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Vegetables as a factory of bio pharmaceuticals: Edible vaccines

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Abstract

Edible vaccine is an application of molecular farming which refers to protein products with clinical or veterinary applications produced in recombinant plant systems. The antigens or therapeutics are expressed in plant tissues, from which the plant tissue can be processed into a form that can be consumed orally. The major advantage of plant biopharmaceuticals over other traditional vaccine production systems is reduced manufacturing cost and its safe delivery. Huge Fermenters and bioreactors are replaced with greenhouses or plants are grown in isolation or is even grown in the field but only with appropriate biological containment of foreign genes, such as maternal inheritance or male sterility, or expression in vegetative tissues with harvest before appearance of any reproductive structure. The downstream cost is also reduced. As it is delivered orally, edible vaccines and biopharmaceuticals have the advantage that there is no need for expensive purification systems and other expenses associated with cold storage, transportation and sterile delivery. Edible vaccines possess both mucosal and systemic immunity. The plant transformation after the development of the transformed plants is validated through various methods *viz.*, PCR, Southern blot, Northern Blot and Western Blot and ELISA. After validation using these methods plants are multiplied.

Keywords: edible vaccines, biopharmaceuticals, vegetables, immunity

Introduction

A vaccine is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters (WHO). Vaccines provides protection against various infectious diseases and vaccination is the most efficient and cost effective means that helps the body to resist the attack. Even though vaccines seems to be cost effective, it is difficult for the people in the developing countries to afford it. The high cost of the traditional biopharmaceuticals is mainly due to the complex production and delivery methods which includes the cost of fermentation, purification and its cold storage, transportation and sterile delivery needs. A promising alternative is the production of vaccines in plants that could be grown locally, as edible, plant based recombinant vaccines as they are inexpensive, safe, and easy to administer (Giddings *et al.*, 2000) ^[10].

Concept of edible vaccines

The concept of edible vaccines evolved from the quotes of the famous Greek physician Hippocrates "Let thy food be thy medicine". Desired genes was incorporated into plants thereby allowing the plants to produce the desired encoded proteins. Edible vaccines differ from the traditional vaccines in the sense that it does not contain any pathogenic genes and contains only the antigenic proteins. Edible vaccines was developed by Arntzen of Texas A & M University in USA in the 1990s his first attempt was the production of hepatitis B surface antigen in tobacco plant (Chaitanya and Kumar.,2006) ^[4]. This novel idea could change the face of the medical field as people no more had to struggle taking the medicine but the same can be taken as a part of their diet. Tobacco was the first model plant in which this bio farming concept was practiced. Later it was then produced in potato to feed experimental animals. The techniques of vaccine production in edible portion have been successfully produced.

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Edible vaccines also known as plant derived vaccines or plant based vaccines is now popular due to its extreme safety profile and low production cost. It is since three decades these vaccines being developed, but is not in use even now (Kim *et al.*, 2016) ^[19].

Properties of an ideal vaccine

It should not be toxic or pathogenic, i.e., it should be safe. It should have very low levels of side effects in normal individuals. It should not cause problems in individuals with impaired immune system. It should produce long-lasting humoral and cellular immunities. The vaccination technique should be simple. The vaccine should be less expensive. Contamination of the environment should not happen. It should be effective and affordable (Das and Deshmukh., 2009) ^[7].

Advantages and Disadvantages

Edible vaccines are comparatively cost effective, as they do not require cold chain storage like traditional vaccines (Nochi *et al.*, 2007) ^[30]. Edible vaccines offer greater storage opportunities as they seeds of transgenic plants contain lesser moisture content and can be easily dried. In addition, plants with oil or their aqueous extracts possess more storage opportunities (Pascual., 2007) ^[32]. It is much safer than the traditional vaccines since it is not produced in the animal tissues and the chances for infection is less. Edible vaccine unlike traditional vaccines. They are much economical as mass production can be undertaken. Purest and safest means of administration as they are taken as a part of diet and there is no need of any external materials like syringes which paves the way for many infectious and dreadful diseases. They can be stored at normal room temperature thereby eliminating the needs for extensive cold storage facilities. It can be produced and prorogated in the native crop species, hence reducing the transportation and storage cost. (Hirlekar and Bhairy., 2016) ^[12].

