Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Metabolic engineering of yeast for lignocellulosic biofuel production

Yong-Su Jin[1,2] and Jamie HD Cate[3,4,5]

Production of biofuels from lignocellulosic biomass remains an unsolved challenge in industrial biotechnology. Efforts to use yeast for conversion face the question of which host organism to use, counterbalancing the ease of genetic manipulation with the promise of robust industrial phenotypes. *Saccharomyces cerevisiae* remains the premier host for metabolic engineering of biofuel pathways, due to its many genetic, systems and synthetic biology tools. Numerous engineering strategies for expanding substrate ranges and diversifying products of *S. cerevisiae* have been developed. Other yeasts generally lack these tools, yet harbor superior phenotypes that could be exploited in the harsh processes required for lignocellulosic biofuel production. These include thermotolerance, resistance to toxic compounds generated during plant biomass deconstruction, and wider carbon consumption capabilities. Although promising, these yeasts have yet to be widely exploited. By contrast, oleaginous yeasts such as *Yarrowia lipolytica* capable of producing high titers of lipids are rapidly advancing in terms of the tools available for their metabolic manipulation.

Addresses
[1] Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana, IL, USA
[2] Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL, USA
[3] Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720, USA
[4] Department of Chemistry, University of California at Berkeley, Berkeley, CA 94720, USA
[5] Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

Corresponding authors: Jin, Yong-Su (ysjin@illinois.edu), Cate, Jamie HD (jcate@lbl.gov)

Current Opinion in Chemical Biology 2017, **41**:99–106

This review comes from a themed issue on **Energy**

Edited by **Matthew Kanan**

https://doi.org/10.1016/j.cbpa.2017.10.025

Engineered *Saccharomyces cerevisiae* for the production of cellulosic biofuels

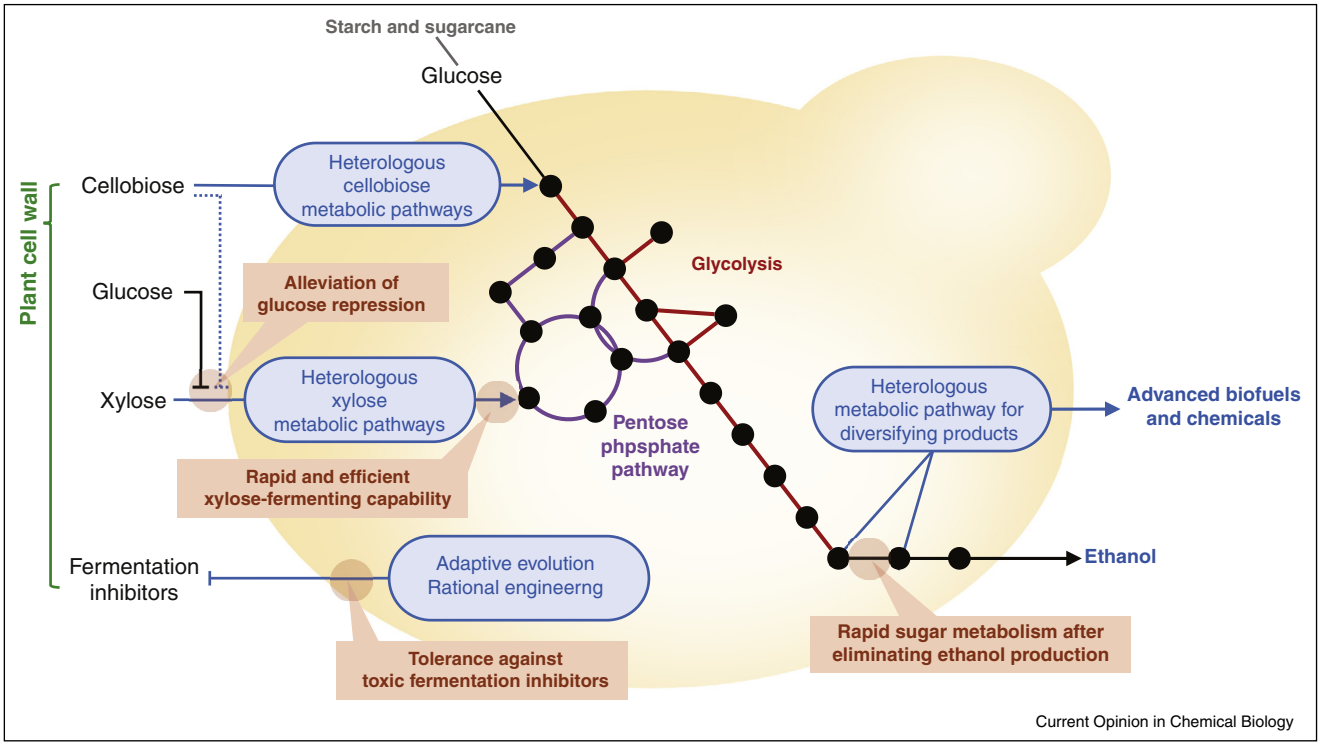
S. cerevisiae has been the workhorse species for the production of ethanol — the only biofuel currently produced at a large scale — from corn and sugarcane. This yeast not only produces ethanol from hexoses at high rates and yields, but also exhibits desirable phenotypes for industrial fermentation, such as high osmotolerance, rapid anaerobic metabolism, and resistance to viral (i.e. phage) infection. Nonetheless, four key issues need to be addressed in order to employ *S. cerevisiae* for the production of cellulosic biofuels (Figure 1). First, an efficient and rapid xylose-fermenting capability needs to be introduced as plant cell wall hydrolysates contain xylose up to 40% of total fermentable sugars in addition to glucose. Second, glucose repression of xylose fermentation needs to be alleviated to increase overall productivity. Third, tolerance against toxic fermentation inhibitors present in cellulosic hydrolysates needs to be improved for fermenting low-cost hydrolysates. Fourth, rapid sugar metabolism needs to be maintained after eliminating ethanol production for producing advanced biofuels. Diverse metabolic engineering approaches to address the abovementioned issues have been undertaken for decades, leading to implementation of nascent commercial operations for the production of cellulosic biofuels.

