Evaluation of Efficacy of Edible Vaccines in the Covid-19 Period

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Abstract:
Vaccination is one of the most efficient and effective ways for human beings to develop immunity against viruses or other pathogens, while vaccines can be delivered both injective and orally. People injecting conventional vaccines suffer from their high production and delivery cost, with the possible reverse reaction of antigen along with the failure of quality tests, the trigger of side effects such as acute myocarditis, and anaphylactic reactions. With both immunities being triggered (mucosal and systematic), plants have shown effectiveness as being regarded as the carrier of antigens travelling through the digestive tract by the protection of bio-encapsulation and expressing antigens on the intestinal surface. In this review, we would discuss the mechanism of edible vaccines including the absorption by either epithelial cells, goblet cells, or Microfold cells, the predicted production methodology of plant-based edible vaccine expressing spike protein on SARS-CoV-2, selection and the reason for the most suitable plant base for this SARS-CoV-2 pandemic, with the discussion on advantages of edible vaccines comparing to the conventional way, followed by some drawbacks on technological and social factors. With analysis on whether edible vaccines could be regarded as one of the solutions for these relatively developing countries or to overcome the barrier of vaccine production, including delivery, administration, and social perspectives.

Keywords
Edible Vaccine; Mucosal immune system; SAR-CoV-2; Transgenic plant

Introduction

Research Background

The vaccine is one of the most important solutions for human beings to defend against the invasion of any highly infectious and dangerous pathogens, which would cause pandemics around the world, damaging the economic and social development of countries. Currently, the spreading of the SARS-CoV-2 and its mutant variants including, Omicron - B.1.1.529, Delta - B.1.617.2, etc. have caused the death of more

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than 6,190,349 deaths and 500,186,525 confirmed cases of COVID-19. Though the development of different kinds of vaccines (mRNA vaccine, live attenuated vaccine, recombinant protein-based vaccines, etc.) have been completed, and a total of 11,294,502,059 vaccine doses have been administered (8:36 pm CEST, 14 April 2022, WHO), can still be hard for some developing countries in Africa to afford the expensive conventional vaccines, as there are only 8,676,141 of them getting vaccinated, which can be a very small proportion compared to the 60,354,591 of the total population (country meters), only about 14% (8:36 pm CEST, 14 April 2022, WHO). Thus, it's urgent to find a possible solution to the vaccine which can be affordable and helpful to trigger resistance to viruses and increase the vaccination rate of these people in Africa, the Eastern Mediterranean, or the Western Pacific.

Name	Total vaccine doses administered	Total vaccine doses administered per 100 population	Persons vaccinated with at least one dose per 100 population	Persons fully vaccinated	Persons Boosted per 100 population
Global	**11,294,502,059**	**144.9**	**65.28**	**4,567,450,177**	**19.97**
+ By WHO Region					
Europe	1,549,014,707	166.01	66.69	585,916,805	27.95
Americas	1,760,380,979	172.12	77.33	684,896,434	30.52
South-East Asia	2,772,362,870	137.15	71.5	1,226,374,998	4.98
Western Pacific	4,234,830,078	215.56	84.77	1,614,540,176	41.12
Eastern Mediterranean	718,687,599	98.34	50.54	311,148,997	9.04
Africa	259,154,722	23.1	17.37	144,546,421	0.85

Figure 1. The tables from WHO list the total vaccine doses administered in the region, total vaccine doses administered per 100 population, the percentage of people vaccinated with at least one dose, and the number of people getting boosted per 100 population. From the table, we are able to deduce that Africa has the lowest vaccine doses administered, which is 259,154,722, and the average vaccine doses administered per 100 people, which is 23.1, much lower compared to 215.56 in West Pacific or 172.12 in America, so do the percentages of one-dose or fully administration (8:36 pm CEST, 14 April 2022, WHO)

Research Objectives

It is becoming obvious now that a pandemic is an international disaster, affecting not only the first and second world but the third world as well. However, through this SARS-CoV2 pandemic, there are still problems of lacking vaccines in the second and third world, which would potentially affect the first world as well, for larger people getting this coronavirus, the more possible mutations may happen, leading to more mutants. For instance, the spreading of wild SARS-CoV-2 in Britain has led to the development of mutant Alpha (B.1.1.7) (Leung, K., Shum, M. H. et al., 2021); the spreading of SARS-CoV-2 in Africa has led to the development of mutant Beta (B.1351) (Tegally, H., Wilkinson, E. et al., 2021); and the spreading of SARS-CoV-2 in Brazil has led to the development of mutant Gamma (P.1) (Faria, N. R., Mellan, T. A. et al, 2021). So, the pathogens leading to a pandemic ought to be the enemy of humanity, and thus may require researchers or investors to develop solutions for every people in this world, both effective and affordable. This paper would discuss one of the possible solutions to pandemics such as SARS-CoV-2 by edible vaccines, which can be good options for these countries in the second and third world.

