Types of Vaccines Used in Aquaculture

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Abstract

Vaccination is the best method for disease prevention. A vaccine is an antigenic preparation intended to produce immunity to disease through stimulation of the production of antibodies and memory cells. An "ideal fish vaccine" should have the potential to generate specific immune response, protection, and memory. There are several methods for vaccine development and application. These methods range from conventional live vaccines to the latest molecular vaccines. Every type of vaccine has its own advantages and disadvantages and the choice of vaccine type depends on the type of target pathogen, immune response, safety of the recipient, and feasibility of the application. Vaccine is classified based on the method of preparation such as live attenuated vaccine, vectored vaccine, inactivated vaccine, and sub-unit vaccine. Live vaccines and killed vaccines are conventional methods of vaccine preparation which has potential for inducing specific immune response in host. However, their applications in aquaculture are limited due to constraint in delivery and uptake. Sub-unit vaccine developed using immunogenic units of pathogen like selected proteins or toxoids hold potential for vaccine development. Recombinant protein vaccine and vectored vaccines such as DNA vaccine, RNA vaccine, edible vaccine, and virus-like

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particles are advantageous because there is no need to culture the pathogen for vaccine production.

Keywords

Types of vaccine · Properties · Live vaccine · DNA vaccine · Peptide vaccine

1 Introduction

Aquaculture continues to be the fastest-growing food-producing sector in the world [1]. However, infectious diseases of bacterial, viral, mycotic, and parasitic origin still remain a major impediment in the intensification of aquaculture. In view of this, fish health management has become a critical component to disease control and is invaluable for improved harvests and sustainable aquaculture. Since the development of the first fish vaccine in the 1940s, vaccination is regarded as the most efficient and economical remedial measure in protecting the health of farmed finfish from various infectious agents [2]. The importance of vaccination is much higher for aquatic animals than those of terrestrial animals, as they are in continuous contact with the microorganisms in their aquatic environment. However, unlike their terrestrial counterpart, fish vaccine development has faced several challenges viz., limited knowledge of the fish immune system, vast diversity of pathogens and their susceptible host species, difficulties in identification and formulation of antigens, selection of efficient adjuvants and vaccine carriers, challenges related to the mode of delivery, and various laws and restrictions related to food fishes. Nevertheless, over the last four decades, fish immunologists have made profound efforts to understand the immune system and the host-pathogen interactions which in turn help to develop vaccination strategies for control of infectious diseases in commercial fish farming.

2 What Is a Vaccine?

A vaccine is an antigenic preparation intended to produce immunity to disease through stimulation of the production of antibodies and memory cells. It works by exposing the immune system of a healthy animal to an antigen and then allowing the host immune system to develop a response and a "memory" to accelerate this response in subsequent infections by the targeted pathogen [3].

3 Properties of Vaccine

An "ideal fish vaccine" should have the potential to generate an immune response. From the commercial and practical point of view, the vaccine needs to have long-term immune response, protection, specificity, and memory. While designing a vaccine, it should also be considered that the vaccine candidates should protect

against a broad range of pathogen strains. The vaccine needs to be user-friendly and cost-effective. Further, the vaccines should be safe for the fish, the person (s) vaccinating the fish, and for the fish consumers.

4 Types of Vaccine

A vaccine is classified based on the approach used to develop the vaccine. Each approach has its own advantages and specific mechanism of action. Vaccines are designed based on the feasibility of manufacturing and nature of infections. The choices for vaccine design are typically based on fundamental information about the microbe, such as how it infects cells and how the immune system responds to it, as well as practical considerations, such as size and value of the fish species to which it is administered. Broadly, vaccines can be classified based on antigen delivery systems: (1) Replicative antigen delivery system: live-attenuated vaccine, DNA vaccine, vector vaccine, and RNA vaccine; (2) Non-replicative antigen delivery system: whole-cell inactivated vaccine, sub-unit vaccine, toxoid vaccine, peptide vaccine, anti-idiotype vaccine, and edible vaccine (Fig. 1). Individual vaccine types are described as follows:

