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Anatomization of fish immune system and enforcement of its vaccination

AUTHORS DETAIL

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Abstract

Immunological responses at the cell level comprise both adaptive and innate mechanisms, involving every subset of leukocytes. Vital processes include cell-mediated cytotoxicity and phagocytosis. Fish immune system is mostly composed of macrophages, cytotoxic T cells dendritic cells, granulocytes, and NK cells. Management of fish health has emerged as a crucial element of disease prevention, with significant benefits to enhanced harvests and aquaculture that is sustainable. Vaccination is widely regarded as the most efficient preventative strategy for the prevention of fish disease, with economic, environmental and social implications. Although Louis Pasteur's "separate, inactivate, and inject" method served as the foundation for the traditional method used to generating fish vaccines, their poor immunogenicity and effectiveness in many situations have caused the emphasis th development of fish vaccine to shift from conventional to modern methods.

Keywords: Fish, vaccine, Adaptive immune system, Innate immune system, Lymphocytes

1. Introduction

Fish make about half of all vertebrate species, making them the most prevalent group on Earth. Because of its excellent digestion and delicious flavor, fish is frequently a mainstay of the human diet. There are about 33,000 fish species exist in the globe. Out of the 531 fish species are exist in Pakistan, 233 are freshwater while the other 298 are marine (Ghouri et al., 2020). Fish can experience stress due to environmental conditions in their native environment, such as low oxygen levels, high stocking densities, and poor water quality, which increases their susceptibility to infectious diseases. With over 10% of all cultivated fish dying each year from infectious diseases, the aquaculture sector still faces a serious problem even if good management techniques and preventative therapies greatly reduce disease susceptibility. Numerous agents are accountable for disease outbreaks in fish farms; of the documented instances, mycotic agents for 3.1%, parasites for 19.4%, viruses for 22.6% and bacterial pathogens accounted for 54.9% (Bjorgen et al., 2022).

2. Immune System of fish

Main purpose of the immune system is to detect and get rid of foreign objects. The adaptive and innate immune systems are its two types (smith et al., 2019).

2.1. Adaptive immune system

Adaptive immunity is a system in which the organism creates immunological memory against the disease by recombination and genetic mutations, even if both subsystems work to protect the body. The immune systems of fish, the earliest jawed vertebrate group to emerge in evolution, are comparable to those of mammals. With the spleen and lymphoid tissue linked with the mucosa as secondary immune organs and the skull, kidney, and thymus as primary immune organs, these early animals have fully developed adaptive and innate immune systems (Mokhtar et al., 2023).

Adaptive immunity, which is aided by memory cells, T-cell receptors, and immunoglobulins, plays a special role in protecting against recurrent infections and eliminating fish pollutants. Fish immune systems heavily rely on epidermal secretions, such as lysozymes, lectins, complement, and C-reactive proteins, which are involved in antigen disruption, phagocytosis, inflammation, and tissue repair (Smith et al., 20219).

2.2. Innate immune system

In consequence to infections, the elements of innate immunity identify pathogen-associated molecular patterns (PAMPs) which is not expressed in host cells. The innate immune receptors identify a number of PAMPs, including phospholipomannan, beta-glucan, chitin, hemagglutinin, lipopolysaccharides (LPS), and lipoteichoic acid (LTA). One of the functional processes of innate immunity, phagocytosis, destroys the pathogens after they have been recognized (Swamy et al., 2024).

2.2.1. Component of innate immune system

Cellular, physical and humoral elements are the three broad categories into which fish's innate immune system components fall (Mokhtar et al., 2023).

2.2.1.1. The physical factors:

The mucus layer, scales and epithelial cells covering the skin, gills, and alimentary canal are examples of physical or surface characteristics that play a crucial part in infection resistance. Additionally, the epidermis's epithelial cells, which are home to lymphocytes, eosinophilic granular cells, and macrophages, block the passage of foreign substances. Maintaining the osmotic equilibrium and getting rid of microbes depend heavily on the quality of these cells (Mokhtar et al., 2023).

2.2.1.2. Humoral immune parameters:

Different soluble chemicals known as humoral immune parameters serve preventive purposes by neutralize the enzymes that the pathogen depends on and preventing the growth of the bacteria. The proliferation of bacteria depends on transferrin, a protein in the acute phase that activates in an inflammatory process to extract iron from damaged tissue. Additionally, it activates fish macrophages. Complement, lysozyme, and antimicrobial peptides are examples of lysins found in fish. Bacterial lysis is caused by lysozyme's action upon the peptidoglycan layer on the cell walls of bacteria. (Saurabh et al.,2008).

