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Non-Invasive Vaccine Delivery System: A Review

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ABSTRACT:
Edible vaccines refer to a new method of administering vaccines where genetically engineered plants are employed to vaccinate against diseases by inducing the desired immune response without having to use needles and syringes to inject the vaccine. In this case, vaccines are introduced into the foods that are consumed by human beings. As plants that contain the antigen are consumed, the antigen induces the desired immune response. The first process involves selecting an immunoglobulin gene from a bacterium. To achieve this, the immunoglobulin gene is then incorporated into a cloning vector. The vector is then introduced into the plant using *Agrobacterium tumefaciens.* The plant is then allowed to grow in plant tissue culture (PTC) to ensure that the antigen is stable. The plant is then allowed to mature. The mature plant is then allowed to produce the required antigen. The plant is then harvested and the antigen extracted. The plant is then allowed to produce the antigen in a bioreactor. This is followed by assessing the quantity of antigen that is being produced. The process of assessing the quantity of protein is carried out in a laboratory. The vaccines are then processed in the laboratory. The goal of this stage is to produce a biopharmaceutical that will be required for vaccination. The vaccine is then tested to ensure that it will be efficient in inducing the desired immune response. In this case, the antigenic protein is assessed to ensure that it is the protein that will stimulate the production of antibodies. During the process of producing an edible vaccine, the gene and the encoded protein are determined by using a suitable method. In this case, the DNA is determined by carrying out Polymerase chain reaction. The process of separating the gene from the rest of the human gene involves carrying out electrophoresis This approach improves vaccine accessibility, particularly in resource-limited regions, eliminating cold chain dependencies and reducing risks associated with injectable vaccines, thereby revolutionizing global immunization strategies.

KEYWORDS: Edible vaccines, Agrobacterium tumefaciens**,** Immunoglobulin gene, Plant Tissue Culture (PTC), Polymerase Chain Reaction (PCR).

INTRODUCTION:

Infectious diseases are still deadly with over a million deaths every year. Let’s see how they can be stopped from killing so many. A lot of the deaths are due to pathogens that are able to penetrate the mucosal linings of mammalian hosts – about 50%. To prevent their spread, new kinds of vaccines are needed, which can block several stages of the disease’s development. Vaccines act as immunological priming agents – that is, prepare the body to resist them beforehand, as opposed to other drug treatments, which are used after the disease begins. And all of this began with Edward Jenner in 1796 and his smallpox vaccine.
Vaccines helps to mitigate the long-term impacts of infectious diseases by providing both quick protection against invading pathogens and the long-term memory of the body's immune system. However, high costs

and operational issues of traditional vaccines, which stem from their heavy reliance on large-scale manufacturing activities, are hindering their wide usage, particularly in areas with limited resources and low to moderate income.

Edible vaccines stand out as a viable alternative with significant practical and financial benefits to address these issues. They are produced in transgenic plants where immunologically relevant antigens are expressed, instead of large-scale bioprocessing. This makes the production and distribution more straightforward, as there is no need for expensive purification and processing. In addition, the fact that plant-based expression is eukaryotic also means that post-translational modifications can potentially enhance the proteins' immunogenicity.

Immunostimulatory properties have also been shown for proteins expressed in bacteria such as Escherichia coli. However, their low level of expression makes the use of mammalian expression systems more expensive and operationally more complex, though these are also capable of more complex post-translational modifications. Due to their high cost and demand for labor, mammalian expression systems have major financial, technical, and scalability challenges, although they can perform more sophisticated post-translational modifications. Given these constraints, edible vaccines may provide a new and effective vaccination method for the promotion of public health in underserved areas.

HISTORY AND DEVELOPMENT OF EDIBLE VACCINES:

In the 1980s, the possibility of utilizing plants to deliver antigens prompted scientists to invent edible vaccines. The notion that plants tissues could work as platforms for producing immune-stimulating proteins and thus result in a new and simple method of vaccination was a remarkable initiative. This concept began to translate in the early 1990s when it was demonstrated that foreign antigens could be expressed in plants. These breakthroughs kicked off the development of edible vaccine vehicles that can deliver antigens like those that combat the Norwalk and the Hepatitis B viruses. Scientists such as Dr. Charles Arntzen have contributed significantly to the advancement of this field, gaining momentum and proof that it could be practicable. He started his studies on edible vaccines by attempting to use tobacco and potato plants to produce the Hepatitis B surface antigen. In 1996, the first live human oral vaccination demonstrative was achieved.

