Data Article

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

Deokyeol Jeong, Suji Ye, Heeyoung Park, Soo Rin Kim *

School of Food Science and Biotechnology, Kyungpook National University, Daegu, 41566, South Korea

**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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* Corresponding author.

E-mail address: soorinkim@knu.ac.kr (S.R. Kim).
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.
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 μg) and donor DNA fragments (4 μg 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 μg/mL nourseothricin sulfate (Gold Biotechnology, St. Louis, MO, USA) and 300 μg/mL hygromycin B (Invitrogen, Carlsbad, CA, USA). Selected transformants were serially sub-cultured in YPD medium supplemented with 100 μg/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.

![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.](image)

### 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 | YGlycerol | YEthanol | PEthanol* |
| D452-2 | D-xylose  | 5.9 ± 0.2                | 0.19 ± 0.01                       | 0.3 ± 0.0 | n. d.    | 0.07 ± 0.02 | n. d. | n. d.  |
|        | L-arabinose | 1.3 ± 0.6                | 0.08 ± 0.03                       | n. d.    | n. d.    | n. d.     | n. d. | n. d.  |
|        | galUA     | < 0.00                   | < 0.00                            | n. d.    | n. d.    | n. d.     | n. d. | n. d.  |
| 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                       | n. d.    | 1.9 ± 0.1 | n. d.    | 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.
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;

**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.

**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(1)   | Substrate consumed (g/L) | galUA consumption rate (g/L/h) | Products (g/L) | Parameters(2)                |
|-------------|--------------------------|--------------------------------|----------------|-----------------------------|
|             |  galUA                   |                                |                |                             |
|             |                          | 6.7 ± 0.7                      | 0.27 ± 0.01    | 0.04 ± 0.01 0.08 ± 0.02 < 0.00 < 0.00 |
| + Glucose   |                          | 3.3 ± 0.2 36.7 ± 0.1           | 0.14 ± 0.01    | 0.06 ± 0.01 0.40 ± 0.01 0.06 ± 0.00 0.66 ± 0.01 |
| + Fructose  |                          | 4.5 ± 0.3 36.1 ± 0.8           | 0.18 ± 0.02    | 0.07 ± 0.00 0.36 ± 0.01 < 0.00 0.65 ± 0.03 |
| + Galactose |                          | 4.6 ± 1.2 25.4 ± 7.3           | 0.17 ± 0.03    | 0.04 ± 0.01 0.05 ± 0.02 < 0.00 < 0.00 |
| + Xylose    |                          | 13.1 ± 0.4 33.3 ± 0.5          | 0.49 ± 0.02    | 0.08 ± 0.00 0.23 ± 0.01 0.01 ± 0.00 0.04 ± 0.00 |
| + Arabinose |                          | 11.9 ± 0.7 28.4 ± 0.1          | 0.32 ± 0.03    | 0.11 ± 0.01 0.11 ± 0.02 < 0.00 < 0.00 |
| + Xylose (X)|                          | 15.3 ± 0.6 33.7 ± 0.1 (X)      | 0.49 ± 0.04    | 0.07 ± 0.00 0.22 ± 0.01 < 0.00 0.02 ± 0.00 |
| + Arabinose (A) |                      | 25.9 ± 4.4 (A)               |                |                             |

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<sub>Glycerol</sub>, glycerol yield (g glycerol/g substrates); Y<sub>Ethanol</sub>, ethanol yield (g ethanol/g substrates); P<sub>Glycerol</sub>, specific glycerol productivity (g glycerol/g cell/h); P<sub>Ethanol</sub>, specific ethanol productivity (g ethanol/g cell/h).
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 | Ref. |
|---------|--------------------------------|------|
| D452-2  | Wild type; *Matα leu2 his3 ura3* | [7]  |
| DY02    | Expressing the heterologous D-xylose pathway; D452-2 αδδδδ::THD3Δ-YXL1-THD3Δ-PGK1Δ-YXL2-PGK1Δ pho13::TEF1Δ-YXL3-TEF1Δ |      |
| YE3     | DY02 int#4::CCW12Δ-gaaA-CCW12Δ |      |
| YE4     | DY02 int#4::PGK1Δ-lgd1-PGK1Δ |      |
| YE5     | DY02 int#4::THD3Δ-gaaC-THD3Δ |      |
| YE6     | Expressing the heterologous D-xylose and D-galacturonic acid pathway; DY02 int#4::CCW12Δ-gaaA-CCW12Δ-PGK1Δ-lgd1-PGK1Δ-THD3Δ-gaaC-THD3Δ |      |
| YE6 YPR1| YE6 CCW12Δ-YPR1 |      |
| YE6 gaaD| YE6 int#6::CCW12Δ-gaaD-CCW12Δ |      |
| YE01    | Expressing the heterologous D-xylose, and t-arabinose pathway; D452-2 αδδδδ::THD3Δ-YXL1-THD3Δ-PGK1Δ-YXL2-PGK1Δ int#1::TEF1Δ-YXL3-TEF1Δ sor1::FBA1Δ-lad1-FBA1Δ-PGK1Δ-Alx1-CYC1Δ | [8]  |
| YE9     | Expressing the heterologous D-xylose, t-arabinose, and D-galacturonic acid pathway; YE6 int#7::FBA1Δ-lad1-FBA1Δ-PGK1Δ-ax1-CYC1Δ |      |

