ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 05, pp.16227-16231, May, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EDIBLE VACCINES: PROPHYLACTIC FOOD

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ARTICLE INFO

Article History:
Received 18th February, 2015
Received in revised form
23rd March, 2015
Accepted 26th April, 2015
Published online 31st May, 2015

Key words:
Edible vaccine, Transgenic plant,
Food vaccine, Immuno-Prophylaxis,
Food vaccine, Future vaccine.

ABSTRACT

Oral delivery of vaccine proteins has been shown more efficient compared to subcutaneous or intramuscular injection vaccines due to the increased chance of provoking mucosal immune responses, which in turn stimulate cell mediated immune responses. Edible vaccines clutch swear as a cost effective, easy to administer, easy to store, readily acceptable vaccine delivery system especially in poor and developing countries. Another advantage of edible vaccine technology is the multi-component ability that is possible due to the crossing of two different plant lines. Resulting multi-component vaccine proteins are known as second-generation vaccines. They allow for several antigens to approach. A multi-component edible vaccine can also be multivalent. It can be designed to confer simultaneous protection against multiple diseases in humans and animals as well. There are several advantages over other methods of biological protein production *viz*. they are efficiently grown on agricultural land, low cost of production with minimum inputs, large scale production is possible by simply increasing the number of plants.

***Citation*:** Pavulraj Selvaraj, Saminathan Mani, Prabakar G and Albert Arockia Raj, P. 2015. "Edible Vaccines: Prophylactic food", *International Journal of Current Research,* 7, (5), 16227-16231.

INTRODUCTION

Preparation of edible vaccines involves introduction of chosen preferred genes into plants genome and inducing these altered plants to assemble the encoded proteins in desired forms. This process is known as transformation and the altered plants are called as transgenic plants. As like conventional subunit vaccines, edible vaccines are comprised of antigenic proteins and are devoid of pathogenic genes assuring no way of establishing infection in host upon immunization. Conventional subunit vaccines preparation protocols are technologically intensive and expensive, further need to be purified, necessitate refrigeration and often yield poor mucosal immune response. In disparity, edible vaccines may enhance compliance, especially in children because of oral administration; eliminate the need for skilled medical personnel. Edible vaccine production is highly efficient and can be easily scaled up to larger production volume. Transgenic plants exhibit good genetic stability. The expressed proteins are heat stable; do not require cold chain upholding. Chance for contamination with animal viruses like mad cow disease which pose a threat in vaccines manufactured from cultured mammalian cells is eliminated, because viruses from plant origin will never infect humans. Edible vaccines provoke both mucosal and systemic immunity when they come in contact with the mucosal lining of digestive which offers a first line defense against invading microbes through mucosal surface. Even in the presence of maternal antibodies, edible vaccines show sero-conversion thus having a potential role in protecting newborn children from infectious diseases. Various foods under study are potato, tomato, banana, rice, etc (Lal *et al.,* 2007). Edible vaccines are currently being developed for a number of human and animal diseases, including measles, cholera, foot and mouth disease, peste des petits ruminant (PPR), porcine transmissible gastroenteritis, bovine rotavirus, bovine viral diarrhea, Norwalk and hepatitis B, C and E (Dus Santos and Wigdorovitz, 2005).

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Preparation of edible vaccines

Selection of the desired gene and plant

First step in developing edible vaccines involves introduction of chosen desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. Development of edible vaccine subunit proteins, selection of

immunodominant epitope regions from the pathogen of interest is the key factor which determines the success of potential edible vaccines (Artnzen, 1997). A successful edible vaccine should be ultimately safe, non-pathogenic and capable of inducing both mucosal and systemic immunity upon entry into the digestive tract. Further, efficacious edible vaccines should able to resist the rigid acidic environment of the stomach and reach the target cells in biologically active form. Selected antigen genes and their required expression machinery should be compatible with the chosen plant type (Walmsley and Arntzen, 2000). Antigens in transgenic plants are delivered by bioencapsulation within the tough outer wall of plant cells. Bioencapsulation of recombinant protein antigens with transgenic plant cell vesicles protects the integrity of the antigens from gastric secretions until the plant cell walls degrade in the intestines. Upon degradation in intestine, antigens are released and released antigens taken up by M cells in the intestinal lining that cover Payer's patches and gut-associated lymphoid tissue (GALT). Subsequent processing of antigen includes passage to antigen processing and presenting cells like macrophages, B-lymphocytes and other local lymphocyte populations. Following vaccination and following exposure to the native pathogen, serum antibody (IgG, IgE) and local IgA responses and memory cells are triggered. Raised Norwalk36es will neutralize the attack by the infectious agent. As like subunit vaccines, edible vaccines are consists of antigenic proteins and are devoid of pathogenic genes of micro-organism. As said earlier, edible vaccines cannot establish infection, assures safety (Pizza *et al*., 2001).

