</i> (<i>ald6::TDH3_p-XYL1-TDH3_p-PGK1_p-XYL2-PGK1_T</i>)			
<i>S. cerevisiae</i>	<i>TDH3_p</i>	Kim626	<u>TAACATACACAAACACATACTATCAGAATACAC</u> TATTTTCGAGGACCTTGTCT
		S00384	<u>TCAACTTAATAGAAGGCCA</u> TTTTAGATCTCCTAGGTTTGGTTTATGTGTGTTTTAT TC
<i>P. stipitis</i>	<i>XYL1</i>	S00385	<u>ATAAACACACATAAAACAACAAACCT</u> AGGAGATCTAAAAATGCCCTTCTATTAAGTTGA AC
		S00386	AAT <u>GCAAGATTTAAAGTAAATTC</u> ACTGTGTTAACCGCATGCTTAGACGAAGATAGGAATCTTG
<i>S. cerevisiae</i>	<i>TDH3_T</i>	S00387	GGA <u>CAAGATTCCTATCTTCG</u> TCTAAGCATGCGTTAACAGTGAATTTACTTTAAATCTTGC
		S00388	ATTCTTTGAAGGTACTT <u>CTTCGAAAAATTCGCGT</u> CTGCTAGCTCTCGCGGAAAAAATTC
<i>S. cerevisiae</i>	<i>PGK1_p</i>	S00389	TTTTAAAGTTTACAAAT <u>GAATTTTTCCGCC</u> AGGAGCTAGCAGACGCGAATTTTTCGAAG
		S00390	CACCAA <u>GGAAGGGTTAGCAGT</u> CATTTTTCTAGATGTTTTATATTTGTGTGAAAAAGTAG
<i>P. stipitis</i>	<i>XYL2</i>	S00391	AAATTAT <u>CTACTTTTTACAACA</u> AAATATAAAACATCTAGAAAAAATGACTGTAAACCTTCC
		S00392	AAAAAATTGAT <u>CTATCGATTTCAAT</u> TCAATTAATACTAGTTTACTCAGGCGCGTCAATG
<i>S. cerevisiae</i>	<i>PGK1_T</i>	S00393	GTCAGGTGCT <u>CATTCAGCGCCCT</u> GAGTAAACTAGTATTGAATTGAATTGAAATCGATAG
		Kim627	GTATATGACGGAAAAGAAATGCAGGTTGGTACA AAATAATATCTCTCTCGAAAG
<i>XYL3</i> expression cassette for deleting <i>PHO13</i> (<i>pho13::TDH3_p-XYL1-TDH3_p-PGK1_p-XYL2-PGK1_T</i>)			
<i>S. cerevisiae</i>	<i>TDH3_p</i>	Kim628	<u>ATGTGACATCTTACTA</u> TTCTCCAGCAGCGTTT CTTCATCGGTATCTTCGC
		S00374	AA <u>TGGGGTAGTGGTCAT</u> TTTTAAGCTTGAATTTCTTGTAAATTAACCTTAGATTAGATTG
<i>P. stipitis</i>	<i>XYL3</i>	S00375	AT <u>CTAATCTAAGTTTTA</u> AITACAAGAATTCAGCTTAAAAATGACCACTACCCCAATTG
		S00376	GCAACTA <u>GAAAAGTCTTATCA</u> ATCTCCGTCGACATCGATTTAGTGTTTCAATTCACITTC
<i>S. cerevisiae</i>	<i>TDH3_T</i>	S00377	CAAGATG <u>GAAAAGTGAATTG</u> AAACACTAAATCGATGTCGACGGAGATTGATAAGAC
		Kim629	<u>CTATAACTCATTATTGG</u> TTAAGGTGTAGATG AAGTTGGGTAACGCCAGG
<i>gaaA</i> expression cassette (<i>int#4::CCW12_p-gaaA-CCW12_T</i>)			
<i>S. cerevisiae</i>	<i>CCW12_p</i>	Kim379	<u>TTCTCCGGGCAGAGA</u> AACTCGCAGGCAACTTG CACGCAAAAAGAAAACCTT

Table 6 (continued)