2.2.1.3. Cellular part of fish innate immune system

There are various types of cells that make up fish innate immune system. Depending on their cell subtype, these immune cells can participate in a variety of responses after being activated when they identify a pathogen through its PAMP. Natural killer cells, granular leucocytes, non-specific cytotoxic cells, mast cells, dendritic cells, lymphocytes, monocytes, eosinophilic granule cells, thrombocytes and macrophages are examples of immune cells found in teleost. Furthermore, fish have Melano macrophage centers and rodlet cells (Firdaus-Nawi et al., 2016).

i. Natural killer (NK) cell

Teleost fish have two main types of NK cell homologs: nonspecific NK-like cells and cytotoxic cells (NCCs). The NCCs was the first identified and most extensively studied killer cell group in teleost. NCCs perform activities similar to those of higher vertebrates, operating on a variety of target cells, such as tumor cells. NCCs also play a role in antimicrobial immunity through stimulating the synthesis and release of cytokines (Mokhtar et al., 2023).

ii. Macrophages

Macrophages play an important part in specific immunological responses because they activate lymphocytes and perform phagocytosis. Macrophages have receptors that recognize β-glucan, allowing immune stimulants to increase leukocytes' respiratory burst and create highly reactive oxygen species having antibiotic properties. In the distal intestine, by acting as antigen-presenting cells, macrophages enable the adaptive immune system to recognize antigens. Several receptors are present on the cell surface of macrophages such as TLRs, CLRs and, PRRs, as well as enhance or killer receptors. Macrophages are also a significant source of chemokines and cytokines, which link adaptive and innate immunity and facilitate effective immune response (Zhang et al., 2024).

iii. Neutrophils

Neutrophils include polymorph nuclear cells found in the bloodstream, the lymphoid organs and peritoneal cavity that may phagocytose cells and foreign particles while also producing a bactericidal, superoxide anions substance. In the immune system's inflammatory response to bacterial, fungal, viral, and protozoan infections, neutrophils are crucial. Macrophages and neutrophils are the two main granulocytes present at the place of injury, and both are impacted by chemotactic chemicals generated by damaged tissue (Sinha et al., 2024).

Iv. Eosinophils, Basophils, Thrombocytes, and Monocytes

Eosinophils are well dispersed cells in connective tissue, especially in the ovaries, circulation, gastrointestinal system and gills, and they provide degranulation just when parasites are found.

Granulocyte are infrequently found in teleost species. Their granules in the cytoplasm contain histamine, an inflammatory mediator. That kind of cells take part in anaphylaxis and allergies. Thrombocytes are agranular, nucleated, and oval-shaped cells. That are capable of both clotting and phagocytosis. Thrombocytes exhibit acid phosphatase activity, causing cell to congregate near the inflammatory site (Wlasow et al., 2019).

v. Dendritic Cells (DCs)

Dendritic cells are antigen-presenting cells present in many organs and activate immune responses that are both acquired and innate. DCs are distinguished by tiny cell bodies having numerous cytoplasmic procedures (dendrites) and were discovered in the skin, gills, ovaries, and gut. Many fish species have populations of cells exhibiting DC-like functions and structure. These cells convey processing antigens to T lymphocytes via MHC class 2 receptors, resulting in a cell-mediated acquired immune response (Firdaus-Nawi et al., 2016).

vi. Lymphocytes

B lymphocytes, also known as efficient antigen-presenting cells, mediate humoral immunity and manufacture antigen-specific antibodies. They additionally create immunological memory through transforming in memory B cells (MBCs). The fish's head kidney is an important site for the creation and growth of mature naïve B lymphocytes. B cells produce immunoglobulins into the plasma, forming antigen-binding complex (Mokhtar et al., 2023).

vii. Mast Cells

Most teleost species have mast cells, which are found near blood arteries in their ovaries, intestine, gills, and skin. The mast cell granules display CD117, S100, and desmin. Different stressors, including pesticide and heavy metal exposure, chronic inflammation, and parasite infections, increase the amount of mast cells in teleost's tissues and organs. Teleost fish also shown release of cytokines, mast cell degranulation, and an inflammatory response following bacterial infection. Mast cells, are the most common granulocytes found in all vertebrate classes, contain piscidins (Uribe et al., 2011).

Viii. Eosinophilic Granule Cells (EGCs)

EGCs are common in several species, including salmonids and cyprinids. EGCs are commonly found in the gills, skin's epidermis, connective tissue around intestinal mucosa and the spinal cord. EGCs can be observed within the connective tissue of stroma that surrounds a bile duct. Because they contain basic proteins, they have spherical granules in the cytoplasm that are brightly marked red with eosin and hematoxylin (HE) (Mokhtar et al., 2023).

ix. Rodlet Cells

Fish contain rodlet cells that are differentiated through thicker capsule-like cell walls and rodlet cytoplasmic inclusion. The rodlet cells in grass carp head kidney exhibit α-SMA. An actin cytoskeleton is present in rodlets cells that help them contract and discharge their material. In the process of expulsion, they undergo a one-way contraction which ultimately damage the cell, resulting in the evacuation of the rodlets and its contents. Rodlet cells commonly found in the kidney, heart, spleen, thymus, gills, liver, skin, pancreas gall bladder, and blood vessel endothelium (Mokhtar et al., 2023).

2.2.4. Mucosal Barriers

Fish may contact with the environment via mucosal barriers and maintaining homeostasis. Mucosal barriers are highly complex tissues have unique adaptations that improve wide range of physiological activities, include their immunity, nutrition, and exchange of gases. Teleosts have four main mucosa-associated lymphoid tissues (MALTs): gill-associated lymphoid tissue (GIALT), gut-associated lymphoid tissue (GALT), and skin-associated lymphoid tissue (SALT). These components of mucosal barriers act like the immune system of host, defending it against infections and several other threats (Ashfaq et al., 2019).

