BIOTECHNOLOGY AND ITS APPLICATIONS

Introduction:

- Biotechnology mainly deals with the industrial-scale production of biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals.
- Its applications include therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment, and energy production.

Biotechnology Research Areas:

- Provide best catalyst in the form of improved organism usually a microbe or pure enzyme.
- Create optimal conditions through engineering for a catalyst to act.
- Downstream processing technologies to purify the protein/organic compound.

Biotechnological Applications In Agriculture:

- Various methods such as agro-chemical based agriculture, organic agriculture, and genetically engineered crop-based agriculture can be used to fulfill the increasing demand of food supply.

REVISE

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- The agro-chemical based agriculture cause environmental pollution and organic agriculture is an expensive process. Thus to overcome these issues, **Genetically Modified Organisms** (GMOs) or GM-crops were developed.
- GMOs are those organisms whose genes have been altered by manipulation. This genetic modification has brought several advantages such as:
  - The crops became more tolerant to abiotic stresses (cold, drought, salt, heat).
  - Development of pest-resistant crops reduces the dependence of plants on chemical pesticides.
  - The post-harvest losses were reduced.
  - The efficiency of mineral usage by plants increased that prevented early exhaustion of fertility of soil.
  - The nutritional value of food was greatly enhanced. **Example: Vitamin A** enriched rice named **golden rice**.

Transgenic Animals:

- These are the animals whose genome (DNA) is manipulated to possess and express an extra foreign gene. Examples: transgenic rats, sheep, etc. Over 95 per cent of all existing transgenic animals are mice.

Benefits of Transgenic Animals:

- **To study regulation of genes and their action on normal physiology and development: Example:** Study

of insulin-like growth factor. Genes (from other species) that alter formation of this factor are introduced and the biological effects are studied. This gives information about biological role of the factor in the body.

- **To study the contribution of genes in the development of a disease and thereby new treatments: Example:** transgenic models for human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's are developed so that investigation of new treatments for the given diseases are made possible.

Transgenic cow, Rosie

- **Biological products:** Transgenic animals can be used to produce biological products by introducing genes which codes for a particular product. They are used to treat diseases such as emphysema, phenylketonuria (PKU), cystic fibrosis, etc. **Example:** human protein **(α-1-antitrypsin)** used to treat emphysema.
  In 1997, Rosie (first transgenic cow) produced human protein-enriched milk (2.4 g/L). It contains human α -lactalbumin. It is nutritionally more balanced product for human babies than natural cow-milk.
- **Vaccine safety testing: Example:** Transgenic mice are used to test the safety of the polio vaccine. If it is

reliable, they can replace the use of monkeys to test the safety of batches of the vaccine.

- **Chemical safety testing (toxicity testing):** Some transgenic animals carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are exposed to the toxic substances and the effects studied. It gives quick results.

Ethical Issues:

- Genetic modification may yield unpredictable results, when such transgenic organisms are introduced into the ecosystem. Therefore, some ethical standards are required to evaluate the morality of all human activities.
- Set of standards which are used to regulate human activities in relation to biological world are called **bioethics**.
- Indian Government has set up organizations like **GEAC** (Genetic Engineering Approval Committee) to make decisions about the validity of GM research and the safety of GM-organisms for public services.

Patent:

- The modification or usage of living organisms for public services has also created problems with patents granted for the same.
- Patent is the right granted by the Government to a producer or inventor to prevent others from commercial use

of his product or invention. Three main criteria for granting the patents are novelty, non-obviousness and utility.

- When the patents are obtained by individuals or groups or country in order to gain exclusive rights for the biological entities and products derived from them, they are called as **biopatents**. Countries like USA, Japan and members of European Union are awarding biopatents.

Biopiracy:

- It is defined as the use of bio-resources by MNCs and other organizations without proper authorization from countries and people concerned without compensatory payment.
- Majority of industrialized and developed nations are economically rich and poor in biodiversity. Hence, they exploit traditional knowledge and resources of poor countries for commercialisation. Therefore, it is mandatory to develop some laws to prevent this exploitation.
- The Indian parliament has recently introduced second amendment of Indian patents bill to deal with these issues.

Controversies in India regarding patent and biopiracy:

- **Basmati rice** is distinct for its unique aroma and flavour and in India 27 documented varieties of Basmati are grown.
- In 1997, an American company Rice Tec got patent rights on Basmati rice through the US Patent and Trademark

office. Having this patent, the company was allowed to sell a 'new' variety of Basmati, in the US and abroad.

- However, the 'new' variety of basmati is formed by crossing the Indian Basmati with semi-dwarf varieties but US company claimed it as an invention or a novelty. Thus, other people selling Basmati rice could be restricted by the patent.
- In 1995, the US Patent Office granted a patent to University of Mississippi Medical Center for 'Use of Turmeric in Wound Healing'.
- In 1996, the European Patent Office, Munich granted a patent to the firm of WR Grace and Co. for 'Fungicidal Uses of neem oil'.
- Therefore, it is necessary to pay attention or counter these patent applications, otherwise other countries or individuals can encash on our rich legacy and we may not be able to do anything about it.

