Chapter - 9

Biotechnology: Principles and processes

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Biotechnology word has been derived from the word "Bio" = Living and technology = technique. Thus biotechnology is the branch of Science in which engineering and technology is used to solve the biological problems related to the requirement of Organism.

→ It was used in ancient time in daily life for fulfilling needs of human life. for example - Curdling of milk, making cheese, preparing wine, bread and vinegar by using activity of various microbes and also selecting Suitable Variety of Crops for cultivation. This may be called as Ancient Biotechnology or classical biotechnology

→ The modern biotechnology in many ways is similar to the older (classical) biotechnology, where we have now developed advanced and modern methods for selective manipulation of genetic material of useful microbes and the genome of higher plants and livestock animals for welfare of human.

→ Thus use of a genetically modified organisms (GMO) for production of new and better products in greater amount for the human life can be called as modern biotechnology. The modern biotechnology includes DNA recombination, gene therapy, production of DNA vaccine, gene Synthesis, and development of test tube baby through in vitro fertilisation.

→ Definition of Biotechnology :→ Biotechnology is the application of scientific and engineering principles for processing of materials formed

by the biological agents such as microbes, whole cells or multicellular organisms to provide goods and service to human.

OR

In simple way, Use of microbes such as bacteria, unicellular animals and plants, different cells, their components and their biological processes to produce useful substances for betterment of mankind is called Biotechnology.

OR

According to European fedaration of biotechnology (EfD) - Biotechnology is the integrated use of biochemistry, microbiology and engineering to obtain technological or industrial application of microorganisms, tissues, cells and organs grown in tissue culture.

* Principles of biotechnology :→ Modern biotechnology is based on two basic principles. These are as follow ⟹

(i) Genetic engineering :→ It deals with the technique to change structural organisation of the genetic material i.e. DNA & and RNA. This technique provides for transfer of desirable genes in to host cell by the process of recombinant DNA technology.

(ii) Bioprocess engineering :→ It deals with methods to grow or culture desired and modified micro-organisms, prokaryotic cells, plant and animal cells ~~under~~ by tissue culture technique. These are further used to obtain

better quality industrial products such as antibiotics, enzymes, pesticides, vaccine etc. in greater amount.

☆ Concept of Recombinant DNA :→ Sexual reproduction in organism is always beneficial over asexual reproduction. In asexual reproduction the heredity of the progeny remains unchanged where as in sexual reproduction genetic variations occur due to Crossing over.

→ The traditional hybridisation technique in plant breeding and animal breeding bring desired as well as undesired genes in the progeny. By use of modern technique of recombinant DNA, gene cloning and transfer of only disered genes is possible in the target Organisms and transfer of undesired genes can be eliminated.

→ The technique of genetic engineering which includes formation of recombinant DNA, use of gene cloning and gene transfer allows us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes in target organism.

→ Incorporation of foreign DNA into the basic DNA of any organism results into modification of the DNA. The modified DNA is reffered as recombinant DNA. Various steps required to incorporate changes in the structure of DNA by inserting foreign DNA.

one of these enzymes adds methyl groups in the DNA molecule and the other enzyme cuts DNA molecule from a specific site. It was called restriction endonuclease.
Werner Arber and Hamilton O. Smith (1976) discovered restriction endonuclease enzyme.

★ Restriction endonuclease enzyme :→

(i) These enzymes cut DNA at specific sites so these are called "molecular scissors".
(ii) These are found naturally in bacteria such as E. coli, Baccilus, Streptococcus, Thermus aquaticus etc.
(iii) Restriction endonuclease enzymes are of three types – (A) Type-I Endonuclease (R-I) (B) Type-II Endonuclease (R-II) (C) Type-III Endonuclease (R-III)
(iv) The first endonuclease - Hind-II, whose functioning depended on a specific DNA nucleotide sequence was isolated and characterised five years later.
(v) 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.
(vi) presently, more than 900 restriction enzymes are known. These have been isolated from more than 230 strains of bacteria. Each of these recognise different sequences of base pairs in DNA molecule.

("Here Draw a diagram which is given on the Page No-196 of Ncert book of Eco-RI")

→ The technique of genetic engineering in which Desirable gene is combined with plasmid DNA or vector DNA and to form a hybrid DNA. Such type DNA is called recombinant DNA. and it is transfer into host cell this techonology is called recombinant DNA technology.

