BIOTECHNOLOGY- PRINCIPLES & PROCESSES

Biotechnology term given by **Karl Ereky.**
Biotechnology deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans.
Types of Biotechnology :-Two types-

(1) Old/traditional:-
Old biotechnology are based on the natural capabilities of micro organisms.
e.g. formation of Citric acid, production of penicillin by Penicillium notatum, making curd, bread or wine, which are all microbe-mediated processes, a form of biotechnology.

(2) New/modem:-
Based on Recombinant DNA technology.
e.g. Human gene producing Insulin has been transferred and expressed in bacteria like E.coli.
According to the European Federation of Biotechnology (EFB) has given a definition of biotechnology that encompasses both traditional view and modern molecular biotechnology. The definition given by EFB is as follows: 'The integration of natural science and organisms, cells, parts thereof and molecular analogues for products and services'.

- **Paul berg (Father of genetic engineering):** He transferred gene of SV-40 virus (simian virus) in to E. coli with the help of 'λ - phage. (Nobel prize - 1980)
- **Stanley Cohen and Herbert Boyer :** First made recombinant DNA by linking an antibiotic resistance gene with a native plasmid of Salmonella typhimurium.

Principles of Biotechnology :-

Among many, the two core techniques that enabled birth of modern biotechnology are :

(i) Genetic Engineering/Recombinant DNA Technology : Techniques to alter the chemistry of genetic material (DNA and RNA). to introduce these into host organisms and thus change the phenotype of the host organism. (i.e. formation of genetically modified organism)

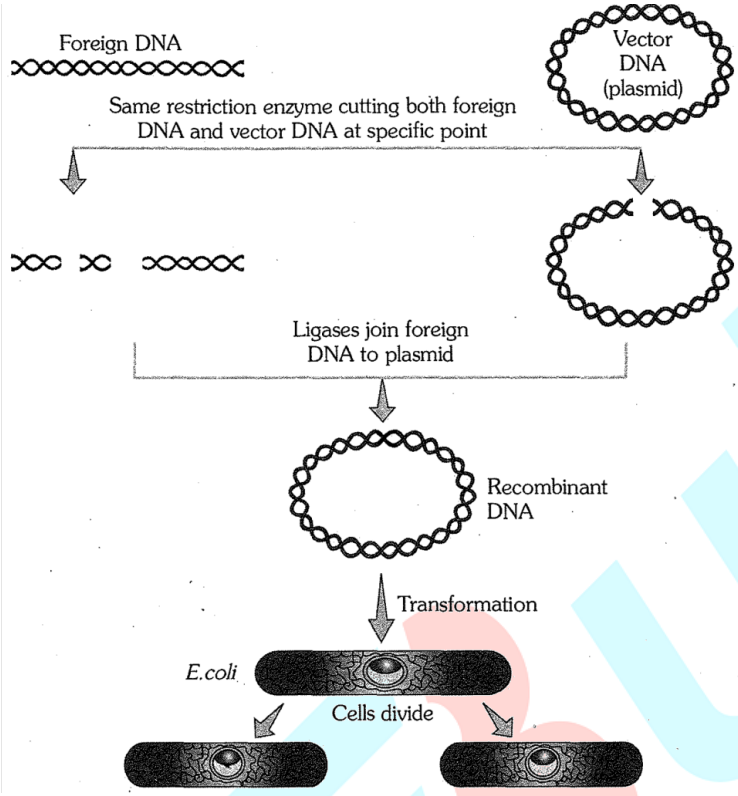
(ii) 'Bioprocess Technology : Maintenance of sterile (microbial contamination-free) ambience in chemical engineering processes to enable growth of only the desired microbe/eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.

The concept of genetic engineering was the outcome of two very significant discoveries made in bacterial research. These were-

(i) presence of extrachromosomal DNA fragments called plasmids in the bacterial cell, which replicate along with, chromosomal DNA of the bacterium.
(ii) presence of enzymes restriction endonucleases which cut DNA at specific sites.
These enzymes are. therefore. called 'molecular scissors'.

The main basis of Recombinant DNA Technology is DNA cloning :- It is making multiple identical copies of any template DNA

There are three basic steps of DNA donning -
(i) identification of DNA with desirable genes:
(ii) introduction of the identified DNA into the host:
(iii) maintenance of introduced DNA in the host and transfer of the DNA to its progeny.



Diagrammatic representation of recombinant DNA technology

TOOLS OF RECOMBINANT DNA TECHNOLOGY:-

Genetic engineering involves cutting of desired segments of DNA and pasting of this D.N.A in a vector to produce a recombinant DNA (rONA). The 'biological tools' used in the synthesis of recombinant DNA include enzymes, vehicle or vector DNA, desired DNA and host cells.

1. **Enzymes :-** A number of specific kinds of enzymes are employed in genetic engineering. These include lysing enzymes, cleaving enzymes, synthesising enzymes and joining enzymes.

(i) **Lysing enzymes** - These enzymes are used for opening the cells to get DNA for genetic experiment.

- **Bacterial cell :** is commonly digested with the help of lysozyme.
- **Plant cell :** is commonly digested with the help of cellulase and pectinase.
- **Fungul cell :** is commonly digested with the help of chitinase.

(ii) **Cleaving enzymes** - These enzymes are used for DNA molecules. Cleaving enzymes are of two types exonucleases and endonucleases.

(a) **Exonucleases** remove nucleotides from the ends of the DNA.

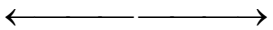
(b) **Endonucleases** make cuts at specific positions within the DNA
eg. Restriction endonucleases

Restriction Endonuclease Enzymes (Molecular scissor or molecular knife)

Restriction enzymes belong to a larger class of enzymes called endonucleases.

Restriction enzymes are used in recombinant DNA technology because they can be used in vitro to recognize and cleave within specefic DNA sequence typically consisting of 4 to 8 nucleotides. This specific 4 to 8 nucleotide sequence is called restriction site and is usually

palindromic, this means that the DNA sequence is the same when read in a 5'-3' direction on both DNA strand



AND MADAM DNA

Restriction enzymes are obtained from bacteria.

What is function of restiction enzymes in bacteria?

They are useful to bacteria because the enzyme bring about fragmentation of viral DNA without affecting the bacterial genome. This is an adaptation against bacteriophages.

These enzymes exist in many bacteria beside cleavage some restriction endonuclease, also have capability of modification.

Modification in the form of methylation, by methylation the bacterial DNA modifies and therefore protects it's own chromosomal DNA from cleavage by these restriction enzymes.

