Volume 3, Number 3, 2026 Pages 1-25

Bioscience Insights

ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)

BiosciInsights.com

CRISPR-BASED FUNCTIONAL GENOMICS OF IMMUNE GENES UNDERLYING PATHOGEN RESISTANCE IN AQUACULTURE SPECIES

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https://doi.org/10.5281/zenodo.23414110

Article Information

Article History

Received:	June 19, 2026
Revised:	August 19, 2026
Accepted:	September 07, 2026
Available Online:	October 16, 2026

Keywords:
CRISPR-Cas9; Aquaculture; Immune-related genes; Pathogen resistance; Functional genomics; Genome editing.

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Abstract

Emerging infectious diseases are a major threat to aquatic animal health, productivity and sustainable food production in aquaculture. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR-Cas9) technology has now become a useful genome editing tool to study the functional role of immune-related genes in pathogen resistance. The recent advances in functional characterisation of immune genes in economically important fishes and crustacean's species through the CRISPR system are critically assessed here. There is an emphasis on gene knockout, targeted mutagenesis and functional validation approaches for the study of innate immune signaling, host-pathogen interactions and disease susceptibility. Toll-like receptor, Jak/Stat and NF-kB are important immune pathways that are studied in relation to antimicrobial responses and pathogen defence. While the application of CRISPR in the characterization of the genes involved in immune function holds great promise, there are several areas of concern that must be addressed, including off target mutations, mosaicism, the variable efficiency of genome editing, genome annotation and the establishment of reliable genotype–phenotype relationships. The transferring laboratory results to disease-proof variants of aquaculture is difficult for ecological safety, animal welfare, and regulatory approval and genetic stability. Integration of CRISPR and transcriptomic/proteomic analysis and with advanced pathogen challenge models may further improve functional validation and mechanism of action. To conclude, CRISPR technologies have tremendous potential in the field of aquaculture, particularly for disease resistance research, but their successful application in aquaculture development requires proper validation and responsible use.

INTRODUCTION

Aquaculture now plays a significant role in food production, job creation and economic prosperity throughout the world (Mair et al., 2023). The risk of infectious diseases due to bacterial, viral, fungal, and parasitic pathogens has risen due to the intensification of aquaculture systems (Gundi et al., 2025). These infections can impact growth, cause animal welfare issues, affect mortality rates, and cause significant economic losses. Conventional disease management tools such as vaccination, better husbandry, biosecurity and using antimicrobials are still required, but their efficacy depends on the disease, cultured species and farming situation. Therefore, elucidation of genetic and molecular basis for disease resistance is one of the key research targets for the sustainable development of aquaculture (Kappes et al., 2023).

Aquatic organisms immune system consists of several molecular pathways which are interconnected to recognize pathogens, control inflammation, and mediate antimicrobial defence (Ghani et al., 2024). The pattern-recognition receptors, Toll-like receptors (TLRs), RIG-I-like receptors, interferon-regulatory factors, cytokines and other signaling molecules are involved in innate immune recognition and antiviral responses in fish (Özcan and Arserim, 2026). The NF-κB and JAK/STAT pathways play additional roles in inflammatory regulation and communication in immune systems, but with species and infection specific functions. The innate immune mechanisms used by crustaceans, such as shrimp and prawns, include pattern recognition, antimicrobial peptides, prophenoloxidase system, and cellular response including phagocytosis (Patnaik et al., 2024). Determination of these pathways at a genetic level is very important to be able to identify immune regulators with a role in modulating susceptibility and resistance to pathogens (Choudhary and Senthil-Kumar, 2022).

Targeted modification of the genome with clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins (CRISPR-Cas) has broadened the possibilities to study the function of genes (Ghosh and Chatterjee, 2024). The development of CRISPR-Cas9 has greatly helped researchers to introduce mutations into specific regions of the genome and study the effects of mutations to gene disruption. This will be complemented by genetic typing of the molecule, gene-expression analysis, transcriptomics, and pathogen-challenge-controlled studies to determine causal relationships between genes and immune phenotypes. A validated loss-of-function experiment can give better evidence that a candidate gene is involved in a process of pathogen resistance/susceptibility or immune regulation and is not just a correlate of expression (Chen et al., 2023).

