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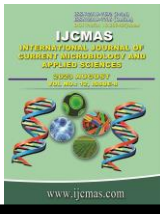

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Role of Crisper-Cas Technique in Bioremediation of Pesticides

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ABSTRACT

Pesticides, insecticides, certain pharmaceuticals, chemically dangerous chemicals which can pollute the environment. Pesticides are primarily used to manage plants, flies, parasitic fungus, and parasites in crop fields. The desire to limit pesticide effects on soil and offsite environments has encouraged research interest in pesticide and related chemical biodegradation. As a result, quick and safe agents for environmental bioremediation, individual decontamination, and therapeutic detoxication are urgently needed. Bioremediation is environment friendly so if we use such kind of technique then more helpful. The ability of microorganisms to degrade complex chemical substances in the environment is referred to as bioremediation. The discovery of CRISPR, mechanism of the CRISPR-based nuclear adaptive immune system "CRISPR associated system, Cas", and its diversion to powerful tools for gene editing revolutionize the field of molecular biology. Brought about and stimulated new and improved gene therapy. By using CRISPR technique transfer desired gene and decrease toxic elements.

Keywords

CRISPR, Cas-9, genome editing, bioremediation, Microorganisms, pesticides

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Introduction

Many toxic substances are used by humans and then release into environment so that it polluted the environment that is why it is necessary to degrade this toxic compounds. Excessive hazardous waste discharge in a clean water and soil disturbances, restricting agricultural production. (Kamaludeen *et al.*, 2003). This is accomplished through falling pest damage to agricultural crops. (Schmidt-Jeffris *et al.*, 2018). Weeds, herbs, bugs, rodent, worm, and microbes are examples of pests like bacteria, fungi, and algae (Bottrell *et al.*, 2018; Duke *et al.*, 2018). Pesticides are categorized according to their desired use and play an important role in enhancing crop productivity and reducing agricultural losses due to pests. (Allmaras *et al.*, 2018).

Rigorous use of pesticides results in contamination of soil, agricultural spills biologically expand water bodies and increased toxicity levels at all trophic stages of the food web such as, DDT -

dichlorodiphenyltrichloroethane. (Thomas *et al.,* 2008; Plattner *et al.,* 2018; Silva-Barni *et al.,* 2018). Apart from that, pesticides also affect the function of organs and damage DNA at the molecular level, adversely affecting health and leading to neurological disorders and cancer. B. Azoxystrobin and atrazine (Singh *et al.,* 2018; Vidartd'EgurbideBagazgoïtia *et al.,* 2018; Fatima *et al.,* 2018). Pesticide residues are removed and decomposed using traditional bioremediation methods (Moorman *et al.,* 1994; John *et al.,* 2018).

Biological agents used by Bioremediation process, mainly microorganisms to clean up polluted soil and water (Strong and Burgess *et al.,* 2008). Bioremediation is a method that utilizes microbes or their enzymes to restore the environment to its original form after it has been contaminated. Different type of gene editing technique was produced like TALEN, ZFN, CRISPR etc. but mostly CRISPR technique is being used because it has high target recognition efficiency, low mutation rate and many more.

CRISPR-Cas-mediated genome editing is easy-to-use method to precisely alter DNA sequences within the genome of living organisms. This method is frequently accepted and further modified as a result of its simplicity and efficiency, resulting in a very strong molecular tool. CRISPR techniques are mostly used for genetic editing to transfer more valuable genes to other organisms so that giving them even more power over toxic chemicals. It could accelerate the procedure of organic bioremediation without adding significant charges or risks.

Bioremediation

To clean up polluted soil and water, bioremediation involves biological agents, mostly microorganisms such as fungus, yeast, or microbes. Microorganisms use pathogens as nutritional or sources of energy in bioremediation processes (Agarwal *et al.,* 1998; Tang *et al.,* 2007). Various enzymes effective for decomposing pesticides has been discovered in environment (Geed *et al.,* 2016; Iyer *et al.,* 2013; Dawson *et al.,* 2008; Pizzul *et al.,* 2009; Brar *et al.,* 2017; Diao *et al.,* 2013; Yair *et al.,* 2008). Bioremediation can be used to target particular toxins like chlorinated insecticides, which are destroyed by microorganisms.

