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Review article

A cross talk between the immunization and edible vaccine: Current challenges and future prospects

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ABSTRACT

Introduction: It is well known that immune system is highly specific to protect the body against various environmental pathogens. The concept of conventional vaccination has overcome the pandemic situation of several infectious diseases outbreak.

Area covered: The recent idea of immunization through oral route (edible vaccine) is vital alternatives over conventional vaccines. Edible vaccines are composed of antigenic protein introduced into the plant cells which induce these altered plants to produce the encoded protein. Edible vaccine has no way of forming infection and safety is assured as it only composed of antigenic protein and is devoid of pathogenic genes. Edible vaccines have significant role in stimulating mucosal immunity as they come in contact with digestive tract lining. They are safe, cost-effective, easy-to-administer and have reduced manufacturing cost hence have a dramatic impact on health care in developing countries.

Expert opinion: The edible vaccine might be the solution for the potential hazard associated with the parenteral vaccines. In this review we discuss the detailed study of pros, cons, mechanism of immune stimulation, various outbreaks that might be controlled by edible vaccines with the possible future research and applied application of edible vaccine.

1. Introduction

The immune system is a dynamic structure in our body that protects us from various pathogens. Immune system continuous tracking of molecules which circulate within the body to detect substances which negatively affect our health. Once the foreign bodies (pathogens) are identified, the immune system attacks to neutralize them with the help of antibodies [1]. Human immune cells are extremely complex and quickly adjusted to overcome every day's challenges. Literarily, the immune system can be described as a complex collection of cells, tissues, organs and process working together to prevent disease. The immune system targets microorganism like a virus, bacteria, pathogen; parasitic worm, toxins, allergens and even sometimes own cell that show unusual characteristics. As microorganism rapidly evolves in a very short period, the immune system has to be prepared to handle massive diversity of antigens (which are commonly identified as an agent that activates immune system) [2].

Generally, each molecule may be an antigen, but researchers have found that carbohydrate and proteins offer the best response, whereas lipids and nucleic acids are poor antigens. The sensitivity and the specificity of the immune system should be taken in to account for the developments of highly specific chemical and cellular tools as it is known that immune system is complex but well organized. As a result, this destroys or kills the invading pathogens, ensuring long-term protection against pathogens and immunological memory for the body that immediately reacts to subsequent encounters with the same antigen [3]. Sometimes body immune system is unable to combat with these pathogens or microorganisms may be due to the resistance causes in the microbe over the long period in which they modified their internal, external structure (modify receptors that present on their surface) or due to the new strain that does not attack before to the human body. To overcome this problem scientists develops vaccines usually, contains whole microbe (either killed or as a live form), microbe small parts (a protein molecule) or toxins that mimic the disease-causing pathogen. These vaccine elements are used to activate the body's immune responses, to identify, kill or prevent further attacks of pathogens such as

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viruses, bacteria, fungi or other foreign bodies [4]. In both infectious and non-infectious disease, vaccines play a crucial role to prevent it.

Nowadays, conventional approaches have successfully developed a vaccine against many infectious diseases particularly by attenuating the pathogen, inactivation of microbes or by sub-units of microbes. Still, the effectiveness, stability, cost and safety of these current conventional vaccines remain important considerations in the production of vaccine, delivery, storage and availability of vaccine. Therefore, it is important to create a new vaccine that is more cost-effective and safer for the benefits of people, relative to the current vaccine. Hence a new alternative approach comes into the picture nowadays that is “edible vaccine” refer to the use of edible plant/vegetable parts or probiotics [5] (live microorganisms) as a vaccine, which is taken orally as whole or parts of plant/vegetable. The concept of “edible vaccine” first given by “Charles Arntzen” in 1990 [6,7]. As per the new research, the probiotics and plant may be genetically modified with the help of gene transfer or transformation and plant virus that contains the human pathogen proteins for the development of an edible vaccine against various life-threatening disease such as cholera, chickenpox, AIDS, malaria, foot and mouth disease (FMD), hepatitis B and C, etc., Some of these diseases require booster vaccination or multiple antigens to promote and retain protective immunity. The plants can express more than one transgene, which allows delivery of multiple antigens for repeated inoculation [8]. Mostly, the oral vaccine is associated with degradation of proteins in the stomach (due to the action of the gastric enzyme and low pH) and gut, which restricts immune response, but due to the rigid protective plant cell wall, these proteins get protection from the stomach acids and enzymes, as human enzymes are incapable of breaking down glycosidic bonds of plant cell wall carbohydrates [9]. But subsequently, release proteins at the gut lumen due to the action of microbial enzymes which break the plant cell wall (bioencapsulation). *Lactobacillus* species are considered intrinsically resistant to acid and the survival rate of probiotic lactobacilli increased in acidic condition when given with glucose [10]. The resistant of probiotic and by encapsulation of antigens in plants, stomach acid or at low pH will provide extra future prospects and tools to overcome the challenges that will be faced by edible vaccines regarding its degradation in the stomach due to gastric enzyme. Now FDA has approved plant cells for cost-effective production of protein drugs (PDs) in large scale current Good Manufacturing Practice (cGMP) hydroponic growth facilities [11]. In freeze-dry (lyophilized) plant cells, protein-drugs (PDs) are more stable at ambient temperature for a longer period and maintain their folding and efficacy [11].

2. Outbreak of diseases

An outbreak occurs when several cases of a particular disease increase more than expected in a specific region over a specific period. In the last three decades numbers of highly transmissible or pathogenic infectious disease like Zika, Ebola, monkeypox, SARS, measles, polio, cholera diphtheria cases have increased in many parts of the globe [12]. This trend further continues with zoonotic spillover events expected to continue as a result of population expansion or overcrowding of cities, unhygienic condition, pollution, and migration to uninhabited areas and effect of global warming on vector distribution. Due to the presence of these factors, it decreases the ability of the individual to combat these diseases. In recent year's demand for pharmaceutical products (vaccine) increase over the world to treat these diseases. However, some counties are unable to manufacture these vaccines due to poor infrastructure or buy these vaccines at higher prices. In these countries, the edible vaccine may be a better option to combat diseases as edible vaccines are cheap and stable for a longer time (Table 1).

2.1. Criteria for the selection of plant or plant part for the development of an edible vaccine

Edible vaccines are the form of medication taken as food by the humans and animals to boost their immunity. Thus, the criteria needed in selecting plants for the production of edible vaccine must meet specific requirements. The far most important criterion of selecting plant is its life cycle [13]. It shouldn't be longer as if it takes long time for maturation then their production and maintenance cost increase. The next criteria are the choice of plant for antigen incorporation, which should not contain any biomolecule that will interact with it. Next important one is the stability of the antigen under high temperature as all vegetable or plant parts are not eaten raw (e.g. potato, rice, pea). Sometime, they are boiled or fried at higher temperature. Plant and plant parts that can be consumed raw are preferable for human immunization. However, banana might be the ideal source of edible vaccines because they are consumed raw even by infants and are a major crop in many developing counties [8]. The one disadvantage of both banana and tomato is the low protein content of the fruit, which might limit the amount of antigen that can be expressed [13]. In contrast, seeds of the plants such as maize and soybean have higher protein content and have been reported to express very high level of foreign proteins.

2.2. Edible vaccine from the plant source

Many plants have been identified and studied for the edible vaccine which was transformed to express antigen for rotavirus, gastroenteritis, cholera, autoimmune diseases and rabies. Moreover, several experiments have used vegetable potato, but potatoes may not be the ideal choice for edible vaccines since frying or boiling will degrade certain antigenic proteins. Certain foods, such as bananas, tomato, carrots peanuts corn and tobacco have a more promising potential as edible vaccines as it can be eaten raw, not only because they are commonly available, but since genetic engineering is efficiently developed these kinds of vegetable plants. The following plant list contains edible vaccines previously studied in animals and which are required to be approved in both human and animal use (Fig. 1).

2.2.1. Potato

Mason et al. were the first persons who conduct the vaccine-based assay produced in potato (*Solanum tuberosum*) to fight against LT-B stain produced by *E. coli* in mice [14]. In that same year, in rats and human volunteers, In the same year, the effectiveness of the antigens produced from potatoes (*Solanum tuberosum*) toward the non-toxic subunit of *Vibrio cholerae* endotoxin and the Norwalk virus capsid pathogen was identified in rats and human volunteers [12,15]. Thanavalas' group proposed in 2005 that potatoes could play a role in human hepatitis B as an oral reinforcement since injectable vaccine cause redness, swelling, or itching at the site of administration. Also, the edible vaccine for the animal has now been developed to replace the injectable vaccine for animal protection [12].

2.2.2. Tomato/tobacco

Tomato (*Solanum lycopersicum*) an appropriate candidate for vaccine development for coronavirus that causes a highly acute respiratory syndrome (SARS). For the development of recombinant SARS-coronavirus (CoV) vaccine, S-spike protein (S-protein) and its truncated fragment are considered as the best choice [9,12]. The genome of tomato and tobacco is incorporated with N-terminal fragment of SARS-CoV protein (S1) used to develop the safe, effective and inexpensive vaccine. When these plant-based vaccines for SARS give to mice, shows significant increase level of SARS-CoV-specific IgA after oral ingestion of tomato, expressing S1 protein. Whereas tobacco-derived S1-protein indicate the presence of SARS-CoV-specific IgG detect by ELISA analysis and Western blot [16]. Tobacco is not an edible plant but play a major

Table 1
Shows the epidemic of many diseases in the last past six years (from 2014 to 2019) around the globe. Data are represented by disease, country, year of outbreak, with the number of cases.

