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Application of CRISPR-Cas9 in Fisheries as a Genetic Tool for Future Aspects

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ABSTRACT

The CRISPR-Cas system, initially discovered in 1987, has revolutionized biotechnology, medicine, and chemical biology since its widespread adoption in 2013. Its unique target specificity, enabled by guide RNA (gRNA), has facilitated diverse applications in genome editing, gene therapy, molecular diagnostics, and basic research. The system consists of CRISPR arrays and Cas proteins, operating through distinct stages of adaptation, expression, maturation, and interference in prokaryotic immune systems. Recent advancements include applications in fisheries, such as pathogen detection and diagnostics for diseases like TiLV and WSSV, as well as genome editing of commercially significant fish species to enhance traits like growth and disease resistance. Notable breakthroughs include the rapid development of genetic mutant breeds and efficient sgRNA design for precise genome editing. Future prospects highlight CRISPR's potential in healthcare and agriculture while emphasizing ethical considerations to mitigate environmental risks associated with genetically modified organisms. The CRISPR-Cas9 system underscores transformative possibilities in research, aquaculture, and conservation biology, necessitating responsible and sustainable applications.

Introduction

In the past five years, CRISPR has brought about revolutionary changes not just in the fields of chemical biology and medicine, but also in biotechnology. Experts from all around the world positively contributed to the advancement of these technologies utilising their specialised knowledge after the first successful use of these technologies in the genetic modification of animals. CRISPR-Cas system has been used as a genome-editing tool because of its target specificity and also ease to target any sequence of interest by changing the gRNA sequence. With this target-specific binding and cleavage of the CRISPR-Cas system, it has been applied to various fields such as genetics, other basic research, gene therapy, and molecular diagnosis. CRISPR Contains guide RNA (small pieces of RNA containing the gene information of the foreign species) and CRISPR-associated (Cas) protein having endonuclease activity. Its functional components include 1. cas genes 2. Leader sequence 3. CRISPR array (Loo et al., 2022). CRISPR loci exhibit several universal features A) Multiple direct repeats with identical or nearly identical, often palindromic sequences B) Non-repetitive similar-sized spacer sequences C) Leader sequence flanking the repeats at one end D). The direct repeats' genetic link with cas genes (Jansen et al., 2002).

In the prokaryotic adaptive immune system, CRISPR works in four main stages, those are 1) Adaptation, where the invader (mainly bacteriophage) is encountered, and an invader-derived short DNA fragment (pre-spacer) is obtained and incorporated in the CRISPR array forming a new spacer. 2) Expression - the CRISPR array is transcribed into a long pre- CRISPR RNA (pre-crRNA) molecule 3) Maturation stage - The pre-crRNA is processed into shorter crRNA molecules each containing a spacer and a part of the repeat sequence 4) Interference - after the crRNA forms a complex with the effector protein, forming a functional RNA guided endonuclease. Under the guidance of the crRNA, this endonuclease hybridises with the target DNA through its spacer sequence upon PAM recognition, ultimately cutting the target DNA sequence (Paul et al., 2020).

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Types of Classes in CRISPR-Cas

CRISPR-Cas system broadly divides into two classes that are class 1 and class 2. This classification is (Bhardwaj et al., 2022), based on the presence of protein complex. Class 1 is found in bacteria and archaea, accounting for 90% of identified CRISPR-Cas/loci and also have heteromeric multiprotein effector complexes. This class 1 is divided into Type 1 which has a multi-subunit complex where Cas3 acts as a signature protein and also the target is ssDNA, Type 3 which has a multi-subunit complex where Cas10 is a signature protein and the target is either DNA or RNA, Type 4 that has also a multi-subunit complex where Cas11 is a signature protein, but the target is still not verified. Class 2 CRISPR-Cas/loci are exclusively present in bacteria accounting for 10% of identified CRISPR- Cas/loci and have single multidomain effector proteins. This class 2 is divided into Type 2 where Cas9 protein is present and targeting the dsDNA, Type 6 where Cas13 is present and targeting ssRNA and also exhibiting collateral cleavage activity, Type 5 has two systems one has Cas12 and targets dsDNA/ssDNA another one has Cas14 protein which is targets ssDNA and also exhibit collateral cleavage activity.