2.2.4.1. Fish Mucus

Several kinds of secretory cells create fish mucus, most common of which being sacciform cells, club cells, and goblet cells (GCs). Goblet cells make mucus granules and contain glycoproteins, which are found at all exterior surfaces, including gills. The first line of defense for disease attack is mucus of the fish. Mucins are the most abundant molecules in mucus which are responsible for its viscosity, physical protection of the skin's surface, pathogen trapping, and signaling on the cell surface. Muccins assist the innate immune system in two key methods. First, they hinder pathogen adherence, persistent colonization of pathogenic bacteria, and parasite invasion by being continually created and continuously shed.

Second, they have a variety of innate immunity-related enzymes and proteins, such as lectins, esterases, transferring, C-reactive protein (CRP), lysozyme, alkaline phosphatase (ALP), antimicrobial peptides (AMPs), complement proteins, immunoglobulins, and proteases, as well as different other peptides and antibacterial proteins, which are responsible of deterioration, inactivation, and control of infection. Fish mucus extracts are increasingly famous for having a large range of biological traits, such as antiparasitic, antibacterial, antiviral, and antifungal properties, that can be used in both fish husbandry and human medicine (Ashfaq et al., 2019).

2.2.4.2 The intestinal mucosa

Leukocytes are found in lamina propria of teleost's intestinal mucosa, which serves as a saturating substrate for them. Proliferating granulocytes, NK cells, and lysozyme are found in these strata, and their combined presence stimulates the synthesis of super oxides and lysozyme. Additionally, the epithelial cells serve as a secondary barrier, allowing appropriate signaling permeability, nutrient flows, and promoting gut microbiota to enhance digestion (Zhang et al., 2024).

2.2.5 Mechanisms of innate defense

Epithelial barriers are the first line of defense consists of structures which provide stable chemical or physical barriers to kill microbes. These barriers include epithelial surfaces like skin, gut, and the gills. It is critical for the fish to retain firmness of covering epithelia, which are essential for osmoregulation and defense. As a result, fish recover wounds astonishingly quickly. Goblet cells secrete a mucus layer that covers normal epithelial cells.

Mucus' primary purpose is to inhibit bacteria, viruses, fungi, and parasites, from attaching with epithelial surfaces. Furthermore, mucus possesses antibacterial properties. The genes encoding antimicrobial peptides, like pleurocidin, were cloned with the winter struggle (Pleuronestes americanus Walbaum); peptide exists in the gut and skin. The gene starts to express on 13 days post-hatch, indicating that this component have a significant function in initial days of fish when acquired immunity develops (smith et al., 2019).

Lectins

Lectins in fish identified as either organic agglutinins or precipitins. They often cross-linking carbohydrates moieties found on outermost layer of bacteria or xenogeneic erythrocytes. They are likely essential for neutralizing immobilizing germs, or bacterial components (e.g. exotoxins) and will promote phagocytosis.
Lectins in fish are structurally unrelated to Ig, although they are similar to invertebrate or plant agglutinins. Lectins in fish have been identified in rainbow trout (Oncorhynchus mykiss) serum, coho salmon (Oncorhynchus kisutch) eggs, and ayu mucous (Plecoglossus altivelis).

C-reactive protein

C-reactive protein (CRP) is a serum part in teleost fish which increases fast in response to experimental infection or bacterial endotoxins with bacterial pathogens. Bacterial cell surface polysaccharide structures react with CRP. It contains capabilities like lectin and can function as opsonin to improve phagocytosis or initiate the complement cascade after attaching with bacterium Vibrio anguillarum (uribe et al., 2011).

Lysozyme

Lysozyme is present in fish eggs, mucus, and serum and can break down the peptidoglycan layer that is part of bacterial cell walls. Macrophages and neutrophilic granulocytes produce lysozyme, which is bactericidal even against dangerous pathogens like Aeromonas salmonicida and Aeromonas hydrophila (Bjorgen et al., 2022).

Interferon

Interferon (IFN) is a cytokine secreted by several cells during response to pathogenic infections. It boosts host cell resistance of many viruses through promoting the production of proteins which impede viral mRNA translation. In teleost, interferon is species-specific; for example, rainbow trout-produced IFN fails to defend cyprinid cells in vitro. During a viral infection, in vivo IFN synthesis peaks after 2-3 days and typically occurs before the virus-neutralizing effects of moving antibodies that manifest one or two week later. Today, interferon activity has observe in several types of fish, including halibut, rainbow trout and Atlantic salmon.

Inflammation

This is an immediate response to tissue damage, typically occur by germs. The onset of inflammation is exceedingly complicated and multifaceted. Different soluble factors (complement system clotting system, and kinin system) and cells (macrophages thrombocytes, and granulocytes) contribute to this process. The procedure is characterized by local vasodilation and an influx of macrophages, monocytes, and granulocytes. A huge inflow of cells provides some preservation by 'screening off' the contaminated part from the remaining parts of the body. In fish, histopathological investigations show inflammatory responses to protozoan, viral, metazoan, fungal, and bacterial, parasite illnesses. Acute inflammatory responses of bony fish are same as mammals. In rainbow trout, granulocyte infiltration appears twelve to twenty-four hours following injection with microorganisms or Freund's complete adjuvant. The number of invading cells (macrophages and granulocytes) increases till 2 to 4 days. Macrophages are encouraged to produce eicosanoids and interleukin-1 (IL-1), which attract and activate different leukocytes, including lymphocytes. These events demonstrate how fish acquired and innate immune systems interact (swamy et al., 2024).