Recent studies have shown the utility of CRISPR-based functional studies to the field of fish immunity. For instance, Qu et al. found that the zebrafish FTR42 gene was sufficient to increase resistance to spring viremia of carp virus, and mechanistic evidence suggested that it does so by degradation of TBK1 protein and messenger RNA decay of IRF7 (Qu et al., 2024). On the other hand, gene disruption of tumor necrosis factor-alpha type I (TNFa-I) in zebrafish made the animals more susceptible to viral infection, demonstrating the critical role of each immune mediator in host defence. These results demonstrate how the outcomes of disease may be affected differently by immune genes, and in some cases in opposing ways. Thus, disease-resistance research should consider the biological impact of each genetic modification and not assume that the knock down of an immune associated gene will lead to increased resistance (Sofyantoro et al., 2024).

In spite of these progress, current functional genome application using the CRISPR tool in aquaculture immunity suffer from species-specific editing efficiency, mosaicism, unexpected genetic modifications, lack of full genome annotation and the lack of genotype to disease phenotype linkage (Robinson et al., 2023). The data from model animals like zebrafish cannot simply be transferred to commercially important animals, or to crustaceans, which have a very different immune organization and genome biology. Laboratory results should also be translated into a practical aquaculture breeding program, which involves assessing genetic stability, growth and reproductive performance, animal welfare, ecological risks, and regulatory requirements (Puthumana et al., 2024).

In this context the present review focuses on the critical evaluation of the CRISPR functional characterization of immune genes related to pathogen resistance in fish and shellfish species utilized in aquaculture. It reviews the evidence around gene knockout and targeted mutagenesis, explores the roles of the major innate immune signaling pathways and makes comparisons between functional results in fish and crustaceans. The review also includes consideration of how to validate the function of genes

using molecular, cellular, transcriptomic, proteomic and pathogen challenge analyses. Finally, it highlights key knowledge gaps and provides suggestions for the future on how genome editing can be combined with functional genomics for developing disease-resistance strategies in aquaculture that are scientifically supported and sustainable.

3. Literature Review

3.1. CRISPR-Based Functional Genomics in Aquaculture

CRISPR-Cas9 genome editing has become a key tool to study the genetic basis of disease resistance in aquatic animals. The identification of candidate immune genes by conventional methods such as transcriptomic profiling, gene-expression analysis and association studies does not always provide information as to whether a gene per se is directly involved in pathogen resistance (Zhu et al., 2024). CRISPR-mediated gene knockout can be used to study the effects of targeting a specific gene and to provide more conclusive evidence for a gene's biological role. Previously, researchers studying the use of CRISPR applications in fish disease control focused on the potential benefits of using it to study host–pathogen interactions and create novel methods for controlling infectious diseases (Bucheeri et al., 2025). More recently, Marnis and Syahputra discussed the growing potential of genome editing using CRISPR in fish disease research, highlighting its use in discovering disease associated genes and in understanding the molecular basis of disease susceptibility. The evidence is not consistent for all aquaculture species, and there are several potential applications that are still in the early stages of development from experimental models to disease-resistant populations.

An associated development is the combination of genome editing with more wide-ranging genetic-improvement programs. Studies on genome editing of edible fish have shown that disease resistance, growth, reproduction, and product quality are important targets for genome modification through CRISPR. But successful Editing is not necessarily better disease resistance (Puthumana et al., 2024). The phenotype depends on the biological function of the targeted gene, its interactions with other immune pathways and the nature of the pathogen. Another caveat is that in some cases, genes from another fish genome may be duplicated, making interpretation of their functions difficult, as one of the duplicate genes may compensate for the loss of the targeted gene. The need for species-specific functional studies, based on genetic confirmation, physiological and disease-related measurements is supported by these considerations (Qin et al., 2022).