During the 2000s, research on improving the effectiveness and efficiency of edible vaccines skyrocketed. The ability to enhance the expression of antigens and immunogenicity due to genetic modification (GM) plants was facilitated by developments in genetic engineering, which laid the technical foundation for broader applications.

During the 2010s, the edible vaccine research area widened to encompass bacterial, parasitic, and even cancer diseases. Some of these candidates have advanced to clinical trials, with research moving from basic to translational. Today, the emphasis has shifted to improving the safety, efficacy, and commercialisation capabilities of edible vaccines. For pursuing these innovative, high-potential vaccines, a burgeoning field of activity is to tackle regulatory barriers and enhance public approval. On a global scale, as the field advances, edible vaccines are deemed to be a revolutionary method of vaccination with worldwide coverage, particularly in low-resource settings where ordinary vaccine delivery may be impractical.

PRODUCTION OF EDIBLE VACCINES:

By the process of genetic engineering, genes are introduced into the plants to make them produce necessary proteins, and this is how edible vaccines are produced. The genetically modified plants are known as the transgenic plant, and the process is called transformation. Just like the usual subunit vaccines, the edible vaccines are also composed of the antigenic proteins present in the pathogen, and they do not have the ability to cause the disease. For people who have poor immunity, these vaccines are safe because they are not in a position to cause the disease, and the immune system will be boosted by producing antibodies. First of all, the gene for the antigenic protein that is to be produced should be extracted from the pathogen to produce the vaccine. The gene is then inserted into a suitable gene vector which is used to introduce the gene into the plant. After inserting the gene, the plant starts to produce the antigen, and the animals or people eat them to develop an immune response.

Two main technologies are used for the vaccination of plant tissues:

1.Stable genomic integration:

The most common method used for the clinical trial of edible vaccines is stable genomic integration where after physically integrating the transgene in the plant genome, it can be propagated vegetatively using cuttings or sexually using seeds. A combination of genes may be introduced with the stable transformation method, potentially resulting in multicomponent vaccine production. Additionally, expression of antigens can be specifically ensured in specific plant tissues or organs by choosing suitable genetic regulatory sequences. The transgene can be incorporated into the nuclear or chloroplast genome of the plant. While biolistic (gene gun) methods of direct DNA injection into plant tissue are also being used commonly, Agrobacterium-mediated transfer is the most widely used means of nuclear transformation. The advantages of chloroplast engineering over nuclear transformation—namely, increased levels of expression and reduced gene flow—have gained increasing recognition in recent times.

2. Transient Expression with Viral Vectors:

This method delivers the vaccine gene into plant cells through recombinant plant viruses. Through systemic infection, the virus causes the plant to produce the antigen. Because transitory expression requires inoculating single plants with the viral vector, it is harder to initiate than stable expression. Nonetheless, since the viral vector can replicate within the plant, amplifying the number of gene copies and elevating antigen expression, transient expression allows for more quantities of antigen production.

COMMON METHODS OF EDIBLE VACCINES:

Agrobacterium tumefaciens-Mediated Transformation:

The *Agrobacterium tumefaciens*-mediated process, a gram-negative bacterium found in soil that naturally infects wounded areas of dicotyledonous hosts and causes crown gall disease, is the most widely used technique for genetic modification of plants. This microbe harbored in its tumor-inducing (Ti) plasmid facilitates the horizontal transfer of a segment of DNA known as T-DNA into the host plant nuclear genome, where the imported genes are integrated and expressed.

In its native state, T-DNA bears oncogenes responsible for tumour induction and opine genes that are unique amino acid derivatives upon which the bacterium derives nutrition. Nevertheless, the Ti plasmid is recombined so as to remove the tumour-inducing structures and incorporate genes specifying therapeutically useful antigens for biotechnological applications such as the production of edible vaccines. After integration, they display stable expression and Mendelian inheritance. Individual altered cells are capable of regrowing whole transgenic plants. Using this technique, studies have shown successful antigen expression in model plants. Extracts of these genetically modified plants had a strong antibody response

when given orally to animals, suggesting that they could be used as edible vaccines. Crucially, many agriculturally important plant species, such as those belonging to the Leguminosae and Graminae families, have evolved Agrobacterium-mediated transformation, expanding its uses in the generation of human and animal vaccines.