Bioremediation, is the process of degrading environmental pollutants towards less hazardous forms using living organisms, typically microbes. It degrades or detoxifies pesticides that are toxic to humans and/or the planet using naturally occurring bacteria, fungus, or flora. Through bioremediation, micro-organisms utilize insecticides as co-substrates in their metabolism with other nutrients, thereby removing them from the surroundings.

The effectiveness of these mechanisms is determined by pesticide features like as dispersion, bioavailability, and land persistence. Pesticide availability to microorganisms must be improved; this is hampered by pesticide adherence to sand grains and their poor water solubility (Ortiz-Hernández *et al.,* 2014). The development of novel microbial bioremediation methods might be sparked by genome editing techniques.

What is CRISPR–Cas?

Even though the discovery of artificially created meganucleases, followed by ZFNs and TALENs, improved genome-editing efficiency, re-designing or re-engineering a fresh set of proteins was necessary to target multiple areas in the genome. The complexity of cloning and protein engineering ZFNs and TALENs so that different types of technique found and adopted which is easy compare to old techniques. By compared to TALENs and ZFNs, CRISPR-Cas is the cheapest, simplest, and easiest gene editing method for scientists to use (Ju *et al.,* 2018).

CRISPR Cas is a site-specific gene editing tool that was recently developed from a naturally occurring RNA-guided endonuclease. Based on the study, several teams developed CRISPR/Cas9, a method that is now employed in most modern biotechnology. The CRISPR gene-editing system is made up of an endonuclease protein with DNA-targeting selectivity and slicing activity that can be controlled by a short guide RNA. Clustered regularly interspaced short palindromic repeat DNA sequences (CRISPR) is an initials for clustered regularly interspaced short palindromic repeat DNA sequences. The CRISPR repeat clusters were separated by non-repeating DNA sequences termed spacers, unlike usual tandem repeats in the genome.

CRISPR-Cas is a genome - editing method that is both efficient and effective (McMahon *et al.*, 2018). Cas is a CRISPR-associated protein that is act like molecular scissors to cut DNA. Cas proteins chop off a section of viral DNA when a virus infects bacteria to insert into the CRISPR region of the bacteria. Obtaining a chemical representation of the infection. Those viral sequences are subsequently replicated into RNA fragments.

This molecule plays various roles in our cells, but in the case of CRISPR, RNA binds to a special protein called Cas9. The resulting complex is act like scouts, latching onto free-floating genetic material and searching for a match to a virus. If the virus attack again, the scout complex immediately recognizes it and Cas9 swiftly destroys the viral DNA. Now CRISPR not only for vial but also any organism DNA is modified.

There are 3 types of CRISPR-Cas systems, namely Types I, II, and III (Zhu *et al.*, 2018), and many other subtypes (Behler *et al.*, 2018). As per system acting, i.e., model organisms, each system has its own Cas (Cooper *et al.*, 2018). Cas9, a DNA endonuclease, is directed by RNA to impede foreign DNA (Mahas *et al.*, 2018). CRISPR consists of a 30–40 bp repetitive sequence separated by a spacer region that complements the foreign sequence, which is subsequently processed and translated into crRNA (Zhang *et al.*, 2018). CRISPRs are then used to generate the gRNA (guide RNA) (Listgarten *et al.*, 2018). CrRNA and Cas protein combine to generate crRNP (ribonucleoprotein), which causes a break in the intruder's DNA/RNA **(**Majumdar *et al.*, 2017). CRISPR's particularity and specificity in function are due to gRNA's exact binding to the target DNA region (Shah *et al.*, 2018). By use of CRISPR/Cas, the gene of interest may be altered (deleted or inserted) from the system by creating a double strand break (DSB) at the target location (Shapiro *et al.*, 2018). The best expression method for achieving the CRISPR-Cas gRNA sequence, the codon-optimized Cas9 variant, and optimum promoters for sgRNA and Cas9 transcription (Rico *et al.*, 2018).

Application of CRISPR-Cas

The CRISPR/Cas9 technology was created for multi-locus editing and biochemical pathways that is rapid, effective, accurate, and simple. Various CRISPR–Cas methods are being used by researchers to identify viruses early, quickly, and efficiently.