Engineered xylose fermenting pathways

Two metabolic pathways — either oxidoreductase or isomerase based — have been implanted into *S. cerevisiae* to enable xylose fermentation. While there have been numerous publications reporting the construction of xylose fermenting *S. cerevisiae*, most of them share the following common engineered features. First, expression levels of enzymes in the xylose assimilating pathways and pentose phosphate pathway (PPP) were optimized to increase the xylose uptake rate and minimize the production of reduced byproducts (xylitol and glycerol). Second, the resulting engineered strains, which had sub-optimal xylose fermenting phenotypes, were subjected to laboratory evolution under xylose conditions to overcome the enzymatic and regulatory barriers.

Zhou *et al.* constructed an efficient xylose fermenting *S. cerevisiae* strain through a combination of rational metabolic engineering and laboratory evolution. Increased dosages of the xylose isomerase (XI) gene, enhanced xylulose kinase (XK) expression, and up-regulated non-oxidative PPP enabled rapid xylose fermentation by these engineered yeast [1]. The highest ethanol yields were

Figure 1



Metabolic engineering of *S. cerevisiae* for the production of advanced biofuels and chemicals from cellulosic sugars. Various metabolic engineering strategies addressing alleviation of glucose repression, rapid and efficient xylose-fermenting capability, enhanced tolerance against fermentation inhibitors and rapid sugar metabolism without ethanol production need to be developed for the economic production of biofuels and chemicals from cellulosic biomass.

achieved through overexpression of a mutant XI and *Scheffersomyces stipites TAL1* along with deletion of *GRE3* and *PHO13* [2]. Engineering xylose assimilation through the oxidoreductase pathway followed similar strategies of combining rational metabolic engineering with laboratory evolution. Additional deletion of *PHO13* and *ALD6* led to enhanced xylose fermentation [3]. As Cas9-based genome engineering is available in yeast, marker and scar-free construction of xylose fermenting *S. cerevisiae* has been demonstrated through CRISPR-Cas9 genome editing [3].

Alleviation of glucose repression on xylose fermentation

As cellulosic hydrolysates are comprised of mixtures of hexose and pentose sugars, such as glucose and xylose, substantial efforts have been made toward engineering yeast capable of metabolizing these sugars efficiently and simultaneously. Simulations based on the kinetic properties of the endogenous yeast transporters indicated that glucose would inhibit the uptake of xylose [4], prompting the search for alternative methods to simultaneously uptake hexose and pentose sugars.

The identification of cellodextrin transporters capable of expression in yeast allowed the import and intracellular hydrolysis of cellulose-derived cellodextrins into glucose monomers [5]. By coupling this advance with xylose utilization, glucose inhibition of xylose uptake could be avoided by hydrolyzing cellodextrins into glucose intracellularly. Ha *et al.* constructed an engineered *S. cerevisiae* capable of efficient fermentation of xylose and cellobiose and demonstrated successful simultaneous consumption of carbon sources within simulated cellulosic hydrolysates [6], establishing co-fermentation of cellobiose and xylose as a viable strategy for bypassing glucose repression of xylose uptake and promising for cellulosic biofuel production.

Later, it was shown that acetic acid — a toxic fermentation inhibitor ubiquitous in cellulosic hydrolysates — can be simultaneously consumed along with cellobiose and xylose. This strategy not only diminished the toxic effect of acetic acid but also increased yield and productivity of biofuel production by using acetic acid as a carbon source [7••].

Following the computational simulations of glucose inhibition on xylose transport [4] and demonstrations of simultaneous cellobiose and xylose consumption, xylose transport was experimentally verified as the overall