A Review On Edible Vaccines: Plant as a Platform For Biopharmaceutical's Production

[1] Sourik Ghosh, [2] Suryakanta Ghosh, [3] Efrit Karim, [4] Bivas Roy
[1]Faculty, [2]Student, [3]Student, [4]Student
[1, 2, 3, 4] Department of Biotechnology, Cyber Research And Training Institute,
The University Of Burdwan, W.B, India.
E.mail: [1]papun.biotech93@gmail.com, [2]suryaghosh07@gmail.com, [3]karimefrit@gmail.com,
[4]bivasroy2000@gmail.com

Abstract: *Infectious diseases are becoming an unembellished public health problem in every part of the domain. In retort to this, transgenic plants have turned into an innovative perceptible platform for the manufacture of fit for human consumption biopharmaceutical proteins, which will be evaluated for their productivity as edible vaccines to shield against several transmittable maladies. Plant-based vaccine technologies encompass the incorporation of the desired genes encoding the antigen protein for precise malady into the genome of plant tissues by several techniques. Agrobacterium mediated gene transfer and transformation via genetically modified plant virus are the communal techniques which have been practiced to yield effective vaccines, through biolistic, electroporation techniques etc. This review attempts to discuss the current status and future of this new preventive modality. Edible vaccines offer exhilarating potentials for the diseases like hepatitis, Tuberculosis, diarrhoea, pneumonia, STDs, HIV, dengue, hook worm, cholera, foot and mouth disease, rabies etc. predominantly in emerging domain where storing and administering vaccines are the foremost glitches. Countless nourishments under exploration for use in edible vaccines comprising certain fruits such as banana; including some vegetables such as potato, tomato and various crops such as rice and maize and other plants also like, tobacco etc. Through this plant-base vaccine method ample type of edible vaccines are produced some are Clostridium tetani vaccine in transgenic plants tobacco, potato, tomato, banana; Anti-Norwalk virus vaccine in GM tobacco and potatoes; Anti-rota viruses vaccine; Cholera toxin in potato; Anti-HAV vaccine, Anti-HBV vaccine, Anti-HPVs vaccine in transgenic tobacco and potato plants; Anti-HIV-1 vaccine, Anti-IBV Vaccine in potatoes; Anti-SIV Vaccine, Anti-RHDV Vaccine in potato. Henceforth, edible vaccines do not require any injectable needles. A prolific track may be accessible where adistinct person get protected from maladies by merely consuming fruits, vegetables and crops. It has passed the chief steeplechases in the path of an evolving vaccine technology. Estimating dosage prerequisite is monotonous in case of plant vaccine. Innumerable technical hindrances, regulatory and non-scientific contests, though all seem conquerable, need to be overcome.*