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Pest Resistant Plants:

- Pest resistant plants act as **bio-pesticides**. These plants prove beneficial as they reduce the need for pesticides and insecticides. Examples: *Bt* cotton, *Bt* corn, rice, etc.

Bt Cotton:

- *Bt* cotton is created using the bacterium *Bacillus thuringiensis*. Some strains of this bacterium produce proteins that kill insects like coleopterans (beetles), lepidopterans (tobacco budworm, armyworm) and dipterans (flies, mosquitoes).
- *B. thuringiensis* forms an insecticidal protein (***Bt* toxin**) crystals during a particular phase of their growth. It does not kill the bacterium itself as it exists as inactive protoxin.
- When an insect ingests the Protien crystals, it becomes active due to alkaline pH of the insect's gut which solubilises the crystals. Toxin binds to surface of mid-gut epithelial cells and make it porous. It causes cell swelling and lysis and finally death of the insect.

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- Specific ***Bt* toxin genes (Crygene)** were isolated from *B. thuringiensis* and incorporated into crop plants such as cotton, tobacco, etc.
- Tabocco is first genetically modified plant for Glyphosate (Herbicide) resistrnce.
- Most *Bt* toxins are insect-group specific and are coded by ***cry* genes**. Examples, proteins encoded by *crylAc* and *crylIAb* control cotton bollworms and of *crylAb* controls corn borer.

Transgenic Cotton Modified To Resist Insects

Nematode resistance in tobacco plants:

- A nematode *Meloidogyne incognita* infects the roots of tobacco plants causing a reduction in its yield.
- This infection can be prevented by **RNA interference** (RNAi) strategy.
- RNAi method prevents the translation of a specific mRNA (silencing) through a complementary dsRNA molecule.
- The source of this complementary RNA is from an infection by RNA viruses or mobile genetic elements (transposons) that replicate via an RNA intermediate.

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- Nematode-specific genes were introduced into host plant using *Agrobacterium* vectors. The introduction of DNA was such that it produces both sense and anti-sense RNA in host cells.
- RNA's being complementary to each other resulted in the formation of double stranded RNA. It initiates RNAi and silences the specific mRNA of nematode.
- Thus the parasite cannot survive in a transgenic host expressing specific interfering RNA.

Biotechnological Applications in Medicine:

- Recombinant DNA technology helps in the mass production of efficient therapeutic drugs which are safe and do not induce unwanted immunological responses.

Genetically engineered insulin:

- Genetically engineered insulin is in great demand due to increase in number of patients with adult onset diabetes.

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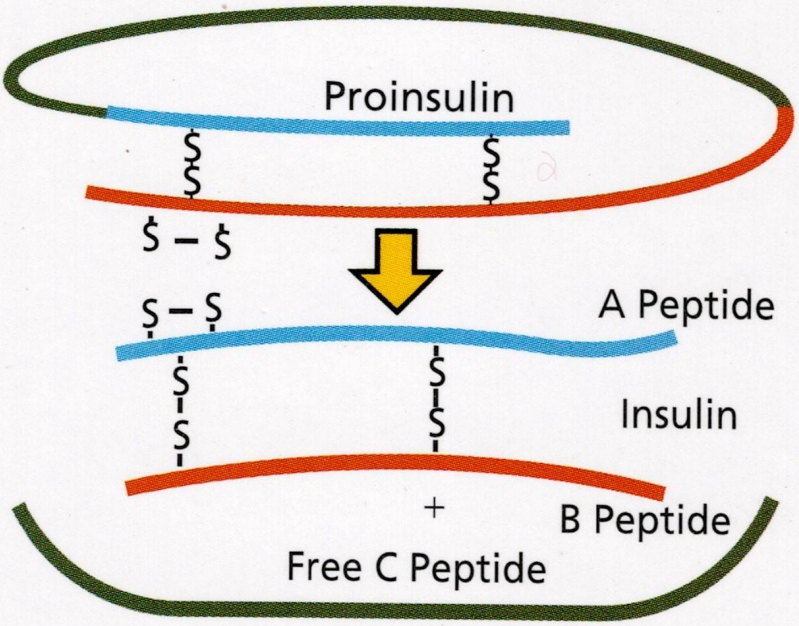
- In earlier time, insulin which was extracted from the pancreas of slaughtered cattle and pigs induce allergy in humans.
- Insulin as a prohormone consists of three polypeptide chains, i.e., A, B, and C.



- Prohormone insulin $\xrightarrow{\text{removal of C peptide}}$ Mature insulin
- In 1983, Eli Lilly, an American company constructed two DNA sequences corresponding to A and B chains of human insulin and then introduced them in plasmids of *E. coli* to produce insulin chains.
- Separately produced chains A and B were extracted and combined by creating a disulphide bond to form mature human insulin (Humulin).

