## UNIT - IX

## CHAPTER – 11 : BIOTECHNOLOGY : PRINCIPLES AND PROCESSES

- Biotechnology deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans.

- The definition given by EFB (European Federation of Biotechnology) is as follows; 'The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services'.

**Principles of Biotechnology:**

- Genetic engineering: 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.
- Maintenance of sterile 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 techniques of genetic engineering which include creation of recombinant DNA, use of gene cloning and gene transfer, overcome this limitation and allow us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into the target organism.

In a chromosome there is a specific DNA sequence called the origin of replication, which is responsible for initiating replication. Therefore, for the multiplication of any alien piece of DNA in an organism it needs to be a part of a chromosome which has a specific sequence known as 'origin of replication'. Thus, an alien DNA is linked with the origin or replication, so that, this alien piece of DNA can replicate and multiply itself in the host organism. This is known as Cloning.

The construction of the first recombinant DNA emerged from the possibility of linking a gene encoding antibiotic resistance with a native Plasmid of *Salmonella typhimurium*.

The cutting of DNA at specific locations became possible with the discovery of the so-called 'Molecular scissors" – restriction enzymes. The cut piece of DNA was then linked with the plasmid DNA with the help of another enzyme called DNA ligase. These

plasmid DNA act as vectors to transfer the piece of DNA attached to it. A plasmid can be used as vector to deliver an alien piece of DNA into the host organism.

"Recombinant DNA technology" or also called "Genetic Engineering" deals about, the production of new combinations of genetic material (artificially) in the laboratory. These "recombinant DNA" (rDNA) molecules are then introduced into host cells, where they can be propagated and multiplied.

**Basic steps in rDNA:**

- Identification of DNA with desirable genes
- Introduction of the identified DNA into the host
- Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

**Tools of Recombinant DNA Technology:**
**Restriction Enzymes (Molecular Scissors):**

Restriction enzymes belong to a larger class of enzymes called Nucleases. These are of two kinds; Exonucleases and Endonucleases. Exonucleases remove nucleotides from the ends of the DNA whereas, endonucleases make cuts at specific position within the DNA.

Example, the first restriction endonuclease – Hind II, always cut DNA molecules at a particular point by recognizing a specific sequence of six base pairs. This specific base sequence is known as the Recognition Sequence for Hind II.

Each restriction endonuclease recognizes a specific Palindromic Nucleotide Sequences in the DNA.

***What are Palindromes?***

These are groups of letters that form the same words when read both forward and backward, eg. "MALAYALAM". The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same.

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 called Sticky ends. The same enzyme cuts both DNA

(vector and foreign DNAs) strands at the same site with sticky ends and these can be joined together using DNA-ligase.

**Separation and Isolation of DNA fragments (DNA of interest):**

- The cutting of DNA by restriction endonucleases results in the fragments of DNA.
- These fragments can be separated by a technique known as Gel Electrophoresis.
- The DNA fragments are separated according to their size.
- The separated DNA fragments can be visualized only after staining the DNA with Ethidium bromide followed by exposure to UV radiation. Now DNA fragments appear bright orange coloured bands.
- The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as *Elution*.
- These DNA fragments are purified and used in constructing recombinant DNA with cloning vector.

**Cloning Vectors (Vehicles for Cloning):**

Vector serves as a vehicle to carry a foreign DNA sequence into a given host cell.

**Salient features of a Vector:**

- It should contain an origin of replication (*ori*) so that it is able to multiply within the host cell.
- It should incorporate a selectable marker (antibiotic resistance gene), which will allow to select those host cells that contain the vector from amongst those which do not.
- The vector must also have atleast one unique restriction endonuclease recognition site to enable foreign DNA to be inserted into the vector during the generation of a recombinant DNA molecule.
- The vector should be relatively small in size.

The most commonly used vectors are – Plasmids and Bacteriophages.

**Identification of recombinants**:

***Insertional inactivation***:

The most efficient method of screening for the presence of recombinant plasmids is based on the principle that the cloned DNA fragment disrupts the coding sequence of a gene. This is termed as Insertional Inactiviation.

For example, the powerful method of screening for the presence of recombinant plasmids is referred to as Blue-White selection. This method is based upon the insertional inactivation of the lac Z gene present on the vector. The lac Z gene encodes the enzyme beta-galactosidase, which can cleave a chromogenic substrate into a blue coloured product. If this lac Z gene is inactivated by insertion of a target DNA fragment into it, the development of the blue colour will be prevented and it gives white coloured colonies. By this way, we can differentiate recombinant (white colour) and non-recombinant (blue colour) colonies.

**Competent Host (Introduction of recombinant DNA into host cells):**

In rDNA technology, the most common method to introduce rDNA into living cells is transformation, during which cells take up DNA from the surrounding environment.

1) Simple chemical treatment with divalent calcium ions increases the efficiency of host cells (through cell wall pores) to take up the rDNA plasmids.
2) rDNA can also be transformed into host cell by incubating both on ice, followed by placing them briefly at 42$^{o}$C (Heat Shock), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.
3) In Microinjection method, rDNA is directly injected into the nucleus of cells by using a glass micropipette.
4) Biolistics / Gene gun method, it has been developed to introduce rDNA into mainly plant cells by using a Gene / Particle gun. In this method, microscopic particles of gold / tungsten are coated with the DNA of interest and bombarded onto cells.
5) The last method uses *"Disarmed Pathogen"* Vectors (*Agrobacterium tumefaciens*), which when allowed to infect the cell, transfer the recombinant DNA into the host.