→ Recombinant DNA was first Constructed by Stanley Cohen and Herbert Boyer in 1972. These Scientists inserted antibiotic resistant gene in to the plasmid (Self replicating extra chromosomal DNA) of Salmonella typhimurium

→ **Steps involved in recombinant DNA technology :→**

There are three basic steps in developing a genetically modified organisms by the use of recombinant DNA technique →

① Identification of DNA having desired genes and its isolation.

② Introduction of the identified and isolated DNA in to host.

③ Maintaining the introduced DNA in the host and it's transfer to the progeny.

☆ **Tools of recombinant DNA technology :→**

The following tools are required to complete the steps involved in recombinant DNA technology.

(i) Restriction enzyme (ii) Cloning vector
(iii) DNA ligase enzyme (iv) Polymerase enzyme
(v) Host organism.

① **Restriction enzyme :→** In the year 1963, two such enzymes were isolated which were responsible for restricting the growth of bacteriophase in Escherichia coli (E-coli).

Nomenclature of restriction endonuclease :-

(i) The first alphabet of the name of enzyme represents the genus from which it has been isolated. This is always written in capital letter.

(ii) The next two alphabets represents the species of that genus. These are written in small letters.
Example - Eco - from Eshcherichia coli
Hin - from Haemophilus influenzae

(iii) The fourth word represent the strain of the genus from which the enzyme has been isolated.

(iv) In case more than on restriction enzymes are isolated from one organism, their number is indicated by Roman numbers in order of their discovery.
Example - Eco-RI (first from R strain of E-coli)
ECO-RII etc. (Second from R, strain of E-coli)

→ Endonucleases enzyme ECO-RI recognizes specific base sequence in DNA molecule and cut it between the base G and A.

→ Restriction enzymes belong to a larger class of enzymes called nuclease. These are of two kinds (i) Exonuclease (ii) Endonuclease

→ Exonuclease remove nucleotides from the ends of the DNA whereas, Endonuclease make cuts at specific position with in the DNA.

→ Each restriction endonuclease functions by recognising a specific base sequence along the length of DNA molecule.

→ The restriction endonuclease binds with the DNA at the recognition site. It then cuts the two strands of DNA helix at specific points

of their Sugar-phosphate backbones. Each restriction endonuclease recognises a specific sequence of nucleotides in the DNA. This specific sequence of nucleotides in the DNA is reffered as palindromic nucleotide sequence.

→ palindrome :→ The groups of letters or alphabets which form the same words on reading forward and backward. Ex- MALAYALAM, SARAS, ~~KANAK~~ KANAK etc.

→ 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. for ex.- the following sequences reads the same on the two strands in 5'→3' direction. This is also true if read in the 3'→5' direction.

5'— GAATTC —3'
3'— CTTAAG —5'

→ Restriction enzymes make cut in the strand of DNA a little away from the centre of the palindrom site and necessarily between the same two bases in the two opposite strands Ex- Eco-RI makes cut in both the DNA strands between G and A bases.

→ This result into formation of Single-stranded portion at the ends of two strands of DNA. These ends are called sticky ends. This Stickiness of the ends facilitates the action of the enzyme DNA ligase.

→ To creat a recombinant molecule of DNA. It is necessary that the foreign DNA and the vector DNA are cut with the same restriction enzyme.

As a result the DNA fragments have the same sticky ends and are complimentary to each other. These ends can be joined together using DNA ligase.

(ii) DNA polymerase :→ This enzyme help in vitro synthesis of complementry templates.

(iii) DNA ligase enzyme :- The ligase enzyme connects or joins the broken segments of DNA. It is used in genetic engineering to connect the foreign DNA with the plasmid DNA.

("Here draw a diagram which is given on the page No - 197 of Ncert book of recombination DNA technique")

☆ Separation and Isolation of DNA fragments :→

→ The cutting of DNA by restriction endonuclease results in the fragments of DNA. These fragments can be separated by a technique known as gel electrophoresis.

→ Since DNA fragments are negative charged molecules they can be separated by forcing them to move towards the positive electrode (anode) under an electric field through medium.

