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Edible Vaccines

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9.1 INTRODUCTION

Vaccines are considered primary tools for health intervention in both humans and animals. Vaccines can be used more widely, especially in developing countries, if their cost of production can be reduced and they can be preserved without refrigeration. In developing countries certain limitations, like vaccine affordability, the need for “cold chains” from the producer to the site of use of the vaccine, and the dependence on injection, are barriers to health care services. Plant-derived vaccines do not face such limitations. Research under way is dedicated to solving these limitations by finding ways to produce oral (edible) vaccines from transgenic plants. Plant-derived vaccines offer increased safety, envisage low-cost programs for mass vaccination, and propose a wider use of vaccination for veterinary use [1].

With the advent of modern molecular biology techniques in the 1980s, new strategies were developed for the production of vaccines. These vaccines are comprised of proteins derived from pathogenic viruses, bacteria, or parasites (generally proteins are produced not by the pathogens themselves, but by expression of the gene encoding the protein in a “surrogate organism”) [2]. In the last decade, it was found that green plants can also be used as the “surrogate production organism” to produce antigens of human pathogens (including HBsAg). These proteins can elicit priming and also can boost the immune response in humans when administered orally. In addition, unlike almost all other cell lines used for production of vaccines, components of plant cells have always been an important part of the normal human diet. Plants, therefore, offer significant new opportunities for making safe and effective oral vaccines [3].

9.2 PRODUCTION OF EDIBLE VACCINES

9.2.1 Selection of the Desired Gene and Plant

The introduction of selected desired genes into plants and then inducing these altered plants to produce the encoded proteins is the primary condition for the development of edible vaccines. This process is known as transformation and altered plants are called transgenic plants. Selection of important epitope region(s) from the pathogen of interest is the one of the key factors that determines the success of potential edible vaccines. Edible vaccine development has been challenged by low expression levels of foreign proteins in transgenic plants. Reported expression rates range from 0.01% to 2% total soluble protein (TSP), which can render edible vaccine proteins less immunogenic. Selection of strong plant-specific super promoters to improve expression levels is another key factor that can determine the success of edible vaccines [4].

9.2.2 Plant Transformation

9.2.2.1 Antigenic Gene Transformation through a Suitable Vector

After transformation of tobacco, great efforts have been made to develop efficient methods for genetic transformation and optimizing expression of foreign genes in plants. The techniques used to introduce foreign genes into plants have been extended to major crops, vegetables, and ornamental and medicinal plants. Various foreign proteins including serum albumin, human α -interferon, human erythropoietin, and murine IgG and IgA immunoglobulins have been successfully expressed in plants. In recent years, several attempts have been made to produce various antigens and antibodies in plants. Antigens or antibodies expressed in plants can be administered orally as any edible part of the plant, or by the parenteral route (such as intramuscular or intravenous injection) after isolation and purification from the plant tissue. The edible part of the plant to be used as a vaccine is fed raw to experimental animals or humans to prevent possible denaturation during cooking, and avoid cumbersome purification protocols [5].

While *Agrobacterium*-mediated transformation still remains the method of choice for dicots, a general method, the biolistics method, of transformation of plants, including