In Fig. 2 different applications are mentioned:

Some of Pesticides which affect the environment

In last few decades, the usage of Pesticides has risen (Huang *et al.*, 2019; Bilal *et al.*, 2019; Lin *et al.*, 2020). Organophosphates, pyrethroids, and carbamates, organochlorines, are the most common pesticides and the threshold for dangerous concentration varies depending on the types of pesticide (Pang *et al.*, 2020; Zhang *et al.*, 2020). Mention some of pesticides:

Organophosphate compounds

Both naturally occurring biomolecules and commercial products such as fertilizers, insecticides, and herbicides include OP (Organophosphates) components. Pest control is among the most important tasks for a good yield (Peshin *et al.*, 2014). Pesticides have become vital as a result, and they are now employed in the manufacture of roughly one-third of all farm products (Zhang *et al.*, 2011). Chemicals and pesticides that are used to kill bugs. Insecticides, herbicides, weedicides, bactericides, fungicides, and larvicides are grouped into numerous classes based on the sorts of bugs they target.

They can be found in all ecosystems, including land, water, and atmosphere, as a result of their overuse. As a result, pollution and numerous illnesses have increased (Chen *et al.,* 2004; Liu *et al.,* 2008; Zhang *et al.,* 2011; Zhang *et al.,* 2017; Zhang *et al.,* 2018).

Organophosphate pesticides contribute for over 70 percent of all pesticides used worldwide, and are thought to be responsible for around 2.5 million toxicosis and accounting for 86.02 percent of all incidents (Zhang *et al.,* 2011). Malathion, parathion, methyl parathion, monocrotophos, chlorpyriphos, dimethoate, dichlorovos, and other organophosphate pesticides are some of the most frequently used. Several microorganisms have been shown to have the ability to degrade these insecticides.

Organophosphate causes inactivation of acetyl cholinesterase it produces toxicity in different organs. It causes bronchorrhoea, bronchoconstriction, bradycardia, miosis in the eye, muscle fasciculations and flaccid paralysis. (Kamanyire and Karalliedde, 2004)

One of the organophosphate, malathion causes nephrotoxicity, hepatotoxicity, neurotoxicity, endocrine disruption, DNA damage and apoptosis. It also produces carcinogenicity, genotoxicity, reproductive organs toxicity. (Badr, 2020)

Xenobiotic compounds

"Xenobiotics" are artificial chemicals that are chemically unique from, molecules found naturally that are derived through biological and abiotic processes. Xenobiotic substances are frequently identified in supplies of toxic waste. Surface runoff, transportation emissions, heating, urban wastes, and natural disasters all contribute to the increased prevalence of these compounds in the environment. The primary cause of large-scale pollution is industry, Medicines, environmental contaminants, food additives, hydrocarbons, Carcinogens, and pesticides are all examples of xenobiotics.

Due to their recalcitrant qualities, xenobiotics such as polychlorinated biphenyls (PCBs), trichloroethylene (TCE), polycyclic aromatic hydrocarbons (PAHs) enter the environment and have become a source of worry contaminants and accumulation. Large businesses including medicines, fossil fuels, pulp and paper bleaching, and agriculture are some of the primary drivers of pollution and xenobiotic introduction into the surroundings.

Carbamates compounds

Carbamates are a kind of insecticide that resembles organophosphate (OP) insecticides in structure and mechanism. Carbamates are N-methyl carbamates made from carbamic acid that promote acetylcholinesterase carbamylation at neuronal synapses and neuromuscular junctions. Trimethacarb, carbaryl, ethinenocarb, fenobucarb, methomyl, oxamyl, pirimicarb, carbofuran, propoxur, and Aldicarb are some of the most common agents that cause hazardous exposure.

In aquatic creatures (frogs, arthropods, and fish), carbamate chemicals produce chronic and acute toxicity by disrupting the biochemical and hematological activities of the gills, liver, blood, and brain (Ghazala *et al.,* 2014; Narra *et al.,* 2016).

Chromosome abnormalities, micronucleus production, sister-chromatid exchange, DNA damage, and apoptosis are all genotoxic consequences of these substances (Chandrakar *et al.,* 2020; Guanggang *et al.,* 2013). In aves and mammals carbamate inhibit brain and plasma cholinesterase. It causes congenital abnormalities in cats and sheep. It also causes significant changes in serum protein, lipid, glucose, AST, ALT levels in mammals (Zaahkouk *et al.,* 2000).

