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Development of microbial cell factories for bio-refinery through synthetic bioengineering

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ABSTRACT

Synthetic bioengineering is a strategy for developing useful microbial strains with innovative biological functions. Novel functions are designed and synthesized in host microbes with the aid of advanced technologies for computer simulations of cellular processes and the system-wide manipulation of host genomes. Here, we review the current status and future prospects of synthetic bioengineering in the yeast *Saccharomyces cerevisiae* for bio-refinery processes to produce various commodity chemicals from lignocellulosic biomass. Previous studies to improve assimilation of xylose and production of glutathione and butanol suggest a fixed pattern of problems that need to be solved, and as a crucial step, we now need to identify promising targets for further engineering of yeast metabolism. Metabolic simulation, transcriptomics, and metabolomics are useful emerging technologies for achieving this goal, making it possible to optimize metabolic pathways. Furthermore, novel genes responsible for target production can be found by analyzing large-scale data. Fine-tuning of enzyme activities is essential in the latter stage of strain development, but it requires detailed modeling of yeast metabolic functions. Recombinant technologies and genetic engineering are crucial for implementing metabolic designs into microbes. In addition to conventional gene manipulation techniques, advanced methods, such as multicistronic expression systems, marker-recycle gene deletion, protein engineering, cell surface display, genome editing, and synthesis of very long DNA fragments, will facilitate advances in synthetic bioengineering.

1. Introduction

The construction of bio-refinery processes to produce various commodity chemicals from lignocellulosic biomass requires the development of useful microbial strains using intensive application of various biotechnologies (Keasling, 2010). The microbial cell factories perform various processes, permitting cost-effective bio-refinery (Hasunuma and Kondo, 2011). Pentose and hexose sugars derived from biomass are converted to target compounds by optimized metabolic pathways with efficiency close to the theoretical yield (Kim et al., 2010; Na et al., 2010). Direct saccharification of biomass and stress tolerance against a harsh condition during the fermentation are also considered in the development of microbial cell factories (Kondo et al., 2010). As these capabilities largely depend on microbial metabolic functions, advanced engineering strategies are required to develop microbial cell factories with optimized performance by the modification, overhaul, and *de novo* construction and integration of metabolic pathways (Bera et al., 2011; Krivoruchko et al., 2011; Na et al., 2010; Zhang et al., 2011).

In this article, we review the current status of synthetic bioengineering in the yeast *Saccharomyces cerevisiae*. As demonstrated for the industrial production of bioethanol, *S. cerevisiae* is an ideal host for bio-refinery processes due to its capacity for cell-recycle fermentation and its remarkable tolerance against various stresses, such as low pH, high temperature, and various inhibitors (Hasunuma and Kondo, 2011). Additionally, *S. cerevisiae* is an extremely well characterized model organism, with a large variety of research tools and resources facilitating metabolic engineering (Cherry et al., 2012; Krivoruchko et al., 2011; Nielsen and Jewett, 2008). Although there is a great deal of information on *S. cerevisiae*, including the complete genome sequence and detailed characterization of metabolic pathways (Cherry et al., 2012; Otero et al., 2010), the engineering of metabolism in this species is still daunting because its metabolic system is too complex to allow researchers to accurately predict the consequences of metabolic modification

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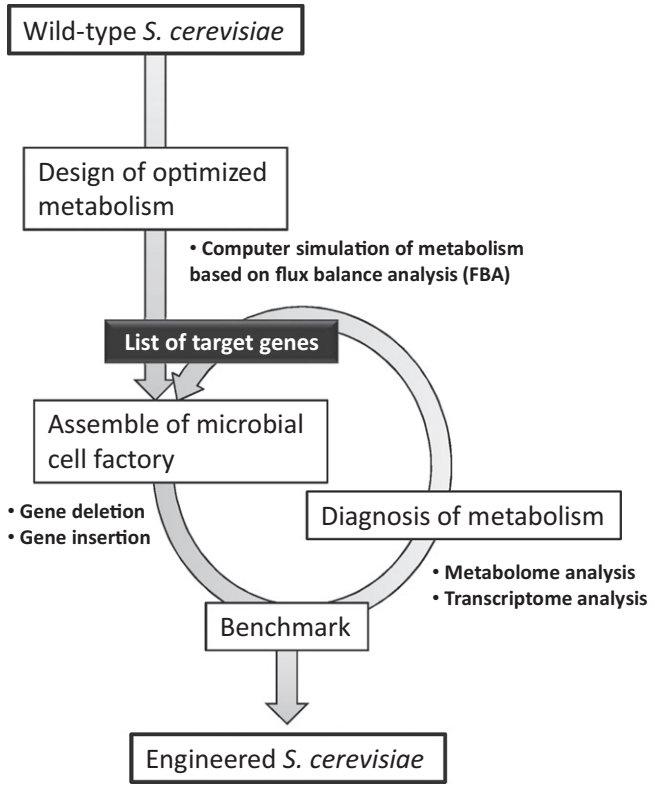

Fig. 1. The research strategy in synthetic bioengineering.

