PLANT-BASED VACCINES IN AQUACULTURE: PROGRESS AND FUTURE OUTLOOK

Abstract

Aquaculture is crucial for food supply, but disease outbreaks and antibiotic use pose risks. Limited fish vaccines are available, highlighting the need for environmentally friendly solutions like vaccination to address disease control and ensure sustainable aquaculture. Plant genetic engineering offers a promising solution for fish vaccine production. It provides cost-effective and safe alternatives to live virus vaccines, with the ability to perform posttranslational modifications similar to natural systems. Plant-based vaccines have been successfully developed for human and animal health, but progress in fish vaccines lags behind. The currentchaptercondenses the growth of piscine vaccines currently in use and the applicability of the plant-production platform for the same.The utilization of plant biotechnology to develop fish vaccines holds great significance for the aquaculture industry, fish health management, food safety and human health risks.

Keywords: Aquaculture, Phyto-vaccine, Biotechnology, Oral administration, One Health, Virus-Like-Particle

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I. INTRODUCTION

In recent decades, aquaculture has undergone extraordinary growth, establishing itself as the leading food industry and surpassing more than half of the total fishery production [1]. In 2022 alone, global aquaculture production achieved an impressive milestone of184.6 million metric tons. This significant contribution aligns with several sustainable development goals (SDGs) of the United Nations, including poverty eradication (G1), zero hunger (G2), good health and well-being (G3), life below water (G14) and life on land (G15) [1]. However, the intensification of aquaculture, characterized by high densities and artificial conditions, has also led to increased risks of infectious disease outbreaks. Globally, around 10% of aquaculture production is lost attributing to disease outbreaks, resulting in substantial economic losses exceeding 10 billion USD [1]. To combat these diseases, antibiotics and chemicals are commonly used, but their indiscriminate use poses threats to ecological balance, human well-being and access to safe food [2]. Antimicrobial resistance (AMR) is a nationwide threat, highlighting the need for alternative strategies such as substituting antibiotics with probiotics and plant-based bioactive substances in aquaculture health management [2]. Ensuring the sustainable development and growth of aquaculture production, fish production has emerged as a highly efficient and eco-friendly approach to safeguard farmed fish from diseases, with minimal impact on the environment. Vaccination has become the standard practice in modern aquaculture worldwide, and momentous progress was seen in the improvement of fish vaccines since the 1980s. Currently, there are 34 commercially produced fish vaccines, and over 140 fish vaccines are approvedworldwide [3]. The main focus of this chapter is to provide an overview of plant-based vaccines in aquaculture and discuss the promising potential of plant biotechnological engineering for advancing vaccine development in this context.

II. NOVEL FISH ORAL VACCINES

In the realm of aquaculture, oral vaccination stands out as the most favourable method for fish and other aquatic animals due to its non-stressful nature and energy saving approach [3]. As of now, there are around 20 commercialized oral vaccines in aquaculture against myriad pathogens [3]. Nevertheless, establishing standardized oral vaccination procedures can present challenges, primarily due to the large surface area of the intestine, which increases the risk of antigen breakdown and local immune responses. Against the age-old oral vaccination techniques, virus-like particles (VLPs), encapsulation and other vaccination strategies have gained popularity in aquaculture [3]. By incorporating the *Escherichia coli*-derived orange-spotted grouper nervous necrosis virus (NNV) VLP vaccine into the commercial feed, oral vaccination achieved a remarkable survival rate of over 50% against NNV infection[4]. Feasibility for vaccination was reported using lyophilized recombinant yeast that produces red grouper NNV capsid protein as a diet for groupers[5]. The selection of the vaccine and delivery method relies on various factors, including the specific characteristics of the farmed fish, the nature of pathogen, the level of protection needed and economic considerations. Future fish vaccines should be economical, environment friendly, suitable for commercial production, and accessible to small fishers. Plant-based biotechnological techniques hold promise for meeting these requirements in fish vaccine development.

III. PHYTOVACCINE DEVELOPMENT

Compared to microbial or human cell expression systems, plant-derived platforms offer several advantages for producing genetically-modified subunit vaccines [6]. Firstly, they are environmentally friendly, harvesting solar energy and sequestering CO_2, resulting in minimal energy demands and zero greenhouse gas emissions. This leads to reduced vaccine production costs. Secondly, they can generate vaccines suitable for oral delivery, which makes them cost-effective. Scaling up production is also more affordable and not constrained by the size or number of available bioreactors. It is considered safe since they lack the safety concerns associated with live vaccines and undesirable components like bacterial endotoxins or hyperglycosylated proteins are absent [6]. More over these systems possess a high capacity for post-translational modifications, such as glycosylation and complex folding, thereby enhancing the vaccines' immunogenicity [6].

Various plant biotechnology platforms have been utilized to produce vaccine antigens, monoclonal antibodies (mAbs), and other biopharmaceuticals [6]. These platforms encompass various methods, such as transient utilizing viral vectors, stable nuclear expression in transgenic plants or cell cultures and stable expression in the plastid (chloroplast) genome of trasnplastomic plants. Each platform has its own advantages and limitations, and the selection of a specific technique depends on the desired production requirements. Additionally, regulatory considerations regarding the production of plant-made recombinant proteins and adherence to good manufacturing practices have been well established in this field [6].