Sl. no.	Infectious diseases	Number of countries affected	Years of outbreak occurs (since 2014)	Total number of cases from 2014 to 2019	Reference
1	Ebola	11	2014, 2015, 2016, 2017, 2018, 2019	28,708	Ebola virus disease. 2020. https://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease (accessed on 14 April 2020)
2	Cholera	121	2014, 2015, 2016	495,121	https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/number-of-reported-cases (accessed on April 2020) https://www.who.int/data/gho/data/indicators/indicator-details/GHO/number-of-reported-deaths-from-cholera (accessed on April 2020)
3	Malaria	105	2014, 2015, 2016, 2017	408,840,562	https://apps.who.int/gho/data/node.main.A1367?lang=en (accessed on April 2020)
4	Measles	165	2014, 2015, 2016, 2017, 2018	11,62,685	https://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencerubella.html (accessed on April 2020)
5	Diphtheria	58	2014, 2015, 2016, 2017, 2018	44,880	https://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencerubella.html (accessed on April 2020)
6	Rubella	132	2014, 2015, 2016, 2017, 2018	123,091	https://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencerubella.html (accessed on April 2020)
7	Poliomyelitis	15	2014, 2015, 2016, 2017, 2018	763	https://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencerubella.html (accessed on April 2020)
8	TB	194	2014, 2015, 2016, 2017, 2018	51,071,942	https://www.who.int/data/gho/data/indicators/indicator-details/GHO/number-of-incident-tuberculosis-cases (accessed on April 2020) https://www.who.int/data/gho/data/indicators/indicator-details/GHO/number-of-deaths-due-to-tuberculosis-excluding-hiv (accessed date April 2020)
9	Pertussis	145	2014, 2015, 2016, 2017, 2018	816,918	https://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencerubella.html (accessed on April 2020)
10	Mumps	114	2014, 2015, 2016, 2017, 2018	2,349,153	https://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencerubella.html (accessed on April 2020)

role in the development of the vaccine as it is used as a proof-of-concept model species for the edible vaccine [1].

2.2.3. Cherry tomatillos

For HBsAg gene of hepatitis B, lines of transgenic cherry tomatillos have been grown. The expression of genes was seen through the whole plant, but it was maximum in the fresh leaves weight of 300 ng/g and with fresh fruit weight of 10 ng/g [12].

2.2.4. Lettuce

Lactuca sativa express the B-subunit of the thermolabile protein of *E. coli*, responsible for both human and animal enteric disease, show the possibility of this vegetable as an edible vaccine. In 2005, the typical swine fear hog pest virus glycoprotein E2 was expressed by lettuce. In Poland, the transgenic lettuce that shows effect against hepatitis B virus is in the development stage [17].

2.2.5. Soybean

In the study, *E. coli* bacteria B-subunit of thermolabile toxin, expression was performed in the endoplasmic reticulum (ER) of soybean (*Glycine max*), which yielded a total antigen level of up to 2.4% of the total soybean seed protein without any problem during drying for further processing. Moreover, when this protein is given orally to rats leads to a rise in systemic IgG and IgA [18,19].

2.2.6. Algae

Chlamydomonas reinhardtii (green algae) has been used as a tool to achieve a large number of proteins specific to both animal and humans for therapeutic purpose [18,19]. The use of algae for the production of vaccines is optimistic, as algae have a very high growth rate, the entire system can be used as a raw material for the development of edible vaccines. Besides, to facilitate the already rapidly growing algae can be cultivated in bioreactors. *C. reinhardtii* contains one chloroplast which facilitates the stability of the desired antigens in the algal line. Notably, the effectiveness of algal vaccines after lyophilization is unchanged, which might promote global delivery of edible algae vaccine [12].

2.2.7. Pea

Based on the expression of the capsid protein Norwalk virus, the transgenic plant was developed. Protein deposition in the un-ripened fruit with a lower accumulation in red fruit was reported up to 8% of soluble protein. Expression in seeds allowed the storage of antigenic peptides, thus creating a plant with a high yield of proteins, with an average protein content of about 20%–40%, which would preclude intensive purification procedure by pharmaceutical industries [12,20]. In addition to the expression of hemagglutinin protein (H), a PA against rinderpest virus (RPV), pea plants were used. The total soluble protein level of expression in leaves was observed to be 0.12%–49% determined by Western blot. Even more, studies are also required to improve the expression of a protein in transgenic peas.

2.2.8. Banana

In banana plants, HBsAg expression was reported with four distinct cassettes (PHER, PHB, pEFEHER and pEFEHBS). At the different level expression of HbsAg were studied with PCR, reverse transcription PCR, and Southern hybridization method. Expression levels reached a height of 19.92 ng/g in the plant, and the antigen was found in banana leaves. However, because of the long period required the shrub needs to grow, the use of this vaccine was denied [21].

2.2.9. Papaya

In 2007, a papaya (*Carica papaya*) vaccine was developed to counter cysticercosis caused by *Taenia solium* by expressing synthetic peptides in 19 transgenic papaya clones. Vaccine was tested in rats, with an immunogenic response of 90% in vaccinated animals. These edible vaccines may offer good relief both in humans and in pigs, which are the two major carriers of the disease [22–24].

2.2.10. Carrot

In an experiment, carrot along with *A. thaliana* was utilized to develop an edible vaccine for surface HIV antigen expression, and in the study, it was reported that rats showed more positive effect compared to those non-treated animals [25]. Carrot (*Daucus carota*) has a positive effect in the treatment of HIV not only because carrots are nutritious

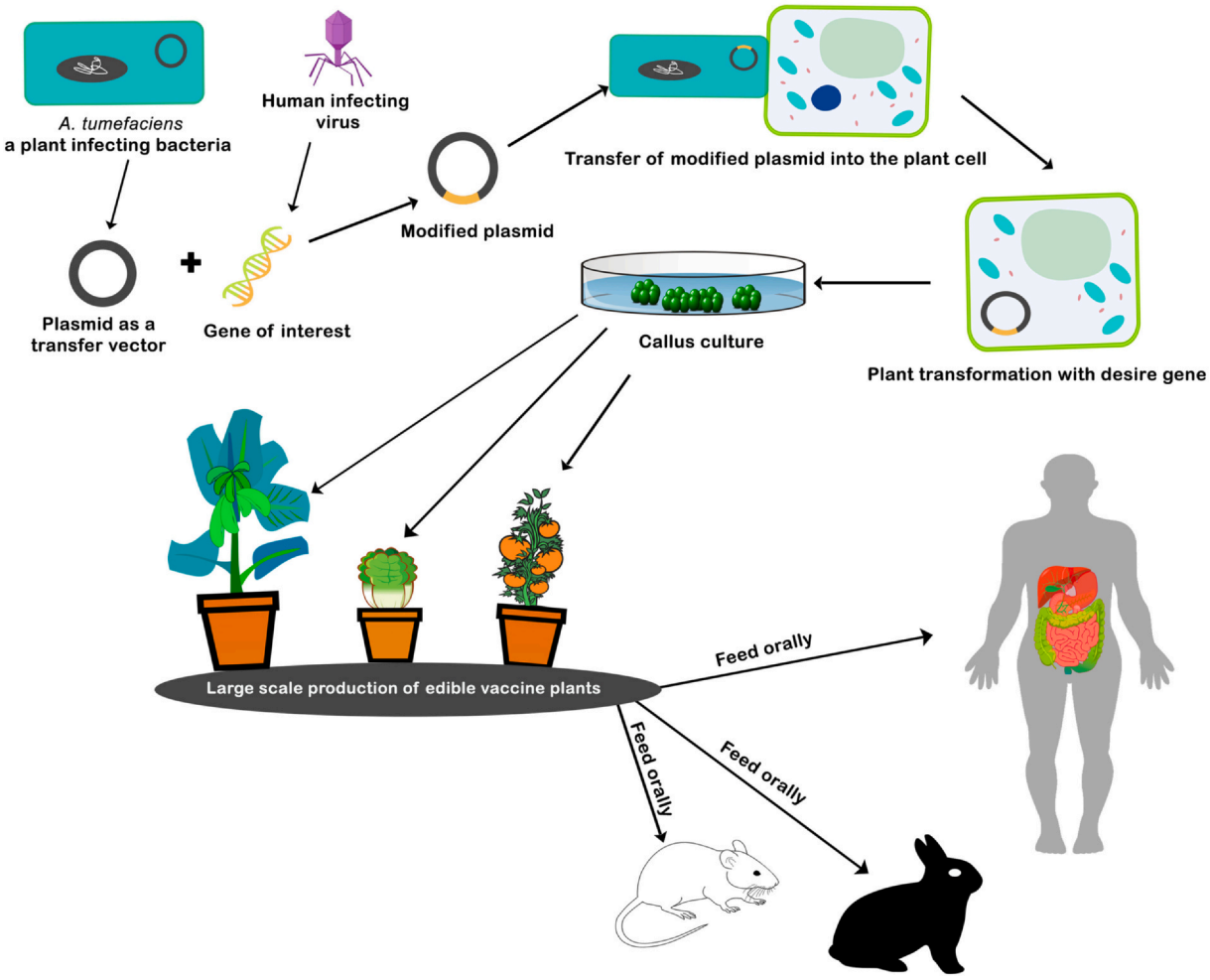

Fig. 1. The procedure involved in the development of the edible vaccine. The development of an edible vaccine starts with the identification of desired genes or proteins which is biotechnologically modified with plant-bacteria or virus plasmid and introduced into it. Then the modified plasmid containing bacteria or virus is introduced to the desired plant cell and cultured in the lab with controlled environment. When plant successfully grew then moves for mass production in the crop field, from where these edible vaccines can be distributed to the whole world. After an edible vaccine has been consumed orally, it will trigger a response to B-cell and T-helper cell and induce an individual immune system as they are the main factors.

and tasty, but because of carrot main chemical constituent carotenoids which on consumption by rats increases monocytes, lymphocytes, and other immune defence. Thus, people with a weakened immune system might benefit from the use of this potential edible anti-HIV vaccine [26]. The efficacy of this anti-HIV vaccine must be confirmed by a clinical trial. In 2010 it has been reported that the UreB subunit of helicobacter pylori was used in transgenic carrots as a potential vaccine. Transgenic carrot expressing the B subunit from *E. coli* thermolabile toxin-induced IgA and IgG production and occurred at the intestinal and systemic level in the rat [27,28].