The first restriction endonuclease-Hind II, whose functioning depended on a specific DNA nucleotide sequence was isolated and characterised five years later. It was found that Hind II always cut DNA molecules at a particular point by recognising a specific sequence of six base pairs. This specific base sequence is known as the recognition sequence for Hind II.

Restriction enzyme (Eco R-I.l was discovered by Arber. Smith & Nathans (1978 Nobel prize).

Besides Hind II, today we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria each of which recognise different recognition sequences.

Nomenclature of enzyme -

- **The first letter :** indicates bacterial genus (In italic)
- **Second** and **third letter :** indicate speceis of bacteria (In italic)
- **Fourth letter :** indicates strain of bacteria (optional)
- **Roman numerical :** signifying the order in which the enzymes were isolated from that strain of bacteria.

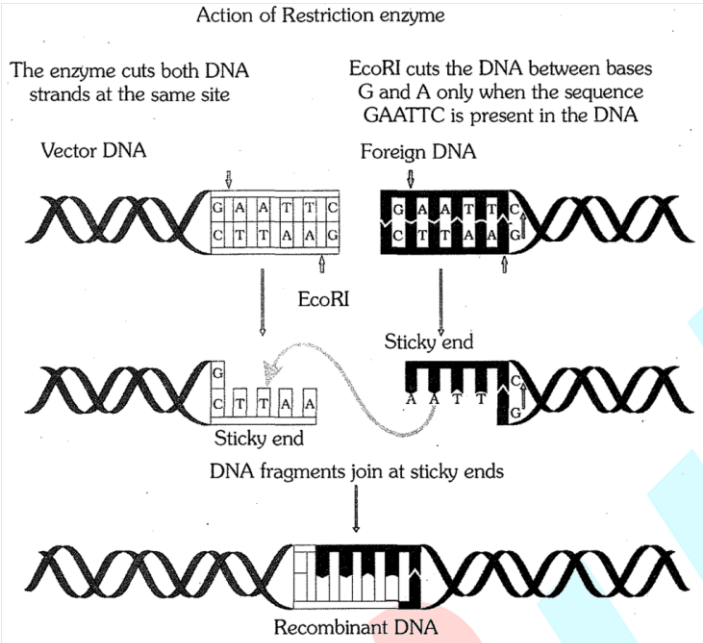
eg. EcoRI comes from Escherichia coli RY 13. In EcoRI, the letter 'R' is derived from the name of strain. Roman numbers following the names indicate the order in which the enzymes were isolated from that strain of bacteria.

Restriction enzymes forms two types of ends on the basis of mode of cutting.

(i) Sticky end (Free end) : Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands.

This leaves single stranded portions at the ends. There are overhanging stretches called sticky ends on each strand. These are named so because they form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends facilitates the action of the enzyme DNA ligase.

eg. EcoRI, Hind III, Bam HI, Sal I



Steps in formation of recombinant DNA by action of restriction endonuclease enzyme - EcoRI

(ii) Blunt end (non sticky end) : some enzymes cleave both strand of DNA at exactly the same nucleotide position, typically in the center of the recognition sequence resulting in blunt end or flush end.

eg. Sma I, EcoRV, Hae III

Sma I (Serratia marcescens)

5' CCC G G G 3' → 5' C C C 3' + 5' G G G 3'

3' G G G C C C 5' ⇒ 3' G G G 5' + 3' C C C 3'

EXAMPLES OF RESTRICTION ENZYME

Recognition sequences of some restriction endonucleases

Name	Recognition sequence	End after cleavage		Source
Eco RI	-G↓AATTC- -CTTAA↑G-	-G -CTTAA	AATTC- G-	*Escherichia coli*
Hind III	-A↓AGCTT- -TTCGA↑A-	-A -TTCGA	AGCTT- A-	*Haemophilus influenzae*
Bam I	-G↓GATCC- -CCTAG↑G-	-G -CCTAG	GATCC- G-	*Bacillus amyloliquefaciens*
Hae III	-GG↓CC- -CC↑GG-	-GG -CC	CC- GG-	*Haemophilus aegyptius*
EcoRV	-GAT↓ATC- -CTA↑TAG-	-GAT -CTA	ATC- TAG-	*Escherichia coli*

(iii) **Synthesizing enzyines.** These enzymes are used to synthesize new strands of DNA, complementary to existing DNA or RNA template. They are of two types; reverse transcriptases and DNA polymerases.

(a) Reverse transcriptases help in the synthesis of complementary DNA strands on RNA templates;

(b) DNA polymerases help in the synthesis of complementary DNA strands on DNA templates.

(iv) **Joining enzymes.** These enzymes help in joining the DNA fragments. For example DNA ligase from Escherichia coli is used to join DNA fragments. Joining enzymes are, therefore, called molecular glues.

2. **Vehicle DNA or Vector DNA.** The DNA used as a carrier for transferring a fragment of foreign DNA into a suitable host is called vehicle or vector DNA.

You know that plasmids and bacteriophages have the ability to replicate within bacterial cells independent of the control of chromosomal DNA.

The following are the features that are required to facilitate cloning into a vector.

(i) **Origin Qf replication (ori) :** This is a seguence from where replication starts and any piece of DNA when linked to this seguence can be made to replicate within the host cells. This seguence is also responsible for controlling the copy number of the linked DNA. So. if one wants to recover many copies of the target DNA it should be cloned in a vector whose origin support high copy number.

(ii) **Selectable marker:** In addition to 'ori', the vector reguires a selectable marker, which helps in identifying and eliminating nontransformants and selectively permitting the growth of the transform ants.

- Normally. the genes encoding resistance to antibiotics such as ampicilin. chloramphenicol. Tetracycline or kanamycin. etc., are considered useful selectable markers for E. coli.
- Enzyme forming gene also act as selectable marker gene. eg. Lac Z gene

Note : The normal E. coli cells do not carry resistance against any of these antibiotics.

(iii) **Restriction sites/Cloning sites :** In order to link the alien DNA the vector needs. recognition sites for the commonly used restriction enzymes. The ligation of alien DNA is carried out at a restriction site present in one of the two antibiotic resistance genes. A vector should have restriction site for many enzyme but only one restriction site for each enzyme, otherwise vector will get fragmented.