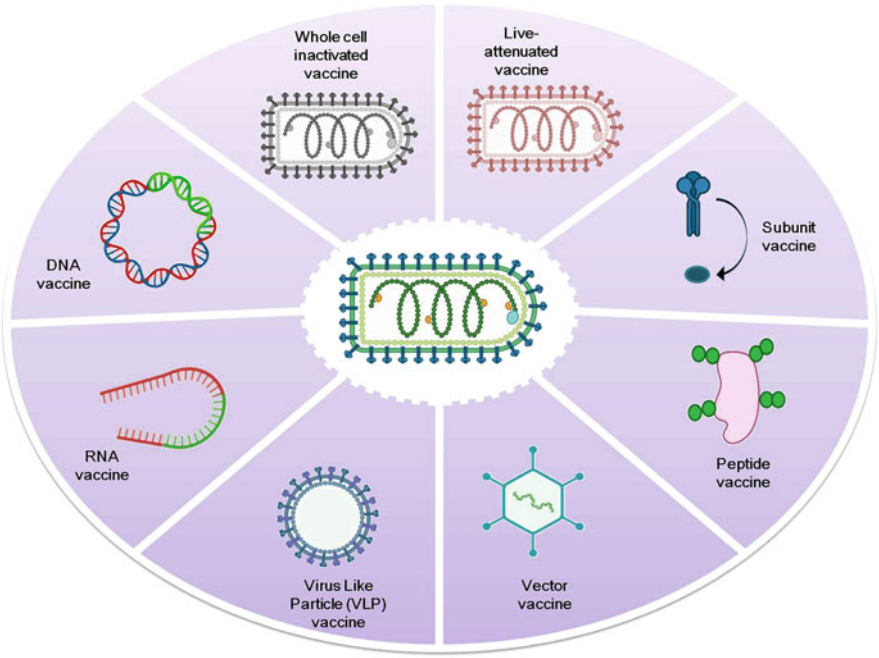

Fig. 1 Types of vaccines

4.1 Live-Attenuated Vaccine

This type of vaccine contains live-attenuated microorganisms which are "weakened" or devoid of disease-causing capacity but still capable of replicating and presenting its immunogenic properties inside the host. These vaccines are prepared by various attenuation methods viz., chemical/heat attenuation, continuous passaging of the pathogen in different heterologous systems (heterologous animals, tissue culture, embryonated eggs), and genetic attenuation (mutation by deletion, disruption, or insertion of the metabolic pathway or virulence gene) [4] (Fig. 2). This vaccine being self-replicating does not need booster immunization and can elicit both humoral and cell-mediated immune responses which in turn help in triggering a high level of long-lasting protective immunity in the host. Live vaccines are the most potent way of active immunization and the results of vaccination are evident in humans and higher vertebrates. Various attenuation strategies have been employed for the development of live vaccines for fish viz., antibiotic mutagenesis for *Flavobacterium* spp., *Vibrio anguillarum*, *Edwardsiella tarda*, and *Aeromonas hydrophila* vaccines [5, 6], mutagenesis using acriflavin dye and novobiocin for attenuation of *Streptococcus agalactiae*, *Streptococcus iniae*, *Edwardsiella ictaluri*, and *A. hydrophila* [7], mutation of koi herpesvirus (KHV) by UV exposure for reducing its virulence and minimizing chances of reversion to pathogenic strain [8], and gene deletion technology used to delete the virulence gene from catfish herpesvirus [9]. Few modified live fish vaccines are licensed in different countries which includes *E. ictaluri* vaccine against enteric septicaemia of catfish (ESC), *Flavobacterium columnare* vaccine against columnaris in catfish [10, 11]; *Arthrobacter* vaccine, licensed in Chile and Canada against bacterial kidney disease (BKD) for use in salmonids having cross-protection against *Rennibacterium salmoninarum* [12]. Among licensed live-attenuated vaccines against viral pathogens, vaccine against viral haemorrhagic septicaemia virus (VHSV) is available in Germany [13], and a live viral vaccine against KHV for carp is available for use in Israel [14].

