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REVIEW

Edible Vaccines: Promises and Challenges

Vrinda M Kurup[1] · **Jaya Thomas**[1]

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Abstract

Vaccines are biological preparations that improve immunity to particular diseases and form an important innovation of 19th century research. It contains a protein that resembles a disease-causing microorganism and is often made from weak or killed forms of the microbe. Vaccines are agents that stimulate the body's immune system to recognize the antigen. Now, a new form of vaccine was introduced which will have the power to mask the risk side of conventional vaccines. This type of vaccine was produced from plants which are genetically modified. In the production of edible vaccines, the gene-encoding bacterial or viral disease-causing agent can be incorporated in plants without losing its immunogenic property. The main mechanism of action of edible vaccines is to activate the systemic and mucosal immunity responses against a foreign disease-causing organism. Edible vaccines can be produced by incorporating transgene in to the selected plant cell. At present edible vaccine are developed for veterinary and human use. But the main challenge faced by edible vaccine is its acceptance by the population so that it is necessary to make aware the society about its use and benefits. When compared to other traditional vaccines, edible vaccines are cost effective, efficient and safe. It promises a better prevention option from diseases.

Keywords Edible vaccine · Genetically modified plants · Mucosal immunity · Biopharmaceuticals · Oral immunization · Molecular farming

Abbreviation

US FDA	United States food and drug administration
IgA	Immunoglobulin A
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
Ti plasmid	Tumour-inducing plasmid
E coli	Escherichia coli
HBsAG	Hepatitis B surface antigen
CT-B	Cholera Toxin B
HIV	Human immuno virus
MSP	Macrophage stress protein
UreB	Urease subunit beta- Helicobacter
LAV	Live-attenuated vaccine
WHO	World Health Organisation
MALT	Mucosa-associated lymphoid tissue
SIgA	Secretory immunoglobulin A
FAE	Follicular-associated enterocytes
APC	Antigen submucosal cells
TMV	Tobacco mosaic virus
PVX	Powder virus
AIMV	Alfalfa mosaic virus
CMV	Cytomegalovirus
VLP	Virus-like-particle
HBsAG	Hepatitis B virus surface antigen
SARS	Severe acute respiratory syndrome
BEVS	Baculovirus expression vector system
HPV	Human papilloma virus
FMDV	Foot-and-mouth-ailment infection
LAB	Lactic acid bacteria
MSP	Merozoite surface protein
CTB	Cholera toxin B
GMP	Good manufacturing practice

Introduction

The greatest achievement of the 19th century was the development of vaccines. The first vaccine to be developed was small pox vaccine by Edward Jenner in 1796 and the work was later continued by Louis Pasteur [1]. The spread of infectious diseases such as diphtheria, tetanus, polio,

✉ Jaya Thomas
jayamarythomas@gmail.com

[1] Department of Pharmacology, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Amrita Institute of Medical Sciences Healthcare, Education & Research, Kochi, Kerala 682041, India

measles mumps rubella and hepatitis was reduced by the administration of vaccines [2, 3]. Our immune system destroys disease-causing germs that we call as pathogens and protects our body from their invasion. If our immune system is not strong enough to fight against the invading pathogens definitely we will get infectious diseases. There comes the importance of vaccination. An antigenic substance prepared from the causative agent of a disease or a synthetic substitute, used to provide immunity against one or several diseases is known as vaccines and the process of administration of vaccines is called vaccination [1]. Vaccines can be prophylactic or therapeutic depending upon the need. When the prophylactic vaccines prevent infections, therapeutic vaccines help to prevent complications of chronic infections such as HIV, hepatitis B and HPV by boosting the immune system and are still under investigation [4]. The three main vaccine production methods are the cell-based vaccines, vaccines developed by investigational manufacturing system and egg-based vaccines [5]. In common, the production of vaccines comprises of four main steps including propagation, isolation, purification, and formulation. Propagation is the multiplication of the living organism used in vaccine. Isolation is the separation of the living organism that is used in the propagation step. Purification is the process of removing unwanted substances mainly the vulnerable ones from the selected living organism for vaccine production. Then, the last step is formulation, in this the purified organism is formulated to vaccine by the addition of suitable preservatives if needed [5, 6].