2.2.11. Rice

A research in 2007 found that transgenic rice (*Oryza sativa*) plants expressing the B subunit of *E. coli* induces significant number of antibodies to this subunit. In the same year, an immune response was found to be caused in chicken by transgenic rice that is a result of the VP2 antigenic protein from infectious bursitis. In 2008, PCR and Southern blot analysis confirmed the functional expression of HBsAg in rice seeds [29,30].

In addition, transgenic rice was developed in 2008 in parallel to express the subunit B of the *E. coli* thermolabile toxin used to convert plant cells using bio-ballistic approach; PCR verified the expression. India and China both are the world two biggest rice producer and have the capability to export these modified rice (vaccine plant) all over the globe [31].

Selected gene encoding: antigen genes from a pathogenic organism (bacteria, viruses or parasites) that have already been identified and for which antibodies are easily accessible can be controlled in a twofold fashion. In one scenario, the whole structural gene is inserted inside the functional elements of the 5′ to 3′ plant transformation vector, allowing the transcription and aggregation of the coding sequence in the plant [32]. For the second scenario, the epitope is identified within the antigen, DNA fragment encoding may be used to create genes through fusion with coated protein gene with the plant virus e.g. CMV or TMV. Then the recombinant virus is then introduced into suitable healthy plants, which produce several new plants (Table 2).

3. Method for the transformation of gene/DNA into selected plants

Essentially there are two types of plant transformation methods but many other approaches have also been utilized to transformation.

3.1. Vector/plasmid carrier system (Agrobacterium tumefaciens mediated gene transfer)

Agrobacterium, a soil bacterium naturally occurring, has been used to transfer a small fragment of DNA into the plant genome and is called transformation [50,51]. In this method, the appropriate recombinant

Table 2
List of known to be an edible vaccine.

Sl. no.	Year	Disease/infectious agents	Antigen	Species	Reference
1.	2014	Human papillomavirus	E7 protein	Algae	[33]
2.	2014	Diabetes	Glutamic acid decarboxylase	Algae	[33]
3.	2014	Hepatitis-B	HbsAg (surface protein of hepatitis B)	Algae	[33]
4.	2012	Infectious bursitis virus	VP2 protein	Quinoa	[34]
5.	2012	Avian flu virus	HPAIV H5N	Tobacco	[35]
6.	2010	Rabies virus	Antigen glycoproteins	Corn	[36]
7.	2010	*Helicobacter pylori*	SubunidadUreB	Carrot	[27]
8.	2008	Hepatitis B	HbsAg (surface protein of hepatitis B)	Rice	[37]
9.	2007	Chicken infectious anaemia	Virus VP1 protein	Tobacco	[38]
10.	2007	*Vibrio cholerae* B toxin	CT-B protein	Tomato	[39]
11.	2007	Hepatitis B	HbsAg (surface protein of hepatitis B)	Tomato	[40]
12.	2007	Infectious bursitis	VP2 protein	Rice	[41]
13.	2007	Cysticercosis caused by *Taenia solium*	Synthetic peptide	Algae	[42]
14.	2007	Swine fever (CSFV) disease	Surface protein E2	Algae	[33]
15.	2006	Norwalk virus	Surface protein	Tomato	[12]
16.	2005	Coronavirus	–	Tomato	[16]
17.	2005	Hepatitis B	–	Potato	[43]
18.	2005	Hog pest virus	Glycoprotein E2	Lettuce	[43]
19.	2005	Hepatitis B	HbsAg (surface protein of hepatitis B)	Banana	[21]
20.	2004	Hog rotavirus (BVR)	Antigen eBRV4	Alfalfa	[44]
21.	2003	Rinderpest virus	Emogglutinin protein (H)	Pea	[45,46]
22.	2003	Hepatitis B	HbsAg (surface protein of hepatitis B)	Cherry tomatillo	[45]
23.	2003	Foot-and-mouth disease virus	Viral structural protein VP1	Algae	[47]
24.	1998	Non-toxic subunit (CT-B) of vibrio	–	Potato	[48]
25.	1998	Norwalk virus capsid	–	Potato	[49]
26.	1998	Enteritis produced by *Escherichia coli*	Heat- labile enterotoxin	Potato	[14]

DNA is inserted into the T-region of disarmed Ti-plasmid of *Agrobacterium tumefaciens* (plant pathogens), which is co-cultured with the plant cells and/or the tissue that will be transformed. The insertion of the exogenous genes and the infection of such a plant tissue into sufficiently modified *Agrobacterium* T-DNA cell contributed to studies of the stable gene incorporation in the genome of the plant, as well as transgenic protein production [32]. However, this technique is sluggish and yield are lower; but the application of this transformation is first limited to tobacco plant and too few other plant species, which extend to most vegetable species including Leguminose and Gramineae.

3.2. Micro-projectile bombardment (Biolistic) method

This is a sophisticated method, based on the micro-projectile bombardment. The selected DNA sequence is precipitated on microparticles of metals (e.g. tungsten, gold) and bombarded by a particle gun at an accelerated rate toward selected plant tissue [52,53]. These metallic microparticles penetrate the cell walls, and the exogenous DNA is emitted into the cell, where it is integrated into the nuclear genome through a process known for the photosynthetic role of cytoplasmic organelles called chloroplast comprising chlorophyll. Particle gun shoots adequately charged metallic particles with selected and processed DNA, which penetrate chloroplast and merge with its genome [32]. Transformation of the chloroplast is an effective alternative to nuclear transformation [54,55].

PDs in chloroplast are more stable when plant cells lyophilized and when preserved at ambient temperature. Therefore, the freeze-drying method improves PDs concentration and prevents bacterial contamination [11].

3.3. Electroporation

In this method, DNA is introduced into the cells to which the electrical pulses of high voltage are released, which are intended to create transitory pores in the plasmalemma. It requires the extra effort to weaken the cell wall as it serves as an efficient barrier to the entrance of DNA into the cell cytoplasm [32].

4. Mechanisms of action of edible vaccines for mucosal and peripheral immune response

4.1. Mucosal and peripheral immunity: a critical issue for oral vaccination

Immune response to the vaccine is affected by the route of immunization. The form of antigen and the active content of vaccine mediate specific tissue tropism. There is now substantial evidence supporting the existence of at least two immune systems, a “peripheral” immune system and a “mucosal” immune system [20]. These systems operate separately and simultaneously in most species including human. Protective immunity acquired during recuperation is usually referred to as “systemic immunity,” but the fact is that it might be dominated by an incomplete form of immunity dictated by a specific pathogen as the Systemic immunity might be a combination of mucosal and peripheral immunity. Lymphocyte traffic patterns, regulated by selective expression of adhesion proteins in peripheral or mucosal lymphatic tissues affect the outcome of an immune response. For example, the same antigen may produce qualitatively different immune responses in lymph nodes, spleen or Peyer's patches. The antigens in the lymph are presented over the fixed antigen-presenting cells in lymph nodes results in “peripheral immunity” characterized by the appearance of specific IgG in the blood. The antigen in the blood is presented in strategic tissue interface in the spleen. This also results in “peripheral immunity”. However, the microenvironment of the spleen is somewhat more complicated as it also accommodates circulating antigen-presenting cells and immunoreactive T- and B cells from other tissues committed to either peripheral or mucosal immunity. Triggers of antigen in the lumens of enteric organs presented on Payer's patches commitment to “mucosal immunity” characterized by the release of specific IgA into the secretions [20,56].

The mucosal surfaces are a popular site for delivering therapeutic small molecules due to the ease of administration and speed of uptake across the large surface areas. Efficacy of the mucosal route of immunization is largely based on the fact that mucous membranes constitute the largest immunogenic organ of the body. This interface is endowed with the well-organized lymphatic structure called MALT (mucosa-associated lymphoid tissue) which constitute T and B cells

(innate and adaptive arms of the immune system). Oral vaccines stimulate the generation of immunity in gut-associated lymphoid tissue (GALT), which includes lymph nodes, Payer's patches (in which lymphocytes are the major component: ~75% are B cells, while ~20% are T cells), and isolated lymphoid follicles in the gastrointestinal tract (GIT). A significant hurdle impacting protein delivery to the GIT that the antigens are rapidly degraded within the harsh environment of the digestive tract is the remarkable challenge for vaccine development. It will also be important to consider the characteristics of the GIT, in which several factors, including proteolytic enzymes, acidic pH, bile salts, and limited permeability that may hinder the induction of a protective immune response [20].

Mucosal immunity system described above have a clear image that induction of mucosal immune response starts with the recognition of an antigen by specialized cells called M-cells located in the mucosal membranes of lymphoid tissues such as Peyer's patches within the small intestine [57]. Then the APC internalize and process the antigen as soon M-cell channel antigens into the underlying tissues causing activation of CD4+ cells [57], which leads to the germinal center development, B-cells maturation and class switching to IgA through CD40/CD40 ligand interaction and cytokine secretion. The antigenic epitopes present on APC then activate B-cells with the help of T-cells [12]. Due to the expression of chemokine hormone receptors like CXCR5 or CXCR10, the B-cells migrate to the mesenteric lymph nodes where they mature into plasma cells and finally migrate to the mucosal membrane and differentiate into plasma cells causing secretion of dimeric and polymeric IgA [12]. On passing through the mucosal epithelial layer toward the lumen, the IgA molecules complex with membrane-bound secretary components to form secretary IgA (sIgA). Transported into the lumen, the sIgA interacts with specific antigenic epitopes and neutralize the invading pathogen. This whole process is elicited in Fig. 2.