4.2 DNA Vaccine

DNA vaccine comprises a self-replicating extra-chromosomal plasmid containing the immunogenic gene of the pathogen (Fig. 3). DNA vaccination involves the

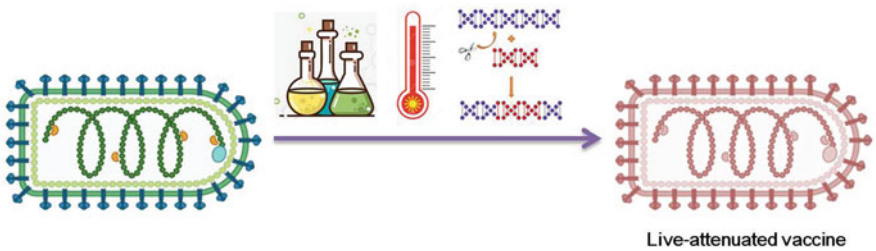

Fig. 2 Live-attenuated vaccine

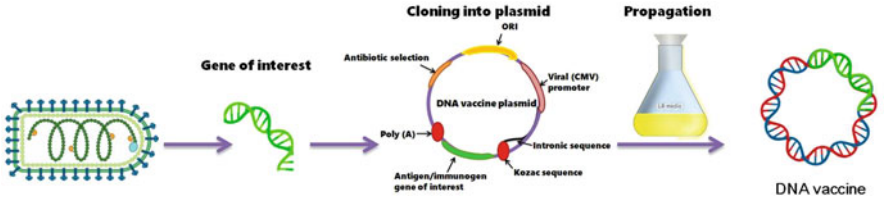

Fig. 3 DNA vaccine

delivery of plasmid DNA (raised in microorganisms such as bacteria) encoding a vaccine antigen to the host [15]. Under the control of eukaryotic promoters, the plasmid DNA expresses itself inside the recipient, first by transcription into mRNA and then by translation into the protein encoded by the gene. The expressed antigenic proteins are recognized by the host immune system as "foreign", inducing strong and long-lasting humoral and cell-mediated immune responses without the risk of inadvertent infection. DNA vaccines have been experimentally tested against several fish pathogens viz., viral haemorrhagic septicemia virus (VHSV) [16–20], infectious hematopoietic necrosis virus (IHNV) [21–25], hirame rhabdovirus (HIRRV) [26–28], spring viraemia of carp virus (SVCV) [29–31], infectious salmon anaemia virus (ISAV) [32, 33], nervous necrosis virus (NNV) [34–36], salmonid alphavirus 3 (SAV3) [37, 38], grass carp reovirus (GCRV) [39, 40], infectious pancreatic necrosis virus (IPNV) [41–45], Koi herpes virus (KHV) [46–49], Channel catfish virus (CCV) [50], Lymphocystis disease virus (LCDV) [51, 52], *E. tarda* [53–60], *Aeromonas* sp. [34, 61], *Vibrio* sp. [62–69], and *Streptococcus* sp. [70–76]. DNA vaccines have also been effective in the prevention of infection caused by intracellular and difficult-to-culture bacteria, like *Mycobacterium marinum* [77]. Despite its effectiveness, several legal restrictions (primarily related to genome integration) for the use of DNA vaccine in food fishes in most of the countries hamper its licensing and commercialization. Two DNA vaccines have been commercialized for use in aquaculture viz., APEX-IHN (Novartis/Elanco) in 2005, for protecting Atlantic salmon against IHNV in British Colombia and CLYNAV (Elanco) in 2017, a polyprotein-encoding DNA vaccine against salmon pancreas disease virus (SPDV) infection in Atlantic salmon (*Salmo salar*) for use within the European Union (EU).

4.3 Vector Vaccine

Vector vaccine utilizes live virus vectors for transferring antigenic genes into the recipient host which in turn express the encoded protein of another pathogenic microorganism, as the vaccine antigen [78] (Fig. 4). The self-assembling ability of viral structural proteins with the resemblance of a native virus has resulted in the development of this class of sub-unit vaccines based on virus-like particles (VLPs) [79]. The baculovirus expression system has proven to be an improved approach for fast expression of plentiful recombinant proteins (VLPs) and is suggested to be an

