Complete and efficient conversion of plant cell wall hemicellulose into high-value bioproducts by engineered yeast

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Plant cell wall hydrolysates contain not only sugars but also substantial amounts of acetate, a fermentation inhibitor that hinders bioconversion of lignocellulose. Despite the toxic and non-consumable nature of acetate during glucose metabolism, we demonstrate that acetate can be rapidly co-consumed with xylose by engineered Saccharomyces cerevisiae. The co-consumption leads to a metabolic re-configuration that boosts the synthesis of acetyl-CoA derived bioproducts, including triacetic acid lactone (TAL) and vitamin A, in engineered strains. Notably, by co-feeding xylose and acetate, an engineered strain produces 23.91 g/L TAL with a productivity of 0.29 g/L/h in bioreactor fermentation. This strain also completely converts a hemicellulose hydrolysate of switchgrass into 3.55 g/L TAL. These findings establish a versatile strategy that not only transforms an inhibitor into a valuable substrate but also expands the capacity of acetyl-CoA supply in S. cerevisiae for efficient bioconversion of cellulosic biomass.
The increasing demands for natural resources and rising concerns on climate change have ignited a broad interest to produce biofuels and chemicals via microbial fermentation using renewable biomass\textsuperscript{1,2}. Second-generation bioenergy and biorefineries based on lignocellulosic plant materials could serve as a sustainable alternative to conventional petrochemical processes\textsuperscript{3,4}. Under this goal, microbial engineers have exerted tremendous efforts to increase bioconversion efficiencies of lignocellulosic carbons into target bioproducts\textsuperscript{5–9}. However, due to the acetylation of hemicellulose and lignin in the plant cell wall, cellulose hydrolysates inevitably contain substantial amounts of acetate, an inhibitor toxic to fermenting microorganisms that negatively influences the bioconversion efficiency of cellulose hydrolysates\textsuperscript{9}. The toxicity of acetate against fermenting microorganisms is one of the major obstacles hampering the commercialization of lignocellulose-based biorefinery.

Although the generation of acetate can be reduced by genetic perturbations to modify plant cell wall\textsuperscript{10}, or prudent design of depolymerization processes of cellulosic biomass, complete elimination of acetate in cellulose hydrolysates is unattainable. Alternatively, a wide variety of approaches have been proposed for physical, chemical, and biological removal of acetate before fermentation\textsuperscript{11,12}, but they impose additional costs to implement. In addition to these pre-fermentation strategies, efforts have been devoted to engineering acetate tolerance in yeast, alleviating but being unable to eliminate the inhibitory and toxic effects of acetate\textsuperscript{13,14}. Previously, we introduced an NADH-dependent acetate reduction pathway into engineered yeast for in situ detoxification of acetate and improved ethanol production from a mixture of xylose and acetate under anaerobic conditions\textsuperscript{7}. However, the capacity of the acetate reduction pathway might be limited by the activities of key enzymes, ATP supply, and intracellular NADH levels\textsuperscript{15}. Besides, the coupled acetate reduction and xylene assimilation also limit its application to cellulosic ethanol production.

Enabling efficient acetate detoxification and valorization by S. cerevisiae would greatly facilitate industrial cellulose biorefineries due to the versatility and robustness of S. cerevisiae as an industrial production platform. Conversion of acetate into acetyl-CoA through a one-step enzymatic reaction, and production of high-value acetyl-CoA-derived molecules, such as fatty acids, sterols, polyketides, and isopenoids have been demonstrated in oleaginous yeasts\textsuperscript{16–19}. While the model yeast S. cerevisiae can produce acetyl-CoA from acetate\textsuperscript{20}, there has been no report of producing acetyl-CoA derivatives from acetate by S. cerevisiae due to toxicity of acetate and slow acetate metabolism. Dedication of S. cerevisiae to fermentative production of ethanol from glucose overshadowed the fact that it can grow on acetate as a sole carbon source under aerobic and neutral pH conditions\textsuperscript{21}. In such cases, acetate is taken up by yeast cells through either Jen1 transporter\textsuperscript{22} or Ady2 symporter\textsuperscript{23}, and converted into acetyl-CoA by acetyl-CoA synthase (Acs1 and Acs2). Acetyl-CoA is then metabolized through the tricarboxylic acid (TCA) cycle or glyoxylate shunt for the generation of ATP and precursors toward glucononeogenesis\textsuperscript{24}. Nonetheless, the respiratory metabolism of acetate as a sole carbon source may lead to unfavorable growth kinetics due to the generation of harmful reactive oxygen species (ROS)\textsuperscript{25} and unbalanced ATP supply-and-demand\textsuperscript{26}. Additionally, acetate is toxic to yeast cells at low pH (pKa = 4.76) conditions due to the ATP-dependent translocation of protons generated by intracellular dissociation of acetic acid\textsuperscript{27,28}. Most importantly, glucose inhibits the transport and metabolism of acetate regardless of aerations so that acetate is trapped as a toxic inhibitor of glucose fermentation\textsuperscript{21}.