Key Words: *Edible vaccine, Biolistic, Electroporation, Ti plasmid, GM food.*

1. INTRODUCTION:

Edible vaccines are the vaccines that one can consume. Edible vaccines are fashioned by first introducing the desired selected genes into plants and then the induction of those plants help produce the encoded proteins. The altered plants are called "transgenic plants" while the process is known as "transformation" **[1].** In case of edible vaccines, this is not a possibility since plant viruses cannot infect humans. The way edible vaccines acts is that, as soon as it meets the digestive tract lining it stimulates the mucosal and systemic immunity, creating a dual mechanism system and providing first-line defence against pathogens attacking through mucosa. **[2].** Now, edible vaccines are Genetically Modified (GM) crops with the purpose of providing additional "immunity" against diseases like TB, hepatitis B, diarrhoea, pneumonia, STDs, HIV etc. These edible vaccines do not contain pathogenic genes but antigenic proteins. Edible vaccines have boundless potential to be a vaccine delivery system that is economical, easy-to administer, easy-to store, fail-safe and socio culturally readily acceptable, especially for the poor developing countries **[1].** Many developing nations are now infested with mayhems and this has now begun to threaten the advances and progresses that have been made in the recent past and to add to that millions are still dying from infectious diseases for which immunization does not exists or if available, are unreliable or too expensive **[3].** As such, when an individual suffering from a particular disease is subjected to vaccines, the vaccine provides resistance against that particular disease by shielding that person's immune systems and stimulating it to fight against any incoming pathogenic invasion. Vaccination is the distribution and administration of vaccines and is also a form of immunization. In the field of biotechnology vaccines are a boon and an invaluable contribution as they provide protection against numerous diseases. Live/attenuated vaccine and killed vaccine are used to prepare the conventional vaccines. However, newer approaches, like with regard to the use of purified antigen vaccines and recombinant vaccines have also been made. The mortality rates are swelling every year despite successful immunization programs **[4].** The concept behind edible vaccines is to make it a part of people's diet. People will be able intake their doses when they consume their food. These Edible Vaccines were fashioned from transgenic plants. An orally active antigen of the target pathogen is expressed in these transgenic plants which is then accumulated and fed to humans. International Patent Co-operation Treaty (1990) saw the publication of the first report of the idea of using a plant expression system for production of an Edible Vaccine. In the report Charles Arntzen and co-workers highlighted the expression of Hepatitis B surface (HBs) antigen in tobacco plants and presented the thought that edible vaccine holds great promise for low cost vaccine production system. Later, by placing multi potent vaccines under second generation edible vaccines, the possibility to be able to provide protection against various diseases came into being. By crossing two plant lines of different antigens multi component edible vaccines can be obtained. This may be restraints on vaccine manufacture, dissemination and transfer. Moreover, the management of vaccine is price effective technique of opposing feast of infections and epidemics **[5].** In 1990, the first report of edible vaccine, (a protein from *Streptococcus* in tobacco) at 0.02% of total leaf protein level, appeared in the form of a patent application under the international patent cooperation treaty **[6].** The production of edible vaccines is done by introducing the desired gene of interest in the selected plants to manufacture proteins encoded with the same gene. The altered plants are called "transgenic plants" while the process is known as "transformation" **[7].** Banana, potato, tomato, lettuce, rice, etc. are some of the food items that are being investigated for the possibility of being used in edible vaccines **[8].**

2. LITERATURE REVIEW:

Edible vaccines may possibly be the answer that will facilitate the spread of the positive effects of vaccines by tackling and reducing some of the potential threats that are associated with parental vaccines such as toxic compounds, allergic responses and risk of attenuated strains reverting to pathogenic strains. Edible vaccines present a means for oral vaccination through banana, potato, tomato, lettuce, rice, etc are some of the food items **[8].** Two methods are used to handle gene encoding antigen from pathogenic organisms such as virus, bacteria, parasites that have been characterized and which have available antibodies. In the first method the entire structural gene is inserted into a plant transformation vector between regulatory elements 5' and 3'. This allows transcription and accumulation of coding sequence in plant. The second method identifies an epitope within the antigen and then using DNA fragment encoding genes are constructed by fusion with a coat protein gene from plant virus such as TMV or CMV. Then the stabilized plants are infected with the recombinant virus which results in the production of the candidate vaccine antigens in plant tissues. Selected desired genes are first introduced into plants and then they are induced to produce the altered protein, thus producing edible vaccines. As such, plant-based vaccine production primarily involves integration of transgene into the plant cells. Before the selected antigen is transferred into the expression system the target sequence of the selected antigen is integrated with the vector. Several techniques, such as *Agrobacterium* gene transfer, biolistic method and electroporation are used to achieve a stable transformation system **[9].**

1) *Agrobacterium* mediated gene transfer method

Various methods are used in plant-based vaccine technologies to achieve the integration of the desired genes, encoding the antigen protein for specific disease into the genome of plant tissues. However, the most common methods that are used to produce effective vaccines are *Agrobacterium* mediated gene transfer and transformation via genetically modified plant virus. *Agrobacterium* is a Gram-negative soil pathogenic bacterium. They naturally infect the plant and transfer their genes (T-DNA) to the nucleus of the plant cells **[10, 11].** The most favored strain for stable expression of the desired protein is *A. tumefaciens.* This is because *A. tumefaciens* carries tumour-inducing plasmid (Ti-plasmid). Ti-plasmid contains genes that are responsible for encoding plant hormones such as auxin and cytokinin synthesis. This in turn induces tumour tissue in plants. During the production of the vaccine these genes will be obliterated to form neutralized Ti-plasmid and then heterologous gene is inserted to form a recombinant plasmid vector **[12].** The recombinant plasmid vector is then transformed into *A. tumefaciens.* Then the transformed bacterium with assistance from *'vir'* gene of the bacterium transfers the introduced heterologous gene and integrates it into the nuclear genomic DNA of the host plant by non-homologous recombination at random sites **[11].** *Agrobacterium* gene transfer method is best suited for di-cotelydenous plants such as tomato, potato, tobacco **[13].**