Biolistic (Gene Gun) Method:

In plants less amenable to Agrobacterium-based methods, the biolistic transformation method—also called particle bombardment or gene cannon technology—provides a viable alternative. In this method, DNA is deposited onto tiny metal beads—most commonly gold or tungsten—before these are propelled into plant tissues with high speed under conditions of partial vacuum.

When these microparticles impact, they penetrate cell walls and membranes, enabling exogenous DNA to enter the cell and potentially become integrated into the nuclear genome. Transformed cells are identified using marker genes such as GUS and are subsequently cultured for potential cloning and propagation.

There are several advantages to this method:

- Since many particles are released, genetic material is introduced into numerous cells at the same time.
- Direct transformation of intact cells to avoid problems related to protoplast isolation.
- Wide applicability and least dependence on the existence, size, or shape of cell walls.

In addition to nuclear transformation, organelle-specific transformations, like those in mitochondria and chloroplasts, may also be accomplished successfully through biolistic delivery. For transgenic expression systems reliant on plastids, this has significant implications.

Chloroplast genetic transformation:

With several molecular and practical benefits, chloroplast transformation has become a strong substitute for nuclear gene integration. All plant cells contain chloroplasts, which are the site of photosynthesis. It is possible to genetically modify these cells to express foreign proteins.

This technique uses a gene gun to deliver recombinant plasmid DNA into chloroplasts. The DNA contains the therapeutic gene, a selectable marker, translational enhancers, and flanking regions for homologous recombination. The plasmid can penetrate chloroplast membranes and incorporate at particular locations within the plastid genome thanks to the high-pressure propulsion.

Regulatory sequences, especially potent promoters like the plastid ribosomal RNA operon promoter (Prrn) and 5′ untranslated sections (5′-UTRs) that improve translation, such as ribosome binding sites (RBS), are essential for effective transgene expression in chloroplasts. These components guarantee high mRNA levels and, as a result, high protein yields.

Other cutting-edge methods, like Galistan Expansion Femto Syringe microinjection and polyethylene glycol (PEG)-mediated transformation, are also used to insert genetic material into chloroplasts in addition to biolistic delivery.

Chloroplast transformation possesses a number of significant advantages, such as:

- Low production cost and high scalability
- By restricting gene flow by pollen, maternal inheritance inherently has genes.
- Homologous recombination for accurate, site-specific integration of genes,
- Elimination of transgene silencing, which is often observed in nuclear modifications,
- The ability to express multiple genes simultaneously during a single transformation event.

Phases of Chloroplast Transformation Development:

- Heteroplasmic Cell Formation: Initial selection step in which transformed and untransformed plastids are present together.

- Generation of Homoplasmic Cells: Plastids in homoplasmic cells all bear the transgene in cells resulting from subsequent selection.
- Multigene expression facilitates co-expression of numerous vaccine-related genes.
- Target Organ Development: Since paternal plastids disintegrate in reproductive organs, inheritance is restricted to maternal lineage, ensuring biosafety.

MECHANISMS OF ACTION OF EDIBLE VACCINES:

The mucosal epithelia of the gastrointestinal, respiratory, and genitourinary tracts—tissues that together make up the greatest immunologically active interface in the human body—are the main points of entry for a wide number of pathogens. The mucosal immune system (MIS), the body's first line of defence, depends on these mucosal surfaces. Consequently, mucosal immunization—especially through the oral and nasal routes—is a very successful method of triggering both systemic and local immune responses against mucosal infections.

By introducing antigens into the edible tissues of genetically modified plants, edible vaccines take advantage of this pathway. The plant cell wall, a strong cellulose barrier that provides defence against enzymatic breakdown and adverse stomach conditions, encapsulates these antigens. Antigenic proteins are protected throughout gastrointestinal transit by this protective process, known as bioencapsulation, and are mostly released in the colon, where plant cell walls are broken down by digesting enzymes and microorganisms.

Specialised epithelial cells called microfold (M) cells, which are found on top of Peyer's patches (PP), which are lymphoid aggregates in the small intestine's ileum and essential parts of gut-associated lymphoid tissue (GALT), sample antigens upon release. The 30–40 lymphoid nodules that make up Peyer's patches contain organised follicles that can trigger immunological responses in response to antigenic stimulation. Antigens can enter these structures through the epithelial barrier and gather in immunologically active areas.

