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PROBING INTO THE EDIBLE VACCINES: NEWER PARADIGMS, SCOPE AND RELEVANCE

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Abstract

Vaccines are proved to be boon for the prevention of infectious diseases and provide acquired immunity against life threatening infections. The lethality of infectious diseases has decreased due to vaccination as it is one of the safe and effective measure to control various infectious diseases. A protein which acts as the vaccine, present in food and consumed as the internal composition of food is known as the edible vaccine. As the name suggests, the term "Edible vaccines" was first used by Charles Arntzen in 1990 and refers to plants that produce vitamins, proteins or other nourishment that act as a vaccine against a certain disease. These vaccines are capable to stimulate the body's immune system to recognize the antigen. Edible vaccines have been the newer form of vaccines which have the power to cover the risks associated with conventional vaccines. 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 are produced by the incorporation of the selected desired genes into the plants and then modified to produce the encoded proteins, providing immunity for certain diseases. Identification, isolation and characterization of a pathogenic antigen is important for making an edible vaccine. At present edible vaccine are developed for various veterinary and human diseases such as cholera, measles, hepatitis and foot and mouth diseases. Current review highlights the importance of edible vaccines which could prove to be cost effective, efficient and safe and would not require refrigeration, making them more accessible to poor people as compared to traditional vaccines.

Key words: Vaccine, Edible, Immunity, Diseases, Protection, Mechanism

Introduction

It is better to prevent a disease than to cure it and the best way to prevent a disease is to have antibodies through immunization while the most effective way to get immunized is by vaccination. Vaccination has been the most cost-effective health care intervention so far. Majority of the vaccines in use today are against viral or bacterial infections. When it comes to the constituents of a vaccine, it mainly comprises of either live attenuated microorganisms, inactivated whole microorganisms or subunit preparations. Some live viral vaccines have been regarded as the most successful of all human vaccines, with even one or two administrations of the same may induce long lasting immunity. Inactivation of microorganisms is the basis of vaccines displaying varying efficacy. Compared to attenuated vaccines, inactivated vaccines need to be administered in substantially larger doses and sometimes even more frequently. In subunit vaccines, the generation of antibodies that prevents infection by both intra and extracellular microorganisms, has been regarded as the prime requirement of a vaccine. The epitopes recognised by such antibodies are usually confined to one or a few proteins or carbohydrate moieties present externally on the surface of the microorganisms. Isolation of such components formed the basis of the first viral and bacterial subunit vaccine (Anderson & May, 1985; Robinson, Farrar, & Wiblin, 2003). Table 1 highlights some of the major vaccines currently approved for therapeutic use in humans.

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Table 1: Existing viral and bacterial vaccines against diseases.

S.No.	Type of Vaccines	Examples	Reference
1.	Live attenuated Live vaccines use a weakened (or attenuated) form of the germ that causes a disease. Immune response is similar to natural infection	Viral-Measles, mumps, rubella, yellow fever, influenza, oral polio vaccine, vaccinia Bacterial- BCG (Bacillus Calmette Guerin), typhoid (Salmonella (Ty21a))	(Arts et al., 2018; Higgins et al., 2016; Jensen et al., 2014; Krone, Kölmel, Henz, & Grange, 2005; Minton, 1973; Morales & Eidinger, 1976; Rosario et al., 2010; Rowland et al., 2012; Tamuzi, Muyaya, & Tshimwanga, 2017) (Miller & McCann, 2000) (Deforest et al., 1988; Farrington et al., 1995; Higgins et al., 2016; Usonis et al., 2005)
2.	Killed or Inactivated vaccines Vaccines use the killed version of the germ that causes a disease. Immune response is mostly humoral	Viral- Inactivated polio vaccine (IPV), Hepatitis-A, Influenza, Rabies, Japanese encephalitis. Bacterial- Pertussis, Typhoid, cholera, Plague	
3.	Toxoids Toxoid vaccines use a toxin (harmful product) made by the germ that causes a disease. In general toxoids are highly efficacious and safe immunizing agents	Tetanus, Diphtheria	
4.	Subunit Vaccines Subunit, recombinant, polysaccharide, and conjugate vaccines use specific pieces of the germ — like its protein, sugar, or capsid (a casing around the germ). Highly Efficacious and safe	Bacterial: Streptococcus pneumonia, Salmonella typhi VI, conjugated toxoids: Clostridium tetani, Viral: H. influenza, type b Conjugated Vaccines: Viral-Measles, Mumps, Rubella (MMR); Bacterial-Diphtheria pertussis tetanus (DPT)	

Expression of bacterial and viral antigens in plants has been well documented in earlier studies (Giddings, Allison, Brooks, & Carter, 2000). In the very first published clinical trial, volunteers were fed raw potato tubers expressing the binding subunit of an *E. coli* heat-labile enterotoxin (Tacket *et al.,* 1998; Tacket *et al.,* 2000). The serum antibodies produced by these volunteers were able to neutralize enterotoxic *E. coli in vitro*.

