**PLANT PRODUCED VACCINES: CURRENT STATUS AND FUTURE PERSPECTIVES**

**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 present chapter summarizes the development of fish vaccines currently utilized and the suitability of the plant-production platform for fish vaccine and then addresses considerations regarding fish vaccine production in plants. Developing fish vaccines by way of plant biotechnology are significant for the aquaculture industry, fish health management, food safety, and human health.

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

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1. **INTRODUCTION**

Aquaculture has experienced remarkable growth in recent decades, emerging as the dominant global fish industry, accounting for over 50% of total fishery production. In 2022 alone, aquaculture production reached 184.6 million metric tons, contributing to several United Nations Sustainable Development Goals (SDGs) including poverty eradication (SDG 1), zero hunger (SDG 2), good health and well-being (SDG 3), life below water (SDG 14), and life on land (SDG 15) [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 cultured aquatic animal production is lost due to infectious diseases, 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 risks to the environment, human health, and food security [2]. Antimicrobial resistance (AMR) has become a global threat, highlighting the need for alternative strategies such as probiotics and plant bioactive compounds to replace antibiotics in aquaculture health management [2]. To ensure the sustainable development and expansion of aquaculture production, fish vaccination has emerged as a highly effective and safe method to protect farmed fish from diseases, with minimal ecological impact. Vaccination has become the standard practice in modern aquaculture worldwide, and significant progress has been made in the development of fish vaccines since the 1980s. Currently, there are 34 commercial vaccines available for use in fish, and over 140 fish vaccines have received approval globally [3]. The current chapter aims at briefing a novel technique of oral vaccination in fish, fish-based vaccines and the potential of plant biotechnological engineering for further development.

1. **NOVEL FISH ORAL VACCINES**

In aquaculture, oral vaccination is considered the most ideal method for fish and other aquatic animals due to its non-stressful and energy-saving administration [3]. As of now, there are around 20 commercialized oral vaccines in aquaculture against myriad pathogens [3]. However, standardizing oral vaccination procedures can be challenging, as the large surface area of the intestine 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]. Oral vaccination along with commercial feed of *Escherichia coli* derived orange-spotted grouper nervous necrosis virus (NNV) VLP vaccine was able to offer over 50% survival against NNV infection [4]. Lyophilized recombinant yeast producing red grouper nervous necrosis virus capsid protein as diet for groupers is reported to be feasible for vaccination [5]. The choice of vaccine and delivery method depends on the characteristics of the farmed fish species, pathogens, and required protection, as well as economic factors. Future fish vaccines should be cost-effective, environmentally friendly, suitable for large-scale production, and accessible to small fish farmers. Plant biotechnological techniques hold promise for meeting these requirements in fish vaccine development.

1. **PLANT-BASED VACCINE DEVELOPMENT**

Compared to microbial or mammalian cell expression systems, plant-based platforms offer several advantages for producing recombinant subunit vaccines [6]. Firstly, they are environmentally friendly, using free solar energy and capturing CO2, resulting in low energy requirements and no greenhouse gas emissions. This leads to reduced vaccine production costs. Secondly, plant-based systems can produce vaccines suitable for oral delivery, eliminating the need for complex bioreactors and downstream processing, making it a more economical option. Scaling up production is also cheaper and not limited by the size or number of available bioreactors. Thirdly, plant systems are considered safe as they do not have the safety concerns associated with live vaccines. Undesirable components, such as bacterial endotoxins or hyperglycosylated proteins, are not found in plant-derived systems. Additionally, plant-based systems have a high capacity for performing post-translational modifications, such as glycosylation and complex folding, which enhance the immunogenicity of the vaccines [6]. However, plant-based platforms still have challenges, including uncertain efficiency of modifications, variability in protein quantity and quality, and difficulties in achieving precise dosage of vaccines in tissues.

