BIOTECHNOLOGY: PRINCIPLE AND PROCESSES

1. Biotechnology

It is an integrated branch of biology which deals with the techniques of using live organisms or enzymes from organisms in order to produce different products and processes useful to humans. The term 'biotechnology' was coined by Karl Ereky in 1917. It encompasses both traditional and modern biotechnology.

2. Principles of Biotechnology

Two core techniques that enabled birth of modern biotechnology are

A. **Genetic Engineering**It refers to the techniques used for modification of chemical nature of genetic material (DNA/RNA) and their introduction into another organism (host) to change the phenotypic characters of that organism. Paul Berg is known as the Father of Genetic Engineering.

B. **Sterilisation Methods** These methods are used to maintain microbial contamination-free conditions in chemical engineering processes to enable the growth and manipulation of only the desired microbes or cells. It is used to get large quantities of products like vaccines, enzymes, antibiotics, etc.

3. **Recombinant DNA Technology** (RDT)

It is a technique of genetic engineering which combines two genes from two different sources to create a new recombinant gene. Recombinant DNA was first created by Stanley Cohen and Herbert Boyer in 1972.

Tools of Recombinant DNA Technology The key tools involved in RDT are

A. Enzymes are involved in genetic engineering which simplify this complex process. The various enzymes involved are

(i) Restriction Enzymes (Molecular scissors) Arber, Smith and Nathan discovered the restriction enzymes. These are enzymes which are used for cutting of DNA at specific locations during DNA technology.

Restriction enzymes belong to a larger class of enzymes called nucleases, which are of two types, i.e. (a) exonucleases that remove nucleotides from the ends of the DNA (either 5 ′ or 3 ′) in one strand of duplex and (b) endonucleases make cuts at specific position within the DNA.

(ii) **DNA Ligases** (Molecular glue) These enzymes repair broken DNA fragments by joining two nucleotides.

(iii) **Alkaline Phosphatase** (AP) This enzyme removes the phosphate group from the 5 ′ end of a DNA molecule, leaving a free 5 ′ hydroxyl group, which prevents unwanted self-ligation of vector DNA molecules during the formation of recombinant DNA.

(iv) **DNA Polymerases** This enzyme helps in in vitro synthesis of complementary DNA (cDNA) strand on DNA templates.

B. **Cloning Vectors**The DNA molecule that can carry a foreign DNA segment and replicate inside the host cell is called as a vector. These vectors have the ability to replicate within bacterial cells independent of the control of chromosomal DNA. Following vectors are commonly used in recombinant DNA technology

(i) **Plasmids**These are small, autonomously replicating usually circular, extrachromosomal double-stranded DNA molecule that occurs in many bacteria and some yeasts, e.g. E. coli containing vector pBR322.

(ii) **Bacteriophages**These are viruses infecting bacteria. They are used as the vector because of very high copy number of their genome within the bacterial cells.

Following features are required to facilitate cloning into a vector

(a) **Origin of replication** (Ori) is a sequence from where replication begins.

(b) **Selectable markers** help in identifying or selecting transformants and eliminating the non-transformants. Transformation is a procedure through which piece of DNA is introduced into the host bacterium.

(c) **Cloning sites** (recognition sites) are generally required to link foreign or alien DNA with vector.

Ligation of foreign DNA is carried at restriction site present in one of the two antibiotic resistance genes. This process results in inactivation of antibiotic resistance gene and called as insertional inactivation.

(iii) **Vectors for Cloning Genes in Plants and Animals** In plants, the Tumour inducing (Ti) plasmid of Agrobacterium tumefaciens (a pathogen of several dicot plants) is used as a cloning vector. The proper vector for cloning in animal cells is SV40.

Similarly, retrovirus, adenovirus, papilloma virus are also used as a cloning vector in animals because of their ability to transform normal cells into cancerous cells.

C. Competent Host Organism

(For Transformation with Recombinant DNA) DNA is a hydrophilic molecule, so it cannot pass through cell membranes. In order to force bacterial cell to take up the plasmid, they must first be made competent.

The competency is the ability of a cell to take up foreign DNA. The cells can be made competent by following methods

(i) Heat shock

(ii) Microinjection

(iii) Biolistic/gene gun

4. Processes of Recombinant DNA Technology

It involves various steps in a specific sequence. Stages of recombinant DNA technology are described as below

(i) **Isolation of Genetic Material** (DNA) In majority of organisms, DNA is the genetic material. The isolation of DNA can be achieved by treating bacterial cells/plant or animal tissues with enzymes such as lysozyme (bacteria), cellulase (plant cells) and chitinase (fungus). RNA and proteins can be removed by ribonuclease and protease.

(ii) **Cutting of DNA at Specific Locations** Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme. This is done at the optimal conditions for that specific enzyme.

(iii) **Separation and Isolation of DNA Fragments**The cutting of DNA by restriction endonucleases results in the formation of fragments of DNA which are separated by a technique known as **gel electrophoresis**.

The separated DNA fragments can be visualised only after staining the DNA with Ethidium Bromide (EtBr) followed by exposure to UV radiation. The bright orange coloured bands of DNA are seen when exposed to UV light.

(iv) **Amplification of Gene Interest using PCR** In this stage, several replicas of useful genes are synthesised by using two sets of primers, a DNA polymerase enzyme and dNTPs by an in vitro method known as Polymerase Chain Reaction (PCR). It was

developed by Kary Mullis in 1985. This method involves three processes, which are as follows

- **Denaturation** at 95°C for 15 minutes which separates the two stands of dsDNA.
- **Annealing** at 40-60°C with two sets of oligonucleotide primers to each separated strand.
- **Extension** where DNA Taq polymerase adds complementary dNTPs to extend the primers by adding nucleotides complementary to the template.

(v) **Ligation of DNA Fragment into a Vector** This process requires a vector DNA and a source DNA. In order to obtain sticky ends, both of these should be cut with the same endonuclease, after which, both are ligated by mixing vector DNA, gene of interest and enzyme DNA ligase to form the recombinant DNA/ hybrid DNA.

(vi) **Insertion of Recombinant DNA into Host Cell/Organisms**

It can be achieved by several methods, before which the recipient cells are made competent to receive DNA.

A recombinant DNA containing gene for resistance to an antibiotic is transferred to host cells to produce ampicillin resistant cells which contain ampicillin resistant gene and are called as selectable markers.

(vii) **Obtaining or Culturing the Foreign Gene Product**The desired protein encoded gene is expressed in the heterologous host, it is called recombinant protein. The cells harbouring cloned genes of interest are grown on a small scale in the laboratory. These cell cultures are used for extracting the desired protein using various separation techniques.

5. Bioreactors

These are the large volume vessels (approximately 100-1000 L), which provide the optimal conditions for achieving the desired product by providing optimum growth conditions like temperature, pH, substrate, salts, vitamins and oxygen.

- The components of a bioreactor include an agitator system, an oxygen delivery system, a foam control system, a temperature control system and pH control system and a sampling part to withdraw culture periodically.
- The most commonly used bioreactors are stirring type bioreactor, simple stirred-tank bioreactor and sparged stirred-tank bioreactor.

6. Downstream Processing

After completion of the biosynthetic phase, 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 of products, which are collectively called the **downstream processing**.