Pyrethroids

Pyrethroids are commonly applied as insecticides in both the work and home, as well as in medicine for the treatment of scabies and headlice. Pyrethroid toxicity is problematic (Ray *et al.*, 2004), and it becomes much more complicated when they are coformulated with piperonylbutoxide, an organophosphorus insecticide, or both, as these substances block pyrethroid metabolism. Pyrethroids primarily affect sodium and chloride channels. As a result, sensitive cells (nerve and muscle) are the primary targets of pyrethroid poisoning, which manifests as dysfunctional function rather than structural failure. Pyrethroid administration may be dentrimental to male fertility. It also causes loss of spermatogonia, more immature germ cells, loss of spermatocytes, apermatids and spermatozoa. Pyrethroid have been shown toxic to aquatic animals e. fish and shellfish (lobster, crayfish). (Chrustek *et al.*, 2018)

Some microorganisms used for biodegradation of pesticides

Bioremediators are microorganisms that fulfill the function of bioremediation. Some of microbial species degrade some pesticides compounds are mention in Table 1:

Table.1 Different xenobiotic compounds produce toxicity (Miglani *et al.*, 2022)

Xenobiotic Compounds	Toxicity
Pharmaceuticals	Reproductive organs toxicity in aquatic and terrestrial animals.
Synthetic polymers	Disruption in food webs, food chain and soil pollution
Halocarbons	Habitat elimination and
Polychlorinated biphenyls (PCBs)	Neurological Abnormalities i.e. Neuronal tissue damage, abnormal reflexes
Polycyclic aromatic hydrocarbons	Oxidative stress, hormonal disorders, genotoxicity, immunosuppresion

Table.2 Different Microorganisam species capable of degrading various pesticides compounds.

Microorganisams	Pesticide degraded	Reference
Pseudomonas sp., Acenetobactor sp., Enterobacter sp.	Methyl parathion and chlorpyrifos	Ravi et al., 2015
Enterobacter	Chlorpyrifos	Niti et al., 2013
Bacillus, Staphylococcus sp.	Endosulfan	Mohamed et al., 2011
Ochrobactrum sp. JAS2	Chlorpyrifos	Abraham et al., 2016
Bacillus subtilis	Cypermethrin	Gangola et al., 2018
Fomitopsis pinicola and Ralstonia pickettii	DDT	Purnomo et al., 2020
Streptomyces rimosus	Deltamethrin	Khajezadeh et al., 2020
Flavobacterium spp	Diazinon	Yasouri *et al.*, 2006
Sphingobium fuliginis	Diazinon, Parathion	Kawahara *et al.*, 2010
Agrobacterium spp.	Deltamethrin, Methamidophos, Methyl parathion, Phoxim, Chlorpyrifos	Wang *et al.*, 2012
Enterobacter spp.	Parathion, Chlorpyrifos, Diazinon, Isazofos, Coumaphos	Singh *et al.*, 2004

Fig.1 Diverse gene editing tools used to decrease toxicity level with the help of Bioremediation.

