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Review Article

CRISPR Genome Editing Technology to Revolutionize Aquaculture and Fisheries

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Abstract

Aquaculture is rapidly replacing wild fish with farmed fish as the principal source of seafood in human diets. Diseases, reduced viability, fertility decline, poor growth, farmed fish escape into wild, and environmental pollution are the major issues faced by the sector. The commercialization of AquaAdvantage salmon and CRISPR/Cas9-developed tilapia (Oreochromis niloticus) initiated using of genetic engineering and genome editing methods, such as CRISPR/Cas, as potential solutions to overcome the challenges. Disease resistance, sterility, and improved growth are among the future features being developed in many fish species. CRISPR/Cas is less expensive, simpler, and more precise than existing genome editing technologies, and it can be employed as a new breeding method in fisheries and aquaculture to address major difficulties. Furthermore, unlike transgenesis, which introduces foreign genes into the host genome and thus alleviates major public safety concerns, CRISPR/Cas genome editing rapidly introduces favourable changes by disrupting genes with targeted minor changes. Although CRISPR/Cas technology has enormous potential, there are a number of technical hurdles as well as regulatory and public issues involving its usage in fisheries and aquaculture. Nonetheless, the exciting point in the CRISPR/Cas9 genome editing is that two CRISPR-edited fish, namely, red sea bream and tiger puffer developed by the Kyoto-based startup, have received approval and are now available for purchase, while another fish, FLT-01 Nile tilapia developed by AquaBounty, is not classified as a genetically modified organism regulatory. However, it is still a long way from revolutionizing and becoming a viable commercial aquaculture breeding technology for aquaculture-important features and species.

Keywords: CRISPR/Cas; Aquaculture; Gene Editing; Growth; Transgenesis

Introduction

The increasing in the global population presents challenges to the global food security. For the global food security, the fisheries sector contributes more than the other animal protein sector. In fisheries sector the capture fisheries getting down due to the environmental pollution, over exploitation and etc. but the aquaculture is contributing more than the capture fisheries [10]. Now a days the aquaculture also facing the lot of problems like disease, slow growth rate, reduce viability, reduce fertility and etc.... so that the researchers were used the genome editing technology to overcome the problems. In earlier days the zinc finger nucleases (ZFNs), and transcription activator-like effectors nucleases (TALENs) genome editing techniques were used but now a days the clustered regularly interspaced short palindromic repeats (CRISPR) genome editing system were widely used in all the fields like plant breeding, anima breeding including fish breeding and etc. [8,18]. Other than the aquaculture species the CRISPR system was also used in the zebrafish animal model. Because Zebrafish have a fully sequenced genome, facile genome manipulation, high fecundity, a short generation time (approximately 3 months), rapid embryonic development (24 hr.), and external fertilization. so that the researchers were used the zebrafish to investigate the development biology and human diseases (including neural disorders, cancer, infectious diseases, cardiovascular diseases, kidney diseases, diabetes, blindness, deafness, digestive diseases, hematopoiesis, and muscle disorders). Despite being an essential biomedical model, zebrafish have significant shortcomings, including organ dissimilarity in the respiratory and reproductive systems. As a result, using zebrafish as a model for human breathing or reproduction is problematic. Furthermore,

because zebrafish live in an aquatic environment, screening of some water-soluble medicines in zebrafish is a constraint.

CRISPR/Cas system

Genome editing is a form of genetic engineering in which DNA in living cells is actively inserted, removed, or modified [30]. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) refers to the distinctive arrangement of short, partially repeated DNA sequences present in prokaryotic genomes. CRISPR and its related protein (Cas-9) is an adaptive immunity technique used by prokaryotes to defend themselves against viruses or bacteriophages [13]. CRISPR has been experimentally demonstrated to be a critical component of prokaryotes' adaptive defense response against viruses. Bacterial cells are vaccinated throughout the adaption process by inserting small segments of viral DNA (spacers) into a genomic region known as the CRISPR array. As a result, spacers act as a genetic memory of earlier viral infections [15]. The CRISPR defense mechanism protects bacteria from repeated viral infections through three basic stages: adaptation (spacer acquisition), crRNA synthesis (expression), and target interference. CRISPR loci are a collection of short repeating sequences found in prokaryotic chromosomal or plasmid DNA. Cas gene is commonly found next to CRISPR, which codes for nuclease protein (Cas protein), which is responsible for destroying or cleaving viral nucleic acid [31].