2) Biolistic Method

Biolistic method is utilized for the transformation of Mono cotyledenous plants with the uses of a gene gun, gene containing DNA coated metal (gold, tungsten) particles are fired at the plant cells **[14].** The plant cells that are able to absorb the DNA are permitted to grow in new plants. Then they are cloned so that they can be used to produce large quantity of genetically identical traits. This method enables DNA to be delivered into cells of plants thus making the gene transfer independent. As such, biolistic method is quite attractive having two types of antigen expression, namely nuclear transformation and chloroplast transformation in transgenic plants. Now, nuclear transformation is achieved by the integration of the desired gene into the nucleus of the plant cells by the way of non-homologous recombination **[15].**

3) Electroporation

In electroporation method, first the DNA is inserted into the cells. Then the cells are exposed to high voltage electrical pulse. This is thought to create transient pores within the plasma lemma. Additionally, this process requires the cell walls to be weakened since the cell wall behaves like an effective barrier against the entry of the DNA into the cell cytoplasm. As such, a mild enzymatic treatment required to weaken the cell wall **[16].**

4) Preparation of Edible Vaccines

The introduction of foreign DNA into the plants genome can be achieved in two ways. First, it can be achieved by bombarding embryonic suspension cell cultures using gene-gun. Second, it can be achieved through *Agrobacterium tumefaciens*, which is most commonly used. *A. tumefaciens*, a naturally occurring soil bacterium, can get into plants through wounds (scratch, etc.), contains a circular "Ti plasmid" (tumor inducing. This enables it to infect plant cells and integrate into their genome, producing a hollow tumor (crown gall tumor), where it can live **[17].** Some steps that are followed for the preparation of edible vaccines are as follows: Foreign DNA can be introduced into the plant's genome either by bombarding the embryonic suspension cell culture using a gene-gun or by the using *A. tumefaciens,* a naturally occurring soil bacterium. This soil bacterium has the ability to get into plants through wounds (scratch etc.). The transformed cells and whole plants that contain foreign gene are identified and selected out using genes for antibiotic resistance. This is done for purpose of expressing the desired product which can then be regenerated from them. The plant genome is integrated at random by the DNA. This results in each independent line having a different antigen expression level. As such 50-100 plants are transformed to get generate time. This allows one to choose the plant that expresses the highest level of antigen and least number of adverse effects. Viral capsid proteins and other such antigens have to self-assemble into VPLs (virus-like particles). VPLs are not infectious since they do not carry DNA or RNA. Every single antigen that have been expressed in the plants need to be checked to see whether they have assembled properly. Animal studies and Western blot can be done to verify proper assembly and can be quantified by Enzyme-Linked Immune Sorbent Assay (ELISA). Foreign DNA can be inserted into the plant genome by exploiting this ability. However, preceding the insertion of the foreign DNA it needs to be ensured that the plasmid does not produce tumor. This can be done by disarming the plasmid by deleting the genes for auxin and cytokinin synthesis. The transformed cells and whole plants that contain foreign gene can be identified and selected out using genes for antibiotic resistance. This is done for purpose of expressing the desired product which can then be regenerated from them. The plant genome is integrated at random by the DNA. This results in each independent line having a different antigen expression level. As such 50-100 plants are transformed to get generate time. This allows one to choose the plant that expresses the highest level of antigen and least number of adverse effects **[18].** Mucosal vaccine can be formulated using different methods. These methods include (i) gene fusion technology, which creates non-toxic derivatives of mucosal adjuvants; (ii) genetic inactivation of antigens by deleting an essential gene; (iii) co-expression of antigen and a cytokine, which controls and modulates mucosal immune response; (iv) genetic material itself, which allows for DNA/RNA uptake and its endogenous expression in the host cell **[19].** The various mucosal delivery systems involve biodegradable micro- and nanoparticles, liposomes, live bacterial/viral vectors and mucosal adjuvants. "Prime-boost" strategy combines different routes of administration and vaccine types. This is done especially where multiple antigens or doses are needed. An example of this could be when a single parenteral dose of MV-H DNA (measles virus haemaglutinin) is followed by multiple oral MV-H boosters. This could prompt greater quantities of MV-neutralizing antibodies than with either vaccine alone **[20].**

