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Advances in cellulosic conversion to fuels: engineering yeasts for cellulosic bioethanol and biodiesel production

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Cellulosic fuels are expected to have great potential industrial applications in the near future, but they still face technical challenges to become cost-competitive fuels, thus presenting many opportunities for improvement. The economical production of viable biofuels requires metabolic engineering of microbial platforms to convert cellulosic biomass into biofuels with high titers and yields. Fortunately, integrating traditional and novel engineering strategies with advanced engineering toolboxes has allowed the development of more robust microbial platforms, thus expanding substrate ranges. This review highlights recent trends in the metabolic engineering of microbial platforms, such as the industrial yeasts *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, for the production of renewable fuels.

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Introduction

Lignocellulosic biomass has gained significant attention as a promising renewable resource for biofuel production from an economic and environmental perspective. With recent technical advances and political and financial support, several companies have launched industrial-scale cellulosic bioethanol production plants with capacities of over 10 million gallons per year over the past few years [1]. Although this progress is encouraging, the economic feasibility of these pioneering facilities is not yet optimal, thus hampering the expansion of the cellulosic bioethanol industry and the production of other cellulosic biofuels.

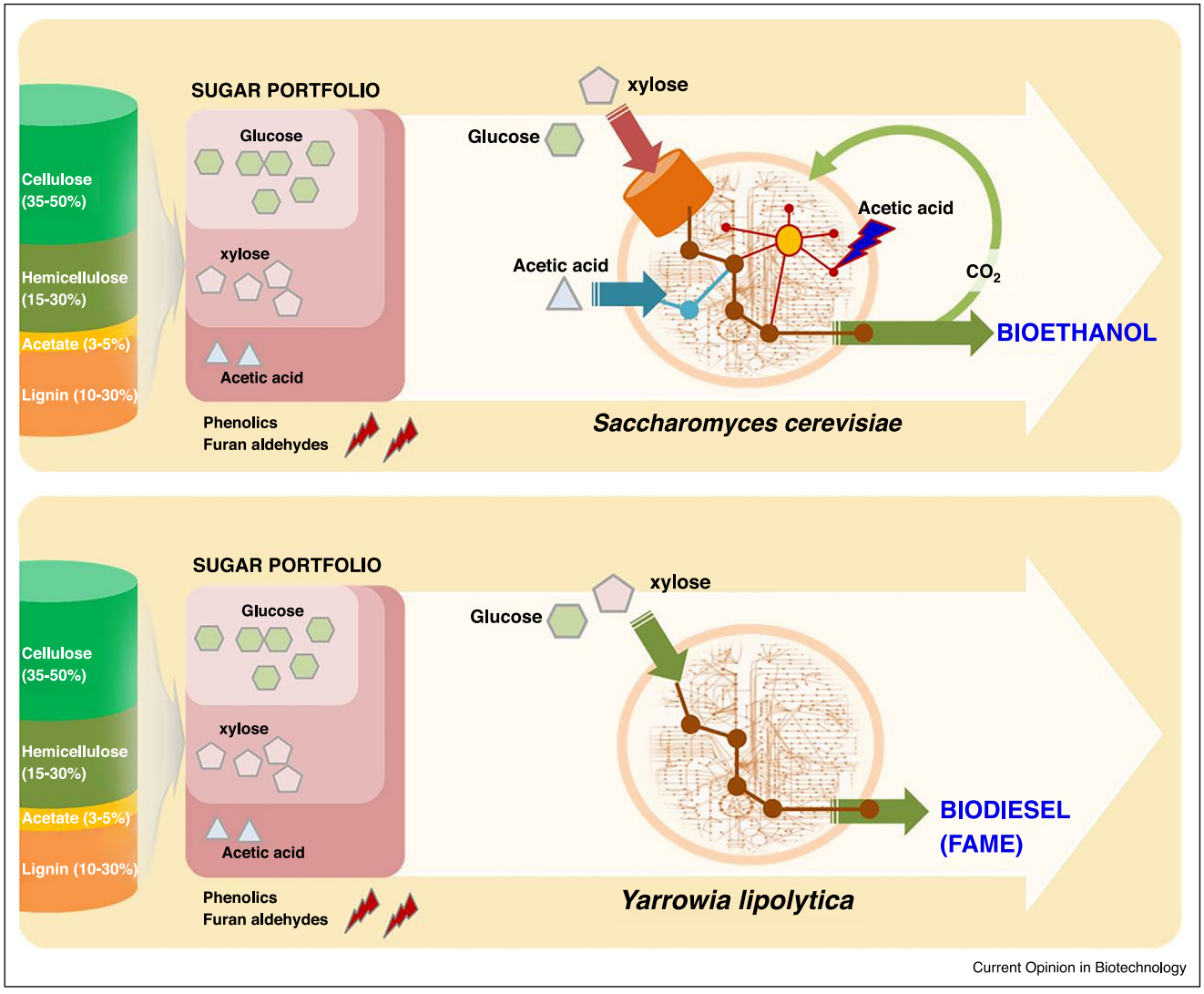
The economic viability of cellulosic biofuel production strongly depends on the performance of the microbial cell in utilizing a broad range of carbon substrates and handling toxic compounds derived from lignocellulosic biomass [2]. The main endeavors in the field of microbial engineering for cellulosic biofuel production have been the exploitation of robust microbial platforms with an expanded or improved carbon source portfolio (*e.g.* glucose, xylose, and acetic acid) and tolerance profiles (*e.g.* acetic acid, phenolics, and furfural); these profiles are directly related to biofuel productivity, titer, and yield [2]. Specifically, recent advances in the development of strains for cellulosic ethanol production have introduced a new era in cellulosic biofuel production by integrating traditional and novel engineering strategies encompassing not only metabolic pathways for the cellulosic biomass conversion into ethanol but also sugar transporters and gene regulatory networks. Moreover, the advanced metabolic engineering toolboxes have spurred the development of more powerful bioethanol producers, resulting in significantly improved production titers and yields. Recently, the cellulosic bioethanol production concept has been expanded to biodiesel production in which oleaginous yeasts are increasingly engineered as emerging biofuel production hosts and to other advanced biofuels (*e.g.* biobutanol) as well. Here, we present an overview of the recent advances and new trends in the engineering of microbial platforms for cellulosic biofuel production with a focus on the conventional biofuels, bioethanol and biodiesel, and their main producers, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* (Figure 1).