Components and mechanism of CRISPR/Cas system

Depending on the function and structure of Cas proteins, CRISPR/Cas system was classified into two classes. Class 1 (TYPE I, III and IV) this consist of multiple subunit Cas protein complex. In class II (type II, V and VI) tis system utilized the single Cas protein. The CRISPR/Cas 9 system structure was relatively simple in type II [27]. In Cas 9 system the guide RNA (g-RNA) and CRISPR associated (Cas) protein are the two vital components. The Cas 9 consist of 2 lobes nuclease lobe and recognition lobe. The nuclease lobe consists of Protospacer Adjacent Motif (PAM) interacting domains, Ruvc and HNH. The PAM specificity is conferred by the PAM interacting domain, which is also responsible for starting binding to target DNA. Each single-stranded DNA is cut using the RuvC and HNH domains [40]. The guide RNA consists of 2 parts CRISPR RNA (Cr RNA) and trans-activating CRISPR RNA (tracr RNA). The Cr-RNA help the target DNA by paring with target sequence its around 18-20 base pairs in size. The tracr-RNA serves as a binding scaffold for Cas 9 nuclease it's a long stretch of loops in structure. The guide RNA is used to target viral DNA in prokaryotes, but it may be synthetically generated in the gene-editing tool by combining crRNA and tracr-RNA to form a single guide RNA (sgRNA) in order to target practically any gene sequence that has to be edited [28].

The CRISPR/Cas-9 genome editing mechanism has three steps: recognition, cleavage, and repair [29]. the formulated small guide RNA command the Cas-9 and detects the target sequence in the gene of interest through its 5crRNA complementary base pair component. Cas-9 nuclease causes double-stranded breaks (DSBs) at three base pairs upstream of PAM [1]. The PAM sequence is a short (2-5 base pair length) conserved DNA sequence downstream of the cut site its size changes depending on the bacterial species. Cas-9 protein, the most extensively used nuclease in genome editing tools, it recognizes the PAM sequence at 5-NGG-3 (N can be any nucleotide base). Once Cas-9 has identified a target site with the suitable PAM, it induces local DNA melting followed by the production of an RNA-DNA hybrid, although the mechanism by which Cas-9 enzyme melts the target DNA sequence is yet unknown. The Cas-9 protein is then activated for DNA cleavage. The HNH domain cleaves the complimentary strand of target DNA, whereas the RuvC domain cleaves the non-complementary strand, resulting in mostly blunt-ended DSBs. Finally, the host cellular machinery repairs the DSB [17,28].

Double-Stranded Break Repair Mechanisms In the CRISPR/Cas-9 system, the two strategies for repairing DSBs produced by Cas-9 protein are non-homologous end joining (NHEJ) and homology-directed repair (HDR) [25]. NHEJ promotes DSB repair by joining DNA fragments via an enzymatic process in the absence of exogenous homologous DNA and is active throughout the cell cycle. It is the most efficient and dominant cellular repair mechanism; however, it is an error-prone system that can result in minor random insertions or deletions (indels) at the cleavage site, resulting in frameshift mutations or premature stop codons [41]. HDR is a highly accurate technique that necessitates the use of a homologous DNA template. It is primarily active the cell cycles of late S and G2 phases. HDR requires a high number of donor (exogenous) DNA templates containing a sequence of interest in CRISPR-gene editing. By inserting a donor DNA template with sequence homology at the expected DSB site, HDR performs the precise gene insertion or replacement [25,41].