**Processes of Reombinant DNA Technology:**

rDNA technology involves several steps in specific sequence such as,

- Isolation of DNA
- Fragmentation of DNA by restriction endonucleases
- Isolation of desired DNA fragment
- Ligation of the DNA fragment into a vector
- Transferring the recombinant DNA into the host
- Culturing the host cells in a medium at large scale and extraction of the desired product

**Isolation of DNA:**

DNA should be isolated in pure form, without macromolecules. Hence cell wall can be broken down by treating the bacterial cells / plant or animal tissue with enzymes such as Lysozyme (bacteria), cellulose (plant cells), chitinase (fungus).

DNA should be removed from its histones proteins and RNAs. This can be achieved by using enzymes ribonuclease for RNA and Proteases for histone proteins. Finally purified DNA precipitates out after the addition of chilled Ethanol.

**Fragmentation of DNA:**

- Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme.
- DNA is a negatively charged molecule, hence it moves towards the positive electrode (anode).
- After having cut the source DNA as well as the vector DNA with a specific restriction enzyme, the cut out gene of interest from the source DNA and the cut vector with space are mixed and ligase is added. This results in the preparation of recombinant DNA.

**Amplification of Gene of Interest using PCR:**

PCR stands for Polymerase Chain Reaction. In this reaction, multiple copies of the gene of interest is synthesized in vitro using two sets of primers and the enzyme DNA polymerase.

The process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times. Such repeated amplification is achieved by the use of a themostable DNA polymerase (*Taq* DNA Polymerase – isolated from a bacterium, *Thermus aquaticus*). The amplified fragment if desired can now be used to ligate with a vector for further cloning.

**Insertion of Recombinant DNA into the Host Cell / Organism:**

There are several methods of introducing the ligated DNA into recipient cells. If a recombinant DNA bearing gene for resistance to an antibiotic (ampicillin) is transferred into *E.coli* cells, the host cells become transformed into ampicillin-resistant cells. If we spread the transformed cells on agar plates containing ampicillin, only transformants will grow, untransformed recipient cells will die. The ampicillin resistance gene in this case is called a selectable marker.

**Obtaining the Foreign Gene Product:**

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.

**Bioreactors :**

To produce in large quantities, the development of bioreactors, where large volume 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).

**Stirred-tank reactor:**

It is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates 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:**

The processes include separation and purification, which are collectively referred to as downstream processing. Strict quality control testing for each product is also required.

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# CHAPTER – 12 : BIOTECHNOLOGY AND ITS APPLICATIONS

The applications of biotechnology include therapeutics, diagnostics and genetically modified crops for agriculture, processed food, bioremediation, waste treatment, and energy production.

Three critical research areas of biotechnology are;

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

## Biotechnological Applications in Agriculture:

The three options that can be thought for increasing food production are,

- Agro-chemical based agriculture
- Organic agriculture; and
- Genetically engineered crop-based agriculture.

The Green Revolution has succeeded in tripling the food supply but yet it was not enough to feed the growing human population. Scientists have decided that use of genetically modified crops is a possible solution.

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

- Made crops more tolerant to abiotic stresses
- Reduced reliance on chemical pesticides
- Helped to reduce post harvest losses
- Increased efficiency of mineral usage by plants
- Enhanced nutritional value of food, eg., Vitamin 'A' enriched rice.

## Bt Cotton:

Some strains of *Bacillus thuringiensis* produce 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 creates pores that cause cell swelling and lysis and eventually cause death of the insect.

Bt toxin genes were isolated from *B. thuringiensis* and incorporated into the several crop plants such as cotton. The toxin is coded by a gene named '*cry*'. There are a number of them, for example, the proteins encoded by the genes *cryIAc* and *cryIIAb* control bollworms and *cryIAb* controls corn borer.

**Pest Resistant Plants:**

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

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 (ds DNA) 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.

**Biotechnological Application 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:**

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 synthesized as a prohormone, 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.

In 1983, Eli Lilly an American company prepared two DNA sequences corresponding to A and B, chains of human insulin and introduced hem 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.

**Gene Therapy:**

Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child / embryo. 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 genet 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.

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 cDNA is then introduced into these lymphocytes, which are subsequently returned to the patient. However, if the gene isolate from marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.

**Molecular Diagnosis:**

Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno-sorbent Assay (ELISA) are some of the techniques that serve the purpose of early diagnosis.

**PCR:**

A very low concentration of a bacteria or virus can be detected by amplification of their nucleic acid by PCR. PCR is now routinely used to detect HIV in suspected AIDS patients. It is being used to detect mutations in genes in suspected cancer patients too.

**ELISA:**

It is based on the principle of antigen-antibody interaction. Infection by pathogen can be detected by presence of antigens or by detecting the antibodies synthesized against the pathogen.

**Transgenic Animals:**

Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as Transgenic Animals.

***Reasons for the production of transgenic animals:***

a) ***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.

b) ***Study of disease:*** Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease, so that investigation of new treatments for diseases is made possible.

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 being developed for use in testing the safety of vaccines before they are used on humans (polio vaccine).

e) ***Chemical safety testing:*** 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.

**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.

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