Phagocytic cells

Phagocytic cell of fish is primarily composed of macrophages and neutrophilic granulocytes. These cells use 'pattern recognition receptors' to recognize evolutionarily conserved epitopes found on bacteria. Fish have been characterized as having many forms of PRRs, including Toll-like receptors. When stimulated by PRRs, these cells phagocytose antigenic material or exhibit cytotoxic activity. The elimination of extracellular or intracellular infections is predicated on the generation of various nitric oxide (NO) and oxygen radical species.

Lymphoid Cells and Organs

Lymphocytes play an important role in acquired immune response as they show T-cell receptor (TCR) and Ig molecules, which serve like antigen-specific recognition units. Lymphocyte heterogeneity (B and T-cells) in fish has proven using hapten carrier investigations, the use of monoclonal antibodies, and functional cell cooperation tests. The lymphoid organs found in cartilaginous fish include the spleen thymus, epigonal organ, Leydig organ, and kidney. There is also evidence of considerable gut associated tissue among these animals.

The skin and gills also contain a large number of leucocytes, indicating that fish have a well- developed mucosal immune system. According to most observations, the spleen of bony fish is a secondary lymphoid and erythropoietic organ, while thymus is primary lymphoid organ that primarily functions in T-cell development. The kidney is likely similar to mammalian bone marrow. Therefore, it may operate as a primary organ (B-lymphocyte differentiation, blood-cell production) as well as a secondary organ (plasma-cell and memory-cell development) (Salinas et al., 2022).

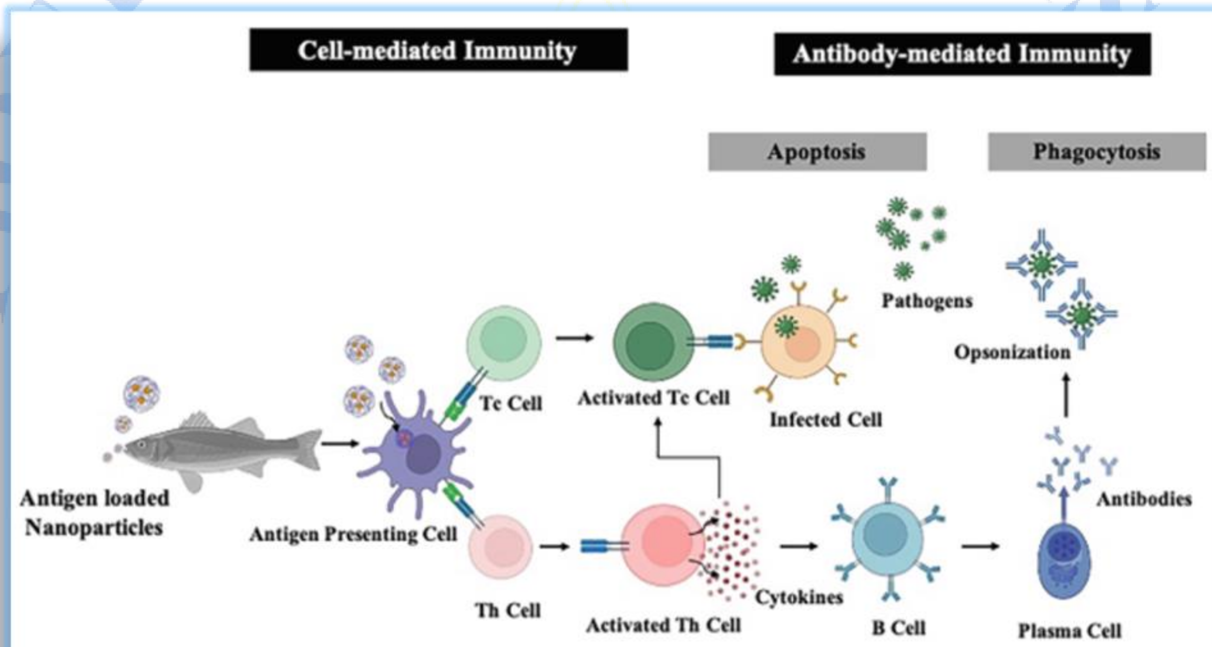

Figure 1. Fundamentals of fish immunity

Fish vaccine

"Vaccine is a preparation of antigen produced by pathogenic organisms, provide non-pathogenic from different methods, which activate the immune system to increase the ability of resistance to disease from viral infection that follows by an infectious agent," To put it simply, vaccinations are biologically based preparations that contain antigens, which might be a single unit of pathogen or the complete pathogen. Their purpose is to create or enhance the immunity of host to a specific illness or collection of several diseases (Evensen, 2016).

PROPERTIES OF VACCINE:

The following characteristics should be included in the "perfect fish vaccine": (Makesh et al., 2020).

1. Immunogenicity:

A vaccine's antigen, or foreign material, should be able to trigger humoral or immune system cell mediated responses in recipient.

2. Immunological memory

Vaccine is considered efficient if it generates long-term protective immune memory, e.g. once regulated, the vaccine must activate the memory B cells of the host, allowing it to identify and resist the antigen quickly in future risk, providing fish protection for at least one production cycle.

3. Safety

Commonly, a vaccination is considered secure if it produces immunity in same way as a natural virus does while without causing clinical sickness or adverse effects in the patient. Furthermore, the vaccine of fish is safe for the next consumer, whether it is a human or another animal, who will eventually ingest the immunized fish.