The antigen bio-encapsulation by the plant cell which avoids degradation and conformational alterations and the enhancement of M-cells uptake of the conjugation of the vaccine antigen with specific ligands will overcome the challenge faced by the conventional vaccine for mucosal immunity stimulation (Fig. 3) [4,58].

4.2. Second generation edible vaccine

The second-generation edible vaccines are multiple-component, protective vaccines against multiple pathogens which can produce more than one antigenic protein by crossing two cell lines containing different antigens. In the same plant, the adjuvant is co-expressible with the same antigen. A trivalent edible vaccine against cholera, enterotoxigenic *Escherichia coli* (ETEC) and rotavirus are the examples that could effectively initiate an immune response [59].

5. Factor affecting the efficacy of an edible vaccine

Antigen loaded in the specific plant tissue is the principle of edible vaccines. Thus, the efficacy and the potency of the vaccines are significantly affected by the nature of "adjuvants". "Adjuvants" are the biomolecules (lectins, saponins) that do not exhibit immunogenic response but potentiate the immune response when co-administered with an antigen. Adjuvants can improve immune and potentiate responses by acting as a depot to guide antigens to relevant sites, protect them from degradation, control release and activate APCs [20]. Immunogenically inert biodegradable adjuvants like lipids, proteins, starch, polysaccharides, or polyesters act as the delivery vehicles for efficient availability at antigen presenting cells (APCs). There are major two methods to associate antigen and particles i.e. encapsulated by the particle by entrapment and linked to the surface by chemical conjugation or physical adsorption. The choice of carrier particles is critical to maximizing the bioavailability of its complex with antigens. Encapsulated antigens are afforded protection against extracellular proteases at the time of transport to the target immune responsive sites [20]. The erosion of the carrier particles leads to the exposure of antigens to APCs including dendritic cells (DCs), macrophages, and monocytes. Thus, a fine balancing act is needed to avoid overprotection of the encapsulated antigen, inhibiting the release of the therapeutic or premature release if protection is inadequate. The above case leads to the reduced bioavailability that weakened the host immunity. Alternatively, the conjugation or linked to the surface by chemical bonding or physical adsorption have efficient therapeutic action as it can facilitate delivery too specific immune responsive sites or cells. The structures like liposomes, virus like particles (VLPs), virosomes, and immunostimulatory complexes (ISCOMs) are well recognized by APCs because they have characteristics, including size, shape, and surface properties that are similar to viral and bacterial pathogens that the immune system has evolved to attack [20] (Fig. 4).

Beyond the factors regarding adjuvants and vehicles for the efficacy of an edible vaccine. These are some factors that significantly affect the efficacy of edible vaccines.

- Antigen selection.
- Efficacy in the model system.
- Choice of plant species.
- Delivery and dosing frequency
- Release pattern (controlled and sustained)
- Public perceptions and attitudes to genetic modification.

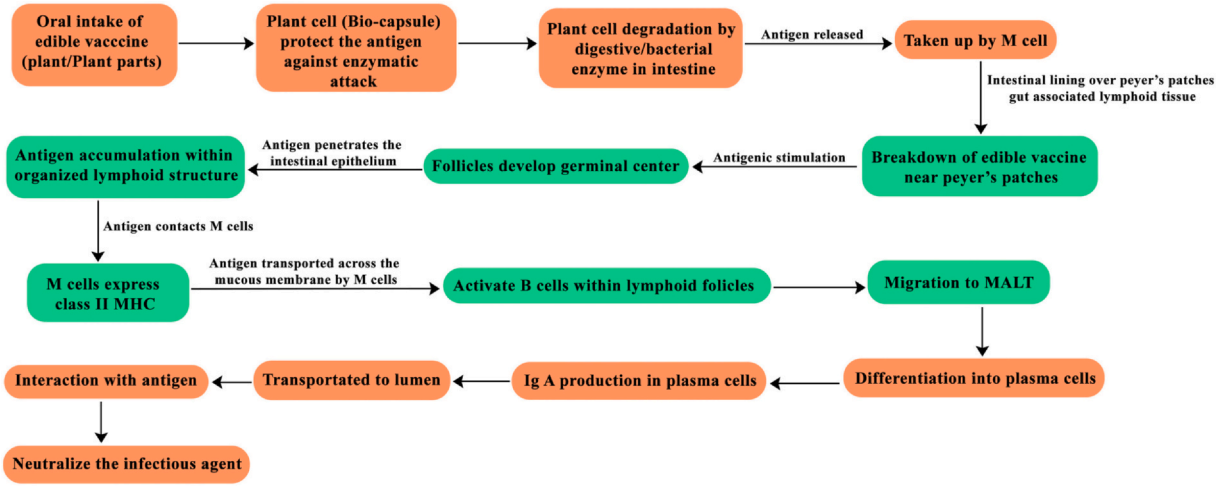

Fig. 2. The steps that stimulates mucosal immunity in a very fine way which is elucidated by this diagram.

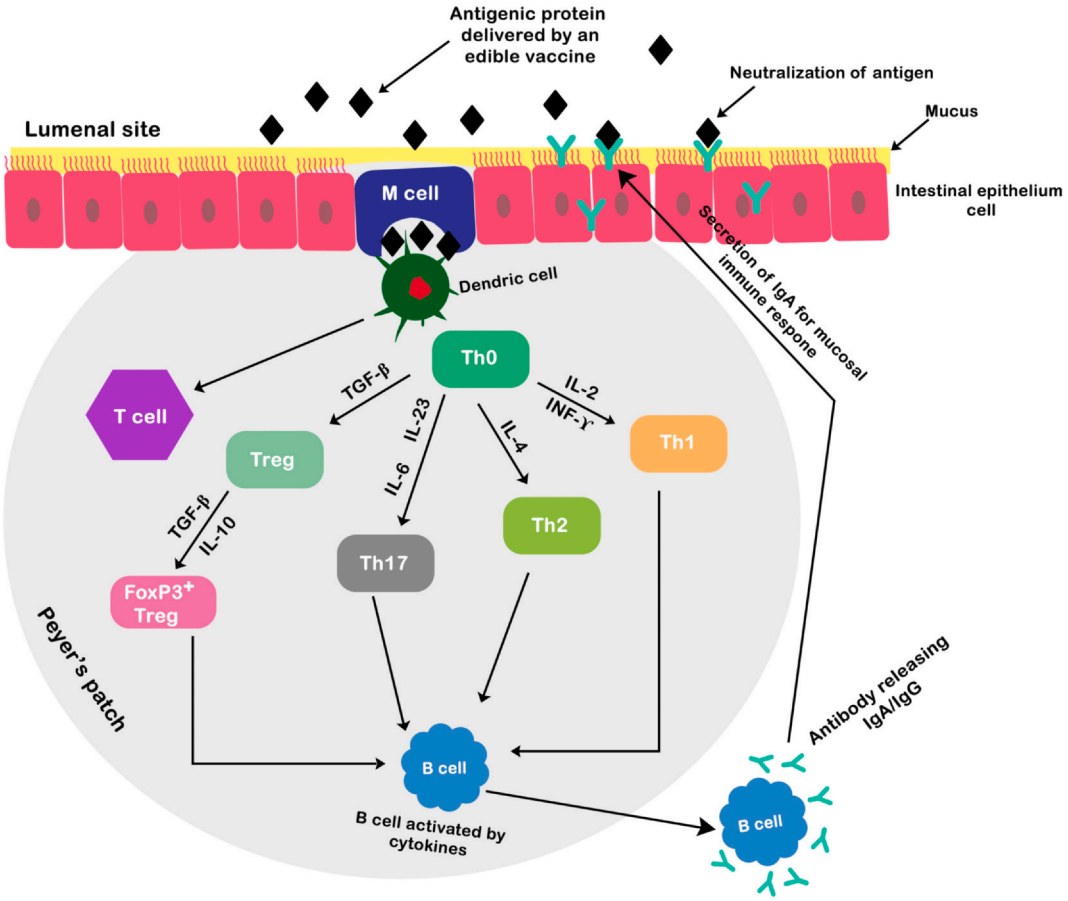

Fig. 3. Edible vaccine containing antigen induces immunity response from the intestine.