Selection of vector depends on :-

(i) Size of desired gene

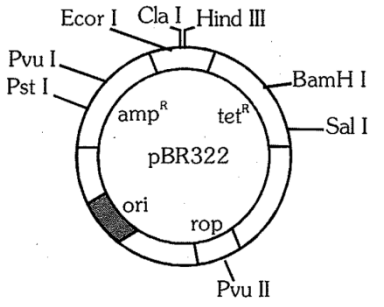
(ii) Type of host

Some examples of vectors :-

(i) **Plasmids:** They are double stranded, circular and extra chromosomal DNA segments found in bacteria which can replicate independently. Plasmids can be takeu out of bacteria and made to combine with desired DNA segments by means of restriction enzymes and DNA ligase. A plasmid carrying DNA of another organism integrated with it, is known as recombinant plasmid or hybrid plasmid or Chimeric plasmid.

eg. pBR 322, PUC 18 (used for gene transfer in bacteria)

Ti plasmid, Ri Plasmid (used for gene transfer in dicot plant)



E. coli cloning vector pBR322 showing restriction sites (Hind III, EcoR I, BamH I, Sal I, Pvu II, Pst I, Cla I), ori and antibiotic resistance genes (ampR and tetR). Rop codes for the proteins involved in the replication of the plasmid.

(ii) Viruses: The DNA of certain viruses is also suitable for use as a vehicle DNA.
Bacteriophage DNA also used as a vector DNA for gene transfer.
Lambda phage (A, phage) has been used for transferring lac genes of E. coli into haploid callus of tomato.
Retro virus - useful for gene transfer in animal cell

	Vector type	Insert size kb
(1)	Plasmid	0.5 – 8
(2)	Bacterophage lambda	9 – 23
(3)	Cosmid	30 – 45
(4)	BAC	50 – 300
(5)	YAC	1000 – 2500

3. Desired DNA / Alien DNA / Foreign DNA / Passenger DNA :-
It is the DNA which is transferred from one organism into another by combining it with the vehicle DNA. The passenger DNA can be complementary, synthetic or random.

(i) Complementary DNA (eDNA)- It is synthesized on mRNA template with the help of reverse transcriptase and necessary nucleotides. eDNA formed through reverse transcription is shorter than the actual or in tdvo gene because of the absence of introns or non-coding regions.

(ii) Synthetic DNA (sDNA)- It is synthesized with the help of DNA polymerase on DNA template.
Kornberg (1961) synthesized first synthetic DNA.
Khorana (1968) synthesized first artificial gene (DNA) without a template. They synthesized the gene coding for yeast alanine t-RNA, which contained only 77 base pairs. However, it did not function in the living system. In 1979, Khorana was able to synthesize a functional tyrosine t-RNA gene of E. coli with 207 nucleotide pairs. Since then a number of genes have been synthesized artificially.

4. Host organism :-
This organism is used for DNA cloning
Host may be plant, animal, bacteria (E. coli), fungi (Yeast).

PROCESSES OF RECOMBINANT DNA TECHNOLOGY:-

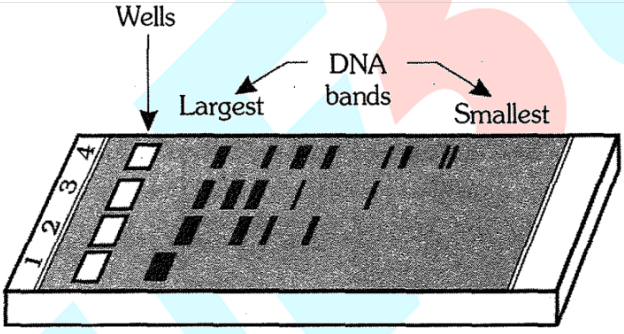
1. Isolation of DNA :-

The DNAs which are to be used as passenger DNA and the vehicle DNA are extracted out of their cells by lysing the cells with the suitable enzyme. The DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA. proteins, polysaccharides and also lipids. This can be achieved by treating the bacterial cells/plant or animal tissue with enzymes such as lysozyme (bacteria), cellulase (plant cells), chitinase (fungus). You know that genes are located on long molecules of DNA interwined with proteins such as histones. The RNA can be removed by treatment with ribonuclease whereas proteins can be removed by treatment with protease. Other molecules can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol. This can be seen as collection of fine threads in the suspension. DNA that separates out can be removed by spooling method.

2. Fragmentation of DNA by restriction endonucleases :-

Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme at the optimal conditions fo! that specific enzyme.

Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion. Both the passenger and vehicle DNAs are then, cleaved by using the same restriction endonuclease so that they have complementary sticky ends.



A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

Note : The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation (you cannot see pure DNA fragments in the visible light and without staining). You can see bright orange coloured bands of DNA in a ethidium bromide stained gel exposed to UV light. The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as elution. The DNA fragments purified in this way are used in constructing recombinant DNA by joining them with cloning vectors.

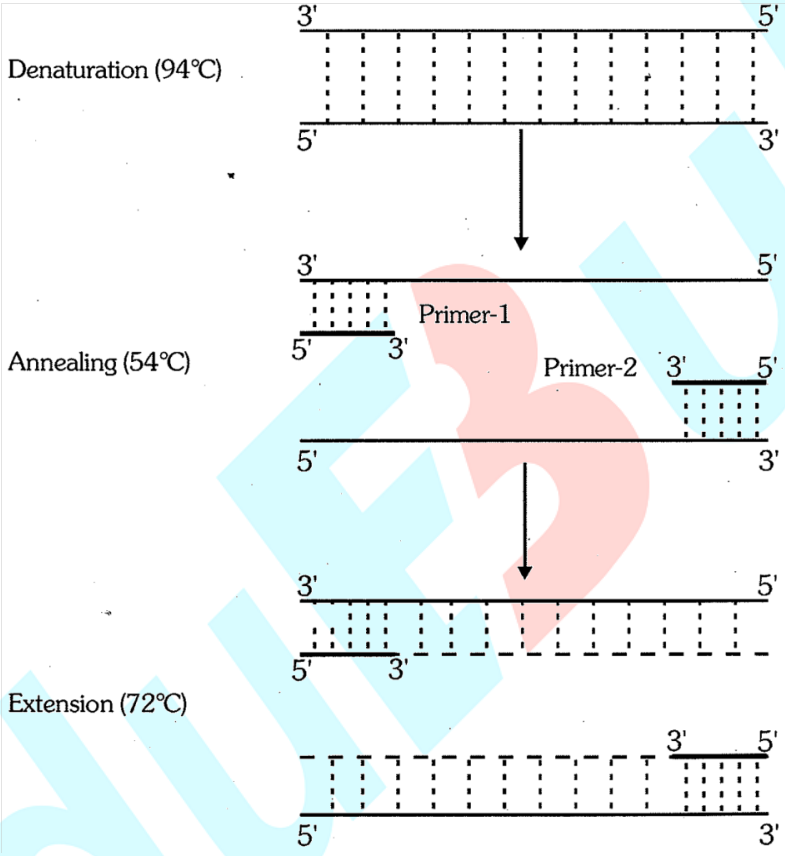
3. Amplification of gene of interest using PCR

Polymerase chain reaction technology (PCR-technology)

- This technique was invented by Kary mullis (1983).
- In 1993 Karry Mullis got nobel prize for PCR(for chemistry)
- PCR is a method for amplifying a specific region of DNA molecule without the requirement for time consuming cloning procedures.
- PCR reaction takes place in Eppendorf tube.