4. Broad spectrum of protection

Because each fish disease contains a large range of stress, a perfect vaccine of a fish should provide good protection from the pathogen's entire spectrum.

5. Different species protection

Different from other animal's infections, that can infect only a few terrestrial species, fish pathogens possess large number of sensitive host species, hence the vaccine of fish should be similarly fruitful in multiple species of fish.

6. User friendly

It is an important factor for the optimum fish vaccination. The vaccine of fish make in a stress-free, mass-immunization, and time-efficient protocol.

7. Cost efficiency

The vaccine of fish is important in the economic aspects of vaccination. It must be affordable so that farmers can easily vaccinate their farmed fish while still profiting from their sales.

Types of vaccine

Vaccine is classed according to the way employed to grow it. The vaccine is designed based on its production feasibility and the type of the infection. Vaccine design decisions are often made depend on basic knowledge of the microbe, like how it damages cells and how immune system give response to it, similar to a practical factor, like value and size of the fish species to whom it would be operated. Vaccines are broadly classified on the base of antigen delivery systems. Replicative antigen delivery systems include live attenuated vaccines, DNA vaccines, Vector vaccines, and RNA vaccines. Non-replicative antigen delivery systems include whole cell inactivated vaccines, Subunit vaccines, toxoids, peptide vaccines, anti-idiotype vaccines, and edible vaccines (Kumar et al., 2024).

1. Whole-Cell Vaccine

Whole-cell vaccinations are defined as a suspension of disease are causing microorganisms which were eliminated (by chemical treatment and heat) and give protection when delivered to a host. This type of vaccinations is inexpensive to create. Vaccines against pathogens like Aeromonas salmonicida Vibrio salmonicida, Vibrio anguillarum, Vibrio ordalli, and Yersinia ruckeri, are administered to fish. This type of dead vaccine is economically accessible like formalin-inactivated whole cell vaccines that can administered with or without adjuvants. Vaccines against lethal fish viruses, such as infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV) and infectious pancreatic necrosis virus (IPNV), spring viremia of carp virus (SVCV), have been developed. Fish are sterilized with formaldehyde.

2. Attenuated Vaccines

Attenuating vaccines are chemically or genetically weakened microorganisms which can elicit detail immune responses of the host. They include living viruses and germs that can cease to cause disease.

3. Recombinant Vaccines

Recombinant vaccines use only pathogen's antigenic regions to grow in a heterologous host and then used like a vaccine. Furthermore, recombinant vaccines can be genetically modified (bacteria or viruses) with less pathogenicity, making them attenuated and recombinant for vaccine production.

4. Synthetic Peptide Vaccines

This type of vaccines can be peptides, antigenic sites, or subunit vaccines that have been shown to produce antibodies against disease causing viruses like rhabdovirus, nodavirus, birnavirus, VHS, IHNV, and IPNV. Fish mRNA vaccines are in high demand as compared to traditional vaccines. The vaccine which successfully reduce the illness is Live-attenuated vaccines (LAVs), but their protection and efficiency remain uncertain. In contrast, the mRNA vaccination is not infectious, making it safer.

5. DNA Vaccine

This type of vaccine is incorporating plasmids carrying an antigen of pathogen have sparked interest like a strong strategy to improve fish immunity to diseases. Researchers in aquaculture increased resistance to IHNV and VHSV by injecting viral genomes producing surface glycoproteins intra-muscularly. Rainbow trout developed an immunological response after being immunized with VHSV glycoprotein and DNA (Ma et al., 2019).

6. Mucosal Vaccine

These vaccinations for pathogenic diseases are investigated because they may induce preservative responses on mucosal surface through reducing the replication of disease-causing microorganisms. Immersion, nasal and oral teleost fish mucosal vaccinations activate T and B lymphocytes, resulting in mucosal and systemic responses. Mucosal vaccines have demonstrated to be effective when they mimic the natural path of disease, like passing through mucosal surfaces (Salinas et al., 2022).

7. Edible vaccines based on plants

Plants may give a cheap base to develop low-cost, live-attenuated, edible, pathogen-free immunizations which can minimize variety of pathogenic illnesses of fish and help to keep a sustainable aquaculture. Plant vaccines can reduce the need for bacteria booster doses or live-attenuated virus. Cell cultures of plants or complete plants may serve as expression of hosts. To show mammalian genes of plants cloned complementary DNA must combined with a plant terminator, regulatory or promoter region. Desired markers aid in detecting recombinants. These types of vaccinations would be simple to absorb orally (Micuchova et al., 2022).

Benefits of plant based fish vaccine

Plant based fish vaccine employed for multifaceted benefits, from which some are given below:

- Plant-derived vaccines are secure and free of hyperglycosylated proteins and toxic metabolites.
- Plants can biosynthesize proteins on a wide scale and perform complex folding and assembly.
- Plants are environmental sustainable, friendly and cost-effective compared to other expression system.
- Vaccines made from plants can be administered orally without the need for complex and time-consuming downstream procedures (Su et al., 2021).

