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Programme: Bachelor of Science (Third year).

Subject: Botany

Paper Code: BOC 109

Title of the unit: Applications of Genetic Engineering**.**

Name of the module: Edible vaccines and industrial enzymes: Protease & Lipase.

Paper Title: Molecular Biology and Genetic Engineering.

Name of the Presenter: Dr. Annie F. D'Souza e Gomes.

INTRODUCTION:

EDIBLE VACCINE

- A pathogen protein gene is cloned.
- Gene is inserted into DNA of plant (potato, banana, tomato)
- Humans eat the plant.
- The body produces antibodies against pathogen protein.
- Humans are immunised against the pathogen.

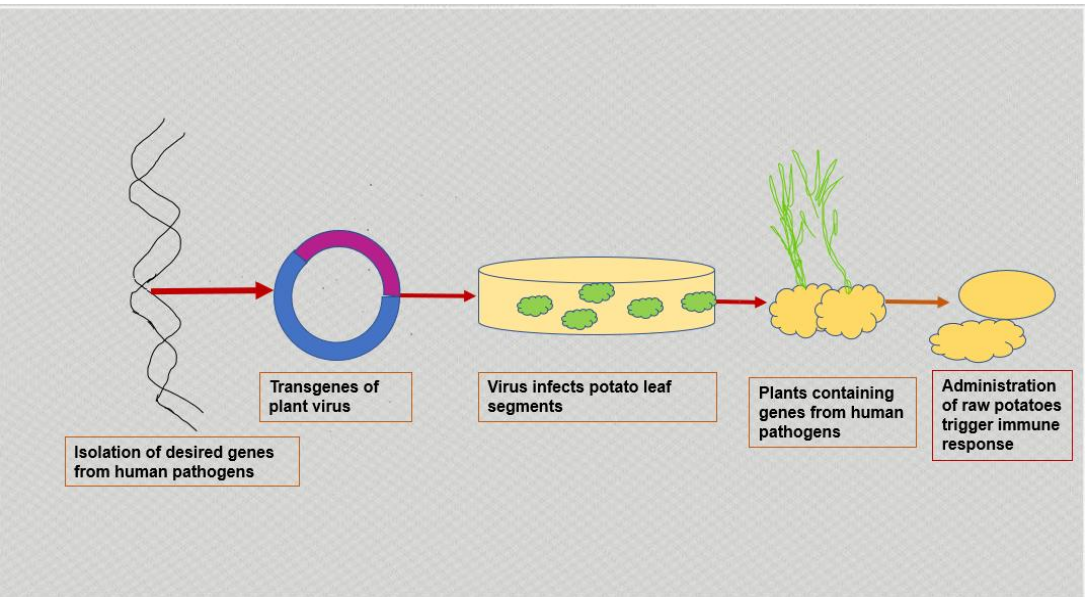
Examples:

a) Diarrhoea (EHEC).
b) Hepatitis B
c) Measles

In the edible vaccine, Transgenic plants are used as vaccine production systems.