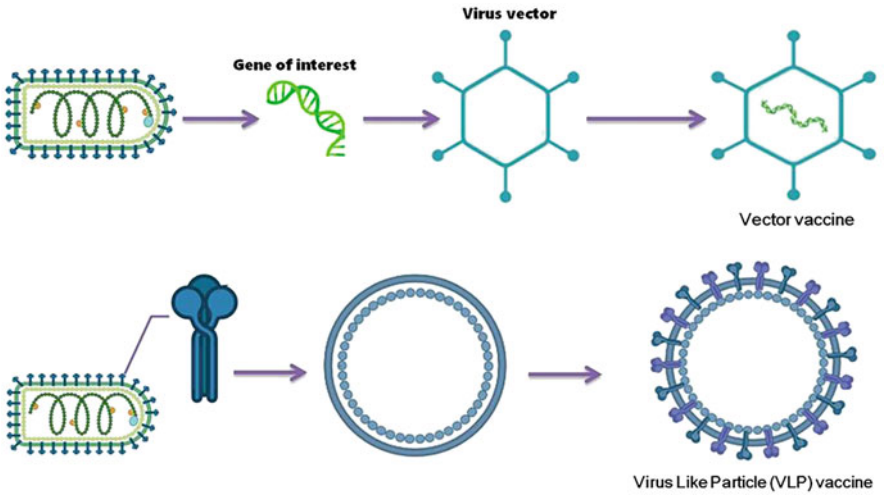

Fig. 4 Vector vaccine

inexpensive and efficient method for producing heterologous proteins [80–82]. The vaccine antigens are capable of stimulating both humoral and cell-mediated immune responses whereas, the vector has the potential to actively replicate inside the host cells, activating the immune system like an adjuvant. VLPs can be produced in competent hosts such as bacteria, plant, or fungi. VLPs are also produced by genetic recombination of an unrelated virus-producing chimera. Few experimental VLPs-based vaccines have been developed in recent years viz., vaccine against infectious pancreatic necrosis, wherein the IPNV capsid protein VP2 expressed in yeast self-assembles into sub-viral particles (SVPs) and induce immune response in Rainbow trout [83]; vaccine against Atlantic cod NNV (ACNNV) for seabass, wherein the coat protein was expressed in plant, *Nicotiana benthamiana* [84]; vaccines against grouper nervous necrosis [85] and viral nervous necrosis [86] were developed for orange-spotted grouper and European seabass respectively, using self-assembly of VLPs. Salmonid alphavirus (SAV) replicon vectors are also commonly used for developing fish vaccines, as these vectors are functional in cells from a wide range of animal classes and express gene of interest (GOI) in the temperature range of 4 °C–37 °C [87, 88]. The alphavirus-based replicon has the advantage that it does not spread/ re-infect other cells after initial replication [88, 89] and also has the ability to improve mucosal immunity [90].

4.4 RNA Vaccine

RNA vaccines are of two types: self-replicating mRNA and non-replicating mRNA. The principle of mRNA vaccine is that the modified mRNA of the target gene is either cloned in a vector or directly injected into the host. This mRNA undergoes

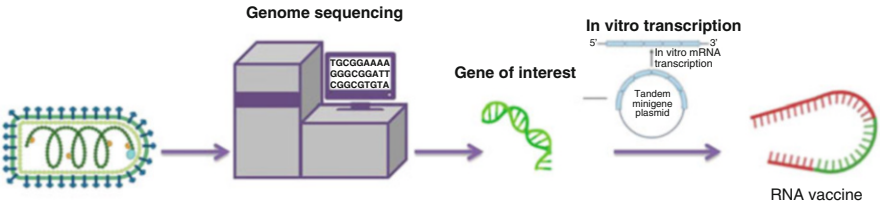

Fig. 5 RNA vaccine

translation of the target protein. The protein is detected as a foreign substance by the host immune system and specific immunity is generated against the pathogen [47] (Fig. 5). Non-replicating mRNA, also called as NRM, are flanked by 5′ and 3′ untranslated regions (UTRs), a 5′-cap structure, and a 3′-poly-(A) tail [91]. Once the NRM enter the cell cytosol, it is immediately translated to protein. The self-amplifying mRNA, also called as SAM, has the same features as that of NRM. Additionally, the construct encodes replicase components which are able to direct intracellular mRNA amplification. SAM particles once delivered in cytosol, replicate to produce multiple copies of mRNA that are ultimately translated into protein. RNA vaccines are more efficient in stimulating antigen-specific cellular immune responses as compared to the conventional plasmid DNA vaccines [92]. With many advantages over DNA vaccine, mRNA vaccine could be developed against important fish pathogens. SAV-based replicon provided significant protection against SAV3. This SAV3 construct can be a future candidate for mRNA vaccine in fish [93].

