The Pharma Innovation Journal 2022; SP-11(3): 110-113

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(3): 110-113

www.thepharmajournal.com
Received: 23-01-2022
Accepted: 25-02-2022

Dr. Pallavi Khajuria
Assistant Professor, Veterinary Physiology and Biochemistry, College of Veterinary Science, GADVASU, Punjab, India

Dr. Gurpreet Singh
Associate Professor, Veterinary Physiology and Biochemistry, College of Veterinary Science, GADVASU, Punjab, India

Dr. Subrat Kumar Dash
Assistant Professor, Veterinary Physiology and Biochemistry, College of Veterinary Science, GADVASU, Punjab, India

Dr. Chetna Mahajan
Assistant Professor, Veterinary Physiology and Biochemistry, College of Veterinary Science, GADVASU, Punjab, India

Dr. Rachna Sharma
Assistant Professor, Veterinary Physiology and Biochemistry, College of Veterinary Science, GADVASU, Punjab, India

Corresponding Author
Dr. Pallavi Khajuria
Assistant Professor, Veterinary Physiology and Biochemistry, College of Veterinary Science, Rampura Phul, GADVASU, Punjab, India

Current status and future prospects of edible vaccines: A review

Dr. Pallavi Khajuria, Dr. Gurpreet Singh, Dr. Subrat Kumar Dash, Dr. Chetna Mahajan and Dr. Rachna Sharma

Abstract
Edible vaccines are alternative and new approach of vaccination as compared to traditional vaccines, they are cheap, heat stable, require no cold chain maintenance. There are many clinical trials which are undergoing for development of edible vaccines to provide protection against many diseases. This may lead to a future of safer and effective immunization. Vaccine is being developed for Measles, cholera and hepatitis B and for many other diseases are in the process of development. For preparation of edible vaccine, molecular farming technique is used in which selected genes are introduced into the plants. The transgenic plant is then induced to manufacture the encoded protein. Also the edible vaccines can be used against emerging COVID 19. Thus by use of suitable plant vector and Spike protein of the corona virus an edible vaccine can be developed against the COVID-19. This aim of this review to provide comprehensive coverage on the importance of edible vaccines to provide protection against various diseases.

Keywords: vaccine, edible, immunization, genetic engineering

Introduction
The most important function of our immune system is to protect our body from invasion of disease-causing germs or pathogens and to destroy them. If our immune system fails to do so, we will be prone to infectious diseases. Vaccination is important against prevention of such infectious diseases. Vaccines contain antigen which is a substance which is prepared from the causative agent of a disease or a synthetic substitute and it provides immunity against one or several diseases. The process of administration of vaccine to prevent a disease is called vaccination (Stern and Markel, 2015) [1]. Propagation, isolation, purification and formulation are major steps involved in production of edible vaccines. Process of multiplication of the living organism for use in vaccine is propagation. Isolation is the process of separating the living organism that is needed for propagation. Purification is the process of removal of unwanted substances from the selected living organism for vaccine production. In formulation, which is the final step in production of vaccine, the purified organism by use of suitable preservatives is formulated to a vaccine form (Greer, 2015) [2].Vaccines can be of two types depending on nature such as prophylactic or therapeutic. The main function of prophylactic vaccines is to prevent infections whereas the therapeutic vaccines boost the immune system and thus prevents complications of chronic infections in the body.

However, the major concern with these conventional vaccines is their safety concern as any failure in the inactivation may result in reversion into the virulent form. Also there is need for trained personnel as they are administered through parenteral route and also multiple doses or addition of an adjuvant are required including specialized storage, refrigeration facility and optimum transport conditions. Contamination of vaccine with undetected virus or bacteria may happen if there is any failure with regard to quality control tests. Parenteral vaccines may cause local inflammation, fever and rarely some hypersensitivity issues may be a concern. Thus edible vaccines are cheap, needleless, convenient, safe, easy to administer and thus a better approach to vaccine production. Edible vaccines are altered or modified plant and animal-based productions that contain agents that trigger an animal's immune response (Kurup and Thomas, 2020) [3]. Edible vaccines can play a very important role compared to traditional vaccines in near future due to its advantages and easy use (Darla *et al.*, 2021) [4].

