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The Application Value Assessment of CRISPR in Screening and Optimizing Bacteria for Industrial Production

Kangjun Yuan[1,a,*]

[1]*College of Life Science and Technology, Huazhong Agricultural University, Wuhan, China*
a. 21sk_ykj@webmail.hzau.edu.cn
**corresponding author*

Abstract: CRISPR is a gene editing technology derived from bacteria and has been widely used in the current biological field. However, the use range of it is still mainly in laboratories. The industrial production field can rarely see its role, such as the screening and optimization of production strains. This article compares the advantages and disadvantages of CRISPR with traditional natural sampling or mutagenesis breeding combined with selective culture medium screening technology in actual production in technical and non-technical fields, emphasizing that CRISPR technology has great advantages in integrating excellent traits and improving breeding efficiency. However, it is still slightly inferior to traditional technology in terms of breeding costs, experimental conditions, and social acceptance. The significance of this study lies in a relatively comprehensive analysis of the various indicators worth comparing when CRISPR technology is actually applied to industrial breeding, which can provide a certain reference value for whether the technology should be officially put into industrial use. However, there are still many defects of CRISPR technology which is difficult to correct; although the direction of its improvement is fairly clear, it's never easy to work, and the development will still need some time.

Keywords: CRISPR, industry, bacteria, assessment.

1. Introduction

These guidelines, written in the style of a submission, show the best layout for your paper using Microsoft Word. If you don't wish to use the Word template provided, please use the following page setup measurements. In modern industrial production, there are lots of substances that can't be synthesized by pure chemical means and require fermentation processes, such as cephalosporins and polyamino acids. In the optimization and iteration of industrial fermentation processes, the most important is to select and breed the production strains. The traditional method for this is combining natural environment sampling with selected media breeding (hereinafter referred to as traditional screening technology), but there are many disadvantages like slow selection speed, suffering large influence of luck, and high susceptibility to interference from other strains. This has imposed certain restrictions on the iterative development of production processes and products.

As a highly efficient gene editing method derived from bacteria, CRISPR technology can effectively improve the efficiency of industrial production strain selection and further optimize related traits. This technology has been used on a large scale in laboratories currently, and there is a lot of practical experience for many different strains. CRISPR technology has the characteristics of

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strong gene targeting and little effect on nontargeted traits, which enables the accurate adjustment of the strains with relatively low risks, allowing researchers to obtain expected results with fewer repeated experiments. This stable and efficient characteristic is exactly what industrial production needs.

However, compared with traditional strain selection programs, CRISPR still has many shortcomings, such as the high cost. Coupled with the existing social stereotype of genetically modified technology, it is difficult to apply CRISPR technology in actual fermentation productions, especially food-related ones. To solve these problems, scientists have begun to explore the optimization direction in reducing cost and improving efficiency to meet the needs of industrial production and carry out publicity and popular science work for society.

This article will introduce CRISPR, analyze the advantages and disadvantages it has in applying to the selection and breeding of industrial production strains when compared with other traditional technologies, and explore the future optimization directions for its disadvantages. Finally, this article will show the future application prospects of CRISPR in the screening and optimization of industrial production strains.

2. Introduction of CRISPR and its application in breeding industrial production strains

The CRISPR system was first discovered in E-coli [1] and quickly proven it could destroy some specific foreign DNA to defend bacteriophages and plasmids as an immune system [2]. Later, researchers found CRISPR can artificially set the sequences to recognize and achieve multiple functions including methylation modification and double-stranded cutting by attaching different Cas enzymes, thereby achieving accurate and efficient editing of the genome of organisms.

The actual application process of CRISPR technology in a laboratory environment includes preliminary preparatory research, primer design, and introduction CRISPR complex. In this process, CRISPR and Cas proteins will function in the cell and begin to cut DNA chains. Specifically, before using CRISPR technology on a specific strain species, it is necessary to determine which genes need to be edited to get the target trait. For this reason, it is necessary to completely study the genome of target microbial species, construct a genomic library, and clarify the correspondence between different genes and traits; this is the most important part of the entire experiment. After work is to determine the upstream and downstream sequences of the target gene, design the guide site of the guide RNA (sgRNA), and construct a complete CRISPR complex. Then the complex can be delivered into the target strain by adenovirus vector transport, liposome embedding delivery, and other methods to achieve gene editing operations [3].

This operation process makes CRISPR difficult to use, but it also brings higher accuracy and efficiency. The latter is exactly what modern industrial production needs, and also what traditional screening technology lacks. As for now, the discovery of wild-type industrial production-used strains is mostly from collecting in field ecosystems that have corresponding natural conditions, with breeding further emphasizing screening conditions in the laboratory to find the most suitable one. This makes the single development cycle of a new strain longer, and due to a certain degree of randomness, the difficulty of reproducing experimental results is higher. But CRISPR can get out from the logical pattern of searching-screening linearity, achieve the automated closed-loop operation of high-throughput screening with the construction-screening then correction-screening circular logic, which successfully improves the efficiency of screening suitable strains industrially, allowing large-scale producing, testing and verifying new strains in standardized processes.