M cells help transcytose antigens to underlying antigen-presenting cells (APCs), like dendritic cells and macrophages, since they express MHC class II molecules. At the same time, interferon-gamma-activated macrophages deliver antigenic peptides to CD4+ T helper cells, promoting immunological activation downstream. Within the Peyer's patch follicles, these interactions encourage B lymphocyte activation and clonal proliferation.

After being activated, B cells go to the mesenteric lymph nodes and mucosa-associated lymphoid tissue (MALT), where they terminally differentiate into plasma cells that secrete IgA. By targeting particular antigenic epitopes, the dimeric secretory immunoglobulin A (sIgA) that is generated is transcytosed over the epithelium and into the mucosal lumen, where it binds and neutralises pathogens to stop colonisation and infection.

Remarkably, B1 cells mediate a sizable amount—roughly 50%—of sIgA synthesis in the gut lumen in a way that is independent of T cells. Because of their polyreactivity, these sIgAs may recognise a wide range of antigens.

One of the main challenges in developing effective oral vaccines is overcoming oral tolerance, a phenomenon where repeated exposure to antigens through the gastrointestinal tract may induce immunological unresponsiveness rather than activation.

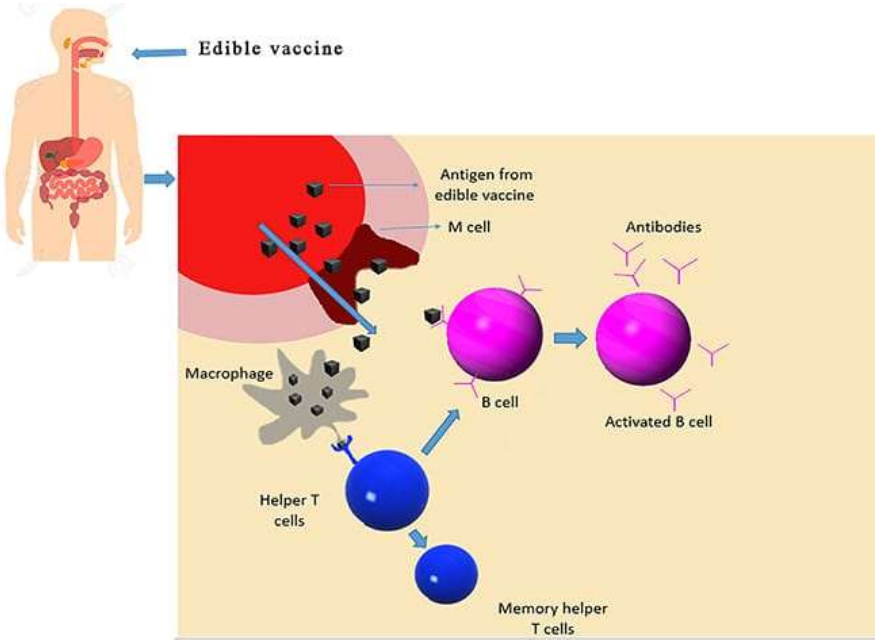

Fig.No:01: Represent the mechanism of action of edible vaccine

COMMONLY USED PLANT FOR EDIBLE VACCINES:

A number of plants are useful for producing edible vaccines because of their storage capacity, ease of growing, and transformation efficiency:

Tobacco: Following Arntzen et al.'s achievement of plant-based immunisation in 1990 by initially expressing HBsAg in transgenic tobacco, the notion of edible vaccination took off. Tobacco was later genetically altered to produce vaccines against enterotoxigenic E. Coli and Vibrio cholerae (through the production of LT-B), hepatitis B, and dental caries antibodies, which are now being tested preclinically. Also, an HPV target vaccine based on tobacco has been developed by Italian scientists at a affordable price tag.

Potato: Potatoes have a bright future as a platform for early development of edible vaccines.

Transgenic potato production has been pioneered at Cornell's Boyce Thompson Institute by Arntzen and Mason. Clinical trials sponsored by NIAID proved that uncooked potato-derived E. coli vaccines stimulated mucosal and systemic immune responses among human volunteers. Additional studies indicated that potato-made vaccines protected against cholera and the Norwalk virus, as mice had vigorous IgA and IgG responses. Heat, however, significantly reduces antigen stability—down to 50% degradation—which highlights the importance of heat-stable antigens or protein amplification for practical applications.