Conventional vaccines have many limitations. One of the major problems is the safety concern. Failures in the inactivation can sometimes lead to reversion of the virulent form. Sometimes, quality control tests can fail and it can lead to contamination of vaccine with undetected virus or bacteria. Need for storage in refrigerated conditions is another limitation. Most of the vaccines are administered by parenteral route and it demands need for trained personnel, multiple doses or addition of an adjuvant, specialized storage and transport conditions. Injectable vaccines are able to stimulate systemic humoural responses only while T cell effector activity and mucosal immunity are essential for the prevention of infectious diseases. Parenteral vaccine administrations sometimes produce some secondary effects such as local inflammation at the inoculation point, fever and rarely some hypersensitivity issues. It is also not possible to develop vaccines against all diseases by conventional methods [7–9]. Due to many pitfalls, the thought of alternative vaccine delivery methods arises out and this paves to the development of plant-based vaccines called 'edible vaccines' [10, 11].

Edible vaccines are nothing but transgenic plant and animal-based production of or those contain agents that trigger an animal's immune response. In simple, plant or animal-made pharmaceuticals are edible vaccines. In 1989, the effort to produce a plant-based vaccine was formulated by Hiatt and co-workers [11]. In 1990, Dr. Arntzen introduced the concept of using transgenic plants to produce and deliver subunit vaccines. This idea of Arntzen proved that the edible vaccine can annihilate the restrictions in the production of traditional vaccines. In tobacco plant, (*Streptococcus mutants*) a surface antigen is expressed from hepatitis B by Mason et al. is the milestone in edible vaccine production. [12] In parallel to the production of edible vaccine in tobacco, they also started the production of hepatitis B and heat-labile toxin B in potato and potato plants [11]. These vaccines have an indefensible advantage of over traditional conventional vaccines. Particularly in the developing world, edible vaccine offer exciting possibilities of reducing the burden of diseases such as hepatitis b and diarrhoea where storing and administering vaccine are often major problems. For production of edible vaccines or antibodies, it is desirable to select suitable plants, algae, yeast, insect cells and lactic acid bacteria whose products are consumed raw to avoid degradation [12, 13].

National Institute of Allergy and Infectious Diseases approved edible vaccine for its remarkable effect of immunogenicity in 1998. This type of edible vaccines offers a cost-effective, needleless, convenient, safe, easy and a better alternative to vaccine production [14–16].There were quite a lot of plant-based vaccines have been developed and most of them are at clinical trial phase [17, 18]. Most of the plant-based vaccines were against viruses and bacteria that infect human, animals as well as poultry which cause fatal illness. So far, there is no edible vaccine that was approved by USFDA because, this type of vaccines were characterised under genetically modified crops. [19, 20] In the light of this sensational research, this review emphasizes on the uses, promises and challenges of edible vaccine in a future perspective.

Mechanism of Action

Edible vaccine mainly stimulates mucosal immunity. This configuration contains both the immune system's innate and adaptive arm (T and B cells). The composition is well structured and these so-called lymphoid mucosal-associated tissues (MALT). SIgA also plays a key role in protecting mucosal surfaces from adhesion for both microbes and toxin activity. The creation of new platforms for the delivery of pathogens or toxin-specific SIgA and systemic IgG is the key to improve vaccine efficacy [21, 22].

Microfold(M) cells are one of the major routes of the capture of the antigen at the intestinal level. M cells are a small amount of follicular-associated enterocytes (FAE) which are mainly found in the gastrointestinal tract. These cells capture a wide range of macromolecules from lumens in the small

intestines to antigen submucosal cells (APCs) on Peyer's patches effectively [23]. Of many APCs, dendritic (DC) cells appear to be the most powerful antigenic cells to trigger an adaptive immune reaction in the priming naive T cells [24]. In an immediate phase, DC is found in a stable state, marked by strong endocytic activity and low capacity for primary naive T cells. DCs, however, mature, increase co-stimulatory molecules and migrate to T-cell areas in lymph nodes under inflammatory situations. There are antigens as well as the release of cytokines to help differentiate the naive antigen-specific T cells into effector cells and migrate to a specific inflammatory site [25]. Intestinal DCs can promote naïve t-cell activation and follicular T-helper differentiation (Tfh) either through direct promotion of Tfh differentiation or through promotion of later transformed T-17 cells into Tfh [26, 27]. Such active B cells leave follicles and move to lymphoid MALT where plasma cells secrete antibodies against immunoglobulin A (IgA) [16]. Those same IgA antibodies are diverted to the lumen in secretions across epithelial cells to interact with antibodies [27]. DCs can also capture luminous antigens specifically through the epithelial cell layer and then through dendrites projection into the lumen [28]. The goblet cell, a type of cell involved in the development of mucins, was a recent mechanism for capturing antigen in the small intestine. Intravital microscopy shows that goblet cells can directly capture and supply intestinal antigens [29]. A reliable, edible vaccine will induce specific responses to T and B cells that will also facilitate long-lasting memory cells for subsequent gatherings during actual infection. Although the advancement of "oral tolerance" refers to the T-cell-mediated paradox involving a decrease in the specific immune response to previously encountered antigen via the oral route, it was one of the controversies on oral vaccine administration [30, 31].Antigens are released in the intestinal immune system due to the lack of inflammation in which immature dendritic cells introduce T cells, which induces tolerance [32]. Secreted cytokines, such as IL-10, and cell-to-cell close contact occurs when regulatory T cells impede the growth and development of dendritic cells to modify their tolerogenic mechanism [33]. Recurrent administration of mucous antigen may also lead to suppression of the immune reaction in humour, and vaccines with reliable levels are difficult to produce [34].