Edible vaccines are currently being developed for a number of human and animal diseases, including measles, cholera, foot and mouth diseases and hepatitis B & C (Giddings *et al.,* 2000). Many of these diseases are likely to require booster vaccinations or multiple antigens to induce and maintain protective immunity. Plants have the capacity to express more than one transgene, allowing delivery of multiple antigens for repeated inoculations (Conrad & Fiedler, 1994).

The research into edible vaccines holds promise for the public health of many developing countries, where diseases like cholera (which are easily preventable through sanitation) kill as many as 10 million inoculated children every year (William HR Langridge, 2000). Initially, cholera outbreaks could be controlled by antibiotics. But since 1980, resistant cholera strains have appeared and the common antibiotics such as ampicillin, streptomycin and tetracycline are not effective against these strains. An injectable cholera vaccine exists, but even inexpensive vaccines are beyond the reach of countries in which the annual public health expenditure average $10 per person (Feikin, Flannery, Hamel, Stack, & Hansen, 2016; Hsiao, Hall, Mogasale, & Quentin, 2018; Jeuland & Whittington, 2009). The new recombinant DNA vaccines are very expensive (A. Sharma & G. Khuller, 2001; A. K. Sharma & G. Khuller, 2001). A new injectable hepatitis vaccine is safer and more effective than conventional treatments, but it can cost up to $100 per person (Lancaster, Elam, & Kaiser, 1989). At the same time, AIDS and the risk of contaminated needles are potential health risk in some third world countries (Heise, 1988; Hu, Kane, & Heymann, 1991). The edible vaccine, in development, would probably be no more effective than most vaccines available today, but they would be cheaper and easier to distribute. Edible vaccines, which aim to increase the vaccination coverage, have to provide clear advantages over the existing ones (Toonen, 1996). Therefore, a new approach has to score better on four criteria of vaccination, *i.e.* it should be easily available, accessible, and acceptable to the consumer and should be effective as well (Nair, 2002).

The success of immunization strategies depends principally on reducing the susceptible proportion of the population to levels below which disease can remain endemic (Gay, Hesketh, Morgan-Capner, & Miller, 1995). Despite advances in medical science, the goal of herd

immunity remains unattainable for most of the world's population, largely because of constraints on vaccine production distribution and delivery. One possible solution may be the production of edible vaccines grown in genetically modified food crops (Giddings *et al.,* 2000). Such plants could be grown locally at a reduced cost, transport requirements and dependence on foreign supply. Vaccine antigens expressed in plant storage organs such as seeds are frequently stable at room temperature, eliminating the need for refrigeration during transport and storage (Mett, Farrance, Green, & Yusibov, 2008; Sala *et al.,* 2003). Oral administration reduces the need for skilled personnel to give injection. In addition, oral vaccination may stimulate both systemic and mucosal immunity (Arakawa, Chong & Langridge, 1998). Another potential advantage is that, plant derived vaccines are subunit vaccines. They contain only a small part of the pathogen and are unable to establish an infection. This offers an additional level of vaccine safety, particularly in immuno-compromised individuals.

Merits of an edible vaccine

An edible vaccine holds a number of advantages over other conventional vaccines such as the scaling up for commercial production of plant-based vaccines has been seen to be faster and less costly. It is envisaged that one gram of raw material could be multiplied to about 40,000 grams in eight months and 8,000 kg in a year. On the other hand, the cost of per gram of raw material from transgenic plants is around 10 cents as compared to $1 for other systems (L.J. Richter, Thanavala, Arntzen & Mason, 2000; Tacket *et al.,* 1998). Moreover, the need for purification of the product, maintenance of lower temperature during transport, use of needles and syringes for administration, are eliminated (Tacket & Mason, 1999; Tacket *et al.,* 1998). Also, the oral intake of medicine has been found to be much more convenient than by injection. The chances of cross contamination with animal or human pathogens are also less likely for production of biomedical materials from plants. Furthermore, farming has been an important and established part of our economy. Growing plants for production of vaccine and other therapeutic agents are certainly going to support global farming and economies (Tacket *et al.,* 2000). The same group of researchers reported that because one plant can express several antigens simultaneously, vaccines against a variety of pathogens can be produced in a single plant (Tacket *et al.,* 2000). In addition, contamination risks associated with mammalian cell lines, yeast or bacterial production system are also eliminated (Shah, Trivedi, Vachhani, & Joshi, 1990).

