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Genetic Engineering for Sustainable Fish Farming: Role of CRISPR

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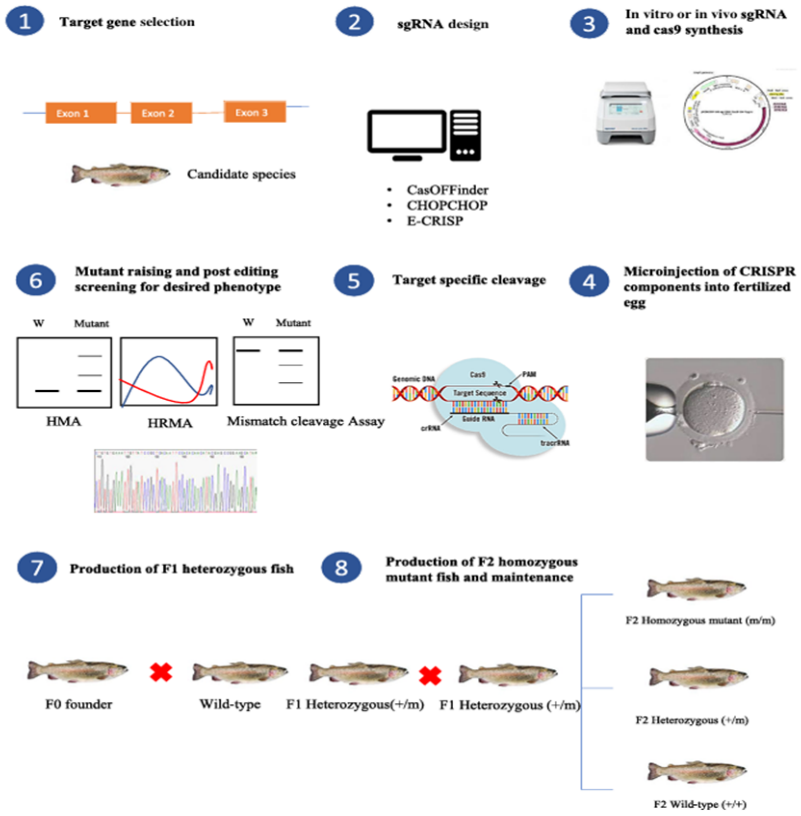
SUMMARY
In sustainable aquaculture, the CRISPR/Cas9 genome editing technology has become a game-changer, enabling precise genetic modifications in fish to enhance desired qualities, including growth rate, disease resistance, and reproductive control. Because this technique introduces specific alterations without integrating foreign DNA, it has substantial advantages over traditional genetic engineering. CRISPR has successfully enhanced muscle mass, immunological responses, and reproductive traits in aquaculture species, including carp, catfish, and tilapia. However, several technological and legal obstacles hinder the use of this technology, including public acceptance, off-target effects, and gaps in genome annotation. Notwithstanding these obstacles, CRISPR in aquaculture has a bright future, potentially improving environmental sustainability and global food security by creating resilient, robust, and rapidly increasing fish supplies.

INTRODUCTION
Biotechnology techniques used to alter the genetic composition of fish to improve characteristics like growth rate, disease resistance, or nutritional value are known as genetic engineering applied to sustainable fish farming. This technique eventually results in aquaculture methods that are more effective and ecologically friendly. This may entail implementing novel technologies like CRISPR. CRISPR is a fantastic technique that offers a quick, simple, and effective high-tech way to edit genomes. It enables targeted genetic changes, such as adding, removing, or changing DNA or turning genes on or off, without exogenous genes. Although CRISPR/Cas editing is a simple and effective novel breeding technique that has recently been used to enhance certain aquaculture species' desirable features, much work needs to be done before it can be used commercially. Here, we'll review what we know about the CRISPR/Cas genome editing technology and its current state, difficulties, and potential uses in aquaculture. CRISPR-Cas9 has been used to boost growth rates in commercially significant species like common carp and tilapia, resulting in more effective production methods (Zhu *et al*., 2024).



Techniques for Sustainable Fish Farming Practices:

There are a few essential steps in the CRISPR/Cas9 genome editing process for edible fish. After looking through the genome database of the targeted species, the desired gene must be selected for targeted mutagenesis. A sgRNA designing tool must be used to create the sgRNA from the targeted region, and oligo synthesis of the sgRNA must be carried out. There are various methods for carrying out genome editing, including ribonucleoprotein (RNP)-based systems, plasmid-based CRISPR/Cas9 delivery systems, in vitro-transcribed sgRNA and Cas9 systems, and systems that can be delivered into a single embryonic cell stage by electroporation or microinjection (Taha *et al.* 2022). The offspring should be thoroughly examined using sequence analysis to find off-target mutations. Selecting off-target free progeny, breeding them with wild type, and producing heterozygous mutants are all critical. Intercrossing the F1 heterozygous FSH population will result in homozygous F2 offspring. Developing genetically enhanced fish species in aquaculture and selecting fish species with desirable phenotypes constitute the final phase.