Here, we focus on detoxifying acetate in cellulose hydrolysates and exploiting its consumption to enhance acetyl-CoA supply in S. cerevisiae for the production of value-added products. We demonstrate that xylose, unlike glucose, does not impose repression on acetate transport and metabolism, which enables efficient co-consumption of xylose and acetate under aerobic conditions. Additionally, the co-consumption substantially improves cell growth and accumulations of lipids and ergosterol, suggesting enhanced supply of acetyl-CoA when xylose and acetate are co-consumed. We, therefore, envision aerobic co-utilization of xylose and acetate, which are major carbon sources in hemicellulose hydrolysates, as a potential strategy for economically producing acetyl-CoA derivatives. In this simple metabolic design, xylose metabolism generates NADPH and ATP to support cell growth and acetate assimilation while acetate is directed toward acetyl-CoA production (Fig. 1). Such a division of duty may also ward off the negative effects of respiratory acetate metabolism while acetate is used as a sole carbon source. As a proof of concept, we choose a polyketide triacetic acid lactone (TAL) and an isopenoid vitamin A as examples of high-value acetyl-CoA derived chemicals and achieve significantly improved titers of both products through co-consumption of xylose and acetate. Thus, we establish a strategy that detoxifies acetate into a valuable substrate, expands the capacity of acetyl-CoA supply in S. cerevisiae, and enables economic conversion of plant cell wall hydrolysates into acetyl-CoA derived fine chemicals.

Results

Xylose enables efficient aerobic co-consumption of acetate in S. cerevisiae. Previously, we constructed an engineered yeast strain SR8 capable of assimilating xylose efficiently through rational engineering and adaptive laboratory evolution\textsuperscript{29}. Based on this strain, we investigated interactions of glucose or xylose with acetate assimilation under aerobic conditions by adding acetate into glucose or xylose cultures at different concentrations. Expectedly, glucose metabolism exhibited strong inhibition on acetate consumption so that acetate was not consumed in the cultures with 40 g/L of glucose (Fig. 2a). Instead, every tested acetate concentration negatively affected cell growth and ethanol re-assimilation in the glucose cultures (Fig. 2a, c). The inhibited ethanol re-assimilation was partially attributed to the disruption of ALD6 gene involved in ethanol utilization as the parental strain SR7 with wildtype ALD6 respired ethanol much faster than the SR8 strain (Supplementary Fig. 1). In contrast to the hampered acetate consumption by glucose, up to 15 g/L of acetate was assimilated by engineered yeast in the xylose cultures (Fig. 2b). Notably, xylose consumption was rather slightly boosted than hindered by the addition of up to 12.38 g/L of acetate, allowing efficient co-consumption of xylose and acetate. These results reveal that xylose metabolism empowers efficient co-consumption of acetate in S. cerevisiae under aerobic conditions.