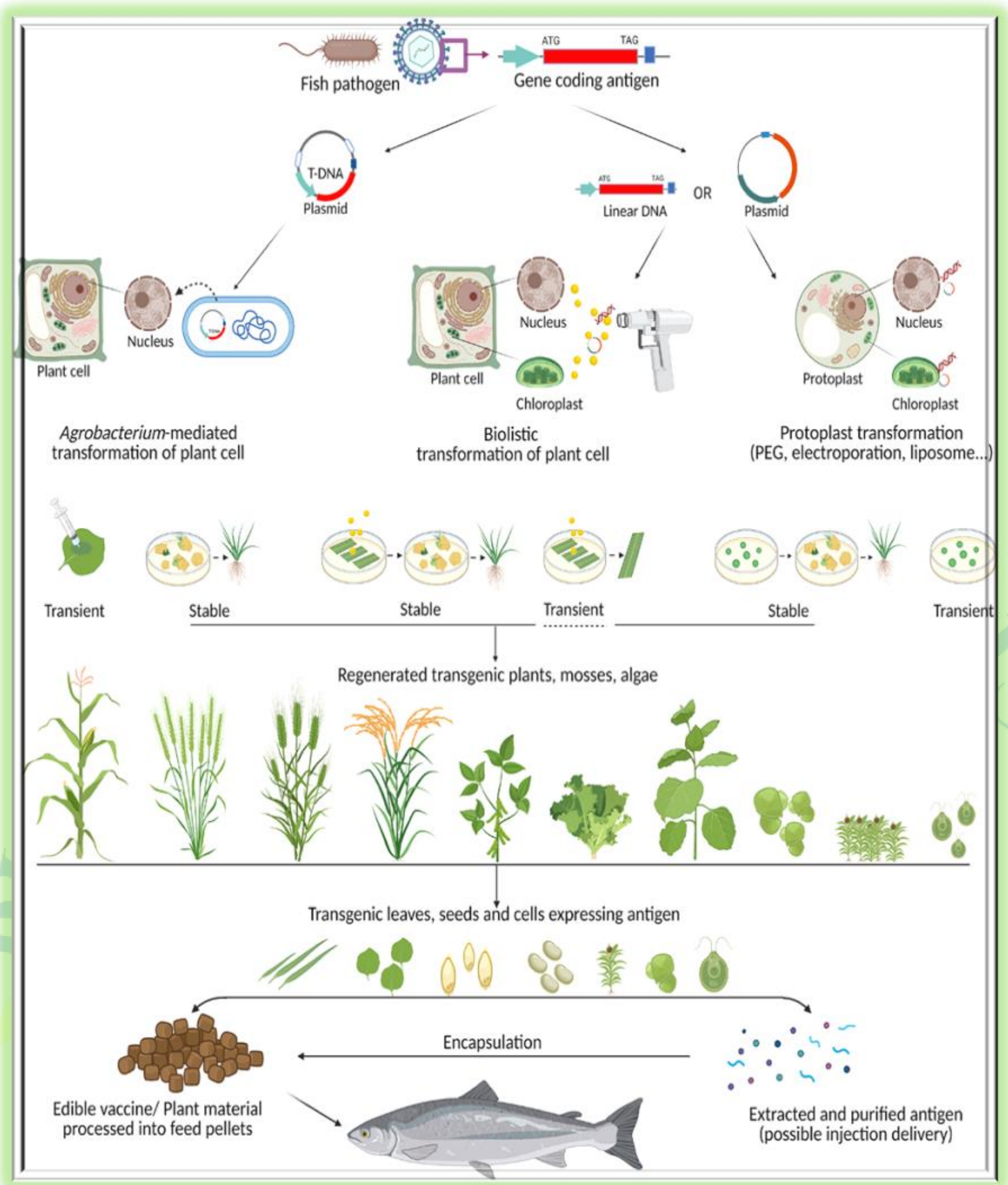

Figure 2 An outline of the steps used to generate a plant-based vaccination of fish.

Fish Vaccine Production against Various Pathogens

The research, manufacture, and commercialization of vaccinations against viral illnesses in fish are currently not available. These processes involve several sources and procedures. Every type of vaccine goes through the following steps: pathogen/disease recognition, process of development, manufacture, validity for using numerous fish individual, documentation, and vaccine licensure of commercial use. These processes are carried out using a variety of resources (Angel Esteban 2012).

Bacterial Hemolytic Jaundice

Bacterial hemolytic jaundice in farmed yellowtails has been proven to produce 5 to 20 % total damage in fish culture, including the population of Japanese amberjack. The bacteria's hemolytic action cause affected skin of fish and muscular tissues to become yellow. Similar infections may occur in natural fish populations. In 1980s this sickness first appeared and has subsequently spread quickly throughout western area of Japan. If less-cost procedures for growth can developed and quality of bacteria remains consistent, an effective vaccine could be developed (Magnadottir 2010).

Bacterial cold-Water Disease

Coldwater disease (CWD) is a bacterial disease that disturb fish living in fresh cold waters below 16 ◦C temperatures. Flavobacterium psychrophilum is the cause of both rainbow trout fry illness and bacterial CWD. In 1990 and 1993, resercher of Japan found bacterial CWD in natural sweetfish and farmed coho salmon, respectively.

Antiserum derived from infected fish of bacterial CWD used for passive vaccination aids in sickness treatment and can be utilized to make an effective vaccine. Use of a formalin-killed cell, oil-based adjuvant, vaccination, has also shown safe effects. (Kumar et al., 2024).