3.2. Functional Characterization of Innate Immune Genes

Many bacterial and viral infections in fish are the first line of defense against which they have to fight is innate immunity. The pattern-recognition receptors, interferon-associated proteins, inflammatory cytokines, transcription factors and antimicrobial effectors are critical elements (Swain and Miryala, 2025). Microbial components are recognised by TLRs and other pathogen recognition receptors that trigger downstream signalling, NF-κB and JAK/STAT pathways are involved in inflammatory control and in cytokine mediated responses. These pathways are well known elements of vertebrate immunity, but the exact function of these pathways in resistance to pathogens may vary among aquatic species. Therefore, changes of gene expression should not be considered evidence that a gene provides resistance. More functional studies are required to determine if an immune gene helps clear pathogens, restricts tissue damage, or, in some cases, aids infection (Chen et al., 2025; Guo et al., 2024; Zhao et al., 2021).

One significant application of CRISPR for characterizing immune-genes was published by Qu et al in 2024. Their research showed that the loss of function in FTR42 in zebrafish increased the survival rate after infection with spring viraemia of carp virus. Mechanistic studies revealed that FTR42 down-regulated interferon immunity by two mechanisms, namely, degradation of TBK1 protein and degradation of the IRF7 mRNA. The results demonstrate the ability to enhance antiviral responses by counteracting a negative regulator of innate immunity. Importantly, this work demonstrates not only that there is a correlation between the amount of a specific gene and the severity of infection, but that this correlation is actually causal, with a specific gene causing a specific infection. The conclusions drawn from the results should not be extrapolated to other fish species or other viral pathogens as the biological effects of the

regulation of interferon may vary depending on the host–pathogen relationship (Guo et al., 2023; Wang et al., 2024).

There have been other studies that have investigated the importance of cytokines in antiviral defenses. Increased susceptibility to viral infection was found when a study in 2025 looked at disrupting tumour necrosis factor alpha type I using the CRISPR/Cas9 system in zebrafish. This is significant because it reveals that the removal of an immune-related gene can have a negative effect on pathogen resistance, and not a positive one. All of this confirms an important rule of immune-gene engineering: the direction of the effect is determined by the role of the gene in immune network. Also, potential targets for boosting certain protective immune responses could be negative regulators while essential protective mediators could need to stay intact. Thus, selection of a gene for targeting should be based on the mechanism of action and then evaluated for pathogen control and possible immune dysregulation(Shanaka et al., 2025).

3.3. Antimicrobial Peptides and Genetic Disease Resistance

AMPs play pivotal role in innate immune defense as they have a role to inhibit microorganisms and help in regulation of host immune responses. Wang et al. discussed the research on antimicrobial peptides engineering and gene editing technologies to enhance disease resistance of fish. They analysed 544 data points from 23 studies, including research on 12 diseases, and found global links between genetic engineering of antimicrobial peptides, a reduction in the amount of bacteria present, a better survival rate following infection, and changes in expression of immune-related genes (Wang et al., 2023). The reviewed evidence, however, comprised various engineering strategies, including transgenic expression and CRISPR associated methods. These approaches should not be confused with each other: induction and over-expression of an antimicrobial peptide gene differs from the deletion of an endogenous gene of the immune system. Furthermore, the effects reported were different depending on fish species, pathogen and antimicrobial peptide (AMP) used. This evidence highlights the possibility of genetic based disease management and the need for careful distinction between genetic experiments involving genome editing versus transgenic and other genetic-engineering studies.

There is also a wider literature that suggests genetic changes might impact a pathogen in different ways. Genetic changes that confer resistance against one bacterial or viral pathogen might not confer the same resistance to another, or boosting immune activation may cause trade-offs in other contexts. Thus, the interpretation of studies on antimicrobial peptides should be made together with the evidence from targeted knockout experiments, pathogen challenge and host health measurement. Future studies would need to explore whether the protection that has been observed is reproducible in a germ-free environment over more relevant disease models and whether it is associated with good growth, development, reproductive performance and welfare.