Factors facilitating xylose and acetate co-consumption. To determine the mechanisms enabling xylose and acetate co-consumption by engineered yeast, we compared transcriptional levels of the genes (ACS1, ACS2, ADY2, JEN1, FPS1, PMA1, and HOG1) related to acetate assimilation under glucose and xylose conditions. The expression levels of ADY2 and JEN1, coding for monocarboxylic acid transporters, were significantly higher under xylene than glucose conditions (Fig. 3), indicating that xylose alleviates glucose repression on acetate transport\textsuperscript{30}. Moreover, ACS1 coding for a major isofrom of acetyl-CoA synthase was highly expressed when cells were grown on xylose as compared to glucose (Fig. 3), facilitating the production of acetyl-CoA from acetate under xylene conditions. Nevertheless, the mRNA level of PMA1, coding for a proton-translocating ATPase in the plasma
The cultures that completely consumed 40 g/L of xylose and 12 g/L of acetate (Fig. 2c). Notably, the cells from the cultures that completely consumed 40 g/L of xylose and 12 g/L of acetate accumulated lipids at a specific content of 71.93 ± 2.07 mg/g dry cell weight (DCW), roughly 48% higher than the cultures with 40 g/L of xylose (48.64 ± 4.70 mg/g DCW) only. Additionally, the cells accumulated 26.90 ± 1.31 mg/g DCW of ergosterol when consuming 12 g/L of acetate with 40 g/L of xylose, roughly 45% higher than the culture grown with xylose as a sole substrate. Considering that lipids and ergosterol are derived from a common precursor, acetyl-CoA, these results indicate that co-utilization of xylose and acetate facilitates an ample supply of acetyl-CoA in S. cerevisiae, which also contributes to enhanced accumulation of cell biomass.

Establishing and improving TAL production in S. cerevisiae. We next aimed to harness this enhanced acetyl-CoA supply during xylose and acetate co-consumption for the production of TAL, a versatile platform chemical for producing various polymers and food ingredients. Specifically, the codon-optimized Gerbera 2-pyrene synthase gene, 2PS, was expressed in a xylose-fermenting S. cerevisiae, resulting in the Tal1 strain capable of producing TAL from glucose and xylose. As expected, the Tal1 strain produced TAL from xylose at a titer (198.46 mg/L) about 6-fold higher than from glucose (33.24 mg/L) (Fig. 4a). By increasing the copy number of the 2PS gene expression cassettes up to four copies, we obtained the Tal4 strain exhibiting enhanced TAL production from both glucose (539.87 mg/L) and xylose (1522.44 mg/L) (Fig. 4a, and Supplementary Figs. 4, 5).

The Tal4 strain was cultured in different single- and mixed-carbon source conditions to investigate their carbon flux distributions toward acetyl-CoA. In terms of single-carbon source conditions, acetate exhibited the highest yield of TAL at 107.81 mg/g acetate, followed by xylose (47.47 mg/g xylose), and ethanol (40.51 mg/g ethanol) (Fig. 4b, and Supplementary Figs. 4, 5). When cultured on 40 g/L of xylose with supplementation of 10 g/L of acetate, the Tal4 strain was able to produce 47.47 mg/g xylose, 40.51 mg/g ethanol, and 47.47 mg/g acetate. The production of TAL was enhanced in the presence of xylose and acetate, indicating that co-utilization of xylose and acetate facilitates an ample supply of acetyl-CoA in S. cerevisiae.
of acetate, the Tal4 strain produced 4936.77 mg/L of TAL at a yield of 90.24 mg/g, roughly a 3-fold and 2-fold improvement over cultures with 40 g/L of xylose as a sole substrate (Fig. 4b, c, and Supplementary Fig. 6c). Even though up to 20 g/L of acetate could be completely depleted with 40 g/L of xylose, the best titer and productivity were obtained in cultures starting with 10 g/L of acetate provided (Fig. 4c, and Supplementary Fig. 6). Therefore, a 4:1 ratio of xylose and acetate concentrations, which is also a typical ratio of xylose and acetate concentrations in cellulosic hydrolysates, was adopted for the following fermentation experiments. These data demonstrate that xylose and acetate co-consumption is an effective strategy to overproduce TAL.

TAL production in a bioreactor with xylose and acetate co-feeding. To maximize the titer of TAL and explore the feasibility of the strategy in a large-scale fermentation, the Tal4 strain was cultured in a 3-L bioreactor with xylose and acetate co-feeding. The fed-batch fermentation produced 23.89 g/L of TAL with a volumetric productivity of 0.29 g/L/h (Fig. 5). As the production level far exceeded the solubility of TAL (8.6 g/L), we noticed precipitation of TAL in the culture during the fermentation and adopted cautious sampling and analysis to avoid errors in TAL measurements. Glycerol at 2.79 g/L remained the only byproduct of xylose and acetate concentrations in cellulosic hydrolysates, was adopted for the following fermentation experiments. These data demonstrate that xylose and acetate co-consumption is an effective strategy to overproduce TAL.
This may be caused by the toxicity of TAL to yeast cells at high concentrations32.

