

Biotechnology and Its Applications

Biotechnological Applications in Agriculture and Medicine:

1. Biotechnology essentially deals with industrial scale production of biopharmaceuticals and biologicals. The applications of biotechnology include therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment and energy production.

2. Biotechnology have the following three critical research areas:

- (i) To provide the best catalyst in the form of improved organism, usually a microbe or pure enzyme.
- (ii) To create optimal conditions through engineering for a catalyst to act.
- (iii) Downstream processing technologies to purify the protein/organic compound.

3. Biotechnological Applications in Agriculture

- (i) Biotechnology applications in agriculture involve following three options:
 - (a) Agrochemical based agriculture.
 - (b) Organic agriculture.
 - (c) Genetically engineered crop-based agriculture.
- (ii) Green revolution increased food production due to the use of:
 - (a) Improved crop varieties.
 - (b) Agrochemicals (fertilisers and pesticides).
 - (c) Better management practices.
- (iii) Genetically Modified Organisms (GMOs) are plants, animals, bacteria and fungi whose genes have been altered by manipulation.
- (iv) Genetic modification in plants have lead to following:
 - (a) Crops became more tolerant to abiotic stresses, such as cold, drought, salt, heat,

etc.

(b) Dependence on chemical pesticides reduced, i.e. pest resistant crops.

(c) Post harvest losses reduced.

(d) Efficiency of mineral usage increased in plants (preventing loss of soil fertility).

(e) Nutritional value of food is enhanced, e.g. vitamin-A enriched rice.

(f) Tailor made plants are created by using GM plants to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

(v) Some of the applications of biotechnology in agriculture are the production of pest resistant plants, which decrease the amount of pesticide used.

Bt toxin is produced by a bacterium and expressed in plants to provide resistance to insects, in effect created a biopesticide, e.g. Bt cotton, Bt corn, golden rice, tomato, potato and soybean, etc.

(a) Bt cotton is created by using some strains of a bacterium, *Bacillus thuringiensis* (Bt is short form,).

(b) This bacterium produces proteins that kill certain insects such as lepidopterans (tobacco, budworm and armyworm), coleopterans (beetles) and dipterans (flies and mosquitoes).

(c) *B. thuringiensis* forms protein crystals during a particular phase of their growth. These crystals contain a toxic insecticidal protein.

(d) Bt toxin protein exist as inactive protoxins, but once an insect ingests the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut, which solubilise the crystals.

(e) The activated toxin binds to the surface of midgut epithelial cells and create pores that cause cell swelling and lysis leading to death of an insect.

(f) Specific Bt toxin genes were isolated from *Bacillus thuringiensis* and incorporated

into several crop plants as cotton.

(g) Most Bt toxins are insect-group specific. The toxin is coded by a gene named cry, e.g. the proteins encoded by the genes cry IAc and cry IIAb control the cotton bollworms and cry IAb controls corn borer.

(vi) Pest resistant plants are developed by using biotechnological processes.

(a) A nematode *Meloidogyne incognita* infects the roots of tobacco plants, which reduces the production of tobacco.

(b) RNA interference (RNAi) process is used for cellular defence. It involves silencing of a specific mRNA due to a complementary dsRNA. It occurs in all eukaryotic organisms as a method of cellular defense.

(c) dsRNA binds and prevents translation of the mRNA (silencing).

(d) The source of this complementary RNA could be from an infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.

(e) *Agrobacterium* vectors are used to introduce nematode-specific genes into the host plant. It produces both sense and anti-sense RNA in the host cells.

(f) These two RNAs are complementary to each other and forms a double stranded RNA (dsRNA) that initiate RNAi and hence, silence the specific mRNA of the nematode.

(g) The parasite cannot survive in transgenic host, expressing specific interfering RNA. The transgenic plant thus, gets itself protected from the parasite.

4. Biotechnological applications in medicine have made immense impact in the area of healthcare by enabling the mass production of safe and more effective therapeutic drugs.

(a) The recombinant therapeutics do not induce unwanted immunological responses

as in case of similar products isolated from non-human sources.

(b) Currently, about 30 recombinant therapeutics have been approved for human use over the world. In India, 12 of these are presently being marketed.