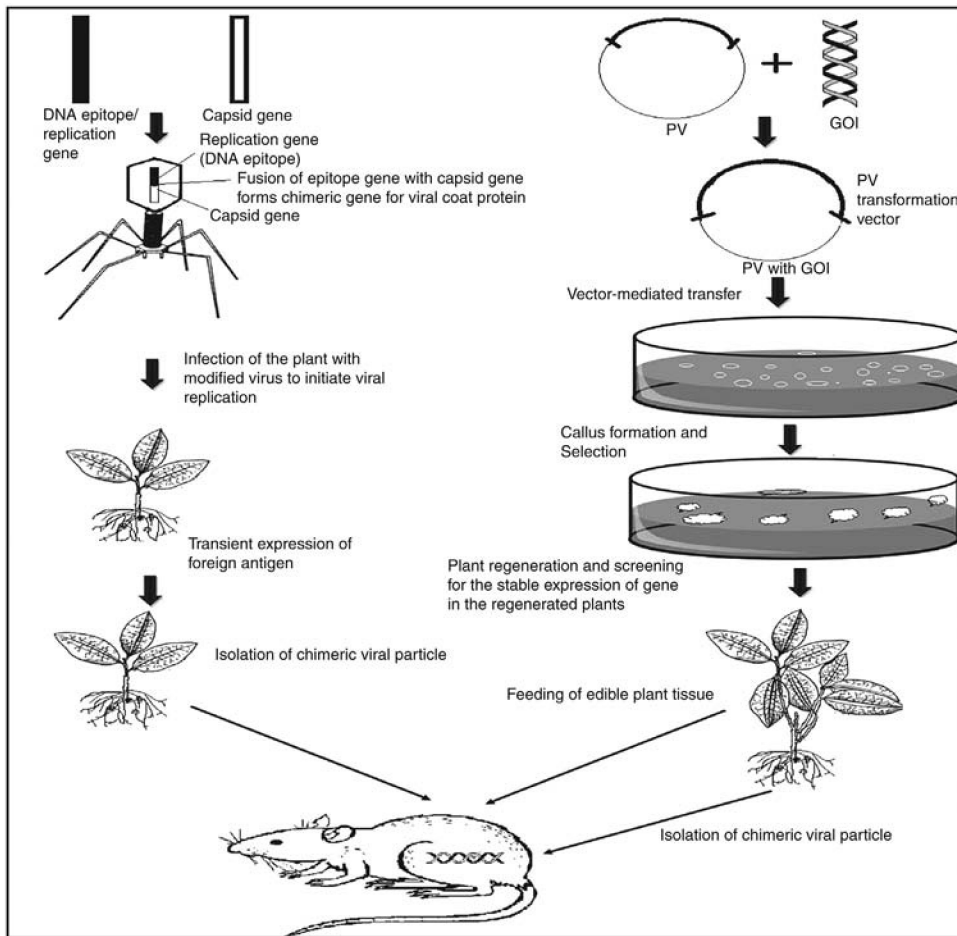


FIGURE 9.1 Strategies for the production of edible vaccine. PV (plasmid vector); GOI (gene of interest).

monocots, has come into existence. Other strategies for expression of foreign genes in plants include use of strong and organ-specific plant promoters, targeting of the protein into endoplasmic reticulum (ER) by incorporating ER-targeting and ER-retention signals, creation of an optimized translation start site context, as well as alteration of codons to suit the expression of prokaryotic genes in a plant. For the production of edible vaccines or antibodies, it is desirable to select a plant whose products can be consumed raw to avoid degradation during cooking. Thus, plants like tomato, banana, and cucumber are generally the plants of choice. While expression of a gene into the genome allows maintenance of the material in the form of seeds, some virus-based vectors can also be used to express the gene transiently to develop the products in a short period (Fig. 9.1). This may have the additional advantage of allowing expression of the product at a very high level; not always attainable in transgenic systems [6].

9.2.2.2 Transformation of Chimeric Gene through Viral Infection

Plus-sense, single-stranded plant RNA viruses have been proposed as an effective alternative to produce vaccine antigens in plants. In this technique, the epitope of interest is engineered into a plant virus, usually within the coat protein gene. Infection of a susceptible non-GM-plant results in intracellular production and accumulation of the epitope. The epitope sequence, as well as the viral genome, never become integrated into the plant genome and hence are only expressed by the generation of infected cells. A recombinant cowpea mosaic virus has shown to elicit protective immunity in mink when engineered to express the antigenic epitope against mink enteritis virus. Recombinant alfalfa mosaic virus (AIMV) has enabled expression of significant quantities of rabies virus and HIV epitopes upon integration of their respective coding sequence into the AIMV coat protein. The extra sequences were found to protrude from the virion surface without interfering with virus assembly. Recombinant AIMV coat protein molecules have also demonstrated the ability to assemble into particles containing three different epitopes from HIV and rabies. This demonstrates the ability of plant viruses to produce multicomponent vaccines [7]. Claimed advantages of transient viral expression of transgenes over transgenic plants are: lesser time for cloning of the foreign gene in the viral genome as compared to time required for transformation of plants; the ease at which antigen production can be scaled up; and the wide host range of plant viruses, which allows use of multiple plant species as biofactories.

9.2.3 Evaluation of the Protein in Animal Models

Each single antigen expressed in plants must be verified by animal studies and Western blots, and quantified by enzyme-linked immunosorbent assay (ELISA).

