Biotechnological Applications

Biotechnology deals with industrial-scale production of bio pharmaceuticals and biological using genetically modified microbes, fungi, plants and animals. It application includes therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bio remediation, waste treatment and energy production

Define the following:

a) **Biopharmaceuticals**: Examines the inter-relationship of physical/chemical properties of drugs doses & route of administration on rate of systematics of drug absorption.
b) **Therapeutics:** The drugs that helps you to relax, feel better and cure illness.
c) **Bioremediation:** Microbes & Bacteria to de contaminate affected area.

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.

Biotechnological Applications in Agriculture

(a) Agrochemical based agriculture.
(b) Organic agriculture.
(c) Genetically engineered crop-based agriculture.

Green revolution increased food (crop) production due to the use of:
(a) Improved crop varieties.
(b) Agrochemicals (fertilisers and pesticides).
(c) Better management practices.

Genetically Modified Organisms (GMOs) are plants, animals, bacteria and fungi whose genes have been altered by manipulation.

What are the advantages of genetic modification in plants?
Ans: (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. Golden Rice (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.

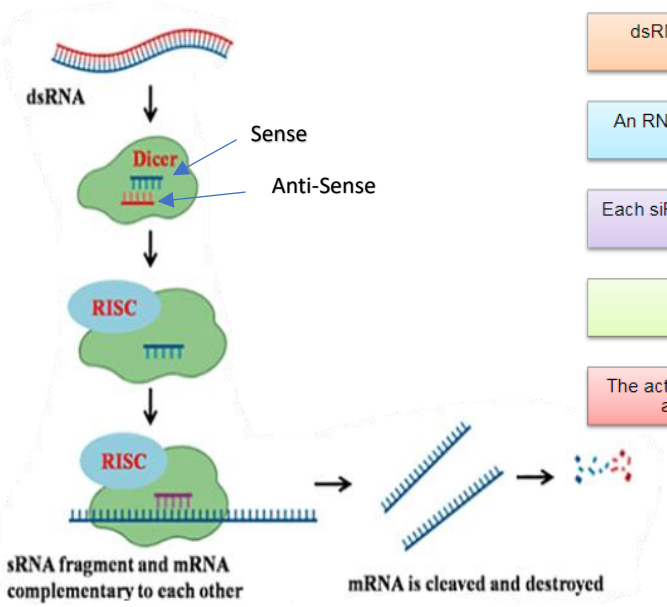
Pest resistant plants:

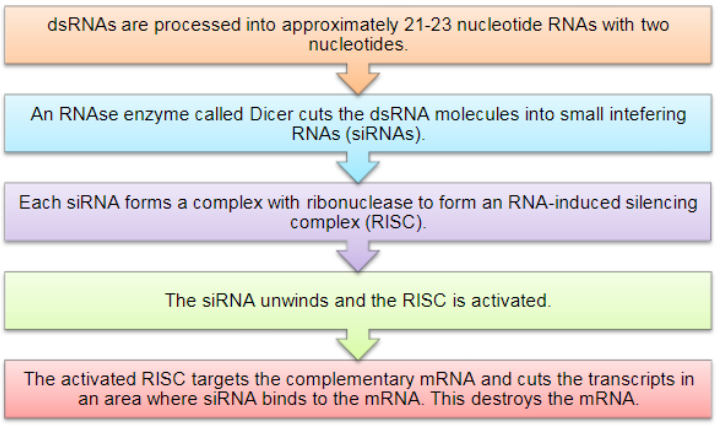
(i) Bt Cotton

- BT toxin gene cloned from bacteria *Bacillus thuringiensis* 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.
- BT -toxin is produced by a bacterium *Bacillus thuringiensis* that kill certain insects like
  Lepidopterens: Tobacco budworm, armyworm, corn borer, cotton bollworm.
  Coleopterans -beetles
  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. thuringiensis has 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 crylAc and cryIIAb control the cotton bollworms, cryIAb controls corn borer.

(ii) Tobacco Plant (Nematode Resistance)

- 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.





