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EDIBLE VACCINE: A PROSPECTIVE SUBSTITUTE FOR BETTER IMMUNIZATION IN FUTURE

RUPALI RAJ KESAR[2], SUMIT KUMAR[1], NIDHI[1] AND UTTAM KUMAR*[1]

*[*1]Department of Agriculture and forestry,Doon (P.G.) college of Agriculture science and technology, Selaqui camp road, Dehradun, Uttrakhand. India.*

[2]Department of community medicine PGI sector -12 Chandigarh, India

ABSTRACT

In this present paper, we make an attempt to insight and assess the methods of preparation, mode of action, advantages, limitations and future prospective of edible vaccines. The study shows that vaccination is a great asset for eradication of infectious diseases in human and animals. Consequently vaccines have reduced mortality rate caused by various infectious organisms. However, vaccines have side-effects on individuals. Recently, a novel approach for developing improved mucosal subunit vaccines has emerged by exploiting the use of genetically modified plants. Edible vaccines can be better substitutes of the traditional vaccines as they can overcome all the problems associated with traditional vaccines. It involves introduction of pre-determined selected desired genes into plants and further inducing these altered plants to manufacture the encoded proteins.

KEYWORDS : Edible vaccines, food crops, plant-based vaccines, oral vaccines and future.

UTTAM KUMAR

Department of Agriculture and forestry,Doon (P.G.) college of Agriculture science and technology, Selaqui camp road, Dehradun, Uttrakhand. India.

Corresponding author

INTRODUCTION

In the whole world maximum illness is due to diseases as per survey of WHO. These diseases are responsible for 20 million deaths annually[1]. Vaccination stimulates the immune system against particular pathogen to block the spreading of infection when it will attack next time[2]. So vaccines help in prevention of diseases and reduces mortality rate. Vaccines have been composed of killed or attenuated species or those whose host differs from the vaccinated species. Vaccines provide immunization of children against six most dangerous diseases, but 20% children all over the world are not immunized and are responsible for approximately two million per year, especially in the remote and undeveloped regions of the world[3]. This is due to the constraints on vaccine production, distribution and delivery. 100% immunization is necessary, because un-immunized populations in remote areas can spread infection in immunized "safe" areas. DNA vaccines are alternative of traditional vaccines for immunization but these are expensive, so poor people cannot afford this approach and there is also chances of failure of the immunization ability of DNA vaccines[4]. So research is going-on for cost-effective, easy-to-administer, easy-to-store, fail-safe and socio-culturally readily acceptable vaccines and their delivery system.

Plants and their edible products provide an attractive alternative for the production of recombinant proteins in a large scale[5]. Plants can be genetically engineered to produce vaccines against diseases such as dental caries, and life-threatening infections like diarrhea, AIDS, etc.[6] Edible vaccines are produced by the process of Transformation. Edible vaccines introduced as a concept about a decade ago, it has become a reality today. A variety of delivery system has been developed. Charles Arntzen, with Hugh Mason and team has done great work on Hepatitis B and heat labile toxin. They used tubers of potato and tobacco plants[7]. Initially thought to be useful only for preventing infectious disease, it has also found application in prevention of autoimmune diseases (Hashimoto's Thyroiditis, Good pasture's syndrome, Insulin-Dependent Diabetes Mellitus), birth control, cancer therapy, etc. There is growing acceptance of transgenic crops in both industrial and developing countries. Resistance to genetically modified foods may affect the future of edible vaccines. They have passes the major hurdles in the path of an emerging technology[4]. Edible vaccines are also useful in production of cheap biopharmaceuticals for human health. Some human proteins such as anticoagulant, Neuroproteins, Growth hormone and collagen can be produced successfully in tobacco plants[8].