- Using PCR-technique very low content of DNA available from samples of blood or semen or any other tissue or hair cell can be amplified many times and analysed. In this technique Taq-Polymerase is used. Taq polymerare enzyme is used in PCR which is a special type of DNA polymerare enzyme which is resistant to high temperature.
- Taq Polymerase is isolated from Thermus aquaticus bacterium.
- Some other examples of polymerase which are used in PCR are -

Pflu Polymerase - Isolated from Pyrococus furiosus bacterium.
Vent Polymerase - Isolated from Thermococcus litoralis bacterium.
Main steps in PCR :-



(i) Denaturation (94°) :- In this step a double stranded DNA molecule is placed at 94°C. So double stranded DNA becomes single stranded & each single stranded DNA functions as a template.

(ii) Annealing/Cooling (54 °) :- In this step two primer DNA are attached at 3' end of single stranded DNA

(iii) Extension (72°) :- In this process Taq polymerase enzyme synthesize DNA strain over template.
PCR is automatic process because Taq. polymerase enzyme is heat resistant.

4. Ligation of the DNA fragment into a vector:-
The complementary sticky ends of the passenger and vehicle DNAs are joined with lipase enzyme. This gives rise to a recombinant DNA

5. Transferring the recombinant DNA into the host (Gene transfer) :-

Transfer of desired genes from one organism into another is an important aspect of genetic engineering.

Gene transfer is achieved by two kinds of transfer· methods:

(i) Indirect method through vectors or carriers and

(ii) Direct or vector less transfer method.

(i) Indirect method :-

(a) Gene Transfer in bacterial cell : Since DNA is a hydrophilic molecule, it cannot pass through cell membranes. In order to force bacteria to take up the plasmid, the bacterial cells must first be made 'competent' to take up DNA. This is done by treating them with a specific concentration of a divalent cation, such as calcium, which increases the efficiency with which DNA enters the bacterium through pores in its cell wall. Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42°C (heatshock), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.

Note: The bacteria to be used as hosts should be without plasmids.
The host cells are treated with clacium chloride or lysozyme.
Transformant : E.coli with plasmid
Non-transformant : E. coli without plasmid

(b) Gene Transfer in plant cell :

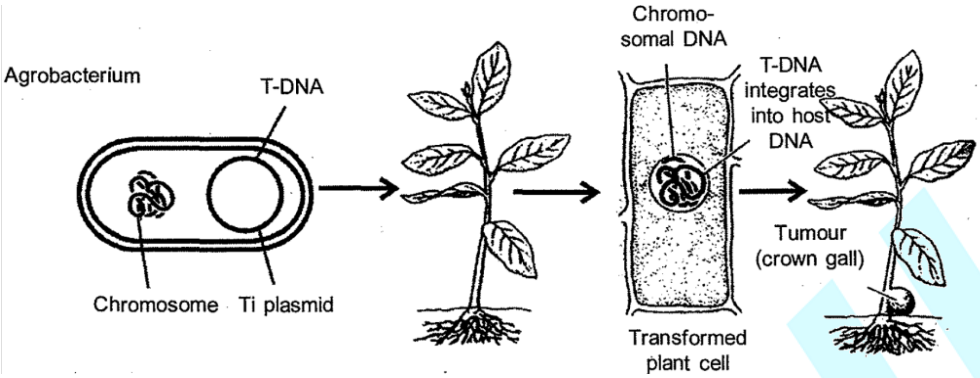
- A plant pathogenic bacterium-Agrobacterium tumefaciens produces crown galls or plant tumours in almost all dicotyledonous plants.
- This bacterium infects all broad leaved agricultural crops such as tomato, soyabean, sunflower and cotton but not cereals.
- Tumour formation is induced by it's plasmid which is therefore called Ti plasmid (Ti = tumour inducing) Agrobacterium tumefaciens naturally transfers some part of Ti-plasmid in to host plant DNA without any human effort so it is called natural genetic engineer of plant. In the transformation process two essential component in Ti-plasmid-
  (a) T-DNA - (Transferred DNA)
  (b) Vir-region - (Virulence region)

  Inside the host plant cell T-DNA is seperated from Ti-plasrnid, and integrated into host plant DNA that causes crown gall tumour.

  Vir-region contains genes which are essential for T-DNA transfer and integration to host plant DNA.

  When we use Ti plasmid as a vector, first we remove the tumour causing gene from T-DNA region. Then desired gene inserted inplace of it. Now, this plasmid is called disarmed plasmid.

  Same as Ri plasmid of A. rhizogenes (causing hairy root disease) also used as vector for gene transfer to plant cell.



(c) Gene transfer in animal :

Retroviruses have also been disarmed and are now used to deliver desirable genes into animal cells.

(ii) Direct method

Foreign genes can also be transferred directly by the following methods:

(a) Electroporation- It creates transients (temporary pores) in the plasma membrane to facilitate entry of foreign DNA.

(b) Chemical mediated genetic transformation- It involves certain chemicals such as polyethylene glycol (PEG), that help in the uptake of foreign DNA into host cells.

(c) Microinjection- It is the introduction of foreign genes mainly into animal cells using micro pipettes or glass needles.

(d) Particle gun/Biolisticmethod- It is a technique in which tungsten or gold particles coated with foreign DNA are bombarded into target cells to facilitate entry of the foreign genes.