Transgenic plants can be easily manipulated to produce immunologically protective proteins against infectious diseases of humans and animals, as well as some autoimmune diseases and cancers. Transgenic maize, rice, potatoes, tomatoes and soybeans have been developed and used in various studies. The results of human and animal trials that have tested several transgenic plant-produced recombinant therapeutic proteins have shown promising responses without major safety concerns. Transgenic plant based vaccines are also being used in veterinary medicine. Livestock animals (bovines) fed transgenic plants, such as *Arabidopsis thaliana*, alfalfa and potato, expressing antigens to protect them from various pathogens, including foot and mouth disease virus (FMDV), bovine rotavirus (BRV) and bovine viral diarrhea virus (BVDV) (Dus Santos and Wigdorovitz, 2005). Ideal plants for edible vaccine production should congregate certain requirements, including production of the antigen of interest in sufficient quantities, preservation of the recombinant antigen immuo-dominant properties and should not interfere antigen processing and presentation.

Vectors with plant-specific super promoters

Edible vaccine development has been hampered by low levels of expression of desired foreign proteins in transgenic plants as expression rates range from 0.01-2% of total soluble protein (TSP), which may make edible vaccine proteins poor immunogenic. Selection of strong plant-specific super promoters to improve expression levels is another key factor that can determine the success of edible vaccines. Expression of several important avian virus proteins over the last ten years includes infectious bursal disease virus (IBDV) VP2 protein, Avian reovirus (ARV) sigma C and Avian influenza virus (AIV) HA antigen (surface glycoprotein). Good expression level was observed in with IBVD as expression levels in vector for VP2 protein of IBDV is 4.8%. The super-promoter consists of a trimer of the octopine synthase transcriptional activating element affix to the mannopine synthase2' (mas2') transcriptional activating element plus minimal promoter sequence. Super-promoter glucuronidase A fusion gene in stably transformed in tobacco maize (*Zea mays*) and *(Nicotiana tabacum*) plants and in transitorily transformed maize Black Mexican Sweet protoplasts. In both maize and tobacco super-promoter activity was much higner in roots than in leaves. In tobacco, superpromoter activity was superior in mature leaves, whereas in maize superpromoter activity differed little among the tested aerial portions of the plant. When compared with other commonly used promoters (cauliflower mosaic virus 35S, mas2' and maize ubiquitin), superpromoter activity was approximately equivalent in both maize Black Mexican Sweet suspension cells and in stably transformed maize plants (Lamphear *et al*., 2004).

Plant transformation

The production of transgenic plants is same as farming normal crops. The changes lie in the transformation process of instilling proteins. At present three methods used to produce transgenic plants *viz*. gene-gun biolistic particle delivery, *Agrobacterium tumefaciens*-facilitated transformation and electroporation with the first two are most common methods being used. Gene-gun transformation inserts the desired DNA into a target plant genome by bombarding embryonic suspension cell cultures. Multi-copy and multi-site transgene insertions resulting in gene silencing is common issue using the gene gun method. *A.tumefaciens*-mediated transformation is the most commonly used in producing transgenic plants. *A. tumefaciens* is a naturally occurring bacterium found in soil that is able to insert segments of foreign DNA into the plant by incoming through wounds such as scratches on surfaces. It has a circular Ti plasmid (tumor inducing), which enables it to infect plant cells, integrate into their genome and produce a crown gall tumor by the way it establishes infection. These attributes can be exploited for insertion of foreign DNA into the plant genome. The Ti plasmid can be disarmed by deleting the genes for auxin and cytokinin synthesis such that tumor formation is eliminated (Lal *et al*., 2007).

