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EDIBLE VACCINE: A NEW PARADIGM IN ORAL IMMUNIZATION

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ABSTRACT

In the past few years edible vaccines have emerged as a brand new concept developed by scientists. Edible vaccines are the vaccines which are produced from a plant. They are also known as subunit vaccines. Selected genes are introduced into the host plants and then the transgenic plants are induced to manufacture the encoded protein. Host plants used for these vaccines include potato, banana, lettuce, corn, soybean, rice and legumes. Edible vaccines present a possibility to reduce various diseases such as measles, hepatitis B, cholera, and diarrhoea. When compared to the traditional vaccines, edible vaccines are needle free (oral dosage), low cost involved, it eliminates the need for refrigeration, it can be stored near to the place of use, it is safe and provides mucosal and systemic immunity to the body. This review discusses the various aspects of edible vaccines such as the food crops required for edible vaccine production, the different types of production methods and the applications of edible vaccines in various diseases.

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INTRODUCTION

The word vaccine is derived from the word 'cowpox', cow means vacca in Latin language. Vaccine is a biological product that helps boost immunity against a particular disease. Vaccine is a boon to mankind as it is effective against various infectious diseases. The idea of edible vaccines was established in the 1990s (Khan *et al.*, 2019). The different types of vaccines available in the market are as follows:

1. Live vaccines consist of a weakened or attenuated form of the virus that causes the disease. Immune response is similar to natural infection. Examples – measles, influenza, oral polio vaccine.
2. Killed or inactivated vaccines consist of the killed version of the virus that causes the disease. Immune response is mostly humoral. Examples – rabies, typhoid, cholera.
3. Toxoid vaccines consist of a toxin made up of the virus that causes the disease. Toxoids are highly efficacious and safe immunizing agents. Examples – tetanus, diphtheria.
4. Subunit, recombinant, polysaccharide and conjugate vaccines consist of specific parts of the virus such as protein, sugar or capsid. It is highly efficacious and safe. Examples – viral – measles, mumps, rubella (MMR), bacterial – diphtheria pertussis tetanus (DPT) (Khadwal *et al.*, 2020; Rosales-Mendoza *et al.*, 2017).

Edible vaccines are developed through genetic engineering. The chosen genes are induced directly into the plants through various methods such as agrobacterium mediated gene transfer, biolistic gene transfer, electroporation and chimeric vectors. The developed transgenic plant contains the coded protein which acts as a vaccine (Kumar and Kiran, 2019). The production of a polio vaccine is an excellent example of an oral vaccine (Streatfield and Howard, 2003a).

HOST PLANT FOR EDIBLE VACCINE

Steps involved in the selection of host plant for Edible Vaccine

1. The choice of the seed is the first step involved in the selection of host plants for edible vaccines. It is based on the physical, biochemical properties of the protein, required function of the vaccine, codon preference, pathways involved and the recombination process.
2. The choice of the promoter is the second step in the selection process. Depending on whether the seed is monocot or dicot, the type of promoter can target the cells and the tissues of the seeds that are used for the expression of the recombinant proteins.
3. In the last step the organelles are targeted which accumulate the recombinant protein. It is done by signal sequences (Yonesi *et al.*, 2020).

A few of the host plants are mentioned below:

Tobacco *(Nicotiana tabacum)*

Tobacco was the first plant from which edible vaccines were produced in 1990. Tobacco can be a potential source for producing edible vaccines to prevent diseases such as diarrhoea, rabies, colon cancer, and hepatitis B. The 'S' gene of hepatitis B virus is used to produce edible vaccines against hepatitis B disease (Rosales-Mendoza *et al.*, 2014).

Banana *(Musa acuminata)*

As bananas can be consumed in its pure form, it is the most preferred fruit for edible vaccines. It can be grown in almost all climates and is cost effective (Kshirsagar and Jawale, 2017).