Tomato: Tomatoes' flexibility for genetic alteration, rapid growth, and edibility make them favorable targets for edible vaccinations.They are easy to convert to paste or dry for dissemination even when their growth in target areas for the vaccine is limited.Transgenic tomatoes expressing antigens against rabies, HIV, Alzheimer's, and malaria have been considered possibilities. Chowdhury and Bagasara (2007) proposed multistage antigen targeting in tomato varieties with varying visual traits to fight malaria. Tomatoes are also being engineered to produce proteins that target beta-amyloid in Alzheimer's disease and offer thermal stability for HIV antigens. Moreover, rabies virus G-protein is produced by UC82b tomato plants that have been engineered to do so using the CaMV 35S promoter.

Banana: With their raw edibility, good child acceptability, and perfect cultivation in tropical regions where immunisation needs are highest, bananas have emerged as a major candidate for edible vaccine production. The fact that they can be taken directly is made more valuable for use as a vehicle for vaccines by the ability of bananas to be easily taken directly compared to heat-processed staples such as rice or potatoes. At dosages estimated at approximately 2 cents compared to $125 for conventional injections, transgenic bananas producing hepatitis B virus (HBV) antigens are likely to greatly reduce immunisation costs .A significant barrier to scalable and timely deployment, however, is the long culture period, which takes approximately a year to produce fruit.

Maize: With the altered varieties that create antigens to human and animal diseases, maize has become an important platform for edible vaccines. In Egypt, scientists have developed maize that can produce the hepatitis B surface antigen (HbsAg), which may benefit over 2 billion people suffering from the disease. In Mexico, genetically modified maize that will carry Newcastle disease virus antigens offers protection to poultry, whereas Iowa State University is attempting to administer flu shots via maize products. Transgenic maize containing rabies virus glycoprotein (G) has shown promising levels of protein expression, while ProdiGene is developing maize that contains HIV-associated proteins. These applications show the potential for maize as a cheap, scalable vaccine production process.

Rice: Rice's room temperature stability, which obviates the need for refrigeration, and resistance to stomach acidity have rendered it a promising candidate for vaccine production. Through the inhibition of allergic immune reactions, certain IgE production, histamine release, and inflammatory symptoms such as sneezing, transgenic rice that produces T cell epitope peptides from Japanese cedar pollen allergens has been found to be effective in oral immunotherapy. In addition, in animal models, rice has been used effectively to express cholera toxin and elicit protective immunity against the diarrhoeal infection. Such developments emphasize rice's use as a cost-effective and sustainable platform for oral vaccine development, particularly in the treatment of infectious diseases such as cholera and allergy.

ALGAE-BASED EDIBLE VACCINES:

Edible vaccines produced from algae, especially those based on green microalgae such as *Chlamydomonas reinhardtii*, have been very promising as an antigen expression platform and offer definite advantages over conventional land-based plant systems. These single-celled organisms are fast-growing, do not need soil or seasonal requirements, and can be cultivated in closed bioreactors, minimizing environmental contamination. The FDA has designated *C.reinhardtii* as Generally Regarded As Safe (GRAS). Its key use is chloroplast transformation, which allows the stable and high-level expression of complex, disulphide-bonded proteins. The lyophilised algae retain immunogenicity over extended periods of time at room temperature, whereas the robust algal cell wall acts as a natural encapsulation system, resisting gastrointestinal degradation of antigens. In spite of its current limitations, such as inefficient glycosylation and low nuclear gene expression, this platform is under investigation for HPV, HBV, and FMDV vaccines. Algal systems offer a cheap, scalable, and valuable solution that is particularly well-adapted to implementation in resource-constrained regions.

Table.No.01: Edible algal vaccines for various diseases

DISEASE	HOST ALGAE
Malaria	*Chlamydomonas reinhardtii*
Foot and Mouth Disease	*Chlamydomonas reinhardtii*

Classical Swine Flu	*Chlamydomonas reinhardtii*
White Spot Syndrome	*Chlamydomonas reinhardtii*
Staphylococcus aureus	*Chlamydomonas reinhardtii*
Human Papilloma Virus	*Chlamydomonas reinhardtii*

PROBIOTICS AS EDIBLE VACCINES:

There are three primary methods by which genetically modified microorganisms are employed to administer vaccines:

1. Live attenuated vaccines, which are nonpathogenic and activate the immune system because the bacteria can't infect mammalian cells because of mutations or deletions in genes.
2. Recombinant protein-producing bacteria isolated and used as antigens during the production of vaccines.
3. Commensal bacteria that present foreign antigens which, when ingested, induce immune reactions without compromising the host.

