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Microbial Biotechnology Based Modified Yeast

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Abstract

The production of heterologous proteins, lipids and chemicals based on modified yeast is a rapidly developing area of microbial biotechnology. Methods of metabolic engineering and new technologies for editing genomes are used to create a fundamentally new high-performance industrially significant yeast strains with new properties.

Keywords: Yeast; Genome-Editing Technologies; Metabolic Engineering

Abbreviations: DSB: Double-Stranded Breaks; ZFN: Zinc-Finger Nucleases; CRISPR: Clustered Regularly Interspaced Palindromic Repeats; TALEN: Transcription Activator-Like Effector Nucleases.

Introduction

Yeast is a convenient model eukaryotic organism for genetic modification: besides their whole genome being sequenced, they are easily cultivated in a bioreactor and recognized as safe organisms. About 20% of all pharmaceutical proteins are obtained using S. cerevisiae [1]. Non-traditional yeast is also actively used as producers [2].

For targeted gene expression in yeasts, genomic editing methods are used. The Cre-loxP system is considered one of the first editing systems, consisting of cre-recombinase (cyclic recombinase) and short DNA sequences-LoxP sites. Cre-recombinase interacts with LoxP sites and specifically cuts a DNA fragment between them [3]. An alternative strategy is the introduction of DNA double-stranded breaks (DSBs) using endonucleases. Due to the negative effect of DSBs on cells, the intracellular repair pathways are aimed at its restoration. A repair matrix can only be used as a donor for homologous recombination provided it shows homology to the gap site. Tools for targeted DSBs include, but not limited to dimeric meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced palindromic repeats (CRISPRs) [4]. These nucleases create site-specific DSBs in the DNA of a specific part of the genome, which are repaired during the recombination process and thus, making targeted mutations successful.

Progress in the development of DNA-editing methods and the improvement of sequencing technologies have contributed to the development of metabolic engineering, which modifies the initial metabolic pathways and their regulation. This in turn makes way for obtaining microorganisms with a high level of target compound production. For instance, more than 100 different proteins were acquired using the yeast Kluyveromyces lactis, which is known to metabolize low-cost carbon sources [5]. Using the CRISPR-Cas9 method, a synthetic pathway for the formation of muconic acid was created for them [6]. The Kluyveromyces marxianus yeast produces ethyl acetate, bioethanol, fuselols and their esters [2]. By disrupting native genes and inducing the expression of heterologous genes, the strain K. marxianus produced ethanol from xylulose at a rate of 2.49 g / l / h [7]. The yeast Yarrowia lipolytica is characterized by its ability to synthesize lipids [8]. Using genomic editing, the yield of triacylglycerides was augmented [9]. By knocking

out the TRP1 gene with the CRISPR-Cas9 system, a new strain producing triacetic acid lactone was developed [10].

The presence of a strong inducible system of protein expression and the nature of their glycosylation facilitated the use of the methylotrophic yeasts Hansenula polymorpha and Pichia pastoris for heterologous expression. Inactivation of the CAT8 gene in H. polymorpha led to an increase in ethanol yield to 12.5 g / L [11]. In H. polymorpha, the CRISPR-Cas9 method was likewise used to introduce the TAL, 4CL, and STS genes for the high yield synthesis of resveratrol of up to 98 mg / L [12]. In P. pastoris, a biosynthesis pathway for the nootkatone synthesis was developed [13]. Genetic modification of amino acid biosynthesis pathway in P. pastoris allowed the strain to synthesize up to 2.22 g / l of isobutanol, which was 43 times higher than the level of its production in the non-mutant strain [14].

With the improvement of sequencing methods and genome-editing tools, it is possible to eliminate the disadvantages of wild-type strains to obtain the desired phenotypes and significantly increase the productivity of industrially significant strains.

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