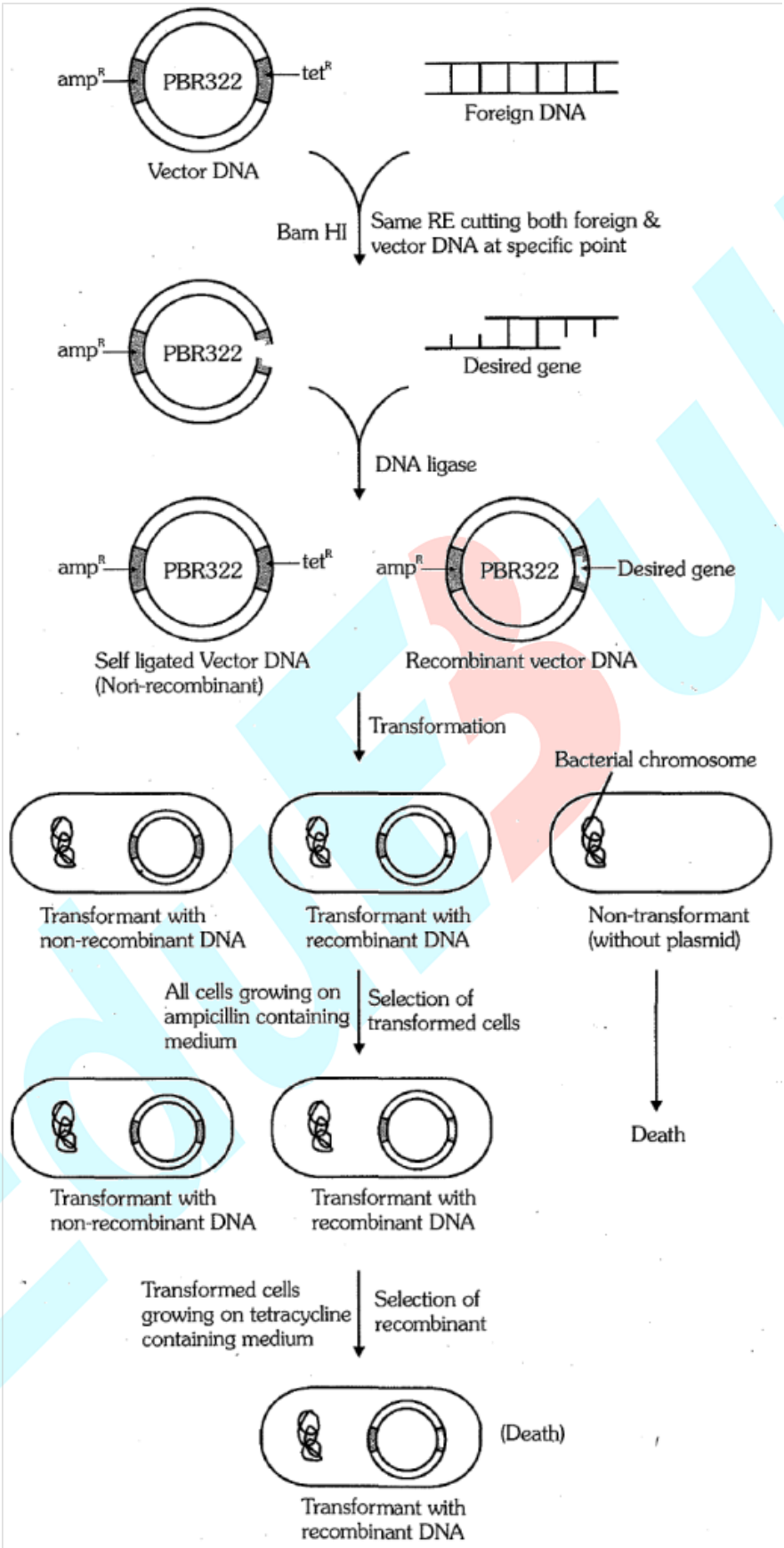
(e) Liposome mediatd gene transfer- In this method DNA encloses within lipid begs. These lipid begs fused with protoplast.

6. Selection of Transformant with recombinant cell :-

(1) Selection by two antibiotic resistant gene

You can ligate a foreign DNA at the Bam H I site of tetracycline resistance gene in the vecter pBR322. The recombinant plasmids will loss tetracycline resistance due to insertion of foreign DNA (insertional inactivation) now. it can be selected out from non-recombinant ones by plating the transformants on ampicillin containing medium. The transformants (plasmid transfer) growing on ampicillin containing medium are then transferred on a medium containing tetracycline. The recombinants will grow in ampicillin containing medium but not on that containing tetracycline. But, non-recombinants will grow on the medium containing both the antibiotics. In this case, one antibiotic resistance gene helps in selecting the transformants.

Note: Insertional inactivation : Due to insertion of desired gene within selectable marker gene of vector, selectable marker gene become inactive or loose their function. This is called Insertional inactivation.



(2) Selection by one Lac Z gene and one antibiotic resistant gene

Selection of recombinants due to inactivation of antibiotics is a cumbersome (troublesome) procedure because it requires simultaneous plating on two plated having different antibiotics. Therefore, alternative selectable markers have been developed which differentiate recombinants from non-recombinants on the basis of their abilily to produce colour in the presence of a chromogenic substrate. In this, a recombinant DNA is inserted within the coding sequence of an enzyme, which is referred to as insertional inactivation. The presence of a chromogenic substrate X-gal gives blue coloured colonies if the plasmid in the bacteria does not have an insert. Presence of insert results into insertional inactivation of the β-galactosidase (reporter enzyme) and the colonies do not produce any colour, these are identified as recombinant colonies.

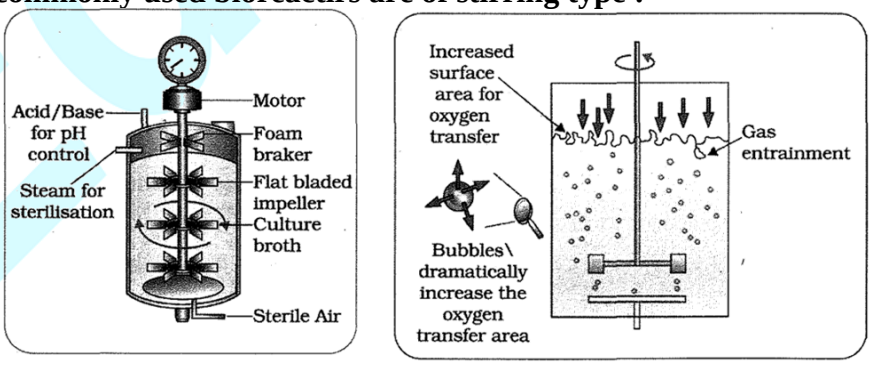
7. Obtaining the Foreign Gene Product :-

When you insert a piece of alien DNA into a cloning vector and transfer it into a bacterial, plant or animal cell, the alien DNA gets multiplied. In almost all recombinant technologies, the ultimate aim is to produce a desirable protein. Hence, there is a need for the recombinant DNA to be expressed. The foreign gene gets expressed under appropriate conditions. The expression of foreign genes in host cells involve understanding many technical details.

After having cloned the gene of interest and having optimised the conditions to induce the expression of the target protein, one has to consider producing it on a large scale. Can you think of any reason why there is a need for large-scale production? If any protein encoding gene is expressed in a heterologous host is called a recombinant protein. The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory. The cultures may be used for extracting the desired protein and then purifying it by using different separation techniques.