9.3 MECHANISM OF ACTION

Most pathogens enter at mucosal surfaces lining the digestive, respiratory, and urino-productive tracts, which are collectively the largest immunologically active tissue in the body. The mucosal immune system is the first line of defense and the most effective site for vaccination against pathogens. Nasal and oral vaccines are most effective for mucosal infections. The goal of oral vaccine is to stimulate both mucosal and humoral immunity against pathogens. Edible vaccines when taken orally undergo mastication, and degradation of plant cells occurs in the intestine due to the action of digestive enzymes. Peyer's patches are an enriched source of IgA producing plasma cells and have the potential to populate mucosal tissue and serve as mucosal immune effector site. The breakdown of edible vaccine occurs near Peyer's patches, which consist of 30–40 lymphoid nodules on the outer surface of the intestine and also contain follicles from which the germinal center develops after antigenic stimulation. These follicles act as a site for the penetration of antigens in intestinal epithelium. The antigen then comes in contact with M-cells. M-cells express class-2 major histocompatibility complex molecules, and antigens transported across the mucous membranes by M-cells can activate B-cells within these lymphoid follicles. The activated B-cells leave the lymphoid follicles and migrate to diffuse mucosal associated lymphoid tissue where they differentiate into plasma cells that secrete the IgA class of antibodies. These IgA antibodies are transported across the

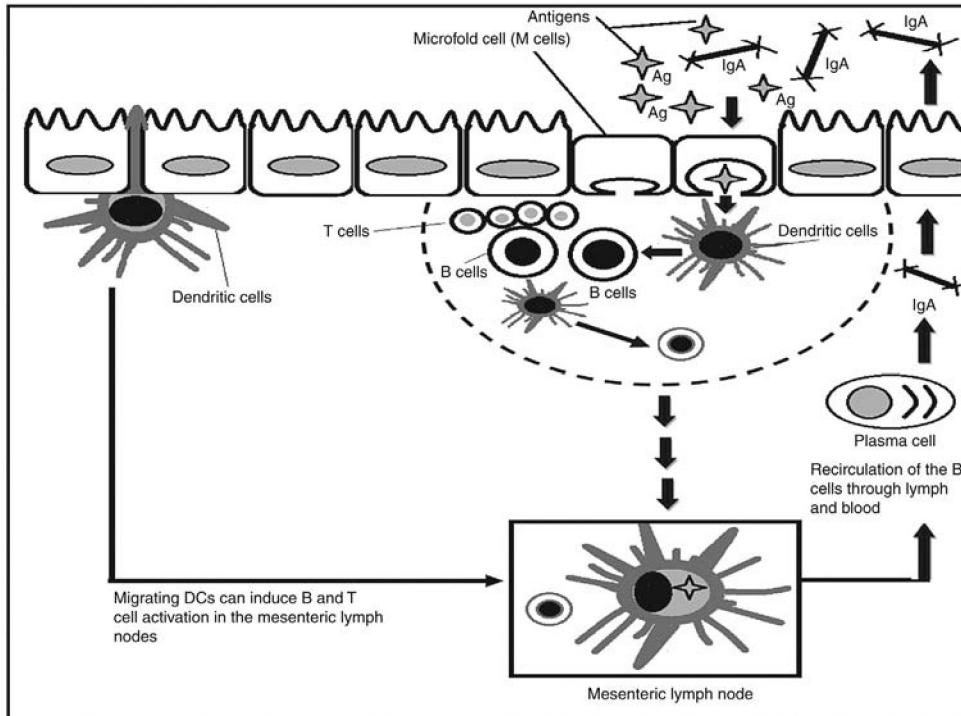


FIGURE 9.2 Mechanism of action of edible vaccines.

epithelial cells into secretions of the lumen where they can interact with antigens present in the lumen (Figure 9.2).