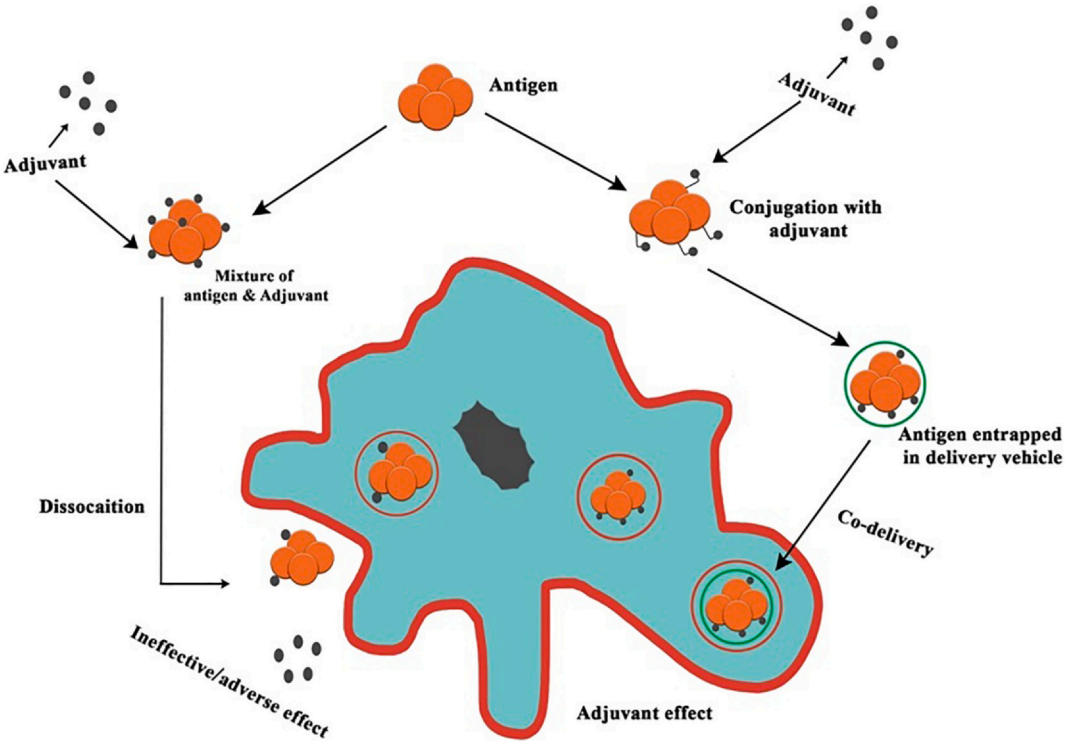

Fig. 4. Effect of adjuvants and delivery vehicle for the efficacy of edible vaccine.

6. The advantage over the conventional vaccine

Due to the use of the oral route, the administration of the edible vaccine is less complicated than the conventional methods that are given through IM, SC, and intra-dermal. Thus, it removes the needs of trained medical personnel and decreases the risk of infection as there is no need for the sterilization of premises and the manufacturing areas.

- Such oral immunization will become a practical key strategy for effective disease prevention in low-income countries, in general.
- Processing, purification, sterilization, packaging or delivery does not require rigorous structure in edible vaccines, minimizing long-term costs in relation to conventional vaccines [32].
- Maintenance and distribution of edible vaccine are easier than the conventional vaccines as it enabling the preservation without the constant cold chain storage.
- Improved storage possibilities for edible vaccines become possible as transgenic plant seeds have lesser moisture content, heat stable and can quickly be dried [32,60].
- A plant containing therapeutically active edible vaccine protein is free of toxins, pathogens and do not have the risk of proteins to reform into the infectious organism [8].
- Improved compliance is particularly related to children's who refuse to take injections of the vaccine.
- Most specifically, in this case, the immunity is activated on the mucosal surfaces of the GI, including those which are the first line of defence on the mouth (mucosal immunity).
- Sophisticated equipment and machinery are not required in edible vaccines since they can easily be cultivated on rich soils and are cost-effective relative to fermenters where the recombinant cell is cultured in a controlled manner [32].

6.1. Limitation

Beyond the several advantages and the convenience over the conventional vaccines, Edible vaccines have certain challenges that have to be overcome for efficient and pure beneficial edible vaccination. Some of them are pointed below:

- Uncertainty in calculating the appropriate oral dosage which might take multiple rounds for a patient to get the effective therapeutic action, and raises the final expense of its application [32].
- The concentration of peptide or protein of edible vaccine varies from generation-to-generation, plant-to-plant, and fruit-to-fruit [32].
- Patient-related factor such as patient age and weight also affected the dose to be administered.
- Repeated intakes of these antigen-bearing plants which stimulate the immune system might over stimulate the immune system itself leads to immune-tolerance to peptide or protein of edible vaccine [61].
- Certain plant or plant parts are not eaten raw, such as potato which is cooked or boiled may destroy or weaken most of the protein or peptide present in its.
- Identification of discrimination line between GMO vaccine plant/plant parts and normal plant/plant parts.
- The function of the edible vaccine can be hampered due to wide glycosylation pattern of plants and humans [32,56].

7. Patents on edible vaccine

GMO are living things developed in the labs by scientists and they are not naturally occurring in nature. Genetic modification is an expensive and involves lengthy process. The government have made several laws to grant patents for these GMO plants (edible vaccines) (Table 3), excluding naturally occurring plants and animals [62].

7.1. Regulatory, ethical aspects and challenges

While considering the edible vaccines a number of issues were observed. It was not clear under which category the edible vaccine should be regulated and what component of it must be licensed whether antigen itself, genetically engineered fruit or transgenic seeds. The regulatory bodies critically examine it to ensure that they do not enter the food supply.

A meeting was held by WHO in January 2005 for the regulatory evaluation of plant-based vaccine [64]. The conclusion was drawn that for the production of the edible vaccine the existing guideline for the development, evaluation, and use of vaccines of a conventional method must be used. Clinical testing of the edible vaccine must be performed under US Investigational new drug application and all the regulatory and GMP requirements must be followed by them [64]. Specific issues of the edible vaccines are present and the future of the edible vaccines depends on various criteria. Acceptance by the population has a major role in the development of the edible vaccine. The society should be aware of the benefits and uses of the edible vaccines. The beliefs of people in some areas are that the genetically modified plants are a threat like evil spirit and may destroy the world [65]. So, the role of the authorities is to awake people from such myths. The next important thing is to check the stability of the modified plants and isolation of the plant is necessary. The transgene may induce allergies sometimes and oral tolerance when administered along with adjuvants to activate the oral mucosal immune system which provokes hypersensitivity with the protein present in various daily food. It is cost-effective and safe and efficient as a vaccine. Better prevention options from diseases are done by an edible vaccine when they are developed in a proper manner when compared to traditional vaccines. During pollination, the genetically modified plants may enter the genetically non-modified plants and cross-contamination may occur which may lead to the aggressiveness of the genetically modified plants [65]. Hence a close and strict monitoring of the plants grown for the edible vaccine is necessary. By the contact of insects and birds with plants which cause contamination of water bodies, DNA or antigen may enter the water bodies. This leads to the entry of the pharmaceutical in the human food chain accidentally and effects the wildlife too. It is necessary to prevent environmental contamination and prevention of side effects.

8. Preclinical and clinical trials

To evaluate and test the interventions such as medication and psychotherapy clinical research is designed called as a clinical trial. These include various biomedical and behavioural researches in human beings to answer various questions on new interventions (Table 4). It gives safety and efficacy data of the drugs or treatment. Clinical trials are only performed after it has gained approval from the Health authority/ethics committee in the country [58]. This includes various phases in which the trials are performed. Due to ethical considerations, direct assessment of the drugs is prevented, except in some cases. Many of the plants have successfully shown antigenic expression, like LT-B (ETEC) in tobacco and potato, rabies virus-G protein in tomato, HBsAg in tobacco and potato, Norwalk virus in tobacco and potato; CT-B (*Vibrio cholerae*) in potato [66].

8.1. Rabies

Antibodies could be induced in mice with the help of rabies antigens expressed in tomato plants. Alternate to this tobacco may also be used. CaMV with the glycoprotein (G-protein) gene of rabies virus (ERA strain) used by tomato plants are shown to be immunogenic in animals [66].

Table 3
List of edible vaccine with their patent/application number.

Sl. no.	Patent/application number	Patent holders	Clam	References
1.	6136320	Prodigene	Production and transfer of recombinant antigen to plant cells through the plasmid-vector system; production of vaccine for hepatitis and transmissible gastroenteritis virus.	https://patents.justia.com/patent/6136320 (accessed on April 2020)
2.	–	Found.Advan. Mil. Med.	Antigen expressed in plant cell, Particularly useful against shigellosis.	[8]
3.	–	Ribozyme-Pharmaceuticals, Inc.	Nucleic acid vaccine for the treatment or prevention of plant or animal viral infection	[8]
4.	–	Rubicon-Lab	Retrovirus expressed in animal or plant cells useful as virus and cancer vaccine.	[8]
5.	6127145, 6066781	Applied Phytologics	Gene construction for susceptibility to disease; production of vaccine in rice, wheat, barley and corn. Construction of a vector containing codon-optimized alpha sub 1 antitrypsin sequence and Production of mature protein (alpha-1 antitrypsin) in plant.	https://patents.justia.com/patent/6127145 (accessed on April 2020) https://patents.justia.com/patent/6066781 (accessed on April 2020)
6.	5977438	Biosource (now Large Scale Biology)	Production of a malarial B-cell, cytotoxic T lymphocyte (CTL) in plant as viral coat protein fusion.	https://patents.justia.com/patent/5977438 (accessed on April 2020)
7.	–	University of Yale	Vaccine against invertebrates (insects, helminths, arachnids, etc.)	[8]
8.	–	University of Texas	Produced Hepatitis B virus core antigen for recombinant vaccine production.	[8,63]
9.	–	Biocem; Rhone-Merieux	Transgenic plant used to produce rabies vaccine.	[8]
10.		Institute Pasteur	In gene therapy attenuated *E. coli* vaccine is used.	[8]
11.	6395964	University of Texas A&M/Tulane University	To boost immunity with the aid of oral administration of antigen and adjuvant with transgenic plant to combat disease like diarrhoea, and cholera.	https://patents.justia.com/patent/6395964 (accessed on April 2020)
12.	7282625, 20030208792	Scripps Research Institute	Production of progeny expressing immunoglobulin heavy and immunoglobulin light chain. Isolation of mRNA coding, immunoglobulin heavy and light chain-coding gene in the transgenic plants. TMV modified CP gene produce HIV GP10 V3 loop fusion protein containing the TMV coat protein, and to overproduce peptides and protein.	https://patents.justia.com/patent/7282625 (accessed on April 2020) https://patents.justia.com/patent/20030208792 (accessed on April 2020)
13.		Cornell University	Increasing foreign protein expression	[58]
14.	7422747 6777546	University Loma Linda	For the treatment of acute gastroenteritis. Transgenic plant vaccine used to treat autoimmune disease.	https://patents.justia.com/patent/7422747 (accessed on April 2020) https://patents.justia.com/patent/6777546 (accessed on April 2020)
15.	–	Agr Genet/Purdue Research Foundation	In against of foot and mouth disease, HIV, and human rhino virus modified virus are used for the production of vaccine.	[8]
16.	10480002	Transalgae Israel Ltd.	Algal based nervous necrosis viruses (NNV) vaccine for white grouper (*Epinephelus aeneus*), and European bass fish; *Salmonella enterica* Serovar enteritidis Flagellin Expressing Algae vaccine for chicken.	https://patents.justia.com/patent/10480002 (accessed on April 2020)
17.	10617751	VisionTech International Limited	The recombinant yeast is used as edible vaccine for preventing white spot syndrome virus (WSSV) infection in shrimps.	https://patents.justia.com/patent/10617751 (accessed on April 2020)
18.	8282936	Asahi Glass company Ltd.	Edible Human papilloma virus type 16 vaccine is produced by Avirulent fission yeast (Schizosaccharomyces pombe) host.	https://patents.justia.com/patent/8282936 (accessed on April 2020)
19.	10030250	Keneth John Piller, Kenneth Lee Bost	Edible vaccine expressed in transgenic soybean used in humans and animals to treat viral, bacterial, fungal, parasitic, or prion related disease, cancer antigens, and toxins. This vaccine can be used to induce tolerance to allergens, and tolerance to autoimmune antigens.	https://patents.justia.com/patent/10030250 (accessed on April 2020)
20.	7354760	University of Central Florida Research Foundation, Inc.	Edible vaccine against *Bacillus anthracis* and *Yersina pestis* to give immunity in mammals.	https://patents.justia.com/patent/7354760 (accessed on April 2020)
21.	6777546	Loma Linda University	CTB-autoantigen chimeric gene construct in plant cells for the development of plant-based vaccine and used in systemic immunosuppressive therapy in autoimmune disease and in transplant.	https://patents.justia.com/patent/6777546 (accessed on April 2020)
22.	5612487	Edible Vaccine, Inc.	Edible vaccine to treat viral infection like hepatitis B by using it surface antigen.	https://patents.justia.com/patent/5612487 (accessed on April 2020)