Research Purposes and Significance

The mechanism of plant-based edible vaccines will be discussed, including how they may be absorbed by either epithelial cells, goblet cells, or Microfold cells, and how they may trigger the immune system to gain resistance toward the antigens expressed. Then I will put it forward by listing down the advantages, disadvantages, and examples of each plant species as an edible vaccine, along with the possible production methodology of a plant-based edible vaccine. The reasons why edible vaccines can be competitive compared to conventional ones and a proposal on the production of edible vaccines

based on tomatoes against all three coronaviruses that have emerged in the past twenty years will also be discussed, and finally listing the possible cautions countries should make before utilizing edible vaccines.

Literature Review

With the pandemic of SARS-CoV-2 spreading across the world, different methodologies have been developed against the invasion of viruses, such as mRNA vaccines, inactivated vaccines, etc. Edible vaccines, ever since the first development in 1990, various edible vaccines based on plants, algae, or insects have been developed, which showed great potential in defending against pathogens, especially in these developing countries. Edible vaccines, unlike conventional injective ones, function quite differently in human bodies. Thus, the targeting pathogens of edible vaccines can be quite different, with unique advantages and disadvantages. Due to the distrust toward GM products, possible technological barriers, and lacking interest and funding, experiments on edible vaccines were not popular in the last decade. However, edible vaccines can become a powerful solution for these developing countries with the requirement of a vaccine that is easy to produce, scale up, deliver and administer.

Underlying Mechanism of Edible Vaccines

Mucosal Immune System

The mucosal immune system, as the largest immunologic organ in the body, is an important site for the induction of immune response to the antigens delivered or tolerance upon antigens ingested, not only because of the large surface area and thus faster uptake rate of antigens, with the ease for administration as well, but also due to the fact that it is the first barrier of antigen invasion, found ubiquitously in the digestive tract, respiratory tract and urine-reproductive tract (Gunasekaran, B.et.al, 2020, Res. 53; Bhatia, S. & Dahiya, R., 2015). With the production of secretory immunoglobin A (SIgA), mucosa-associated lymphoid tissue (MALT) B and T cells along with the specific lymphocytes on mucosal layers, both innate and adaptive immune systems can be triggered. Thus, a vaccine that can deliver antigens to mucosal layers and trigger the secretion of SIgA is an alternative vaccine compared to conventional ones, with the first edible vaccines being developed.in tobacco expressing a surface protein from Streptococcus in 1990.

Mechanism of Action of Edible Vaccines

After being chewed by the teeth in the mouth and ingested, the edible vaccine passes through the acidic atmosphere and digestive enzymes in the stomach by the protection of bio-encapsulation, with the entire antigen being absorbed in the intestine and getting into the flow of lymphatic tissues in lymph vessels.

Epithelial Cells

As the antigens arrived at the highly folded surface of the small intestine, they will be absorbed into the epithelial cells by facilitated diffusion, then by the exit of basal surfaces and absorption of capillaries, antigens enter into capillaries between mesenteric arteries and the hepatic portal vein, where they further diffuse into the tissue fluid. Macrophages are not seen in tissue fluid normally, however, by the secretion of interferon-gamma by the activated CD4+ cells, monocytes can be stimulated to diffuse out from blood vessels into the tissue fluid and derive into macrophages. When macrophages encounter antigens, phagocytosis will occur, with the antigen being engulfed and broken down into fragmented peptides by lysosomes. However, unlike the destruction of peptides like neutrophils, a class II MHC protein binds to the fragment and expresses it on the surface of the

macrophage to the helper T cells to trigger further immune responses to secrete antibodies (Johansen, F.E. et.al, 1999).

Goblet Cells

Goblet cells (GCs) are a major secretory cellular lineage in the intestinal epithelium that produces mucus, which is composed chiefly of mucins and inorganic salts suspended in water. Antigens can be delivered through the small intestine GCs functioning as the passage from the lumen to the underlying CD103+ Lamina propria dendritic cells (LP-DCS), while two predominant subsets lying on the LP: when antigens encounter and bind to the epitopes on the CD103+ LP-DC, IgA production will be induced, with the imprint gut homing on lymphocytes and induction of regulatory T cells being triggered. On the other hand, CD103-CX3CR1+ DCs would trigger the promotion of tumour necrosis factor-alpha (TNF-alpha), colitis, and T-Helper 17 cells (TH17 T cells), which can secrete interleukin 17 (IL17), causing TH17s to differentiate inhibiting Treg differentiation (McDole, J.R. et.al, 2012).