9.4 ADVANTAGES AND DISADVANTAGES OF EDIBLE VACCINES

9.4.1 Advantages of Edible Vaccine

- Edible vaccines are effective as a delivery vehicle for immunization because adjuvants that enhance the immune response are not required.
- Edible vaccine can elicit mucosal immunity, which is not observed in traditional vaccines.
- Edible vaccines are also cost effective in availability, storage, preparation, production, and transportation. Vaccines produced by biotechnological methods are stable at room temperature, unlike traditional vaccine, which needs cold chain storage, which multiplies the yearly cost to preserve vaccines. Moreover, the seeds of transgenic plants could be dried as there is less moisture content in seeds and the plants with oil or their aqueous extracts possess more storage opportunities. Manufacturing cost is low as there is no need for special premises to manufacture them. Edible vaccine can be easily produced at mass level in comparison to an animal system.

- Edible vaccines are well tolerated, as they do not require administration by injection unlike traditional vaccines. Thus, there is also a reduced need for medical personnel and risk of contamination is low. The feasibility of oral administration compared to injection is also an advantage.
- Plant-derived vaccines could be the source for new vaccines combining numerous antigens. These multicomponent vaccines are called second generation vaccines as they allow for several antigens to approach M-cells simultaneously.
- Edible vaccines are subunit preparations, do not involve attenuated pathogens, and improve the safety of individuals as compared to traditional vaccine since there is no possibility of proteins reforming into infectious organisms.
- The separation and purification of vaccines from plant materials is very easy and pathogenic contamination from animal cells can be effectively prevented.

9.4.2 Disadvantages of Edible Vaccines

- There is the possibility of development of immunotolerance to the vaccine protein or peptide.
- Consistency of dosage form differs from plant to plant and generation to generation.
- Protein content varies from plant to plant and generation to generation.
- Ripeness also affects the proteins that are present in form of antigens in the fruits.
- Limitations of methods for standardization of plant material/product.
- Stability of vaccine differs from plant to plant.
- Some food cannot be eaten raw (e.g., potato) and needs to be cooked, which will denature or weaken the protein present in it.
- Variable conditions for edible vaccine are also a major problem. Potatoes containing vaccine can be stored at 4°C for longer time, while tomato does not last long at this temperature. Thus, these vaccines need to be properly stored to avoid infection through microbial spoilage.
- Another concern regarding edible vaccine is the need for proper distinguishing characters to identify between “vaccine fruit” and “normal fruit” to avoid maladministration of vaccine, which could lead to tolerance.
- The glycosylation pattern of plants and humans is different, which could affect the functions of vaccines.

9.5 MOST POPULAR EDIBLE VACCINES PRODUCED BY PLANTS [8,9]

9.5.1 Tobacco

The first report of the production of edible vaccine (a surface protein from *Streptococcus*) in tobacco, at 0.02% of total leaf protein level, appeared in 1990 in the form of a patent application published under the International Patent Cooperation Treaty. Subsequently, a number of attempts were made to express various antigens in plants (Table 9.1). Since acute watery diarrhea is caused by enterotoxigenic *Escherichia coli* and *Vibrio cholerae* that colonize the small intestine and produce one or more enterotoxin, an attempt was made to produce edible vaccine by expressing heat-labile enterotoxin (LT-B) in tobacco.

TABLE 9.1 Benefits Envisaged in Plant-Derived Edible Vaccines

Benefit	Characteristics
Oral delivery	The plant cell wall, consisting essentially of cellulose and sugars, provides protection in the stomach and gradual release of the antigen in the gut
Use as raw food or dry powder	The vaccinogenic plant tissue may be used as raw food or, alternatively, proteins may be partially or fully purified and administered in capsules as dry powder
No need for “cold chain”	The vaccinogenic plant parts or plant extracts can be stored and shipped at room temperature
Mucosal and serum immune response	Plant-derived vaccines are primarily designed to trigger the mucosal immune system (IgA)
Cost efficiency	Production cost will be reduced 100–1000 times as compared with that of traditional vaccines
Optimized expression system	Plants may be engineered to accumulate the antigen in convenient intracellular compartments (endoplasmic reticulum, chloroplast)
Ease of genetic manipulation	Procedures essentially rely on established molecular and genetic manipulation protocols; these are already available
Ease of production and scale-up	GM plants can be stored as seeds. Unlimited vaccine quantity can be produced from these in limited time; production and management are suitable for developing countries
Safer than conventional vaccines	Lack of contamination with mammalian pathogens
Ideal to face bioweapons	Safety and cost efficiency plant-derived vaccines act as an ideal tool to face bioterrorism
Ideal for veterinary use	Cost affordable and ready for use as food additive