Demerits of edible vaccines

Several technical and logistical problems are required to be resolved before an edible plant vaccine becomes a reality in practice. Most transgenes are expressed at very low level in plants for which development of efficient promoters are the prerequisite especially to target the production of proteins into edible part of plants (Chaitanya & Kumar, 2006; Jacob, Cherian, Sumithra, Raina & Sankar, 2013; Jelaska, Mihaljevic & Bauer, 2005). The stability of vaccine proteins when transgenic fruits or leaves are stored at ambient conditions is another cause of concern. Another problem encountered with edible vaccines is the dosage as it may vary depending upon the conditions where and when they are grown. Optimal dosage levels are required to be developed in order to fix this issue (Shah *et al.,* 1990). Another complication with oral vaccines is the oral tolerance especially when the antigen is taken up in food repeatedly (Strobel & Mowat, 1998), resulting in the suppression of the antibody production. The induction of oral tolerance is both time and dose-dependent. The antigenic dose necessary to induce protection is generally smaller than that required to produce tolerance (Chaitanya & Kumar, 2006; Tsuji, Mizumachi, & Kurisaki, 2001).

Insight into the mechanism of action of edible vaccines

Mucosal immunity is known to be stimulated as a result of edible vaccine with the involvement of both the adaptive and innate arms of T and B cells. Primarily there is activation of the mucosal immune response system (MIS) against a pathogen forming the first line of defence. Almost all human pathogens invade at mucosal surfaces via gastrointestinal, respiratory and urogenital tract (McGhee & Fujihashi, 2012; Mor, Gómez-Lim & Palmer, 1998). The antigen gains entry into the gut mucosal layer through M cells and macrophages. M cells transport the antigens to the T cells (Siebers & Finlay, 1996). M cells are known to capture a wide range of macromolecules from lumens in the small intestine to antigen submucosal cells (APCs) on Peyer's patches effectively (Mabbott, Donaldson, Ohno, Williams, & Mahajan, 2013). Dendritic (DC) cells have been shown to be powerful antigenic cells to trigger an adaptive immune reaction in the priming naive T cells (Mildner & Jung, 2014). The antigenic epitopes present on the APC surface along with the assistance of helper T cells, further activate B cells. Upon activation, activated B cells migrate to the Mucosal associated lymphoid tissue (MALT) where they mature into plasma cells and to secrete immunoglobulin A (IgA) (Scadding, 1990; A.K. Sharma, Verma, Tewari, & Khuller, 1999). IgA then forms the secretory IgA, which is then transported into the lumen where they interact with

antigens and neutralize the invading pathogen Fig. 1 (Brandtzaeg, Kiyono, Pabst, & Russell, 2008; Walmsley & Arntzen, 2000). Not only the lymphoid mucosal-associated tissues (MALT) but secretory immunoglobulin (SIgA) also plays a vital role in protection against microbial adhesion and toxins of mucosal surfaces. Therefore the challenge lies in fact that new platforms are needed for the delivery of pathogens or toxin-specific SIgA and systemic IgG which is extremely important for improving the vaccine efficacy (Dietrich, Griot-Wenk, Metcalfe, Lang, & Viret, 2003).

Edible vaccines versus diseases

Edible vaccine against diarrhoeal disease

Transgenic potatoes expressing β-subunit of the E. coli heat-labile toxin were fed to human volunteers in 1997 for the first time which resulted in increase in serum antibodies to about 4-fold in 80% of the volunteers [20]. Similarly, in another trial at the Boyce Thompson Institute (Cornell University, USA) in which potatoes containing the Norwalk virus responsible for vomiting and diarrhoea, 95% volunteers were reported to have induced antibodies in serum [20]. Another study revealed that transgenic tomatoes expressing protein specific to Norwalk virus could induce antibodies in a mice model.

In the recent studies at US (Cornell University), researchers have established their research on the transgenic tomatoes especially against Norwalk virus (causing severe diarrhoea). It was observed that the tomatoes produce a specific surface protein. In mice, the same tomatoes have shown an enhanced immune reaction in response to the virus (Chaitanya & Kumar, 2006; L. Richter, Mason, & Arntzen, 1996). Similarly, banana also has been explored as another source, due to its ease of availability and being a locally grown plant. The expression of a protein in banana will depend on the identification of a tissue specific promoter. Other examples include rabies glycoprotein being expressed in viral vectors in spinac and hepatitis B surface antigen in lettuce and potato. (Kapusta *et al.,* 1999; M. Sharma & Sood, 2011; Yusibov *et al.,* 2002). Main issue with potato-based edible vaccine is that they are required to be eaten raw as cooking results in protein denaturation, making it ineffective. Therefore, Banana was tried as an edible vaccine successfully as it does not require cooking and its ease of availability.