Rice *(Oryza sativa)*

Rice is used as a potential host plant for edible vaccines as it can be stored at room temperature it doesn't require refrigeration and it does not dissolve in the stomach due to the acids present. It is used for cancer treatment. More research is going on for its wider use (Arakawa, 1998).

Maize (*Zea mays*)

Maize plants have been genetically engineered to produce a protein known as HBsAg (hepatitis B surface antigen) which is used for the development of hepatitis B virus vaccine. It is cheaper than the traditional vaccines and does not require refrigeration. A major drawback of this vaccine, which the scientists are trying to overcome, is that maize needs to be cooked for consumption which causes degradation of proteins. Maize has been made to express rabies virus glycoprotein of the Vnukovo strain. Ubiquitin promoter and CaMV promotor are fused to the coding region of the rabies gene. This vaccine is also being developed by the gene gun method (Rosales-Mendoza *et al.*, 2017; Lamphear *et al.*, 2002).

Potato *(Solanum tuberosum)*

Potato - based vaccine is used against the Norwalk virus. This virus leads to diarrhoea, nausea and stomach cramps. Potato is also used as a vehicle for diabetes - related proteins, the vaccine against a strain of *Escherichia coli* and cholera vaccine. The advantages of the potato - based vaccine are that it can safely stimulate antibodies, it is affordable and it can be stored for a long period of time without refrigeration. Cooking the potato can denature the antigen and decrease the immunogenicity (Arakawa *et al.*, 1998; Domansky *et al.*, 1995).

Tomato *(Solanum lycopersicum)*

Tomato is one of the most used food crops which can produce edible vaccines. It is a vector to develop the vaccines against anthrax, rabies and AIDS (Acquired Immunodeficiency Syndrome). The advantages of tomato crop are that it grows quickly, is heat-stable, can be produced in almost all parts of the world and is high in vitamin A which boosts immunity (Hirlekar and Bhairy, 2017; Concha *et al.*, 2017).

Lettuce *(Lactuca sativa)*

Lettuce expresses beta subunit of the thermolabile protein of *E. coli*, that is the cause for many diseases in humans. Therefore, researchers are experimenting with this plant to find its potential as an edible vaccine. The protein which was detected in the leaves corresponded to the required antigen. Hepatitis B virus can be treated with this vaccine. The advantage of lettuce being a host plant is that it can be consumed raw (Concha *et al.*, 2017).

Spinach *(Spinacia oleracea)*

Spinach is known to be an edible vaccine for anthrax and a vehicle for HIV-1 (Human Immunodeficiency Virus) Tat protein. The other food crops which hold the potential to be developed as edible vaccines are wheat, peanuts, sweet potato, watermelon, carrot, alfalfa and soybean (Kumar *et al.*, 2018).

COMMON TECHNIQUES USED IN EDIBLE VACCINES PRODUCTION

***Agrobacterium* mediated gene transfer method**

Agrobacterium mediated gene transfer method is the introduction of an appropriate gene of interest into the T-DNA region of a disarmed Ti plasmid of *Agrobacterium*. The recombinant DNA is placed into *Agrobacterium* and plant pathogen which is also cultured with other plant cells or tissues to be transformed. This method mainly holds good results in dicotyledonous plants. examples, potato, tomato and tobacco (Rajangam, 2018).

Biolistic gene transfer method / Gene gun method

In the biolistic gene transfer method or gene gun method, a gene containing DNA is coated with metal particles such as gold or tungsten. This gene is induced into the plant cell. The plant cells which take up the metal coated gene containing DNA grow into new plants. They are later cloned to produce a large number of genetically identical plants. The major limitation of this method is the cost factor. This method requires costly devices to carry out the procedure as compared to other methods of edible vaccine production (Rajangam, 2018).

Electroporation

In electroporation method, the plant cells are exposed to a high voltage electric pulse for a short period of time. This results in a transient pore in plasma lemma. Plasma lemma acts as a barrier for the introduction of DNA. As the pores open, the DNA is introduced into the cytoplasm of the plant cell (Rajangam, 2018).