Production of Edible Vaccines

Edible vaccines can be produced by incorporation of transgene in to the selected plant cell. The integration of the transgene can be done without combining with vector by direct gene delivery method or by combining with the vector by indirect gene delivery method. The transgene can be expressed in the plants by two transformation system depending on the site where antigen should be merged with the cells (stable transformation and transient transformation system) [35–37]

Direct Gene Delivery Method

Direct gene delivery is the simple method. In this the selected DNA or RNA is directly introduced in to the plant cell. The most commonly used direct gene delivery method is the biolistic method and it is also known as gene gun or micro-projectile bombardment method. This is a vector-independent method. This is done when gene transfer through agrobacterium species-mediated transformation is not possible [38–40].

In this transformation method, the DNA or RNA is coated with gold or tungsten which acts as a micro-carrier. Then, the coated DNA is placed in to the gene gun and is exposed to high pressure of Helium gas. The coated DNA will move due to high pressure and gets penetrated in to the targeted plant cell. This method requires very high cost and can harm the plant. [14, 41]

Nuclear transformation and chloroplast transformation can be done by biolistic method. These were the two types of antigen expression method [42]. Incorporating desired gene in to the nucleus of the plant cell through non homologous recombination is called nuclear transformation and the gene is injected to the chloroplast to increase the protein expression is called chloroplast transformation. Most commonly adopted method for the production of edible vaccine is chloroplast transformation. [43–46]

Examples of vaccines produced by biolistic methods are cholera, Lyme disease, anthrax, tetanus, plague, rota virus and canine parvovirus. [47]

Indirect Gene Delivery

This is a vector-mediated gene delivery. In this method, the desired plant cells were infected with plant bacteria or plant virus to produce the protein of interest [48]

Agrobacterium Mediated Gene Transfer

Agrobacterium is a gram –ve bacteria that attacks the plants and transfer their genes to plant nucleus. Agrobacterium tumefacians and Agrobacterium rhizogenes were the two species that are commonly used. Agrobacterium tumefaciens carries tumour-inducing Ti plasmid and agrobacterium rhizogenes carries root-inducing plasmid Ri plasmid [49]. The genes coded for auxin and cytokine in Ti plasmids were removed for vaccine production. This method is used to yield a stable integration of the antigen in to the plant genome. This is a slow process and the yield is low. But it is simple and cost effective. Examples for vaccines produced

by this method were diarrhoea, TB, dengue, avian flu virus, ebola [50]

Genetically Engineered Plant Virus

This process modifies an appropriate plant virus to make a viral coat protein chimeric gene. It is therefore a vector for the delivery of genetic components in plant cells. In plants, this technique leads to transient antigen expression [12, 51]. The recombinant virus is a product of viral replication throughout viral infection in plants that expresses the intended protein or peptide. Furthermore, vaccine epitopes can be synthesized and accumulated by changing viral capsid proteins [51, 52]. Plant virus-induced infection has many benefits, including a large degree of recombinant protein expression shortly after disease, easy generation of multiple antigenic duplicates on layer of the viral particle and the permission of big-scale infections in the plant [45]. However, viral reproduction products must first be purified from the infected plants before they are vaccinated. This technique of manufacturing also causes plant death following infection. Therefore, when the vaccine is caught, another plant needs to be infected with the virus and this reinfection method needs to be continuously made to produce sustained vaccines [53].

The previous advancement includes mainly RNA viruses, such as Tobacco Mosaic Virus (TMV), Powder Virus (PVX), Alfalfa Mosaic Virus (AIMV), CMV and CMV as an expression vector. Plant Virus expression system mainly includes engineered viruses such as RNA Virus (CMV) [54]. Such viruses do not reproduce in mammalian cells and are, therefore, an acceptable alternative vector for human and veterinary production. Additionally, as most expression mechanisms explain, the vaccine antigen obtained is protective against threat infection. DNA viruses such as Gemini viruses are also constructed as one of the most advanced plant expression systems with advances in plant virus molecular biology. Gemini viruses have a small single stranded DNA replicated by a double-stranded intermediate DNA in the nucleus of healthy cells [55].