The CRISPR/Cas9 genome editing process in farmed fish is shown in the diagram. First, a target gene is chosen, and then a specific sgRNA is designed using programs like CHOPCHOP. One-cell stage embryos are microinjected with the Cas9 protein and the synthesised sgRNA. Gene alterations result from the targeted DNA breaks that Cas9 subsequently introduces. Assays such as HRMA or HMA are used to screen for mutations. F1 heterozygous offspring are created by breeding successfully altered F0 fish, and these are then crossed to produce F2 homozygous mutants. This technique allows commercial aquaculture to create genetically enhanced fish lines with desired characteristics like disease resistance and faster growth (Puthumana *et al.*,2024).

Role of the CRISPR Technique:

1. Growth enhancement:

Some of these fish with altered genomes have shown superior characteristics, such as increased growth or resistance to disease. For instance, the mean body weight of myostatin-knockout channel catfish was 29.7% higher than that of control fish. Several studies have effectively altered genes linked to muscle growth and development, yielding encouraging outcomes in animals like common carp, channel catfish, and red sea bream. One important field of study is the manipulation of genes linked to growth hormone. For instance, it has been demonstrated that changes to the myostatin (mstn) gene adversely affect muscle growth and increase muscle mass and growth in various fish species. Disrupting the mstn-gene increased the blunt snout bream's body weight and muscle mass, but identical changes in the olive flounder resulted in a notable increase in muscle mass. Growth hormone genes have been overexpressed in species such as Atlantic salmon by transgenic techniques, leading to faster growth rates and higher biomass production. Such genetic improvements are essential to supplying the increasing demand for fish protein worldwide. Beyond conventional genetic modification, CRISPR/Cas9 technology has become a potent instrument for accurate genome editing.

2. Disease resistance:

The ability of a fish species to withstand internal and external stressors that may impair its physiological or morphological capabilities is known as disease resistance. Disease is a major obstacle to the growth of aquaculture and has a detrimental effect on total output yield, which lowers fish farmers' profitability. CRISPR/Cas9 can reduce the number of bacterial colony-forming units in fish tissues, improve post-infection survival rates, and change the expression of immune-related genes by incorporating vector-engineered antimicrobial peptide genes (AMGs). CRISPR/Cas9 has been used to modify immune response-related genes in tilapia, increasing their resistance to bacterial infections such as Aeromonas hydrophila and Streptococcus agalactiae. Similarly, CRISPR/Cas9 has been used to target genes in catfish that control immunological pathways, increasing their survival rates after exposure to pathogens.

3. Reproduction:

Reproduction. Because of the benefits of the CRISPR/Cas9 method, researchers are now primarily focusing on the genes linked to fish reproduction to comprehend the genetics governing sex differentiation and sex determination. They are where germ cells differentiate and derive from primordial germ cells (PGCs) in embryos. Dead end (dnd), nanos C2HC-type zinc finger 2 (nanos2), and nanos C2HC-type zinc finger 3 (nanos3) are the genes known to contribute to PGC creation and maintenance; therefore, knocking out these genes may cause fish to become sterile. The crucial role of dnd in germline development was further confirmed when the CRISPR/Cas9-mediated gene silencing of the dnd gene in Atlantic salmon produced an F0 mutant with loss of pigmentation and gonad germ cell loss. In addition, the tilapia species experienced sex reversal when sex-determining genes were disrupted. For instance, male-to-female sex reversal was brought on by inhibiting AMH (anti-Mullerian hormone).

CRISPR/Cas9 technology has also been used to characterise several sex differentiation genes, including dmrt1 (double sex and mab-3-related transcription factor 1), sf-1 (steroidogenic factor 1), and gsdf (gonadal somatic cell-derived factor). Gonadal dysgenesis and XY feminisation were observed in F0 tilapia when SF-1 was knocked out. Furthermore, cyp19a1a (cytochrome P450, family 19, subfamily A, polypeptide 1a), foxl2 (forkhead box L2), and serum estradiol-17 were all expressed less in XX fish when sf-1 was disrupted, but the former genes were expressed more in XY fish. Additionally, it was shown that whilst estradiol-17 only partially restored the gonadal differentiation of sf-1-disrupted XX fish, 17-methyltestosterone restored the gonadal differentiation of sf-1-disrupted XY fish. It showed that SF-1 plays a significant part in controlling tilapia reproduction and steroidogenesis. The cyp17a1 gene was recently silenced in common carp by CRISPR/Cas9-based gene knockout, producing all females (Zhai *et al*. 2022).