- 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. It produces both sense and anti-sense RNA in the host cells.
- 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
- The parasite could not survive in a transgenic host expressing specific interfering RNA.

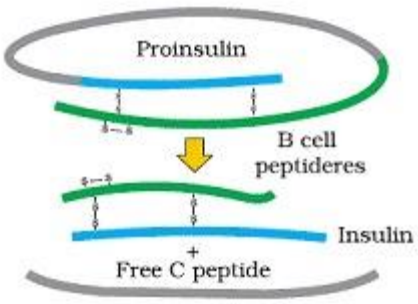
Biotechnological Applications in Medicine

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.

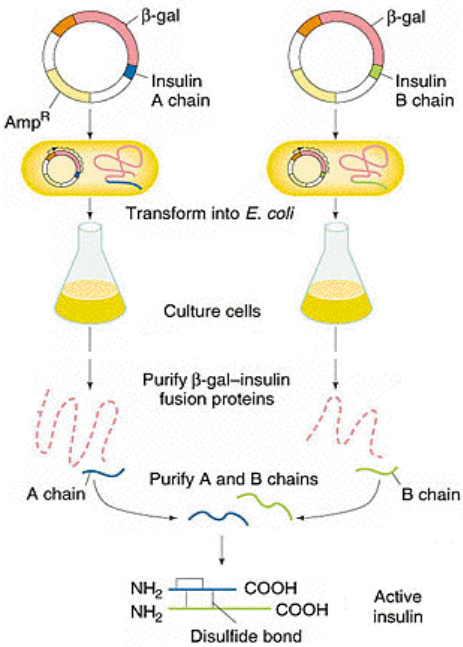
(b) Currently, about 30 recombinant therapeutics have been approved for human use over the world. In India, 12 of these are presently being marketed.

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



(a) Earlier Insulin was extracted from the pancreas of slaughtered cattle & pigs which may causes allergy or other reactions in some patients.

(b) Active Insulin consists of 2- short polypeptide chains, i.e. **chain-A** (21 amino acid) and **Chain- B** (30 amino acid), linked together by disulphide bridges. (C-peptide removed during maturation).