→ Now days, the most commonly used medium is agarose which is a natural polymer extracted from sea weeds.

→ The DNA fragments separate according to their size through sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther it moves.

→ Agarose gel electrophoresis is employed to check

The progression of a restriction enzyme digestion this process is repeated with the vector DNA also.

→ DNA is a negatively charged molecule, hence it moves towards the positive electrode. The process is repeated with the vector DNA also.

→ 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 can see bright orange coloured bands of DNA in a ethidium bromide (EtBr) stained gel ~~expo~~ 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.

("Here draw a diagram which is given on the page No. 198 of electrophoresis in ncert book.")

(iv) Cloning vector :→ The plasmids found in the bacterial cell and bacteriophase can replicate in the bacterial cells independent of the DNA of bacterial chromosome. Hence plasmid and bacteriophase are the most suitable vectors used presently.

→ Bacteriophases because of their high number per cell, have very high copy numbers of their genome with in the bacterial cells. Some plasmid

may have only one or two copies per cell whereas others may have 15-100 copies per cell.

→ If we are able to link an alien piece of DNA with bacteriophage or plasmid DNA. We can multiply its numbers equal to the copy number of the plasmid or bacteriophage.

→ Essential features of vector :→

(i) It can be inserted easily into host cell and can be isolated easily.

(ii) It should freely replicate in host cell.

(iii) It should have one marker gene or marker site which can help in selection of recombinant cells.

(iv) Transformation by it should be easy and perfect.

→ Some important Vectors used in E. coli are as follows —

(i) Plasmid :→ These are small, extra chromosomal double stranded, usually circular DNA molecules found in bacterial cells. The plasmids have the ability of replicate independent of bacterial chromosomal DNA.

(ii) Bacteriophage :→ These are viruses infecting bacteria. Once inside the bacterial cell, the bacteriophage genome multiplies independent of the bacterial chromosome. The Bacteriophage may form very high number of copies of their genome in the bacterial cell.

☆ Features required to facilitate cloning into Vector :→

(i) Origin of replication (ori) :→ This is the sequence of nucleotides from where replication of DNA starts. When any fragment of DNA is linked with it, this sequence, it is replication can take place

in the host cell. This sequence is also responsible for the number of copies of linked DNA. Hence to recover many copies of the target DNA. It should be cloned in the vector which has such sequence which supports high number of copies.

(ii) Selectable marker :→ The vector requires a selectable marker, which helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants.

Transformation is a processes through which a piece of DNA is introduced in a host cell.

→ Normally the genes which code for resistance to antibiotics such as ampicillin, tetracyclin, Chloramphenicol, Kanamycin etc. are considered useful selectable markers in case of E. coli. The normal cells of E. coli do not possess resistance against any of these antibiotics.

(iii) Cloning sites or recognition sites :→ The vector must possess very few, preferably only one site which may be recognised by the commonly used restriction enzymes.

→ The foreign or alien DNA is linked or cloned on this site. presence of more than one recognition sites in the vector may generate several fragments and the gene cloning may become complicated.

→ The ligation of foreign DNA is done at a site present in one of the two antibiotic resistant genes. In case of pBR322 the foreign DNA is cloned at BamHI site i.e. tetracycline resistance gene.

Selection of recombinants or transformants formed by incorporating the foreign gene (DNA) can be done by one of the following methods →

(A) Inactivation of antibiotic resistant gene :→ If a foreign DNA is closed at the Bam HI site in the vector pBr322 the recombinant plasmid will lose resistance to tetracyclin ~~de~~ due to presence of foreign DNA. The recombinants can be selected by plating them on medium containing ampicillin. The recombinants will grow on the medium containing ampicillin but not on the medium which contains tetracyclin.
on the other hand, non recombinants will grow on the medium containing both the antibiotics. In this case, one antibiotic resistance gene helps in selecting the transformants, whereas the other antibiotic resistance gene gets inactivated due to insertion of foreign DNA and helps in selection of recombinants.

(B) Insertional inactivation :→ The selection of the recombinants by inactivation of antibiotic resistant gene is a slow and complicated process. Because it requires simultaneous plating on two plates having different antibiotics. Therefore an alternative selectable markers have been developed to differentiate between recombinants and non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substrate.