Conversion of hemicellulose fractions of switchgrass into TAL.

As xylose and acetate are predominant components of hemicellulose hydrolysates which can be readily obtained from lignocellulose materials, we next attempted bioconversion of hemicellulose fractions of switchgrass into TAL. Without any detoxification steps, the obtained switchgrass hemicellulose hydrolysates were used to cultivate the Tal4 strain. When cultured in two times concentrated hydrolysate, the Tal4 strain completely consumed xylose, acetate, and glucose within 115 h, and produced 3545.74 mg/L of TAL with a yield of 67.45 mg/g substrates (Fig. 6). Notably, 4.92 g/L glycerol was accumulated by the end of fermentation, possibly due to osmotic stress caused by the concentration and neutralization procedures33. When cultured in unconcentrated hydrolysate, the strain did not accumulate glycerol at the end of fermentation, leading to an overall higher yield of TAL (86.53 mg/g substrates) (Supplementary Fig. 7). We did not observe xylitol accumulation in this and all other xylose culturing experiments, suggesting sufficient oxygen supply to eliminate xylitol production. These results demonstrate the end of fermentation, possibly due to osmotic stress caused by the concentration and neutralization procedures33.
repression even in the presence of oxygen. Apart from molecules to examine this strategy, TAL and an isoprenoid vitamin A were selected as testbed production of acetyl-CoA derived chemicals. The simplest polyketide Nevertheless, the specific content of (pro-)vitamin A was significantly enhanced, indicating enhanced acetyl-CoA availability when acetate is co-consumed with xylose.

**Enhanced vitamin A production via co-utilization of xylose and acetate.** After demonstrating efficient TAL production from hemicellulose fractions, we investigated the effectiveness of this strategy for the production of vitamin A, another valuable acetyl-CoA derivative. Vitamin A is an essential human micronutrient that has been widely used in dietary supplements, animal feed, cosmetics, and pharmaceuticals. A previously constructed xylose-fermenting, vitamin A producing strain SR8A was used, and cultured on glucose or xylose with supplementation of acetate at varied concentrations. The co-consumption of 5 g/L and 10 g/L of acetate with 40 g/L of xylose resulted in 68.38% and 97.83% improvement in (pro-)vitamin A (vitamin A and β-carotene) titers as compared to the culture using xylose as a sole substrate (64.38 mg/L) (Fig. 7, and Supplementary Fig. 8). The best titer obtained here (127.37 mg/L) was 38.90% higher than the previous report of (pro-)vitamin A production in a xylose flask culture with dodecane in situ extraction. The improvement was partially derived from the increased cell biomass titers. Nonetheless, the specific content of (pro-)vitamin A was significantly enhanced, indicating enhanced acetyl-CoA availability when acetate is co-consumed with xylose.

**Discussion**

In this study, we present efficient xylose-acetate co-consumption and its implementation as a metabolic engineering strategy to expand acetyl-CoA supply in *S. cerevisiae* for the enhanced production of acetyl-CoA derived chemicals. The simplest polyketide TAL and an isoprenoid vitamin A were selected as testbed molecules to examine this strategy.

Acetate transport and metabolism are both subject to glucose repression even in the presence of oxygen. Apart from glucose repression, high concentrations of ethanol derived from glucose fermentation were found to constrain acetate assimilation. Accordingly, up to 12.38 g/L of acetate was co-assimilated with 40 g/L of xylose at a rate of 0.23 g/L/h (Fig. 2b). The capacity and rate of acetate consumption are roughly 7-fold and 11-fold higher, respectively than those of the anaerobic acetate reduction pathway coupled with xylose assimilation which reduced acetate into ethanol.

*S. cerevisiae* has been widely used for ethanol production for thousands of years, but this yeast is recognized to bear a limited supply of acetyl-CoA from glucose due to the Crabtree effect. Employing xylose as a carbon source instead of glucose offers an approach to address this limiting factor for enhanced production of acetyl-CoA-derived chemicals. Inspired by the enhanced accumulation of lipids and ergosterol in the cells grown on a mixture of xylose and acetate (Fig. 2c), we speculated that aerobic co-consumption of xylose and acetate might further synergistically improve acetyl-CoA supply. Specifically, xylose metabolism sustains cell growth and provides ATP to support the conversion of acetate to acetyl-CoA as a metabolic shortcut (Fig. 1). On the other hand, the consumption of acetate increases medium pH and slightly boosts xylose consumption in return (Supplementary Fig. 3). Such division of duty also wards off the negative effects of acetate respiration when used as a single substrate.