Edible vaccines against measles

Globally, measles causes over 8,70,000 deaths every year. However, mortality rate was reduced by 60%, to 345 000 deaths during 1999-2005 because of pneumonia or encephalitis (Wolfson *et al.,* 2007). Currently available measles vaccine holds promise in terms of its effectivity (95% seroconversion rate) and being safe (Cutts, Henao-Restrepo, & Olive, 1999; Simons *et al.,* 2012). However, the measles live attenuated vaccine (LAV) has no oral efficacy and could be destroyed upon maintenance of a "cold-chain" of refrigeration posing challenges as far as distribution and storage is concerned (Oyefolu *et al.,* 2007). Another challenge is that the efficacy of the above Live attenuated vaccine (LAV) gets reduced by the presence of maternal antibodies. These challenges and

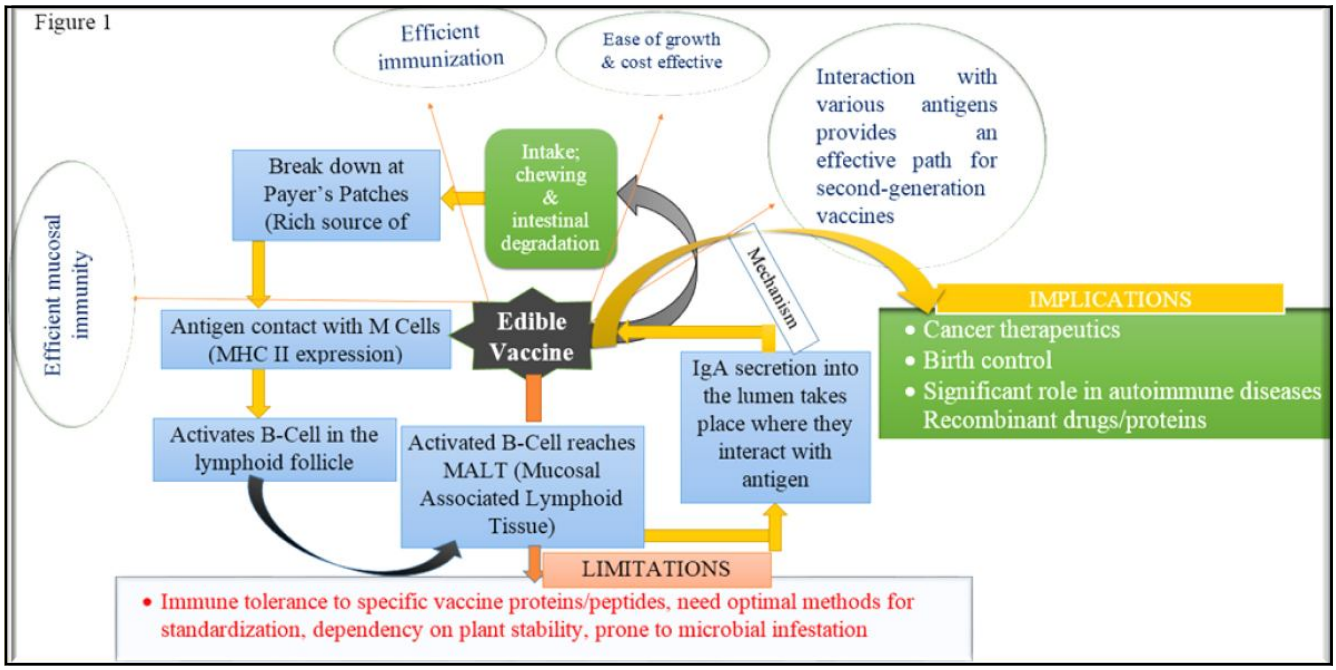

Fig. 1: Potential advantages and limitations of edible vaccines along with the proposed mechanism of action.

hinderances are attributed for not able to achieve the complete eradication of measles (Osterhaus, van Amerongen, & van Binnendijk, 1998). The very first step to develop an edible vaccine comprises of choosing an antigen which is to be expressed in plants. Subsequently, antibodies are produced which have the MV-neutralising activityas has been seen in earlier studies where hemagglutinin (H) antibodies tend to have the predominant neutralizing effect that can be well-correlated with immunoprotection against measles. There are two major surface proteins i.e. hemagglutinin (H) and fusion proteins reported in measles virus which have the potential to be developed as edible vaccines (R. T. Chen *et al.,* 1990). The attenuated Edmonston Vaccine strain derived from H protein subunit was shown to provide immunoprotection, hence forming the basis for an edible vaccine against measles (Das, 2009). There are number of ways reported in literature for the generation of transgenic plants. Typical example of the use of *Agrobacterium tumefacien*s, a naturally occurring soil bacterium to transfer the DNA segment into the plant genome through a process named as transformation leading to the generation of whole plant from an individual plant cell. Transgenic plants generation is known to be species dependent and moreover, it is a time-consuming process usually taking 3-9 months (Huang, Dry, Webster, Strugnell, & Wesselingh, 2001). There was another study where Measles virus, MV-H gene was expressed in the tobacco plant and upon orla administration resulting in the induction of serum antibodies production in mice. The study affirmed the retention of immunogenicity by the plant derived MV-H protein. Tobacco plant has been extensively exploited in the plant biotechnology laboratories as it is easy to grow in most soils and climates as well. Moreover, it also provides large amounts of tissue and it is easier to process and inexpensive as well. Furthermore, tobacco is an established product in global economy with higher gross profit and relatively more stability in terms of price, making tobacco as a good model system as far as evaluation of transgenic or recombinant proteins are concerned.