Outline of Edible Vaccines

Edible vaccines offer a better choice predominantly in developing countries because they are cost-effective, easily administrable, no storage issues and bio-friendly. Edible vaccines provide mucosal activity along with systemic immunity. Plant-based vaccines are comparatively more easier to manufacture, while normal vaccine production requires highly sophisticated and expensive techniques for bulk production as in mammalian and microbial cell culture . The statistics show that the entire population in China requires only 40 acres of land for production of hepatitis B edible vaccines annually. As per this, 200 acres of plot is required for the production of edible vaccine for all infants in the world [15]. Various edible products like plants, algae, insect cells, whole yeast and lactic acid bacteria are used as alternative agents for parenteral vaccines [15].

Plant-based Edible Vaccines

In recent times, plants have also been widely used to design new biopharmaceutical systems, which facilitate the suitable folding of exogenous proteins and are economically viable [56, 57]. This is also known as "molecular farming," where marketing-value biomolecules of genetically engineered crops were generated. Numerous on-going clinical trials are underway using purified antigens temporarily generated in injectable vaccine formulations from tobacco plants (*Nicotiana benthamiana*). For e.g., a Phase II clinical trial has lately been performed using a quadrivalent flu vaccine (VLP) derived from plants and declared in recent times that a phase III clinical study has been undertaken [58]. The idea that plants are edible led to their use for oral vaccinations in the beginning of the 90s. They could be a delivery vehicle, as well as bio factories, being edible. Research studies have attempted in recent times in the growth of edible products to resolve the weaknesses of traditional vaccines [59]. When the idea was launched, several potential benefits have been apparent in using plants to produce vaccines. First, as the biopharmaceutical industry showed, plant vaccines would probably be lower production costs and it could be easy to scale. The victory of the experimental Ebola drug ZMapp manufactured in Nicotiana plants led to the gain of visibility in molecular farming [60, 61]. Unlike the production of biomolecule, however, the edible vaccine formulations require no pre-administration treatment or purification, which further lowers the cost involved with production.The most studies have utilized cultivated potatoes but, as cooking or boiling can weaken most of antigenic proteins, potatoes might not be the best choice in edible vaccines [62]. More than a generalized application, but a successful design and development of genetic transformation methods, plants including tomatoes, maize, tobacco, bananas, carrots and peanuts will have a more bright future as edible vaccines. Till date, a large number of edible species, such as lettuce, tomato, potato, papaya, carrot, quinoa and tobacco has been converted into vaccine antigens. In animal models the appropriate folding and improved expression of the antigens obtained in these processes have been screened [59, 63].

Major Plant Species Used as Vaccine Models

Potato

Potato is an appropriate model for producing vaccines against tetanus, diphtheria, hepatitis B and Norwalk virus.

The first attempt to develop edible vaccine in potato is for enteritis caused by E.coli strain. Potato may also have a role as an oral strengthening to the hepatitis B vaccines in humans [64]. An edible vaccine against mink enteritis virus attack was developed in potatoes. Potato edible vaccine also tried against rabbit haemorrhagic virus in wild rabbits. The main benefit of producing edible vaccine from potato is the ease of transformation and propagation. There is no need of refrigerators for storing and one of the main disadvantage is cooking leads to denature of antigens [65].

Rice

Rice is the other plant species used for the development of edible vaccines. Advantages over other plants were commonly used in baby food and high expression of antigen. But it grows slowly and requires glasshouse condition. In 2007, a study conducted in transgenic rice called *Oryza sativa* persuades significant amount of antibodies against E coli. Functional expression of HBsAg in rice seeds was confirmed in 2008. Vaccines developed from rice plant will have a massive power on the public health where rice is the major source of food [65, 66].

Banana

Banana is the commonly used plant species in the production of edible vaccine. It does not need cooking. Proteins were not destroyed even after cooking. Inexpensive when compared to other plants. Banana plants express HBsAg. The leaf contains antigen. The main disadvantage is it takes 2–3 years to mature and spoils fast after ripening [67].