3.4. Evidence from Crustaceans and Major Research Gaps

Shrimp and prawns are economically important crustacean species in aquaculture and are highly reliant on innate immunity as an effective defense mechanism against pathogens. They have antimicrobial peptides, pattern recognition molecules, the prophenoloxidase system and hemocyte-mediated cellular defenses as parts of their immune system (Kulkarni et al., 2021). While CRISPR approaches have many potentials uses in studying these mechanisms, there is less direct functional evidence for their use in crustaceans and less evenly distributed among species than is reported elsewhere in the genome editing literature in fish. Another aspect covered in the fish disease management review was the use of RNA-targeting approaches, as well as the potential for using CRISPR technologies for aquatic pathogens. But, attacking pathogen nucleic acids is conceptually different from editing host immune genes and needs to be considered as a different research route (Ferdous et al., 2022).

An important constraint is the lack of transferability of work performed in model organisms to commercially reared fish and crustaceans. There may be variation in the efficiency of editing due to differences in genome organization, gene duplication, reproductive biology, embryo manipulation, and immune-system architecture, which can impact the interpretation of the phenotype. Other problems are mosaicism in modified individuals, unintended changes in the genome, an incomplete genome annotation and the challenge of maintaining stable and beneficial traits across generations. The problems

pointed to the need to validate specific genetic changes at the level of the DNA, to determine the impact on the function of the genes and on the function of the proteins, and to assess the effect of the pathogen through appropriate controls (Lin et al., 2025).

The literature shows that, overall, CRISPR functional genomics can provide insights into the function of the select genes in aquatic immune defence, but does not support the assumption that all modifications of immune genes will enhance disease resistance. There are several research gaps to be addressed in comparative studies between fish and crustaceans, validation under the appropriate pathogen challenge conditions, incorporation of multi-omics data, and evaluation of long-term consequences on the biology. This review will therefore be focused on the difference between direct and indirect evidence from CRISPR, comparing immune-gene modification and resistance and susceptibility, and identifying methods that may enhance the reliability and applicability of future disease-resistance studies in aquaculture.

4. CRISPR Technologies in Aquaculture

CRISPR genome editing has proved to be a powerful molecular tool in understanding gene function and understanding the resistance mechanism in aquaculture species. Using a guide RNA, the CRISPR-Cas9 system guides the Cas9 nuclease to a specific sequence in the genome of the target cell, where DNA is cut through cellular DNA repair mechanisms, causing insertions or deletions. These mutations could affect the way in which the genes are working, enabling scientists to explore the function of immune genes in the human body. CRISPR-Cas9 can be used in the functional genomics, host–pathogen interaction studies, and detection of genetic determinants in bacterial and viral resistance in aquatic organisms (Gutási et al., 2023; Zhu et al., 2024).

There are multiple genome editing approaches in aquaculture research, such as gene knockout, targeted mutagenesis, and sequence editing. One of the most important applications of gene knockout is to determine the ability of a given immune regulator gene to confer resistance to a pathogen or susceptibility to infection. CRISPR-mediated disruption of FTR42 in zebrafish improved resistance to spring viraemia of carp virus, for instance, showing the potential of targeted genome editing to investigate antiviral immunity (Qu et al., 2024). CRISPR can also be integrated with transcriptomic, proteomic and molecular data to pinpoint changes downstream of genetic modification and to elucidate immune response mechanisms. Efficient editing, reliable genotyping and validation of the resulting biological phenotype, however, are required for successful application (Quyoom et al., 2026; Wang et al., 2025).

There are several hurdles to overcome in the aquaculture application of CRISPR technologies: off-target mutations, mosaicism, editing efficiency variability, incomplete genome annotation, and potential for unwanted effects on growth, reproduction, and other physiological functions. Standardized approaches to editing are further complicated by the differences in reproductive biology and genome organization between fish and crustaceans. Thus, molecular validation of CRISPR findings, pathogen challenge experiments, and animal welfare and ecological safety studies are required before it could be considered for use in commercial aquaculture.