4.5 Whole-Cell Inactivated Vaccine

Whole-cell inactivated vaccines are based on the principle of Louis Pasteur's "isolate, inactivate, and inject" [94]. These vaccines contain killed microorganisms (virus/bacteria/parasite) that have been inactivated through physical or chemical processes such as heat, formaldehyde, or radiation treatment (Fig. 6). The inactivated pathogens lose their ability to cause disease but remain antigenic or immunogenic to the host. The host in turn recognizes the foreign structure of the killed pathogen, and subsequently activates its immune system (mainly humoral immune system). However, being inactivated, these vaccines induce relatively weaker immune responses than live vaccines so they require suitable adjuvant as well as several booster doses for maintaining adequate level of protective immunity over longer time. Commercial inactivated vaccines have been reported for carps and salmon globally. The first report on vaccine trial in fish was on an inactivated vaccine against *Aeromonas salmonicida*, and an oral vaccine, attempted in cutthroat trout *Oncorhynchus clarkia* [95]. Inactivated vaccine recorded successful immune protection against *Yersinia ruckeri* and this was the first commercially licensed fish vaccine [96]. Following the success of killed vaccine, research on developing killed vaccines increased especially against the infections of high-value fish species such as Atlantic salmon

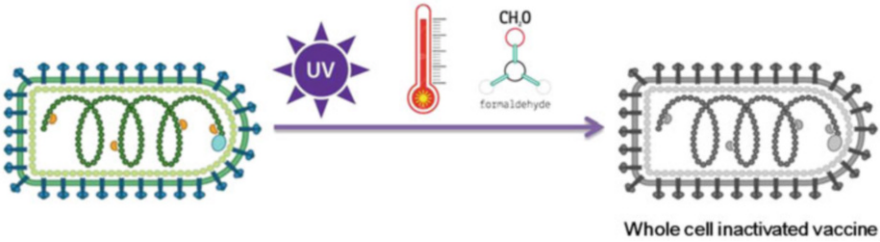

Fig. 6 Whole-cell inactivated vaccine

[97]. Although this method was effective for developing vaccine against some fish pathogenic bacteria, its utility faced major obstacle for developing vaccine against most other fish pathogens, especially viruses. Nevertheless, the first inactivated viral vaccine for fish, against a carp rhabdovirus, causing spring viremia of carp (SVC) was produced by a Czechoslovakian company (Bioveta) in 1982.

4.6 Sub-Unit Vaccine

Sub-unit vaccine uses the recombinant technology where only the immunogenic target regions of a pathogen are expressed in a heterologous host from which the protective antigen is purified and used in vaccine formulation [78] (Fig. 7). Biotechnological tools are used for recognition and designing of the gene sequence of pathogen's protective antigen. After designing, the antigenic genes are inserted into prokaryotic [98] or eukaryotic [99] production hosts and are cultured on a large scale under strictly controlled laboratory conditions by fermentation technology, with the aim to produce the antigenic protein. The production hosts include bacteria [98], cell culture [100], yeast [101], insect cells [99], microalgae as well as transgenic plants [102]. However, in the case of fish vaccines, the administration of the recombinant antigens produced through fermentation was found to be inefficient in inducing protective immunity, which might be due to poor immunogenicity of the antigens [103, 104]. Molecular techniques enabled the expression of highly antigenic proteins of the target pathogen in bulk and subsequent delivery of the purified antigen as a vaccine. Although initial works on sub-unit vaccines in aquaculture were not successful due to the rapid degradation of the protein during production and transport, or in the gut of the animals, improvements were made to stabilize the antigens and many sub-unit vaccines have been developed. Most of the sub-unit vaccines are developed by expressing the sub-unit protein in *Escherichia coli*-based prokaryotic expression system. One of the most successful examples is a sub-unit vaccine against infectious pancreatic necrosis (IPN), comprising of fused IPN-VP2 gene. ISAV vaccine containing recombinant hemagglutinin-esterase protein is available as an oral vaccine in the name of Centrovet in Chile. Baculovirus system and yeast expression system have been used for the vaccine against viral haemorrhagic septicaemia and IHNV [105]. Although there are many reports on sub-unit vaccines for fish, they are not commercially available for use in aquaculture [6]. The major