The cells can also be multiplled in a continuous culture system wherein the used medium is drained out from one side while fresh medium is added from the other to maintain the cells in their physiologically most active log/exponential phase. This type of culturing method produces a lamer biomass leading to higher yields of desired protein. Small volume cultures cannot yield appreciable quantities of products, To produce in large quantities, the development of bioreactors. where large volumes (100-1000 litres) of culture can be processed, was required. Thus, bioreactors can be thought of as vessels in which raw materials are biologically converted into specific products. individual enzymes etc,. using microbial plant, animal or human cells. A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).

The most commonly used bioreactirs are of stirring type :-



(a) Simple stirred - tank bioreactor **(b) Sparged stirred - tank bioreactor**

A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer faciliates even mixing and oxygen availability throughout the

bioreactor. Alternatively air can be bubbled through the reactor. The bioreactor has an agitator system, an oxygen delivery system and a foam control system. a temperature control system. pH control system and sampling ports so that small volumes of the culture can be withdrawn periodically.

Downstream Processing-

After completion of the biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product. The processes include separation and purification, which are collectively referred to as downstream processing.
The product has to be formulated with suitable preservatives. Such formulation has to undergo through clinical trials as in case of drugs. Strict quality control testing for each product is also required. The downstream processing and quality control testing vary from product to product.

BEGINNER'S BOX-1

1. Transfer of any gene into a completely different organism can be done through
(1) Genetic engineering (2) Tissue culture
(3) Transformation (4) RNA interference

2. DNA probe is used in :
(1) Gel electrophoresis (2) Northern blotting
(3) DNA finger printing (4) Interferon synthesis

3. First artificial gene synthesized by khorana was a gene of
(1) Arginine (2) Lysine
(3) Alanine t-RNA of yeast (4) Valine t-RNA

4. PBR 322 is an artificial gene vector which does not have
(1) Amphiciline marker gene (2) Cos site
(3) Restriction site for cia I enzyme (4) Ori

5. The thermos table enzyme Taq & Pflu isolated from thermophilic bacteria are :-
(1) RNA polymerase (2) DNA primers
(3) DNA polymerases (4) DNA ligase

BIOTECHNOLOGY AND ITS APPLICATION

Biotechnology, as you would have learnt from the previous chapter, essentially deals with industrial scale production of biopharmaceuticals and biologicals using genetically modified microbes. fungi, plants and animals. The applications of biotechnology include therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment, and energy production.

(A) BIOTECHNOLOGICAL APPLICATION IN AGRICULTURE:

Let us take a look at the three options that can be thought for increasing food production

(i) agro-chemical based agriculture;
(ii) organic agriculture; and
(iii) genetically engineered crop-based agriculture.

The Green Revolution succeeded in tripling the food supply but yet it was not enough to feed the growing human population. Increased yields have partly been due to the use of improved crop varieties, but mainly due to the use of better management practices and use of agrochemicals (fertilisers and pesticides). However, for farmers in the developing world, agrochemicals are often too expensive, and further increases in yield with existing varieties are not possible using conventional breeding. Is there any alternative path that our understanding of genetics can show so that farmers may obtain maximum yield from their fields? Is there a way to minimise the use of fertilisers and chemicals so that their harmful effects on the environment are reduced? Use of genetically modified crops is a possible solution.

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Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO).

GM has been used to create tailor-made plants to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

Use of gentically modified (GM) plant:-

1. To enhance nutrientional quality of food
eg. Golden rice : Vitamin A enriched rice (In this rice gene of ~-carotene is transfered)

2. Made crops more tolerant to abiotic stresses (cold, drought, salt, heat)

3. Helped to reduce post harvest losses
eg. Flavr Savr Tomato : Transgenic variety of Tomato- Flavr Savr due to the inhibition of polygalacturonase enzyme which degrades pectin. So that tomato variety remains fresh and retain flavour much longer. Flavr Savr Tomato develop by anti-sense tenchnology.

4. Increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).

5. To produce biopharamaceutical product

eg. Production of Hirudin: Hirudin is a protein that prevents blood clotting. The gene incoding hirudin was chemically synthesized and transferred into Brassica napus. Where hirudin accumulates in seeds. The hirudin is purified and used as medicine.

6. To produce herbicide resistant plant

eg. First transgenic plant was tobacco. It contains resistant gene against weedicide (Glyphosate).

7. **Pest-resistant crops :** reduced reliance on chemical pesticides.

(i) **Insect resistant plant**

eg. Bt cotton : Some strains of Bacillus thuringiensis produce proteins that kill certain insects such as lepidopterans (tobacco budworm. armyworm). coleopterans (beetles) and dipterans (flies. mosquitoes). B. thuringiensis forms protein crystals during a particular phase of their growth. These crystals contain a toxic insecticidal protein.

The Bt toxin protein exist as inactive protoxins but once an insect ingest the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which solubilise the crystals. The activated toxin binds to the surface of midgut epithelial cells and create pores that cause cell swelling and lysis and eventually cause death of the insect.

Bacillus thuringiensis. produces crystal [Cry] protein. This Cry protein is toxic to Larvae of certain insects. Each Cry protein is toxic to a different group of insects. The gene encoding cry protein is called "cry gene". This Cry protein isolated and transferred into several crops. A crop expressing a cry gene is usually resistant to the group of insects for which the concerned Cry protein is toxic. There are a number of them, for example, the proteins encoded by the genes cryiAb and cryiiAb control the cotton bollworms. that of cryiAb controls com borer.

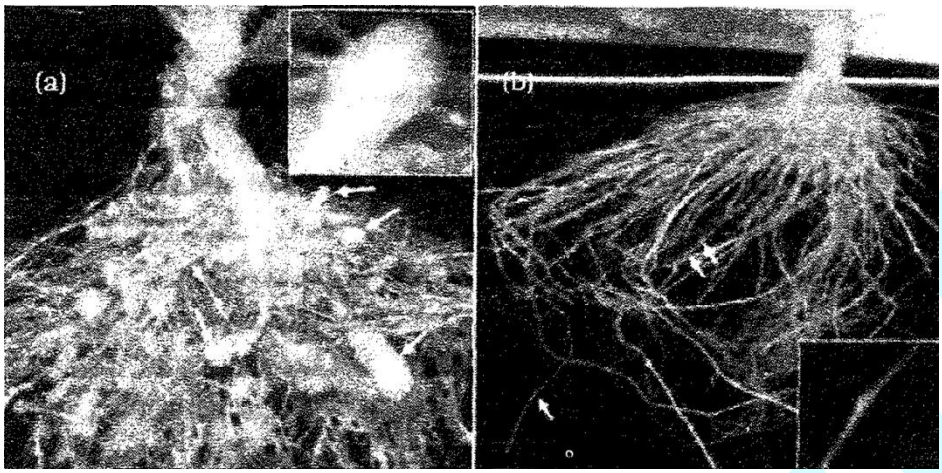
However, gene symbol italics, e.g., cry. The first letter or the protein symbol, on the other hand, is always capital and the symbol is always written in roman letters, e.g., Cry.