Transgenic plant screening

Genes for herbicide and antibiotic resistance are used as markers to select for transformed cells and whole plants, which contain the foreign genes and for expressing the desired product, at which time selected (transformed) cells or plants can be regenerated. The gene of interest integrates randomly into plant genomes, resulting in a various antigen expression level for each independent line. About 50-100 plants can be transformed simultaneously and plants expressing the highest levels of antigen and least number of adverse effects can be selected for further analysis. Production of transgenic plants is species-dependent and may takes 3-9 months. Reducing this time to 6-8 weeks is becoming possible by using real-time

quantitative PCR (qPCR), a genetic approach that can help accelerate the selection process. Some antigens, like viral capsid proteins, require post translational modification and self-assembly into VLPs (virus like particles). These VLPs mimic the virus without carrying DNA or RNA and therefore are not pathogenic (Streatfield, 2005).

Evaluation of the protein in animal model

Each single antigen expressed in plants must be tested for its proper folding and assembly, which can be confirmed by animal experiment, western blots analysis and quantified by enzyme linked immune sorbent assay (ELISA). Specific protocols for orally administering high-value proteins like pharmaceutically interesting substances produced in plants to humans and farm animals requires added scientific study in order to future use of these compounds in large scale. Formulations need to be optimized for production in the forms of tablets and it should retain the biological activity of the prophylactic protein (Streatfield, 2005). But still**,** there are several concerns which need to be answered before edible vaccines can begin to gain a niche in the clinical and pharmaceutical markets of humans and animals *viz*. selection of antigen, choice of plants, delivery system, efficacy in host, dosage, safety, communal perception, quality control and licensing procedures.

Advantage of edible vaccines

Edible vaccines are effectual as a vehicle for immunization because adjuvants which conventionally used to increase the immune response are not necessary. Edible vaccine can elicit mucosal immunity which is lack in traditional vaccines. Edible vaccines are also cost effective in terms of storage, preparation, production and transportation. Vaccines produced by biotechnological method are stable at room temperature, unlike conventional vaccine which may cold chain.

The seeds of transgenic plants can be dried and stored. As edible vaccines are produced from flora, they are easily available. Manufacturing units does not require sterile facilities which make manufacturing cost as low as possible (Charmi Shah *et al*., 2011). Edible vaccines may be readily acceptable as they do not require administration by injection unlike traditional vaccines. Risk of human infection and contamination of environment is low. Plant origin vaccines could be the source for novel vaccines by combining several antigens. These types of multi-component vaccines are called as second generation a vaccine which allows for several antigens to approach M cells at given time. Edible vaccine may improve the safety of individual as compared to conventional vaccine since there is no possibility of proteins to reform into infectious disease causing organism.

Currently developing edible vaccines against viral diseases of human beings and animals

Virus	Plant used for expression	Target species	Route of administration	References
Enterotoxigenic E.coli	Tobacco	Humans	Oral	Joensuu *et al*., 2004
Enterotoxigenic E.coli	Potato	Humans	Oral	Tacket *et al*., 1998
Enterotoxigenic E.coli	Maize	Humans	Oral	Streatfield *et al*., 2003
Vibrio cholera	Potato	Humans	Oral	Arakawa et al., 1997
HIV	Potato	Humans	Oral	Horn *et al*., 2003
Hepatitis-B virus	Potato	Humans	Oral	Thanavala *et al*., 1995
Hepatitis-B virus	Tomato	Humans	Oral	Richter *et al*., 2000
Hepatitis-B virus	Lettuce	Humans	Oral	Prakash *et al*., 1996
Norwalkvirus	Tobacco	Humans	Oral	Mason *et al*., 1996
Norwalkvirus	Potato	Humans	Oral	Tacket *et al*., 2000
Rabies virus	Tomato	Humans	Intact glycoprotein	Prakash *et al*., 1999
Rabies virus	Tobacco	Humans	Oral	Brown.edu.com
Human cytomegalovirus	Tobacco	Humans	Immunological protein	Wright *et al*., 2001
Rabbit hemorrhagic disease virus	Potato	Rabbit	Injection	Castaon *et al*., 1999
Transmissible gastroenteritis coronavirus (TGEV)	Maize	Swine	Oral	Lamphear *et al*., 2004
TGEV	Tobacco	Swine	Injection	Sciencedaily.com
TGEV	Arabidopsis	Swine	Injection	Sciencedaily.com
FMD	Arabidopsis	Bovine	Injection	Wigdorovitz *et al.1999*
FMD	Alfalfa	Bovine	Oral or injection	Dus Santos *et al* 2004
Bovine viral diarrhea virus	Alfalfa	Bovine	Oral	Aguirreburualde *et al*., 2013
Bovine rotavirus	Alfalfa	Bovine	Oral	Wigdorovitz, *et al*., 2004
Peste des petits ruminants virus (PPRV)	Pigeon pea	Small ruminants	Oral	Prasad *et al*., 2004