→ In this process a recombinant DNA is inserted in the plasmid in the coding sequence of an

enzyme - β-galactosidase. This foreign DNA inactivates the gene for synthesis of this enzyme. This is reffered as insertional inactivation. The bacterial colonies having inserted (recombinant) plasmid show no colouration while those having normal plasmid (non-recombinant) show blue colouration. In
→ this process the enzyme β-galactosidase functions as selectable marker, the presence of which differentiates between recombinants and non-recombinants.

(iv) Vectors for cloning genes in plants and animals :→ Bacteria and viruses are known to transfer their genes in to plants and animals. These microbes are able to transform the eukaryotic cells where they multiply by using the machinery of the host cells. for Example. Agrobacterium tumefaciens is a pathogen of several dicot plants. and it is able to introduce a fragment of it's DNA (T-DNA) in to the genome of host. As a result the normal plant cell is transformed in to tumour cells and form chemicals required by the pathogen.

→ Similarly, retroviruses transform the normal cells of animals in to Cancerous cells.

→ A better understanding of how these pathogens transform their eukaryotic host cells is being used now in biotechnology. The useful cloning vectors are used for introducing genes of interest into plants and animals for the benefit of human. The tumor inducing (Ti) plasmid of Agrobacterium tumefaciens has been now modified into a cloning

Vector which is not pathogenic to plants. Similarly retroviruses have also been modified into non pathogenic form and are now used to deliver or introduce desirable genes into animal cells.

→ Now it is possible to ligate or join a gene or DNA fragment of interest in to a suitable vector and transfer it in to a bacterial plant or animal host cell where it multiplies.

(V) Competent host (for transformation with recombinant DNA) :— The DNA molecule can not pass through the cell membrane because DNA is a hydrophilic molecule. Hence the bacterial cell must be made competent to take up the DNA (plasmid

Note :→ Competency is the ability of a cell to take up foreign DNA.

→ The host cell can be made competent by two methods

(i) Chemical method :→ In this method the host cell are treated with a specific concentration of ~~divalent~~ divalent cation, such as calcium (Ca^{+2}). This increases ~~effien~~ efficiency with which the DNA enters the bacterium through the pores in the cell wall. After this these cells are incubated with recombinant DNA on ice, followed by placing them at 42°C for a short time (heat shock) These are then again put on ice. By this treatment the bacteria become competent to take up the recombinant DNA.

(ii) physical method :→ The foreign DNA can be inserted in to the host cell by the following physical methods :→

(A) Micro-injection :→ In this method foreign DNA or genes are directly injected in to the nucleus of animal cell. A glass needle of 0.5-1.0mm diameter or a micropipette is used for injecting the recombinant DNA.

(B) Biolistics or gene gun :→ By this technique foreign DNA or genes can be introduced in to intact plant cells. In this process the target cells are bombarded with high velocity microparticles of gold or tungston coated with desired DNA. The microparticles of gold or tungston coated with desired DNA are shot in the target cells with the help of microprojectile. This technique is now used in all type of plants all over the world to develop transgenic plants.

(C) Disarmed pathogen vectors :→ In this method the pathogenic vectors are first transformed in to non pathogenic form. These disarmed vectors are used to transfer the desired recombinant DNA in to their hosts. for example retroviruses, papilloma virus in case of animals and Agro-bacterium tumefaciens in case of plants.

☆ Processess of recombinant DNA technology :→ Recombinant DNA technology involves various steps in Specific sequence. These are as follows-

(i) Isolation of the genetic material (DNA).
(ii) Cutting the DNA at Specific location.
(iii) Amplification of gene of interest using PCR.
(iv) Insertion of recombinant DNA into the host cell/organism
(v) Obtaining the foreign gene product.
(vi) Down stream processing.

① Isolation of the genetic material (DNA) :→
In majority of organisms, the genetic material is DNA. In order to cut the DNA with restriction endonuclease, it should be present in pure form, and should be free from other macromolecules.
→ As the DNA is enclosed within the membranes, it is necessary to break open the cell to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and lipids.
→ The bacterial cells/plant or animal cells are treated with enzymes, such as lysozyme (in case of bacteria), Cellulase (In case of plant cell), Chitinase (in case of fungus) to separate nucleic acid from these macromolecules.
→ The RNA can be removed by treatment with ribonuclease and histone proteins are removed by treatment with protease. Other molecule can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled (-20°c) ethanol.
This can be seen as collection of fine threads in the suspension.