In recent years, many research institutions in countries have used CRISPR and other gene editing technologies as core to build automated synthetic biology factories (or engineered infrastructure/cloud laboratories) [4,5] to verify and implement the above-mentioned circular process. In 2019, the Global Biofoundry Alliance (GBA) was established in Kobe, Japan, hoping to build

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international unified standards for the intersection of gene editing and other synthetic biology technologies with industrial production [6]. This also proves that CRISPR as one of the commonly used gene editing technologies, will have a high degree of acceptance and a wider application environment in the future.

3. Advantages of CRISPR compared with traditional screening technologies

Compared with traditional screening technology, there are many advantages of CRISPR in the process of industrial new strain construction.

3.1. Create the needed characteristics controllably

CRISPR allows to addition of new genes to existing DNA of the strain according to real production requirements to create new desired traits. As early as 2012, Patrick J. Westfall et al. adjusted some genes in the mevalonate (MVA) pathway of Saccharomyces cerevisiae CEN.PK2, getting an almost 1:10 ratio to produce artemisinin and its precursor, amorpha-4,11-diene during conventional fermentation [7]. This makes large-scale industrial production of artemisinin possible, providing a guarantee for the global prevention and treatment of malaria.

3.2. Can focus on optimizing a specific trait

CRISPR allows to optimization of a certain trait of strain in a specific direction. But in traditional screening technology, it's hard to optimize the trait initiatively, only being able to search for existed dominant one passively; and the mutagenesis method also cannot control the direction of change to get specific traits. The team of Shuling Yang et al. worked at the basic species E004 of Yarrowia lipolytica which had completed the pre-modified regulation of the erythritol degradation pathway, greatly increased the erythritol production of the strain by overexpressing the ZWF and TKL genes by CRISPR/Cas9, and constructing a related gene expression disk based on the 8UAS1BXPR2-PTEFin promoter; secondly, they knocked out the related genes in the mannitol synthesis pathway, reducing the output accumulation of the byproduct mannitol by 59.4%. Finally, the best-engineered strain E326 selected in the research successfully achieved an erythritol titer of 256 g/L in a 5L bioreactor [8].

3.3. Minimize the impact on the original dominant traits of the strain

The principle of CRISPR brings the characteristic of strong directivity, which means less influence on other genes when modifying a particular one. The wild-type strains obtained through traditional screening technology usually need to compromise the survival or productional capabilities, with one side advantageous and the other side disadvantageous; however, using CRISPR to edit genes can complete and optimize the disadvantageous capabilities with minimally influenced advantageous capabilities.

3.4. Produce new strains faster

The CRISPR application process is standard and, thus has many advantages such as suffering less interference from accidental events and low difficulty in reproduction. That condition allows editing multiple genes in parallel and conducting a new experiment round quickly by simply replacing the sgRNA in actual production applications, which traditional screening technology lacks. In 2019, the team of Yueping Zhang et al. used gRNA-tRNA array CRISPR, creatively achieve to destroy 8 genes in only a single operation in Saccharomyces cerevisiae at an efficiency of 87% [9]. This is a great

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example to prove CRISPR greatly increases the efficiency of gene editing of strains and the speed of constructing new strains.

The above advantages make CRISPR more efficient in constructing strains meeting requirements when working in industrial scenarios than traditional screening technology, and currently traditional screening technology may not be able to retain multiple advantageous traits in a strain at the same time, but CRISPR technology can avoid this situation to a certain extent. This is very important in the industrial production process.

4. Existing problems with CRISPR technology

Although CRISPR has many advantages as mentioned above, it has not yet been used in large-scale industrial production. One of the important reasons is that compared with traditional screening technology, CRISPR still works worse in terms of economy and difficulty of application.

4.1. Higher using cost than tradition

As a gene editing technology, the production difficulty, production cost, and patent fees of CRISPR-related materials and reagents make the overall cost very high in actual application. In contrast. Traditional screening technology takes almost null cost when sampling in the natural environment; not only less difficult the operations of configuration and sterilization for screening culture media are, but the material cost is also lower; In comparison, CRISPR is far from the concept of reducing costs and increasing efficiency in industrial production. This also means that the microbial industry will only consider gene editing methods including CRISPR to create new strains until traditional screening technology cannot select suitable strains.

4.2. Require preliminary research to find target genes

CRISPR is a technology to manipulate genes, which means finding out which is the target gene and how to manipulate it in advance is the major premise when using it. This depends on preliminary research extremely, including the analysis of the gene library of the original strain, the perception of specific metabolic links, etc.; thence for some special strains that have not gotten enough studied, it will be difficult for CRISPR to play a role. In contrast, traditional screening technology is mainly aimed at screening existing strains, and the technical reserves required are only simple contents such as the ratio of culture medium materials. This allows researchers to focus on the traits exhibited by the strains, reducing the additional cost expenditure of early technology accumulation, and is more suitable for the selection and breeding of species with less gene library research.