9.5.2 Potato

Transgenic potatoes were created and grown by Charles Arntzen Hugh S. Mason and their colleagues at the Boyce Thompson Institute for Plant Research, an affiliate of Cornell University. Transgenic potatoes containing enterotoxin stimulated strong immune responses in animals. An edible vaccine could stimulate an immune response in humans. Volunteers ate bite-sized pieces of raw potato that had been genetically engineered to produce part of the toxin secreted by the *E. coli* bacterium, which causes diarrhea. Ten of the 11 volunteers (91%) who ingested the transgenic potatoes had four-fold rises in serum antibodies at some point after immunization, and six of the 11 (55%) developed four-fold rises in intestinal antibodies. The potatoes were well tolerated and no one experienced serious adverse side effects (Table 9.2).

9.5.3 Tomato

Tomatoes serve as an ideal candidate for this HIV antigen because unlike other transgenic plants that carry the protein, tomatoes are edible and immune to any thermal process, which helps retain its healing capabilities. Even more importantly, compared to bananas tomatoes were found to grow at a high rate of success in Russia.

TABLE 9.2 Advantages and Disadvantages of Different Plants as Transgenic Bioreactors

Plant/Fruit	Advantage	Disadvantage
Tobacco	<ul style="list-style-type: none"> • Good model for evaluating recombinant proteins • Low cost preserving system • Easy purification of antibodies stored in the seeds • Large harvests 	<ul style="list-style-type: none"> • Produces toxic compounds* (toxic alkaloids incompatible w/oral delivery) • Potential for outcrossing in field
Potato	<ul style="list-style-type: none"> • Dominated clinical trials • Easily manipulated/transformed • Easily propagated from its "eyes" • Stored for long periods without refrigeration 	<ul style="list-style-type: none"> • Relatively low tuber protein content • Unpalatable in raw form**; cooking may cause denaturation and poor immunogenicity of vaccine
Banana	<ul style="list-style-type: none"> • Does not need cooking • Proteins not destroyed even if cooked • Inexpensive • Grown widely in developing countries 	<ul style="list-style-type: none"> • Trees take 2–3 years to mature and transformed trees take about 12 months to bear fruit • Fruits spoil rapidly after ripening and contain very little protein, so unlikely to produce large amounts of recombinant proteins
Tomato	<ul style="list-style-type: none"> • Grows quickly • Cultivated broadly • High content of vitamin A may boost immune response • Overcomes the spoilage problem by freeze-drying technology • Heat-stable, antigen-containing powders[†], made into capsules • Different batches blended to give uniform doses of antigen 	<ul style="list-style-type: none"> • Relatively low fruit protein content • Acidic fruit may be incompatible with some antigens or for delivery to infants • No <i>in vitro</i> system to test fruit expression
Rice	<ul style="list-style-type: none"> • Commonly used in baby food because of low allergenic potential • High expression of proteins/antigens • Easy storage/transportation • Expressed protein is heat stable 	<ul style="list-style-type: none"> • Grows slowly • Requires specialized glasshouse conditions
Lettuce	<ul style="list-style-type: none"> • Fast growing • Direct consumption 	<ul style="list-style-type: none"> • Spoils readily
Soybean and alfalfa	<ul style="list-style-type: none"> • Relatively efficient transformation system • High protein content in leaves • Leaves edible uncooked • Ideal system for animal vaccines 	<ul style="list-style-type: none"> • Potential for outcrossing in field • Deep root system problematic for cleaning field
Legumes or cereals	<ul style="list-style-type: none"> • Production technology widely established • High protein content in seeds • Stable protein in stored seeds • Well suited for animal vaccines • Industrial seed processing well established 	<ul style="list-style-type: none"> • Inefficient transformation systems • Heating or cooking for human use may cause denaturation and poor immunogenicity of vaccine (corn meal is exception) • Potential for outcrossing in field for some species

* Currently, therapeutic proteins in tobacco are being produced.

** Some kinds of South American potatoes can be eaten raw. Although some studies show that cooking does not destroy full complement of antigen in potatoes.

[†]Freeze-dried tomato powder containing NV capsid and LT-B was found immunogenic.

