REVIEW ARTICLE

Botanical Defence: Exploring Plant-Derived Vaccines for Animal Health – An Overview

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ABSTRACT

Plant-based vaccines have emerged as a revolutionary approach in the field of immunization, offering numerous advantages over conventional vaccine production methods. This article explores the concept of plant-derived vaccines and their potential applications in animal health and sustainable agriculture. Utilizing plants as bioreactors, plant-based vaccines produce specific antigens that trigger a robust immune response upon ingestion by animals. These vaccines are pathogen-free and do not require refrigeration, making them cost-effective and accessible, especially in resource-limited regions. Moreover, plant-based vaccines are safer than traditional vaccines, as they contain no live pathogens, and they have demonstrated high stability and scalability in antigen production. Maintaining dosage consistency and adhering to Good Manufacturing Practice (GMP) standards are essential for ensuring vaccine quality and efficacy. Plant-based vaccines represent a paradigm shift in vaccine production and offer a pathway to a healthier and more resilient future. With further advancements and collaboration among stakeholders, plant-based vaccines have the potential to impact global health, addressing disease challenges in animals and humans alike.

Key words: Antigens, Bioreactors, Cost-effective, Goats, Plant-based vaccines.

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INTRODUCTION

In an ever-changing world where animal health is paramount to sustainable agriculture and human well-being, innovative approaches to vaccination are gaining significant attention. One such pioneering avenue is the utilization of plant-based vaccines for animals, a cutting-edge field that holds immense promise in revolutionizing veterinary medicine. Traditional methods of vaccine production have primarily relied on the use of live attenuated or inactivated pathogens. While effective, these vaccines may pose some challenges, including the risk of pathogen reversion, cold chain requirements, and concerns regarding safety. In contrast, plant-based vaccines offer a sustainable and environment friendly alternative with the potential to address these limitations. Amidst the ever-evolving landscape of animal health and agriculture, a remarkable paradigm shift is underway in the realm of vaccination. A novel and eco-conscious approach, plant-based vaccines for animals, has emerged as a beacon of hope, promising a greener and more sustainable future for veterinary medicine (Streatfield and Howard, 2003).

The significance of immunization in safeguarding animal populations from infectious diseases cannot be overstated. Traditional vaccine production, while effective, has been associated with certain challenges, Plant-based vaccines are recombinant protein subunit vaccines (Streatfield, 2005), which were conceptualized as antigenic formulations derived from transgenic plant biomass expressing specific antigens (Garcia-Alonso *et al*., 2014). Mason and Arntzen in the year 1995 developed the concept of transgenic plants for the development of vaccines and demonstrated for the first time in the publication of a patent application by Roy Curtiss, III and Guy A. Cardineau, who achieved the production of transgenic tobacco plants capable of expressing a colonization antigen of *Streptococcus mutan* (Garcia-Alonso *et al*., 2014). The first licensed vaccine was

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produced against Newcastle disease virus infection in poultry manufactured in tobacco suspension (Tacket, 2007; Yusibov and Rabindran 2008). The COVIFENZ® COVID-19 vaccine produced in *Nicotiana benthamiana* has been approved in Canada, and several plant-made influenza vaccines have undergone clinical trials (Su *et al*., 2023). Plant recombinant technologies are being constantly improved and diversified (Hefferon, 2014).

Production of Plant-Based Vaccines

The development of plant-based vaccines begins with the identification, isolation, and characterization of a specific pathogenic antigen (Laere *et al*., 2016). This antigen is the key component that will trigger the desired immune response in animals upon consumption of the plant-based vaccine. Once the target sequence of the antigen is identified, it is integrated with a suitable vector, often a plasmid or viral vector, to facilitate its transfer into the plant's expression system. This vector carries the genetic information of the antigen and acts as a delivery vehicle to introduce the antigen's genetic material into the plant cells. The gene encoding the antigen is then cloned into a transfer vector, a step that involves inserting the genetic material into the vector in a precise and controlled manner. This cloning process ensures that the genetic information of the antigen is correctly integrated into the transfer vector (Concha *et al*., 2017). Subsequently, the transfer vector carrying the antigen gene is transferred into the plant cells, where it becomes integrated into the plant's genome. This integration can occur either through stable transformation or transient transformation. In the stable transformation system, the genetic material is introduced into the plant's germline cells, such as pollen or ovules, which results in the transmission of the transgene to subsequent generations of the plant. This heritable transmission ensures that the antigen will be expressed in the fruit or plant tissues throughout the plant's growth and development (Laere *et al*., 2016).