Template genomic DNA ^a	Donor DNA fragments	Primers	Sequences (5'-)
<i>A. niger</i>	<i>gaaA</i>	Kim380 Kim381 Kim382	TCAACA <u>CAGCTGGGGAGCCATTTTTTATTGATATAGTGTTTAAGCGAAT</u> TCTGTC <u>ATTCGCTTAAACACTATATCAATAAAAAATGGCTCCCCAGCTG</u> TAGA <u>ATGTATAAATAATAATAAACTAAGTCTACTTCAGCTCCCACTTCC</u>
<i>S. cerevisiae</i>	CCW12 _T	Kim383 Kim384	GGAT <u>GGAAAGTGGGAGCTGAAGTAGACTTAGTTTATTATTATTATACAT</u> <u>TGTGAGGGCCGATTATGCAGGCCTAGA</u> TGTTCTAGTGTGTTTATATTATC
<i>lgd1</i> expression cassette (int#4::PGK1_p-<i>lgd1</i>-PGK1_T)			
<i>S. cerevisiae</i>	PGK1 _p	Kim385 Kim386	<u>CCTCGGGCAGAGAACTCGCAGGCAACTTG</u> GTGAGTAAGGAAAGAGTGAG <u>CTGATGGTGACTTCAGACATTTTTTTGTTTTATTTTGTGTA AAAAGTAG</u>
<i>T. reesei</i>	<i>lgd1</i>	Kim387 Kim388	<u>CTACTTTTTACAACAATATAAAAACAAAAATGCTGGAAGTCACCATCAC</u> ATTGATCTAT <u>CGATTTCAATTC AATTCAATTCAGATCTCTCTCCGTTCA</u>
<i>S. cerevisiae</i>	PGK1 _T	Kim389 Kim390	CTGCCATCT <u>TGAACGGAGAGAAGATCTGAATTGAATGAATTGAAATCG</u> <u>CTCTGTGAGGGCCGATTATGCAGGCCTAGA</u> AAATAATATCCTTCTCGAAA
<i>gaaC</i> expression cassette (int#4::TDH3_p-<i>gaaC</i>-TDH3_T)			
<i>S. cerevisiae</i>	TDH3 _p	Kim391 Kim392	<u>CTCGGGCAGAGAACTCGCAGGCAACTTG</u> GAATAAAAAACACGCTTTTTTC GACTCCGGGGCG <u>GAGCGGGTAAAGGCAATTTTTTTGTTTGTATGTGTGTT</u>
<i>A. niger</i>	<i>gaaC</i>	Kim393 Kim394	TTCGAATA <u>AACACACATAAACAAACAAAAAAATGCCTTTTACCCCGTCC</u> ATTTAAAT <u>GCAAGATTTAAAGTAAATTCACCTAAGCAATATCCGGCAACG</u>
<i>S. cerevisiae</i>	TDH3 _T	Kim395 Kim396	TGAGAAGT <u>CGTTGCCGATATTGCTTAGGTGAATTACTTTAAATCTTGC</u> <u>CTCTGTGAGGGCCGATTATGCAGGCCTAGA</u> ATCCTGGCGAAAAAATTC
<i>gaaA</i>, <i>lgd1</i>, and <i>gaaC</i> expression cassettes (int#4::CCW12_p-<i>gaaA</i>-CCW12_p-PGK1_p-<i>lgd1</i>-PGK1_T-TDH3_p-<i>gaaC</i>-TDH3_T)			
<i>S. cerevisiae</i>	CCW12 _p - YE3	Kim410 Kim411	<u>TCITTTAGGTTAATTGTCGCTGTTATTGCTCA</u> GATTTTTCTCGGAGATGG TAGTTC <u>CTCACTCTTCTTACTCACTGTTCTAGTGTGTTTATATTATCC</u>
<i>S. cerevisiae</i>	PGK1 _p - YE4	Kim412 Kim413	AGCCAA <u>GGATAATATAAACACACTAGAACA</u> GTGAGTAAGGAAAGAGTGAG AAACTCGAA <u>CTGAAAAGCGTGTTTTTTATTCCCGATTATGCAGGCCTAG</u>
<i>S. cerevisiae</i>	TDH3 _p - YE5	Kim414 Kim415	TATTATTTT <u>CTAGGCCCTGATAATCGGGAATAAAAAACACGCTTTTTCAG</u> <u>CTACTCTCTCTAGTCGCCGGTGTGT</u> GAAAGTTAATTGTGGGTTTTTC
<i>lad1</i> and <i>alx1</i> expression cassettes (int#7::FBA1_p-<i>lad1</i>-FBA1_T-PGK1_p-<i>alx1</i>-CYC1_T)			
<i>S. cerevisiae</i>	FBA1 _p - <i>lad1</i> - YE01	Kim553 Kim554	<u>CTTACACTGTGTAATGACAAATGTTTTT</u> TGAACAACAATACCAGCCTTC <u>TGTTTTACGTTATCAAGATTATGTCACTATT</u> GGCCGAAAATTAAGCCT
Overexpression of YPR1 (CCW12_p-YPR1)			
<i>S. cerevisiae</i>	CCW12 _p	Kim537 Kim538	<u>GTAACITTTGCAATATAATCAGGTCGCAAAAT</u> CACGCAAAAGAAAACCTT <u>GAAGAATTCITTTAACGTAGCAGGCAT</u> TATTGATATAGTGTTTAAGCGAAT
<i>gaaD</i> expression cassette (int#6::CCW12_p-<i>gaaD</i>-CCW12_T)			
<i>S. cerevisiae</i>	CCW12 _p	Kim541 Kim542	<u>CGGAGGAGACCGCTATAACCGGTTTGAATTTA</u> CACGCAAAAGAAAACCTT TA <u>ACCTTCTTCCGAGAGACATTTTTTATTGATATAGTGTTTAAGCGAAT</u>
<i>A. niger</i>	<i>gaaD</i>	Kim543 Kim544	TC <u>ATTCGCTTAAACACTATATCAATAAAAAATGCTCTCCGAAAGAAAGGT</u> GT <u>ATAAATAATAATAAACTAAGTTTATTAACAACATCACCTTATGACCAGC</u>
<i>S. cerevisiae</i>	CCW12 _T	Kim545 Kim546	TG <u>GTCAATAAGGTGATTGTTTAAATAAACTT</u> AGTTTATTATTATTATACAT <u>CTTGCTGTGCTCAAACTTCTGAGTTG</u> TGTTCTAGTGTGTTTATATTATC