Chimeric viruses

Chimeric virus is a man - made virus. It is created by joining nucleic acid fragments of two or more different microorganisms. Here at least two of the fragments contain the required genes for replication. In this method, plant viruses are genetically engineered to produce the required genes. These viruses are used to infect their natural hosts. Here, the cloned genes are expressed to varying degrees in different parts of the plant, including the edible parts. A few types of viruses are redesigned to express the fragments of antigenic surface proteins, such as TMV (Tobacco mosaic virus), CPMV (Cowpea mosaic virus), Alfa mosaic virus. Overcoat technology and epicoat technology are used in this method. The entire protein of the plant is produced in overcoat technology. Only the foreign proteins are expressed in epicoat technology (Rajangam, 2018).

MECHANISM

The objective of oral vaccination is to stimulate mucosal and systemic immunity against viruses or bacteria. Edible vaccines are administered orally. They undergo the mastication process which is the chewing process in the mouth and the high amount of plant cell degradation process in the intestine. This results in the action of digestive or bacterial enzymes on the vaccine. The essential source of IgA (immunoglobulin A) producing plasma cells are peyer's patches (pp). They have the ability to populate mucosal tissue. Also, they act as a mucosal immune effector site. The collapse of edible vaccines near peyer's patches consisting of 30-40 lymphoid nodules on the outer surface intestine and follicles. These follicles act as an area from which antigen penetrates into the intestinal epithelium. Which results in the accumulation of antigen within the lymphoid structure. The antigen is then linked with the M cell. The M cell passes the antigen to macrophages and B cell. The T cells are activated by B cells to provide an immune response. Therefore, immunity is activated by the edible vaccine (Kumar and Kiran, 2019; Dhama *et al.*, 2013).

ADVANTAGES OF EDIBLE VACCINE

- Oral administration so no need of medical personnel and syringes
- Can be stored near the site of use
- Bioencapsulation can guarantee antigen protection
- Multiple antigens can be delivered
- It can be integrated with other vaccine approaches
- Mass production is economical
- No serious side effects (Martins M, 2020; Kurupa and Thomas, 2020; Aryamyally A, 2017; Lamphear and Howard, 2002; Mason H, 2002).