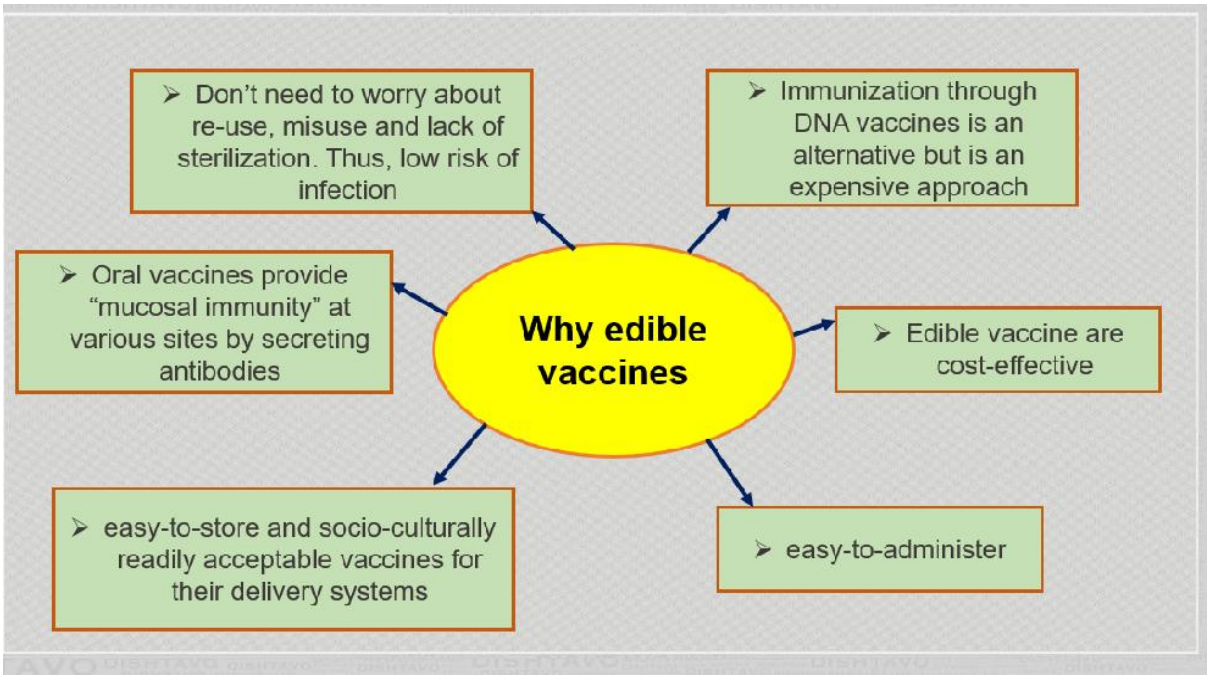
- The genes encoding antigens of bacterial and viral pathogens can be expressed in plants in a form in which they retain native immunogenic properties.
- Initially thought to be useful only for preventing infectious diseases, it has also found application in prevention of autoimmune diseases, birth control , cancer therapy, etc.
- Edible vaccines are currently being developed for a number of human and animal diseases.
- Introduce genes of interest into plants (Transformation) Genes expressed in the plant tissues edible parts (Transgenic plants).

- Genes encode putatively protective vaccine antigens from viral, bacterial, and parasitic pathogens that cause disease in humans and animals Ingestion of the edible part of the transgenic plant.



IDEAL PROPERTIES EDIBLE VACCINES:

- Nontoxic or Non-pathogenic very low levels of side effects
- Not cause problems in individuals with impaired immune system
- Long lasting humoral and cellular immunities
- Vaccination should be Simple Not contaminating the Environment Should be effective and affordable
- Most importantly, they trigger the immunity at the mucosal surfaces such as mouth which is the body's first line of defence.
- Needs no purification.
- Edible vaccine activates both mucosal and systemic immunity • Heat-stable; do not require cold-chain maintenance.
- If the local/native crop of a particular area is engineered to produce the vaccine, then the need for transportation and distribution can be eliminated



MECHANISM OF ACTION

The goal of oral vaccination is to stimulate the mucosal and systemic immunity against pathogen .

Edible vaccine when taken orally undergoes the mastication process and the majority of plant cell degradation occur in the intestine as a result of action of digestive or bacterial enzyme on edible vaccine .

. Peyer's patches (PP) small masses of lymphatic tissue found throughout the ileum region of the small intestine.

Monitor intestinal bacteria populations and preventing the growth of pathogenic bacteria in the intestines. are an enriched source of IgA producing plasma cells and have the potential to populate mucosal tissue and serves as mucosal immune effector site.

The breakdown of edible vaccine near PP, consisting of the 30-40 lymphoid nodules on the outer surface of intestine and contain follicles.

