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Genom Editing of Saccharomyces Cerevisiae Using CRISPR-Cas9 System: A Review Study

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Abstract

Saccharomyces cerevisiae is an important yeast has been used for a long time to produce alcohol or bread. Moreover, genetically engineered S. cerevisiae cells continue to be exploited as industries for production of biofuels, pharmaceutical proteins and food additives. Genetically modified strain of S. cerevisiae created using traditional methods is laborious and time consuming. Recently, originally a system of immunity in archaea and bacteria, Clustered regularly interspaced short palindromic repeats "CRISPR" and CRISPR-associated "Cas" have been exploited as a flexible means for editing of genome. Until now, this tool has been applied to many organisms including yeast. Here, we review the significance of S. cerevisiae as an industrial platform and the use of CRISPR/Cas system and its uses in research and industry of this yeast.

Introduction

Hershey and Chase discovered that the material of genetic is DNA in 1952 (*Hershey & Chase, 1952*). This was followed by the description of DNA structure and revelation of the double helix of DNA (Watson and Crick, 2003). Subsequently, DNA manipulation *in vitro* has begun with enzymes including polymerases (Kornberg et al., 1956) ligases (Weiss and Richardson, 1967), and restriction endonucleases (Danna and Nathans, 1971). All these discoveries put the basis for molecular biologists to manipulate the DNA outside the living cell by Recombinant DNA technology (Cohen et al., 1973). Chain-termination inhibitor or dideoxynucleotides technique used for DNA sequencing of øX174 phage a breakthrough ease determination of nucleotide sequence on a certain DNA fragment (Sanger et al., 1977).

Renowned as one of the most significant scientific developments in molecular biology of the 20th century, polymerase chain reaction (PCR) is a technology, which easily and quickly produces limitless copies of specified DNA flanked by two primers from just one template strand in a few hours. The isolation of *Taq* DNA polymerase from *Thermus aquaticus* (Chien et al., 1976) and the invention of thermocycler manufactured by Perkin-Elmer Cetus together with PCR establish the gold triangle of this technology for research today. Another revolutionary development is Next-generation sequencing (NGS) technique that permits parallel sequencing of about two terabases in a single sequencing run (Illumina, 2017). Accordingly, the cost of base sequence decreased continuously which allow researchers with a simple laboratory to do sequencing on a daily basis for different basic biological and medical applications (Qi, 2017).

Genome editing or engineering technology can be defined as the process of making perpetuation in a specific location of a certain cell genome ranged from bacteria to humans. Double strand breaks (DSBs) is DNA damage due to cleaving of both strands by breaking down phosphate bonds of both strands. DSBs might be done in a certain cell due to some physiological and pathological states. DSBs induced mutation, which could be lethal to cell

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however, there are two mechanisms involved in DNA damage repair, which are homologues, directed repair dependent on the endogenous or exogenous DNA strand, which led to prices sequence repair (knock-in). The second method is a non-homologues directed repair in which a ligation process led to the addition or deletion (indels) of the targeted break (Knockout). Restriction endonucleases (Type II) can cut specific DNA at a specific location in vitro. Similarly, genome editing engineered endonucleases such as meganucleases (MNs) (Smith et al., 2006), zinc finger nucleases (ZFNs) (Urnov et al., 2005), transcription activator-like effector nucleases (TALENs) (Moscou and Bogdanove, 2009) and clustered regularly interspaced short palindromic repeat (CRISPR)/ CRISPR-associated nuclease (Cas9) (Mali et al. 2013) could cut targeted DNA at specific location but this time *in vivo*.

Saccharomyces cerevisiae considered as a valuable yeast in biotechnology. Furthermore, as a model organism, it broadens our knowledge of many cellular processes (Botstein and Fink, 2011). On biotechnology perspective, this yeast has enormous contributions for human benefits for its production of food, beverages, fuels, heterologous protein, pharmaceuticals, and chemicals (Hong and Nielsen, 2012).