Microfold Cells (M cells)

Microfold cells are derived from the cycling Lgr5+ stem cells the dome-associated crypts surrounding the follicle-associated epithelia (FAE) and the villous crypts on the base of villi dependent on the secretion of RANKL (TNFSF11) secreted by the stromal MCi cells residing underneath the FAE of GALT. Due to the high endocytic and transcytotic capacity of M cells, antigens can be delivered through follicles to the mucosal lymphoid tissues by being detected by a variety of "immunosurveillance" receptors on M cell's apical surfaces (GM1-ganglioside receptor, Glycoprotein 2 (GP2), Uromodulin (Umod), activated Beta 1 integrin, etc.), Later, when they encounter mononuclear phagocyte which has migrated to T cell area by dendrites projection, carrying on the promotion of maturation of naïve T cells and differentiation of follicular T-helper (Tfh) cells, phagocytosis occurs just like the macrophage mentioned before, with the antigenic epitopes being expressed on the surface of the antigen-presenting cell (APC), reacting with the specialized T cells receptors in the conjunction between class II MHC molecules secreted by activated M cells (Rescigno, M. et al., 2001; Julius, M. C, Robert E. L, and Huan Wang, Mabbott, N.A. et.al, 2013). The maturation of B cells will be enhanced at the same time due to the expression of chemokine hormone receptors like CXCR5 or CXCR10, turning them into plasma cells. Secretion of dimeric and polymeric immunoglobin A (IgA) would then occur after the migration and differentiation of these plasma cells from the lymph nodes to mucosal membranes (Concha, C. et al., 2017). Secretory IgA (sIgA) is then formed by these IgA, followed by transportation to the lumen SIgA are polyreactive chemicals used to recognize and neutralize these foreign pathogens by reacting with the specific antigenic epitopes (Walmsley AM, Arntzen CJ, 2000).

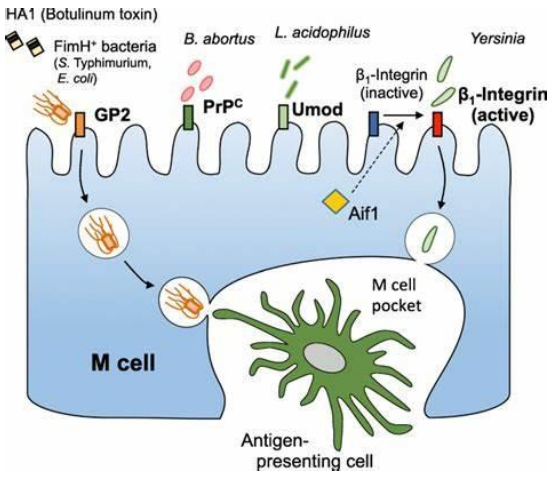

Figure 2. Different antigen uptake receptors on the apical surface of M cell (Nakamura, Y., Kimura, et al., 2018)

Production of Edible Vaccines

For the production of antigens of a specific pathogen, the gene of interest should be selected, along with the promotor and fusion protein. Polymerase chain reaction (PCR) is then carried on to amplify the gene of interest. The gene sequences obtained would then be verified and purified, with restriction enzyme being used, leading to the transfer of the gene sequence into the plasmid, with the selection of an appropriate cloning site along with the antibiotics to select bacteria, followed by the growth of bacteria carried out under optimum condition.

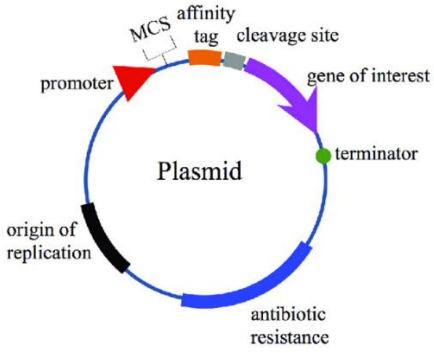

Figure 3. A typical plasmid map listing the sites for the promoter, MCS, an affinity tag, cleavage site, the gene of interest, terminator, antibiotic resistance, and origin of replication (https://www.researchgate.net/publication/326626998/figure/download/fig1/AS:652653633525 80@1532611804879/Schematic-plasmid-map-showing-the-major-features-present-in-common-expression-vectors.png)

Either direct way (biolistic method) or indirect way (agrobacterium method) can be used to deliver the plasmid into the plant cells, the use of Agrobacterium tumefaciens is preferred although it is inefficient, due to the fact that the gene gun being used may harm the plant candidates with agrobacterium can infect a large number of plant species while being cost-effective. Vacuum agroinfiltration can be applied to boost the specificity and efficiency of the process (Chen, Q. et.al., 2013), while syringe agroinfiltration should not be used due to it may damage plant cells as well.