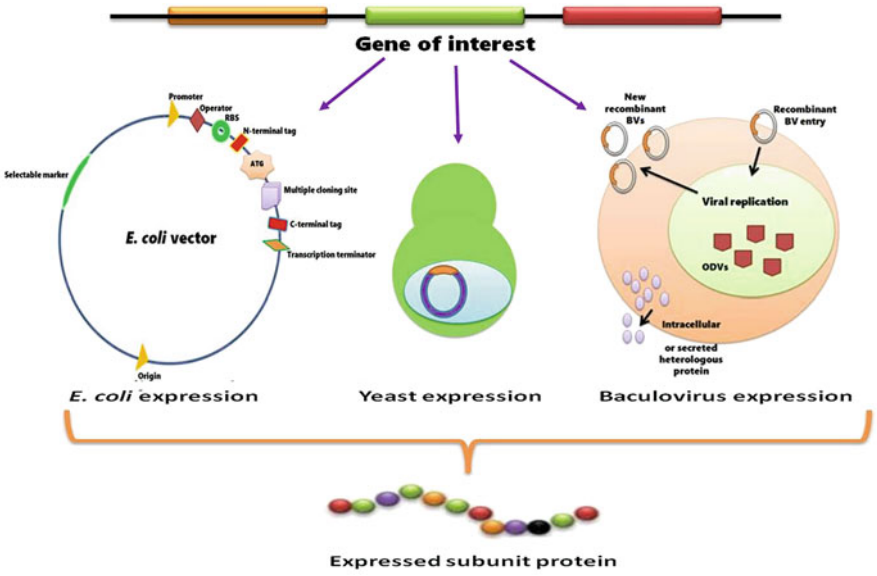

Fig. 7 Sub-unit vaccine

issue with recombinant vaccines is the environmental safety and regulatory clearance. Thus, recombinant protein-based vaccines need to prove their environmental safety for field testing [106].

4.7 Toxoid Vaccine

Toxins (exotoxin and endotoxin) are components that are secreted by bacteria as part of their pathogenic response. Toxoid vaccine is generally developed from exotoxin. When toxicity of the toxin is inactivated or reduced by chemical or heat treatment, while maintaining its immunogenicity, it is called a toxoid (Fig. 8). Toxoid has a capacity to trigger the immune response and mount immunological response and memory. When the immune system receives a vaccine containing a harmless toxoid, humoral immune system is activated and produces antibodies that lock onto and block the toxin. This is also termed as anatoxin. In aquaculture, few reports of experimental trial of toxoid vaccine with low antibody response are available. Toxoid-enriched inactivated vaccine containing *Photobacterium damselae* subspecies *piscicida* was reported to give 37–41% protection. The toxoid vaccine has also been tried against *A. salmonicida* [107].

4.8 Peptide Vaccine

Peptide vaccines are synthetic peptides or small amino acid domains on the surface of a carrier protein, which have the capacity of generating immune responses in the