(ii) **Nematode resistant plant :**

Several nematodes parasitise a wide variety of plants and animals including human beings. A nematode Meloidegyne incognitia infects the roots of tobacco plants and causes a great reduction in yield. A novel strategy was adopted to prevent this infestation which was based on the process of RNA interference (RNAi). RNAi takes place in all eukaryotic organisms as a method of cellular defense.

This method involves silencing of a specific mRNA due to a complementary dsRNA molecule that binds to and prevents transfation of the mRNA (silencing). 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.

Using Agrobacterium vectors, nematode-specific genes were introduced into the host plant. The introduction of DNA was such that it produced both sense and anti-sense RNA in the host cells. These two RNA's being complementary to each other formed a double stranded (dsRNA) that initiated RNAi and thus, silenced the specific mRNA of the nematode. The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant therefore got Itself protected from the parasite.



Host plant-generated dsRNA triggers protection against nematode infestation : (a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism.

(B) BIOTECHNOLOGYCALAPPUCATIONS IN MEDICINE

(1) Genetically Engineered Insulin

It is a proteinaceous hormone having 51 Amino acids arranged in two polypeptides A and B having 21 and 30 Amino Acids, respeCtively and joined by S-S disulphide bridges.

Sir Edward sharpy-Shafer (1916) was the first to note that diabetes of some persons was because of failure of some islands of pancreas to produce a substance which he called insulin (Derived from the latin, insula, meanning island).

Banting and best (1921) were the first to isolate insulin from dog's pancreas and used it to cure diabetes in man.

The first genetically engineered insulin obtained by recombinant DNA technique with the help of E-Coli was produced by the American firms, Eli-Lilly on July 5, 1983. It has been given the trade name humulin and has been approved for clinical use.

Insulin used for diabetes was earlier extracted from pancreas of slaughtered cattle and pigs. Insulin from an animal source, though caused some patients to develop 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. In mammals, including humans, insulin is synthesised as a prohormone (like a pro-enzyme, the pro-hormone also needs to be processed before it becomes a fully mature and functional hormone) which contains an extra stretch called the C peptide.

This C peptide is not present in the mature insulin and is removed during maturation into insulin. The main challenge for production of insulin using rONA techniques was getting insulin assembled into a mature form. In 1983, Eli Lilly an American company 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 disulfide bonds to form human insulin.

(2) Gene Therapy :

- A new system of medicine gene therapy, may develop to treat some hereditary diseases such as SCID, haemophilia etc.

- Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child/embryo. Here genes are inserted into a person's cells and tissues to treat a disease. Correction of a genetic defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene.
  The first clinical gene therapy was given in 1990 to a 4-year old girl with adenosine deaminase (ADA) deficiency. This enzyme is crucial for the immune system to function. The disorder is caused due to the deletion of the gene for adenosine deaminase. In some children ADA deficiency can be cured by bone marrow transplantation; in others it can be treated by enzyme replacement therapy, in which functional ADA is given to the patient by injection. But the problem with both of these approaches that they are not completely curative. As a first step towards gene therapy, lymphocytes from the blood of the patient are grown in a culture outside the body. A functional ADA eDNA (using a retroviral vector) is then introduced into these lymphocytes, which are subsequently returned to the patient. However, as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes. However, if the gene isolate from marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.

(3) Medical Diagnosis of Disease (Molecular diagnosis)

You know that for effective treatment of a disease, early diagnosis and understanding its pathophysiology is very important. Using conventional methods of diagnosis (serum and urine analysis, etc.) early detection is not possible. Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immunosorbent Assay (EUSA) are some of the techniques that serve the purpose of early diagnosis.

Presence of a pathogen (bacteria, viruses, etc.) is normally suspected only when the pathogen has produced a disease symptom. By this time the concentration of pathogen is already very high in the body. However, very low concentration of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected by amplification of their nucleic acid by PCR. PCR is now routinely used to detect HN in suspected AIDS patients. It is being used to detect mutations in genes in suspected cancer patients too. It is a powerful techqnique to identify many other genetic disorders.

A single stranded DNA or RNA, tagged with a radioactive molecule (probe) 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 hence not appear on the photographic film, because the probe will not have complimentarity with the mutated gene.

EUSA 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 that have had their DNA manipulated to possess and express an extra (foreign) gene are know as transgenic animals. Transgenic rats. rabbits. pigs. sheep. cows and fish have been produced. although over 95 percent of all existing transgenic animals are mice.

(i) Normal physiology and development : Transgenic animals can be specifically designed to allow the study of how genes are regulated. and how they affect the normal functions of the body and its development. e.g,. study of complex factors involved in growth such as insulin-like growth factor. By introducing genes from other species that alter the formation of this factor and studying the biological effects that result. information is obtained about the biological role of the factor in the body.

(ii) **Study of disease :** Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease. These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible. Today transgenic models exist for many human diseases such as cancer cystic fibrosis, rheumatoid arthritis and Alzheimer's

(iii) **Biological products :** Medickles required to treat certain human diseases can contain biological products, but such products are often expensive to make. Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as human protein (α-1-antitrypsin) used to treat emphysema. Similar attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis. In 1997, the first transgenic cow, Rosie. produced human protein-enriched milk (2.4 grams per litre). The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies that natural cow-milk.

(iv) **Vaccine safety :** Transgenic mice are being developed for use in testing the safety of vaccines before they are used on humans. Transgenic mice are being used to test the safety of the polio vaccine. If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of the vaccine.

(v) **Chemical safety testing :** This is known as toxicity/safety testing. The procedure is the same as thatused for testing toxicity of drugs. Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are then exposed to the toxic substances and the effects studied. Toxicity testing in such animals will allow us to obtain results in less time.