As a proof of concept, we established TAL production from xylose in an engineered *S. cerevisiae* strain and observed enhanced production by co-utilization of xylose and acetate in both shake flask and bioreactor cultures. This methodology led to an increase in TAL titer (23.89 g/L) and overall productivity (0.29 g/L/h) by greater than six-fold and fourteen-fold, respectively, compared to the titer and productivity reported previously using engineered *S. cerevisiae*. Furthermore, the productivity achieved here is over two-fold higher than a previous report using an engineered *Yarrowia lipolytica*, which is believed to harbor high flux through acetyl-CoA. We also employed this strategy for the production of vitamin A (Fig. 7), indicating its versatility and effectiveness for overproducing many other high-value acetyl-CoA derived bioproducts in *S. cerevisiae* such as n-butanol, (poly) hydroxybutyrate, fatty acids, flavonoids, and other polyketides and isoprenoids.

More importantly, this strategy can be seamlessly integrated into cellulosic biomass-based biorefineries as xylose and acetate are predominant components of hemicellulose hydrolysates; we found that hemicellulose fractions of plant cell wall hydrolysates can be completely converted into acetyl-CoA derived fine chemicals. Instead of acting as an inhibitor, acetate becomes a valuable substrate when coupled with its hemicellulose counterpart under aerobic conditions. Following this concept, cellodextrins could also allow acetate co-consumption considering intracellular hydrolysis of cellodextrins into glucose might alleviate glucose repression on acetate transport, enabling a more attractive bioconversion of cellulosic biomass. Moreover, these results demonstrate the feasibility of coupling sugar metabolism with organic acid assimilation in *S. cerevisiae*, opening the door to many potential applications. For instance, acid whey, a byproduct from the yogurt and cheese industries that contains substantial amounts of lactose and lactic acid could be harnessed to produce value-added products in engineered yeast. Additionally, the cytosolic accumulation of citric acid and an inactive overflow metabolism are required to shift the metabolism of *S. cerevisiae* from ethanol fermentation to lipogenesis, we envision that
c coupling citrate assimilation with xylose metabolism may facilitate lipoid overproduction in S. cerevisiae. Metabolic optimization using a mixture of substrates has been proposed as a unique strategy to enhance the performance of microbes. Together with current metabolic engineering approaches, the co-substrate strategy would circumvent many bottlenecks associated with the complex and highly regulated sugar metabolism facilitating enhancements in yield and productivity. In the case of using sugar and organic acid as mixed-carbon sources, sugar acts as a primary substrate to support cell growth while organic acid is added as a second substrate that provides a shortcut towards key metabolic intermediate such as acetyl-CoA. With proper design of sugar-organic acid combinations and feeding patterns, the principal biosynthetic components, carbon, ATP, and reducing equivalents, could be better balanced for an optimal production level exceeding the theoretical maximum of a single substrate fermentation.

Collectively, this work demonstrates a versatile strategy that deployed a ubiquitous inhibitor in plant cell wall hydrolyses into a valuable substrate, overcoming the limit of native metabolism for the supply of acetyl-CoA in S. cerevisiae, and enabling economically sustainable conversion of cellulosic carbon into high-value acetyl-CoA derived bioproducts. Based on these results, we envision that co-utilization of sugars and organic acids could be a paradigm of bioconversion in addition to traditional sugar-only or organic acid-only utilization for the production of biofuels, chemicals, and bioproducts.

Methods

Plasmid and strain construction. The strains, plasmids, and oligonucleotides used in this study are listed in Supplementary Table 1, 2, 3, respectively. Standard protocols were used for molecular biology procedures. The amino acid sequence of Gerbera hybrida 2-pyrone synthase obtained from UniProtKB (accession number P48391.2) was codon-optimized and synthesized as a gBlock by IDT (Integrated DNA Technologies, USA). The amino acid sequence was amplified using primers 2PS-Amp-U and 2PS-Amp-D and cloned into the Xhol and BamHI sites of the pRS426-PCW12 plasmid, resulting in the plasmid pRS426-2PS. The 2PS expression cassette flanked by a strong constitutive Ccw12 promoter and CYC1 terminator was cloned into yeast integrative plasmids pRS403, pRS404, pRS405, and pRS406 using CloneEZ2 PCR Cloning Kit (GeneScript Biotech, USA), resulting in pRS403-2PS, pRS404-2PS, pRS405-2PS, and pRS406-2PS, respectively. The primer sets Insert-U/Insert-D and Vector-U/Vector-D were used for the PCR cloning.