Arntzen in the 1990s was the first to introduce the concept of edible vaccines. *Streptococcus mutans* was the first bacterium to be used for expression of a surface antigen in tobacco. Bacterium causes dental caries, and protection against tooth decay can be obtained by the

stimulation of a mucosal immune response that would prevent the bacteria from colonizing the teeth (Chaitanya and Jonnala, 2006) [5]. For preparation of edible vaccines, by use of process called "transformation, encoded proteins are manufactured by selection of desirable gene and its introduction into plants. These altered plants or modified plants containing gene of interest and capable of manufacturing encoded proteins are known as "transgenic plants".

Mechanism of Action

Technique of bio-encapsulation is used to deliver the antigen in transgenic plants. The tough outer wall of plant cells protects the antigen gastric secretions, and thus antigen is finally released in the intestines. M (Microfold) cells in the intestinal lining that overlie payer's patches and gut-associated lymphoid tissue (GALT), take up the released antigen and pass it on to macrophages, other antigen-presenting cells and local lymphocyte populations, there by generating serum IgG, IgE, local IgA response and memory cells, which are able to neutralize the attack by the real infectious agent (De Aizpura and Russell Jones, 1988) [6]. Mucosal as well as systemic immunity are activated by edible vaccines, when they come in contact with the digestive tract lining.

Production of edible vaccine

There are various methods of production of edible vaccine but most commonly used methods are as follows:

1. By use of genetic engineering proteins of interest are expressed in plant viruses and these plant viruses are genetically engineered to express the desired proteins. The resultant recombinant virus is then inoculated into the plants which are grown and chimeric viruses are extracted and purified. These plant edible vaccines are used to generate the immune response.
2. Another technique is "Transformation", in which gene of interest is integrated with plant vector. Various techniques are used for introduction of these transgene into plant cell which are grouped into following categories:

a) Agrobacterium mediated gene transfer: In this technique, T-region of a disarmed Ti plasmid of Agrobacterium is used as site where appropriate gene construct is inserted. The recombinant DNA is placed into Agrobacterium; a plant pathogen which is co-cultured with the plant cells or tissues to be transformed (Streatfield, 2006) [7]. But this method gives low yield and the process is also slow. This method has shown good results for dicotelydenous plants like potato, tomato and tobacco. Genes are expressed by this method in experimental animals and plants (Mariotti *et al*., 1989) [8]. Examples of vaccines produced by this method were diarrhea, TB, dengue, avian flu virus, ebola (William, 2002) [9].

b) Biolistic method: Gene containing DNA coated metal (e.g. gold, tungsten) particles by use of a gene gun is fired at the plant cells (Taylor and Fauquet, 2002) [10]. DNA is taken up by the plants, which are then allowed to grow in new plants, and genetically identical crops in large numbers is produced by cloning. This method is quite attractive because DNA can be delivered into cells of plant which makes gene transfer independent of regeneration ability of the species. The only concern with this method is requirement of costly device particle gun. Vaccines produced by biolistic methods are Lyme disease, anthrax, tetanus, plague, rota virus, cholera and canine parvovirus (Wu *et al.*, 2003) [11].

c) Electroporation: In this technique, the cell wall of the plants is weakened by use of mild enzymatic treatment, so as to allow the entry of DNA into cell cytoplasm and then a high voltage electrical pulse is used to induce transient pores in the plasmalemma, thereby allowing introduction of DNA into cells.

Plant Species Used as Vaccine Models

The major advantage of using plant based models for development of edible vaccines is that they have least tendency of contaminating as they are not much infective to human beings or animals. For developing vaccines against diseases such as diphtheria, tetanus, hepatitis B and Norwalk virus, potato is used as a candidate. Enteritis caused by E.coli strain was the first disease in which potato was used as a vaccine model. Potato can also play a role in oral strengthening to the hepatitis B vaccines in human beings (Concha *et al.*, 2017) [12]. Potatoes were also used to develop an edible vaccine against mink enteritis virus attack. Also against rabbit hemorrhagic virus in wild rabbits, potato edible vaccine is being used. The main benefit of using potato as a candidate for edible vaccine is the ease of transformation and propagation.

Tobacco is not an edible plant but it is also used as a model for the development of edible vaccines. Norwalk virus causes gastroenteritis and a vaccine was developed in tobacco in 1996 against this virus. Transgenic tobacco expresses VP1 protein against chicken infectious anemia. Tobacco has the ability to express a polypeptide related to hepatitis B. Potato is also used as a candidate to develop vaccine against coccidiosis.