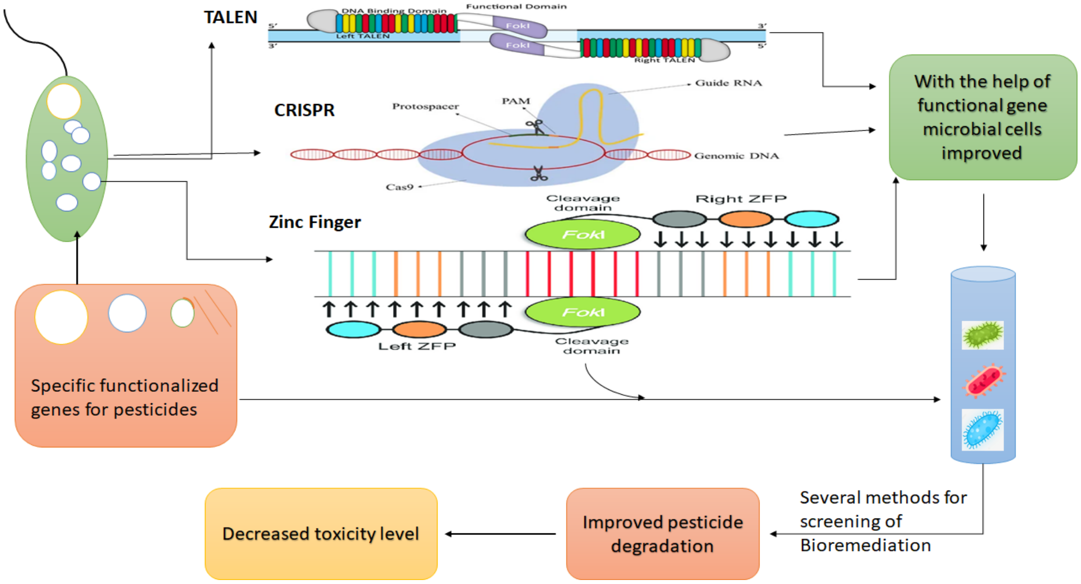
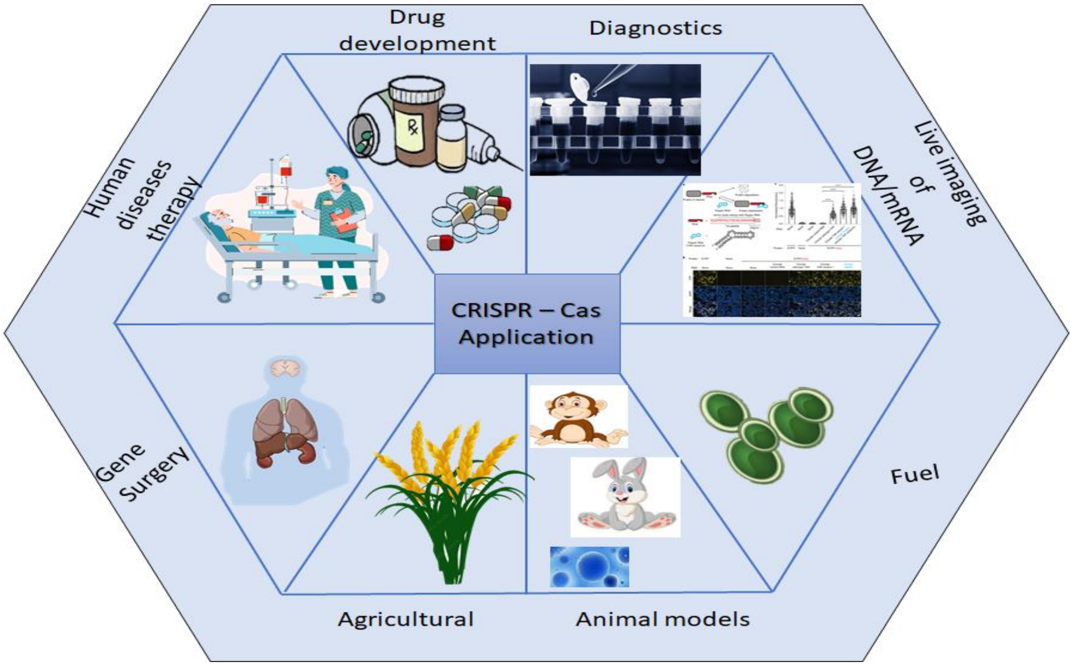


Fig.2 Applications of CRISPR – Cas technique.



How Pesticides is degraded by CRISPR

CRISPR is a strong genetic modification technique. It enables researchers to readily modify DNA sequences and change genetic expression in bacteria, which can be used to create microbes that degrade organic pollutants. To achieve the goal of bioremediation, microbial groups can be manipulated utilising a variety of genetic engineering technologies (Li *et al.*, 2020). Various microbial strains (e.g., *Achromobacter*, *Dehalococcoides*, *Pseudomonas*, *Pseudomonas*, *Burkholderia*, *Rhodococcus*, *Comamonas*, *Alcaligenes, Sphingomonas, and Ralstonia*) have been created to speed up the biodegradation of Synthetic pollutants (Bilal *et al.*, 2020).