Table 4
Clinical trial status of some edible vaccine.

Sl. no.	Pathogen	Antigen	Host	Diseases	Clinical trial status	References
1.	*Vibrio cholerae*	CTB	Rice	Cholera	Phase 1	[67]
2.	Norwalk virus	CP	Potato	Diarrhoea	Early phase 1	[68]
3.	*Enterotoxigenic* E. coli	LT-B	Potato	Diarrhoea	Early phase 1	[69]
4.	HBV	HBsAg	Potato	Hepatitis B	Phase 1	[70]
5.	*Enterotoxigenic* E. coli	LT-B	Maize	Diarrhoea	Early phase 1	[71]
6.	HBV	GP/NP	Spinach	Rabies	Early phase 1	[36,68]
7.	HBV	HBsAg	Lettuce	Hepatitis B	Early phase 1	[36,68]

8.2. Cholera

CT-B gene of *Vibrio cholerae* when transferred to the potato was seen effective in mice. Administration of a single potato a week for one month with periodic boosters was seen providing immunity. Mutant cholera toxin subunit A (mCT-A) and LT-B in crop seed, when expressed together, has shown to be effective by nasal administration and is practical [66].

8.3. Hepatitis B

When human trials of the potato-based vaccine of hepatitis B were performed, it has shown remarkable result. Specific antibodies were induced in mice by VLPs. In a single potato expression of a single dose of HBsAg can be achieved [66]. Protective level of 10 mIU/mL of specific antibodies exceeded significantly. Higher expression of plasmid HBsAg subtype ayw was seen in roots as compared to leaves tissue of transgenic potato when cloned with CaMv (cauliflower mosaic virus) [66]. Further studies need to be done for increasing the antigenic expression in potato by using promoter like patatin promoter. A superior plant material then yeast-derived antigen for both priming and boosting immunity in mice was observed. When mice were given a single parenteral dose of yeast-derived recombinant HBsAg followed by transgenic potatoes lead to the development of antibodies that immediately peaked at > 1000 mIU/mL and were maintained at > 200 mIU/mL for five months. This was a prime-boost strategy in mice which useful in developing countries. The guarded greenhouse was used to grow plants of transgenic tomatoes and around 4000 vaccine doses were obtained from just 30 tomato plants. Lettuce plant is also being developed.

8.4. Norwalk disease

When transgenic potatoes expressing Norwalk virus antigen were fed to 20 people, out which 19 people were observed developing antibodies. Bananas and tomatoes expressing Norwalk virus are also being engineered [58,66].

8.5. Measles

When tobacco expressing MV-H (measles virus haemagglutinin from Edmonston strain) was fed to the mice antibody were developed which were five times of the levels considered protective for humans and they secreted IgA in their feces. The titers were increased 20 times the level protective for human by using a prime booster strategy of combining parenteral and subsequent oral MV-H boosters [66]. Significantly greater titers were observed than with either vaccine administered alone. The successful experiments in mice provoked a similar experiment with primates. When a dose of 35–50 g MV-H lettuce is given with CT-B (adjuvants) it is enough but an increased dose is demanded when it is given alone [66].

8.6. ETEC

Raw transgenic potatoes expressing LT-B were fed to eleven volunteers. Out of these 10 were seen with neutralizing antibodies and 6 developed mucosal responses. First published successful human trial was by Charles Arntzen at Boyce Thompson Institute, USA, in 1997 [13,66].

8.7. STDs

When BLB/c mice were given Human papillomavirus type-11 (HPV-11) recombinant VLPs produced in insect cells, it was found immunogenic to them. The response is dose-dependent, conformationally-dependent and genotype-restricted [66]. Thus, VLPs may be effective oral immunogens for the prevention of anogenital HPV disease.

8.8. Anthrax

A protein identical to the major protein present in existing vaccines could be expressed when tobacco leaves bombarded with pag gene (anthrax protective antigen - PA) using a gene gun [66]. Oedema factor and a lethal factor which were responsible for toxic effects were absent in these vaccines. A numerous anthrax antigen could be produced. Tomato plants are being used to put the same anthrax antigen. Transformation of spinach by inoculating it with TMV-expressing PA is also being developed by scientists as spinach is safer as a vaccine [58].

8.9. Others

Research works of developing a various vaccine is undergoing and are being tested regularly for their safety and efficacy. Clinical trials of all the vaccines are done so that a safer and effective drug can be obtained. Due to the complex multistage life cycle of the parasite, its inaccessibility to study and by its large genome advances in the vaccine of malaria are hindered. However chimeric coat proteins of CPMV expressing malarial and foot-and-mouth disease epitopes have been reported [66]. Work for the development of vaccine of rotavirus and Streptococcus is being carried out in various developing countries. High titers of IgG and mucosal IgA in mice could be induced by transgenic potatoes expressing VP7 in case of rotavirus. A fusion protein consisting of LT-B and early secretory antigen ESAT-6 of *Mycobacterium tuberculosis* [66] were produced by transgenic *Arabidopsis thaliana*, demonstrating both the antigens by ELISA. Respiratory syncytial virus (RSV) pneumonia has been lethal to small children of the age of 2 years. RSV expressed in transgenic tomato and transgenic potatoes have shown good results in mice. Development of RSV based apple juice is also being done.

8.10. Bio-terrorism

Many scientists discussing about the development of new edible vaccine from genetically modified plants with their risks and benefits, or regulation. Plant based edible vaccine has many advantages over conventional vaccine but, inappropriate management of a transformed plant or fruit carrying a vaccine may cause serious problems to public health all over the globe, whether due to mismanagement or due to intentional reasons (bio-terrorism), as the production can be easily

scaled up for millions of doses within a limited period of time [12,30,66]. To overcome this problem in collaboration with U. S. Army, developed a vaccine to address bioterrorism of Ebola virus with its surface glycoproteins which can be transiently expressed in plants [12].

9. Comparative evaluation of novel edible vaccine and conventional vaccine

Unlike the conventional vaccine, the edible vaccine is capable to bring mucosal immunity. But there is a remarkable limitation of the conventional vaccine over the edible vaccine. The latest novel approach for vaccination has several benefits and the formulation technique, transportation and mode of administration is so convenient that it can be practised without sophisticated technology and specialized medical expertise. It also doesn't require subsidiary elements to stimulate the immune system [32]. The conventional vaccines are safe as it contains attenuated and heat-killed pathogens hence do not present any risk of proteins to reform into the infectious organism [32]. Regarding the economy for production and the quantity to be manufactured, edible vaccines are much more convenient as it minimum cost and its process for production can be scaled rapidly by breeding [61], but the concentration of the protein depends on the weather condition (sunlight, rainfall, altitude etc.), whereas conventional vaccine is produced in the controlled environment. The route of administration and delivery of the vaccine improves compliance than conventional vaccination.

10. Application of edible vaccine in humans and animals' diseases

The basic concept that is involved in the development of vaccines is to select desired genes and then incorporation of these genes into plants and then encoded proteins are produced by the altered plants after enabling it. Various edible vaccines from plants like potato, banana, tobacco, etc. are produced against various diseases like cholera, measles, hepatitis and foot and mouth disease. Following are a few applications of edible vaccines.

10.1. Role in autoimmune diseases

Some of the autoimmune diseases that are under study for the development of edible vaccine include; lupus, rheumatoid arthritis, transplant rejection and multiple sclerosis. The developmental stage of growing self-antigen production in plants is in process. A clinical study was carried out which included a strain of mouse susceptible to diabetes. In this trial, the mouse was fed with the potatoes capable of expressing insulin and protein GAD (glutamic acid decarboxylase), linked to CTB subunit. In this study, delayed onset of high blood sugar level and immune attack suppression was proved successful [7,32,58].