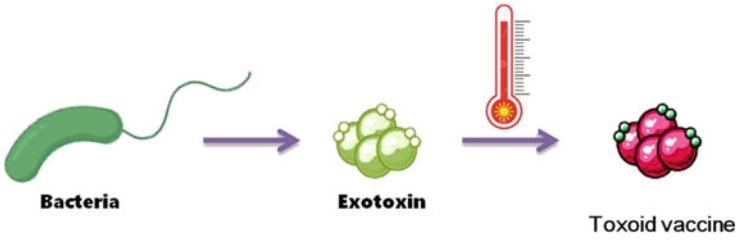

Fig. 8 Toxoid vaccine

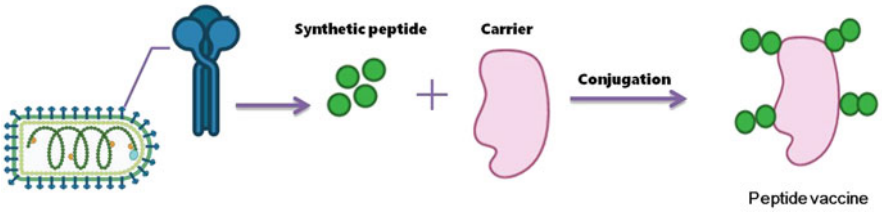

Fig. 9 Peptide vaccine

recipient host (Fig. 9). The small amino acid domain that has the potential to generate immunogenicity is first identified using bioinformatic tools such as Predict Protein, Prosite, SwissProt and Epitope mapper. The peptide is then synthesized and the synthetic peptide is used as a vaccine to generate the immune response. These are referred to as peptide vaccines as they have the potential to generate immune response and memory. The short peptides are bound to some surface carrier proteins and used as a vaccine. Although, they are very simple and safe, due to low immunogenicity their applications are limited in fish.

4.9 Anti-Idiotype Vaccine

This vaccine comprises of antibodies that have three-dimensional immunogenic regions, designated as idiotopes that consist of protein sequences which can bind to cell receptors (Fig. 10). Idiotopes are aggregated into idiotypes, specific to their target antigen. Thus, anti-idiotypes are antigen-mimics that can trigger immune response in the host. These anti-idiotypes can be purified from serum or can be designed using bioinformatics-based molecular docking approach and used as antigen replacement. However, this is yet to be explored in fish vaccination.

4.10 Edible Vaccine

Edible vaccines are plant-based vaccines prepared by molecular farming where whole plants or plant cells/tissues are cultured in vitro for the production of

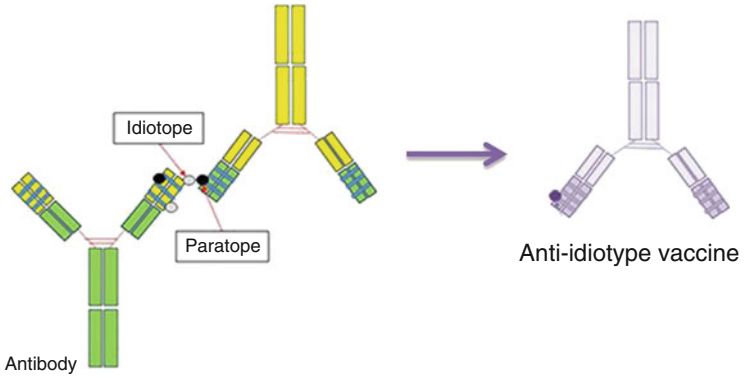

Fig. 10 Anti-idiotype vaccine

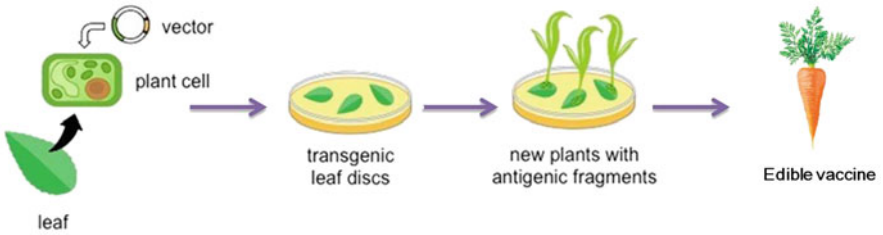

Fig. 11 Edible vaccine

immunogenic proteins [108] (Fig. 11). These are potentially cheap to produce and are viable alternative to mainstream production systems. Edible vaccines, after consumption, expresses the antigenic proteins, which are then transported via specialized M-cells to the dendritic cells subsequently activating a coordinated immune response involving B-cells and T-helper cells. This vaccine technology is at an early stage for fish vaccines [109] but likely to develop in the near future.

