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Current Trends in Edible Vaccine Development using Transgenic Plants

Tae-Geum Kim and Moon-Sik Yang*

Division of Biological Sciences, Chonbuk National University and Jeonju Center, Korea Basic Sciences Institute, Jeonju 561-756, Korea

Abstract Immunology textbooks currently report orally administered antigens as inducing immune tolerance rather than immune stimulation. Nevertheless, current plant-based edible vaccine technology, if sufficiently developed, may offer several advantages. For example, it is easy to apply, store, and transport. It could also induce both mucosal and systemic immune responses, which cannot be achieved using an injection vaccine. Plant-based vaccines are also anticipated to prove quite useful in the animal industry, since the cost of injection is a significant burden in the animal industry. Although no commercial plant-based edible vaccines are currently available, several candidate vaccines are undergoing clinical trials. Consequently, many scientists are anticipating that a commercial plant-based edible vaccine will be available in the near future. © KSBB

Keywords: plant-based vaccine, transgenic plant, edible vaccine

WHAT IS A PLANT-BASED EDIBLE VACCINE?

Plant-based edible vaccines are plant materials administered *via* the oral route, prepared by using transgenic plants that express antigen proteins capable of inducing protective immunity against various human and animal diseases to induce specific immune responses. Usually, vaccination is a process by which immune responses against pathogens are induced without real infection and can protect the hosts against pathogenic infection.

Vaccines are materials used for vaccination. Vaccines can be categorized based on their methods of production, *e.g.* live vaccines, killed vaccines, attenuated vaccines, and recombinant vaccines. Vaccines can be also categorized based on their method of application such as the injection method, which is the most popular method, as well as aerosol application and oral administration. The pattern of immune response induction is known to be profoundly influenced by the route of antigen administration, since the pattern of immune response induction is determined by the type of antigen-presenting cells in the antigen administration.

Among the various categories of vaccine described above, the recombinant vaccine is generally regarded as the most economical and safe type of vaccine. Additionally, oral vaccines are considered the easiest vaccine administration method, based on the feasibility and cost of vaccine application. Additionally, based on a 1992 WHO report asserting that vaccines for children should be inexpensive, easily applicable, and easily containable under ambient conditions, it is conceivable that plant-based edible vaccines may constitute one of the best and most effective candidates for vaccines to be administered to children.

In order to obtain transgenic plants for the development of edible vaccines, two different strategies such as nuclear transformation and transient expression using plant virus and/or *Agrobacterium* are usually applied. The initial effort to introduce heterologous genes into plants dates from the early 1980's, during which *Agrobacterium tumefaciens* was utilized. *A. tumefaciens* is a gram-negative soil bacterium capable of introducing its own T-DNA and transforming plant cells. The introduced bacterial genes encode for plant hormones and induce tumor tissues in plants, such as crown gall. In this event, the bacterium utilizes the Ti (tumor-inducing) plasmid for gene transfer. The principle of gene transfer *via* T-DNA made it possible to use the plasmid as the vehicle for heterologous gene transfer into plants. In detail, the recombinant plasmid vector, in which the hormone gene was removed and the heterologous gene was inserted, is initially transformed into *A. tumefaciens* and the recombinant bacterium transfers the introduced heterologous gene into the nuclear genomic DNA of the host plant with the help of the *vir* gene product in *Agrobacterium*. *Agrobacterium*-mediated plant transformation is done primarily with dicotyledons, but there have been recent reports asserting that the