Tomato

An effective vaccine against acute respiratory syndrome, SARS caused by coronavirus was first established in tomato. It produces better effect against Norwalk virus than vaccines produced from potato. The leaves, stem, fruits, and other tissues has the ability to express CT-B proteins from *Vibrio cholera* B toxin [68]. Tomatoes have also been used to express HBsAg. An effective vaccine against the Alzheimer's disease was developed in this plant by the expression of beta-amyloid proteins. The vaccines for pneumonia, septicaemia, and bubonic plagues were developed from tomatoes. It grows quickly and can cultivate broadly. High content of Vitamin A in tomatoes may boost immune response. But it readily spoils [69, 70]

Lettuce

This plant is an effective model system against enteric diseases in both animals and humans caused by E coli. Glycoprotein E2 expressed lettuce for classical swine fear hog pest virus was developed. This plant is mainly used up in the raw form and it produces beneficial effects against hepatitis B virus. It is the utmost effective plant that can be used as an edible vaccine [71, 72].

Tobacco

Tobacco is not an edible plant. It is used as a model for the development of edible vaccines. A vaccine was developed in tobacco for Norwalk virus in 1996 that causes gastroenteritis. Transgenic tobacco expresses VP1 protein against chicken infectious anaemia. Tobacco has the ability to express a polypeptide related to hepatitis B. It is also used to develop vaccine against coccidiosis [73–75].

Alfalfa

Alfalfa is the plant used to develop edible vaccines mainly for veterinary purposes. Transgenic alfalfa containing hog pest virus glycoprotein E2 was developed in 2005. Alfalfa plants was developed to express Eeg95-EgA31 of *Echinococcusganulosus* [75].

Carrots

Carrots were not only healthy and delicious but also can be consumed in the form of edible vaccines. Vaccines against HIV, E coli, Helicobacter pylori shows potential effects when it is produced in transgenic carrots. People having weak immune system gets proper benefit by consuming this type of antigen containing carrot edible vaccine [20, 76, 77].

Algae-Based Edible Vaccines

Green microalgae have turned out to be profoundly valuable protein generation stages for an assortment of industrial and treatment applications, particularly for complex or heavily disulfide-reinforced proteins. Unicellular green algae have all the positive traits of plant frameworks, in addition to a few novel focal points over terrestrial plants as vaccine. Algal biomass accumulation is very quick, and the whole of the biomass can be used for vaccine production. Green microalgae such as Chlamydomonas reinhardtii as a feasible alternative for vaccine generation [78]. Notwithstanding, a few impediments of plant-derived vaccines, for example, low-expression levels and unsuitable glycosylation of antigen proteins, have been depicted. So far, just chloroplast transformation is possible, and just one organelle is available, regardless of whether it possesses half of the cell volume stable transformed lines of green algae are easily available and can prompt expanded yield of expressed antigens. In reality, unicellular green growth had all the positive

attributes of plant frameworks, in addition to novel favourable circumstances over earthbound plants. Their development neither has occasional limitations nor depends on soil fertility [79]. Cross-contamination of adjacent yields ca nnot happen, as green algae can be grown with encased bioreactors. Moreover, with respect to regulatory perspectives, green algae, for example, C. reinhardtii, are commonly perceived as safe (GRAS) by the FDA. At long last, algae can be effectively lyophilized and, when dried, can be stored at room temperature for as long as 20 months without losing antigenic efficacy. Actually, the algae cell wall guarantees the bio encapsulation, as it was demonstrated to counteract antigen degradation by proteins of the GIT [80, 81].

These qualities demonstrate that algae would be a perfect host for vaccine. Therefore, as officially portrayed for plant-inferred palatable immunizations, the ease and less difficult strategic as far as assembling, stockpiling, conveyance, and organization of the green growth-based innovation make it a perfect framework with regards to asset-restricted settings contrasted with traditional antibody details [82]. There are algae-based vaccines right now in clinical trials; be that as it may, preclinical details against human papillomavirus (HPV), HBV, and foot-and-mouth ailment infection (FMDV) are a work in progress to defeat some specialized issues like low-expression level from the atomic genome and absence of glycosylation following chloroplast expression [78, 83]. Research to date show that algae such as Chlamydomonas can produce complex antigens that can stimulate immunogenic responses and are suitable to be developed as vaccines [78].

Insect Cell-Based Vaccines

As a result of fast improvement, the rising BEVS and insect cell culture innovation was acknowledged as an option for the generation of recombinant proteins, including subunit vaccines. The increasing advancement of BEVS and also the culture of insects as an alternative of recombinant proteins such as subunit vaccines have been recognized as a result of fast improvements. Baculoviruses and insect cell cultivation technology were mainly confined to research laboratories to develop targeted drug proteins. BEVS / Insect cell technology is the multipurpose network for either the production of intended vaccine candidates [83]. BEVS could efficiently be used to produce recombinant proteins monomeric or oligomeric and complex protein frameworks, such as coated and uncoated vlp. Because of its well-documented safety profiles and the capacity to transduce mammalian cells efficiently, baculoviruses are also tested as alternatives to vaccine antigen distribution. In all these vaccination techniques, the BEVS / insect cell technology does not allow a biocontainment program. BEVS was often used to efficiently produce adeno-related vectors for gene production and immunization. The BEVS is presently the system of choice of production for recombinant hybrid proteins in various immunization strategies. Most of these vaccines were available in the early or progressed phases. Insect cell systems are extensively used for anything other than their ability to manufacture high protein rate and make cotranslation and post translation amendments, along with glycosylation, phosphorylation and protein treating [84].