Various plant biotechnology platforms have been utilized to produce vaccine antigens, monoclonal antibodies (mAbs), and other biopharmaceuticals [6]. These platforms include transient expression using viral vectors, stable nuclear expression in transgenic plants or cell cultures, and stable expression in the plastid (chloroplast) genome of transplastomic 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].

1. **APPLICATION OF PLANT-BASED VACCINE**

Plant molecular farming can utilize various plant species for protein production. Plastid genome engineering has been successfully employed in edible 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 production of oral vaccines [6]. Transgenic plants have been used to produce vaccines against viruses, bacteria, and parasites. Another system for transient expression is the production of vaccines and biopharmaceuticals in algae. Microalgae, such as *Chlamydomonas* *reinhardtii*, *Dunaliella* *salina*, 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-made vaccines have also been developed against 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. Additionally, plant-made veterinary vaccines have been studied for mink, dogs, and cats [8]. These examples highlight the potential of plant molecular farming for vaccine production in both human and veterinary medicine.

1. **THE PROS AND CONS OF PHYTO-VACCINES**

Plant-based vaccines are well-suited for oral administration, offering a simpler manufacturing process without the need for additional injection devices. These vaccines provide high purity, making vaccination more convenient and eliciting stronger immune responses. When ingested orally, vaccine antigens pass through the gastric environment and reach the intestine, where they are absorbed by M cells in the follicle-associated epithelium, triggering both mucosal and systemic immune responses. Edible plants like lettuce, potato, tomato, corn, and rice serve as ideal bases for these vaccines, offering a needle-free, convenient, and easily administered route. 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].

1. **THE POTENTIAL OF PLANT-BASED VACCINES IN AQUACULTURE**

In the development of new fish vaccines, economic cost plays a crucial role, considering the high-density farming and large scale of aquaculture. Plant-based production platforms offer a cost-effective, efficient, and safe approach for vaccine production in this context. Despite having a higher initial cost compared to antibiotics, plant-based vaccine systems are promising in providing 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 the diversity of fish species, target diseases, scalability, resources, biosafety concerns, commercial potential, feeding habits, and management feasibility should be considered. Oral vaccination, which provides a non-stressful and energy-saving administration for both fish and farmers, is considered an ideal method for fish vaccination. Vaccination through oral delivery using edible plants reduces the need for costly fermentation, purification, cold storage, transportation, and sterile delivery associated with other production methods. Oral vaccines produced in edible crops offer distinct cost advantages and antigen stability at room temperature, making them an attractive option in terms of simplicity and safety. Fish vaccines made in edible plants offer great potential for oral vaccination in aquaculture. A plant-generated recombinant subunit vaccine could also provide several antigen proteins simultaneously [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) | Nicotiana benthamiana | Intraperitoneal | [10] |
| Salmonids | Atlantic cod nervous necrosis virus (ACNNV) | Nicotiana benthamiana | Intramuscular | [11] |

1. **CONCLUSION**

Current research on plant-based vaccines primarily focuses on enhancing antigen quantity and purity in transgenic plants to elicit sufficient immune responses. Optimal targeting of the subcellular compartment in plant cells is also crucial for achieving the desired quantity and quality of antigens. Despite facing several challenges, the potential and promise of improved plant-based vaccines are highly attractive. Plant-based vaccines are considered an emerging vaccine type, and it is anticipated that they will eventually receive regulatory approval. Given the growing global population and increasing demand for food safety, effective management of aquatic health is essential worldwide. Plant-based fish vaccines have the potential to offer low-cost, high-safety, and effective protection for farmed fish species. However, the development of plant-made fish vaccines lags behind efforts in producing plant-made vaccines for humans and non-aquatic animals. This review has provided an overview of the current status of fish vaccine management and the application of plant genetic engineering in fish vaccine production. The utilization of plant-made recombinant protein vaccines with oral administration presents distinct advantages in terms of production and vaccination. However, it is important to note that plant-made vaccines for oral delivery have not yet received approval.

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