OVERVIEW OF EDIBLE VACCINES

Edible vaccines provide a cost-effective production system with a safe and efficacious delivery system. Vaccines are very cost effective and less in mass production so, the alternative solution to vaccine is "Edible vaccines". Edible vaccines were first tested on humans in 1997, when scientists asked volunteers to eat Anti-diarrheal transgenic potatoes produced by Boyce Thompson Institute at Cornell University. After consuming the potatoes, almost all the volunteers produced antibodies in their bodies just as if they had received a traditional anti-diarrheal vaccination and there were no side-effects of potatoes. Volunteers are also testing raw potatoes engineered to produce a Hepatitis B antigen at the Roswell Park Cancer institute in Buffalo, New York. Hugh Mason, an associate research scientist in edible vaccines at the Boyce Thompson Institute, hopes to develop "methods to increase production of foreign protein in plant cells and to engineer protein antigen that will enhance their potential as human and animal vaccines." If the FDA approves, "This technology will be a big plus for the developing world"[5].

GENERATION OF EDIBLE VACCINES

In 1992, research team of Mason described the expression of Hepatitis B surface antigen (HBsAg) in Tobacco plant. Thanavala characterized the recombinant product which assembled into virus like particles and could evoke specific immune responses in mice upon parenteral delivery. To prove that plant-derived HBsAg can stimulate mucosal immune responses via the oral route, in 2000, Richter group switched to potato tubers as an expression system and optimized it to increase accumulation of the protein in the plant tubers.

Multicomponent vaccines can be obtained by crossing two plant lines having different antigens. Adjuvant may also be co-expressed along with the antigen in the same plant. B subunit of Vibrio cholera toxin (VC-B) tends to associate with copies of it forms a doughnut-shaped five-member ring with a whole in middle[9]. This feature can be bring several different antigens to M cells at one time-for example, a trivalent edible vaccines against cholera, ETEC (Enterotoxigenic *E. coli*) and rotavirus could successfully elicit significant immune response to all three[10].

METHODS FOR THE PREPARATION OF EDIBLE VACCINES

The desired gene which codes for antigen can be inserted into plants by two several methods-

In one method, desired plant virus is genetically modified to express the desired proteins. Then this modified virus is inoculated into the plant. These resultant edible vaccines are used for immunological purposes. In other method, the desired gene is first inserted into plasmid by transformation. The desired gene can be inserted into plants by following methods-

Vector mediated gene transfer:-

Agro bacterium mediated gene transfer: - The desired gene is inserted into T-region of Ti plasmid of agrobacterium. The recombinant DNA is placed into agro bacterium, which is a plant pathogen and is co-cultured with the plant cells for transformation. The major limitation of this method is that it gives low yield and the process of genetically modification is slow.

Vector-less DNA transfer Methods:-

Gene Gun method- In this method the desired gene containing DNA coated metal (e.g. - gold. tungsten) particles are fired at the surface of plants by using gene gun[11].

Electroporation- The desired gene or DNA is inserted into plant cells by exposing them to high voltage electric pulse for long period[12]. The cell wall is has to be weakened by enzymatic treatment, so that it allow the entry of DNA into the cell.

PREPARATION OF EDIBLE VACCINE

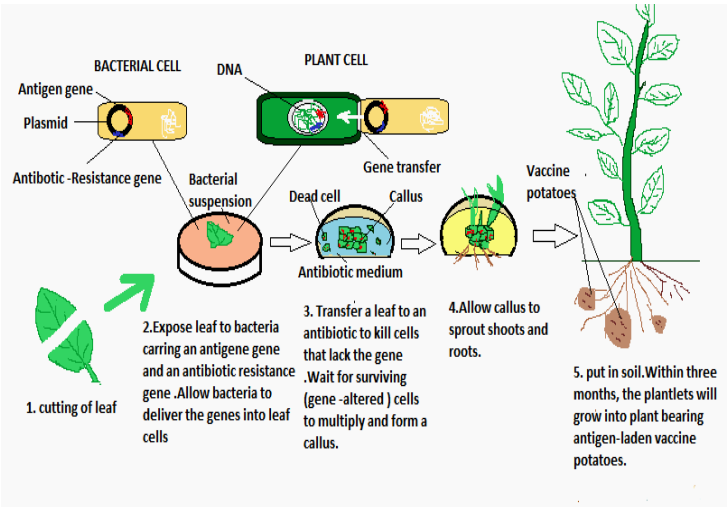

Figure 1

Preparation of Edible Vaccine[3].