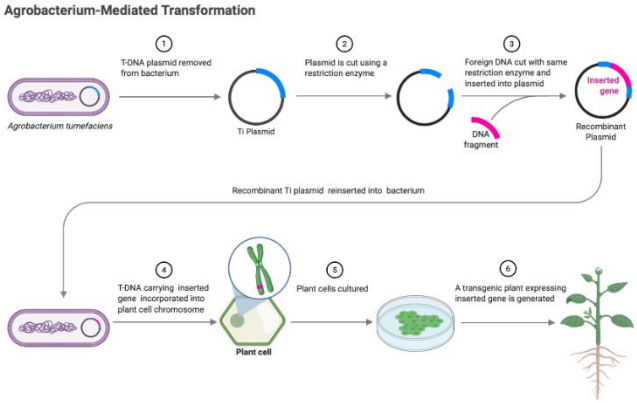

Figure 4. Flow chart of Agrobacterium-mediated Transformation, from the first step of TDNA plasmid removal to the expression of the gene of interest step by step (BioRender (2022). Agrobacterium-Mediated Transformation. Retrieved from https://app.biorender.com/biorender-templates/figures/5c8c7ba9d4f2ef3300632942/t-5f2aac122c88bf00b025a76c-agrobacterium-mediated-transformation)

Comparisons between direct and indirect ways are also shown in the table below:

Table 1. Table comparing the two methods of delivering genes into plant cells

	Agrobacterium method	Micro-projectile bombardment (Biolistic) method
Way of transform	Indirect	Direct
Medium	Plant bacteria (usually A. tumefaciens)	Charged microparticles of metals such as tungsten or gold
Vector-dependence	Vector-dependent	Vector-independent
Advantages	The whole process is simple and cost-effective.	Can be useful in co-transformation and when agrobacterium is not available.
	Agrobacterium can infect a wide range of plant candidates.	The freeze-drying condition can boost the concentration and efficacy of the protein drugs (PDs) in chloroplast.
Disadvantages	Relatively inefficient	Expensive
	Low yield obtained	Harmful to the candidate itself

Selection of Plant-base in Edible Vaccines

Edible vaccines can be based on various hosts, including plants, algae, insects, etc. Plants gained interest in being a base for recombinant expression systems in the late 1980s. The plant

served not only as biofactories but also as delivery vehicles of antigens into the mammalian immune system, which led to their role as bases in edible vaccines as early as the 1990s. For the selection of the plant candidate, the choice can vary from vegetables to fruits and even staple food we eat every single day. Tomato may be the optimum choice for the plant-based edible vaccine for this SARS-CoV-2 pandemic.

Despite the quick ripening rate, the high content of Vitamin A in tomatoes may boost immune response, which indicates that tomatoes can also be a potential base for plant-based edible vaccines for pandemics like covid-19, while tomatoes can also be cultivated broadly and easily (Lou, X. M., et. al., 2007). Transgenic tomato (Solanum Lycopersicum) is suitable also due to the secretion of S-spike protein and its truncated fragment is the best choice for the antigen present in the vaccine (Concha, C., et al., 2017). By means of incorporation of an N-terminal fragment of SARS-CoV protein (S1), a species of edible vaccine based on tomatoes can be developed, which would trigger the boost in SARS-CoV SIgA in mice after oral ingestion (Pogrebnyak, N. et al., 2005). The table below shows some examples of edible plant-based vaccines that have been developed based on various vegetables or fruits.

Table 2. Table listing the plant bases, and examples of expression of antigens against various pathogens causing pandemics

Plant candidate	Name of disease	Pathogen	References
Tobacco (Nicotiana bethamiana)	AIDS, Hepatitis B, Influenza (H5N1, H7N9, H1N1, H3N2, Highly Pathogenic Avian Influenza (HPAI)), Japanese encephalitis	Human immunodeficiency virus (HIV) Hepatitis B virus (HBV), Influenza A virus subtype H5N1, H7N9, H1N1, H3N2, avian influenza (HPAI), Japanese encephalitis virus	Kumagai, M.H., et al, 1993; Huang, Z., et al., 2005; D'Aoust, M.A., et al., 2008; Pillet, S., et al., 2015; Shoji, Y., et al., 2008; Kanagarajan, S., et al., 2012; Appaiahgari, M. B., et al., 2009
Potato	Cholera, Diarrhea, Enteritis, Hepatitis B, Norwalk disease, Rotavirus	Vibrio cholerae, Rotaviruses (RV), Enterotoxigenic E. coli (ETEC), Hepatitis B virus (HBV), Norwalk virus, VP7 (Human rotaviruses)	Arakawa, T., et al., 1997; Zhang, Y., et al., 2003; Mason, H.S., et al., 1998; Shulga, N.Y., et al., 2004; Mason, H.S., et al., 1996; Wu, Y.Z., et al.,2003
Banana	Hepatitis B	Hepatitis B virus (HBV)	Kumar, G.B.S., et al., 2005
Carrot (Daucus carota)	AIDS, Diphtheria–tetanus–pertussis (DTP), Rabies, Tuberculosis	HIV-1 subtype, Corynebacterium diphtheriae, Clostridium tetani, and Bordetella pertussis, Rabies virus, Mycobacterium tuberculosis	Lindh, I., et al., 2009; Brodzik, R., et al., 2009; Rojas-Anaya E., et al. 2009; Uvarova, E.A., 2013
Tomato (Solanum Lycopersicum)	Cholera, Hepatitis B, Norwalk disease, Rabies, pneumonia, septicemia, and bubonic plague, SARS-CoV	Vibrio cholerae, Hepatitis B virus (HBV), Norwalk virus, Rabies virus, Streptococcus pneumoniae, A. hydrophila, Yersinia pestis, corona virus	Jani, D., et al., 2002; Kumar, G. B. S., et al., 2005, Salyaev, K., et al., 2007; Zhang. X., et al., 2006; McGarvey, P.B., et al., 1995, Lou, X.M., et al., 2007, Concha, C., et al., 2017, Pogrebnyak, N. et al., 2005
Soybean	Enteritis, Genital herpes, breast, ovarian, colon and lung cancer	E coli,	Sahooa, A., et al., 2020, Takagi, H., et al., 2005, Jan, N., et al., 2016
Lettuce	Hepatitis B, Influenza (Highly Pathogenic Avian Influenza (HPAI)), Swine edema disease	Hepatitis B virus (HBV), avian influenza (HPAI), Shiga toxin 2e-producing Escherichia coli	Legocki, A.B., et al., 2005; Liu, C.W., et al., 2012; Matsui, T., et al., 2009
Pea	Norwalk disease, Rinderpest	Norwalk virus, rinderpest virus (RPV)	Concha, C., et al., 2017, Bhatia, S., Dahiya, R., 2015, Gao, Y., et al., 2003, Satyavathi, V.V., et al., 2003
Rice	AIDS, Avian chlamydiosis, Hepatitis B, Enteritis	Human immunodeficiency virus (HIV), Mycoplasma pneumonia; and rhinoviruses. Hepatitis B virus (HBV), E coli	Vamvaka, E., et al., 2016; Zhang, X., et al., 2009; Qian, B., et al., 2007, Nochi, T., et al., 2007