Saccharomyces cerevisiae

A eukaryotic microorganism, single-celled, that is commonly named as brewer or baker yeast (Duina et al., 2014). For more than 7000 years, this yeast has been domesticated by a human for their fermentation ability of grapes fruit converting it to alcohol (Mortimer, 2000). This yeast attracts huge attention due to many valuable features that are important on both research and industry. The characteristics of the yeast such as the longtime of safe use make it generally regarded as safe (GRAS) by USA Food and Drug Administration (FDA), a huge knowledge about its genetics, physiology and Biochemistry (Nevoigt, 2008), the availability of great genetic manipulation tools applied with ease such as high transformation rate procedures (Gietz and Schiestl, 2007), and the high homologues recombination rate which facilitate the gene manipulation within the cell chromosomes (Klinner and Schafer, 2004) as well as the presence of large amount of vectors for cloning and expression (Demain et al., 2011). This yeast served as a model organism for basic and applied research as well as a cell factory to produce valuable industrial and health products.

Sacchromyces cerevisiae genome was fully sequenced on April 1996, to be the first eukaryotic genome sequencing project (Goffeau et al. 1996). Three years later, Winzeler et al created a precise deletion for 2026 Open reading frame (ORF) which occupy more than one-third of the whole genome (~ 6000 ORF). Of these, 17% of the ORF were essential for the yeast viability whereas 40 % showed a growth defect of the yeast (Winzeler, et al., 1999). By 2014 and according to Saccharomyces Genome Database (SGD), about 85% of ORF (5067 ORF) were annotated and their gene functions were known. All above features make this yeast a valuable model organism to study many basic biological phenomena such as cell cycle ageing (Murakami and Kaeberlein, 2009), (Alberghina et al., 2012), apoptosis (Yue et al., 2013) unravelling the complexity of human diseases like drug discovery (Delneri, 2010), neurodegeneration (Pereira et al., 2012), cancer (Burdelski et al., 2015) and much more.

Many industrial products massively depend on *S. cerevisiae* for their production. Moreover, until today it is the most used yeast for production of valuable industrial products. Traditionally, this yeast used to produce beer, wine, sake and bread making (Steensels et al., 2014). Due to its high tolerance to adverse industrial environments such as high substrate concentration , low pH, inhibitors and working anaerobically all these features make this yeast suitable to produce a high quantity of ethanol, carbon dioxide and other products. This yeast is applied to produced more valuable products other than that mentioned above such as

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epidermal growth factor (Chigira et al., 2008), antibody production (Rakestraw et al., 2009), nutraceuticals such as vanillin (Brochado et al., 2010), fuels such as biobutanol (Schadeweg and Boles, 2016), fatty acids (Ghosh et al., 2016) and chemicals such as 1,2-propanediol (Jung et al., 2011), succinic acid (Ito et al., 2014), 2,3- butanediol (Kim and Hahn, 2015) and xylitol (Kogje and Ghosalkar, 2016).

Saccharomyces cerevisiae as a model and microbial cell factory has improved through history by classical methods that occur naturally by natural diversity, sexual reproduction, genetic recombination, transposon integration into the genome, spontaneous mutation, horizontal gene transfer, changes on ploidy level. The other methods, which are largely dependent on recombinant DNA technology such as metabolic engineering that include rational inverse and evolutionary engineering (Steensels, et al., 2014). Within this and the past decades, new genome manipulation tools have been recognized for accurate editing of *S. cerevisiae* genome such as ZFNs, TALENS, and CRISPR-Cas systems.

CRISPR-Cas genome editing system

In archaea and bacteria, CRISPR-Cas DNA digestion systems offer adaptive immunity against attacking plasmid DNAs or phages (Barrangou et al., 2007). These organisms frequently capture a DNA sequence of ~20 bp from invaders and these sequences " protospacers" were added into their genome and are headed by an AT-rich nucleotides leader sequence forming CRISPR array (Jansen et al., 2002). CRISPR can be classified into three dissimilar CRISPR systems types (I, II and III) (Haft et al., 2005). The types I and III comprise multiple Cas proteins to simplify the identification and cleaving of target DNA (Hale et al., 2009). The type II system, however, contains a small number of Cas proteins (Barrangou et al., 2007).

The damage of external DNA contains spacer similar sequences has 3 steps: gaining of spacer, creation of CRISPR RNA (crRNA) and interference. During phage infection, its DNA is recognized, digested and added into the CRISPR array. Subsequently, the sequence is transcribed to yield a pre-crRNA, which go through a subsequent processing forming a crRNA. The final step, an effector complex contains a DNA cutting enzyme that exploit crRNA to identify any DNA and destruct the complementary similar to the spacer sequence (Singh et al., 2016).