***Corresponding author**
Tel: +82-63-270-3339 Fax: +82-63-270-4334
e-mail: mskyang@jbnu.ac.kr

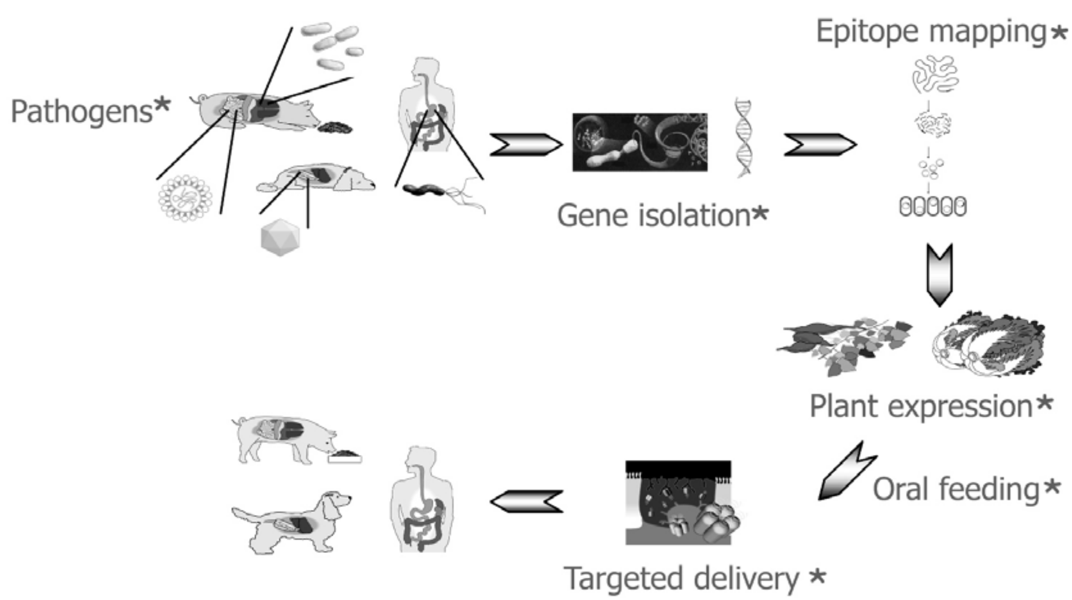

Fig. 1. General principle for the development of a plant-based edible vaccine.

method could be applied to monocotyledons. When *Agrobacterium*-mediated transformation is not feasible, such as in the case of monocotyledons, a transformation procedure using gene gun technology is applied. In order to introduce the heterologous gene using a gene gun, metal particles 1~3 μm in diameter are coated with plasmid vectors harboring the genes to be introduced and the particles are applied to plant cells using a gene gun at high pressure. This method has an advantage in that it allows the introduction of more than two heterologous genes, although it also has the disadvantage of requiring expensive experimental tools and also that the introduced genes are rather unstable. Nevertheless, since the advent of the portable and inexpensive gene gun, this method may be used for the introduction of large amounts of heterologous genes in a short period of time. However, according to recent reports regarding the successful introduction of heterologous genes into monocotyledons using *Agrobacterium*, *Agrobacterium*-mediated gene transfer is a more favorable method for the introduction of heterologous genes into monocotyledons than direct gene transfer using gene gun technology.

There are some problems with *Agrobacterium*-mediated gene transfer for heterologous gene expression using transgenic plants. For example, the establishment of transgenic plants *via* the nuclear transfer method requires a long time. Additionally, the expression level of heterologous protein in transgenic plants is usually not particularly high. In order to overcome these problems, new technology that combines the technology for plant viral vector systems with *Agrobacterium*-mediated gene transfer has been introduced [1,2] and is currently being used for the transient expression of large quantities of heterologous proteins in *Nicotiana benthamiana*. This method, referred to as the magnifection method [3], involves the application of a viral vector system combined with the *Agrobacterium*-mediated gene transfer method and can be utilized to generate abundant proteins within a period of several days. This method is useful for the production of heterologous proteins, and can be easily and frequently used for the production of monoclonal antibodies in plants.

SIGNIFICANCE OF PLANT-BASED EDIBLE VACCINE TECHNOLOGY

Plant-based edible vaccines are one of the more recently proposed vaccine technologies [4,5]. Plant-based edible vaccine technology has been developed based on the notion that the vaccine can be readily applied and is safe, similarly to recombinant vaccines. The main objectives of plant-based edible vaccine technology are the transformation of antigen genes into plants, the production of prorecombinant antigen proteins in transgenic plants, and the oral application of the transgenic plant (possibly in food) to induce introduced antigen-specific immune responses (Fig. 1). Plant-based edible vaccines are generally more useful than conventional injection vaccines. For example, the nature of the plant-based edible vaccine for easy application makes it beneficial for vaccines to be administered to children. Since the plant-based edible vaccine can efficiently induce mucosal immune responses, which is not feasible with a conventional injection vaccine, the technology is quite useful for the development of vaccines against gastrointestinal infectious diseases. Plant-based edible vaccines do not require a cold chain to preserve the vaccine; hence, the technology is very useful for vaccines to be used in countries without concrete health infrastructure. Finally, as the cost for vaccine injections in the animal industry is related closely to the productivity of the industry,

Table 1. Status of plant-based edible vaccine development

Company	Plant	Grown in	Product	Disease	Status
Plant Biotechnology	Tobacco	Field	Secretoty antibody vaccine	Tooth decay	E.U. approved
Dow AgroScience	Tobacco	Cell culture	Poultry vaccine	Newcastle disease	USDA approved
CIGB, Cuba	Tobacco	Greenhouse	Vaccine purification antibody	Hepatitis B	On market
Arizona State University	Potato	Greenhouse	antigen	Hepatitis B	Phase II trial
Large Scale Biology	Tobacco		antigen	Non-Hodgkin's lymphoma	Phase II trial
Arizona State University	Potato	Greenhouse	antigen	Norwark virus	Phase I trial
Thomas Heferson	Spinach		antigen	Rabies virus	Phase I trail
ProdiGene	Maize		antigen	Diarrhea	Phase I trial

Modified from Kaiser, J. [32]

plant-based edible vaccines represent a viable alternative for injection vaccines from the perspective of production cost. There are currently no commercially available plant-based edible vaccines on the market [5].