CHALLENGES FOR EDIBLE VACCINE

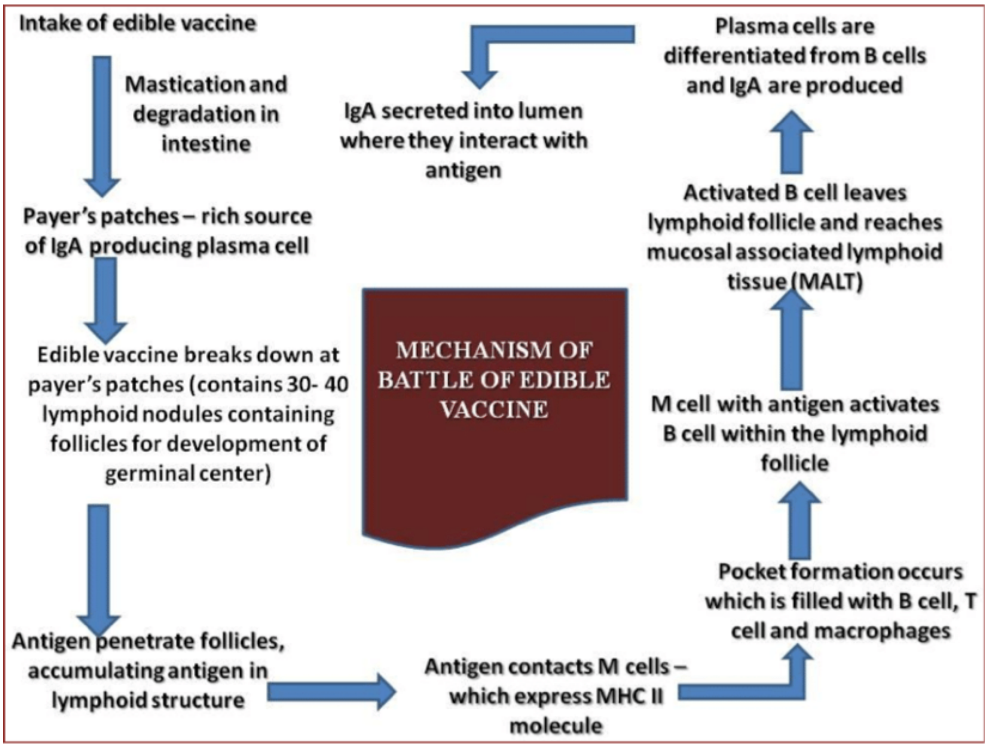

Figure 1. A schematic diagram on Mechanism of Edible Vaccine (Source: Dhama *et al.*, 2013)

- Consistency of dosage from fruit to fruit, plant to plant, lot to lot, and generation to generation is not the same
- Selection of best plant is difficult
- A few foods are not consumed raw and cooking the food might weaken the medicine present in it.
- Not convenient for infants
- People could intake too much or too little of the vaccine, which could be toxic
- Degradation of proteins can take place in the stomach due to low pH and gastric enzymes (Kurup and Thomas, 2020; Miranda *et al.*, 2020; Wang *et al.*, 2020; Khan *et al.*, 2019; Chaitanya and Kumar, 2006; Streatfield and Howard, 2003b).

TECHNIQUES TO OVERCOME THE CHALLENGES

- Expression in plasmids
- Plant viruses expressing foreign genes
- Coat-protein fusions
- Viral-assisted expression in transgenic plants
- Promoter elements can be later substituted with target antigens (Jan and Majid, 2016).

APPLICATION OF EDIBLE VACCINES

The various therapeutic and diagnostic applications of edible vaccines are mentioned in Table 1 (Tripurani and Rao, 2003; Arakawa and Langridge, 1998; Domansky *et al*, 1995; Kumar and Ling, 2021).

CONCLUSION

The efficiency of edible vaccines depends on both the effectiveness of the vaccine and its proper dosage to individuals who are able to respond to it. Researchers are working on how to reduce the challenges such as public opinion about genetically modified products. A few people think that genetically modified products are toxic to humankind. A few other obstacles which need to be looked upon are low expression levels of the antigen and denaturation of protein upon heating. Once these obstacles are overcome, the next step would be to maintain stability and uniform expression in the plant, which will help in eliminating the dosage problem. The burgeoni-

-ng development of plant biotechnology in the recent decades has made the use of transgenic plants as viable alternatives to cell culture systems for the production of edible vaccines possible. Along with the number of advantages comes the equal number of challenges. With more research in this field, the success of edible vaccines will be guaranteed.

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Table 1. Application of edible vaccines in various diseases

Pathogen/Disease Condition	Host Plant used as Edible Vaccine	Disease cured using Edible Vaccine
Norwalk virus	Tobacco, Potato	Diarrhoea, Nausea, Stomach cramps
Rabies virus	Tobacco, Spinach	Rabies
Hepatitis B virus	Tobacco, Potato, Lettuce	Hepatitis B
HIV virus	Tomato	AIDS
Vibrio cholerae	Potato	Cholera
Rabbit haemorrhagic disease virus	Potato	Haemorrhage
Transmissible gastroenteritis coronavirus	Tobacco, Maize	Gastroenteritis
Cancer treatment	Wheat, Rice	Cancer
Colon cancer treatment	Tobacco, Potato	Colon cancer
Herpes simplex virus – 2	Soybean	Herpes
Enterotoxigenic Escherichia coli (ETEC)	Potato, Maize	Bacterial diarrhoea
Paramyxovirus	Tobacco, Carrot	Measles
Plasmodium falciparum	Tobacco	Malaria
Alzheimer's disease	Tomato	Alzheimer's disease
Type - I Diabetes	Potato	Type - I Diabetes

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