Applications of Recombinant DNA products

Medically useful recombinant products	**Applications**
Human insulin	Treatment of insulin - dependant diabetes
Human growth hormone	Replacement of missing hormone in short stature people.
Calcitonin	Treatment of rickets.
Chorionic gonadotropin	Treatment of infertility.
Blood clotting factor VIII/IX	Replacement of clotting factor missing in patients with Haemophilla A/B.
Tissue Plasminogen activator (TPA)	Dissolving of blood clots after heart attacks and strokes.
Erythropoetin	Stimulation of the formation of erythrocytes (RBCs) for patients suffering from anaemia during dialysis or side effects of AIDS patients treated by drugs.
Platelet derived growth factor	Stimulation of wound healing
Interferon	Treatment of pathogenic viral infections, cancer
Interleukin	Enhancement of action of immune system
Vaccines	Prevention of infectious diseases such as hepatitis B, herpes, influenza, pertusis, meningitis, etc.

Application of Genetically Engineered Microbes

Microbes	Applications
Escherichia coli (gut bacterium)	Production of human insulin, human growth factor interferons, interleukin and so on.
Bacillus thuringiensis (soil bacterium)	Productions of endotoxin (Bt toxin), highly potent, safe and biodegradable insecticide for plant protection.
Rhizobium meliloti (bacterium)	Nitrogen fixation by incorporating "nif" gene in cereal crops.
Pseudomonas putida (bacterium)	Scavenging of oil spills by digesting hydrocarbons of crude oil.
Bacterial strains capable of accumulating heavy metal	Bioremediation (cleaning of pollutants in the enviroment).
Trichoderma (fungus)	Production of enzyme chitinases for biocontrol of fungal diseases in plants.

Transgenics and their potential applications

Transgenic	Useful appications
Bt Cotton	Pest resistance and high yield.
Flavr Savr Tomato	Increased shelf-life (delayed ripening) and better nutrient quality
Golden Rice	Vitamin A and Fe - rich
Cattles (cow, sheep, goat)	Therapeutic human proteins in their milk
Pig	Organ transplantation without risk of rejection

GOLDEN KEY POINTS

1. First transgenic animal was mouse contain gene for growth hormone. This enlarged mouse was known as Supennouse.
2. First introduced transgenic crop in India (2002) is Bt-cotton.
3. Charles Weismann of university of Zurich, obtained interferon through recombinant E.coli (1980)
4. Microbes have been engineered to produce Human growth Hormone (HGH) for curing dwarfism.
5. Vaccines which are produced by genetic engineering e.g., for Hepatitis-Band Herpes virus.
6. Nitrogen fixation genes may be transferred from bacteria to the major food crops to boost food production without using expensive fertilizers.
7. **Bioremediation :** In pollution control, microbes have been engineered to break up the crude oil spills. Dr. Ananda Mohan Chakraborthi introduced plasmids from different strains into a single cell of Pseudomonas putida. The result was new genetically engineered bacterium which would clean the oil spills called. "Superbug" (Oil eating bug). He transferred four types of genes/plasmids in this bacteria. These are OCT, XYL, CAM & NAH.
8. **Genetic modified food** - The food is prepared from genetically modified crop [transgenic] is called genetically modified food or G.M.Food.

ETHICAL ISSUES

The manipulation of living organisms by the human race cannot go on any further. without regulation. Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms.

Going beyond the morality-of such issues, the biological significance of such things is also important. Genetic modification of organisms can have unpredicatable results when such organisms are introduced into the ecosystem.

Therefore, the Indian Government has set up organisations 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.

The modification/usage of living organisms for public services (as food and medicine sources, for example) has also created problems with patents granted for the same.

GM crops are already in cultivation in U.S.A, Europe and several other countries. In India, some insect resistant cotton varieties expressing cry genes have reached the farmers, fields. It has been argued that transgenic crops may be harmful to the environment. The two points, Firstly, the transgene may be transferred through pollen from these crops to their wild relatives secondly GM crops may pollute the envionnent.

BIO - PATENT

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

(A) An invention [including product]
(B) An improvement in an earlier invention
(C) The process of generating products and
(D) A concept or design

There is growing public anger that certain companies are being granted patents for products and technologies that make use of the genetic materials, plants and other biological resources that have long been identified, developed and used by farmers and indigenous people of a specific region/country.

Rice is an important food grain, the presence- of which goes back thousands of years in Asia's agricultural history. There are an estimated 200.000 varieties of rice in India alone. The diversity of rice in India is one of the richest in the world. Basmati rice is distinct for its unique aroma and flavour and 27 documented varieties of Basmati are grown in India. There is reference to Basmati in ancient texts, folklore and poetry, as it has been grown for centuries. 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. The patent extends to functional equivalents, implying that other people selling Basmati rice could be restricted by the patent. Several attempts have also been made to patent uses, products and processes based on Indian traditional herbal medicines, e.g,. turmeric neem. If we are not vigilant and we do not immediately counter these patent applications, other countries/individuals may encash on our rich legacy and we may not be able to do anything about it.

BIO-PIRACY

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

Most of the industrialised nations are rich financially but poor in biodiversity and traditional knowledge. In contrast the developing and the underdeveloped world is rich in biodiversity and traditional knowledge related to bio-resources. Traditional knowledge related to bio-resources can be exploited to develop modern applications and can also be used to save time,. effort and expenditure during their commercialisation.

There has been growing realisation of the injustice, inadequate compensation and benefit sharing between developed and developing countries. Therefore, some nations are developing laws to prevent such unauthorized exploitation of their bio-resources and traditional knowledge. The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill, that takes such issues into consideration, induding patent terms emergency provisions and research and development initiative.

BEGINN BOX-2

1. Bt toxin kills the insect by
   (1) Blocking the nerve conduction (2) Damaging the surface of trachea
   (3) By creating pores in the tracheal system (4) By creating pores in the mid gut

2. Which is not an application of modern biotechnology?
   (1) Production of humulin (2) Developing a DNA vaccine '
   (3) Gene therapy (4) Production of cheese and butter

3. Transgenic Brassica napus has been used for the synthesis of :-
   (1) Hirudin (2) Heparin (3) Polgalacturonase (4) Cry protein

4. Transgenic tobacco plant was developed by the process of RNAinterference, was resistant against the infection of :-
   (1) Algae Scenedesmus (2) Fungi Fusarium
   (3) Bacteria : Bacillus thurigenesis (4) Nematode : Melodigyne incognita

5. The first clinical gene therary was given for treating ,
   (1) Rheumatoid arthritis (2) Adenosine deaminase defeciency
   (3) Diabetes (4) Chicken pox

ANSWER KEY

BEGINNER'S BOX-1

1.	(1)	**2.**	(3)	**3.**	(3)	**4.**	(2)	**5.**	(3)

BEGINNER'S BOX-2

1.	(4)	**2.**	(4)	**3.**	(1)	**4.**	(4)	**5.**	(2)