However, from vaccine delivery perspective, it is unsuitable because of its tendency to produce many toxic compounds (Tacket *et al.,* 2000). Other model plants such as potatoes and lettuce have also been exploited for antigenic expression, resulting in the induction of immunological responses (Kapusta *et al.,* 1999; Tacket *et al.,* 2000). More so rice, has been commonly used in baby foods because of its lower allergenicity. Moreover, rice is easily mixed with baby foods, water and breast milk, hence can be conveniently delivered to infants. Ease of storage, transport and stability in terms of protein expression in rice are some other positive attributes of using rice (Hanba, Wada, Osaki, & Nakamura, 1996; Webster, Thomas, Strugnell, Dry, & Wesselingh, 2002). Stoger et al. further reported that mammalian proteins can be successfully expressed at higher levels in the transgenic rice (W.H. Langridge, 2000). On the negative side, rice has a slower growth profile and requires specialised glass house conditions, making it a restrictive species for preliminary studies.

Oral vaccination requires higher antigenic dose than either intranasal or parental vaccination. Three successful human trails have shown that adequate doses of antigen can be achieved with plant based vaccines (Kapusta *et al.,* 1999; Streatfield *et al.,* 2001; Tacket *et al.,* 1998). Preliminary analysis of MV- H transgenic lettuce plants suggested that 35-50 g of lettuce should be sufficient to deliver doses of MV-H protein comparable to those used in clinical trials (Lal, Ramachandran, Goyal, & Sharma, 2007; J. Saxena & Rawat, 2014). MV-specific immune responses upon vaccination with plant extract could be further induced and made more consistent by the use of mucosal adjuvant (Giddings *et al.,* 2000; Huang *et al.,* 2001). Actually the mucosal adjuvant may enhance immunological responses at mucosal surfaces and simultaneously reduces the oral dosage required to induce an immune response. Moreover, edible vaccine can be more effective in terms of immunogenenicity if the delivery system employed is intact such as using intact plant material. Hence, encapsulation of an antigen within a biological plant material (such as within the tough plant cell wall and membrane compartments) provides increased protection from intestinal degradation (Kwon, Verma, Singh, Herzog, & Daniell, 2013; Modelska *et al.,* 1998). Not only the encapsulation but also the route of administration and vaccine types could play a pivotal role in increased protective immune responses. This was evident in a study when a single dosage of MV-H DNA inoculation given orally followed by multiple MV-H boosters, could induce MV neutralising antibodies significantly (with enhanced neutralizing titres >20 folds) in comparison to vaccination with a DNA or plant derived vaccine alone (Huang *et al.,* 2001; Stittelaar *et al.,* 2002).

Edible vaccine versus foot and mouth disease

Though vaccinations have kept a significant check on the spread of this dreadful livestock, foot and mouth viral (FMDV) disease, still with its recent outbreak has really questioned the efficacy and safety of the available inactivated and attenuated edible vaccines. VP1, one of the structural proteins of FMDV harbours key epitope

regions responsible for inducing VP1 specific antibodies (Brown, Sprecher, & Keller, 1991). Transgenic constructs have been used extensively for the development of an edible vaccine in order to treat the disease. Many transgenic vaccines have been reported in the past few years including transgenic alfalfa plant with structural proteins of FMDV, tobacco mosaic virus infected plants genetically modified with FMDV structural protein (Wigdorovitz *et al.,* 1999) or bacterial plasmids containing genes coding for FMDV proteins (Wong *et al.,* 2000). For efficient vaccine delivery, genetically modified (GM) organisms were employed and proven effective in protecting farm animals. However, the protocols followed are quite cumbersome and suffers from many limitations. Oral tolerance has been attributed to be one of the major complications in case of oral vaccines. This is evident from the fact that when the antigen is taken up repeatedly in food, the systemic immunity gets suppressed as the antigen may suppress or lower down the production of antibodies. Some studies revealed that the trans placental exposure to the edible vaccine may cause the foetus to be viral tolerant and thus, becoming a carrier of the virus without showing symptoms (Lal *et al.,* 2007; J. Saxena & Rawat, 2014).