Discussion

Drawbacks of Conventional Vaccines

High Production Cost

Production requires higher costs, more time, and better infrastructure. (Sohrab, S.S., et al., 2017). The cost of production can be dramatically high, with the high production of mRNA and deactivated vaccines due to the high requirement of the complicated techniques and infrastructure required for conventional vaccine production, along with the time-consuming purification and downstream process, which can increase the difficulty in scaling up and quick response by these developing countries.

High Delivery and Storage Costs

Delivery and storage of edible vaccines require refrigeration all along the process to avoid denaturation of antigens. While refrigeration is needed to prevent antigens in vaccines to denature, which will boost the cost of delivery, along with the accompany of trained personnel by means of aircraft from the pharmaceutical

companies to these developing countries. Contamination due to the use of sprays or aerosol to store the live vaccines requires further money to deal with as well. (SHAKOOR, S., et al., 2019)

Potential Failure and Side Effects

While accidents such as failure of quality tests, triggers of acute myocarditis, and anaphylactic reactions may happen after the injection of edible vaccines. Failure of quality control tests may lead to the injection of undetected pathogens when inactivated vaccines are being injected. The report by National Health Surveillance Agency in Brazil claimed that the quality inspection of the inactivated vaccine (Covaxin) against SARS-CoV-2 is not carried on properly, thus causing a lot of people in Brazil to catch the covid-19 directly. While various side effects would be caused by the injection of the mRNA vaccines, for example, it is now proven that the second dose of injection of BNT162b2 mRNA Covid-19 Vaccine may trigger Acute myocarditis after the injection of the smallpox vaccine in teenagers (Mansour, J., et al., 2021). Possible brain, spinal cord, peripheral nervous system, and cardiac inflammation may be triggered as well, causing loss of sight, dysmetria, gait instability, paresthesia, sphincter disturbance, and limb weakness in the people taking the injection (Khoei, K., et al., 2022). Though it is predicted that 1 in one million would get anaphylactic reactions (McNeil, M.M., et al., 2016), the reality indicates that the actual possibility may be much higher: for two to three cases within days after the approval of administration (Mueller, B., 2020). Caused by the release of histamine and other mediators from mast cell granules (Dreskin, S., et al., 2020), an anaphylactic reaction can affect the dermatologic, respiratory tract and gastrointestinal, and can be quite fatal due to the asphyxiation caused by the upper airway angioedema or bronchospasm, or hypotension. Local inflammation may be triggered as well at the inoculation point, with fever along with further hypersensitivity being induced. (Vrinda, M.K., Thomas, J., et al., 2019, p. 80-90; Ruggeberg, J., et al., 2007).

Possible hindrance of functions by physical barriers (mucosal surfaces) or absence of T cell effector and mucosal immunity can deactivate conventional vaccines as well, which makes them less suitable for a pandemic (Vrinda, M.K., Thomas, J., et al., 2019).

In conclusion, though conventional vaccines have technology that is more advanced compared to edible ones, the high requirement for techniques and infrastructure for production, delivery, and administration prevent the widespread usage of vaccines in developing countries. While immunological problems can also be triggered due to individual differences, including inflammation and Anaphylactic reactions being triggered more usual than the prediction probability.

Advantages of Plant-based Edible Vaccines

Edible vaccines are highly efficacious alternatives that trigger our mucosal and systematic immune response while having lots of advantages in not only production but also administration and mechanism.