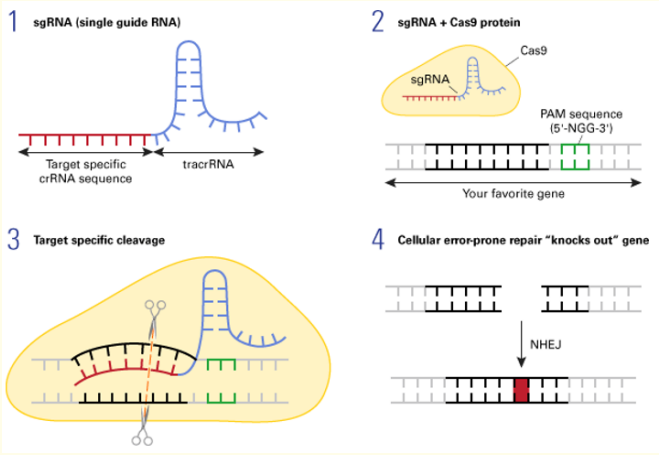

Figure 1: The basic idea behind CRISPR/Cas9-mediated gene disruption. (1) A single guide RNA (sgRNA) sequence composed of a crRNA sequence unique to the DNA target and a tracr RNA sequence that interacts with the Cas9 protein, (2) interacts to a Cas9 protein recombinant with DNA endonuclease activity, (3) The resulting complex will cause double-stranded DNA cleavage specific to the target (4). The cleavage site will be repaired by the nonhomologous end joining (NHEJ) DNA repair mechanism, which is prone to errors and can result in insertions/deletions (INDELs) that affect gene function [14].

Crispr/Cas-genome editing in aquaculture and fisheries

Crispr/Cas technology has been experimented in multiple/varied aquaculture scenarios and they are summarized in table 1. The aim was to increase the overall performance of fish by focusing on commercially important traits like growth, diseases resistance, reproductive performance, sterility, pigmentation and nutrient value.

Growth

In Red sea bream (*Pangrus major*), the myostatin gene (Mstn) was knocked out by CRISPR/Cas 9, which resulted in significant increase of muscle mass and better overall growth [21]. Also in other species like Common carp (*Cyprinus carpio*) [44], Channel catfish (*Ictalarus punctatus*) [19], mud loach (*Misgurnus anguillicaudatus*) [37], olive founder (*Paralichthys olivaceus*) [20], Pacific oyster (*Crassortrea gigas*) [42] and blue snout bream (*Megalobrama amblycephala*) [35], the myostatin gene was knocked out by CRISPR/Cas9 which also resulted in significant overall growth of the fish species. In Tiger puffer (*Takifugu rubripe*), CRISPR/Cas 9 system was used to knock out the leptin receptor gene, which made the fish to eat more and resulted faster growth [21,33]. In Rainbow trout (*Oncorhynchus mykiss*), the IGF binding proteins (IGFBP-2b) was disrupted by using CRISPR/Cas 9, which increased the somatic growth [4].

Species name	Gene	Role of the gene	Trait of the gene	Reference
Red sea bream (*Pangrus major)*	Myostatin (mstn)	It's the transforming growth factor β (TGFβ) superfamily, and it is a key regulator of skeletal muscle growth	Growth	[21]
Common carp (*Cyprinus carpio)*	Myostatin (mstn)	It's the transforming growth factor β (TGFβ) superfamily, and it is a key regulator of skeletal muscle growth	Growth	[44]
Channel catfish (*Ictalurus punctatus*)	Myostatin (mstn)	It's the transforming growth factor β (TGFβ) superfamily, and it is a key regulator of skeletal muscle growth	Growth	[19]
Olive founder (*Paralichthys olivaceus*)	Myostatin (mstn)	It's the transforming growth factor β (TGFβ) superfamily, and it is a key regulator of skeletal muscle growth	Growth	[20]
Mud loach (*Misgurnus anguillicaudatus*)	Myostatin (mstn)	It's the transforming growth factor β (TGFβ) superfamily, and it is a key regulator of skeletal muscle growth	Growth	[37]
Pacific oyster (*Crassostrea gigas*)	Myostatin (mstn)	It's the transforming growth factor β (TGFβ) superfamily, and it is a key regulator of skeletal muscle growth	Growth	[42]
Tiger puffer (*Takifugu rubripe*)	leptin receptor gene	controls the appetite, causing fish to eat more	Growth	[21,33]
Blunt snout bream (*Megalobrama amblycephala*)	Myostatin (mstn)	It's the transforming growth factor β (TGFβ) superfamily, and it is a key regulator of skeletal muscle growth	Growth	[35]