MODE OF ACTION OF EDIBLE VACCINES IN HUMAN BODY

The antigens in Edible vaccines are delivered through Bio-encapsulation, i.e., the hard outer wall of plant cells, which protects antigens from gastric secretions, and finally breakup in intestines. Then these antigens are revealed and consumed by M cells in intestine. Then antigens passed to macrophages, other antigen presenting cells and lymphocyte cells. These cells generate antibodies like IgE, IgG and IgA and neutralize the antigens by attacking on them[3].

According to the figure no. 2, an antigen in a food vaccine gets up by M cells in the intestine (below, left diagram) and passed to various immune-system cells, which then launch a defensive attack- as if the antigen were a true infectious agent, not just part of one. That response leaves long-lasting "memory" cells able to promptly neutralize the real infectious agent if it attempts an invasion (right diagram).

PROTECTION PROVIDED BY EDIBLE VACCINES

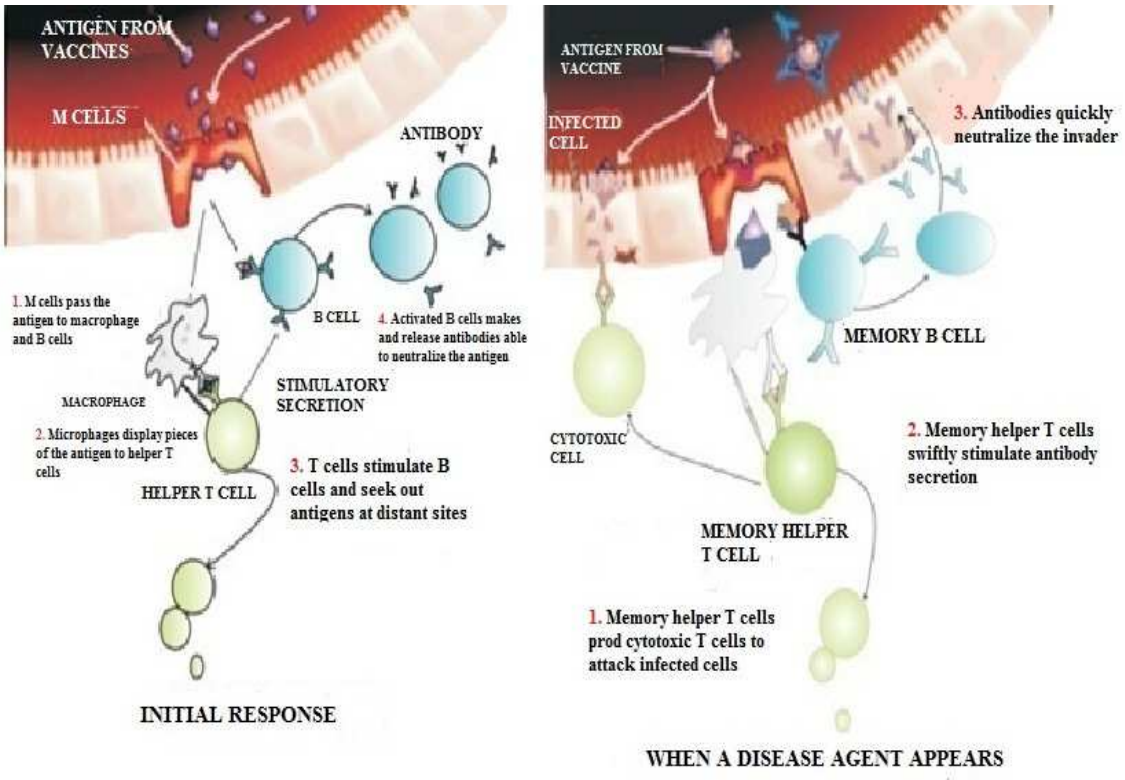

Figure 2

Protection provided by Edible vaccines. It is showing initial response (left) and response when an antigen enters into the body (right)[3].