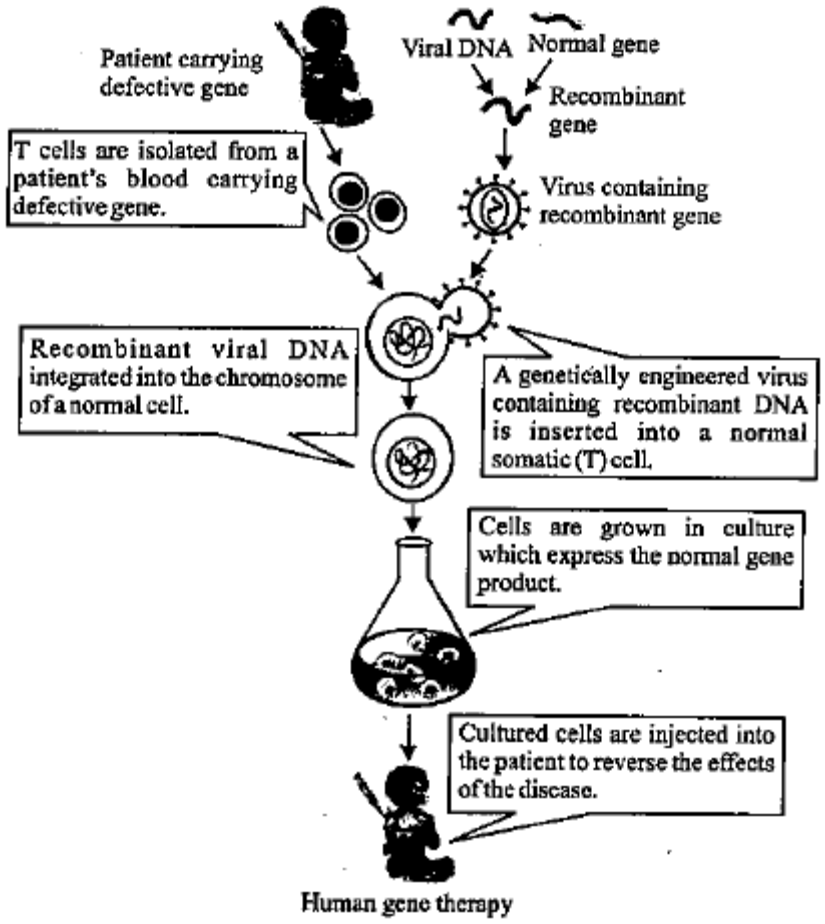


(c) In mammals, insulin is synthesised as a prohormone (needs to be processed before it becomes a fully mature and functional hormone) which contains an extra stretch called the C-peptide.

(d) 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.**

Que: Why can insulin not be taken orally in the form of tablets?
Ans: Insulin is made of protein, if it is given orally in the form of tablets it will be digested by the digestive enzymes, and may not function as Hormone.

Gene therapy: Collection of methods of 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 (enzyme for immune system function).
•ADA deficiency is an inherited disorder that damages the immune system and causes severe combined immunodeficiency (SCID). [Both B lymphocytes (Provide Antibody mediated immunity) & T lymphocytes (Provide cell mediated immunity) gets damage]
• In some children, ADA deficiency can be cured by bone marrow transplantation and enzyme replacement therapy, but they are not completely curable.
Steps involved are as follows:
1) Collect Lymphocytes from patient blood & grown in a culture outside the body.



2) Introduce functional ADA cDNA (using retroviral vector) into these lymphocytes & subsequently returned to patient.
3) Patient requires periodic infusion of such genetically engineered lymphocytes as these cells are not immortal.

- 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.

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) Some molecular diagnosis techniques 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 but 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**
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 followed by autoradiography detection.
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.

Transgenic Animals

Animals with DNA manipulated to possess and express a foreign gene . Eg: Transgenic mice, rats, rabbits, pigs, sheep, cows and fish etc. First Transgenic Animal is Mice, as well as 95 % transgenic animals are mice.

Common reasons for development of transgenic animals-

a) **Normal physiology and development**– To study of gene regulation, their effect on normal function like physiology and development of body. Ex: insulin like growth factor.

b) **Study of disease**–To study contribution of gene in the development of disease. Eg: Transgenic model for many human disease like cancer, cystic fibrosis, rheumatoid arthritis, Alzheimer's disease etc.

c) **Biological products**–Produce useful biological products by introduction gene which codes for a particular product.

Eg: Human protein (alpha – 1-antitrypsin) used to treat emphysema.

Similarly, product for treating Phenylketonuria & cystic fibrosis.

First transgenic cow, Rosie contains human alpha-lactalbumin, produced human protein-enriched milk (2.4 gm / litre).

d) **Vaccine safety**– Transgenic mice used in testing the safety of vaccine before they are used on human. Eg: Polio vaccine was tested on transgenic mice and then on monkey.

e) **Chemical safety testing**– They carry genes which make them more sensitive to toxic substances than non-transgenic animals. Exposed to toxin & results studied in less time.

Ethical Issues: Genetic modification may cause unpredictable results when such organism are introduced into ecosystem. Hence, modification of Human race can not go on any further, without regulation.

GEAC (Genetic Engineering Approval Committee): Organization set up by The Indian Government which will make decisions regarding the validity of GM research and the safety of introducing GM-organisms for public services.

Bio-patent: A patent is the right granted by a government to an inventor to prevent others from making commercial use of his invention.

Indian parliament has cleared the second amendment of Indian Patent Bills that takes issues into consideration , including patent term emergency provision & research & development initiative.

Biopiracy: The use of bio-resources by multinational companies and other organizations without proper authorization from the countries and people concerned without compensatory payment.

Industrialised nation with poor diversity generally does this in under developing nation with rich biodiversity.

There are estimated 2 lakhs variety of rice in India alone (richest rice diversity).

In India there are 27 variety of Basmati which has unique aroma & flavour.

Eg: An American company (1997) 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 (Which had actually been derived from Indian farmer's varieties by crossing 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.