On the other hand, transient transformation involves introducing the antigen gene into non-germline cells of the plant. This results in the expression of the antigen in a limited area or for a specific period. Transient expression systems are often used for rapid and temporary production of the antigen, particularly in the research and development stages. Regardless of the transformation method used, the plant cells begin to produce the antigen as a result of the integrated transgene. The antigen is then synthesized and accumulated within the plant tissues, usually in the leaves or fruits. Upon ingestion of the plant-based vaccine by animals, the antigens are released from the plant cells and delivered to the digestive system. S1 glycoprotein gene of chicken infectious bronchitis virus (IBV) has been introduced into potato (Zhou *et al*., 2004). High neutralizing antibody titers against IBV were obtained in chicken serum after three oral immunizations with transgenic potato tubers (5 g; 12.45 µg of S protein) or two or three intramuscular immunizations with transgenic potato extracts. *In vitro* confirmation of spleen cell formation showed that the potato-based vaccine produced protective immunity. Rice and tobacco were shown to express the protective antigen VP2, which effectively shielded chickens from a highly virulent strain of the infectious bursal disease virus (IBDV) (Gomez *et al*., 2013; Wu *et al*., 2007).

Transformation System in Plant-Based Vaccine Production

Stable transformation system can be achieved through nuclear or plastid integration. Biolistic and genetically modified *Agrobacterium* strain can lead to the formation of stable transfection. Transient transformation system involves the production of desired protein or antigen soon after the heterologous gene resides transiently in the host cells. The transgene is not incorporated into the genome of the plant cells. Two most commonly used methods that would achieve transient expression of a desired protein in plants are the *Agrobacterium*-mediated transformation of genetically modified plant virus and particle bombardment (Laere *et al*., 2016).

(A) Direct Gene Delivery Method

The direct gene delivery method simply means the direct introduction of DNA or RNA into the plant cells (Sanford, 1990; Korban, 2005). The most common direct gene delivery approach is biolistic method (gene gun or microprojectile bombardment method). It involves the use of gold or tungsten as a microcarrier to coat the DNA. The coated DNA will then be placed on top of the macrocarrier, inserted into the gene gun, and subjected to high pressure of helium gas (Shah *et al*., 2011; Saxena and Rawat, 2014). Due to the high pressure, the coated DNA will travel at a high speed within a vacuum and penetrate into the cells of the targeted plant (Vasil and Vasil, 2006).

(B) Indirect Gene Delivery Methods

Indirect gene delivery involves the utilization of plant bacteria, particularly the *Agrobacterium* species and plant viruses, which naturally infect the plant cells and are able to integrate the gene of interest into the plant genome (Chen and Lai, 2015).

a. Agrobacterium-Mediated Gene Transfer

Agrobacterium is a Gram-negative soil pathogenic bacterium that naturally infect plants and transfer their genes (T-DNA) to the nucleus of the plant cells (Gomez *et al*., 2010). Two strains of *Agrobacterium* species that have been commonly used as a biological vector are *A. tumefaciens* and *A. rhizogenes*. The main difference between these two species is the plasmid that they carry. *A. tumefaciens* carries tumour-inducing plasmid (Ti-plasmid), while *A. rhizogenes* carries root-inducing plasmid (Ri-plasmid) (Sharma and Sood, 2011).

In the Ti-plasmid, genes encoding for plant hormones such as auxin and cytokinin synthesis will induce tumour tissue in plants. However, for vaccine production, these genes will be deleted to form disarmed Ti-plasmid and a heterologous gene is inserted forming a recombinant plasmid vector. The recombinant plasmid vector is transformed into *A. tumefaciens* and with the help of *vir* gene of the bacterium, the introduced heterologous gene is transferred by the transformed bacterium and integrated into the host plant nuclear genomic DNA (Laere *et al*., 2016).