Table 1

***List of some plant/ fruit preparing edible vaccines Advantages and its limitations*[1]**

Plant/Fruit	Advantages	Limitations
Potato	Resistance to diseases caused by fungi and bacteria such as dry rot, late blight and pink rot.	During cooking antigens may be denatured and decrease immunity
Tomato	Suitable carrier for an oral vaccination against Alzheimer's disease, Can be eaten raw	Spoils rapidly
Golden rice	Cures Vit. A deficiency, Climate change resistance, enhance the nutrition	Environmental impact, exess intake of vitamins and minerals
Banana	Protect against Hepatitis B, measles, and the dysentery-causing Norwalk virus, no need to be cooked, worldwide popularity, abundance and baby-friendliness	Need long time for growing a tree and ripening, Rapidly spoil after ripening
Tobacco	Low cost for storage and have numerous seeds, easy purification of antibodies from seeds	Toxic compounds production, so these can be harmful for humans and animals
Lettuce	Fastly growing, direct consumption	Spoils readily
Carrot	Rich in β carotein, production of Insulin	
Wheat	Large number of seeds help in increased harvest	Need cooking
BT-Corn	Less pesticides, More yields, Cost efficiency	Long term effects on environmental and human health

CLINICAL TRIALS AND APPLICATIONS OF EDIBLE VACCINES

Clinical trials on various transgenic plants and animals are undergoing. The vaccines have also been examined in humans. But some of the challenges are yet to improve before commercialization of edible vaccines. In china, Ying Ma and colleagues have done the expression of ORF2 partial gene of hepatitis E virus in tomato[13]. In 1995, Domansky has worked on expression of HbsAg in roots of transgenic potatoes[14]. Some examples and applications of edible vaccines are in table 1 and table 2 respectively[1].

AUTOIMMUNE DISEASES: Some proteins have been identified that can induce autoimmunity in peoples which have Type I diabetes. Edible vaccine can be made from potato and Tobacco plants against the Diabetes.

***GASTROINTESTINAL DISEASES*:** WHO demonstrated that Cholera vaccine is able to provide cross protection against Enterotoxic *E. coli* heat labile Enterotoxin (LT-B). Transgenic potatoes which express LT-B can be used for these types of diseases. It is also proved that the antigens in potatoes are not killed when it is heated for ripening[6].

Table 2

***Examples of edible vaccines*[1]**

Name of the vaccines	Plant vector	Causing Agent
Rabies	Tobacco	Rabies virus
Hepatitis B	Potato, tobacco	Hepatitis B virus
Gastroenteritis	Maize	Corona virus
HIV	Tomato	HIV virus
Cholera	Potato	*Vibrio cholera*

Table 3
***Applications of edible vaccines*[1]**

Plants	Application
Wheat, Rice	Treatment of Cancer
Nicotiana tabacum	Dental caries
Soyabean	Herpes
T. benthamiana	Colon cancer

PROSPECTIVE AND FATE MAP OF EDIBLE VACCINES

The future of edible vaccines depends on the feasibility of producing sufficient quantities of immunogenic vaccines. The edible vaccines that innately possess immunogenicity and do not require additional adjuvant will probably be the first successful vaccines for human or livestock use. The grain-based vaccines will be the most likely candidates because pollen dispersion or potential contamination of normal food supplies with transgenic pollen can be controlled. The future use of these vaccines also will depend on the development of stable transgenic lines that effectively maintain the vaccine expression for subsequent plant generations. In addition, edible vaccines may have to withstand food processing and possibly cooking.