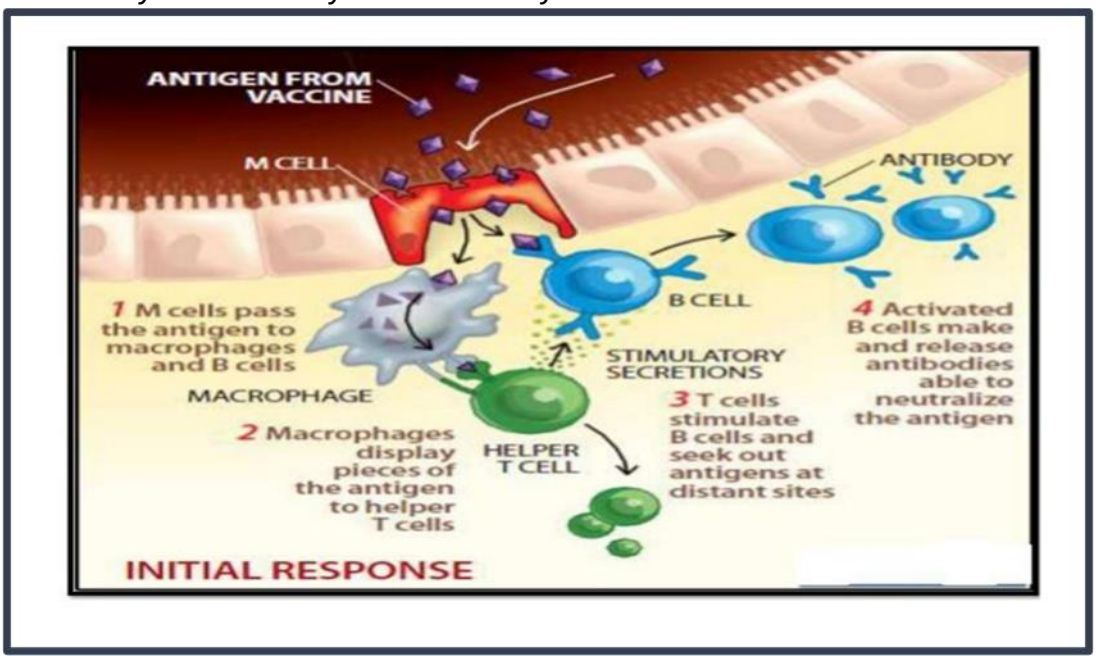
These follicles act as the site from which antigen penetrates the intestinal epithelium ,thereby accumulating antigen within organized lymphoid structure .

The antigen then comes in contact with M-cell.

M cell passes the antigen to macrophages and B cell.

These B cell activates the T cell to provide immune response .

In this way the immunity is activated by the edible vaccine.



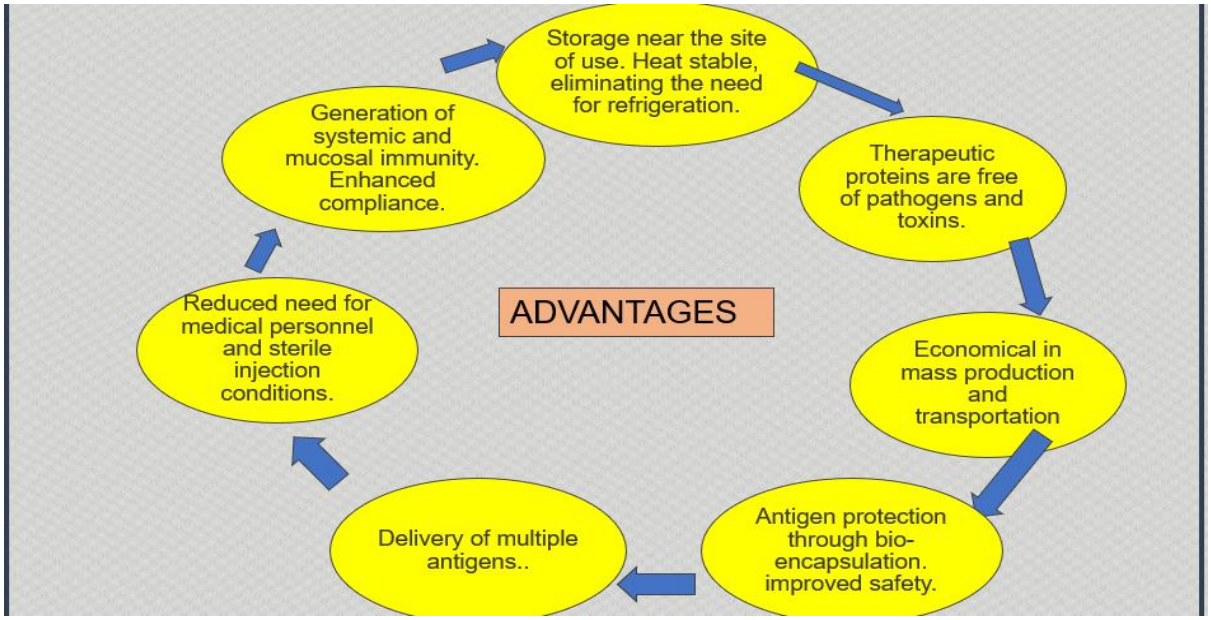
Method of Developing an Edible Vaccine

Gene encoding antigen from pathogenic organisms (virus, bacteria or parasites) that have been characterized and for which antibodies are available, can be produced in the edible parts of the plants in two ways.