5. Immune Genes and Pathogen Resistance

Pathogen recognition, immune signaling, inflammatory regulation, and antimicrobial defense are some of the key functions of immune-related genes in aquaculture species. In fishes, the pattern-recognition receptors such as Toll-like receptors (TLRs) and RIG-I-like receptors recognize microbial components and initiate downstream signaling pathways which play roles in the clearance of pathogens. The NF-κB pathway is involved in the regulation of inflammatory responses, while the JAK/STAT pathway is involved in cytokine signaling and immune regulation. Moreover, interferon related genes, like IRF7, play a role in antiviral defence by controlling interferon stimulated genes. The functional annotation of these genes could provide insights into the responses of aquatic organisms to viral and bacterial infections and inter-specific variation in susceptibility (Saleh et al., 2024).(Swain and Miryala, 2025)

Table 1. Immune-related genes and their potential roles in pathogen resistance in aquaculture species

Immune gene/pathway	Main biological function	Relevance to pathogen resistance	Research application
FTR42	Regulates interferon-associated immunity	Its disruption enhanced resistance to spring viraemia of carp virus in zebrafish	CRISPR-mediated functional characterization
TNF-α	Regulates inflammation and immune responses	Supports host defence against viral infection in zebrafish	Cytokine-function investigation
TLR family	Recognizes pathogen-associated molecular patterns	Initiates innate immune signaling against microbial pathogens	Host–pathogen interaction studies
IRF7	Regulates interferon-related gene expression	Contributes to antiviral immune responses	Analysis of interferon signaling
STAT family	Mediates cytokine-dependent signaling	Participates in immune regulation and antimicrobial defence	Investigation of JAK/STAT signaling
Antimicrobial peptide genes	Encode peptides with antimicrobial activity	Can contribute to defence against bacterial and other pathogens	Functional analysis of antimicrobial mechanisms

The table summarizes established or proposed biological roles. Direct CRISPR evidence is specifically indicated for FTR42; the remaining entries should not be interpreted as proof of CRISPR-based validation in aquaculture species.

CRISPR experiments have shown that different individual immune regulators can have significantly different effects on pathogen resistance. Qu et al (2024) found that genetic disruption of FTR42 in zebrafish by CRISPR strengthened interferon-mediated immunity and increased resistance to SVC. By contrast, a 2025 study found increased susceptibility to viruses in zebrafish with disruption of tumor necrosis factor alpha type I, with this inflammatory mediator acting as a protective agent. These results show that immune-gene modification does not necessarily enhance disease resistance and that it requires consideration of the biological function of the target gene, as well as the interactions with other immune pathways (Gundi et al., 2025; Quyoom et al., 2026).

Shrimp/prawns rely on the innate immune system in the crustacea, which includes antimicrobial peptides, pattern recognition proteins, hemocytes and prophenoloxidase system. Understanding genes involved in these processes could lead to understanding molecular factors of bacterial and viral pathogens. The functional evidence using direct CRISPR, however, is not consistent among crustaceans and results obtained in fish do not necessarily apply to them(Puthumana et al., 2024). Thus, future study should integrate targeted genome editing and molecular validation, transcriptomic and proteomic studies, and pathogen-challenge experiments. This integrated approach can deepen genotype–phenotype relationships, and uncover novel genes in the immune system that can drive disease resistance while simultaneously maintaining growth, reproduction, and animal welfare.

6. Applications of CRISPR Technologies in Fish and Crustaceans

There is significant potential for the use of CRISPR based genome editing technology in investigating immune mechanisms and pathogen resistance in economically important fish species. CRISPR-Cas9 can be applied to disrupt candidate immune genes in fish such as zebrafish, common carp, and other cultured species to test the role of these genes in the susceptibility to bacterial and viral infections. Zebrafish is especially valuable for the study of innate immune signalling and host–pathogen interactions. Qu et al. (2024) have shown that disruption of FTR42 could improve the resistance of zebrafish to spring viraemia of carp by inducing interferon-associated immunity. These results form the basis for pursuing similar studies on immune regulators in economically important fish. However, specific validation is required as

there are inter-species differences in genome organization, immune responses, and pathogen biology that can affect the results of the genetic modification(Qu et al., 2024; Robinson et al., 2023).