4.3. Higher environment requirement with susceptibility to interference

The operation of CRISPR needs fairly clean experimental conditions, and related reagents also have certain temperature and humidity requirements for storage. In the real application of CRISPR, the environment must be strictly kept constant and clean in multiple parts including sgRNA preparation and bacterial transfection. Slight interference may lead to abnormalities like RNA chain breakage, Cas enzyme inactivation, etc., and ultimately cause the failure of the whole work. But traditional screening technology only has stringent requirements for bacterial preservation and sterile operation, other requirements such as for the overall laboratory environment are relatively lower.

4.4. Exist off-target effects

The off-target effect is an inherent question in CRISPR, which may lead to effects on unexpected genes. This is a great damage that may be caused in the later bacterial cloning and expansion process.

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At present, the off-target effects can only be detected after operation over and mitigated, never eliminated from the root, and most schemes to optimize have only achieved good results in vitro. In 2023, the team of Rui Tian et al. constructed the AID-seq method to detect low-frequency off-targets by CRISPR nucleases in vitro and established an off-target detection model from experimental data through the CRISPR-Net deep learning method [10]. However, this method is still limited to the vitro environment and has not been verified in the cell environment. In addition, also it can only detect without the ability to correct the off-target that occurs.

As a young technology, CRISPR still has many flaws. It can be considered that CRISPR gets higher gene editing efficiency and the ability to precisely manipulate specific traits of a strain at the expense of higher costs and technical barriers. Not only the future optimization direction of CRISPR it is, but also a must solved problem before fully industrialized.

5. Possible problems in the industrial Application of CRISPR

CRISPR belongs to the gene editing technology; this type of technology always suffers some problems in practical applications even at the non-technical level.

5.1. Product safety needs to be tested

When applying CRISPR in strains, it is hard to guarantee whether non-target metabolic pathways are affected. This may lead to unexpected by-products in the actual production process, causing potential safety issues. During the author's research in the microbial fermentation workshop, there was clear commentary explaining if the primary fermentation product is the final product without additional processing (such as yogurt), the used strains must be the wild-type or screened from the wild-type, gene-edited, or mutagenized species is illegal. But if the final product is the secondary fermentation product with post-processing this limit is not suitable.

5.2. Low social acceptance of CRISPR products

In some countries (such as China), society generally remains skeptical of biological technology, especially genetic modification technology. In addition, due to the lack of popular science work, the public tends to confuse gene editing technology with genetic modification technology, making it difficult to promote products that use gene editing technologies, including CRISPR in the market. At the same time, some countries have stricter implementation standards for such products using gene editing technology [11], which makes the promotion of such products more difficult and costly than traditionally produced ones, causing weaker market competitiveness.

Although CRISPR has great potential, for this non-technology part of the problem, even though the directions for improvement are fairly clear, there are still many objective limitations in the actual operation. So predicting the specific solution time and implementation results is almost impossible. However, believing with the development of technology and the continuous application of CRISPR, these problems will be improved to a certain extent.

6. An Example on optimizing industrial bacteria using crispr technology

The yeast ADH2 gene encodes an alcohol dehydrogenase which converts ethanol into acetaldehyde. The team of Ting Xue et al. designed two specific sgRNAs that targeted the ADH2 gene sequence 220 bp upstream of the start codon and 180 bp downstream of the stop codon to completely remove the ADH2 gene from the yeast genome by CRISPR/Cas9. Finally got ADH2-deficient strain had a 99.4% decrease in ADH2 relative gene expression and a 74.7% increase in ethanol production under

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the same fermentation conditions [12]. This study fully verified the great potential of CRISPR technology in optimizing industrial.

7. Conclusion

After many years of development, CRISPR has gained sufficient laboratory application experience, but its industrial application is still in its infancy. Compared with traditional screening technology, CRISPR technology has great advantages in constructing the traits of the desired strains, but it still has many defects in terms of application conditions, cost, and the social impact of the final product.

As society's cognition of things will continue to change with the development of modern technology rapidly developing, it is difficult to predict the real direction of future improvements in CRISPR. In terms of the current adaptability gap between this technology and industrial applications, the recent improvement direction may focus on reducing the cost and off-target probability, while other aspects need to be adjusted according to actual needs.

Overall, CRISPR is still one of the excellent means to screen and optimize industrial production strains in the future. However, this technology is still mainly regarded as an unconventional means, only got small-scale usage. If hope to achieve large-scale industrial application, there are many improvements necessary. For example, reduce the price of CRISPR technology-related reagents, improve the gene editing efficiency of the CRISPR system per unit mass, reduce the frequency of off-target effects, and so on. In short, although CRISPR is currently used in a narrow range, it is still promising in the future.

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