Disadvantages

Dosage of a particular vaccine cannot be controlled as it varies with the age of patient, weight and ripeness of the fruit. Standardization of the edible vaccine is to be done as the large doses causes immune tolerance and low doses doesn't produce the required antibody. Edible vaccines have short shelf life (eg: lettuce as an edible vaccine). Certain strategies must be developed to distinguish between the normal fruit and vaccine fruit as it causes misadministration (Jan *et al.*, 2016) ^[15]. Edible vaccines get destroyed or denatured on cooking (Moss *et al.*, 1999) ^[29].

Mode of Action

The main advantage of the edible vaccines is that it protects the antigenic protein from the acidic pH of the stomach and prevents the digestion and thus ensures the safe and efficient delivery of the antigen to the intestine. The principle of bio encapsulation plays a major role in this, as the tough cellwalls of the plants helps in safeguarding the antigenic protein within it (Lossl and Waheed., 2011) ^[26]. Mucosal immunity, the first line of defense mechanism against most of the pathogens entering through mucosa like mycobacterium tuberculosis and agents causing HIV, diarrhea, pneumonia is being efficiently ensured in the edible vaccine mechanism. The majority of the human pathogens causes infection at the mucosal surfaces of gastrointestinal, urinogenital and respiratory tracts (Tacket *et al.*, 1999) ^[39]. As the principle of

bio encapsulation acts upon it and these ensure mucosal immunity, antibody mediated immune response and cell mediated immune response. The antigen which is safely delivered to the intestine is taken up by the Peyer's patch, where the digestion of the plant vaccines and release of the antigens takes place. These antigens are taken up by the M cells and presented to the B cells. The antigen presenting cells aids this complex process. The activated B cells ultimately differentiates to give plasma cells which in turn secretes IgA class of antibody thereby ensuring the mucosal and humoral immunity (Pant and Sanjana., 2014) ^[31].

Second generation edible vaccines

Multi-component vaccines that provide protection against several pathogens, which have the ability to develop more than one antigenic proteins are called as second generation edible vaccines. These multi-component vaccines are obtained by crossing two plant lines containing different antigens. Adjuvant may also be co-expressed along with antigen in same plant. B subunit of Vibrio cholera toxin (VC-B) tends to associate with copies of itself forming a doughnut shaped five-member ring with a hole in middle (Landridge., 2000) ^[21]. This features can bring several different antigens to M cells at once. For example, a trivalent edible vaccine against cholera, ETEC (Enterotoxigenic *E.-coli*) and rotavirus could successfully elicit significant immune response to all three.

Conventional vaccines vs. edible vaccines

Conventional vaccines are synthesized by attenuated pathogens. The antigenic proteins are made through mammalian cell culture in huge reactors and suspension tanks and thus are prone to chances of pathogenic contamination like the mad cow disease or mammalian viruses, blood-borne pathogens, oncogenes and bacterial toxins (Ferrante *et al.*, 2001) ^[9]. Edible vaccines are produced through bio farming from the transgenic plants which requires the sophisticated structures like green house, polyhouse etc. and hence are not prone to any contamination.

Methods for edible vaccine production

The edible vaccines can be produced the desired antigen coding gene is isolated from the microbes and handled in 2 ways:

Transient method

In this method of edible vaccine production, the plant viruses are genetically engineered to get a desired protein. These viruses are then allowed to infect the plants and thus within the plants the protein is being produced. This is meant for short term production only as the heritability cannot be assured

Transformation method

Here the gene of interest from the desired antigen is integrated into the vector and these vectors are then transferred into the plant via Agrobacterium Mediated Gene transfer or Biolistic Method

Agrobacterium mediated gene transfer

The suitable gene of interest is extracted and it is inserted into the T – region of Ti plasmid of agrobacterium. This agrobacterium is allowed to infect the explant by allowing the co cultivation. The live bacteria is then killed using the bacteriostatic medium and then transferred to the selective

medium which favours the transformants and the non transformants are eliminated (Streatfield, S. J., 2006) [37]. The transformants are then cultured on the suitable media and then evolved into mature plants through a series of biotechnological techniques.

Biolistic Method

It is also called as gene gun method where in the DNA particles are coated with the heavy metals like gold and tungsten and are fired into the plant in a projectile motion (Taylor, *et al.*, 2002) [41]. Transformed plants are allowed to grow into new plants and undergo cloning to give rise to new plants. The cost economics of this methods limits its use.

Candidates for Edible vaccines

Edible parts of the plants are used to express the desired antigen of interest. The plants used varies like the cereals including the rice and maize, fruits like banana, leaves of many plants like that of tobacco, alfalfa, peanut leaves and the vegetable crops like potatoes, tomatoes, soybean seeds, cowpea, pea, carrot and lettuce. Plants are selected on the basis of the criteria like hardiness and palatability, accessibility to the crop and easy transformation (Ferrante *et al.*, 2001) [9].