CRISPR technologies can be used to explore the function of genes in antimicrobial defence, pathogen recognition, hemocyte activity and immune signalling in crustaceans, such as shrimp and prawns. These mechanisms will be relevant for the understanding of susceptibility to the main aquaculture diseases, such as white spot disease (by white spot syndrome virus) and bacterial infections (due to Vibrio species). The functional characterization of candidate genes could provide insight into the effects of crustacean immune responses on pathogen replication, survival and disease development (Patnaik et al., 2024; Saleh et al., 2024). While the use of CRISPR in crustaceans presents other challenges, such as having more complex genome organization in some species, efficient delivery of genome-editing components, mosaicism, and the challenge in creating stable edited lines. Thus, results of gene expression or RNA interference experiments should not be claimed as direct evidence of CRISPR.

In general, applications of CRISPR for fish and crustaceans should be considered based on the strength of the experimental evidence, with a clear distinction between gene modification (direct) and functional studies (indirect) or proposed future applications. Combining genome editing, molecular genotyping, transcriptomics, proteomics and pathogen-challenge experiments would be beneficial for understanding immune-gene function. Validated findings may be used to create a breeding program and other disease-management measures in the longer-term. Additional testing and evaluation of genetic stability, growth, reproduction, animal welfare, ecological risk and regulatory issues are required prior to commercial application (Ferdous et al., 2022).

7. Functional Validation and Multi-Omics Integration

To determine if CRISPR genetic modifications have a meaningful impact on immune response and disease resistance in aquaculture species, functional validation is important. After genome editing, the gene selected for editing can be confirmed as being edited and as having the required mutation by using molecular methods like PCR, DNA sequencing, and targeted genotyping. But just getting it edited doesn't show a working effect on immunity(Swain and Miryala, 2025). Thus, the genetic analysis must be performed in conjunction with measurements of gene-expression, protein-level, immune-response, and controlled pathogen-challenge. Relevant outcomes can be pathogen load, pathogen survival, inflammatory responses, tissue damage, and expression of immune related genes. Genuine gene-specific effects should be differentiated from unintended mutations and from experimental variation, which can only be done with appropriate controls and independent validations (Lin et al., 2025; Zhu et al., 2024).

Integrating CRISPR technology with multi-omics approaches can provide a more comprehensive understanding of the molecular mechanisms underlying disease resistance. Transcriptomics can be used to determine changes in gene-expression networks after targeted gene disruption, while proteomics can help determine alterations in immune-related proteins and signaling pathways. Metabolomics can also be used to analyse changes in biochemical processes caused by infection, inflammation and cellular stress. Integrating these data could be useful for detecting downstream pathways, compensatory mechanisms and possible biomarkers linked to resistant or susceptible phenotypes. For instance, comparing the transcriptomes or proteomes of edited and non-edited fish after viral challenge may be useful to find out if the disruption of an immune regulator increases antiviral signaling or if it has an unintended inflammatory effect. These datasets can then be integrated with bioinformatic approaches for identification of candidate genes and molecular networks for further experimental validation (Kulkarni et al., 2021).

Although the integration of multi-omics has potential, it is not without its difficulties: high experimental costs, complex data analysis, differences in sampling conditions, and challenges in establishing causal relationships. Variations in gene expression or protein levels may be secondary effects and not direct effects of the edited gene (Robinson et al., 2023). As such, specific molecular experiments must be performed to validate the computational results and disease-related phenotypes must be reproducible. The experimental designs and statistical analysis must be standardized between species and pathogens for comparisons to be made. Combining CRISPR-based functional genomics with multi-omics techniques could provide a powerful combination of evidence testing immunity associations of specific genes with

pathogen resistance, and inform the development of scientifically sound strategies for managing disease in aquaculture.