10.2. In cancer therapy

For effective cancer therapy agents, many plants have been engineered to generate monoclonal antibodies. Soybean (BR-96) is an effective agent in curing breast cancer, ovarian cancer, colon cancer and lung tumours [32].

10.3. In birth control

When TMV is administered, a protein is produced which is found in *Mousezona pellucida* (ZB3). Due to resulting antibodies, the protein can prevent fertilization of eggs in mice [32,72].

10.4. Recombinant drugs/proteins

Apart from the production of vaccines and antibodies, plant compositions are altered by engineered viral inoculations to produce enzymes; drugs (serum protease, albumin and interferon). For example-tobacco plants produce glucocerebrosidase (hGC) to treat Goucher's disease, interleukin-10 to treat Crohn's disease. This method is cost-effective [32].

10.5. Rabies

The causative agent of rabies is a single-stranded negative-sense RNA virus. The name of the virus is lyssavirus of the family Rhabdoviridae, which is having a cylindrical shape with bullet-shaped virus particles. In concern of rabies, the transgenic tomato was noticed with stable expression of the rabies surface protein but it lacked immune-protective ability. A synthetic gene coding for the surface glycoprotein (G-protein) of rabies virus identifies the major antigen that induces protective immunity was strategically designed to achieve high-level expression in transgenic plants for immune-suppression by rabies vaccines glycosylation of G-protein is required [73].

10.6. Foot and mouth disease

Foot and mouth disease are caused by a picornavirus, foot and mouth disease virus. It is a type of disease which causes high fever which lasts for two to six days and then blisters inside the mouth and feet. Mainly cloven-hoofed animals including domestic and wild bovids are affected by this disease. The structural protein VP1 (viral protein) of FMDV carries epitopes which cause the induction of protective neutralizing antibodies. This can be expressed as an immunogenic antigen in '*Arabidopsis thaliana*' alfalfa and potato. These are used to express a virus specific-protective antibody response [20,61,74].

10.7. Hepatitis

Hepatitis B virus is the causative agent for the infectious disease hepatitis B. In this the liver of the humans are affected and inflammation occurs called serum hepatitis. The gene encoding hepatitis B surface antigen (HBsAg) that was linked with a constitutive promoter transformed the tobacco plant genetically [75]. The presence of HBsAg in extracts of transformed leaves was seen by the enzyme-linked immunoassays using a monoclonal antibody directed against human serum-derived HBsAg at levels that correlated with mRNA abundances. This led to a conclusion that no major inherent transcription or translation of this foreign gene in plants was found. Purification of this recombinant HBsAg from the transgenic plant was done by immune-affinity chromatography and then is examined by electron microscopy. When negatively stained preparation applied a spherical particle of 22 nm was observed. Similar physical properties were observed in recombinant HBsAg and human serum-derived HBsAg due to sedimentation of transgenic plants in sucrose and caesium chloride density gradients [76,77]. As the similarity was observed the conclusion from it can be drawn that transgenic plants hold promise as low-cost vaccine production systems.

10.8. Rota viral disease

Rota viral disease is a common disease occurring in infants and young children. At least once by the age of five, nearly every child in the world is infected with rotavirus. This causes diarrhoeal disease among children. It is very less likely to occur in adults. There are 10 species of the genus, referred to as A, B, C, D, E, F, G, H, I and J [6]. More than 90% of rotavirus infections are caused by most common rotavirus namely rotavirus A. Synthesis and insertion of a codon-optimized gene (sVP6) encoding the VP6 protein of human group A rotavirus was done into the alfalfa genome using Agrobacterium-mediated transformation [6]. When oral immunization with pBsVP6-transgenic alfalfa was done provides a potential means of protecting children and young animals from severe acute rotavirus-induced diarrhoea [61].

10.9. Cholera

Cholera is a diarrhoeal disease caused by bacteria *Vibrio cholerae*, a bacterium of comma shape. It colonizes the intestine and produces enterotoxin cholera toxin B (CTB) and causes acute watery diarrhoea [78,79]. When taken orally CTB acts as a mucosal immunogen. A mucosal response to pathogens was obtained due to the CTB binding to the eukaryotic cell surfaces via the GM1 ganglioside receptors present on the epithelial surface of the intestines. An experiment was carried out by Daniel et al. in which a construction of the chloroplast expression vector, pLD-LH-CTB, was carried out. Using immunoblot assay the production of CTB in *E. coli* was observed. The bombardment of the plasmid DNA (pLD-LH-CTB) in Nicotiana tobacco leaves was carried out. In a medium containing a selection marker, in this case, streptomycin, the transformed leaves were cut and grown. It was analysed using PCR analysis followed by southern blot analysis. Quantification of the CTB protein produced was carried out using Western blot analysis and ELISA. A strong affinity for GM1 ganglioside was demonstrated by both the chlorophyll-synthesized CTB and the bacterial CTB in GM1 ganglioside assay [78]. The growth rate, flowering, and seeding in transgenic tobacco are not expressed due to high levels of constitutive expressions, unlike when expressed in the nuclear genome.

10.10. Malaria

Malaria is a disease caused by plasmodium parasite and is spread by female Anopheles mosquito called “malaria vectors.” 5 parasite species have been reported that cause malaria in humans, out of which 2 of the species - *P. falciparum* and *P. vivax* - cause the greatest threat [59,80]. Symptoms of malaria include fever, headache and chills - may be mild and difficult to recognize as malaria. If it is not treated within 24 h the *P. falciparum* may grow progressively and cause severe illness, often leading to death. After many efforts of developing a vaccine for malaria recently, 3 antigens have been selected for development of vaccine of malaria. The antigens were obtained from Plasmodium parasites namely - merozoite surface protein (MSP) 4 and MSP 5 from *P. falciparum* and MSP4/5 from *Plasmodium yoelii.* Induced antibody responses against the blood-stage parasite were seen when recombinant MSP 4, MSP 4/5 and MSP1 was orally administered as a supplementary therapy with cholera toxin B (CTB) in mice [59,80]. For expression of minimum antigen large amount of antigen must be incorporated in plants. For proper protection against various stages of malarial infection, a multiple of 10–15 antigen targets will be required. An anti-malarial edible vaccine in transgenic tomato plants was developed to overcome the problem of transport and the logistic of vaccination which would be difficult to achieve under current fiscal constraints [59,80].

10.11. Measles

Measles is a disease caused by a virus and is highly contagious. It occurs mainly in children and remains an important cause of death among young children globally, despite the availability of a safe and effective [8]. The transmission of measles occurs through droplets from the nose, mouth or throat of infected persons. High fever, a runny nose, bloodshot eyes, and tiny white spots on the inside of the mouth are the symptoms that occur 10–12 days after infection. In 2018, around 140,000 measles deaths were observed globally, mostly among children under the age of five, even after the availability of a safe and cost-effective vaccine. The oral efficacy of measles oral live attenuated vaccine is absent and when maintained on a cold chain of refrigeration they are destroyed. Due to the presence of maternal antibody, the effectiveness is reduced. Among the two surface proteins present namely - haemagglutinin (H) and fusion protein, H protein infected with wild-type measles virus [9,81]. Animals vaccinated with MVH were observed and the results indicated that the fecal sample of the animal shows the presence of IgA antibodies [60]. For measles vaccine, according to some studies, the transgenic carrot is the best choice for the measles vaccine.

10.11.1. Probiotic oral vaccines

Probiotics are living microorganisms, according to the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nation, that gives the host health benefits when administered in adequate amount [82,83]. Another definition says probiotics must contain at least 106 CFU/g of visible and active microorganisms in food, while freeze-dried supplements demonstrate better results with 107 to 1011 viable microorganisms per day [83–85]. The most frequently used probiotic bacteria are in specific strains belonging to *Lactobacillus*, *Enterococcus*, *Saccharomyces boulardii*, *Bifidobacterium* and *Escherichia coli* that are the most predominant and subdominant groups of gastro-intestinal microbiota [82,86,87]. However, other stains such as *Leuconostoc*, *Streptococcus*, *Predicoccus*, and *Lactococcus* are also used as probiotics [83]. *Saccharomyces boulardii* is the only yeast that used as probiotic and used in the prevention and therapeutic agent for GI disorder and diarrhoea in many countries [84,85]. Use of these yeast and live bacteria to generate an immune response or to carry a vaccine component is a new concept in the development of vaccines. Probiotic bacteria target inductive site of the host immune system such as mucosal surface and antigen-presenting cells (APCs) on macrophages. Still lot of probiotic vaccine under clinical trials (Table 5) and probiotic based vaccine mention in Table 6.

10.11.2. Benefits of probiotic vaccine vectors

Stable colonization and survival in vivo, particularly in the harsh gut environment [88].

Improve the efficacy of the vaccine.

Other beneficial effects include anti-inflammatory activity and antibacterial activity [88].

10.11.3. Limitations

The major limitation of probiotic vaccine is oral tolerance and difficult to monitor the immune complexity [88]. Another major factor innate low immunogenicity of probiotics.

10.11.4. Mechanism of action

The specific mechanism by which probiotics accomplish their beneficial actions have not been well established. However, many postulated mechanisms of action include increased adhesion to intestinal mucosa, concomitant inhibition of pathogen adhesion, enhancement of the epithelial barrier, production of anti-microorganism substances, competitive exclusion of pathogenic microorganism and modulation of the immune system explain most of their beneficial effects.