Production

Production of edible vaccines, unlike the conventional ones, turns out to be cheaper and easier to scale up. Production of edible vaccines tends to be affordable as the production of edible vaccines requires no pre-administration treatment, purification, or downstream processing (Vrinda, M.K., et al., 2020), while the low requirement of sterilization of premise and manufacturing areas decreases the cost to produce edible vaccines, along with the abandon of sophisticated equipment and machinery

(Sahooa, A., et al., 2020) Comparing to conventional vaccines, edible vaccines can easily be cultivated on rich soils and are cost-effective relative to fermenters required by conventional ones in a controlled manner (Vrinda, M.K., et al., 2020). Production of edible vaccines can be scaled up easily, as it only takes 40-acre of land to grow edible vaccines that can vaccinate the whole people living in China, and 200-acre for all babies in the world (http://www.moleculafarming.com/plant derived-vaccines.html.). Plant-based edible vaccines can also be environmental-friendly as they can grow by requiring no external carbon source as they are fueled by photosynthesis (Goddijn, O.J.M., Pen, J., 1995), which decreases the implicit cost of production.

Administration

Edible vaccines are beneficial for developing countries to develop due to their easier maintenance and distribution of the vaccine and its seeds, with alternatives able to be applied without the requirement of refrigeration, such as freeze-drying along with larger compliance. The maintenance and distribution of edible vaccines can be much simpler compared to conventional ones, for the processing, purification, sterilization, packaging, and delivery do not claim rigorous structure. (Jan, N., et al., 2016). As edible vaccines are heat-stable and able to exhibit good genetic stability, which can be beneficial for developing countries to produce edible vaccines by themselves, storing them near the site of use at room temperature (Lal, P., et al., 2007). Edible vaccines are outstanding because alternative storage pathways are possible when storing them (Jan, N., et al., 2016), For example, leaves of the edible vaccines can be lyophilized to store, which turns out to have better long-term stability, antigen content, and microbial contamination than the freshly grown one (Criscuolo, E., et al., 2019). Freeze-dried cholera toxin B subunit exendin-4 (CTB-EX4) expressing leaves showed stability for up to 10 months even at room temperature, while lettuces expressing the protective antigen from Bacillus anthracis could be stored up to 15 months at room temperature, with lyophilized lettuce had no detectable microbes, with 6000 cfu/g microbes in fresh leaves (Kwon, K.-C., et al., 2013). Seeds of transgenic plants can be preserved easily as well as have lesser moisture content, have higher heat stability, and can be dried quickly (Sahooa, A., et al., 2020). While improved compliance can also be acquired, edible vaccines can be intake by the body needless, which is an advantage for those who refuse to take injections of vaccines, while eliminating the use of needles can also reduce the chances of infection (Sahooa, A., et al., 2020). Edible vaccines offer exciting possibilities for fighting against diseases such as hepatitis B and diarrhea where the storing and administration of conventional vaccines become the major problem (Vrinda, M.K., et al., 2020, p.79-90; Webster, D.E., et al., 2002).

Applications

Edible vaccines can produce multiple antigens which are powerful against mucosal-infected diseases, while harmless to human beings at the same time. Edible vaccines can be extremely powerful to fight against pathogens invading through the mucosa, such as Mycobacterium tuberculosis, SARS-CoV-2, etc. (Lal, P., et al., 2007) Plant-based vaccines produce proteins that are free from hazardous, the absence of toxins, pathogens, and the potential of the reformation because the plant pathogens are not capable of infecting human beings (Webster, D.E., et al., 2002; Mishra, N., et al., 2008) With bioencapsulation, advanced transient expression technology and activation of both mucosal and systematic immunity boost the effectiveness of edible vaccines also. Inheritance can also be

applied between mothers and the fetus-in-utero by means of the transplacental transfer of maternal antibodies or breast milk between mothers and infants. While it is becoming possible to express multiple genes in a single operon, because expression of foreign genes in plant cells is possible, which can be beneficial to the development of multivalent vaccine development (Criscuolo, E., et al., 2019; Oey, M., et al., 2009), for example, there is a trivalent edible vaccine against cholera, ETEC (Enterotoxigenic E. coli) and rotavirus can successfully elicit an immune response to all three (Yu, J., Langridge, W.H., 2001). Edible vaccines may also be possible solutions to these rare diseases such as dengue, hookworm, rabies, etc., with combinations of other vaccination methods or antigens to enhance the effectiveness of the vaccination, its strong potential to produce monoclonal antibodies to treat cancer and autoimmune diseases in sufficient amount further indicates that edible vaccines can be worth developing (Lal, P., et al., 2007):

Disadvantages of Edible Vaccines

Poor Efficiency

The inefficiency in producing transgenic plants may become a big problem when edible vaccines are being used as weapons against serious pandemics. It may take as long as 3-9 months to develop one single species (Lal, P., et al., 2007), while during the covid-19 pandemic, it took around two months (from Jan.11th to Mar.18th) for mRNA and inactivated vaccines against SARS-CoV-2 to be put into clinical trials. Regulatory problems would prevent the edible vaccines or having lot-to-lot consistency, distribution of dosage, and purity, as accurate dosage difficult to determine. With reduced choices of crops due to the denaturation of antigens under high temperatures of cooking. Mucosal tolerance may be triggered after the injection by turning on suppressor cells, leading to the failure to respond to antigens by the immune system. While the larger demands on the intake of vaccines make the situation even more severe, the misidentification and misadministration of species also induce troubles.