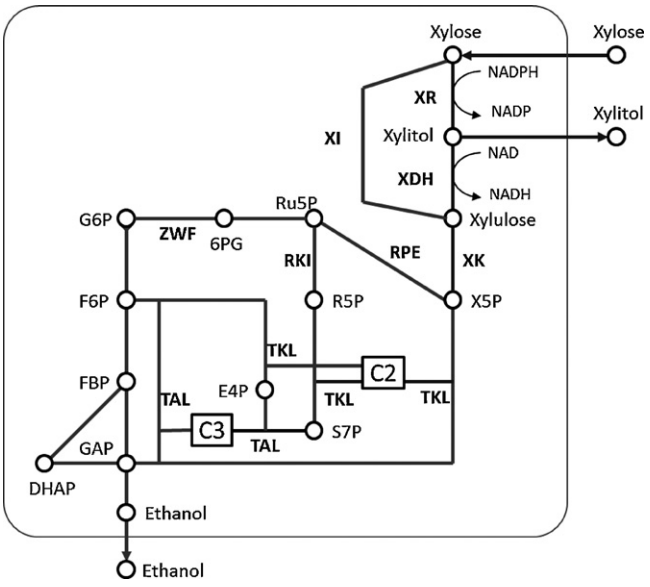

Fig. 2. The xylose assimilation pathway in metabolically engineered *S. cerevisiae*. *Abbreviations*: DHAP, dihydroxyacetonephosphate; E4P, erythrose-4-phosphate; FBP, fructose-1,6-*bis*phosphate; F6P, fructose-6-phosphate; GAP, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; 6PG, 6-phosphogluconate; R5P, ribose-5-phosphate; Ru5P, ribulose-5-phosphate; S7P, sedoheptulose-7-phosphate; X5P, xylulose-5-phosphate. Enzymes are indicated by their gene symbols: TAL, transaldolase; TKL, transketolase; RPE, ribulose-5-phosphate 4-epimerase; RKI, ribose-5-phosphate isomerase; XI, xylose isomerase; XK, xylulokinase; XDH, xylitol dehydrogenase; XR, xylose reductase; ZWF, glucose-6-phosphate dehydrogenase.

(Kim et al., 2012). Due to this challenge, the metabolic engineering of *S. cerevisiae* has generally been performed on a trial-and-error basis. After various modifications are tested, the most promising approaches are implemented to increase target production. Although success has been achieved using this method, a novel and rational strategy has recently been pursued for more radical and system-wide modification of metabolism (Cherry et al., 2012; Krivoruchko et al., 2011; Nielsen and Jewett, 2008). The novel approach, termed *synthetic bioengineering*, is essential for creating yeast cell factories for target compound production (Fig. 1). The synthetic bioengineering approach is, however, not special as a research and development program as shown in Fig. 1. In the beginning of the engineering work, an optimized metabolic pathway is designed by aid of a computer simulation. Based on the blueprint of metabolic pathway, a list of target genes to be deleted or inserted is determined. A customized yeast cell factory is assembled with using advanced gene manipulation techniques. Following a benchmark of prototype strains, its problems diagnosed from a metabolomic and transcriptomic data are fixed for further improvement of its performance. This review consists of three sections. In the first section, the achievements of metabolic engineering to improve xylose assimilation, and glutathione and butanol production are discussed to provide an overview of the current state of the field. The second section deals with the roles of metabolic simulation, transcriptomics, metabolomics and fine-tuning of metabolic functions in synthetic bioengineering. In the final section, advanced gene manipulation techniques for the construction of yeast cell factories are described, and future prospects are discussed.

2. Metabolic engineering of yeast

2.1. Metabolic engineering of yeast strains for xylose fermentation

The improvement of xylose fermentation has been an important goal of metabolic engineering in *S. cerevisiae* because xylose is the second most abundant pentose sugar in lignocellulosic materials. Most efforts have focused on manipulation of the initial xylose assimilation pathway (Fig. 2) (Hahn-Hagerdal et al., 2007; Matsushika et al., 2009; Nevoigt, 2008; Van Vleet and Jeffries, 2009). Anaerobic xylose fermentation in *S. cerevisiae* was first achieved by heterologous expression of xylose reductase (XR) and xylitol dehydrogenase (XDH) genes from *Scheffersomyces stipitis* along with the overexpression of endogenous xylulokinase (XK) gene (Matsushika et al., 2008) (Fig. 2). Heterologous expression of XR and XDH genes gave higher specific ethanol production as a result of a higher xylose consumption rate. However, the ethanol yield was less than the theoretical yield because a significant amount of the by-product xylitol was produced and released into the fermentation medium. This was likely due to an intracellular redox imbalance caused by the difference in coenzyme preference between *S. stipitis* XR and XDH, which preferentially use NADPH and NAD^+, respectively. The excess $NADP^+$ and NADH formation may result in the xylitol by-production. To overcome this hurdle, the xylose isomerase (XI) gene, derived from bacteria and anaerobic fungi, was introduced into *S. cerevisiae* (Hahn-Hagerdal et al., 2007). Since XI converts xylose to xylulose in one step, ethanol yield close to theoretical can be achieved by avoiding cofactor imbalance (Fig. 2) (Karhumaa et al., 2007b). However, the xylose consumption rate of the XI-expressing strain was lower than that of the XR/XDH-based strain because of the low activity of XI in the recombinant yeast (Madhavan et al., 2009).

A series of studies demonstrated that the introduction of xylose utilization pathways can cause problems in *S. cerevisiae* metabolism that hamper efficient ethanol production. For example, in recombinant xylose-utilizing *S. cerevisiae* strains, xylose is transported by non-specific hexose transporters with poor affinity (Saloheimo et al., 2007). The expression of heterologous xylose transporter genes has enhanced xylose utilization in recombinant xylose-fermenting *S. cerevisiae*. Enhanced xylose fermentation to ethanol was observed when *S. stipitis* Sut1p and a glucose/xylose facilitator