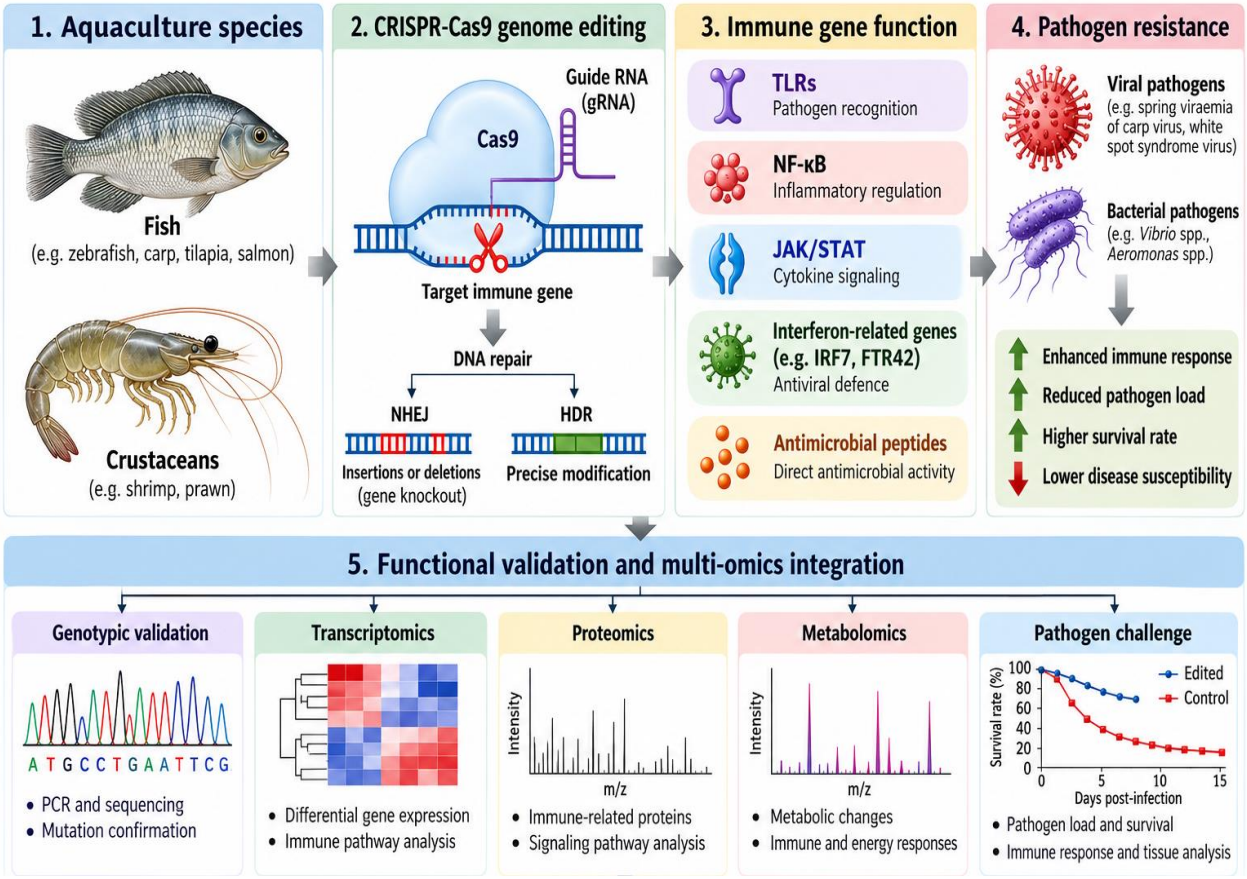


Figure 1. Schematic representation of CRISPR-Cas9-mediated functional characterization of immune genes associated with pathogen resistance in aquaculture species