Banana

Bananas are reproductively sterile due to the mechanism of parthenocarpy which restricts the gene transfer and thus is a good choice as the candidate plants. Since it is eaten raw and does not require cooking the reliability on banana has increased. It is inexpensive too and rich source of vitamin A which boosts the immune response (Ferrante *et al.*, 2001) [9].

Potato

Potato has served as a vehicle to compact against the diseases like cholera, dengue and porcine respiratory diseases etc. (kim *et al.*, 2017, chen *et al.*, 2011) [27, 5]. The main advantages of the potato is the affordability by the common and its long shelf life even without refrigeration (kim *et al.*, 2017) [27]. The need for cooking limits the use as it denatures the antigen and reduces its immunogenicity (Hein *et al.*, 1996 and Arakawa *et al.*, 1997) [11, 1].

Maize

It is cheaper and does not need to be refrigerated. A major disadvantage of this vaccine is to be cooked for use which causes degradation of proteins (Arakawa *et al.*, 1998) [1].

Tomato

Tomato is one among the widely cultivated vegetables and is quick growing too. Like banana it is also rich in vitamin A which increases its immunogenicity. In general tomatoes show great oral vaccine potential as they contain tomato lectin, which act as an adjuvant and a safe vehicle for the gut antigen delivery and also the alpha tomatine which is a powerful plant endogenous adjuvant (Carreno – Gomez *et al.*, 1999; Morrow *et al.*, 2004) [3, 28].

Vegetables as candidate plants

Vegetables have the capacity of inducing immune response in both blood serum and in intestinal mucosa. Vegetables are hardy and palatable plant with high nutritive value and protein content. Most of them can be consumed raw as salads and thus the question of reduction in immunogenicity is eliminated.

Table 1

Disease	Expression system	Expression level	Reference
Diarrhea	Carrots Water cress	0.3% TSP 1.3% TSP	Rosales-Mendoza <i>et al.</i> (2008) [34] Loc <i>et al.</i> (2010) [25]
Cholera	Tomato	0.081% TSP	Jiang <i>et al.</i> (2007) [16]
Hepatitis B	Potato Potato	8.5 µg g-1 FW Up to 0.05% TSP	Thanavala <i>et al.</i> (2005) [42] Rukavtsova <i>et al.</i> (2015) [36]
Gastroenteritis	Potato tubers Tomato fruit and potato tubers	0.3 – 0.4% TSP 8% and 0.4% TSP	Li <i>et al.</i> (2006) [23] Pogrebnyak <i>et al.</i> (2005) [33]
AIDS	Carrot	90 ng g-1 FW in carrot	Lindh <i>et al.</i> (2014) [24]
Cervical cancer	Tomato	0.05-0.1% TSP	Monroy-García <i>et al.</i> (2014) [27]
Malaria	Rapeseed mustard	Not mentioned	Lee <i>et al.</i> (2011) [22]
Tuberculosis	Potato Potato	Not mentioned 0.033% of TSP	Zhang <i>et al.</i> (2012) [44] Jose <i>et al.</i> (2014) [17]
Haemophilus B	Lettuce	0.48-0.63% TSP	Su <i>et al.</i> (2015) [38]
Dengue	Potato Tomato	0.005% of TSP 0.0155% of TSP	Kim <i>et al.</i> (2017) [20] Kim <i>et al.</i> (2016) [19]
Porcine reproductive and respiratory syndrome	Potato	2.5-4.7 µg g-1 in leaves 0.8-1.2 µg g-1 in tubers	Chen <i>et al.</i> (2011) [5]
Chronic gastritis	Carrot	25 µg g-1 of roots	Zhang <i>et al.</i> (2010) [43]
Bubonic and pneumonic plague	Lettuce	0.08% TSP	Rosales-Mendoza <i>et al.</i> (2010) [35]

Regulatory aspects/issues

There were many objections raised to the use of plants for manufacturing of the recombinant Pharmaceuticals. Care is be taken to regulated and isolate the edible vaccines from contaminating the food, medicine or agricultural products. This is ensured by the use of greenhouses and other separate bodies that make sure the antigenic proteins are not being released to the external environment by any means (Tacket. C.O., 2009) [40]. Transgenes may spread by sucking insects, pollen and transfer to soil microbes during plant wounding or breakdown of roots and may pollute surface and ground water. The labelling of edible pharmaceutical plants to preserve their identity, facilitate traceability and avoid the contamination of the food supply. As a proof of this concept, transgenic tomato plants expressing neutralizing IgA antibodies against rotavirus were crossed with another

transgenic line expressing the Antirrhinum majus Roseal and Delila transcription factors in the fruit, thus activating anthocyanin biosynthesis and generating purple fruits (Butelli *et al.*, 2008) [2].