Bioethanol: a path forward for the most conventional biofuel

New trend in the engineering of S. cerevisiae, the common bioethanol producer

The yeast *Saccharomyces cerevisiae* is a particularly attractive ethanol-producing microbial platform due to its well-characterized physiology, the availability of genetic tools and already proven industrial feasibility [3]. However, owing to its inability to assimilate xylose, the transition from 1st to 2nd generation bioethanol production has shown limited practical success, even with intensive metabolic engineering efforts. Over the past two years, the engineering strategies have expanded beyond the traditional concept of optimizing catabolic pathways, which focuses on certain key steps in the xylose catabolic pathway, to further improve cellulosic ethanol yield and/or productivity. The underscored

Figure 1



Current focuses on the engineering of yeasts for cellulosic biofuel production. Engineering approaches of *S. cerevisiae* to enhance bioethanol production from complex lignocellulosic biomass sources, including (a) transcription factor engineering for improved xylose catabolism and/or stress tolerance; (b) the development of xylose-specific transporters; and (c) non-sugar carbon source (acetate) catabolism. For cellulosic biodiesel production, *Y. lipolytica* is actively engineered to introduce the xylose catabolic pathway.

engineering strategies include (1) transporter and transcription factor engineering to boost the efficiency in carbon utilization and/or to improve stress tolerance and (2) reconsideration of acetic acid as a carbon source rather than an inhibitor. These transitions were largely contributed from evolutionary engineering in the era of affordable genome sequencing. Through this journey, unconventional engineering targets have also been revealed that have reinforced applicable engineering strategies. In the following section, we highlight the recent trends in the engineering strategies to develop more powerful cellulosic ethanol-producing strains of *S. cerevisiae* (Table 1).

Development of powerful mixed sugar co-fermenting yeast strain

Despite the improved xylose catabolic pathways, the limited xylose utilization led to engineering strategies being redirected to increase intracellular xylose transport. Directed evolution has led to the development of mutant xylose transporters with significantly improved xylose utilization rates and alleviated glucose repression. CiGXS1 $FIVFH_{497*}$, a mutant glucose/xylose co-transporter evolved from CiGXS1 of *Candida intermedia*, enabled *S. cerevisiae* to have a slightly faster xylose transfer rate than that of glucose while retaining the glucose transport rate at the wild-type level [4•]. Directed