5) Edible vaccine as fruit

I) Banana (*Musa acuminate*)

Boyce Thompson Institute for Plant Research at Cornell University has been genetically engineering fruits to produce edible vaccines. Bananas grow extensively in many parts of the developing world and as such they are particularly alluring as vaccines **[21].** Aside from this, the administration of this vaccine will not require any syringes and as such it would cut down costs and prevent infections from contaminated syringes. This vaccine can prevent or perhaps even erase autoimmune disorders such as type I diabetes, multiple rheumatoid arthritis, and sclerosis **[22].** Though banana can be consumed without cooking, its drawback is that banana has low protein content and the banana trees take at least two years to mature and it also spoils fairly rapidly after ripening **[23].**

6) Edible Vaccine as vegetable

I) Potato (*Solanum tuberosum*)

Norwalk virus (Stomach virus) is spread through contaminated food and water and causes acute abdominal pain and diarrhea. A vaccine based on potato has been made to combat this virus. It has been observed that an effective way of developing immunity to *E. coli* toxin could be achieved by eating of potato. This potato based vaccine is safe and has several advantages apart from stimulating antibodies with ease. It is affordable, effective, and is easily propagated from the 'eyes' of the potato. Further it only takes a few months to grow and can be stored for long periods of time without refrigeration. In 1997, the first human trial for edible vaccine took place and in this trial, the volunteers ate transgenic potatoes. These transgenic potatoes contained the subunit of the *E. coli* heat-labile toxin, which causes diarrhea **[24].** However, the drawback of this potato-based vaccine is that it needs cooking which can denature antigen and decrease immunogenicity **[25].**

II) Tomato (*Lycopersicones culentum*)

Using tomato as a vector, edible vaccine can be developed to combat Anthrax, Rabies and HIV/AIDS. It has few advantages, for example it is extensively cultivated and grows quickly. Further it also tastes good. Its only drawback being that it rots easily **[26].**

7) Edible Vaccine as crop

I) Rice (*Oryza sativa*)

Edible vaccines developed by genetically modifying rice can be used against cholera. The vaccine can be administered orally and does not require any purification or refrigeration **[27].**

II) Maize (*Zea mays*)

Edible vaccines developed by genetically modifying rice can be used against cholera. The vaccine can be administered orally and does not require any purification or refrigeration **[27].**

8) Plant base vaccine against disease

Clostridium tetani vaccine is produced into potato, tomato, banana **[28].** Anti-Norwalk virus vaccine is produced into tobacco and potato. Cholera toxin (CT) B and A2 subunit complementary DNAs (cDNAs) merged to a rotavirus enterotoxin and enterotoxigenic *Escherichia coli* fimbrial antigen genes and transferred into potato for the production of Anti-rotaviruses vaccine**.** Fusion antigens are synthesized in altered tuber tissues and amassed into cholera holo toxin-like arrangements that recalled enterocyte-binding affinity **[29].** Anti-HAV vaccine and Anti-HBV (HBsAg protein) vaccine can be produced through *Agrobacterium* mediated gene transfer into the epicotyl segment of citrus plant and tubular edifice of potato respectively **[28, 30].** Anti-HPV Vaccine, Anti-infectious bronchitis virus (IBV) Vaccine, Anti-rabbit hemorrhagic disease virus (RHDV) Vaccine and Anti-SIV Vaccine are prepared by using *A. tumefaciens* mediated transformation method. The fusion gene was transferred into the potato cells and the transformed plants successfully regenerated **[19, 31].**