Rice is the other plant species used for the development of edible vaccines. Rice can be easily used in baby food and also there is high expression of antigen in rice. Disadvantage associated with use of rice as a candidate is that there is requirement of glasshouse conditions for growth and also it grows slowly. In 2007, a study was conducted in Oryza sativa, a variety transgenic rice, and it was observed that significant amount of antibodies against *E. coli* were produced (Qian *et al.*, 2008) [13].

Banana is another candidate used in the production of edible vaccine. The main advantage of banana as a vaccine species is that it does not need any cooking and also proteins are not destroyed even after cooking. Banana is also inexpensive as a candidate when compared to other plants and also expresses HBsAg. The only disadvantage is it takes plant 2–3 years to mature and spoilage is faster after ripening when compared with other vaccine species (Mason, 1996).

Severe acute respiratory syndrome, SARS is a respiratory illness caused by corona virus is Tomato is an effective vaccine candidate against SARS. It has also shown better results against Norwalk virus than vaccines produced from potato. CT-B protein have been expressed in parts of tomato such as leaves, stem, fruits, and other tissues from vibrio cholera B toxin (Kumar *et al.*, 2005)[14]. Vaccine against the Alzheimer's disease have been developed by expressing HBsAg and Beta-amyloid proteins in tomatoes.

Alfa alfa plant is used to develop edible vaccines mainly for veterinary purposes and it was used to express Eeg95-EgA31 of *Echinococcus granulosus* (Wigdorovitz *et al.*, 1999) [15].

Transgenic carrots have been used against HI, *E. coli*, Helicobacter pylori and have shown potential effects (Yan *et al.*, 2010) [16]. *Nicotina benthamiana* have been used as vector against Inflenza virus (Lavanchy, 2004) [17] and Spinach is used as vector against Rabies in various clinical trails (De Muynck *et al.*, 2009) [18].

Green microalgae as a valuable protein generation stages for an assortment of industrial and treatment applications have been used particularly for complex or heavily disulfide-reinforced proteins. Unicellular green algae have all the positive traits of plant frameworks, in addition to a few novel focal points over terrestrial plants as vaccine. Algal biomass accumulation is very quick, and the whole of the biomass can be used for vaccine production.

Green microalgae such as *Chlamydomonas reinhardtii* is a feasible alternative for vaccine generation. *Dunaliella salina* algae have been used against Hepatitis B infection (Mclaughlin-Drubin and Munger, 2010) [19] and *Chlamydomonas reinhardtii* algae have been used against Malaria (Gozar and Kaslow 1998) [20], FMD (Alonso *et al.*, 2002) [21], classical swine flu (Smal *et al.*, 2012) [22], White spot syndrome (Hormaeche *et al.*, 1970) [23], Staphylococcus aureus infection (Vilar, 2003) [24], HPV(Medina and Guzma, 2001) [25] and in treatment of hypertension(Yap and McKenzie, 1978) [26] in clinical trials.

Whole cell based yeast vaccines have also shown promising results as Saccharomyces cerevisiae have been used against influenza and *Actinobacillus pleuropneumoniae* (Mattanovich *et al.*, 2012) [27].

Probiotics can also be used for edible vaccines as Lactic acid bacteria's such as *Lactobacillus spp* have been found to serve as potential vectors for preparation of edible vaccines. Promising results have been shown by *Lactobacillus casei* against Anthrax *and Streptococcus gordonii* against HIV virus (Oggioni *et al.*, 1999) [28]. Virulence plasmid of *Yersinia entercolitica* can be used for synthesis of recombinant proteins (Sory and Cornelis, 1988) [29].

Research trails are currently in progress regarding the use of these bacteria's against various diseases. Studies have shown that spores of *Bacillus subtilis* spores expressing desireable proteins were effective aginst Helicobacter infection (Roland *et al.*, 2005) [30].

Advantages and major applications of edible vaccine

The major advantage of edible vaccines is that they don't require adjuvants which enhance immune response. Also these vaccines can elicit mucosal as well as systemic immunity. They are cost effective in storage, preparation, production and transportation. These vaccines can be given orally, thus, there is no requirement of skilled persons for administration. There are many diseases which spread through reuse of infected needles. So, by use of edible vaccines the risk of spread of infectious diseases is also lowered.

The first human trial for an edible vaccine was started in 1997 in which Volunteers were fed transgenic potatoes containing the b-subunit of the *E. coli* heat-labile toxin, which causes diarrhea. There was four fold increase in serum antibodies in ten out of eleven volunteers. The development of edible vaccines against various diseases is currently in progress. For development of vaccine against malaria three antigens merozoite surface protein (MSP) 4 and MSP 5 from Plasmodium falciparum and MSP4/5 from Plasmodium yoelii are being used. Transformation was done into tobacco plant by plasmid/vector of measles virus hemagglutinin (MV-H) from the antigen edmonston strain and when it was fed to mice MV-H could attain antibody titers 5 times the level is protective for humans and secretory IgA in their feces were also present.

Also the edible vaccines can be used against emerging COVID 19. The Spike protein of the corona virus can be used to develop a vaccine against the COVID-19 by cloning into a plant expression vector and the desired plant like tomato and theses vaccines can be given orally. Research is ongoing with regard to this. Development of such a protective vaccine will be important to prevent and control the spread of this deadly virus. Spike (S) protein gene can be cloned into a plant expression vectors (Sohrab, 2019) [31] and thus by use of this plant based platform edibles vaccine in future prospects can also be developed for COVID 19.

Table 1: Developmental status of edible vaccines in clinical trials

Pathogen	Antigen	Host	Use	Trial	Reference
Enterotoxigenic *E. coli* Early	LT- B	Potato	Diarrhoea	Phase 1	(Zhou *et al.*, 2015)
Enterotoxigenic *E. coli* Early	LT- B	Maize	Diarrhoea	Phase 1	(Tacket *et al.*, 2000)
Norwalk Virus	CP	Potato	Diarrhoea	Early phase 1	(Tacket *et al.*, 1998)
Rabies Virus	GP/ NP	Spinach	Rabies	Early phase 1	(Tacket *et al.*, 2004)
Vibrio cholerae	CTB	Rice	Cholera	Phase 1	(Thanavala, 2005)
HBV	HBV	*Saccharomyces cerevisiae*	Chronic HBV	Phase 2	(Yuki *et al.*, 2013)

Conclusions

Edible plant-derived vaccines may lead to a future of safer and more effective immunization. Resulting therapeutic products would overcome some of the difficulties associated with traditional vaccines, like costly production, distribution and delivery. Edible vaccine studies demonstrate encouraging progress toward resolving major hurdles in these emerging commercial vaccine technologies. However, there are main challenges of edible vaccine regarding its approval from the public domain as there are opinions such as genetically modified products may harm environment and society. Also there is need for close monitoring while growing plants for the production of edible vaccines in order to prevent cross contamination in molecular farming during pollination between genetically modified plants and non-genetically modified plants. Potential benefits of edible vaccines are more promising enough to overcome its side effects, thus, proper research and development in this area lead to better control over infectious diseases.

References

1. Stern AM, Markel H. The history of vaccines and immunization: Familiar patterns, new challenges. Health Affairs. 2005;24:611-21.
2. Greer AL. Early vaccine availability represents an important public health advance for the control of pandemic infuenza. BMC Research Notes. 2015;8:1.
3. Kurup VM, Thomas J. Edible vaccines: Promises and