b. Agroinfiltration Approaches

Agroinfiltration is a method that involves the infiltration of *A. tumefaciens* suspension into the intracellular spaces of desired parts of the plants by using a syringe and results in transient expression of desired protein or transgene. It can improve the expression level of antigen protein in plant cells (Kim *et al*., 2016). There are two methods of agroinfiltration, which are syringe infiltration and vacuum infiltration. Syringe infiltration is the simplest method where, by using a needleless syringe, the transformed *A. tumefaciens* is injected into the leaf (Chen *et al*., 2018). Another method of agroinfiltration is vacuum infiltration which involves the submerging of the leaves in the infiltration buffer containing transgene-carrying *A. tumefaciens*. A negative atmospheric pressure is subjected to the submerged leaves in the vacuum chamber with the aim of withdrawing the air present in the interstitial spaces of the leaves and occupying the space with the transformed *A. tumefaciens* (Chen and Lai, 2015).

c. Genetically Engineered Plant Virus

In this method, a suitable plant virus is modified in order to create chimeric gene for viral coat protein. Thus, it acts as a vector to deliver genetic materials into the plant cells. This method results in transient expression of antigen in plants. The recombinant virus will express the desired protein or peptide as a by-product of viral replication activity during viral infection in plants (Yu and Langridge, 2000). Plant virus expression system involves mostly the engineered RNA viruses such as tobacco mosaic virus (TMV), potato virus X (PVX), alfalfa mosaic virus (AIMV), cucumber mosaic virus (CMV), and cowpea mosaic virus (CPMV) as expression vector. These viruses are not known to replicate in mammalian cells; hence they act as an excellent alternative to replicating vaccine vectors for development of both human and veterinary vaccines (Fujiki *et al*., 2008).

IMMUNITY INDUCED BY PLANT-BASED VACCINE

There are two options for vaccine administration: injection (intramuscular or subcutaneous) and mucosal (oral or nasal) administration. Injection-type vaccines elicit strong protective immunity by preferentially inducing IgG production. They are most suitable against pathogens that infect via a systemic or respiratory route; however, the antigens have to be purified before administration. These vaccines are often produced in tobacco plants using transient expression. Oral- or nasal-type vaccines induce mucosal and systemic immunity (Azegami *et al*. 2014; Lamichhane *et al*. 2014). Once these vaccines pass through the gastric environment and reach the small intestine, antigens are incorporated into M cells in the follicle-associated epithelium (FAE) for the induction of mucosal and systemic immune responses (Holmgren and Czerkinsky, 2005; Azegami *et al*., 2014).

ADVANTAGES OF PLANT-BASED VACCINES

The utilization of plants as bioreactors for producing plant-based vaccines offers numerous advantages over conventional vaccine production methods. These advantages have the potential to revolutionize the field of immunization and contribute to a more sustainable and efficient approach to protecting animal health and public health. Some of the key advantages include:

1. ***High Yield of Recombinant Proteins:*** Plants have the remarkable ability to produce high amounts of recombinant proteins efficiently. This high yield of proteins makes them ideal bioreactors for vaccine production, enabling the generation of a significant quantity of antigens needed for large-scale vaccine manufacturing.
2. ***Pathogen-Free and Safer Vaccines:*** Plant-based vaccines are produced using recombinant subunit technology, which means they contain only specific antigens and not live pathogens. As a result, they are considered safer than traditional vaccines, as they do not carry the risk of causing the disease they are designed to protect against. The absence of live pathogens in plant-based vaccines eliminates any potential risk of pathogenic contamination (Takeyama *et al*., 2015).
3. ***Storage Stability and Cost-Effectiveness:*** Plant-based vaccines offer advantages in terms of storage stability and cost-effectiveness. These vaccines do not require refrigeration during storage, reducing the cost and logistical challenges associated with maintaining a cold chain for transportation and storage. This characteristic is especially beneficial for regions with limited access to reliable refrigeration facilities.
4. ***Eco-Friendly and Sustainable Production:*** The production of plant-based vaccines is environment friendly and sustainable. It relies on renewable resources (plants) to serve as natural bio-factories for vaccine antigen production. This approach reduces the carbon footprint associated with vaccine production and aligns with sustainable agricultural practices (Yusibov and Rabindran 2008).
5. ***Rapid Production and Time-Efficiency:*** The plant-based vaccine production process allows for rapid antigen synthesis and vaccine development. Compared

to conventional methods, which can involve time-consuming steps in pathogen cultivation and inactivation, plant-based vaccines can be produced more quickly and efficiently.

