Biotechnology and its Applications

1 - INTRODUCTION

Biotechnology: Essentially deals with industrial scale production of biopharmaceuticals and biologicals using GM microbes, fungi, plants and animals.

Applications of biotechnology include:

- Therapeutics
- Processed food
- Diagnostics - Bioremediation
- Genetically modified crops for agriculture
- Waste treatment
- Energy production

Three critical research areas of biotechnology:

- Providing best catalyst in the form of improved microbes or pure enzymes
- Creating optimal conditions through genetic engineering
- Downstream processing technologies for purification

2 - BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE

Food production could possibly be increased by three ways:

- Agrochemical based agriculture
- Organic agriculture
- Genetically engineered crop-based agriculture

Green revolution resulted in tripling of food production:

Reasons for success of green revolution:

- Improved crop varieties
- Agrochemicals (fertilisers + pesticides)
- Better management practices

Problem Area and Hinderances:

- Enhancement in food production by green revolution was still not enough to feed growing population
- **Agrochemicals** are often too **expensive** for farmers of **developing world**
- **Increase in yield** with existing varieties is **not possible** using **conventional breeding**

Genetically Modified Organisms

Organisms including plants, bacteria, fungi and animals whose **genes have been altered by manipulation** are called genetically modified organisms **(GMO)**.

Applications of genetic modification:

- Made crops more tolerant to abiotic stresses (cold, drought, salt, heat).
- Reduced reliance on chemical pesticides (pest-resistant crops).
- Helped to reduce post harvest losses.
- Increased efficiency of mineral usage by plants (prevents early exhaustion of fertility of soil).
- Enhanced nutritional value of food, e.g., golden rice, i.e., Vitamin 'A' enriched rice.

Tailor Made Plants:

Plants have been developed to supply alternative resources to industries in the form of starches, fuels and pharmaceuticals.

3 - INSECT RESISTANT PLANTS

- Provides resistance to insects without the need for insecticides (bio-pesticide)
- Examples of biopesticides are Bt cotton, Bt corn, rice, tomato, potato and soyabean etc.

Bt cotton

Bacillus thuringiensis (A bacterium)

Source of the **cry gene**

Codes for **Bt toxin**

- Produced during a particular phase of their growth
- Inactive protoxins (Protein crystals)
- Endotoxin

Sequence of events

Cry gene from *Bacillus thuringiensis*
↓
Bt cotton (cells express inactive protoxins)
↓
Ingested by cotton bollworms
↓
Alkaline pH in the midgut of insects solubilities protein crystals
↓
Active toxin
↓
Create pores in midgut epithelial cells
↓
Swelling of midgut epithelial cells and thereby lysis
↓
Death of insect

Choice of gene depends on:

(i) Target pest

(ii) Crop

- Cry I AC and cry II Ab
- Cotton bollworm
- Cry I Ab
- Corn borer

Bt toxins are insect group specific :

- **Lepiddoptera:** Tobacco budworm, armyworm, cotton bollworm
- **Coleoptera:** Beetles
- **Diptera:** Flies and mosquitoes

4 - PEST RESISTANT PLANTS

- Method of cellular defense seen in all eukaryotes against pest infestation.
- Technique responsible : RNA interference (RNAi)
- Based on post transcriptional silencing of mRNA
- Translation of mRNA coded from pest specific genes is silenced/prevented due to formation of complementary dsRNA

Source

I.Viruses with RNA genome

II. Mobile genetic elements Transposons replicating via an RNA intermediate

The case of nematode resistant transgenic tobacco:

- Pest causing **roots knot** disease in tobacco plant : ***Meloidogyne incognita*** (Nematode/helminth)
- **Nematode specific gene** is introduced in host plant (tobacco), by using Ti plasmid (vector) of Agrobacterium tumefaciens, in such a manner that it produces both sense and **antisense RNA** in the host cells.
- Sense RNA and antisense RNA being complementary form **dsRNA** that initiates **RNAi**.
- Parasite could not survive in a **transgenic host** expressing **specific interfering RNA**.
- **Host plant – generated dsRNA triggers protection against nematode infestation.**

5 - BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE

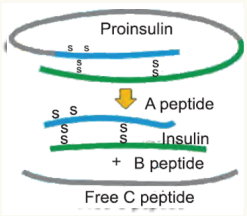
Advantages of Recombinant Therapeutics:

- 30 recombinant therapeutics have been approved for human use the world over. In India, 12 of these are presently being marketed.
- Mass production of safe and effective drugs.
- Do not induce unwanted immunological responses.