8. Limitations, Biosafety, and Future Directions

Although the power of the CRISPR/Cas system for genome editing has opened the possibility of functional genomics in aquaculture, there are several technical and biological challenges that need to be resolved. A primary issue is the presence of off-target mutations where mutations occur at unintended locations in the genome. Another issue is that of mosaicism, especially in early-generation edited organisms, where various genetic edits can occur in individual cells, further hindering genotypic and phenotypic interpretations. Moreover, the efficiency of editing, the lack of genome annotation, gene duplication and compensatory mechanisms may impact the functional characterization inaccuracy. An unplanned physiological effect can also be seen when a gene involved in an immune system disorder is disrupted; growth, reproduction, development, or susceptibility to other disease agents can all be affected. Thus, molecular confirmation of genome editing, controls and reproducible functional analyses are necessary before reaching a conclusion about the role of a gene in disease resistance.

Table 2. Applications, limitations, and validation strategies for CRISPR technologies in aquaculture

Application	Potential benefit	Major limitation	Recommended validation
Immune-gene knockout	Identifies genes involved in disease resistance or susceptibility	Compensatory gene functions	Genotyping and functional assays

Targeted mutagenesis	Investigates specific genetic variants	Unintended mutations	DNA sequencing and off-target assessment
Antiviral immunity research	Clarifies interferon and antiviral signaling mechanisms	Pathogen-specific effects	Controlled viral challenge and pathogen-load analysis
Antibacterial defence research	Identifies immune regulators involved in bacterial responses	Variable effects across pathogens	Bacterial burden and survival measurements
Transcriptomic integration	Identifies altered immune pathways	Expression changes do not establish causality	Differential-expression and pathway analyses
Proteomic integration	Reveals changes in immune-related proteins	Complex data interpretation	Protein-level confirmation and pathway validation
Disease-resistant aquaculture development	May inform genetic improvement strategies	Ecological, welfare, and regulatory concerns	Multigenerational assessment and biosafety evaluation

Another point to consider when assessing the use of CRISPR in aquaculture is biosafety. Potential for interbreeding, competition, or alteration of ecological interactions with the environment could lead to environmental release of edited aquatic organisms affecting wild populations (Gundi et al., 2025). Genetic changes that confer pathogen resistance can also affect susceptibility to other pathogens or interactions between the host and the microbiome. In this context, genetic stability over generations, animal welfare, ecological effects and the possibility of unforeseen effects should be taken into account for biosafety assessment. For the development and/or assessment of genome-edited aquatic organisms, measures are needed to ensure containment, environmental risk assessment, and compliance with national and international rules and regulations. With regard to responsible use of CRISPR, there should be a balance between the potential benefits for aquaculture productivity and risks to biodiversity and ecosystem health (Robinson et al., 2023).

Further studies should focus on increasing the accuracy of editing strategies, increasing genome annotation, and increasing reliability of genotype–phenotype correlations. Combining CRISPR with transcriptomics and proteomics and other functional-genomics tools may help to better understand the molecular networks in pathogen resistance. There is also a need to pay more attention to commercially important fish and crustaceans, since the results obtained from model organisms do not always translate to farmed fish. To determine if beneficial immune phenotypes are stable over time, without impacting growth, reproduction or other pathogen responses, long-term studies should be carried out. In conclusion, the successful integration of robust experimental validation, clear biosafety evaluation, and responsible regulatory oversight will be critical to bringing CRISPR-inspired innovations into the realm of aquaculture applications that are viable and sustainable.

Conclusion:

Genome Editing in Aquaculture using CRISPR has great potential to explore immune genes that exhibit resistance to pathogens. This will allow the functional elucidation of immune signaling pathways in host pathogen systems, as well as targeted gene modification. But issues like off-target mutations, mosaicism and variable editing efficiency need to be taken into account. The combination of CRISPR, transcriptomics, and proteomics and functional validation can enhance the understanding of disease-resistance mechanisms. Researches to be conducted in the future should focus on species specific research, genetic stability, animal welfare and ecological safety. With proper scientific validation and responsible application, CRISPR technology can be a valuable tool for sustainable aquaculture.

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