6. ***Simplified Delivery:*** Plant-based vaccines offer a simplified delivery system. Once the antigens are expressed within the plant tissues, the vaccines can be administered orally, eliminating the need for injections. This administration method may improve vaccination acceptance and coverage, particularly in animals and wildlife.

CHALLENGES OF PLANT-BASED VACCINES

While plant-based vaccines offer significant advantages, they also present several challenges that need to be addressed to fully realize their potential. Overcoming these challenges is crucial to ensure the successful development, manufacturing, and widespread adoption of plant-based vaccines. Some of the key challenges include:

1. ***Antigen Selection and Host Compatibility:*** Selecting the appropriate antigen and the compatible plant host is a critical challenge in plant-based vaccine development. The antigen must be carefully chosen to ensure its immunogenicity and ability to elicit a strong and specific immune response in the target animals. Additionally, identifying the suitable plant host that can efficiently produce and express the antigen is essential for achieving optimal vaccine efficacy (Rigano and Walmsley, 2005; Sharma and Sood, 2011).
2. ***Consistency and GMP Manufacturing:*** Ensuring consistency in vaccine dosage and adhering to Good Manufacturing Practice (GMP) standards are vital aspects of vaccine production. Maintaining uniformity in antigen expression across different batches of plant-based vaccines is essential to achieve consistent immunogenicity and efficacy. Meeting GMP requirements is essential for ensuring the quality, safety, and reproducibility of vaccines, and it may involve overcoming challenges related to scalability and standardization (Laere *et al*., 2016).
3. ***Enhancing Immunogenicity of Oral Vaccines:*** Oral vaccines delivered through plant-based systems face challenges related to achieving sufficient immunogenic activity. To enhance the effectiveness of oral vaccines, specific adjuvants or delivery systems may be required to augment the immune response and induce robust protection against the targeted pathogens. Identifying suitable adjuvants that do not compromise safety while enhancing immunogenicity is a crucial area of research (Mestecky *et al*., 2008).
4. ***Regulatory Compliance:*** Regulatory guidelines set forth by government agencies, such as the US Department of Agriculture (USDA) and the Food and Drug Administration (FDA), are essential for ensuring the safety, efficacy, and quality of plant-based vaccines. Navigating these regulatory frameworks, especially with regard to the growth of transgenic plants, manufacturing processes, and clinical trials, requires strict adherence and compliance. Addressing any potential concerns related to biosafety, environmental impact, and public health is paramount for gaining regulatory approval and market acceptance (Laere *et al.,* 2016).

CONCLUSION

Plant-based vaccines represent a transformative and promising approach in the field of immunization, offering numerous advantages for the prevention and treatment of both human and animal diseases. The utilization of plants as bioreactors provides an innovative solution to overcome the limitations of conventional vaccine production methods. With their higher therapeutic value and safer characteristics, plant-based vaccines have the potential to revolutionize the landscape of healthcare and agriculture. The success of plant-based vaccines lies in selecting the right antigen and compatible host plant to ensure optimal expression and immunogenicity. Through advancements in chloroplast transformation via biolistic or particle bombardment gene delivery methods, researchers have found a very promising alternative for enhancing the production efficiency of plant-based vaccines. By harnessing the natural immune response of plants, plant-based vaccines have the ability to produce high amounts of recombinant proteins without the need for live pathogens. This advantage makes them safer and more cost-effective compared to traditional vaccines. Additionally, the storage stability of plant-based vaccines and their reduced dependence on refrigeration further improve accessibility, particularly in resource-limited regions. As with any transformative technology, challenges must be addressed to fully realize the potential of plant-based vaccines. Overcoming obstacles related to antigen selection, dosage consistency, GMP manufacturing, and regulatory compliance is essential for achieving widespread adoption and acceptance. Moreover, efforts to enhance the immunogenicity of oral vaccines through appropriate adjuvants are crucial to maximize their effectiveness. The success of plant-based vaccines also relies on raising awareness and building public trust regarding their safety, efficacy, and benefits.

Thus, the emergence of plant-based vaccines represents a significant leap towards a greener, safer, and more sustainable approach to immunization. As research and development in this field continue to progress, the world stands on the cusp of a new era in vaccine technology, one that has the potential to transform the health and well-being of both humans and animals alike. Embracing the power of plant-based vaccines, we embark on a journey towards a future where the boundless potential of nature converges with the ingenuity of science to create a healthier and more resilient world.

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