- In one case, the entire structural gene is inserted into a plant transformation vector. This will allow transcription and accumulation of coding sequence in plant.
- In second case, epitope within the antigen are identified, DNA fragment encoding these can be used to construct genes by fusion with a coat protein gene from plant virus, e.g., TMV (Tobacco mosaic virus) or CMV (Cytomegalovirus).

The recombinant virus is then used to infect stabilized plants.

The resultant edible plant vaccines are utilized for further immunological studies.



PROTEASE ENZYME:

Proteases are enzymes which catalyze the hydrolysis of peptide bonds present in proteins and polypeptides.

They are widely used in detergent and pharmaceutical, followed by food industries.

They represent 60% of industrial enzymes on the market. they find applications in leather processing as well as bioremediation processes.

Proteases can be classified based on their origin, catalytic activity and nature of the reactive group in the catalytic site. The major sources of protease enzymes are animals, plant and microorganisms (both bacterial and fungal).

Proteases are divided into **two groups**: exopeptidases and endopeptidases, based on the site of action on polypeptide chains.

- The exopeptidases act on the ends of polypeptide chains and endopeptidases act randomly in the inner regions of polypeptide chains.
- The endopeptidases are further classified into six groups, based on the catalytic residue present in the active site:

serine, aspartic, cysteine, metallo, glutamic acid and threonine protease .

- Plant proteases such as bromelain, ficin and papain are widely used in food industry for various applications such as brewing, tenderization of meat, coagulation of milk and as a digestive aid .
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Dairy

- Proteases are also used to improve the flavour, nutritional value, solubility and digestibility of food proteins as well as to modify their functional properties including coagulation and emulsification . Proteases are widely used in baking industry for the production of bread, baked foods, crackers and waffles.
- These enzymes are used to reduce the mixing time, decrease dough consistency and uniformity, regulate the gluten strength in bread and to improve the texture and flavour.
- The acid protease from *Aspergillus usamii* has been successfully employed for the improvement of functional properties of wheat gluten.
- The addition of protease could release sufficient peptides and amino acid levels in the wort to get a proper fermentation.
- Acidic fungal proteases are used in improving fermentation of beer as they are efficient even at low pH by balancing the amino acid profile of beer .

LIPASES

- Lipases are enzymes which catalyze the hydrolysis of long-chain triglycerides.
- They are naturally present in the stomach and pancreas of humans and other animal species in order to digest fats and lipids. Microbial lipases are produced by bacteria, fungi and yeast
- This enzyme finds application in various industries including food, biofuel, detergents and animal feed.
- It is also used in leather, textile and paper processing applications. In the food and beverage industry, lipases find major application in dairy, baking, fruit juice, beer and wine industries. Commercial lipases are mainly used for flavour development in dairy products and processing of other foods containing fat.
- They can improve the characteristic flavour of cheese by acting on the milk fats to produce free fatty acids after hydrolysis.
- Different types of cheese can be made by using lipases from various sources, *e.g*. Romano cheese using kid/lamb pre-gastric lipase, Camembert cheese using lipase from *Penicillium camemberti* and cheddar cheese using *Aspergillus niger* or *A. oryzae*.
- Lipase catalysis could improve the texture and softness of cheese.
- Lipases are also used as flavour development agents in butter and margarine, also to prolong the shelf life of various baking products.
- They are used to improve the quality of cocoa butter, which has a melting point of 37 °C due to the presence of palmitic and stearic acids and can easily melt at 37 °C.

- This lipase finds application in the production of ice cream, single-cell protein, carbohydrate esters and amino acid derivatives Lipases also find application as a biosensor in food industry.
- Immobilized lipase was successfully used for the determination of organophosphorous pesticides with a surface acoustic wave impedance sensor by lipase hydrolysis.
- It may also be used in the determination of triglycerides and blood cholesterol.
- In alcoholic beverages such as wine, the aroma can be modified using lipase.
- In addition to this, lipase could also be used in the processing of different waste streams that are released from food industries.