This endonuclease cuts a target DNA sequence (23-bp) containing a guide sequence (20-bp), (protospacer), and the sequence of 5′-NGG-3′ called protospacer adjacent motif (PAM) (Mali et al., 2013). Coupling of crRNA and tracrRNA to form a single guide RNA (sgRNA) ease the creation of CRISPR/cas9 (Jinek et al., 2012).

CRISPR/Cas9 is restricted by its need to the PAM sequence. Therefore, 5′-X20NGG-3′ is the target sequences (X20 is the 20-bp crRNA sequence). This site is also restricted by the need of a guanine base at the 5′ end. Preventing this restriction was made by creating guide RNA with one or two extra G at its 5′ end (Cho et al., 2014).

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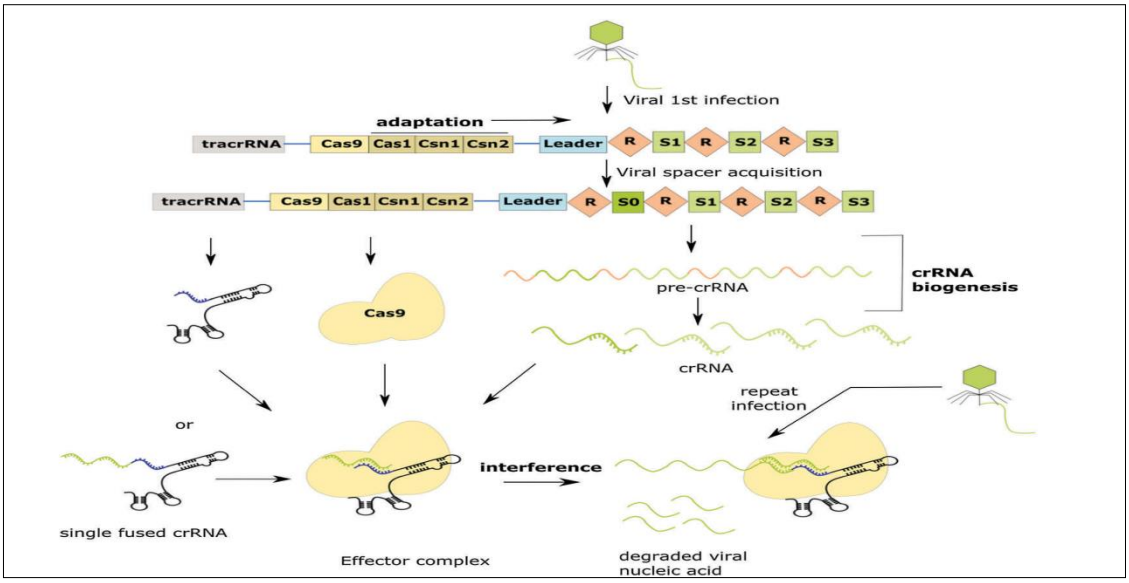

Figure 1. *Components of the CRISPR system. R: bacterial repeats; S0, S1, S2, S3: virus sequences (Singh et al., 2016).*