3. CONCLUSION:

Vaccines are fashioned to prompt an immune response without causing any disease. Edible vaccines provide for oral administration of vaccination. The development of oral vaccines using plant-based vaccines is truly an exciting and noble innovative strategy. For the efficacious and affordable pharmaceuticals, edible vaccines are the emerging innovations in medical science and plant biology. The production of edible vaccines is done by introducing the desired gene of interest in the selected plants to manufacture proteins encoded with the same gene. The altered plants thus produced are called "transgenic plants". While the process used is known as "transformation" which is achieved through some of selected gene transfer methods, mainly *Agrobacterium* mediated method. Edible vaccines have provided the world with exciting new possibilities. These vaccines hold promise to significantly decrease the burden of diseases like hepatitis, Tuberculosis, diarrhoea, pneumonia, STDs, HIV, cholera, foot and mouth disease, dengue, hookworm, rabies etc. especially in the developing countries that are still trying to overcome problems of storing and administering vaccines. Edible vaccines in combination with other vaccination programs can also be used against exceptional diseases like dengue, hookworm, rabies, etc. by facilitating multiple antigen delivery. Various foods are under investigation for their use in edible vaccines. In terms of fruit, banana has become a major player. While considering vegetables, potato, tomato shows great promise and in terms of crops, maize and rice has become major contenders. Plants like tobacco etc. are also being investigated to be used in edible vaccines. Many edible vaccines are being produced using this plant base vaccine process. Some examples of such vaccines could be *Clostridium tetani* vaccine in transgenic plants tobacco, potato, tomato, banana; Anti-Norwalk virus vaccine in GM tobacco and potatoes; Anti-rota viruses vaccine; Cholera toxin in potato; Anti-HAV vaccine, Anti-HBV vaccine, Anti-HPVs vaccine in transgenic tobacco and potato plants; Anti-HIV-1 vaccine, Anti-infectious bronchitis virus (IBV) Vaccine in potatoes; Anti-SIV Vaccine, Anti-rabbit hemorrhagic disease virus (RHDV) Vaccine in potato. As such, edible vaccines are on the path to provide a bright future where injectable needles will no longer be required to administer vaccine. People could get vaccinated and be protected against diseases by simply eating foods like fruits, vegetables and crops, that comprise their regular diet. Edible vaccines have overcome the major hurdles on becoming an emerging vaccine technology. Consistency of dosages for edible vaccines differs from fruit to plant and generation of the plant while stability of vaccine in fruits is still unknown. There are yet several technical obstacles, regulatory and non-scientific challenges that need to be overcome, which all seem surmountable.

4. FINANCIAL DISCLOSURE:

We declare that we have no conflict of interest.

5. ACKNOWLEDGEMENT:

The authors are thankful and fortunate enough to get constant encouragement support and guidance from the management of Cyber Research And Training Institute, Burdwan, The University Of Burdwan, W.B.