To construct TAL producing strains, TRP1 was disrupted by Cas9-based gene modification34 as an extra auxotrophic marker in the CT2-auxo strain. Donor DNA was amplified using primers TRP1Donor-U and TRP1Donor-D. The plasmid CAS9-NAT (Addgene #64329) was transformed into the CT2-auxo strain followed by the guide RNA plasmid gRNA-trp-HYB and donor DNA transformation. Cells were selected on yeast extract-peptone (YP) plate (10 g/L yeast extract, 20 g/L peptone) containing glucose (YPD) supplemented with 120 μg/mL nourseothricin and 300 μg/mL Hygromycin B (YPDHB). The positive colonies with TRP1 deletion were confirmed by sequencing using primers TRP1-Seq-U and TRP1-Seq-D and designated as the CT2-4 strain. The integrative plasmids pRS403-2PS, pRS406-2PS, pRS405-2PS, and pRS404-2PS were linearized using restriction enzymes NdeI, Ncol, AflII, and PmlI (New England Biolabs, USA), respectively, and sequentially transformed into CT2-4 strain. Cells were selected on synthetic complete (SC) plate minus the appropriate auxotrophic compounds, yielding Tal1, Tal2, Tal3, and Tal4 strains with different copy numbers of the 2PS gene. The empty plasmids pRS403, pRS404, pRS405, and pRS406 were used to construct a prototrophic strain CT2-con.

Media and culture conditions. For shake flask fermentation, yeast cells were routinely pre-cultured in YPD medium and inoculated into main fermentations. The initial cell optical density (OD500nm) of fermentations for TAL production in defined medium or unconcentrated switchgrass hemicyellose hydrolysate was ~1. All other flask fermentations started with an initial OD500nm of ~10. Main fermentations were conducted with 50 mL of modified Verduyn medium37 containing glucose, xylose, acetate (potassium acetate), or ethanol as a carbon source in 250 mL baffled flasks at 30 °C and 300 rpm. Appropriate amino acids were added while culturing auxotrophic strains. Fermentation media were buffered with 50 mM potassium hydrogen phthalate at pH of 5.5. Fed-batch fermentation for TAL production was performed at the initial OD500nm of ~1.5 by automatically by pump and continuous feeding of HCl. The temperature was held constant at 30 °C with agitation adjusted from 3 Lpm (lines per minute) to 5 Lpm to maintain aerobic conditions according to the cell densities. Anilox 204 (Sigma-Aldrich, St. Louis, US) was added to prevent the formation of foam.

Preparation and conversion of switchgrass hydrolysate. The switchgrass was harvested from the University of Illinois Energy Farm and shredded into small pieces, washed, and then dried at 60 °C for 24 h. The resulting dry biomass was ground to ~2% sulfuric acid. The hydrolysate was conducted using a sterilization cycle of 121 °C for 60 min in a laboratory steam sterilizer (Amresco Lab 250, Steris, Ireland). The liquid fraction was separated from solids by filtration and neutralized using calcium hydroxide to pH 5.5. The resulting hemicellulose hydrolysate was either concentrated two times before or directly used for the production of TAL by the Tal4 strain in a 250 mL baffled flask with supplementation of 2 g/L yeast extract and 2 g/L peptone as a nitrogen source. The two times concentrated hydrolysate contained 40.00 g/L of xylose, 6.26 g/L of acetate, 6.31 g/L of glucose, 2.01 g/L of furfural, and 0.42 g/L of hydroxymethylfurfural (HMF). The fermentation was conducted in 50 mL of the fertilized hydrolysate at 30 °C and 300 rpm with an initial cell optical density of ~1 for nonconcentrated hydrolysate or ~10 for two times concentrated hydrolysate.

