## **CHAPTER-12 BIOTECHNOLOGY AND ITS APPLICATIONS**

Biotechnology deals with industrial scale production of biopharmaceuticals and biological using genetically modified microbes, fungi, plants and animals. It application includes therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment and energy production. The main three critical research areas of biotechnology includes -

I. Providing the best catalyst in the form of improved organism usually a microbes or pure enzyme.

II. Creating optimal conditions through engineering for a catalyst to act.

III. Downstream processing technologies to purify the protein or organic compounds.

Biotechnological Applications in Agriculture- food production can be increased by

a) Agro-chemical based agriculture

b) Organic agriculture

c) Genetically engineered crop-based agriculture.

- Green revolution successfully increased the food production many folds by using better management practices and use of agrochemicals, fertilizers and pesticides. Further increase in production is not possible by using these methods. To overcome this genetically modified crop is used.
- Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO). GM plants have many applications-
- Made crops more tolerant to abiotic stresses
- Reduced reliance on chemical pesticides
- Helped to reduce post harvest losses

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- Increased efficiency of mineral usage by plants
- Enhanced nutritional value of food, eg., Vitamin 'A' enriched rice.

## Application of Biotechnology in production of pest resistant plants-

Pest resistant plants decrease the amount of pesticides used. Bt toxin is produced by a bacterium called *Bacillus thuringiensis*.Bt toxin gene has been cloned from the bacteria and been expressed in plants to provide resistance to insects without the need for insecticides; in effect created a bio-pesticide. Examples are Bt cotton, Bt corn, rice, tomato, potato and soyabean etc

Bt cotton- Bacterium Bacillus thuringiensis produce proteins that kill certain insects like lepidopterens, colepterans (beetels) and dipterans (flies, mosquitoes).

- *B. thuringiensis* produce crystals that contain a toxic **insecticidal protein.** This toxic protein present in bacterium as inactive protoxins but as soon as insect ingest the inactive form due to alkaline pH of gut, it converted into an active form of toxin and bind to surface of midgut epithelial cells and create pores that cause cell swelling and lysis and eventually death of insect.
- The gene from B. thuringiensishas been incorporated into several crop plants like cotton, maize, rice etc. The toxin is coded by a gene named **cry**. The protein coded by the genes crylAb and cryIIAb control the cotton bollworms, cryIAb controls corn borer.

#### Pest Resistant Plants

• Nematodes like *Meloidegyne incognitia* infects the roots of tobacco plants and causes reduction in yield. The infestation of these nematodes can be prevented by the process of RNA interference (RNAi). RNAi is present in all eukaryotic organisms as cellular defence by silencing of specific mRNA due to complementary dsRNA molecules that bind to and prevents translation of the mRNA.

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- The source of complementary dsRNA may be from an infection by viruses having RNA genomes or mobile genetic elements that replicate through RNA intermediate.
- Nematode specific genes were introduced into host plant using Agrobacterium vectors. The parasite could not survive in a transgenic host expressing specific interfering RNA.

## **Biotechnological Applications in Medicine**

The rDNA technological processes have made immense impact in the area of healthcare by enabling mass production of safe and more effective therapeutic drugs. At present, about 30 recombinant therapeutics have been approved for human use the world over. In India, 12 of these are presently being marketed.

#### **Genetically Engineered Insulin**

Adult –onset diabetes can be controlled by taking insulin at regular intervals. The main source of this insulin was isolation of insulin from animals. Now a day's insulin can be obtained from bacterium using techniques of biotechnology.

- Insulin was earlier extracted from pancreas of slaughtered cattle and pigs but insulin from these sources develops allergy or other types of reactions to the foreign protein.
- Insulin consists of two short polypeptide chains- chain A and chain B, that are linked together by disulphide bridges.

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- In humans, insulin is synthesised as a prohormone, which contains an extra stretch called C peptide, which is absent in mature insulin. The main challenge for production of insulin using rDNA technique was getting insulin assembled into a mature form.
- An American company, Eli Lilly in 1983 prepared two DNA sequence corresponding to A and B chain of human insulin and introduced them in plasmids of E.coli to produce insulin chain. Chain A and Chain B were produced separately, extracted and combined by creating disulphide bonds to form human insulin.



#### **Gene Therapy**

It is a collection of methods that allows correction of a gene defect that has been diagnosed in a child or embryo. This method is applied in a person with a hereditary disease. In this method, genes are inserted into a person's cells and tissues to treat a disease.



- The correction of gene defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for non-functional gene.
- The first clinical gene therapy was done in 1990 to a 4 year old girl with adenosine deaminase (ADA) deficiency. This disorder is caused due to the deletion of the gene for adenosine deaminase that is essential for immune system to function. This defect can be treated by enzyme replacement therapy in which functional ADA is given to the patient by injection or bone marrow transplant.
- In gene therapy method lymphocytes from the blood of the patient are grown in culture medium outside the body. A functional ADA cDNA is then introduced into these lymphocystes and returned to the patient. In this method periodic infusion of such genetically engineered lymphocytes is needed. If gene isolated from bone marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.



#### **Molecular Diagnosis**

Conventional method of diagnosis such as serum or urine analysis is not able to early detection of disease causing pathogens or virus. Following methods can be used to diagnosed earlier-

- I. Recombinant DNA technology
- II. Polymerase Chain Reaction (PCR)
- III. Enzyme Linked Immuno-sorbent Assay (ELISA).
  - Symptoms of disease appear only when the concentration of pathogen get increased significantly. Low concentration of bacteria and virus can be detected by

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amplification of nucleic acid by PCR. It detects the mutation in the gene in cancer patient. PCR is routinely used to detect the HIV in suspected AIDS patients. Genetic disorder can be also detected by using PCR technique.

- A single stranded DNA or RNA having radioactive molecule is allowed to hybridise to its complementary DNA in a clone of cells followed by detection using autoradiography. The clone having the mutated gene will not appear on the photographic film.
- ELISA is based on the principle of antigen-antibody interaction. Infection by pathogen can be detected by the presence of antigens like proteins, glycoproteins etc. or by detecting the antibodies synthesised against the pathogen.

#### **Transgenic Animals**

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Animals that have had their DNA manipulated to possess and express a foreign gene are known as transgenic animals. Transgenic mice, rats, rabbits, pigs, sheep, cows and fish have been produced. Common reasons for development of transgenic animals-

a) **Normal physiology and development**- they are designed to allow the study of gene regulation, their effect on normal function of body. By introducing genes from other species that alter the formation of this factor and studying eh biological affects that results.

b) **Study of disease**- a number of transgenic animals are designed to increase our understanding of how genes contribute to the development of disease. Transgenic model has been developed for disease like cancer, cystic fibrosis, Alzheimer's disease etc.

c) **Biological products-** .Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (gene) which codes for a particular product such as human protein (alpha – 1-antitrypsin) used to treat emphysema. The first transgenic cow, Rosie, produced human protein-enriched milk (alpha-lactalbumin - 2.4 gm / litre).

d) **Vaccine safety**- transgenic mice are developed for used in testing the safety of vaccine before they are used on human. Polio vaccine was tested on transgenic mice and then on monkey.

e) **Chemical safety testing**- transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. It gives us the results in less

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time.

#### • Ethical Issues:

The Indian Government has set up organizations such as GEAC (Genetic Engineering **Approval Committee**), which will make decisions regarding the validity of GM research and the safety of introducing GM-organisms for public services.

#### **Biopatent:**

A patent is the right granted by a government to an inventor to prevent others from making commercial use of his invention. Now, patents are granted for biological entities and for products derived from biological resources.

#### **Biopiracy:**

It is the term used to refer to the use of bio-resources by multinational companies and other organizations without proper authorization from the countries and people concerned without compensatory payment.

In 1997, an American company got patent rights on Basmati rice through the US Patent and Trademark Office. This allowed the company to sell a 'new variety of Basmati, in the US and abroad. This 'new' variety of Basmati had actually been derived from Indian farmer's varieties. Indian Basmati was crossed with semi-dwarf varieties and claimed as an invention or a novelty.

Several attempts have also been made to patent uses, products and processes based on Indian traditional herbal medicines, e.g., turmeric and neem.