Rainbow trout (*Oncorhynchus mykiss*)	IGF-binding proteins IGFBP-2b	IGF-binding proteins (IGFBP) binds to IGF and acts as a negative regulator of somatic growth.	Growth	[4]
Common carp (*Cyprinus carpio)*	Cytochrome P450 17A1 (cyp17a1)	It regulates the sex steroids biosynthesis	Sterility and reproductive Confinement	[43]
Atlantic salmon (*Salmo salar)*	Dead end (dnd)	It plays the role in germ cells production	Sterility and reproductive Confinement	[12,22]
Sterlet (*Acipenser ruthenus)*	Dead end (dnd)	It plays the role in germ cells production	Sterility and reproductive Confinement	[3]
Gibel carp (*Carassius gibelio)*	foxl2 homeologs (Cgfoxl2a-B, Cgfoxl2b-A, and Cgfoxl2b-B)	It determined and maintained ovarian development via oestrogen biosynthesis	Sterility and reproductive Confinement	[11]
Yellow catfish (*Pelteobagrus fulvidraco*)	PDZ domain-containing gene, pfpdz1	It involves in male sex differentiation	Sterility and reproductive Confinement	[5]
Nile tilapia (*Oreochromis niloticus*)	Doublesex- and mab-3-related transcription factor (dmrt6 and dmrt1)	It involves in male germ cells proliferation and testicle differentiation	Sterility and reproductive Confinement	[23]
Nile tilapia (*Oreochromis niloticus*)	Forkhead box L2 (foxl2)	It determined and maintained ovarian development via oestrogen biosynthesis	Sterility and reproductive Confinement	[23]
Nile tilapia (*Oreochromis niloticus*)	Nanos C2HC-Type Zinc Finger (nanos2 and nanos3)	It's required for the continuous production of oocytes	Sterility and reproductive Confinement	[23]
Nile tilapia (*Oreochromis niloticus*)	Steroidogenic factor-1 (Sf-1)	Its major regulator of steroidogenesis and reproduction in fish	Sterility and reproductive Confinement	[38]
Nile tilapia (*Oreochromis niloticus*)	Gonadal soma-derived factor (Gsdf)	It involves in male germ cells proliferation and testicle differentiation	Sterility and reproductive Confinement	[16]
Atlantic salmon (*Salmo salar)*	Δ5 and Δ6 desaturases	These critical enzymes in the pathways for the biosynthesis of the polyunsaturated fatty acids arachidonic, eicosapentaenoic, and docosahexaenoic acids.	Omega-3 production	[7]
Atlantic salmon (*Salmo salar)*	Fatty Acid Elongase 2 (elovl2)	It encodes an enzyme involved in the elongation of long-chain omega-3 and omega-6 polyunsaturated fatty acids (LC-PUFAs)	Omega-3 production	(6]
Rohu (*Labeo rohita*)	*Toll-like receptor TLR22*	Play a distinctive role in immune defence	Disease resistance	[2]

Prawn (*Exopalaemon carinicauda*)	*Carotenoid isomerooxygenase (EcNinaB-X1)*	It might play a key role in immune system	Disease resistance	[36]
Yellow croaker (*Larimichthys crocea*)	Tyrosinase (tyr)	catalyze melanin biosynthesis in pigment cells and play important roles in determining vertebrate coloration	Pigmentation	[24]
Chinese lamprey (*Lethenteron morii*)	*Slc24a5*	responsible gene for the "golden" phenotype in fish	Pigmentation	[45]
Loach (*Paramisgurnus dabryanus*)	Tyrosinase (tyr)	catalyze melanin biosynthesis in pigment cells and play important roles in determining vertebrate coloration	Pigmentation	[39]
White Crucian carp (*Carassius auratus cuvieri*)	Tyrosinase (tyr)	catalyze melanin biosynthesis in pigment cells and play important roles in determining vertebrate coloration	Pigmentation	[26]
Nile tilapia (*Oreochromis niloticus*)	slc45a2 (solute carrier family 45 member 2)	It is a membrane transporter that mediates melanin biosynthesis	Pigmentation	[32]
Atlantic salmon (*Salmo salar)*	Tyrosinase (tyr)	catalyze melanin biosynthesis in pigment cells and play important roles in determining vertebrate coloration	Pigmentation	[9]
Atlantic salmon (*Salmo salar)*	Solute carrier family 45, member 2 (slc45a2)	It is a membrane transporter that mediates melanin biosynthesis	Pigmentation	[34]

Table 1: List of aquaculture species used in the CRISPR genome editing studies.