Essentially, the expression scheme of baculovirus was not restricted to cultivated cells alone. The larvae or pupae of insects can be used in the manufacture of proteins. *Bombyxmori* larvae or pupae were used in the mass production of recombinant proteins and as a sustainable vaccine delivery method in the light of edible vaccines with larvae or pupae silkworm. A process of expression of the baculovirus-silkworm can make cotranslational and post-translational changes and thereby achieve high quantities and numerous proteins [59, 85]. Baculovirus ought not to be regarded GRAS as is incapable of replicating to vertebral animals. In addition, protease inhibitors and bio capsule-like fat might be in silkworms to prevent enzyme digestion in the GIT by recombinant proteins [86]. The absence of mammalian-specific promoters, however, prevents replication, because baculoviruses can destroy the mammalian cells. Baculoviruses are thus usually regarded as secure, non-infectious as well as human non-pathogenic. Recombinant baculoviruses were produced as effective production systems for gene therapy in mammalian cells by inserting mammalian expression cassettes into the baculovirus genome [87].

Whole-Cell Yeast-Based Vaccines

The industrial application of yeast cells to heterologous protein production has been well defined. The ability to create translational changes to this system, the status of the GRAS and the cell wall that could protect antigen throughout the GIT make yeast a fascination for vaccine delivery [88, 89]. The main problem with this mechanism is the hyper glycosylation of recombinant proteins, but impaired N-glycosylation strains of yeast were already resolved. The capacity of whole-cell yeast-oriented vaccines to generate an immune response has been studied [59, 90]. Notable evidence showing that this system can induce a protection of the mucosa is found from several preclinical studies based on orally administered Saccharomyces cerevisiae and developed for various influenza agents such as HPV, Actinobacillus pleuropneumoniae [91, 92].

In addition, the increased immunogenicity of this mechanism may be due to adjuvant activity on the β-glucans yeast cell wall showing the immune modulated and adjuvant effects of innate pathogen receptor binding on macrophages, DCs and neutrophils [93, 94]. There are currently

two clinical trials being developed: GS-4774 for HBV treatment and GI-5005 for hepatitis C virus treatment [95].

Lactic Acid Bacteria-Based Vaccines

Lactic acid bacteria (LABs) are gram-positive, nonsporulating and non-pathogenic bacteria used for generations for production of food, preservation and the expression of the treated genes of heterologous antibodies (scFV-m9, dAb-m36 and dAb-m36.4 [96, 97]. These bacteria were considered potential candidates for the mucosal vaccine vector since the ability of LAB to generate specific immune responses to recombinant foreign antigens. This delivery system can provide protection against deterioration of the antigen and trigger both innate and adaptive immune responses [98, 99]. Many LABs, in short *Lactobacillus spp* and *Bacillus subtilis*, were used in preclinical studies against various communicable diseases. This research has produced several results, but they have all shown an elicited immune response. Oral B. subtilis spores expressing Helicobacter pylori urease B that protect against Helicobacter infection is one of the example of these type of edible vaccine [100]. LAB has its natural adjuvant and immunomodulatory effects as an important characteristic, but its molecular mechanism is not fully understood. Additionally, other studies reported a dendritic cell maturation effect and cytokine secretion induction [101]. Notwithstanding the hopeful nature of recombinant LABs as vectors for mucosal vaccines and the promising results obtained from murine tests, some characteristics have to be considered, namely that the vaccine strains, although mentioned as GRAS, could not be labelled as avirulent due to the potential transition of antibiotic selection markers into microbes [102]. The advancement of LAB based vaccines requires considereation of various aspects like the role or location of each antigen expressed and route of administration (as different routes will have different immune effects). In general, more studies and clinical trials are required to develop effective LAB-based vaccines (Table 1) [103].