Gene transfer in the environment

Different approaches are suggested to stop the flow of gene from edible vaccines (GM crops) to broader environment. Two important techniques of preventing it from occurring are:

- Physical isolation and
- Genetic containment

Physical isolation is tough and expensive and needs to be carried out frequently (at each stage of production). The crop is grown in isolation and the small and large scale field trials are carried out in isolated areas. The seed and commercial crops can be grown in contained greenhouse conditions or in

places free of weed or food crop relatives. Moreover, the earth growing GM crops and the surrounding fields ought to be left to 'lie fallow' for some time ensuring no seeds to remain and grow in the upcoming crop cycle. The genetic containment was achieved at different levels through technological means. Existing infertility and incompatibility systems helps in limiting the transfer of pollens may be used as well as Genetic Use Restriction Technologies (GURTS) that hinder with fertility or seed formation. Another method was the use of chloroplast transformation wherein the foreign genes were transferred into chloroplast genome as chloroplasts are inherited maternally and not confined in pollen. Another way to minimize the transfer is to concentrate on the non-food crops like tobacco or the recombinant biopharmaceuticals must attain the GRAS (Generally Recognized As Safe) status and using the right agricultural techniques for production (chow *et al.*, 2016) [6].

Future and current status of edible vaccines

Protalix, an Israel based biopharmaceutical company has been successful in producing and manufacturing the edible vaccines. Some of the edible vaccines being manufactured is:

- **Taliglucerase alfa (Elelyso):** it is a recombinant glucocerebrosidase enzyme produced from transgenic carrot cell cultures, also known also as Elelyso, taliglucerase has won approval from the U.S. Food and Drug Administration in May 2012 as an orphan drug for the treatment of Type 1 Gaucher's disease. The Brazilian National Health Surveillance Agency, known as ANVISA, granted regulatory approval for Elelyso (Uplyso) to treat adults with Gaucher disease in March 2013, and extended that approval to children in December 2016.
- **Alidornase alfa (PRX-110):** A proprietary plant cell-expressed recombinant form of human deoxyribonuclease I (DNase I). DNase I is part of current Cystic Fibrosis therapy, intended to reduce sputum viscosity that accumulates in the lungs of Cystic Fibrosis patients, which exposes patients to recurrent infections and compromises lung function. AIR DNase (alidornase alfa), developed to make mucus in the lungs of cystic fibrosis patients less sticky, and showed remarkably good results in 2017. Alidornase alfa is being developed to treat all cystic fibrosis patients and expected to replace Pulmozyme® in 2020.
- **Pegunigalsidase alfa (PRX-102):** A plant cell culture expressed and a chemically modified version of the recombinant alpha-Galactosidase-A protein. Protein subunits are covalently bound via chemical cross-linking using PEG chains, resulting in a more active and stable molecule than the current available versions. Protalix is currently conducting Phase 3 clinical trial for PRX-102 for the treatment of Fabry Disease. Pegunigalsidase alfa is being developed to replace Fabrazyme and interim data shows potential superiority in efficacy.
- **OPRX-106:** plant cell-expressed recombinant human tumor necrosis factor receptor II fused to an IgG1 Fc domain (TNFR2-Fc), in development for oral administration. If successful, OPRX-106 will be the first ever oral enzyme treatment as currently there are no other oral enzyme treatments available.
- **PRX-105:** a recombinant human Acetyl cholinesterase, produced from genetically modified cell line of tobacco cells (*Nicotiana tabacum*), which can be used as a

counter-measure against nerve agents attack. PRX-105 completed exploratory Phase I clinical trials now.

Conclusion

Edible plant-derived vaccines present a better possibility of safer and more efficient immunization in the future. Limitations linked with traditional vaccines, like production, distribution and unsafe delivery can be eliminated by the use of edible vaccines. Edible vaccines can wipe away the major issues in medical field. The time is not so far when there is need for an economical, safer and efficient delivery system to be developed at a larger scale in the form of edible vaccines. Edible vaccines are the ray of hope to all the developing and under developed countries who cannot afford purchasing the costly medicines. Hence, edible vaccines provide a greater opportunity in the near future when no longer injectable needles are to be used and an individual can simply take his medicine as a part of diet.

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