There are a variety of vaccines available for the treatment of FMD (foot and mouth disease) including inactivated vaccines such as oil-emulsion, aqueous, or aluminum-based vaccines. These vaccines are monovalent, bivalent, or multivalent in nature but have been reported to be unstable. These vaccine formulations could be stored and kept unspoiled for a longer period of time in liquid nitrogen medium (Kamel, El-Sayed, & Vazquez, 2019). BHK-21 cells were used extensively for the preparation of mouse-attenuated live FMD vaccines, which have been utilized efficiently for immunization of cattles (G. Mowat, Brooksby, & Pay, 1962). However, another study revealed that the live attenuated vaccine used for the treatment of FMD, resulted in development of lesions in animals. Recently introduced attenuated FMD vaccines, have been proven to be more stable as they have lesser risk of reverting to virulence than traditional ones (Kamel *et al.,* 2019).

DNA and Peptide vaccines have also been shown to have a positive impact on the treatment of FMD. DNA vaccines expressing B-cell and T-cell epitopes were shown to protect mice from FMDV infection despite the lack of a specific humoral response upon challenge (Borrego *et al.,* 2006). On the other hand, the peptide vaccines have additional advantages of being cost effective and relatively more stable against infectious FMDV. Such vaccines have been shown to have a single linear peptide structure corresponding to FMDV capsid proteins or those containing T-cell and/or B-cell epitopes (Wang *et al.,* 2002). On VP1 protein of FMDV type A, a conformational neutralizing epitope named "135YxxPxxxxxGDLG147" has been characterized and used for the epitope-based vaccination (Liu, Yang, Wang, Liang, *et al.,* 2017; Liu, Yang, Wang, Wang, *et al.,* 2017; Soria *et al.,* 2017). A CTBVP1 fusion protein which showed weak but significant binding affinity for GM1 ganglioside, was successfully expressed in *Chlamydomonas* in bulk quantities. Hence, this fusion protein holds promise to be used as a potential mucosal vaccine (M. Sun *et al.,* 2003).

Edible vaccines versus autoimmune disorders

Antigens are known to be produced in the target tissues resulting in diseases such as arthritis, diabetes and multiple sclerosis which are also termed as autoimmune disease conditions (A.M. Mowat, 1987). Feeding the target antigen for example, collagen in arthritis may relieve or suppress the symptoms of autoimmune conditions. Therefore, current research is focusing on introducing target antigens for autoimmune diseases into crop plants so as to express them suitably and manage the symptoms of autoimmune disorders. Again the issue of oral tolerance comes into the picture when the oral vaccine is regularly being taken through general food supplies which may suppress immunity to the disease normally protected by the vaccine (Weiner, 1997). Therefore, one must take appropriate measures so that food vaccines could be prevented from dissemination to the general food supply.

Edible vaccines against human papilloma viral disease

Human papilloma virus (HPV) disease is one of the most common sexually transmitted diseases worldwide. HPV is known to be a major cause of cervical cancer in women as well. Urgent attention is required to develop an edible vaccine which could confer protection against HPV. A study revealed the isolation of a genetic sequence for the synthesis of HPV protein envelope and virus like particles (VLPs) were generated using this sequence. Moreover, these VLPs were reported to be non-infectious in nature and were speculated to be effective oral immunogens for the prevention of HPV disease (Rose *et al.,* 1999).

Edible vaccines versus dental caries

Transgenic plants are the majorly considered for vaccine production system especially in case of the edible vaccines which have wider implications against variety of diseases including dental cariosity. The expressed genes

encoding the antigens of viral and bacterial pathogens, are known to maintain the inherent immunogenic characteristics. Moreover, these vaccines have major composition of antigenic proteins while pathogenic genes are deficient. Edible vaccine after ingestion, as the vaccine gets digested, the protein move into the blood stream where infectious protein is neutralized due to the immune response (Khan *et al.,* 2019). Plants have been extensively modified to produce many drugs such as albumin, serum protease and interferon which are otherwise difficult or expensive to produce (Daniell, Streatfield, & Wycoff, 2001; Fernández San Millán, Mingo Castel, Miller, & Daniell, 2003; Loesche, 1986; Philip, Suneja, & Walsh, 2018). Similar strategy was adopted in order to produce antibodies in transgenic plants against S*treptococcus mutan*s, which is a common tooth decay bacterium. The plant produced specific antibodies could easily neutralize the pathogen providing protection against the dental disease. However, in order to get effective protection against dental disease, individual transgenic plant producing single antibody chains are required to be developed initially which can further be hybridized to develop a plant producing complete antibodies consisting of heavy and light chains.