CRISPR-cas System in Fisheries

Based on the CRISPR/Cas12a system, Zhang et al. (2020) created a unique, visible, and specific detection approach for V. parahaemolyticus with a detection limit of up to 1.02 x 102 copies/µL. As little as 200 WSSV copies per reaction can be detected using CRISPR-Cas12a in conjunction with PCR or RPA without causing cross-reactivity with other shrimp DNA viruses (Chaijarasphong et al., 2019). The RPA-Cas12a requires less than an hour to operate at a steady temperature of about 37 °C, doesn't require complicated equipment, and may be used for field diagnosis. A fully field-deployable diagnostic technique was developed by combining the CRISPR-Cas13-based SHERLOCK approach for WSSV detection with lateral flow colorimetric reporting and paper matrix nucleic acid extraction (Sullivan et al., 2019). Targeting the ptp2 gene, the CRISPR-Cas 12a for EHP detection can identify as little as 50 DNA copies without exhibiting cross-reactivity with closely related microsporidians (Kanitehinda, 2020). The viral adenosine triphosphatase (SDDV-ATPase) gene, which has a high specificity for SDDV and a target detection limit of 40 copies per reaction, is used in the CRISPER-RPA application (Sukonta et al., 2021). A CRISPR-Cas13a system-based quick RGNNV detection technique is sensitive enough to identify RGNNV at a minimum of 102 fM copies (Huang et al., 2022). Additionally, segment 9 of TiLV has the lowest detection of 200 copies and the highest sensitivity (Sukonta et al., 2022); the technique is created employing Cas12a for the detection of pirVPA and pirVPB fragments of AHPND. The occurrence of TiLV from Nile tilapia was first reported in India (west Bengal and Kerala) during 2018 (Behera et al. 2018). The suspected cause of huge mortality in tilapia farms was confirmed as TiLV outbreaks by molecular detection followed by cell culture isolation of the virus. Further association of bacterial pathogens such as Lactococcus garvieae with TiLV infection (Swaminathan et al., 2021) and Aeromonas (Rao et al., 2021) explains the co- infection as a major cause of TiLV driven heavy mortality at tilapia farms. Permissibility of the cell lines of Nile tilapia, Oreochromis niloticus such as OnlB and liver OnlL (Thangaraj et al., 2018); OniLH (Yadav et al., 2021; heart and gill cell (Nanthini et al., 2019) was tested as a useful tool for TiLV isolation and testing. Transcriptome analysis of TiLV infected liver shows significant information on the gene regulation of infection with key findings of down regulation of regulation MAP3K7, IFIT1 and TRIM25 genes and upregulation, namely BCL2L1, NFKBIA, TRFC, SOCS, EPOR, PI3K and AKT genes (Sood et al., 2021). Reassortment and evolutionary dynamics of tilapia lake virus genome segments describes the rate of nucleotide substitution for each gene segment in the order of $1– 3 \times 10^{-3}$ nucleotide substitutions per site per year (Dev et al., 2022).

CRISPR-cas related to Genetics Technology

The CRISPR-Cas technology has also been used more widely. Gene drives, antiviral, in vivo directed evolution, chromatin structure manipulation, and other uses have all been made possible by genetic engineering. Systems where one of two alleles is inherited more than 50% of the time are generally referred to as "gene drive" (Bier, 2021). The gene drive allele can replace its homolog by HDR, resulting in super-Mendelian inheritance, thanks to the effective and adaptable targeted DNA cleavage that CRISPR-Cas can do. Both plants and animals have benefited from the application of CRISPR-based gene drive technology (Bier, 2021). The EvolvR system, which continuously diversifies all base pairs within a tunable window in a targeted locus, is created by fusing DNA polymerases with Cas9 nickase. CRISPR-Cas9 has also been used to create virus resistance in eukaryotic cells (Chen, 2021) and for directed evolution (Halperin et al., 2018). Additionally, the so-called CRISPR-GO system can regulate the spatial location of genomic loci by combining dCas9 with a protein unique to a certain compartment (Wang et al., 2018).