RNA sequencing. The rationally engineered xylose-fermenting S. cerevisiae SR7 which is the parental strain of SR8 and has a clear genetic background was used for RNA sequencing experiments to rule out the potential effects of the evolved mutations in SR836. The SR7 strain was cultured in 50 mL YP media containing either 40 g/L glucose or 40 g/L xylose at initial OD500nm of ~0.1. The cultures were incubated in 250 mL flasks at 30 °C and 100 rpm. Cells were sampled in triplicate when OD500nm reached 1 for RNA extraction. By the time of sampling, there was around 37 g/L glucose or 33 g/L xylose remained in the media (Supplementary Fig. 9). Total RNA was extracted using YeastRNA Kit (Zymo Research, USA), and the quality of the RNA sample was evaluated via gel electrophoresis. Transcript patterns of SR7 strain on glucose and xylose were determined through RNA sequencing which was conducted on an Illumina HiSeq 2000 system at the W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign. QIAGEN CLC Genomics Workbench 6.3 was used to map the RNA-sequencing data to the reference SR7 genome generated via gBlock synthesis and mapped to the S. cerevisiae S288C reference genome. The total number of exonic reads per kilobase million (RPKM) from three biological replicates of xylose culture were compared to those of glucose culture. Statistical significance of the differences was evaluated using two-tailed Student’s t-test.

Quantitative analysis. Cell growth was monitored by measuring OD600 using a spectrophotometer (BioMate 5; Thermo Fisher Scientific, Waltham, USA). A conversion factor of 0.41 was adopted to determine DCW from optical density. The concentrations of glucose, xylose, xylitol, acetate, glycerol, and ethanol in culturing media were detected by high-performance liquid chromatography (HPLC, Agilent 1200 Series, Agilent Technologies, Wilmington, USA) equipped with a refractive index detector and the Rezex ROA-Organic Acid H+ (8% column (Phenomenex Inc, Torrance, CA). Culturing media were diluted and analyzed at 50 °C with 0.005 M H2SO4 as the mobile phase. The flow rate of the mobile phase was set at 0.6 mL/min. Data were collected by Agilent ChemStation B.03.

Total lipid weight was determined following a previously reported protocol. Specifically, 2 mL cell cultures with cell optical density of 10 were centrifuged at 21,130 × g for 1 min. Cell pellets were transferred into 15-mL glass tubes and crushed using BeadBuster with 6 mL of chloroform/ methanol (1:1 volumetric). The samples were then mixed with 1.5 mL water and vortexed for 1 min. After centrifugation, the organic layer was collected, washed with 1.5 mL of 0.1% (v/v) NaCl water solution, and dried overnight at room temperature in a preweighed tube. The residue was further dried at 50 °C by purging nitrogen to constant weight to determine lipid content. Total lipid weight was calculated from the final tube weight by subtraction of the original tube weight. Total lipid weight was divided by DCW to obtain specific lipid content.

For ergosterol quantification, cell pellets were saponified and extracted using n-heptane for HPLC measurement. Briefly, cell extracts from 2 mL of fermentation broth were mixed with 0.6 mL extraction solution (50% KOH: C2H5OH = 2:3) and incubated at 85 °C water bath for 2 h. Afterward, the saponified mixtures were extracted with 0.6 mL n-heptane. n-heptane was evaporated by vacuum centrifugation. Dried samples were dissolved in 0.5 mL of acetone for the analysis using Shimadzu HPLC system equipped with UV detector (Shimadzu SPD-20A) and C18 column (Phenomenex Kinetex 5 μm C18). The mobile phase was 100% acetonitrile at a flow rate of 2 mL/min. Ergosterol was detected at 280 nm. Data were collected by Shimadzu LabSolutions CS C191-E020. TAL was quantified by HPLC (LC-20A, Shimadzu, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD-20A, Shimadzu, Kyoto, Japan). Specifically, the fermentation broth was diluted at an appropriate rate, mixed thoroughly, and centrifuged at 21,130 × g to remove cells. The supernatant was injected into HPLC.
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Author contributions

L.S. and Y.S.J. designed research; L.S., J.W.L., S.Y., Z.Q.S., and S.R.K. performed research; L.S., S.R.K., and S.L. analyzed data; and L.S., S.L., and Y.S.J. wrote the paper. All authors read and approved the paper.

Competing interests

The authors declare no competing interest.

Additional information

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