Applications of Edible Vaccines

Malaria

Countless efforts and many policies tried to develop a vaccine for malaria. Recently, three antigens are selected for the development of edible vaccine for malaria. That were merozoite surface protein (MSP) 4 and MSP 5 from Plasmodium falciparum, and MSP4/5 from Plasmodium yoelii. Oral administration of recombinant MSP 4, MSP 4/5 and MSP1, as an adjunctive therapy with cholera toxin B (CTB) in mice, induced antibody responses against the blood-stage parasite. Large quantity of antigen should be incorporated to the plants to express a minimum amount of antigen [16, 40]. By conventional wisdom, the immune mechanism responsible for protection against malaria will require a multiple of 10–15 antigen targets for proper protection against various stages of malarial infection. Due to antigenic competition, large number of target would not be appropriate to be used for vaccination as single dose [114]. If immunisation schedules could be arranged the stability of vaccines carrying different malarial antigens, their transport and the logistic of vaccination would be an almost impossible task to achieve under the current fiscal constraints so a unique way to circumvent these difficulties, an anti-malarial edible vaccine in transgenic tomato plants were developed

Measles

Measles causes 8000000 deaths over globally every year. The measles live-attenuated vaccine (LAV) has no oral efficacy and is destroyed on maintenance of a cold chain of refrigeration. Maternal antibody presence in the LAV reduces its effectiveness [115]. Two surface proteins are

Table 1 Developmental status of edible vaccines in clinical trials

Pathogen	Antigen	Host	Use	Clinical trial status	References
Enterotoxigenic E. coli	LT- B	Potato	Diarrhoea	Early phase 1	[104]
Enterotoxigenic E. coli	LT- B	Maize	Diarrhoea	Early phase 1	[105]
Norwalk Virus	CP	Potato	Diarrhoea	Early phase 1	[106]
Rabies Virus	GP/ NP	Spinach	Rabies	Early phase 1	[107]
HBV	HBsAg	Lettuce	Hepatitis B	Early phase 1	[108]
HBV	HBsAg	Potato	Hepatitis B	Phase 1	[109]
Vibrio cholerae	CTB	Rice	Cholera	Phase 1	[110, 111]
HBV	HBV	*Saccharomyces cerevisiae*	Chronic HBV	Phase 2	[112]
HCV	HCV	*Saccharomyces Cerevisiae*	Chronic HCV	Phase 2	[113]

there hemagglutinin (H) and fusion proteins, H protein infected with wild-type measles virus. The outcome indicated that the faecal samples of the animals vaccinated with MV-H shows the presence of IgA antibodies. Studies revealed that transgenic carrot plant is the best choice for measles vaccines [115, 116].

Hepatitis

Based on WHO estimates, two billion people around the world have evidence of past or current HBV infections. Over 360 million individuals are persistently infected, and there are over 600,000 deaths from HBV-related diseases—liver cirrhosis or hepatocellular carcinoma. Hepatitis B surface antigen known as HbsAG is used for the production of edible hepatitis B vaccine. Potato is the plant of choice for the development of edible vaccine for hepatitis. The HbsAg expression is seen more in roots than other parts of the plant [117–120].

Autoimmune Diseases

In the case of autoimmune diseases such as type 1 diabetes, it is very much useful to take self-antigens. Damage of beta cells and fails to produce insulin is the main reason for diabetes. Insulin is the drug of choice in type 1 diabetes and it cannot cure the disease completely. Potatoes contain insulin or glutamic acid decarboxylase along with innocuous B subunit of the Vibrio cholera toxin shows a better improvement in diabetic mice. It could supress the immune responses and maintains the level of insulin [121, 122].

Diarrhoeal Diseases

The third leading cause of mortality among Indian children is diarrhoea. GIT infection is the main cause of diarrhoeal disease. Bacteria, virus, and parasitic organisms were the pathogens responsible for the infection. Many oral vaccines have been developed for the prevention of diarrhoeal disease indeed only a few mucosal active vaccines against pathogen got licence. For the successful oral immunization the must pass through the harsh atmosphere of the stomach and intestine. This can be achieved by edible vaccine against enter toxigenic Escherichia coli (ETEC), cholera, and norovirus have been developed. In a study by Haq et al., gene-encoding LT B is transferred to potato and tobacco leaves through Agrobacterium tumifaciens and this is fed to mice. Those mice which consumed these potatoes and tobacco leaves developed serum IgG and mucosal IgA anti-LT-B [16].

Transgenic corn expressing LT-B is also used as a candidate for edible vaccine against enterotoxigenic E. coli infection. Protein expression is stable in corn and the time required for the development of corn is less. In preclinical studies, the transgenic corn activates IgG and IgA responses in mice [123, 124].