Utilising CRISPR-Cas systems as tools for genomic alteration has transformed biological research. The CRISPR-based gene editing toolbox has significantly grown beyond the basic cutting of two strands of DNA, and numerous CRISPR-Cas nucleases and their modified variants have been generated for various parts of biological research. In the meantime, new functional components will undoubtedly be added to the currently existing CRISPR-Cas systems in the future to produce an ever-expanding toolkit for genome engineering research.

Development of CRISPER

Ishino et al (1987) identified some mysterious repetitive sequences downstream of the iap gene on the chromosome of Escherichia coli K1. Later, four CRISPR-associated (Cas) genes were identified in CRISPR-containing prokaryotes (Jansen et al., 2002). The CRISPR spacers are derived from preexisting sequences, either chromosomal or within transmissible genetic elements such as bacteriophages and conjugative plasmids (Mojica et al., 2005) which has a role in prokaryotes' immune system (Barrangou et al., 2007). Especially Cas1 and Cas2 from Escherichia coli form a complex that is essential for spacer acquisition during the CRISPR-Cas adaptive immunity (Nunez et al., 2014). Cpf1 (Cas12a), a putative class 2 CRISPR effector is involved in Cpf1 mediated DNA interference with features distinct from Cas9. According to Zetsche et al. (2015), Cpf1 is a single RNA- guided endonuclease that uses a T-rich protospacer-adjacent motif and lacks tracrRNA.

CRISPR Applications

Various applications of CRISPER Cas9 as genome editing tool are explained (Jinek et al., 2012; Cong et al., 2015). However efficient reactivity of CRISPER Cas9 for sensitive detection of Zika virus. Using CRISPR/Cas9 cleavage and nicking endonuclease (NEase) mediated nucleic acid amplification, Pardee et al. (2016) further developed the unique CRISPR/Cas9 initiated isothermal exponential amplification reaction (CAS-EXPAR) approach, which is site-specific and quick (Huang et al., 2018). More than 20 commercially significant fish species, such as Atlantic salmon, tilapia, common carp, and grass carp, have had their genomes edited using CRISPR-based technologies (Figure 1) (Lu et al., 2021; Yang et al., 2022). These genome editing experiments examine a variety of aquaculture properties, like as growth, muscle development, reproduction, pigmentation, disease resistance, and feed utilisation. Also found that the species and genes affected by CRISPR-Cas9 significantly affect its mutation efficiency. According to Edvardsen et

al. (2014) and Liu et al. (2019), the effectiveness of the tyr gene is 22% in Atlantic salmon but 60% in white crucian carp. Muscle growth-related genes called myostatin a and b, which stop skeletal muscle from forming, seem to have a relatively high mutation efficiency of 94% and 88%, respectively (Kishimoto et al., 2018). The most notable example among many is the complete removal of the muscle growth inhibitor (Pm-mstn) using CRISPR-Cas9, which enabled the development of a novel red seabream breed. Two years were required to generate the pure genetic mutant breed, which is far less time than is required with conventional breeding techniques (Kishimoto et al., 2018).