Edible vaccine versus cholera

The gene encoding cholera toxin antigen was inserted into cells of an organism that cause a plant disease called crown gall (Osterhaus *et al.,* 1998). New genes were transformed into the alfalfa plant by infecting it with the transformed crown gall disease and the cells of the new infected plant were cultured containing the cholera antigen and the alfalfa plant was regenerated from that infected cells. The transgenic plant was reported to confer protective immunity to cholera in animals that eat the alfalfa plant (Arakawa, Yu, & Langridge, 1999; Walmsley & Arntzen, 2000). In another study, the fusion of PA20, ipaD and CTxB was generated and transformed into tomato plants, for the production of vaccine against cholera. The expression analysis of the antigens revealed that the green fruits had the highest expression of the recombinant proteins, which were further subjected to immunogenicity analysis for a suitable vaccine candidate (Davod, Fatemeh, Honari, & Hosseini, 2018).

Edible vaccine against Hepatitis-B

Hepatitis B virus (HBV) infection is probably the single most important cause of persistent viremia in humans affecting more than 350 million people worldwide (Gunasekaran & Gothandam, 2020; Lavanchy, 2004). The HPV infection may cause a serious liver infection resulting in jaundice, cirrhosis and liver cancer as well (Moss, Cutts, & Griffin, 1999). Currently, there are two forms of injectable HBV vaccines, including the one purified from the serum of infected individuals while the other comprises of a recombinant antigen expressed and purified from yeast. Hepatitis B surface antigen HBsAg (usually isolated from high-titer patients) has been widely used as a vaccine for a while now. The conventional hepatitis B vaccine utilises a single protein HBs Ag, produced in yeast. The protein after polymerization forms a complex which can mimic the structure of the actual virus. After injection, this protein complex is known to triggers the immune system providing protection against hepatitis. However, a study reported that upon oral administration, production of vaccine antigen in plants often fails to meet the minimum level required to produce an immune response (Conrad & Fiedler, 1994). Towards overcoming this limitation, another group of researchers studied a variety of ways to increase plant production of the HBs Ag and with its ability to initiate an immune response when injected into mice (Khamsi, 2005; Washam, 1997). Another study successfully analysed the ability of plant produced HBsAg to trigger an immune response when administered orally (Cutts *et al.,* 1999). It was realized that for the development of hepatitis B virus (HBV) vaccine, the key determinant is to identify an immunogenic HBV protein that could stimulate the human immune system to produce protective antibodies (L.J. Richter *et al.,* 2000). Moreover, one has to optimize and establish a quantitative measure of the success of vaccination for which optimisation of dosage levels and timings are quintessential.

A group of researchers took a lead to extract HBs Ag from transgenic tobacco leaves containing virus like particles (VLPs).Upon parenteral immunization of mice with the above extract, it was found to elicit B and T-cell responses, endorsing protective effects similar to those of a commercial vaccine (Guan, Guo, Huo, Guan, & Wei, 2010; A.K. Sharma *et al.,* 1999; Thanavala, Yang, Lyons, Mason, & Arntzen, 1995). A study reported that the HBsAg human antibody CL4mAb, could be successfully expressed in an algal expression vector, *Phaeodactylum tricornutum (Yano, Maeda, & Takekoshi, 2004)*. Another study looked into the transformation of the HBsAg gene into algal strain *Dunaliella salina through electroporation. The study observed large scale expression of HBsAg by D.salina with ability to induce immune responses (Y. Chen, Wang, Sun, Zhang, & Li, 2001; Geng, Wang, Wang, Li, & Sun, 2003).*

Edible vaccines against other diseases

The E2 protein of Classical swine flu virus (CSFV) was reported to have major antigenic properties which

could effectively neutralize its respective antibodies (He *et al.,* 2007; Markowska-Daniel, Collins, & Pejsak, 2001). Upon expression in *Chlamydomonas reinhardtii, and further analysis, it was observed that this protein had immunogenic properties a*s evident from the increase in serum antibody against CSFV when the extract was administered subcutaneously (He *et al.,* 2007; Markowska-Daniel *et al.,* 2001).

Fibronectin-binding protein expressed by *S. aureus* was also found to be important for its pathogenicity which was fused with the cholera toxin B (CTB) resulting in improvement of the antigen-specific systemic and mucosal immune response upon expression in chloroplast of the microalgae, *C. reinhardtii* (Dreesen, Charpin-El Hamri, & Fussenegger, 2010; J.B. Sun, Holmgren, & Czerkinsky, 1994). As we know that *Staphylococcus aureus is a human pathogen responsible for bacteremia after infecting the nasal mucosa and skin along with* secondary infections such as endocarditis, pneumonia, meningitis etc (Moreillon & Que, 2004).