However, based on a report asserting that phase III human trials are currently meeting with success, it is conceivable that commercial plant-based edible vaccine products may become available in the near future. Additionally, since this technology utilizes the most advanced modern vaccine manufacturing and vaccination technologies, the social and economical impact of the technology might prove to be enormous.

One of the concerns regarding plant-based edible vaccine technology is that the orally administered antigen generally induces immune tolerance, rather than immune stimulation. In other words, the vaccinated individual is not capable of responding to the pathogen, even after vaccination. Fortunately, however, plant substances including lectins that are incorporated into the vaccination materials appear to function as vaccine adjuvants. Additionally, the enhancement of antigen levels in transgenic plants should also be ensured prior to the practical application of plant-based edible vaccines. Several approaches have been used to enhance the antigen expression levels in transgenic plants. For example, strong promoters can be applied to improve antigen expression in transgenic plants. Additionally, other factors that might be influential in augmenting antigen gene expression have been searched extensively. The optimization of the amino acid codons in plants is also considered as a strategy, by which antigen gene expression in transgenic plants can be improved. More importantly, the chloroplast transformation method [6] and the use of a viral expression vector system [2] are considered useful methods for the high-level expression of antigen genes introduced into transgenic plants due to the high copy number characteristics of the expression systems. Those efforts were reported to be successful protocols for the enhancement of antigen gene expression in transgenic plants. As an alternative approach to improve the vaccination efficacy of plant-based edible vaccines, it might be possible to improve the efficiency of antigen delivery into the mucosal immune system. One such strategy is the use of a toxin with known binding affinity to the mucosal surface [7]. Enterotoxins are composed of two subunits one for binding and the other for toxicity, and the binding subunit might be employed as an antigen-targeting material. For example, the binding subunit of enterotoxin can be used as a vaccine carrier and/or an adjuvant, either conjugated to the antigen or in the form of a co-administered material. The toxin subunits are the cholera toxin B subunit and the enterotoxigenic *Escherichia coli* heat-labile enterotoxin B subunit. Those toxin subunits have been well documented for their adjuvant activity for orally-administered antigens. If an efficient immune response induction can be achieved *via* any one of these several approaches, plant-based edible vaccines might take their place as among the most advanced available vaccination protocols.

There are several factors used to decide on the target plant for vaccine production, including vaccine type, vaccine storage, and the part of the plant to be employed. Banana, potato, tomato, corn, rice, wheat, and barley are considered as possible target plants due to the efficiency with which they can be stored and transported, as well as the fact that the plants are largely consumable. In particular, alfalfa and corn, which are major ingredients in animal feed, are considered good target plants for the development of plant-based edible vaccines for use in animals. Those plants have been shown to be feasible in the antigen gene transformation of *Agrobacterium*-mediated and gene gun methods.

CURRENT STATUS OF PLANT-BASED EDIBLE VACCINE DEVELOPMENT

The production of medically useful proteins using transgenic plants initially drew public attention when Nature published a manuscript in 1989 describing the production of monoclonal antibody from the tobacco plant [8]. Monoclonal antibodies are utilized for a variety of purposes, including the treatment of arthritis and cancer. The production of monoclonal antibody *via* a plant expression system has lowered the price of the production of 1 Kg of monoclonal antibody from 3 million dollars to 100 dollars. Additionally, the production of medically useful proteins using a plant expression system provides an additional benefit in that unwanted contaminants that might be included by using an animal cell culture system to generate the protein could be eliminated.

Among the medically useful proteins, the production of vaccine material and its efficacy was reported in Science in 1995 by the Biodesign Research Team at Arizona State University, led by Dr. Charles Arntzen [9]. They expressed the heat-labile toxin B subunit of enterotoxigenic *E. coli* (LT-B) in the potato, orally administered it to mice and confirmed the efficient induction of immune responses. This report published by Haq *et al.* [9] drew great attention from the public, as the vaccine material is extremely precious for people in underdeveloped and developing countries, to whom the vaccine materials are not available due to economic issues. Since then, there have been many publications regarding plant-based edible vaccines against animal [10-13] and human diseases [14-30]. Extensive review of research articles on plant-based edible vaccines was published by S. Tiwari *et al.* [31]. Current status of development on plant-based edible vaccine in clinical trials is summarized in Table 1. Although no plant-based edible vaccines are currently commercially available, a secretory antibody vaccine was approved in the EU, a poultry vaccine against Newcastle disease was approved by the USDA, and a hepatitis B virus vaccine using a tobacco plant has been approved in Cuba. As shown in Table 1, both a potato-based vaccine against the hepatitis B virus and a plant-based vaccine against the rabies virus are undergoing human trials, and thus a plant-based edible vaccine is expected to be commercialized within a couple of years. Additionally, Rybicki [5] described that big companies such as Bayer and Dow Agro Sciences are also entering the fray, which is a good sign for the future of plant-based edible vaccines.

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