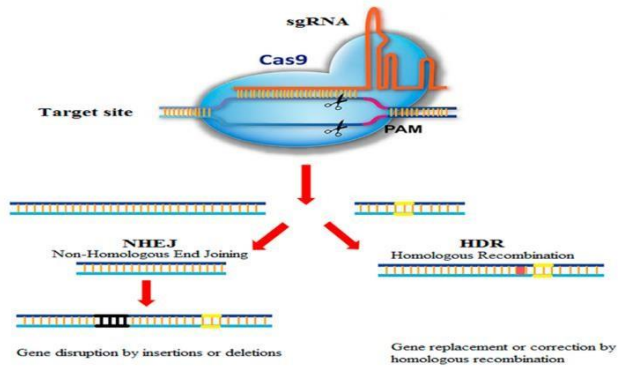

Figure 1. Schematic of CRISPR-Cas9 genome editing system (Source: Tavakoli et al., 2021)

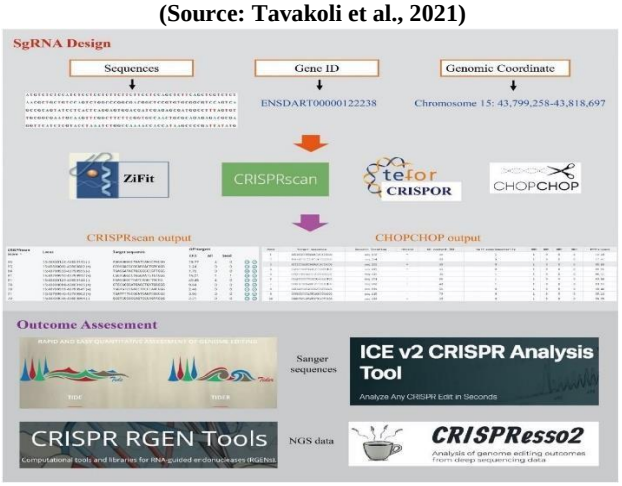

Figure 2: An example pipelines of sgRNA design and outcome assessment of genome editing in aquaculture, with prediction programs denoted (Source: Luo et al., 2022).

Most likely, the most important stage in CRISPR-Cas9 research is designing the sgRNA (Figure 2). Finding the PAM site, or 5′-NGG-3′, and figuring out the 20 nucleotides upstream should be sufficient to forecast sgRNA candidates in theory (Doudna and Charpentier, 2014; Jinek et al., 2012). However, in actual genome editing, only a limited number of sgRNA may effectively target DNA on the desired spot, and off-target editing may occur due to nucleotide mismatches between sgRNA and the PAM motif (Doench et al., 2016; Hsu et al., 2013). Therefore, while editing the genome with CRISPR-Cas9, it is crucial to evaluate the cleavage effectiveness of sgRNA as well as the possibility of off-target activity (Doench, 2018; Hanna and Doench, 2020).

Future Aspects

Since its discovery as a defence mechanism in prokaryotes, CRISPR-Cas systems have undergone several milestones in their evolutionary history. The polythetic categorization approach was used to categorise CRISPR-Cas systems due of their complex and dynamic evolution. Precision genome engineering has made major improvements with CRISPR-Cas9, which present new opportunities to transform the fields of farming and healthcare. By utilising its potential, researchers may imagine a future in which genetic disorders that were previously incurable can be cured, agricultural produce become more durable and productive, and our knowledge of the genetic basis of life is expanded. To optimise the benefits and reduce any hazards, people must carefully consider the moral effects of using CRISPR-Cas9 prior to embrace its future.

Conclusion

In conclusion, CRISPR/Cas9 has enormous potential to transform aquaculture and fisheries management. It provides accurate and focused genetic alterations in fish species, supporting conservation efforts, the development of desired traits, and disease resistance. However, to guarantee the ethical and sustainable application of this technology in fisheries, rigorous assessment of the implications for the environment and ethics is essential. Opportunities for conservation efforts are also created by the use of CRISPR/Cas9 in fisheries. Through the introduction of advantageous genetic variations that improve fish survival and reproductive performance, it can be used to restore endangered fish populations. The use of CRISPR/Cas9 in fisheries, however, brings up moral and environmental issues. Ecosystems may suffer unexpected effects if genetically modified fish are released into the wild. Therefore, to guarantee responsible use and avoid detrimental effects on native species, stringent laws and risk assessment procedures must be in place.

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