Erythrocyte Inclusion Body Syndrome

The causative agent of erythrocyte inclusion body syndrome in Oncorhynchus kisutch is porcine orthoreovirus II. This sickness occurs when temperature of water falls below 10◦C, leading to widespread anemia disease and mortality. A DNA-based genetic approach may aid in vaccine development. Haatveit et al. investigated PRV-1 DNA vaccination in fish and found a medium protective effect. Because of the relationship between PRV-2, and PRV-1, a recombinant vaccine may be effective in treating erythrocyte inclusion body syndrome (Kumar et al., 2024).

Parasitosis

The "Neobenedenia girellae, Benedenia seriolae and skin-parasitic capsalid monogeneans" infect farmed marine fish in Japan. The latter is highly specific to Seriola species, whereas the former parasitizes a wide range of species of fish and has a moderate specificity to host. Although praziquantel bath treatments and oral administration with hydrogen peroxide solutions or freshwater effectively control parasite but they are costly and time-consuming. Miamiensis avidus, a ciliate, causes scuticociliatosis in a fish different types of marine species, such as Japanese flounder. Because there is no effective therapy, numerous nations, like Malaysia and Japan, require a vaccine for scuticociliatosis infection (Kumar et al., 2024).

Autogenous Vaccines

Autogenous vaccines are not permitted in Japan's aquaculture. Where there are no reports of commercial effective fish vaccines, autogenous vaccines derived from strains of pathogen in a specific fish farm may give protection. When breeding diverse fish species, a variety of diseases and variances might occur. A fish vaccine development may be facilitated by introducing autogenous vaccines in countries such as Japan, where diverse species of fish are grown. Therefore, more study on fish vaccinations is now needed (Kumar et al., 2024).

Mode of Administration of Vaccine in Fish

The development of fish vaccines depends on additional factors, such as the mode of delivery, such as injection or mucosal surface vaccination (oral or immersion), even though the types of vaccines based on the antigen's nature—that is, replicating vaccines, non-replicating or and recombinant technologies. These are several ways that vaccines may be administered: (Bedekar et al., 2022).

1. Injection Vaccine

The traditional method of administering vaccines to fish is through injection. While the intramuscular route is advised for DNA vaccinations, the intraperitoneal route is utilized for the majority of vaccines, including live attenuated, whole-cell destroyed, and subunit vaccines. The injection approach is also the most vigorous, providing the recipient host with high levels of long-term immunity. The depot effect (antigen retention at the site of injection) is thought to be responsible for the long-term safety provided by injectable vaccines.

2. Immersion vaccines

These vaccines involve immersing fish in water have vaccine antigens, which are then take up by the gills, skin, or gut and prepared through the immune system, resulting in a safe immunological response. These vaccination consists of two methods one is bath immunization (diluted vaccine for a longer amount of time) and dip vaccination (high dose vaccine for a short period of time). This method has been shown to be highly effective for bulk vaccination of fry (<0.5g) and small fish that are adaptively immunocompetent (Bedekar et al., 2022).

3. Oral Vaccination

The oral form of vaccinations to fish appears to be the best way because this type is non-stressful and effective for all sizes of fish. However, developing effective and safe oral vaccinations has been difficult due to a number of important limitations. The combination of oral vaccinations with feed presents some obstacles, such as the vaccine's ability to tolerate the extremely high temperatures and pressures related with feed manufacture and extrusion. Furthermore, the antigen of oral vaccine must be stable throughout the extremely acidic gut environment (foregut and stomach) without breakdown before reaching the hindgut, where immune cells can process it.

As a result, the majority of oral vaccinations produced against fish infections demonstrated only short-term protective efficacies and did not provide long-term protection (Bedekar et al., 2022).