⑪ Cutting the DNA at Specific location :→
The purified DNA molecules so collected are incubated with restriction enzyme at suitable conditions. Agarose gel electrophoresis is used to check progress of digestion of DNA by restriction enzyme. Because DNA is a negatively charged molecule, So it moves towards positive electrode (anode). this process is repeated with the vector DNA also. After cutting the desired DNA as

well as the vector DNA, the gene of desired DNA and the cut DNA of vector are mixed and ligase enzyme is added to suspension. As a result of this recombinant DNA is formed.

(iii) **Amplification of gene of interest using PCR :→** Kary mullis (1985) developed a technique by which multiple copies of DNA and gene of interest can be synthesised in vitro. This technique is known as polymerase chain reaction (PCR). This technique involves three steps which are cycled repeatedly and by repeating 20-30 times up to 1 billion copies are made. The three steps involved in PCR technique are as follows :—

(A) **Denaturation :→** The double stranded DNA is denatured by using high temprature of 95°C for 15 seconds. Now each separated single strand acts as a template for DNA synthesis.

(B) **Annealing :→** Two sets of oligonucleotide primers complementry to the single stronds are annealed (hybridised). This is carried out at 50-60°C relatively slightly lower temperature.

(C) **Extension :→** The enzyme DNA polymerase extends the primers using the nucleotides provided in the reaction and the genomic DNA acts as template. The thermostable enzyme Taq DNA polymerase (enzyme isolated from Thermus aquaticus bacterium) is used in the reaction. The amplified fragments can now be use to ligate with a vector for further cloning.

("Here draw a diagram of PCR which is given on the page no. 202 of NCERT book)

(iv) Insertion of recombinant DNA in to the host cell/ organism :→ When the recipient cells become competent to receive, they take up the DNA present in their surrounding. Hence if recombinant DNA containing ampicillin resistant gene is introduced in E. coli. The E. coli cells are transformed in to ampicillin resistant cells. When these E. coli cells are cultured on agar plate containing ampicillin antibiotic, only the transformed recipient cells will grow and the untransformed recipient cells will die. Thus transformed cells of E. coli can be selected. In this case the ampicillin resistance gene is called Selectable marker.

(v) Obtaining the foreign gene product :→ The foreign gene present in the recombinant DNA expressed it self under appropriate conditions. When any protein encoding gene expresses itself in a host cell, it is called recombinant protein. The cells containing cloned genes may be grown in the laboratory on a small scale. These cultures can be used for extracting desired protein and the protein can be purified by different separation technique.

→ These cells can also be multiplied in a continuous culture medium. The old medium can be drained out and fresh medium can be added to the system. This help in increasing the physiological activity of cells and a higher yield of desired protein is obtained. Large quantity of protein on commercial basis can be produced by the use of bioreactors.

☆ **Bioreactors :→** Bioreactors are large sized vessels in which large volumes (100-1000ml) of culture can be biologically processed and converted in to specific products. for achieving desired products, individual enzymes and optimal growth conditions (such as temperature, pH, substrate, salts, vitamins, oxygen etc.) are provided in the bioreactors. Microbial cells, plant cells, animal cells or human cells having desired recombinant DNA are used for obtaining desired products. The most commonly used bioreactors are of stirring type.

→ **Components of bioreactors :→** (i) An agitator system (ii) An oxygen supply system (iii) A foam control system (iv) A temprature control system (v) pH control system (vi) And sample ports for periodically withdrawl of small volume of culture.

→ A simple stirred tank bioreactor has a provision for evenly mixing and uniform availability of oxygen through out the contents of the bioreactor. Sparged stirred tank bioreactor has the provision for bubbling of air throughout the content of bioreactor.

(vi) **Downstream processing :→** The products of bio-synthesis are subjected to a series of processes so that the finished products are available for marketing. The processes include separation and purification which are collectively referred to as downstream processing. The products of biosynthesis are separated and purified and suitable preservatives are added to the products. In case the product has medicinal value and is to be used as drug clinical trials are carried out before the same is released for marketing.