Plasmodium falciparum, a parasitic protozoan is known to cause malaria responsible for more than 100 million deaths annually (Gunasekaran & Gothandam, 2020). The RTS, S/ASO2A is a vaccine currently available against malarial sporozoite. Granule bound starch synthase (GBSS) fused to three malarial vaccine candidates were expressed in the microalgae, *C. reinhardtii r*esulting in accumulation of starch-antigen in the chloroplast sufficient to provide protective immunity against otherwise lethal doses of *Plasmodium falciparum* in the mice model (Dauvillee *et al.,* 2010). Moreover, starch provided extra stability to the vaccine as well. Furthermore, this microalga has a GRAS status (Generally regarded as safe) with ease of scaling up and cultivation as well. In another study, pfs25 and pfs28 malarial subunit (malaria transmission blocking candidates) vaccines were expressed in *C. reinhardtii (Gregory et al., 2012; A.K. Saxena et al., 2006)*. Another study concluded that a-pfs25 displayed significant malarial transmission-blocking capabilities in comparison to a-pfs28 (Gozar *et al.,* 2001; Gozar, Price, & Kaslow, 1998). Keeping in view of the above findings one could speculate that future belongs to these edible vaccines having the potential to wipe-out a variety of diseases including diarrhoea, cholera, malaria, autoimmune and viral diseases etc.

Clinical studies of edible vaccine

Human clinical trials of an edible vaccine which is incorporated into the genome inside a food (raw potatoes) have indicated some promising outcomes against diseases as evidenced through the induction of protective immune response upon its consumption. Researchers are hoping to wipe out wide-spectrum of diseases including diarrhoea, cholera, FMD, malaria etc. by using vaccines raised in transgenic plants that require no refrigeration (Walker, Steele, Aguado, & Committee, 2007). In the first phase of human testing (trials approved by the Food and Drug Administration, the potatoes eaten by volunteers contained a vaccine against travel diarrhoea, a common enteric condition resulting from intestinal infection by *E. coli* (Steffen, Castelli, Dieter Nothdurft, Rombo, & Jane Zuckerman, 2005). Volunteers were given to eat 50g or 100g of genetically altered potatoes in a six monthly double-blind trial with each volunteer consuming three servings of potatoes over a period of three-weeks (Oakes, Shewmaker, & Stalker, 1991). No side effects were noticed in the study upon consumption of transgenic potatoes. Antibody secreting cells were found in the blood serum of the volunteers who ate the genetically modified potatoes. Moreover, antibodies were reportedly found in both blood and stool samples. Techniques to create edible vaccine in bananas are now under way at Boyson Thomson Institute (BTI) (Park Jr *et al.,*), but the crop takes much longer to mature and produce edible fruit. A delicious vaccine containing food, particularly bananas, a favourite fruit among children could be inexpensive and plentiful (Mibei, Ambuko, Giovannoni, Onyango, & Owino, 2017). Delivery of vaccine in plant cells may provide additional protection to the antigen as it passes through the acid environment of the stomach.

Challenges and future perspectives

Plant cultivation with desired bioactivity has been the mainstay of modern medicines with extended medicinal applications including delivery of vaccines. Edible vaccines have shown promise in terms of their heat stability, ease of administration and cost effective with regards to production having merits over traditional vaccines in terms of production, administration and delivery. Moreover, a successful edible vaccine could potentially transform the health policy and practice of any nation. However, there are challenges how to manage the issue of oral tolerance, safety issues of genetically modified organisms and optimal vaccine dosages.

An edible vaccine harbours DNA fragments coding for proteins present more often on the surface of the pathogen, stimulating the body's immune response. The biggest challenge is whether the antigen withstands the hostile and acidic environment of the human stomach and trigger the immune system in the righteous manner. Another challenging aspect is about the vaccine dosages and its regulation, as at higher doses it may induce

tolerance. Genetically modified foods and the complications associated with them have further posed a challenge for a successful edible vaccine.

Undoubtedly vaccines have been realized as the major public health initiatives of the modern era However resistance towards genetically modified foods is a major threat to the commercial use of edible vaccines. Contamination encountered during transgenesis is another challenge and a concern to be dealt with utmost care. There are other hurdles as well such as WHO certifications before the launch of the vaccine for human applications along with ascertaining quality, efficiency and associated environmental effects.

Conclusions

Advancements in the vaccine technology, with the advent of oral DNA vaccines (A. Sharma & G. Khuller, 2001; Woo, Wong, Zheng, & Yuen, 2001), internal delivery (Plante *et al.,* 2001) and plant-derived edible vaccines, may further lead to safe, easy to produce, distribute, ease of delivery and cost effectiveness of a vaccine overcoming the drawbacks of traditional vaccines. Hence one could foresee a promising healthcare future in terms of using plant derived vaccines because of their safety and efficient immunization. The need is to develop an efficient, economical and safe delivery system at a larger scale in the form of edible vaccines, paving the way for protecting individuals from diseases by simply eating a fruit or vegetable.

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Conflict of interest

The authors claim no conflict of interest in publication of this manuscript in Plant Archives Journal.

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