Other therapeutic applications in current research

Disease condition	Plant used for expression	References
Auto-immune Type I diabetes	Potato and tobacco	Ma *et al*., 1995
Enterotoxic *E. coli* heat labile enterotoxin (LT-B)	Potato	Mason *et al*., 1998
Measles	Tobacco	Huang *et al*., 2001
Cancer	Rice, Tobacco	Ma *et al*., 1998; Torres *et al*., 1999
Dental caries	N. tabacum	Ma *et al*., 1995, 1999
Hepatitis B	Potato	Domansky, 1995; Richter *et al*., 2000
Colon cancer	T. benthamiana	Verch *et al*., 1998
Herpes virus	Soyabean	Zeitlin *et al*., 1998
Norwalk virus	Banana, tomato	Carter *et al*., 2002
Anthrax	Tomato, spinach	Sciencedaily.com
Respiratory syncytial virus (RSV)	Tomato, potato	Sandhu *et al*., 2000

Edible vaccines can be scaled up to large volume production by breeding as compared to conventional animal system (Pizza *et al*., 2001; Streatfield *et al*., 2001).

Major troubles associated with edible vaccines

Stability of dosage form may differs from one plant to another plant and generation to generation, protein content in given volume, weight, age, ripeness of the fruit and quantity of the food to be eaten in lack of availability of standardization methods for plant product. More chance for development of Norwal-tolerance to the vaccine proteins. Larger dose may produce immune tolerance and low doses yields less antibody production result in poor immune response. Stability of vaccine may vary from plant to plant. Some food like maize, potato needs cooking before eating which may denature or fade the quality of the expressed proteins. Variable conditions for edible vaccine are also a major problem. Potatoes containing vaccine need to be stored at 4^{o}C and could be stored for longer time but a tomato does not last long. These food vaccines need to be stored properly to avoid microbial spoilage.

Another major concern with edible vaccine is require of appropriate distinguishing characters to recognize normal fruit and vaccine fruit to avoid misadministration of food vaccine which may lead to tolerance. Glycosylation pattern of plants and humans protein is different which may affect or alter the functions of vaccines (Streatfield *et al*., 2001, Streatfield, 2005).

Brief mechanism of Action

Antigens in transgenic plants are delivered through bio-encapsulation, the tough outer wall of plant cells, which protects them from acidic gastric secretions and finally break up in the intestines. The antigens are released in intestine, taken up by M cells in the intestinal lining which lining the Payer's patches and gut-associated lymphoid tissue (GALT), passed on to antigen processing and presenting cells and local lymphocyte populations. Then it will generate serum IgG, IgE responses, local IgA response and create memory cells, which neutralize the attack by the real infectious agent during exposure with infectious agents (Walmsley and Arntzen, 2000).

Conclusion

Transgenic plants expressing vaccine proteins are cost-effective, easily reproduced, can package vaccine proteins in seed or fruit and cannot be contaminated by mammalian pathogens. Multi-component vaccines could be produced through gene stacking. Plant tissues can be directly fed to patients as edible vaccines, which eliminates the need for purification and refrigeration, takes advantage of bio-encapsulation and could stimulate mucosal immunity. Local agricultural production and processing facilities could be utilized. The expression of antigens in transgenic plants has been increasingly used in the development of experimental vaccines, particularly oriented to the development of edible vaccines. This technology becomes highly suitable to express immunogenic proteins from pathogens. Foot and mouth disease virus, bovine rotavirus and bovine viral diarrhea virus are considered to be the most important causative agents of economic loss of cattle production. They are optimal candidates for alternative means of future immunization. Hence the forthcoming era of this next generation vaccine may lead to a future of safer and more effective immunization.

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