The future of edible vaccines may be affected by resistance to GM foods, which was reflected when Zambia refused GM maize in food aid from the United States despite the threat of famine. Before endorsing such vaccines for human use, the WHO's concerns of quality assurance, efficacy and environmental impact need to be addressed. Random insertion of genes can destabilize the genomes of its plant and animal hosts and the effects could ricochet through the neighboring ecosystem. By facilitating horizontal gene transfer/recombination, genetic engineering may contribute to emergence and re-emergence of infectious, drug-resistant diseases, rise of autoimmune diseases, cancers and reactivation of dormant viruses. Bacteria may take up transgenic DNA in food in human gut. Antibiotic resistance marker genes can spread from transgenic food to pathogenic bacteria, making infections very difficult to treat. Minor genetic changes in pathogens can result in dramatic changes in host spectrum and disease-causing potentials and inadvertently plants may become their unintentional reservoirs. There is also the risk of creating altogether new strains of infectious agents, like super viruses.

CONCLUSION

Prospective biotechnology approaches for vaccinating people with edible plants is a new idea that appears to hold great promise. Current research is focused at mixing viral or bacterial DNA in a formula, which is then inserted into soil bacteria. When a plant takes on the bacteria, therapeutic DNA becomes stitched into the plant's genetic makeup and as the plant grows, its cells start to produce whatever proteins the new genes are designed to make. When the plant or fruit is eaten, immunization starts, prompting the body to produce the appropriate antibodies.

Edible vaccines hold great potential, especially in Third World countries where transportation costs; poor refrigeration and needle use complicate vaccine administration. While research is also being conducted with laboratory animals, diabetics may someday benefit from an edible form of insulin. Researchers have developed technologies that permit the introduction of a hybrid gene that produces human insulin in potatoes. Creating edible vaccines involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. This process is known as "transformation," and the altered plants are called "transgenic plants." Like conventional

subunit vaccines, edible vaccines are composed of antigenic proteins and are devoid of pathogenic genes. Thus, they have no way of establishing infection, assuring its safety, especially in immune compromised patients. Conventional subunit vaccines are expensive and technology-intensive, need purification, require refrigeration and produce poor mucosal response. In contrast, edible vaccines would enhance compliance, especially in children and because of oral administration, would eliminate the need for trained medical personnel. Their production is highly efficient and can be easily scaled up. For example, hepatitis-B antigen required to vaccinate whole of China annually, could be grown on a 40-acre plot and all babies in the world each year on just 200 acres of land! They are cheaper, sidestepping demands for purification (single dose of hepatitis-B vaccine would cost approximately 23 paisa), grown locally using standard methods and do not require capital-intensive pharmaceutical manufacturing facilities. Mass-indefinite production would also decrease dependence on foreign supply. They exhibit good genetic stability. They are heat-stable; do not require cold-chain maintenance; can be stored near the site of use, eliminating long-distance transportation. Non-requirement of syringes and needles also decreases chances of infection. Fear of contamination with animal viruses - like the mad cow disease, which is a threat in vaccines manufactured from cultured mammalian cells - is eliminated, because plant viruses don't affect the animals.

Edible vaccines activate both mucosal and systemic immunity, as they come in contact with the digestive tract lining. This dual effect would provide first-line defense against pathogens invading through mucosa, like *Mycobacterium tuberculosis* and agents causing diarrhea, pneumonia, STDs, HIV, etc. Scientists place high priority on combating the diarrheal agents - Norwalk virus, Rotavirus, *Vibrio cholerae* and Enterotoxigenic *E. coli* (ETEC) - responsible about three million infant deaths/year, mainly in developing countries. Administration of edible vaccines to mothers might be successful in immunizing the *fetus-in-uterus* by transplacental transfer of maternal antibodies or the infant through breast milk. Edible vaccines seroconvert even in the presence of maternal antibodies, thus having a potential role in protecting infants against diseases like group-B *Streptococcus*, respiratory syncytial virus (RSV), etc, which are under investigation. Edible vaccines would also be suitable against neglected/rare diseases like dengue, hookworm, rabies, etc. They may be integrated with other vaccine approaches and multiple antigens may also be delivered. Various foods under study are banana, potato, tomato, lettuce, rice, etc. Edible vaccines are currently being developed for a number of human and animal diseases, including measles, cholera, foot and mouth disease and hepatitis B, C and E.

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