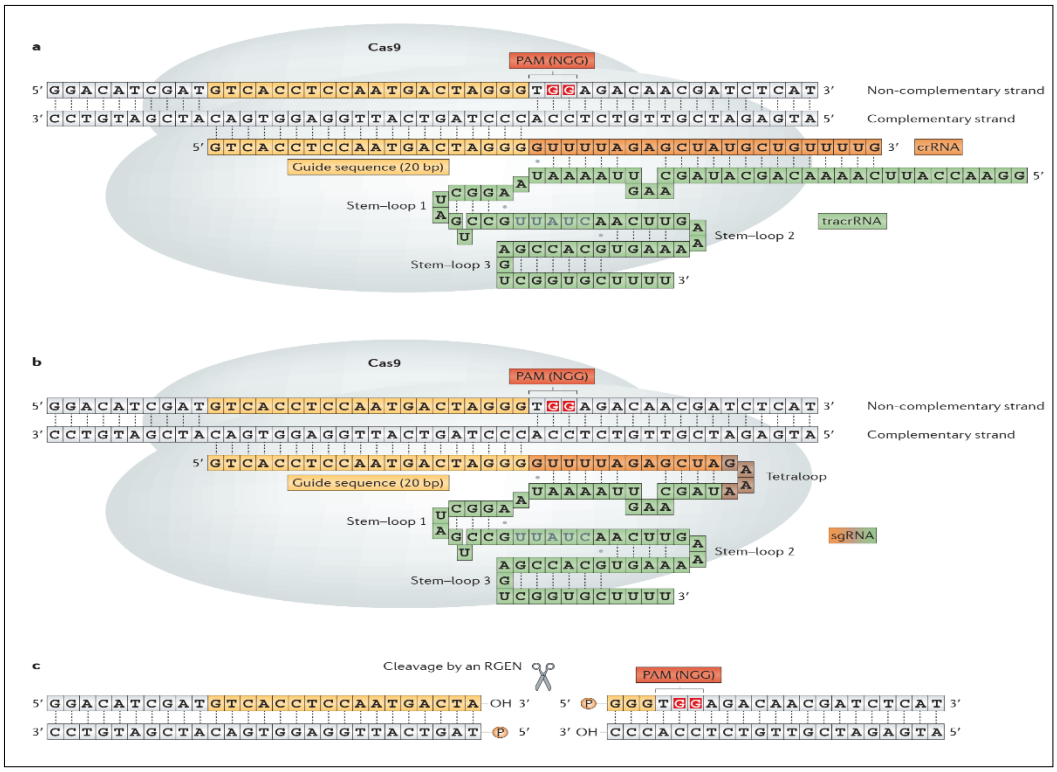

Figure 2. *Schematic representations of CRISPR-Cas9. A. It contains a crRNA, tracrRNA and Cas9 endonuclease. B Alternatively, CRISPR-Cas9 comprises sgRNA and Cas9 (Kim and Kim, 2014).*

Saccharomyces cerevisiae and genome editing using CRISPR-Cas9 system

Yeasts are extensively exploited for research and industrial bioprocesses for a long time. Among them, *S. cerevisiae* is non-pathogenic and used for a long time to make alcohol and bread (Mitsui et al., 2019).

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Saccharomyces cerevisiae has been exploited as a microorganism to decode eukaryotes biological processes (Botstein and Fink 2011) and as metabolic engineering platform (Nielsen *et al.* 2013). Heterologous expression and adaptation of metabolic pathways in *S. cerevisiae* needs the introduction of multiple genetic alterations and rewiring metabolism network. Genetic modifications continue to take a long time and laborious process, as each one involves cycles of transformation, selection and confirmation. Additionally, consecutive alteration may be restricted by the availability of selection markers. These limitations enhanced wide search to find new genetic markers for *S. cerevisiae* (Siewers, 2014).

Alternative methods have been used for genome editing such as meganucleases, ZFNs and TALENs. Bacteria have established some systems to cut foreign DNA such as CRISPR-Cas system. This system has frequently been used in *S. cerevisiae* to make efficient knock-outs and knock-ins of genes without a selective marker (Ronda et al., 2015) as well as the addition of multiple genes (Sasaki et al., 2019). It is also used for engineering of metabolic processes such as the addition of pathways or reducing byproduct formation by suppression of multiple gene expressions and improving *S. cerevisiae* stress tolerance (Shi et al. 2016; Mitsui et al. 2019).

Conclusion

The importance of *S. cerevisiae* as a factory for production of various important products and the use one of the genome editing tool (CRISPR-Cas) to make gene disruptions, multiple gene insertions, and metabolic engineering in *S. cerevisiae* were discussed in this review. CRISPR/Cas9 system is widely used when compared with other genomic editing tools such as ZFNs and TALENS due to simple requirements. These involved the need of PAM sequence that is extensively existed in the genome, therefore; most of genes can be modified. The second characteristic, CRISPR/Cas9 system comprises simple constituents “sgRNA and Cas9 proteins”. The third feature, the system can be placed on a single or multiple-vectors. The system can modify multiple genes simultaneously in one transformation improving the efficacy of genome editing. Nonetheless, limitations such as the effect of off-target, requirements to enhance the efficiency of editing, and the need of a carrier system, which is appropriate to many yeasts. Many researchers tried different approaches to solve these problems. In future, this system could be used to develop this important yeast and other microbes for research and biotechnology.

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