Salmonella species, Yersinia enterocolitica, and Listeria monocytogenes are among the most frequent bacterial vectors selected for use for their good safety profile and potential to enhance immunity.

Table.No.02: Edible probiotic vaccines for various disease

DISEASE	CARRIER ORGANISM
Influenza	*Listeria monocytogenes*
HIV	*Streptococcus gordonii*
Anthrax	*Lactobacillus casei*

INSECT CELL BASED VACCINES:

Baculovirus Expression Vector Systems (BEVS) and insect cell culture technology offer an efficient and safe method for producing recombinant proteins, including subunit vaccines. BEVS can generate complex proteins, such as virus-like particles (VLPs), with high yields and essential post-translational modifications. Insect larvae, like Bombyx mori, are also used for large-scale protein production, providing a sustainable approach to edible vaccines. Baculoviruses, with their non-infectious, non-pathogenic nature, are regarded as safe for gene therapy and vaccine development.

ADVANTAGES AND DISADVANTAGES:

Advantages of edible vaccines:

- Compared to traditional vaccines, edible vaccines are more affordable and have a lower manufacturing cost.
- Their manufacture is simple and doesn't call for sophisticated biotechnological facilities or complicated infrastructure.
- They are particularly well-suited for deployment in environments with limited resources because to their ease of transport and storage, which eliminates the requirement for refrigeration.
- These vaccinations, which come from plant sources, are safer by nature because there is no chance that they will be contaminated by infections that come from animals. When given orally, they eliminate the need for sterile injection facilities and skilled medical personnel, making mass vaccination campaigns easier.

- Because plant-based therapeutic proteins are non-toxic, they are often well-tolerated and linked to few side responses.
- All things considered, edible vaccines are a viable, secure, and easily obtainable immunization approach that has the potential to revolutionize public health, especially in underprivileged areas.

Disadvantages of edible vaccines:

- Uncertain Dosage: Because plant generations, individual plants, and antigen concentrations vary, it is still unclear what the ideal dosage should be and how long it should last manufacturing Consistency: Because antigen concentrations vary among fruits and plants, it is difficult to standardize large-scale manufacturing.
- Unknown Long-Term Effects: It's yet unclear how edible vaccines will affect people in the long run.
- Pesticide Issues: Applying pesticides to plants that produce vaccines may harm the plants as well as the people who use them.
- Transgenic Escape: Although controlled growth methods can reduce the chance of transgenic plants contaminating the environment, there is still a chance.
- Impact of Cooking: Although some studies indicate that some vaccines can withstand cooking if dosages take temperature and cooking time into account, cooking may break down vaccine proteins.
- Stomach Degradation: The vaccination proteins may be broken down by the stomach's acidic environment before they have a chance to elicit an immunological response.
- Glycosylation Variations: The efficacy of the vaccination may be impacted by variations in the glycosylation of plant proteins and human cells.

CONCLUSION:

Despite the fact that the global burden of disease has been significantly reduced by traditional vaccines, around 30 million children are deprived of immunization annually, resulting in an estimated three million preventable deaths. An innovative solution is offered by edible vaccines based on genetically modified plants, which allow for scalable and cheap immunization, which is particularly beneficial in disadvantaged and poor regions.

The recent advances, for example, initiation of the initial human clinical trials, demonstrate an important achievement in the industry. Nevertheless, numerous challenges exist, such as antigen expression elevation, maintaining the integrity of antigens after being drawn, and performing extensive testing on long-term safety.

Methods for augmenting oral immunogenicity, like co-expression or genetic conjoining of antigens with molecular adjuvants, are showing promising results. Edible vaccines are not only applicable in human medicine but have also yielded positive results in veterinary medicine, particularly for those infections that pass through mucosal surfaces.

Plant-based vaccines are emerging as a powerful tool with the potential to revolutionize public health by making vaccination more accessible, affordable, and equitable on a global basis as scientific progress continues to accelerate.

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