Organophosphate compounds degraded by using CRISPR

Organophosphate compound is very toxic to the environment and human health also. For the degradation many technique is available like physical, Chemical, Biodegradation, Catabolic, Co-catabolic, CRISPR system and many other tools are also available but among this all, nowadays CRISPR like new method is available and also easy to use because CRISPR is gene editing tool system and easily edit the genes of insecticides. CRISPR uses a variety of genes, including Cas proteins (Cas9 protein), which attach to DNA and chop it, thereby turning off the targeted gene. The so-called CRISPR array is preceded by an AT- rich leader sequence. Unlike other technologies, CRISPRs do not require the use of separate cleaving enzymes. They may also be quickly paired with custom "guide" RNA (gRNA) sequences that direct them to their DNA targets.

Create a guide RNA that matches the organophosphates gene, which is hazardous, and then alter and connect it to Cas9. Cas9 is directed to the target gene by the guide RNA, and the protein molecular scissors cut the DNA. This is the secret to CRISPR's power: we can alter any gene in the genome simply by injecting Cas9 linked to a short piece of tailored guide RNA. When a cell's DNA gets cut, it will try to repair it. Nucleases are proteins that trim the broken ends and put them back together.

However, this kind of repair, known as non-homologous end joining, is prone to errors, which can result in excess or missing bases. The resultant gene is frequently rendered useless and switched off. Cellular proteins can conduct a distinct DNA repair method termed homology directed repair if we add a new sequence of template DNA to their CRISPR cocktail. This approach is highly effective in organophosphate degradation since it allows us to alter any genome and replace it with the required gene.

Xenobiotic compounds degraded by using CRISPR

Microorganisms can adopt to xenobiotics presented into the environment via gene transfer. This technique can be modified further to change microbe metabolic pathways in order to have them digest dangerous xenobiotics at a quicker speed under specified environmental circumstances. Both genetically manufacturing microorganisms and isolating naturally occurring xenobiotic degrading bacteria are examples of bioremediation mechanisms.

Xenobiotic contaminants can be degraded by endophytic microorganisms. *Cycloclasticus sp*., a PAH-degrading bacteria Isolated and capable of breaking down xenobiotic hydrocarbons like as naphthalene, pyrene, phenanthrene, and others. Cas9 (or CRISPR-associated) is an enzyme protein that functions as a pair of molecular scissors capable of cutting DNA strands (Mahas *et al.*, 2018). CRISPR comprises a complementary 30–40 nucleotide direct repeat sequence that is further processed into crRNA (Majumdar *et al.*, 2017; Zhang *et al.*, 2018). CRISPR can also produce guided RNA (Listgarten *et al.*, 2018). Cr ribonucleoproteins are made by combining crRNA with the Cas protein (RNPs). This crRNP causes cleavage in the intruder's nucleic acid (DNA/RNA) (Jaiswal *et al.*, 2019).

Carbamates compounds degraded by using CRISPR

Many Carbamates compounds are accessible, such as methomyl, carbofuran, aldicarb, propoxur, oxymyl, carbaryl etc., and they destroy the environment, soil, water, and human health, causing microorganisms such as *Sphingomonas sp., Paracoccus sp. YM3, Sphingbium sp. CFD-1, Cupriavidus sp.* ISTL This microorganism is used to minimize toxicity and create innocuous compounds, and the correct method to do it is through the CRISPR approach. By using this CRISPR system it cuts the toxic gene and add the desired gene in specific location. As above mentioned in organophosphate, xenobiotic compound is also degraded using this technique. According to that compound is degraded micro-organism choose and desired gene is inserted so that less harmful product is produced.

Future Perspectives

Pesticides and improved planting products have been more popular in both developed and developing countries. Unfortunately, all of these chemicals are hazardous to varying degrees and have negative consequences for human health and the environment. Pesticide use in agriculture has become an unavoidable practice as the world's population and food demands have grown. Because of their extensive usage as pesticides and significant human toxicity, some microbes can degrade many chemicals Pesticides. Biodegradation, also known as bioremediation, has been proven to be an effective way to decrease pesticide contamination in the environment. Bioremediation is a method of removing pollutants by accelerating natural biodegradation processes. CRISPR-Cas systems have emerged as a breakthrough genome editing technology in recent years, accelerating the advancement of life science and our knowledge of life. These new technologies promise that gene editing will become much more efficient and easy in the future.

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