5 Conclusion

Vaccination is the best method for disease prevention, and there are several options for vaccine development and application. These methods range from conventional live vaccines to the latest molecular vaccines. Every type of vaccine has its own advantages and disadvantages and the choice of vaccine type depends on the type of target pathogen, immune response, safety of the recipient, and feasibility of the application. The advantages and disadvantages of each type of vaccine are given in Table 1. Vaccination and developing a strategy for successful vaccination in fish have various challenges which can be addressed by modern vaccine methods such as a recombinant protein-based vaccine, VLPs, and synthetic peptides. In the present scenario of emerging diseases which cause serious impact on aquaculture

Table 1 Advantages and disadvantages of vaccines

Vaccine type	Advantages	Disadvantages
Live-attenuated vaccine	Being self-replicating does not need booster immunization Provides long-lasting protective immunity to the host Can be administered easily through oral or immersion method	Possesses the risk of recombination of different strains resulting in the emergence of the new strains Has the risk of reverting to virulent strain Causes serious threat to off-target animals and the aquatic environment Not suitable for immuno-compromised animals as they work on an active immune system
DNA vaccine	Induces strong and long-lasting protective immunity to the host Possesses no risk of inadvertent infection DNA vaccines are stable in dried powder or in a solution and do not need a cold chain The vector can encode for multi-valent vaccine against multiple diseases, which could be given in a single administration DNA vaccines are relatively cheap and are easy to produce via identical production processes	Legal restrictions (primarily related to genome integration) for the use of DNA vaccine in food fishes in most of the countries hampers its licensing and commercialization
Vector vaccine	Apart from the antigen, the vector has the potential to replicate inside the host cells actively and can activate the immune system like an adjuvant The alphavirus-based replicon has the advantage that it does not spread/re-infect other cells after initial replication The alphavirus replicon has the ability to improve mucosal immunity	Pre-existing antibodies against the vector virus can neutralize or inhibit the viral vector, thereby reducing the targeted immune response against the foreign antigen Vector vaccine technology is still new to fish vaccine development and has been tested to a minimal extent
RNA vaccine	RNA vaccines are not made from pathogen particles or inactivated pathogen, so are non-infectious Unlike DNA vaccine, RNA vaccine does not integrate itself into the host genome and gets degraded once the protein is made Limited clinical trial results indicate that these vaccines generate a strong immune response and are well-tolerated by healthy individuals	Very new technology, so tested to a very limited extent in finfish vaccinology
Whole-cell inactivated vaccine	Unlike live attenuated vaccines, the inactivated vaccines does not carry the risk of mutating back to their disease-causing state Do not require cold chain for storage and can be easily transported in freeze-dried	Being inactivated, these vaccines induce relatively weaker immune responses, so they need several booster doses for maintaining adequate level of protective immunity over a longer time

(continued)

Table 1 (continued)

Vaccine type	Advantages	Disadvantages
	form These vaccines are easy to manufacture and are economically feasible	To maximize their effectiveness, they require suitable adjuvant Mostly injection mode of delivery is effective
Sub-unit vaccine	Have no live components, thus no risk of inducing disease Safe, stable, and easy to manufacture	Although very effective against human and animal pathogens, in the case of fish vaccine, the administration of the recombinant antigens is found to be inefficient in inducing protective immunity Poor immunogenicity of the antigens, induces a less strong immune response Often a response can be elicited, but there is no guarantee that immunological memory will be formed in the correct manner
Toxoid vaccine	Toxoid has the capacity to trigger an immune response and mount immunological response and memory These are extremely safe methods of immunization and are less likely to induce any side effects They can also work in immuno-compromised individuals	May require several doses and usually need an adjuvant Relatively low antibody responses are reported from the limited experimental trial of toxoid vaccine in aquaculture, reducing its applicability
Peptide vaccine	They are very simple and safe.	Due to low immunogenicity, their applications are limited in fish vaccinology
Anti-idiotype vaccine	Anti-idiotypes can be purified from serum or can be designed using bioinformatics-based molecular docking approach and is used as an antigen replacement	Yet to be explored in fish vaccination
Edible vaccine	These are potentially cheap to produce and are a viable alternative to mainstream production systems such as microbes and mammalian cells cultivated in large-scale bioreactors Unlike other recombinant technologies, they are free from undesirable components, e.g., endotoxins in bacteria, and hyperglycosylated proteins produced by yeast There is no limit to their production scale, and the cost of scaling up is low	This vaccine technology is at an early stage for fish vaccines but likely to develop in the near future.

production, it is important to focus more on developing effective vaccines so that infectious diseases can be prevented and production losses can be minimized.

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