6. REFERENCES:

1. Goyal R., Sharma R., Lal P., Ramachandran V., (2007): Edible vaccines, Current status and future: *Indian Journal of Medical Microbiology*, 85-93.
2. Langridge W H., (2000): Edible vaccines: *Scientific American*, 283, 66-71.
3. Tacket C O., Mason H S., Losonsky G., Clements J D., Levine M M., Arntzen C J., (1998): Immunogenicity in humans of a recombinant bacterial-antigen delivered in transgenic potato: *Nat Med*, 4, 607-609.
4. Landridge W., (2000): Edible vaccines: *Scientific Am*, 283, 66-71.
5. Lal P., Ramachandran V G., Goyal R., Sharma R., (2007): Edible vaccines: *Current status and future*, 25, 93-102.
6. Mason H S., Arntze C J., (1995): Transgenic plant as vaccine production system: *Trends Biotechnol*, 13, 388-392.
7. Sibila J., Snjezana M., Natasa B., (2010): Production of biopharmaceuticals, antibodies and edible vaccines in transgenic plants: *Current studies of biotechnology*, 121-127.
8. Giddings G., Allison G., Brooks D., Carter A., (2000): Transgenic plants as factories for biopharmaceuticals: *Nat Bio technol*, 18, 1151-1155.
9. Ma H., Chen G., (2005): Gene transfer technique: *Nature and Science*, 3, 25-31.
10. Laere E., Ling A., Wong Y., Koh R., Mohd M., Hussein S., (2016): Plant-Based Vaccines, Production and Challenges: *Journal of Botany*, 1-11.
11. Gómez E., Zoth S C., Carrillo E., Berinstein A., (2010): Developments in plant-based vaccines against diseases of concern in developing countries: *Open Infectious Diseases Journal*, 4, 55-56.
12. Sharma M., Sood B., (2011): A banana or a syringe, journey to edible vaccines: *World Journal of Microbiology and Biotechnology*, 27, 471-477.
13. Mariotti D., Faontana G S., Santin N., (1989): Genetic transformation of grainlegumes: *Phaseolus vulgaris* L. and *P. coccineus*: *Journal of Genetic Breed*, 43, 77-82.
14. Taylor N J., Fauquet C M., (2002): Microparticle bombardment as a tool in plant science and agricultural biotechnology: *DNA Cell Biology*, 21, 963-977.
15. Gómez E., Zoth S C., Berinstein A., (2009): Plant-based vaccines for potential human application: *Human Vaccines*, 5, 738-744.
16. Jan N., Shafi F., Hameed O B., Muzaffar K., Dar S M., Majid I., (2016): An Overview on Edible Vaccines and Immunization: *Austin Journal of Nutrition and Food sciences*, 4, 1078-1084.
17. Charles J A., (1997): Edible Vaccines: *Public Health Reports Science*, 112, 190-197.
18. Webster D E., Thomas M C., Strugnell R A., Dry I B., Wesselingh S L., (2002): Appetising solutions, An edible vaccine for measles: *Med J*, 176, 434-437.
19. Mason H S., Haq T A., Clements J D., Arntzen C J., (1998): Edible vaccine protects mice against Escherichia coli heat-labile enterotoxin (LT), Potatoes expressing a synthetic LT-B gene: *Vaccine*, 16, 1336-1343.
20. Tacket C O., Mason H S., Losonsky G., Clements J D., Levine M M., Arntzen C J., (1998): Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato: *Nat Med*, 4, 607-609.

21. Moss W J., Cutts F., Griffin D E., (1999): Implications of human immunodeficiency eradications of measles: *Infect Dis*, 29, 4452-4455.
22. Moss W J., Cutts F., (2005): Implications of human immunodeficiency: *Infect Dis*, 27, 4582-4587.
23. William S., (2002): A review of the progression of transgenic plants used to produce plant bodies for human usage: *Journal of Young Investigators*, 4, 56-61.
24. Nemchinov L G., Liang T J., Rifaat M M., Mazyad H M., Hadidi A., Keith J M., (2000): Development of a plant-derived subunit vaccine candidate against hepatitis C virus: *Arch Virol*, 145, 2557-2573.
25. Arakawa T., Chong D., Langridge W., (1997): Expression of Choleratoxin B subunit oligomers in transgenic potato plants: *Transgenic*, 6, 403-413.
26. Doshi V., Rawal H., Mukherjee S., (2013): Edible vaccine from crop, Current and future scope: *Journal of Pharmacology Science Innovation*, 2(3), 1-6.
27. Thanavala Y., Yang Y., Lyons P., Mason H., (1998): Edible vaccine from GM crop: *Immunity*, 73, 8266-8274.
28. Tacket C O., Mason H S., Losonsky G., Clements J D., Levine M M., Arntzen C J., (1998): Immunogenicity in humans of a recombinant bacterial-antigen delivered in transgenic potato: *Nat Med*, 4, 607-609.
29. Rigano M M., Alvarez M L., Pinkhasov J., Jin Y., Sala F., Arntzen C J., (2004): Production of a fusion protein consisting of the enterotoxigenic *Escherichia coli* heat-labile toxin B subunit and a tuberculosis antigen in *Arabidopsis thaliana*: *Plant Cell Rep*, 22, 502-8.
30. Korban S S., Krasnyanski S F., Buetow D E., (2002): Foods as production and delivery vehicles for human vaccines: *J Am Coll Nutr*, 21, 212-217.
31. Modelska A., Dietzschold B., Sleysh N., Fu Z F., Steplewski K., Hooper D C., (1998): Immunization against rabies with plant-derived antigen: *Proc Natl Acad Sci USA*, 95, 2481-2845.