Cholera is a bacterial diarrhoeal disease with symptoms similar to that of ETEC. Vibrio cholera O1 and O139 were the main pathogens. The vaccine-induced protection against Vibrio cholera is due to anti-cholera toxin responses. Transgenic potato contain CT-B induces the production of intestinal and serum anti-CT-B antibodies in mice [125–127].

Norovirus are enteric viruses that comes under the family of Calciviridae and which is the major cause of gastro enteritis. Norwalk virus is one among this species. Potato and tobacco expressed with Norwalk virus were developed by Mason et al. In preclinical studies in mice with these transgenic plants expressing Norwalk virus faecal and serum antibody responses were observed [128, 129].

Anthrax

Biolistic transfer of Pag gene to tobacco plant expresses PA antigen and it can be used in the treatment anthrax disease of cattle. This Pag gene is also injected in tomatoes and spinaches for future studies [130].

Regulatory, Ethical Aspects and Challenges

In January 2005, WHO conducted a meeting regarding the regulatory evaluation of plant-based vaccine. The meeting ended up by concluding that the existing guidelines for development, evaluation, and use of vaccines made by conventional methods can be applied in the production of edible vaccines. There were specific issues related to edible vaccine [27, 131]. Plant-derived vaccines should be clinically tested under US investigational new drug application, and also must follow all the regulatory and GMP requirements [132, 133].

The future of edible vaccine depends on many criteria. It should be well approved by the population so that it is necessary to make aware the society on the use and benefits of edible vaccines. In some areas, it is believed that genetically modified plant and products were a threat like evil spirits and destroy the world so there is a crucial role to awake the people from this myth of evil spirit by the authorities. The next important benchmark to check is the stability of the genetically modified plants and proper isolation of the plant is essential [134, 135]. Sometimes the transgene causes allergies. Plant-made oral vaccines might induce allergic reactions during post-translational modifications, and oral tolerance when co-administered with oral adjuvants to mostly activate the mucosal immune system may provoke hypersensitive responses to other proteins contained in the daily food. Recurrent delivery of plant-made edible oral vaccines can boosts regulatory T-cell stimulation contrary to vaccine antigen causes hypersensitivity reactions in case of

pollen allergy or food allergy. Prevention of environmental contamination and prevention of serious side effects is mandatory. When compared to other traditional vaccines, edible vaccines promises a better prevention option from diseases if they are developed in a proper manner. This is a cost-effective, efficient and safe model as a vaccine [136–138]. Growing plants for edible vaccine production requires close monitoring. The safety and quality of the genetically modified plants will be a difficult task even though manufacturing of genetically modified plants are regulated. Cross-contamination between genetically modified plants and non-genetically modified plants may occur during pollination and the genetically modified plants themselves become aggressive. Sometimes the DNA or the antigen may release into the water sources by the contact of insects or birds with the plants which causes the contamination of water bodies. The pharmaceutical could accidently enter in to the human food chain and also affect the wildlife population [139–141].

Conclusion

Vaccines play an important role in the prevention of infection. Vaccines helped human to get exposed to many infectious agents without falling ill. The dangerous diseases such as polio and measles has badly hunted the life of old generation and became under control now only due to the development of vaccines. Vaccines are capable of reducing the antibiotic use tremendously and can play a crucial role in an era where antibiotic resistance is becoming a major challenge. Conventional vaccines are very expensive, requires refrigeration, mostly administered by parenteral route and it produces only modest mucosal response. Constant research activities can improve the presently available vaccination techniques and better prevention of infectious diseases can be ensured. Discovery of edible vaccine is one of the major break-through in the branch of biotechnology and its success requires wide acceptance and attention. Compared to the traditional vaccines, edible vaccines do not require sophisticated equipment and machines for the vaccine production. Edible vaccines are safer and do not demand sterile injection conditions and storage facilities etc. Edible vaccines stimulate both systemic and mucosal responses.

The main challenge of edible vaccine is its approval from the public as there are some opinions such as genetically modified products harm the society as well as environment. Close monitoring is required while growing plants for the production of edible vaccines as there is chance of cross-contamination in molecular farming between genetically modified plants and non-genetically modified plants during pollination. There is a possibility of accidental entry of pharmaceutical in to the human food chain and may also affect the wildlife. Edible vaccine can produce complex multimeric proteins that cannot be expressed by microbial system and is a safe and effective method of vaccination. As the benefits of edible vaccines are prominent enough to overcome its side effects, proper research and development in this area is required and it can bring about an era of better control over infectious diseases.

Compliance with Ethical Standards

Conflict of interest There is no conflict of interest among the authors

Informed Consent Not applicable

Research Involving Human and Animal Participants The article being a pure review, It does not involve any human volunteers or animals.

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