Challenges. Mol. Biotech. 2020;62:79-90.
4. Darla R, Chikitha G, Poojitha TL, Prasanna GD, Sirisha A. Edible vaccines: Modern Approach for Immunization. I. J. Pharma. Res. 2021;5:60.
5. Chaitanya VK. Jonnala UK. Edible Vaccines. Sri Ramachandra J Med. 2006;1:33-34.
6. De-Aizpura HJ. Russell-Jones GJ. Oral vaccination. Identification of classes of proteins that provoke an immune response upon oral feeding. J Exp. Med. 1988;167:440-51.
7. Streatfield SJ. 2006 Mucosal immunization using recombinant plant based oral vaccines. Methods. 2006;38:150-7.
8. Mariotti D, Fontana GS. Santin F. Genetic transformation of grain legumes: Phaseolus vulgaris L. and P. coccineus. J Genet. Breed. 1989;43:77-82.
9. William S. A review of the progression of transgenic plants used to produce plant bodies for human usage. J Young Investig. 2002;4:56-61.
10. Taylor NJ and Fauquet CM. Microparticle bombardment as a tool in plant science and agricultural biotechnology. DNA Cell Biol. 2002;21:963-77.
11. Wu L, Jiang, L, Zhou Z, Fan J, Zhang Q, Zhu H, Han Q, Xu Z. Expression of foot-and-mouth disease virus epitopes in tobacco by a tobacco mosaic virus-based vector. Vaccine. 2003;21(27-30):4390-8.
12. Concha C, Canas R, Macuer J, Torres MJ, Herrada AA. Disease prevention: An opportunity to expand edible plant-based vaccines. Vaccine. 2017;5:14.
13. Qian B, Shen H, Liang W, Guo X, Zhang C, Wang, W. Immunogenicity of recombinant hepatitis B virus surface antigen fused with preS1 epitope sex pressed in rice seeds. Transgenic Res. 2008;17:621-631.
14. Kumar GBS, Ganpati TR. Revathi CJ. Srinivas L, Bapat VA. Expression of hepatitis B surface antigen in transgenic banana plants. Planta. 2005;222:484-93.
15. Wigdorovitz A, Perez Filgueira D M, Robertson, N, Carrillo C, Sadir AM. Morris T J. and Borca M V. Protection of mice against challenge with foot and mouth disease virus (FMDV) by immunization with foliar extracts from plants infected with recombinant tobacco mosaic virus expressing the FMDV structural protein VP. Virol. J. 1999;264:85-91.
16. Yan-Ju YE, Wen-Gui LI. Immunoprotection of transgenic alfalfa (*Medicago sativa*) containing Eg95-EgA31 fusion gene of *Echinococcus granulosus* against Eg protoscoleces. J Trop. Med. 2010;3:10-13.
17. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral. Hepat. 2004;11:97-107.
18. De Muynck B, Navarre C, Nizet Y, Stadlmann J, Boutry M. Different subcellular localization and glycosylation for a functional antibody expressed in *Nicotiana tabacum* plants and suspension cells. Transgenic Res. 2009;18:467-482.
19. Mclaughlin-Drubin ME. Munger. The human papillomavirus E7 oncoprtein. Virology J. 2010;384: 335-44.
20. Gozar MM. Price VL. Kaslow DC. Saccharomyces cerevisiae-secreted fusion proteins pfs25 and pfs28 elicit potent *Plasmodium falciparum* transmission-blocking antibodies in mice. Infect. Immunit. 1998;66:59-64.
21. Alonso LG, Garcia-Alai MM, Nadra AD, Lapena AN, Almeida FL, Gualfetti P. High-risk (HPV16) human papillomavirus E7 oncoprotein is highly stable and extended, with conformational transitions that could explain its multiple cellular binding partners. Biochemistry. 2002;41:10510-10518.
22. Smal C, Alonso LG, Wetzler DE, Heer A, De Prat Gay G. Ordered self-assembly mechanism of a spherical oncoprotein oligomer triggered by zinc removal and stabilized by an intrinsically disordered domain. PloS One. 2012;7:e36457.
23. Hormaeche CE, Joysey HS, Desilva L, Izhar M, Stocker BA. Immunity induced by live attenuated Salmonella vaccines. Res. Microbiol. 1990;141:757-764.
24. Vilar M, Barrrientos F, Almeida M, Thaumaturgo N, Simpson A, Garratt R. An experimental bivalent peptide vaccine against schistosomiasis and fascioliasis. Vaccine. 2003;22:137-44.
25. Medina E, Guzma, CA. 2001. Use of live bacterial vaccine vectors for antigen delivery: potential and limitations. Vaccine. 200;19:1573-1580.
26. Yap K, Ada G, McKenzie IF. 1978. Transfer of specific cytotoxic T lymphocytes protects mice inoculated with influenza virus. Nature. 1978;273:238-239.
27. Mattanovich D, Branduardi P, Dato L, Gasser B, Sauer M, Porro D. Recombinant protein production in yeasts. Methods Mol. Biol. 2012;824:329-358.
28. Oggioni MR, Medaglini D, Romano L, Peruzzi F, Maggi T, Lozzi, L. Antigenicity and immunogenicity of the V3 domain of HIV Type 1 glycoprotein 120 expressed on the surface of Streptococcus gordonii. AIDS Res. Hum. Retroviruses. 1999;15:451-459.
29. Sory M, Cornelis G. Yersinia enterocolitica O:9 as a potential live oral carrier for protective antigens. Microb. Pathog. 1988;4:431–442.
30. Roland KL, Tinge SA, Killeen KP and Kochi SK. Recent advances in the development of live, attenuated bacterial vectors. Curr. Opin. Mol. Ther. 2005;7:62-72.
31. Sohrab SS. An edible vaccine development for coronavirus disease 2019: the concept. Clin. Exp. Vaccine Res. 2019;9:164-168.