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Gene Therapy:

- This method helps in the correction of a gene defect in a child or embryo.
- In this technique, normal genes are inserted into a person's cells and tissues to treat a hereditary disease. It compensates for the non-functional gene.
- First clinical gene therapy (1990) was given to a 4-year old girl with **adenosine deaminase (ADA) deficiency**.
- This disorder is caused due to the deletion of a gene for **adenosine deaminase** (essential enzyme for the proper functioning of immune system). In some children, it can be treated by **bone marrow transplantation** or by **enzyme replacement therapy**. Both of these are not completely curative.
- Steps of **Gene therapy** for ADA deficiency: Collect lymphocytes from the patient's blood and grow in a culture → Introduce a functional ADA cDNA into lymphocytes (using a retroviral vector) → They are subsequently returned to the patient.
- This technique should be periodically repeated as lymphocytes are not immortal.

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- If the ADA producing gene from marrow cells is introduced into cells at early embryonic stages, it could be a permanent cure.

Molecular Diagnosis:

- Early diagnosis of diseases is made possible by techniques such as Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA).
- Extremely low concentration of a bacteria or virus can be detected by amplification of their nucleic acid by Polymerase Chain Reaction.
- PCR technique is used to detect HIV in suspected AIDS patients and also to detect mutations in genes in suspected cancer patients.
- In Recombinant DNA technology, a single stranded DNA or RNA, tagged with a radioactive molecule (probe) is hybridized to its complementary DNA in a clone of cells. It is detected by autoradiography. The clone having mutated gene will not appear on photographic film, because the probe does not show complementarity with mutated gene.
- ELISA is based on **antigen-antibody interaction**. In this, infection by pathogen can be detected by the presence of **antigens** (proteins, glycoproteins, etc.) or by detecting the **antibodies** synthesized against the pathogen.

TEST

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1. Nematode specific genes were introduced into the tobacco host plant by using the vector:
   (a) plasmid
   (b) bacteriophage
   (c) pBR322
   (d) *Agrobacterium*
2. Which one of the following is not the product of transgenic experiments?
   (a) Pest-resistant crop variety
   (b) High nutritional value in grains
   (c) Production of insulin by rDNA technique
   (d) Drought-resistant crops
3. Which kind of therapy was given in 1990 to a four-year-old girl with ADA (Adenosine Deaminase) deficiency?
   (a) Radiation Therapy
   (b) Gene Therapy
   (c) Radiation Therapy
   (d) Immunotherapy
4. The maximum number of existing transgenic animals is of _____.
   (a) Fish **(b)** Mice
   (c) Cow **(d)** Pig

TEST

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Solutions:

1. **Option (d) is correct.**
   Nematode specific genes were introduced into the tobacco host plant by using the vector *Agrobacterium*. This introduction of DNA leads to the production of both sense and anti-sense RNA in the host cells.

2. **Option (c) is correct.**
   In transgenic experiments, genes of the organisms have been altered by manipulation. This modification leads to the production of crops which are more tolerant to abiotic stresses (cold, drought, etc.), development of pest-resistant crops and it also enhances the nutritional value of food.

3. **Option (b) is correct.**
   In 1990, first clinical gene therapy was given to a four -year old girl with ADA deficiency. Steps of Gene therapyare: Collection of lymphocytesfrom patient's blood and grow in a culture → Introduce a functional ADA cDNAinto lymphocytes (using a retroviral vector) → They are subsequently returned to the patient.

4. **Option (b) is correct.**
   Over 95 per cent of all the existing transgenic animals are mice. They are present in maximum number.

5. The first ever human hormone produced by recombinant DNA technology is _____.

   (a) Progesterone

   (b) Insulin

   (c) Estrogen

   (d) Adrenaline

6. In *Bt* cotton, the *Bt* toxin present in plant tissue as pro-toxin in converted into active toxin due to _____.

   (a) Acidic pH of the insect gut

   (b) Alkaline pH of the insect gut

   (c) Presence of conversion factors in insect gut

   (d) Action of gut microorganisms

Solutions:

5. Option (b) is correct.

The first ever human hormone produced by recombinant DNA technology isInsulin. In 1983, Eli Lilly, an American company constructed two DNA sequences corresponding to A and B chains of human insulin and then introduced them in plasmids of *E.coli* to produce insulin chains. Separately produced chains A and B were extracted and combined by creating a disulphide bond to form mature human insulin (Humulin).

6. Option (b) is correct.

In *Bt* cotton, the *Bt* toxin present as pro-toxin. When an insect ingests this toxin, it becomes active due to alkaline pH of the insect's gut. Toxin binds to surface of mid-gut epithelial cells and make it porous. Ultimately, it causes cell swelling and lysis which leads to death of the insect.

7. Polymerase Chain Reaction is not used in:

   (a) confirming presence of a pathogen during early infection

   (b) identification of mutated genes in suspected cancer patients

   (c) isolating the gene of interest from host DNA to be cloned by recombinant procedures

   (d) detection of the presence of HIV in suspected AIDS patients

8. Which of the following transgenic human protein product has been used to treat emphysema?

   (a) α-1 antitrypsin

   (b) α-1 globulin

   (c) Casein

   (d) Plasminogen activator