Genetically Engineered Human Insulin

- Problem : Insulin extracted from slaughtered cattle and pigs could cause allergy.
- **Solution:** Production of humulin

Proinsulin
A peptide
Insulin
B peptide
Free C peptide

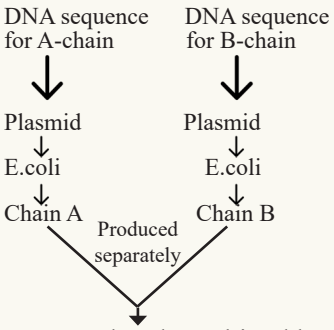


Maturation of proinsulin to insulin

- Recombiant insulin manufactured by Eli Lilly, an American company, in 1983
- The main challenge for production of insulin using rDNA techniques was getting insulin assembled into a mature form

Sequence of events:

Artificially synthesised



Extracted and combined by creating disulphide bonds

↓

Human insulin

Insulin is a peptide hormone and can be degraded by proteases in our gut

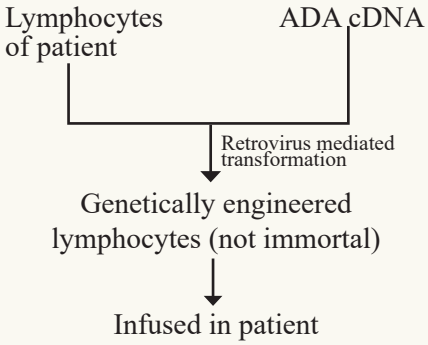
6 - GENE THERAPY

- Insertion of genes into an individual's cells to treat diseases by
  - (i) Replacing a defective mutant allele with a functional one
  - (ii) Gene targeting which involves gene amplification.
- **First clinical gene therapy** was conducted in **1990** in a **4 year old** girl to treat adenosine **deaminase (ADA) deficiency**. ADA enzyme is crucial for immune system to function

Treatment for ADA Deficiency

1. Enzyme replacement therapy
   - Functional ADA is given by injection
2. Bone marrow transplantation in children
3. Gene therapy - Could be a permanent cure if bone marrow transplantion is done at early embryonic stages.

Steps in gene therapy



7 - MOLECULAR DIAGNOSIS METHODS

PCR (Polymerase Chain Reaction):

- **Basis:** Nucleic acid amplification
- **Advantage:** Detection of very low concentration of a bacteria or virus
- **Uses:** Detection of HIV infection, mutations in genes in cancer patients, genetic disorders

ELISA (Enzyme Linked Immuno-Sorbent Assay):

- **Basis:** Antigen - antibody interaction
- **Uses:** Detect the presence of antigens or antibodies synthesized against pathogens

Autoradiography

- **Probe:** Radioactive ssDNA or ssRNA that hybridises with complementary DNA
- **Probe will not hybridise with mutated gene**, hence mutated gene will not appear on the photographic film due to lack of complementarity.

Parameters	Conventional	Modern
• Early detection	Not possible	Possible
• Examples	Serum and urine analysis	RDT, PCR, ELISA

8 - TRANSGENIC ANIMALS

- Possess manipulated DNA and express foreign gene
- Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced
- 95% of transgenic animals are mice.

Uses of Transgenic Animals

- To study how genes are regulated and how they affect the normal functions of body, e.g. study of insulin – like growth factors
- Transgenic models exist for study of diseases like cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's

Biological products

- **1 antitrypsin -** To treat **emphysema**.
- Similar attempts are made for treatment of PKU (Phenylketonuria) and cystic fibrosis.
- First transgenic cow: **Rosie** developed in **1997** producing human protein enriched milk (2.4 grams per litre)
- The milk contained **alpha-lactalbumin**: More balanced product for human babies than natural cow milk

Vaccine Safety

- **Transgenic mice** are being used to test the safety of **polio vaccine** to replace the use of monkeys.
- **Chemical safety testing**
- Transgenic animals are made **more sensitive to toxic** substances to obtain results in less time.

9 - ETHICAL ISSUES

Genetic modification of organisms (GMO) can have unpredictable results when such organisms are introduced into the ecosystem.

- Genetic manipulation of living organisms by humans has to regulated for moral and biological significance.
- GEAC (Genetic Engineering Approval Committee) : Makes decisions regarding the validity of GM research and the safety of introducing GMO for public services
- Developing in biodiversity countries are rich and traditional knowledge related to bio-resources
- Biopiracy : Refers to the use of bio-resources by multinational companies and other organisations without proper authorization from the countries and people concerned without compensatory payment.

Controversies regarding patents and biopiracy:

(i) Basmatic rice :

- 2,00,000 varieties of rice in India. 27 documented varieties of Basmati rice in India
- In 1997, an American company got patent rights on Basmatic rice through the US patent and Trademark office.

Basmati rice × Semi-dwarf variety of rice

↓

New variety of Basmati rice

(ii) Turmeric

(iii) Neem

The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill.