



Microbes with a Mission: How CRISPR Is Cleaning Our Waters

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ABSTRACT

The CRISPR-Cas9 gene-editing technology became a revolutionary gene-editing technology in microbial biotechnology with new measures of sustainable wastewater treatment and pollution control. CRISPR can be used to develop engineered microbes through refined and effective genetic changes to increase; pollutant breakage, heavy-metal detoxification, and overcoming antibiotic resistance in aquatic systems. Outside of genome editing, CRISPR-based biosensors including Cas12 and Cas13 enable rapid pathogen and antimicrobial resistance gene detection in wastewater, and have a fast and sensitive diagnostic efficiency. Combination of CRISPR with metabolic engineering, artificial intelligence (AI), and high-throughput screening leads to its increased potential, the way to intelligent, adaptive, and sustainable wastewater management. Although deliveries are still facing some challenges like off-target effects, delivery inefficiencies, and ethical issues, improved CRISPR 3.0 technologies with base and primer editing can be seen as a new dawn in efficient and environmentally friendly wastewater management in future environmental protection.

KEYWORDS

CRISPR-Cas9; Microbial genome editing; Wastewater bioremediation; Antimicrobial resistance

Introduction

Over the decades, microorganisms have been critical in the process of eliminating pollutants that threaten ecosystems and human health like pesticides, heavy metals and persistent organic pollutants (POPs). These microbes aid in the restoration of the environment balance through their processes of natural detoxification and decompositions. Nevertheless, Modern pollutants (especially heavy metals) are increasingly complex and persistent and thus may exceed the ability of natural microbial systems to cope. The conventional form of bioremediation methods, whereby naturally occurring microorganisms are used to degrade harmful of substances, are slow, ineffective, and very much environmental dependent. Although recent developments in the field of molecular biology and metagenomics have helped to improve our knowledge of microbial diversity and functional roles, the new technologies have failed to keep up with the growth rate and complexity of environmental contamination. The groundbreaking technology of gene-editing known as CRISPR-Cas9 has changed microbial engineering. CRISPR enables researchers to control the specifics of genomic editing of microbes, improving or remaking bacteria that can degrade pollution in a more effective way. Using these targeted genetic modifications, engineered microbes are now capable of being customized to clean up heavy metals, degrade organic contaminants, and act in synergy in microbial consortia to address a number of contaminants at the same time. Combining environmental science and innovative biotechnology offers an extremely strong and effective solution to wastewater treatment and overall pollution reduction in the world.

CRISPR-Cas9

CRISPR-Cas9 is one of the most disruptive innovations of modern biotechnology that transformed the process of genetic modification in a vast variety of organisms. First found in bacteria

and archaea as a component of their adaptive immune system, allows microorganisms to have memories of viral infections through segments of viral DNA stored in their own genome. These stored sequences are referred to as CRISPRs, which conduct the Cas9 enzyme to cut and inactivate the genetic code of the invader with accuracy in case they recur.

In essence, CRISPR-Cas9 uses a guide RNA to guide the protein of Cas9 that is applied as molecular scissors to cut DNA at a specific point. Also, new variants of Cas9 can switch genes on and off without any incisions into the DNA, opening new prospects of controlling biological processes.

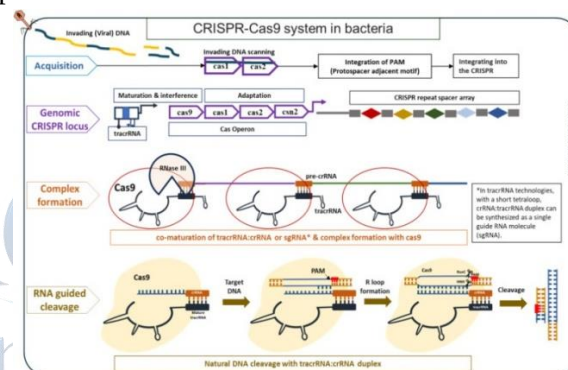


Fig: CRISPR-Cas System in bacteria (Bhanu et al., 2024)

Adaptation of CRISPR-Cas9 for Microbial Genome Editing

The use of CRISPR-Cas9 technology in the editing of the microbial genome has generated important developments in environmental biotechnology. In order to make the most of CRISPR in microbes, a number of important elements of the system have been optimized:

- 1) **Vector Design:** CRISPR-Cas9 elements are generally transferred into microbial cells through the plasmids. These plasmids will be designed to express either the Cas9 protein or the single guide RNA (sgRNA), or it can be integrated in the microbial genome to be expressed over time.
- 2) **Target Gene Selection:** With the aid of bioinformatics programs, including CHOP-IT and

CRISPRdirect scientists can make highly specific sgRNA so that only the target gene sequence is altered.

3) Delivery Mechanisms: CRISPR-Cas9 factors are incorporated into cells of microorganisms by electroporation, conjugation, or transformation. In more complex applications, to deliver whole libraries of guide RNAs, such systems as piggyBac transposons are applied to facilitate massive genetic modifications.

Advanced Gene Editing Techniques

With the ongoing development of the CRISPR-Cas9 technology, researchers have devised new gene-editing methods to increase the accuracy and flexibility of microbial engineering. Recombineering is one such strategy that combines recombinational engineering with CRISPR-Cas9 to make editing much more efficient. Under this method, microbial genomes are exposed to short DNA fragments so as to have accurate genetic modifications. An example is the Single-Strand DNA Recombineering (SSDR) system that uses single-stranded DNA fragments to fine-tune individual genes, and Double-Strand DNA Recombineering (DSDR) that uses two-stranded DNA to afford superior and reliable genome editing- especially in bacterial organisms such as *Escherichia coli*.

Non-Homologous End Joining (NHEJ) is another breakthrough which is a DNA repair mechanism that cements pieces of broken DNA strands, without requiring a homologous template. NHEJ is not as specific as homologous recombination, but comes in handy especially to produce targeted gene knockouts in microbes that inherently lack an efficient DNA repair mechanism.

Application prospects in wastewater bioremediation

The combination of CRISPR technology with metabolic engineering has given rise to engineered microbes with the ability to survive in severe environmental conditions and also to degrade toxic substances effectively. CRISPR-Cas9 has massive potential in the next-generation

wastewater treatment due to its accuracy, low cost, and high performance. CRISPR technology will turn microbes into effective environmental partners with an augmented detoxification and resistance capability, which can help achieve a more sustainable and less polluted environment.

In addition to gene editing, CRISPR-Cas is redefining the detection of pathogens using their marvelous speed, accuracy, and ease. Due to their enhanced ability to identify and cleave certain genetic sequences, including CRISPR-Cas12 and Cas13 have become potent biosensing platforms with great potential of conducting real-time molecular diagnostics. CRISPR-based assays have been effectively established to detect viral RNA, such as SARS-CoV-2, in wastewater and also pathogenic bacteria such as *Salmonella*, *E.coli*, *Listeria monocytogenes*, and *Mycobacterium tuberculosis*.

Application prospects in pathogen detection

It is possible to combine CRISPR technology with nucleic acid amplification methods to enable scientists to detect pathogens with high sensitivity at the point of care. It is a combination of these that makes an accurate and fast detection of infectious agents that gives early signs of disease outbreaks. In addition, it is an effective instrument in managing and evaluating the efficiency of wastewater treatment systems with a colossal potential to the community health and environmental security.

Application prospects in ARG/AMR control

Antibiotics have extensively been used and thus, this has resulted in the prevalence of antibiotic-resistant bacteria and antibiotic resistance genes (ARGs) in water bodies and is a major threat to global health. CRISPR-Cas system is an effective innovative approach in the detection and combating antimicrobial resistance (AMR). High sensitivity biosensors based on advanced solutions such as Cas12 and Cas13 have been created to amplify detection of AMR genes in wastewater, with CRISPR-Cas9 able to be

programmed to cut and silence multi-drug resistance plasmids. Innovative methods like the incorporation of CRISPR into portable diagnostics or the use of the system together with the isothermal amplification technique make it possible to conduct quick on-site testing of water samples. Also, it is also possible that researchers will use engineered bacteriophages to introduce CRISPR components to wastewater systems, which selectively target and cause the elimination of resistant bacteria.

Challenges, Limitations, and Ethical Considerations

Although the CRISPR-Cas technology has great potential, there are several limitations to its application, which need to be resolved to use it safely and effectively. The first is the potential of off-target effects, i.e. Cas enzymes accidentally cleaving non-target DNA sequences, which leads to random mutations. The genetic diversity, repetitive DNA areas and the lack of efficient delivery contribute to additional complications of microbial genome editing and decreases the accuracy and safety. Resistance of microorganisms to CRISPR based treatment may also develop with time.

A host immune response to Cas9 proteins is another challenge as most organisms such as human beings have immunity that precedes the exposure to Cas9 proteins thus reducing the efficiency of editing. In addition, the size of Cas9 is very large, not to mention the dangers of immune elimination and protein degradation, thus it is difficult to deliver CRISPR fragments to target cells safely and effectively. Besides, the possibility of introducing genetically modified microbes into the environment and due to the possible misuse, there are important ethical and biosafety issues. To address these challenges, novel methods of delivery systems like nanoparticles or modified vectors and the method of mitigating immune reactions and off-target

toxicity must be developed to make CRISPR usage in environmental biotechnology less risky and less target specific.

Future Directions

The future of CRISPR technology in microbial engineering is in the use of CRISPR technology with automation, artificial intelligence (AI), and high-throughput screening (HTS). Robotic automation and HTS are able to screen engineered microbial strains in a rapid manner, saving a lot of time and money in industrial applications. Meanwhile, machine learning can improve the accuracy of genome editing to reduce off-target effects and forecast complicated metabolic pathways.

The introduction of CRISPR 3.0 technology, such as base and prime editors, CRISPRa/i systems, and CRISPRa/i systems have reached the stage of precision, reversibility, and multiplexed genome-wide alterations with no generation of double-strand DNA breaks. These advances enhance the creation of microbial cell factories with enhanced production capability, ability to endure stresses and biosynthetic functions. In the future, the development of next-generation intelligent fermentation will rely on synthetic microbial consortia, CRISPR-based biosensors and AI-controlled bioreactors.

Conclusions

The accuracy, flexibility, and effectiveness of CRISPR-Cas9 have revolutionized the entire field of microbial genome engineering by making it easy to create engineered microbes that can generate industrial enzymes, pharmaceuticals, and biofuels. Although the combination of AI and machine learning enhances the accuracy of guide RNA (gRNA) design and reduces off-target effects. The base and primer editing tools and the CRISPRa/i systems are emerging and expanding in their use. Despite the challenges in the use of CRISPR technology such as off-target effects and

ethical issues, the rapid development of this technology has continued to push it towards broader, safer, and more sustainable applications in biotechnology, agriculture, and healthcare.

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