*XYL1, XYL2, and XYL3 are derived from *Pichia stipitis*; gaaA, gaaC, and gaaD are derived from *Aspergillus niger*; lgd1 and lad1 are derived from *Trichoderma reesei*; ax1 is derived from *Ambrosiozyma monospora*.
Table 4
Guide RNA (gRNA) plasmids.

| gRNA     | Target cut site | gRNA and PAM sequences (5'-) | Plasmid name         |
|----------|----------------|-----------------------------|----------------------|
| ALD6.1   | ALD6           | GTCAAGATCACACTTCCAAAAAGTTTAGACGAAATATCAAG | pRS42H-ALD6.1       |
| PHO13.1  | PHO13          | TCCCTATCTATATACTTCCGG        | pRS42H-PHO13.1       |
| YPR1.1   | YPR1           | CATCTGACATTTATATCTGC        | pRS42H-YPR1.1        |
| INT#4    | Intergenic region upstream ASF1 | CTCTCGAAGTGGTCACGCTCG GGG | pRS42H-INT#4         |
| INT#6    | Intergenic region upstream ATG33 | TTTGTCACAGTGTACATCAGC GGG | pRS42H-INT#6         |
| INT#7    | Intergenic region downstream YGR190C | GATACTTATCATTAAGAAAA TGG | pRS42H-INT#7         |

Table 5
Primers used for construction of guide RNA plasmids.

| Plasmid name | Primers | Sequences (5'-) |
|--------------|---------|-----------------|
| pRS42H-ALD6.1 | Kim044  | AAGATCACACTTCCAAAAAGTTTAGACGAAATATCAAG |
|              | Kim045  | TTGAAGTGGTCACGCTCG GGG |
| pRS42H-PHO13.1 | Kim624  | ATAAACACACATAAACAAACAAACCTAGGAGATCTAAAAATGCCTTCTATTAAGTTGAAC |
|              | Kim625  | SOO376          |
| pRS42H-YPR1.1 | Kim535  | GCTGACATTTATATCTGC |
|              | Kim536  | SOO387          |
| pRS42H-INT#4 | Kim310  | SOO388          |
|              | Kim311  | SOO389          |
| pRS42H-INT#6 | Kim314  | SOO391          |
|              | Kim315  | SOO392          |
| pRS42H-INT#7 | Kim486  | SOO393          |

Table 6
Primers used for construction of donor DNA fragments.

| Template genonic DNA | Donor DNA fragments | Primers | Sequences (5'-) |
|---------------------|---------------------|---------|-----------------|
| XYL1 and XYL2 expression cassettes for deleting ALD6 (ald6::TDH3p-XYL1-TDH3-T-pGK1-pXYL2-pGK1p) |
| S. cerevisiae       | TDH3p-XYL1-TDH3-T-pGK1-pXYL2-pGK1p | Kim626  | SOO384          |
| P. stipitis         | XYL1                 | SOO385  | SOO386          |
| S. cerevisiae       | TDH3-T-pGK1p         | SOO387  | SOO388          |
| P. stipitis         | XYL2                 | SOO390  | SOO391          |
| S. cerevisiae       | XYL2                 | SOO392  | SOO393          |
| gaaA expression cassette (int#4::CCW12p-gaaA-CCW12) |
| S. cerevisiae       | CCW12p-gaaA-CCW12    | Kim629  | SOO374          |
| P. stipitis         | XYL3                 | SOO375  | SOO376          |
| S. cerevisiae       | TDH3p-gaaA-CCW12     | SOO377  | SOO378          |
| gaaA expression cassette (int#4::CCW12p-gaaA-CCW12) |
| S. cerevisiae       | CCW12p-gaaA-CCW12    | Kim629  | SOO374          |
| P. stipitis         | XYL3                 | SOO375  | SOO376          |
| S. cerevisiae       | TDH3p-gaaA-CCW12     | SOO377  | SOO378          |
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].
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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