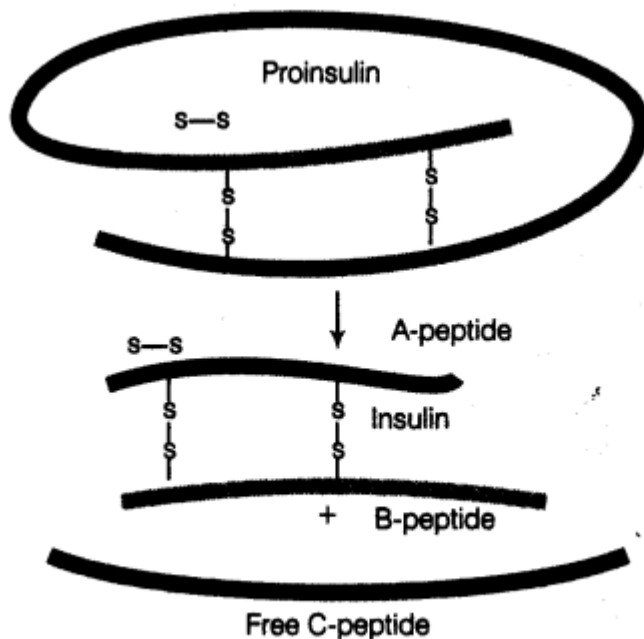
I. Genetically engineered insulin leads to sufficient availability of insulin for the management of adult-onset diabetes.

(a) Insulin used for diabetes was earlier extracted from the pancreas of slaughtered cattle and pigs. This caused allergy or other reactions in some patients.

(b) Insulin consists of two short polypeptide chains, i.e. chain-A and B, linked together by disulphide bridges.

Maturation of proinsulin into insulin (simplified)

(c) In mammals, insulin is synthesised as a prohormone (needs to be processed before it becomes a fully mature and functional hormone) which contains an extra stretch called the C-peptide.



Maturation of proinsulin into insulin (simplified)

(d) C-peptide is not present in the mature insulin and is removed during maturation into insulin. Thus, the main challenge for the production of insulin using

rDNA techniques was getting insulin assembled into a mature form.

(e) Eli Lilly an American company in 1983, prepared two DNA sequences corresponding to A and B-chains of human insulin and introduced them in plasmids of E. coli to produce insulin chains. Chains-A and B were produced separately, extracted and combined by creating disulphide bonds to form human insulin.

II. Production of vaccines through genetic engineering such vaccines are called recombinant vaccines also called '**subunit vaccines**' or '**second generation vaccines**', e.g hepatitis-B. These are of two types:

(a) Protein vaccines use of specific protein produced by rDNA in vaccine.

(b) DNA vaccines use of genetically engineered DNA to be injected as vaccine to produce an immunological response.

Hepatitis vaccine contains the viral envelope protein, hepatitis-B surface antigen (HBs Ag). This gene is isolated from yeast vectors.

Some protein coding genes isolated from pathogens are also incorporated and expressed in plants produce antigens and are also called edible vaccines.

III. Gene therapy is a collection of methods that allows correction of gene defects, diagnosed in a child or embryo.

(a) Genes are inserted into a person's cells and tissues to treat a disease.

(b) Correction of a genetic defect involves delivery of a normal gene into the individual or embryo to take over the function and compensate for the non-functional gene.

(c) First gene therapy was given to a four year old girl with Adenosine Deaminase (ADA) deficiency by M Blease and WF Andresco in 1990s.

- ADA is caused due to the deletion of the gene for adenosine deaminase.

- In some children, ADA deficiency can be cured by bone marrow transplantation and

enzyme replacement therapy, but they are not completely curable.

(d) Steps involved are as follows:

- In first step of gene therapy, lymphocytes from the blood of the patient are grown in a culture outside the body.
- A functional ADA cDNA (using a retroviral vector) is then introduced into these lymphocytes, which are subsequently returned to the patient.
- As these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes.
- If the gene isolated from bone marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.
- Some other diseases that can be treated by gene therapy are haemophilia, cystic fibrosis, Parkinson's disease, etc.

IV. Molecular diagnosis helps to solve the problem of early diagnosis and treatment of diseases.

(a) Using conventional methods of diagnosis (serum and urine analysis), early detection of diseases is not possible.

(b) To overcome this problem, some molecular diagnosis techniques were developed that provide early detection of diseases. These are as follows:

- Polymerase Chain Reaction (PCR) helps in early detection of diseases or pathogens by the amplification of their nucleic acid.

Low concentration of pathogens (bacteria, viruses, etc) in the blood does not allow its detection.

PCR can amplify nucleic acids of such pathogens even when their concentration is very low.

PCR technique can be used for detecting HIV in suspected AIDS patients, genetic

mutation in suspected cancer patients and in identifying genetic disorders.

• **Recombinant DNA technology is a modern molecular diagnostic technique. It is done in the following steps:**

A single stranded DNA or RNA tagged with a radioactive molecule called probe, is allowed to hybridise to its complementary DNA in a clone of cells.

The cells are then detected by autoradiography.

The clone having mutated gene will not appear on the photographic film, because the probe will not have complementarity with the mutated gene.

• Enzyme Linked Immuno Sorbent Assay (ELISA) is based on the principle of antigen-antibody interaction. Infection by pathogen can be detected by the presence of antigens (proteins, glycoproteins, etc) or by detecting the antibodies synthesised against the pathogen.