Sterility and reproductive confinement

In Common carp (*Cyprinus carpio*) [43], the cytochrome p45017A1 gene was knocked out by CRISPR/Cas 9, which regulated biosynthesis of sex steroids, to produce all female common carps. In Atlantic salmon (*Salmo salar*) [22], the dead end (dnd) gene was knocked out by CRISPR/Cas 9 to produce germ-cell free salmons, which did not undergo puberty. In Sterlets (*Acipenser ruthenus*) [3], the dead end (dnd) gene was knocked out, which resulted in the sterile sterlet hosts, which were used for surrogate production. In Gibel carp (*Carassius gibelio*) [11], the Cgfoxl2a-B, Cgfoxl2b-A and Cgfoxl2b-B genes were knocked out, the genes which maintained ovarian development through oestrogen biosynthesis and resulted the sterile fish. In Yellow Catfish (*Pelteobagrus fulvidraco*) [5], the PDZ domain containing gene (pfpdz1) was knocked out, which produce the sterile male fish. In Nile Tilapia (*Oreochromis niloticus*), five different genes namely (dmrt6 and dmrt 11), (foxl2), (nanos2 and nanos3), (sf-1) and (Gsdf) were knocked out by CRISPR/Cas 9, which resulted as follows: (i) Doublesex and mab-3 related transcription factor (dmrt6 and dmrt11)- which produce the sterile male fish (ii) Forkhead box L2 (foxl2)- which produce the sterile female fish (iii) Steroidogenic factor-1 (sf-1)- lack of steroidogenesis and reproduction in fishes [38]. and (iv) Gonadal Soma Derived Factor (Gsdf)- which produce the sterile male fish [16].

Omega-3-production

In Atlantic salmon (*Salmo salar*) [7], Δ5&Δ6 desaturases and elovl2 were knocked out by CRISPR/Cas9. The elovl2- knocked out fish showed reduced levels of 22:6n-3 and accumulation of 20:5n-3 and docosapentaenoic acid (22:5n-3).

Disease resistance

In Rohu (*Labeo rohita*) [2], the TLR-22 gene was knocked out by CRISPR/Cas 9 genome editing system, to investigate about its role against pathogenic double stand RNA viruses, bacteria and parasites. In Prawn (*Exopalaemon carinicauda*) [36], CRISPR/Cas 9 was employed to knockout the carotenoid isomerooxygenase (EcNinaB-X1), which resulted in lower mortatlity of prawns, after bacterial challenge, when compared to wild type.

Pigmentation

The CRISPR genome editing system was employed in species like Yellow croaker (*Larimichthys crocea*) [24], Loach (*Paramisgurnus dabryanus*) [39], white crucian carp (*Carassius auratus cuvieri*) [26] and Atlantic salmon (*Salmo salar*) [9], to knocked out Tyrosine gene it results the low vertebrate coloration. Because the Tyrosine gene play a role in catalyzed the melanin biosynthesis in pigment cells and also determined vertebrate coloration. In Nile Tilapia (*Oreochromis niloticus*) [32], the slc45a2 gene was knocked out, which mediated melanin biosynthesis and produced an albino variant with red skin and eyes. In Chinese lamprey (*Lethenteron morii*) [45], the slc45a5 gene was knocked out, which resulted in the 'Golden phenotype' of the fish. Also, in Atlantic salmon (*Salmo salar*) [34], the slc45a2 gene was knocked out, which mediated melanin biosynthesis and produced graded range of phenotypes.

Conclusion

CRISPR/Cas approach is the most advanced gene editing technologies available now and thus the majority of the genetically engineered fish items for commercialization will be CRISPR/Cas products. The approach can deliver far-reaching answers to the numerous problems confronting the fish farming industry viz disease resistance, improved growth, reproduction rates and nutrient values, sterile breeding, and targeted therapy. For CRISPR/Cas to play a greater role in aquaculture as a new breeding tool, trait-related genes and signal pathways must be identified and functionally annotated. CRISPR genome editing products will be easier for consumers to accept than GMOs since CRISPR/Cas technology-derived items are labelled "genome edited" rather than "genetically modified," and this shift in nomenclature will save many negative reactions that occurred previously for GMOs. The world's first CRISPR/Cas genome-edited fish (red sea bream) are now available for purchase and have reached the customer in Japan. Other than that, the tiger puffer fish also approved for marketing in Japan.

Future uses and consequences for CRISPR-related research in aquaculture species are now being contested, often passionately, in a range of scientific and social settings. One thing is certain: we will continue to get a better understanding of the genomes and complicated physiological connections of many key aquatic species, allowing for more precisely targeted gene editing to optimize production characteristics. All with the potential to reduce genetic impacts on wild fisheries.

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