Negative Social Impacts Genetically

Possible bioterrorism may be triggered when the transgenic process is allowed by ill-mentioned purpose or mismanagement triggering leakage or explosion (Zapanta, P.E., et al., 2014), with the creation of super viruses becoming possible and simple by shuffling DNA and used as bio-weapons. Antibiotic resistance may be developed by bacteria by means of the intake of marker genes, with other minor genetic changes in pathogens leading to possible changes in the host spectrum or mechanism causing the disease (Lal, P., et al., 2007). DNA pollution, unlike chemical pollutants, can be taken up by cells of all types, leading to further replication and mutation, mainly caused by the cross-contamination between transgenic plants and non-genetically modified plants and leakage of antigen into water sources through insects or birds (Harlé, J. R., et al., 2010; Hirlekar, R., & Bhairy, S., et al., 2017; Twyman, R. M., et al., 2005).

Lack of Funding and Interest

Small technology companies, international aid organizations, and governments are the main funders of the research of edible vaccines as it targets mostly developing countries, thus a large number of programs remain underfunded. Lacking interest in investigation is another problem due to the limiting number of research and development (R&D) personnel in these pharmaceutical companies, with the fact that cheap conventional ways have already been developed against diseases like diphtheria, tetanus, etc. which would indicate little incentive

to develop edible vaccines for them (Lal, P., et al., 2007). The table below shows the current clinal trials on edible plant vaccines:

Table 3. Edible plant vaccines clinical trials:

Pathogen	Host	Status	Clinical Trial ID	Reference
Enterotoxigenic E. coli	Potato	Early Phase I	restricted cohort study design	Tacket, C. O., et al., 1998
Enterotoxigenic E. coli	Maize	Early Phase I	restricted cohort study design	Tacket, C. O., et al., 2004
Norwalk virus	Potato	Early Phase I	restricted cohort study design	Tacket, C. O., et al., 2000
Rabies virus	Spinach	Early Phase I	restricted cohort study design	Yusibov, V., et al., 2002
HBV	Lettuce	Early Phase I	restricted cohort study design	Kapusta, J., et al., 1999
HBV	Potato	Phase I	NCT01292421	Thanavala, Y., et al., 2005
Vibrio cholerae	Rice	Phase I	UMIN000009688	Nochi, T., et al., 2009; Yuki. Y.. et al., 2013; Kashima. K., et al., 2016; Kurokawa, S., et al., 2013

Social Resistance Against GM Products

Resistance against GM products has been carried out consistently, with full or partial bans in 38 countries due to their bad public image. While the debate on GM food has always been carried on by scientists, in 2012, Gilles-Éric Séralini in Springer carried out the study of the effect of NK-603 Roundup Ready® Maize (NK-603 RR Maize) on rats, and the same experimental setup has been used as an earlier Monsanto safety study to gain maize approval (Hammond B et al., 2004). After the significant chronic kidney deficiencies representing 76% of altered parameters, 3-5x higher necrosis and liver congestions in males, 2-3 folds increase in mortality of females, and higher tumor incidences, he believed the that Monsanto study overexpressed in NK-603 RR Maize thus called out for long-term toxicity studies on the edible GM crops (Séralini, G., et al., 2014). Due to the flawed experimental design, animal types used, statical analysis, and data presentation, his paper was retracted (Zdziarski, I., et al., 2014). However, in 2014 he republished the paper to debate further on the GM crop issues.

Though we have to admit that no matter whether GM crops may harm animals and the environment or not, GM techniques remain imperfect, with the potential haphazard toxicity, allergenicity caused by either potential pleiotropic effect of the insertion of genetic products or disruption of the natural gene of crops when the transgenic process is carried on or both (Bawa, A., et al., 2013). The example of Starlink maize has shown failure at an elementary level, with the unwanted leakage from the animal diet into human diet in 2000 in USA, EU, Japan, etc. due to the approval of usage of Starlink maize in animal feed by US Environment Protection Agency (EPA) in 1998 (Carter, C., Smith, A., 2007).

In conclusion, inefficiency, regulatory problems, lessened choices of crops, and triggers of mucosal tolerance will reduce the efficacy of edible vaccines. While possible bioterrorism and DNA caused, little funding available with lessened interest from researchers and resistance to GM products developed in society with accidents happening before becoming the obstruction of development of plant-based edible vaccines.

