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Genome and metabolic engineering in non-conventional yeasts: Current advances and applications

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ABSTRACT

Microbial production of chemicals and proteins from biomass-derived and waste sugar streams is a rapidly growing area of research and development. While the model yeast *Saccharomyces cerevisiae* is an excellent host for the conversion of glucose to ethanol, production of other chemicals from alternative substrates often requires extensive strain engineering. To avoid complex and intensive engineering of *S. cerevisiae*, other yeasts are often selected as hosts for bioprocessing based on their natural capacity to produce a desired product: for example, the efficient production and secretion of proteins, lipids, and primary metabolites that have value as commodity chemicals. Even when using yeasts with beneficial native phenotypes, metabolic engineering to increase yield, titer, and production rate is essential. The non-conventional yeasts *Kluyveromyces lactis, K. marxianus, Scheffersomyces stipitis, Yarrowia lipolytica, Hansenula polymorpha and Pichia pastoris* have been developed as eukaryotic hosts because of their desirable phenotypes, including thermotolerance, assimilation of diverse carbon sources, and high protein secretion. However, advanced metabolic engineering in these yeasts has been limited. This review outlines the challenges of using non-conventional yeasts for strain and pathway engineering, and discusses the developed solutions to these problems and the resulting applications in industrial biotechnology.

Contents

Abbreviations: HR, homologous recombination; NHEJ, nonhomologous end-joining; DSB, double strand break; CRISPR, Clustered regularly interspaced short palindromic repeats; TALEN, transcription activator-like effector nucleases; sgRNA, short (or single) guide RNA; PAM, protospacer adjacent motif.

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1. Introduction

The microbial production of fuels and chemicals from biomass and other renewable carbon sources is an attractive alternative to petroleum-derived products. One of the largest scale example of this is ethanol production by the yeast *Saccharomyces cerevisiae*—in 2015, over 25 billion gallons were produced worldwide from starch, waste sugar streams, and biomass-derived sugars (www.afdc.energy.gov/data/10331). *S. cerevisiae* is the organism of choice because of its high rate of production and tolerance to ethanol titers upwards of 120 g L^{-1} [1,2]. These phenotypes, among others, have led to the widespread study of *S. cerevisiae* and its development as a model eukaryotic host for chemical biosynthesis. A valuable approach to metabolic engineering is identifying organisms with desirable phenotypes and developing new synthetic biology tools to enhance these phenotypes. Bioethanol production in *S. cerevisiae* is a good example of this, and illustrates the potential of identifying other hosts and phenotypes to synthesize bioproducts other than ethanol. A number of examples of this strategy already exist in industry, where non-conventional yeasts with unique and advantageous phenotypes are used to produce proteins, lipids, and commodity chemicals. Metabolic engineering in these yeasts is, however, more challenging in comparison with *S. cerevisiae*, because less is known about their metabolism and genomics, and advanced genetic engineering tools are limited.

In this review, we focus on six non-conventional yeasts (Table 1): *Kluyveromyces lactis, K. marxianus, Scheffersomyces (Pichia) stipitis, Yarrowia lipolytica, Hansenula polymorpha, and Pichia pastoris.* In contrast to *S. cerevisiae,* these yeasts are Crabtree negative and favor respiration over fermentation; phenotypes that are particularly useful for protein production as well as the biosynthesis of chemicals other than ethanol [3]. *K. lactis* is discussed here because of its capacity to metabolize inexpensive substrates such as waste whey and because of its use as a host for heterologous protein production in the food, feed, and pharmaceutical industries [4]. The *Kluyveromyces* species *K. marxianus* is also industrially relevant because of its wide substrate spectrum, fast growth characteristics, and thermotolerance to ~50 °C [5,6]. Native strains of *K. marxianus* are also known to synthesize ethyl acetate at rates above 2 g L^{-1} h^{-1} in aerated bioreactors [7,8]. *S. stipitis* is capable of fermenting xylose at high rates compared to other yeasts and has been widely studied for ethanol production from biomass-derived sugars [9,10]. *Y. lipolytica* is a well-studied oleaginous yeast and has attracted interest due to its ability to synthesize and accumulate high levels of intracellular lipids [11–13]. The methylotrophic yeast *H. polymorpha* has been studied as a model system for peroxisome function as well as for its methanol and nitrate assimilation pathways [14,15]. Significant efforts have gone into heterologous protein production in *H. polymorpha* due to its efficient secretion pathways, effective glycosylation machinery, and tightly controlled expression systems [16]. *H. polymorpha* is also thermotolerant to temperatures comparable to *K. marxianus* and can assimilates various substrates, thus making it a potential alternative host for ethanol production [17]. The methylotrophic yeast *P. pastoris* has similar protein secretion and glycosylation capabilities to *H. polymorpha* and has been widely used for heterologous protein production [18]. Its capacity to grow to extremely high cell densities and high capacity for membrane protein expression also provide inherent advantages over other yeast hosts [19,20].

Despite these many advantages, metabolic engineering of non-conventional yeasts is limited by a lack of sophisticated genome editing tools and an incomplete understanding of their genetics, metabolism, and cellular physiology. In this review, we discuss the challenges and solutions that have arisen in engineering non-conventional yeasts for metabolic engineering and synthetic biology applications. We begin our review with a discussion of the challenges to genetic engineering, followed by a discussion of strategies for improving genome and pathway engineering. Finally, we discuss representative examples of metabolic engineering in

Table 1
Overview of non-conventional yeast species, their industrially-relevant phenotypes, common uses in biotechnology, and comparison with *S. cerevisiae*.

Yeast	Beneficial Phenotype	Products	Reference
K. lactis	High protein secretion Growth on lactose	Proteins for food and feed industry Pharmaceutical enzymes	[4]
K. marxianus	Thermotolerance Fast growth characteristics High ethyl acetate production Growth on a range of sugars	Ethanol and volatile acetate esters	[5]
S. stipitis	High ethanol production from xylose	Ethanol fermentation from biomass derived carbohydrates	[21]
Y. lipolytica	Efficient production of lipids Growth on glycerol and alkanes	Lipids and oleochemicals	[12]
H. polymorpha	Thermotolerance Tightly regulated expression system Beneficial glycosylation for therapeutics	Heterologous protein High temperature ethanol fermentation	[17,18]
P. pastoris	Tightly regulated expression system High cell density on minimal media Beneficial glycosylation for therapeutics Efficient production of membrane proteins	Pharmaceuticals and industrial enzymes	[18]
S. cerevisiae	High ethanol production High HR capacity Well known genomics and physiology Advanced synthetic biology tools	Ethanol in fermented beverages and as biofuel Commodity and specialty chemicals Pharmaceuticals	[2,22]

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