The flanking region is underlined.

^a *Saccharomyces cerevisiae* D452-2; *Pichia stipitis* CBS 6054; *Aspergillus niger* CBS 120.49; *Trichoderma reesei* ATCC 5676.

2.3. HPLC analysis

Quantitation of glucose, fructose, galactose, xylose, arabinose, galacturonic acid, glycerol, and ethanol was performed by high-performance liquid chromatography (HPLC; Agilent Technologies, 1260 series, USA) device equipped with a RI detector and a Rezex-ROA Organic Acid H+ (8%) (150 mm × 4.6 mm) column (Phenomenex Inc., Torrance, CA, USA). The column was eluted with 0.005 N H₂SO₄ at 0.6 mL/min and 50°C [1,6].

Table 7
Primers used for confirmation of correct assembly and integration.

Primers	Sequences (5'-)	Primers	Sequences (5'-)
Introduction of D-xylose pathway		Introduction of D-galacturonic acid pathway	
Kim049	GGAACGGTGAGTGCAACG	Kim322	GCCATCTATTTGCCGTC
Kim427	AAACTGTTCACCCAGACACC	Kim397	GCTGGGGGAGCCATTTTTATTG
Kim194	AGCCAACTACAGAGAACAGG	Kim398	GTGGGAGCTGAAGTAGACTTAG
Kim100	CGGCACCGTCGAACAATCTG	Kim323	TCACGACACACCTCACTG
Kim101	CCGCTTACTCTTCGTTCCGGTCC	Kim399	CCTGTGATGGTGACTTCAGAC
Kim193	CTCAGCATCCACAATGATACAG	Kim401	GAACGGAGAGAAGATCTGAATTG
Kim426	GCGCTATTGCATTGTTCTTGTC	Kim400	ACAGCCTGTTCTCACACAC
Kim547	AGGTATGCGATAGTTCCTCAC	Kim402	GCGGGGTAAAAGGCATTTTTTTG
Kim125	TGCAGCTCCAATTCGTCAC	Kim408	GCCGGATATTGCTTAGGTG
Kim630	GAGGTGACACCCCTTACCAAC		
Kim631	CTGTACTACACCTTCAACTC	Introduction of L-arabinose pathway	
Kim632	CGCTGAACCCGAACATAGAAATATC	Kim490	GGCACTAGGAGCATTGTGCG
Kim633	TCGATATTTCTATGTTCCGGTTCAG	Kim304	GCTTCGCTAATCCAGAGGTC
Kim078	GATTGGAATTGGTTCGCAGTG	Kim400	ACAGCCTGTTCTCACACAC
Kim048	GAGGAAGACGTTGAAGGTGG	Kim491	GTCCCTTAGGGTGCCTATAATG
Kim149	TTTGAAGTGGTACGGCGATG		
Kim577	CACCCAAGCACAGCATAAC	Overexpression of YPR1	
Kim634	TGGTTCGATAACGAAGATTCAG	Kim539	CAATCCGTGAAACCCTTTCTT
Kim635	GTCTTGTAGATTGAGAAGTGGTCC	Kim540	CTGCCAACTTCTTCTTCAATCAA
Kim636	TCTATGAGGCAAGTAAAGGCAC		
Kim492	AACAGGCGACAGTCCAAATG	Introduction of <i>gaad</i> gene cassette	
Kim077	TGGAGTCAAACCTGGCCAG	Kim326	GGTCTGACTCCTACTGAGC
		Kim093	GCAAAGATAGCGGCGTAGGTG
		Kim549	GCATCCTTTGCTCCCGTTC
		Kim327	AGCATCGAGTACGGCAGTTC

Table 8
Chemical composition of pectin-rich biomass.

Source	Arabinose	Galacturonic acid	Ratio	Reference
Orange peel hydrolysate (g/L, ~ 10% solid loading)	32.6	13.2	2.47	[9]
Sugar beet pulp hydrolysate (g/100 g dry matter)	22.5	22.5	1.00	[10]

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dib.2020.105359>.

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