Conclusion

Future Perspective

For plant-edible vaccines to function well in the next pandemic like the SARS-CoV-2, crops that can be eaten raw with a high level of protein expression may be chosen. Tomato may be a good choice due to the expression of N-terminal fragment of SARS-CoV-2 protein (S1) in tomatoes against coronavirus in 2005 (N. Pogrebnyak, et al., 2005). Researchers also found that the gene sequence of SARS-CoV-2 is 96% identical to other coronaviruses transmitted in bat populations (Zhou, P., et al., 2020; Wu, F., et al., 2020).

In the past 20 years, there have already been

three pandemics caused by beta coronaviruses, i.e., SARS-CoV-1 in 2003, MERS-Cov in 2012, and SARS-CoV-2 in 2019 (CDC). Thus, developing an edible vaccine effective for pathogens belonging to Betacoronavirus is necessary, which only leads to the alteration of the gene of interest. Researchers would have to select the homologous gene sequence in all viruses to obtain the antigens which can trigger the immune response against most of them. The following figure indicates the genome structures of the SARS-CoV-2, SARS-CoV, and MERS-CoV.

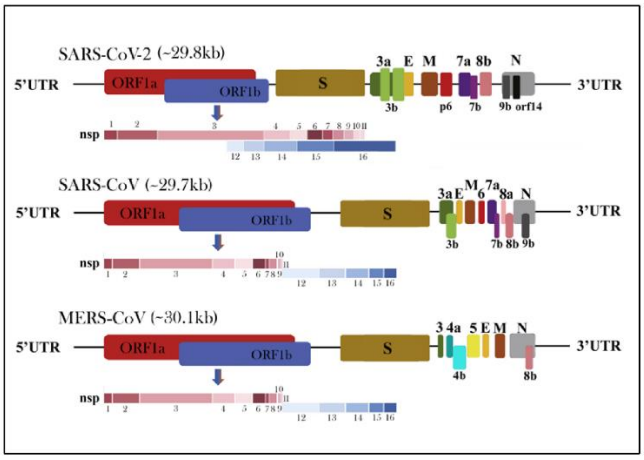

Figure 5. Graphic genome structures of SARS-CoV-2, SARS-CoV, and MERS-CoV (Hozhabri, H., et al., 2020)

Low Production Cost and Easy Scaling Up

As mentioned before, the production cost for this transgenic tomato can be very low and can be scaled up easily in these developing countries so that millions of people can intake the spike protein and develop immunity against the virus in a relatively short period of time. This is quite beneficial not only for those who lived in developing countries but also for those who lived in developed ones, since the more people this SARS-CoV-2 infected, the easier mutations would occur, with the potential invalidation of vaccines that most people have injected. Though cautions need to be taken to avoid the leakage of DNA fragments or the transfer of these fragments by birds or insects, which means certain avoidance needs to be done to keep them away from the plant. Possible solutions include building a specific huge greenhouse that is far away from rivers or soils, with only transgenic tomatoes being implanted in certain conditions (29 degrees in Celsius, 400-500 µmol m-2 s-1 in light intensity, and 14h per day is the optimum growing condition for tomatoes) (Tomato Seed Germination, Time Period, and Procedure | Agri Farming; Wolska, G., et al., 2009).

Cheaper Delivery

Delivery of transgenic tomatoes can be much cheaper compared to the conventional one, which involves long-distance importation with refrigeration needed throughout the process. Tomatoes can be sterilized and freeze-dried at the greenhouse and send to the sites, thus there is no need to worry about pests with large amounts of edible vaccines to be delivered, while examination of the quality of tomatoes and protein contents can be done in advance, which can be more efficient.

Oral Consumption

Edible vaccines can be beneficial in administration because they can be consumed orally, without the use of needles, which appears to be relatively difficult in developing countries to stay sterilized throughout the injection process. Sterilized warm water is needed for these tomatoes to be eaten; people should eat the vaccines without taking any meals so that fewer antigens would be denatured when passing through the stomach.

Standardization in Industry and Education on GM Products

Tomatoes should be examined strictly and thoroughly before they are consumed orally,

while standardization is required throughout the process. Education should be given to people as well for them to understand the danger and potential of this technology, thus fewer people would have negative thoughts about GM products.

Conclusion

In conclusion, this dissertation lists the mechanism of how antigens can be delivered to the intestines through the acidic atmosphere of the stomach by the protection of bio-encapsulation and by being absorbed by either epithelial cells, goblet cells, or M cells, along with the trigger of their specific immune responses. The production procedure of general edible vaccines and the selection of an appropriate plant base based on its advantages, disadvantages, and example of expression before has also been introduced. Discussion on the significance of edible vaccines has also been carried out by comparing the disadvantages of conventional vaccines and illustrating of advantages and disadvantages of edible vaccines, followed by the prediction of possible edible vaccines based on tomatoes, which may help the immune system to develop resistance against the whole beta coronaviruses genre, and evaluation on whether edible vaccines could be regarded as one of the possible solutions to developing countries during pandemics or not, along with the rules the governments may have to obey when applying oral intake of vaccines.

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