To understand the impact of adjuvant strategies on the immune response to lactic acid bacteria (LAB) mucosal vaccine, it's essential to explore the endogenous immune activating mechanisms possessed by LAB [89,90]. The features that make LAB particularly attractive to be used as mucosal vaccine vector is that it ability to stimulate innate immunity response through its Gram-positive cell wall of lipoteichoic acid and peptidoglycan that activate pattern-recognition receptors such as nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family, toll-like receptor (TLR) 2, and C-type lectin receptors. Different LAB species can activate TLR9, TLR6, and TLR3 as well as stimulate interferon responses [90]. In addition, some LAB stains can bond to intestinal mucus and mucosal epithelium or microfold (M) cells leading to mucosal colonization and increase uptake and transport to the mucosal immune induction sites such as Peyer's patches and tonsillar crypts. Lactic acid bacteria can interact with APCs such as induced IgG and SIgA and dendritic cells [90]. The mechanism of activation of dendritic cells and the resulting immune response depends on the lactic acid stain. For example, the response of murine DCs can respond differently depending on LAB stains and is further complicated by the fact that these responses can be different even between DC subtypes. It shows the complexity of choosing a suitable LAB stain as a vaccine

Table 5
List of oral probiotics under clinical trial.

Sl. no.	Condition	Intervention	Phase	Reference
1.	Asthma	Probiota Bifido (Bifidobacterium)	Not applicable	https://clinicaltrials.gov/ct2/show/record/NCT03157518?term=oral+probiotic&draw=3&rank=2 (accessed on August 2020)
2.	Gingivitis	Probiotic tablet [mixture of Lactobacillus rhamnosus PB01, DSM 14869 and Lactobacillus curvatus EB10, DSM 32307, 1 * 10(9) CFU]	Not applicable	https://clinicaltrials.gov/ct2/show/NCT04071210?term=oral+probiotic&draw=3&rank=3 (accessed on August 2020)
3.	Bacterial vaginosis	Lactobacillus rhamnosus and lactobacillus acidophilus Lactobacillus rhamnosus and Lactobacillus plantarum	Not applicable	https://clinicaltrials.gov/ct2/show/NCT03116789?term=oral+probiotic&draw=3&rank=4 (accessed on August 2020)
4.	Hyperbilirubinemia, neonatal	Lactobacillus salivarius AP-32 Bifidobacterium animalis subspecies lactis CP-9	Not applicable	https://clinicaltrials.gov/ct2/show/NCT03876678?term=oral+probiotic&draw=3&rank=5 (accessed on August 2020)
5.	Bacterial vaginosis and vaginal candidiasis at pregnancy	L. acidophilus, L. acidophilus, *L. paracasei*, *L. rhamnosus*, Streptococcus thermophilus and Bifidobacterium bifidum	Not applicable	https://clinicaltrials.gov/ct2/show/NCT02795845?term=oral+probiotic&draw=3&rank=9 (accessed on August 2020)
6.	Chronic periodontitis	Lactobacillus rhamnosus SP1	Phase 2	https://clinicaltrials.gov/ct2/show/NCT02283736?term=oral+probiotic&draw=2&rank=19 (accessed on August 2020)
	Mucositis oral	Bifidobacterium animalis subsp. lactis	Phase 3	https://clinicaltrials.gov/ct2/show/NCT04187222?term=oral+probiotic&draw=3&rank=107 (accessed on August 2020)
7.	Anti-biotic resistance	L. rhamnosus HN001	Phase 2	https://clinicaltrials.gov/ct2/show/NCT01321606?term=oral+probiotic&draw=3&rank=109 (accessed on August 2020)
8.	Dental plaque	Lactobacillus reuteri DSM 17938 and PTA 5289	Phase 2	https://clinicaltrials.gov/ct2/show/NCT02311218?term=oral+probiotic&draw=3&rank=114 (accessed on August 2020)
9.	Sepsis	Lactobacillus plantarum	Phase 2	https://clinicaltrials.gov/ct2/show/NCT00518596?term=oral+probiotic&draw=5&rank=142 (accessed on August 2020)
10.	Rhinitis	Lactobacillus paracasei LP-33	Phase 3	https://clinicaltrials.gov/ct2/show/NCT01096615?term=oral+probiotic&draw=5&rank=152 (accessed on August 2020)

Table 6
Probiotic based vaccine.

Sl. no.	Vehicle	Vaccine target	Antigen	Immune response	Outcome	Reference
1.	L. lactis	*Streptococcus pneumoniae*	PppA (LPA+)	Mucosal IgA antibody and serum antibody	Boosted resistance to Intranasal infection with virulent pneumococcal serotypes	[91]
2.	L. lactis	Rotavirus	VP8	Intestinal IgA antibody and serum antibody	Suppressed the viral infection (100%) in MA-104 cells	[92]
3.	L. lactis	*Helicobacter pylori*	UreB	Serum antibody and fecal IgA	Protected mice from gastric infection with *H. pylori* strain SS1.	[93]
4.	L. casei	Porcine epidemic diarrhoea virus (PEDV)	Core neutralizing epitope (COE)	High level of SigA and IgG, and a Th2 immune response	Protected piglets (60%) 96 h post-infection of PEDV	[92]
5.	EcN	Enterotoxigenic *Escherichia coli* (ETEC)	Stx B-subunits, OspA and OspG proteins	Heterogeneous immune response	Insufficient to mediate protection	[94]
6.	S. gordonii	*Bordetella pertussis*	S1 subunit of pertussis toxin (PT)	SigA in saliva and IgG	Long-term oral colonization and maintenance of recombinant S. gordonii in BALB/c mice	[95]
7.	*B. subtilis*	*E. coli*	LTB	Serum IgG and fecal IgA	50% protection to mice challenged with ETEC heat labile toxin (LT)	[96]
8.	B. subtilis	*Clonorchis sinensis*	*C. sinensis* enolase	A mix of IgG1/IgG2a	Rats immunized have worm reduction rate and egg reduction rate of 61.07% and 80.67% respectively	[96]

vector.

11. Conclusion

The conventional vaccine previously developed have a great role in reducing the annual death toll from several infectious diseases, yet these discoveries mask the tragic gaps in drug delivery process and fail to achieve the required therapeutic effect especially in the remote and improvised part of globe due to lack of availability of vaccine. Due to the non-existence, unreliable or too costly immunization technique and non-effective drug delivery system, millions of people are still dying from infectious diseases. This is worrisome for the entire globe.

12. Expert opinion

The very new technology for the immunization via the oral route is the overcome for all the challenges faced by the conventional vaccine. The edible vaccine might be the solution for the potential hazard associated with the parenteral vaccines. The encapsulation of a particular gene of interest in the plant cell, which produces the desirable encoded protein is the basic principle of an edible vaccine. The convenient manufacturing process and easy transportation guidelines make it easier for the outreach of vaccines for crucial infectious diseases in developing and underdeveloped countries. The induction of mucosal and peripheral immunity and easy administration of vaccine has created a large future scope in medical research. The production of the plant-based vaccine will eventually eradicate the outbreak of the infectious disease throughout the globe.

12.1. Key issue

1. The concept of conventional vaccination has overcome the pandemic situation of several infectious diseases outbreak.
2. It is important to introduce the genes encoding mucosal adjuvants into transgenic plants used for creation of edible vaccines and/or use plants containing the secondary metabolites capable of playing the role of mucosal adjuvants.
3. Edible vaccines have significant role in stimulating mucosal immunity as they come in contact with digestive tract lining.
4. Highly efficient systems of transplastomic plants or transient expression systems using plant virus-based vectors can be an alternative for production of antigens for an inexpensive and safe subunit vaccine.
5. Combined use of injection and mucosal vaccines against various infectious disease can provide the most reliable protection against this viral infection.

12.2. Limitation

The major limitation of this manuscript is that as in date, neither the allergy nor the tolerance has been studied in humans.

Abbreviations

FMD	foot and mouth disease
FMDV	foot and mouth disease virus
AIDS	acquired immunodeficiency syndrome
FDA	Food and Drug Administration
PDs	protein drugs
cGMP	current Good Manufacturing Practice
LT-B stain	heat-labile endotoxin strain
ETEC	enterotoxigenic *Escherichia coli*
SARS	highly acute respiratory syndrome
CoV	coronavirus
ER	endoplasmic reticulum
IgG	immunoglobulin G
IgA	immunoglobulin A
HbsAg	hepatitis B surface antigen
PCR	polymerase chain reaction
CMV	cytomegalovirus
TMV	tobacco mosaic virus
DNA	deoxyribonucleic acid
mRNA	messenger ribonucleic acid
MALT	mucosa-associated lymphoid tissue
GALT	gut-associated lymphoid tissue
GIT	gastrointestinal tract
MHC	histocompatibility complex
IM	intramuscular
SC	subcutaneous
GMO	genetically modified organism
GAD	glutamic acid decarboxylase
RPV	rinderpest virus
CTB	cutaneous tuberculosis
VP1	viral protein 1
hGC	human chronic gonadotrophin
APC	antigen presenting cells
VLPs	virus like particles
DCs	dendritic cells
LAB	lactic acid bacteria
NOD	nucleotide-binding oligomerization domain
TLR	toll-like receptor
SigA	secretory immunoglobulin
CFU	colony forming unit
CD40	cluster of differentiation 40
CXCR5	CXC chemokine receptor 5
WHO	World Health Organization
GMP	Good Manufacturing Practice
ELISA	enzyme linked immunosorbent assay
MSP	merozoite surface protein
NLR	NOD like receptor
ISCOMs	immunostimulatory complexes
CaMv	cauliflower mosaic virus
ERA	Evelyn-Rokitnicki-Abelseth strain of rabies virus
HVP-11	human papillomavirus type-11
RSV	respiratory syncytial virus

Declaration of competing interest

All the authors declare none conflict of interest.

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