IV. APPLICATION OF PHYTOVACCINES

Plant molecular farming can utilize various plant species for protein production. Plastid genome engineering has been successfully employed in consumable plants such as lettuce, tomato, potato, and cabbage to produce a wide range of foreign proteins. The chloroplast expression system, in particular, has shown promise for the manufacture of oral vaccines [6]. Vaccines against viruses, bacteria and parasites have been successfully produced using transgenic plants. Another system for transient expression is the production of vaccines and biopharmaceuticals in algae. Microalgae, such as *Chlamydomonasreinhardtii*, *Dunaliellasalina*, and cyanobacteria, offer potential benefits as a protein production and oral delivery system for vaccinating fish [7]. Microalgae possess digestibility, high nutrient content, and immunogenicity, making them suitable as functional feed additives. However, further research is needed to establish effective and mature genetic manipulation systems in many microalgae species used in the aquaculture industry [7].

Plant-based vaccine development has been extensively reviewed, although commercialization of human plant-based vaccines is still pending. Numerous attempts have been made to produce subunit vaccines in transgenic plants, demonstrating the potential of this approach. Examples include the production of hepatitis C virus antigens in lettuce, which successfully induced immune responses in mice [8]. Plant-derived vaccines have been developed to combat various diseases, including rabies, porcine reproductive and respiratory syndrome virus, porcine post-weaning diarrhea and Newcastle disease in poultry [9]. The only plant-made vaccine approved by the United States Department of Agriculture to date is a tobacco-made Newcastle disease vaccine for poultry. Furthermore, research has explored the development of plant-made veterinary vaccines for dogs and cats [8].

These examples highlight the potential of plant molecular farming for vaccine production in both human and veterinary medicine.

V. THE PROS AND CONS OF PHYTO-VACCINES

Plant-derived vaccines are well-suited for oral administration, offering a simpler manufacturing process without the need for additional injection devices. These vaccines offer excellent purity, making the vaccination process more convenient and eliciting robust immune responses. When administered orally, vaccine antigens pass through the gastric environment and reach the intestine, where M cells in the follicle-associated epithelium absorb them, triggering both mucosal and systemic immune responses. Edible plants such as lettuce, potato, tomato, corn, and rice provide ideal foundations for these vaccines, presenting a needle-free, convenient, and easily administered delivery method. Lettuce, in particular, has shown promise as a plant base for oral vaccines, with successful expression of several human therapeutic proteins at high levels in lettuce chloroplasts. Notably, oral vaccines from lettuce chloroplasts against diseases like dengue fever have been investigated, demonstrating the potential of this approach [7,8,9].

VI. THE POTENTIAL OF PHYTOVACCINES IN AQUACULTURE

Plant-based production platforms offer a cost-effective, efficient, and safe approach for the development of new fish vaccines in the context of aquaculture's high-density farming and large scale. Despite higher initial costs compared to antibiotics, plant-based vaccine systems show promise in delivering effective and safe vaccines to protect fish health and support sustainable development in aquaculture globally. When selecting a plant expression system for fish vaccine production, factors such as fish species diversity, target diseases, scalability, resources, biosafety concerns, commercial potential, feeding habits, and management feasibility should be considered. Oral vaccination emerges as an ideal method, providing a non-stressful and energy-saving administration for both fish and farmers. Vaccines delivered orally using edible plants reduce the need for costly fermentation, purification, cold storage, transportation, and sterile delivery associated with other methods, offering distinct cost advantages and antigen stability at room temperature. Fish vaccines made in edible plants hold great potential for oral vaccination in aquaculture, and plant-generated recombinant subunit vaccines could offer the simultaneous delivery of multiple antigen proteins, further enhancing their efficacy[3]. However, no plant-produced fish vaccine has been commercialized to date (Table 1). Utilizing a plant expression system, VLPs composed of viral capsid proteins that mimic natural viral structures have emerged as promising subunit vaccine candidates for fish [3]. Based on the success of human vaccines produced in plants, the development of fish vaccines in edible or non-food crops for oral vaccination holds significant potential. Thus the need for further research efforts in plant biotechnology is imminent to advance cost-effective fish health management and foster a sustainable aquaculture industry.

Table 1: Plant-Based Vaccines Which Have Been Reported In Aquaculture

Host species	Disease/Pathogen	Plant species	Delivery route	Reference
Grouper	Red grouper nervous necrosis virus (RGNNV)	Yeast	Oral	[5]
Salmonids	Cardiomyopathy syndrome (PMCV)	Nicotianabenthamiana	Intraperitoneal	[10]
Salmonids	Atlantic cod nervous necrosis virus (ACNNV)	Nicotianabenthamiana	Intramuscular	[11]

VII. CONCLUSION

Researchers are currently working on plant-based vaccines, aiming to make the antigens they produce in plants more potent in triggering immune responses. They also want to target specific parts of plant cells to get the right amount and quality of antigens. Though there are challenges, these improved plant-based vaccines have a lot of potential. People believe they will eventually get approval from regulators. As the world's population grows, it's crucial to protect aquatic health in fish farming. Plant-based vaccines for fish can be a cheap and safe way to do this. However, making vaccines for fish from plants is not as advanced as making vaccines for humans and other animals. This review gives an overview of how fish vaccines are managed and how plant genetic engineering is used to make them. Using plant-made vaccines that are taken by mouth has some great benefits for production and vaccination. But, it's important to know that these oral plant-based vaccines are not approved yet.

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