Challenges in Fisheries:

1. Technical acceptance:

A thorough knowledge of genetic backgrounds and genome sequences is necessary for CRISPR editing in aquaculture species. In many species, gene activities are unclear. Since only a few genes linked to economically relevant qualities like growth, disease resistance, and robustness have been found in aquaculture species, this lack of functional genetic information is a significant restriction. This makes it difficult to create efficient gRNAs that target particular genes and limits the use of CRISPR/Cas. Before being used in vivo, CRISPR/Cas constructs should ideally be validated in cell culture systems to guarantee accurate and efficient gene editing (Yang *et al.*, 2022). However, the paucity of established and well-characterised cell lines for many aquaculture species limits this strategy. CRISPR/Cas constructs are typically introduced into fertilised fish eggs at the one-cell stage for in vivo editing (Yang et al., 2022). The most popular technique, microinjection, is labour-intensive and necessitates an expensive, dedicated platform. It is challenging to inject a lot of eggs in a short time, and access to freshly fertilised embryos is a significant limitation in certain species, such as prawns. Furthermore, the egg membrane poses difficulties for effective microinjection, particularly in oviparous fish species. There is currently no recognised gene editing platform for ovoviviparous fish. Numerous aquaculture species, including teleost fish, have undergone whole genome duplication (WGD) events. Because many copies of a gene might affect targeting precision, this gene duplication makes CRISPR editing more difficult. To overcome this difficulty, comparative research between gene copies is required. Additional optimisation is necessary to increase editing efficiency, decrease off-target effects, and restrict mosaicism, even in species where CRISPR/Cas9 has already been used. Unintentional genomic alterations brought on by off-target mutations may have unfavourable effects on the organism and raise concerns about food safety.

2. Regulatory and consumer acceptance:

Gaining public trust and regulatory permission is one of the most difficult tasks facing CRISPR/Cas genome editing in aquaculture. While CRISPR technology has much potential to improve aquaculture qualities like growth, health, and sustainability, its practical application is still primarily restricted to the research stage. This is mostly due to cautious public perception and uneven development of international regulatory frameworks. Even though CRISPR is fundamentally distinct from genome editing, many people still confuse the two. There is no introduction of foreign genes in CRISPR-based editing. This method closely resembles what could happen organically via generations of selective breeding. There are currently no standardised international guidelines. While some classify CRISPR products as non-GMOs and exempt them from stringent biosafety inspections, others continue to undergo stringent evaluations. Consumers are expected to embrace CRISPR-derived products more favourably, partly because the term "genome edited" is less contentious than "genetically modified." However, effective public acceptance is largely dependent on open and honest communication. People must comprehend the use of CRISPR, its rationale, and the ideals it upholds, including safer, healthier food, better animal welfare, and more environmentally friendly aquaculture methods.

Prospects:

In the coming decades, commercial aquaculture and fish breeding operations should benefit from the technical advancements of the CRISPR/Cas9 technology and favourable and suitable public and regulatory perspectives. With the benefits provided by the CRISPR/Cas9 system, it is possible to build commercial fish with various advantageous traits, including accelerated growth rate, high nutritional value, disease resistance, and tolerance to environmental stress. However, before genetically modified stocks are put on the market, careful attention must be taken to eliminate unintentional pleiotropic effects, develop an inducible and stable editing

mechanism for traits conferring sterility and maturation to prevent accidental gene spills into the environment, and enable multiple edits simultaneously in broodstock populations to target various characteristics.

CONCLUSION:

A revolutionary technique in sustainable aquaculture, CRISPR/Cas9 genome editing allows for precise genetic alterations to improve fish species' growth, disease resistance, and reproduction ability. It has great potential for developing fish strains that can flourish in harsh environmental settings while lowering dependency on pesticides and antibiotics. Technology is growing quickly despite major technical and legal obstacles. Increased public and regulatory acceptance, decreased off-target effects, and better genome annotation are necessary for its successful integration into commercial aquaculture. CRISPR has the potential to revolutionise fish aquaculture worldwide and support future environmental sustainability and food security if used responsibly and communicated openly.

REFERENCES:

Taha, E. A., Lee, J., & Hotta, A. (2022). Delivery of CRISPR-Cas tools for in vivo genome editing therapy: Trends and challenges. *Journal of Controlled Release*, *342*, 345-361.

Yang, Z., Yu, Y., Tay, Y. X. and Yue, G. H. (2022). Genome Editing and Its Applications in Genetic Improvement in Aquaculture. Rev.. Aquac. 14, 178–191. doi: 10.1111/raq.12591.

Zhai, G., Shu, T., Chen, K., Lou, Q., Jia, J., Huang, J., ... & Yin, Z. (2022). Successful production of an all-female common carp (Cyprinus carpio L.) population using cyp17a1-deficient neomale carp. *Engineering*, *8*, 181-189.

Zhu, M., Sumana, S. L., Abdullateef, M. M., Falayi, O. C., Shui, Y., Zhang, C., ... & Su, S. (2024). CRISPR/Cas9 Technology for Enhancing Desirable Traits of Fish Species in Aquaculture. *International Journal of Molecular Sciences*, *25*(17), 9299.