TABLE 9.3 Antigens Produced in Transgenic Plants

Protein	Plant	Carrier
Hepatitis-B surface antigen	Tobacco	–
Rabies virus glycoprotein	Tomato	–
Norwalk virus capsid protein	Tobacco	–
<i>E. coli</i> heat labile enterotoxin β -subunit	Potato	–
Cholera toxin β -subunit	Potato, tobacco	–
Mouse glutamate decarboxylase	Potato	–
VPI protein of foot and mouth disease virus	<i>Arabidopsis</i>	–
Insulin	Potato	–
Glycoproteins of swine-transmissible gastroenteritis corona virus	<i>Arabidopsis</i>	–

9.5.4 Banana

For vaccines or subunit vaccinations, bananas seem to be the desired vector. The advantage of bananas is that they can be eaten raw as compared to potatoes or rice, which need to be cooked, and bananas can also be consumed in a pure form. Research is leaning toward the use of bananas as the vector since most third world countries, which would benefit most from edible vaccines, are in tropical climates that are suitable for growing bananas.

9.5.5 Maize

Egyptian scientists have genetically engineered maize plants to produce a protein used to make the hepatitis-B virus vaccine. A team of researchers led by Hania El-itriby, director of Cairo's Agricultural Genetic Engineering Research Institute, developed genetically modified (GM) maize plants that produce the protein known as HBsAg, which elicits an immune response against the hepatitis-B virus and could be used as a vaccine (Table 9.3).

9.5.6 Rice

When predominant T-cell epitope peptides, which were derived from Japanese cedar pollen allergens, were specifically expressed in rice seed and delivered to the mucosal immune system, the development of an allergic immune response of the allergen-specific Th2 cell was suppressed. Furthermore, not only were specific IgE production and release of histamine from mast cells suppressed, but the inflammatory symptoms of pollinosis, such as sneezing, were also suppressed. These results suggest the feasibility of using an oral immunotherapy agent derived from transgenic plants that accumulate T-cell epitope peptides of allergens for allergy treatment (Table 9.4). When the seed expression system is used as a platform for foreign protein production, substantial amounts of recombinant proteins can be accumulated, because the seed is a natural storage organ for accumulating the starch, protein, and oil required for seedling growth. Also, artificial peptides or proteins accumulate in seed, which is in remarkable contrast with other tissues.

TABLE 9.4 Transient Production of Antigens in Plants After Infection with Plant Virus Expressing Recombinant Gene

Influenza antigen	Tobacco	TMV
Murine zona pellucida antigen	Tobacco	TMV
Rabies antigen	Spinach	AIMV
HIV-1 antigen	Tobacco	AIMV
Mink enteritis virus antigen	Black-eyed bean	CPMV

9.5.7 Safety and Public Acceptance [8–10]

Plant-derived vaccines are free from animal pathogen contaminants. Furthermore, plant DNA is not known to interact with animal DNA and plant viral recombinants does not invade mammalian cells. Further safety of plant-derived vaccines can be achieved by following similar regulations established for traditional vaccines. Nevertheless, the present concern over the use of GM plants is now affecting research in this important field, especially in Europe. One of the fears is that GM pollen may outcross with sexually compatible plants (related crops or weeds) and affect biodiversity. In order to address this alarm, several pollen containment approaches have been developed. These are essentially based on the exploitation of different forms of male sterility (suicide genes, infertility barriers, apomixis). An alternative way of solving the problem is engineering vaccines into the chloroplast DNA (cpDNA), which is not transmitted to the sexual progeny through the pollen grains. An additional safety feature would be the recognition of GM plants that produce vaccines by the addition of genes encoding colored plant pigments. It is important to recognize that plants that produce vaccines are medicinal plants and should be grown, processed, and regulated as pharmaceutical products. In the majority of earlier papers, level of antigen accumulation in the plant organ was in the order of 0.1–0.4% of total soluble protein, while the more recent developments on cpDNA integration promises to increase this value to 30% or more. At the latter value, land requirements for industrial plant-derived vaccine production will be in the order of a few thousand square meters. This will definitely enable vaccine-producing plants to be set apart from field grown crop plants and offer added safety when engineered plant viruses are used for transient antigen expression. A further point of public concern in GM plants is the presence of antibiotic resistance genes (used as selective marker in most transgenic plants). Approaches have now been developed to generate GM plants (with both nuclear or cpDNA integration) that do not carry these genes.

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