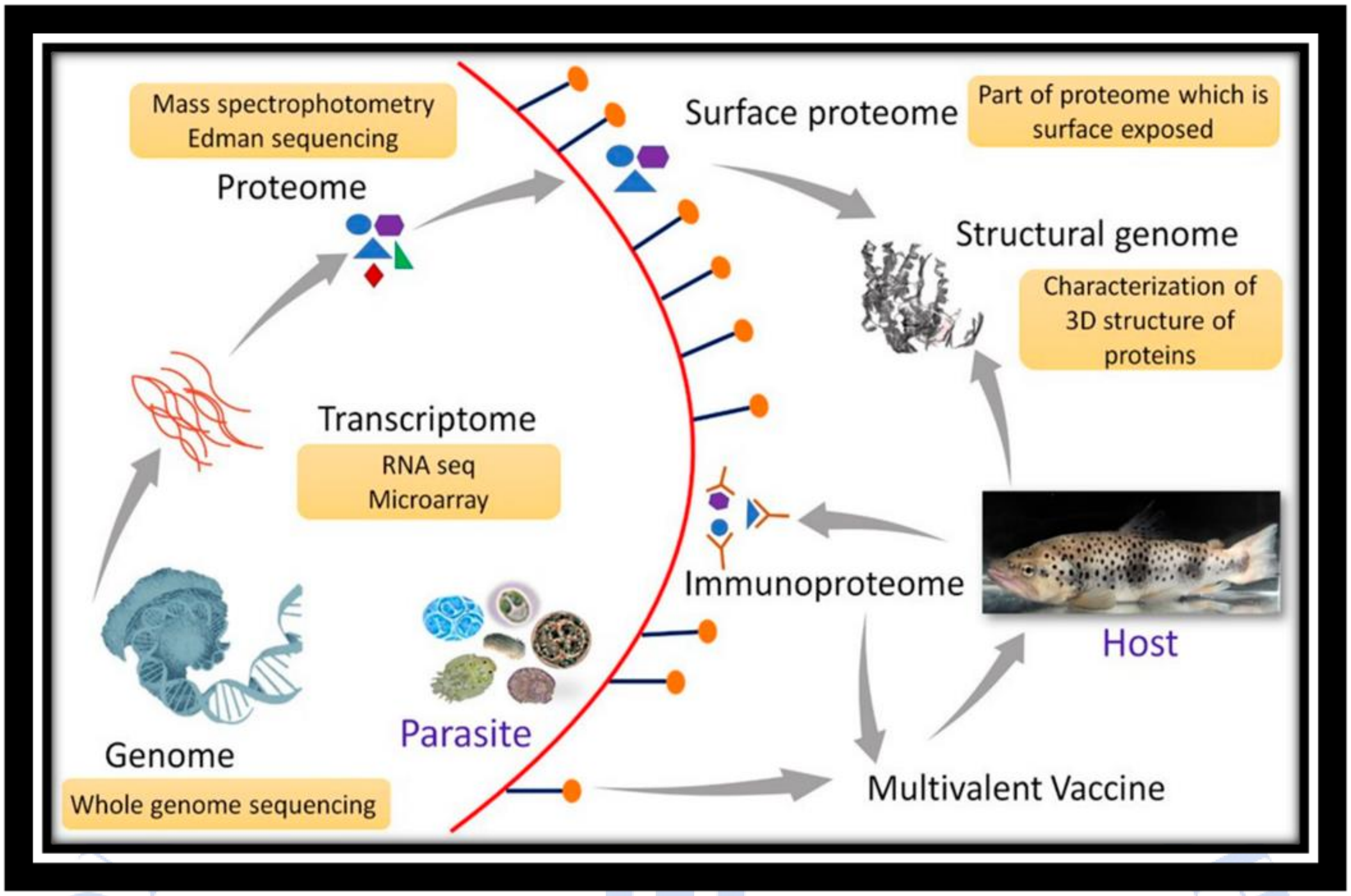

Figure 3. Contagious form of fish vaccination development

Limitations and Challenges in Developing Vaccines

An effective vaccination delivers wide-term immunity, easy to distribute, and cost-effective. The recommended route of delivery is oral vaccination because it is versatile and easy to use, can be administered in large amount at once. It is not stressful for fish. However, while oral vaccination is observed as a future prospect, it has limits. Because of its entry method, this type of vaccination may induce oral tolerance. Oral tolerance is the reduction of humoral cellular and immune responses, is the major challenges to preventing the creation of effective oral vaccinations (Rathor et al., 2024).

1. Types of Pathogens

Piscine pathogens are exceedingly varied in living world, with wide spectrum of antigenic determinant. Even though most effective commercial fish vaccines use preparations of killed whole-cell bacteria that are injected intraperitoneally, this method seems to be limited for other types of fish pathogens, especially intracellular or complex pathogens (like viruses and parasites) that are costly or challenging to cultivate (Mondal et al., 2022).

2. Fish species:

A wide range of species of fish is a barrier in development of vaccine. Because many fish infections contain a broad spectrum of sensitive hosts and every fish species behaves uniquely, it is important to understand host-pathogen interactions. As a result, there is no uniform formula for producing vaccine to kill a virus that will be equally productive in all vulnerable hosts (Rathor et al., 2024).

3. Administrative methods:

The most difficult obstacle to overcome is determining the best way to administer vaccines. Some innovative vaccines under development are safe, but modern vaccination and administration methods regimens are insufficient for optimal effectiveness (e.g., booster/prime immunization). (Evenson., 2016).

4. Antigen production method:

The selection of protective antigens is critical for the development of a successful vaccination. Additionally, to selection, the antigen cost manufacturing must be considered so that prepared vaccines are affordable to middle-income and low-income fish farmers.

5. Challenge models

The efficacy of vaccinations must be evaluated using standardized the disease in living organisms challenge models that keenly resemble the essential infection route. Cohabitation bath challenges efficiently meet the criteria of common exposure, although they are most difficult to standardize and control as compare to injection challenge approaches. Furthermore, injection is not a suitable way of testing a mucosal vaccination e.g., dip immersion delivered to fry (Yoshizawa et al., 2024).

Advancements in Vaccine Development

New advancements in biotechnology have permitted a rapid manufacture of vaccinations to treat a variety of infections. In the bioinformatics techniques, software describe the gene that will yield recombinant proteins suitable for vaccinations.

A functional analysis and computer assessment might reach to the conclusion that a successful organism's gene exists, which can be categorize for development into a vaccine to control the disease (Rathor et al., 2024).

Conclusion

The fish's immune system includes both adaptive and innate immune responses. Due to delayed adaptive immune system response, the innate immune system response is critical about attacking infections and establishing disease defiance. Fish vaccine development has made significant progress in recent decades, but there is still much work to be done.
The results presented here will help to improve our understanding, willpower, and modulation of protective immune pathways about fish health. Furthermore, knowledge about immunology of fish will aid in creation of vaccine.

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