BIOTECHNOLOGY

- The term Biotechnology was given by **Carl Ericay**.
- It is based on the principles of **Molecular Genetics**, **Microbiology** and **Biochemistry**.
- In the familiar examples of Biotechnology such as Making curd and bread, Microorganisms are exploited.
- During 1913 – 1915, **Acetone** and **Butanol** were produced from *Clostridium acetobutylicum*. It was understood that biochemical products could be synthesised at industrial level by using microorganisms.
- During **Second World War** (1940), **Penicillin** was produced by using *Penicillium notatum*.
- In 1970's, **restriction enzymes** were discovered and **recombinant DNA technology** has progressed. It revolutionised the conventional breeding methods.
- **E**uropean **F**ederation of **B**iotechnology defined Biotechnology as ' *a science of utilising the properties and uses of microbes or to exploit cells and cell constituents at industrial level for generating useful products essential to life and human welfare*.

Scope and Applications of Biotechnology

- Biotechnological methods are applied in the production of medicines, veterinary products, chemicals, foods, energy, pollution control and waste management. Some of the areas of its application are:

1. Microorganisms like **Bacteria**, **Actinomycetes** and **Fungi** are used for the production of *antibiotics*. Much improvement in the methods of processing and production was achieved due to biotechnological advances. Now – a- days, it is possible to produce penicillin at a cost of **1%** of total cost of production in 1945.
2. Methods of Biotechnology are used to create and commercially produce pharmaceutical drugs like **anticancer agents, interferon, human insulin** and **growth hormones**.
3. **Sugar substituting agents** are produced and marketed. This has shown much impact on international sugar market. Similarly **oil palm** plants are cloned and cultured on large scale.
4. Commercial production of **Enzymes** is increased. These enzymes are used in starch industry. *Immobilized Glucose Isomerase* is used for mass production of High Fructose Corn Syrup.
5. Food supplement agents such as **Monosodium Glutamate** (Flavouring agent), **Aspertate, Nucleotides** and **Lysine** are produced in large amounts.
6. Genetically engineered microorganisms are used for the production of **Rennet**. It is used for commercial production of *cheese*.
7. Biotechnology helped for the production of non conventional proteins known as **Single celled proteins**.
8. *Bacillus thuringiensis* and **Baculoviruses** such as **NPV** are commercially produced and used as **biopesticides** to protect the crops. Similarly, many improvements are made in the production of **biofertilizers**.

GENETIC ENGINEERING

- Genetic engineering or **Recombinant DNA technology** helps in transferring the genes from one organism to altogether different organism.
- **Watson** and **Crick's** discovery of DNA molecular structure and **Nathans** discovery of *Restriction endonucleases* laid foundations for genetic engineering.
- Recombinant DNA technology uses **Vectors** to transfer the genes from one organism to another organism.
- Genetic engineering is a laboratory technique of *gene manipulation*, which brings about **novel** combination of genes.
- It helps in obtaining multiple copies of desired gene, which is known as **Gene cloning**. Gene cloning is carried out in a computerised machine known as **Thermocycler** that uses the method called *Polymerase Chain Reaction*.

Method

- There are 4 stages in the method of genetic engineering known as

1. Isolation of Gene.
2. Insertion of gene into suitable vector.
3. Introduction of recombinant vector into suitable host.
4. Selection of transformed host cells.

1. Isolation of Gene

- Plant cells are subjected to **cell wall degradation** by using enzymes to obtain protoplasts.
- Unit membranes of the cell protoplasts are dissolved by using **detergents** to obtain protoplasm mass.
- Protoplasm is treated with **phenols** and **nucleases** and later subjected to *gradient centrifugation* to obtain pure DNA.
- DNA is subjected to **restriction endonucleases** treatment to cut into respective genes or DNA fragments.

- **Gel Electrophoresis** separates these DNA fragments on the basis of their relative length or size.
- From a group of genes of the same lengths, the gene with desired sequence is separated by **Southern Blotting technique**.

Restriction Endonucleases

- These are also known as *molecular scissors*.
- About more than **250** restriction enzymes have been discovered.
- These enzymes identify *specific nitrogen sequence* present between adjacent genes and cut the DNA between two specific adjacent bases of that sequence.
- These were discovered by **Nathans**.
- There are two types of Restriction Endonucleases. The first type of enzymes make straight cuts in the DNA molecule. Hence the DNA fragments formed have **blunt** or **Flush** ends.
- The second type of enzymes make *staggered* cuts. Hence the DNA strands are cut at different points of specific sequence known as **Palindromic sequence**. Hence at one end of the fragment *one strand protrudes more* than the other and at another strand the other one protrudes more than the earlier one. Such single stranded protruding ends of the Double stranded DNA fragments are known as **Sticky ends** or **Cohesive ends**. Hence the DNA fragments of different organisms generated by the action of same restriction enzyme can be joined with their sticky ends. This property of restriction enzymes has immense value for the formation of recombinant DNA.
- In the restriction enzyme ***Eco* RI**, ***E*** indicates the genus name *Escherichia*, ***co*** indicates the species name *coli*, **R** indicates the type of **plasmid** that has the genes for the enzyme and the roman numerical **I** indicates the series of restriction endonucleases obtained from that organism with that plasmid.
- This enzyme recognises the **GAATTC** sequence on both the strands of DNA and cuts the two strands between **G** and **A**.

2. Insertion of the isolated gene into a suitable Vector

- The desired gene is inserted into suitable vector to produce indefinite number of copies of gene. It is known as **gene cloning**. The vector acts as a vehicle to carry the gene.
- A vector with the characters such as ***1. Low molecular weight, 2. Having sites for the action of restriction enzymes, 3. Ability to replicate inside the host cell and 4. Having genes for antibiotic resistance***.
- **Plasmids, Bacteriophages** and **Cosmids** are some of the commonly used vectors in Genetic engineering.

Phage vectors

- λ (Lambda) **Phage** is the most commonly used vector among viral vectors. The advantage of using it is that most of the phage DNA is not needed for its replication. In place of it the desired gene of donor organism can be inserted.

Plasmids

- These are *extrachromosomal DNA rings* present in almost all bacterial species.
- They are *inheritable* and carry few genes. These genes determine variety of functions.
- These are very *easy to isolate* and *reintroduce* into bacterial cell.
- Besides natural vectors, artificially modified plasmids such as **pBR 322** (*Boliver & Rodriguez*) and **pUC 19, 101** (*University of California*) are commonly used.
- For isolation of plasmid, the bacterial cell is treated with **E**thylene **D**iamine **T**etra **A**cetic acid and **lysozyme** to dissolve the cell wall. Then the bacterial cell is subjected to *centrifugation* in **Sodium lauryl sulphate** solution to separate the plasmid.

Method

- The isolated plasmid is *cut and opened* by subjecting to **restriction endonuclease** treatment. It is made *linear* with sticky ends.
- The isolated gene is joined to the sticky ends of opened plasmid.
- The enzyme **DNA ligase** forms ester bonds between the ends of DNA strands of plasmid and the isolated gene to form a *circular hybrid* called **Recombinant DNA** or **Chimeric DNA**.

3. Introduction of Recombinant Vector into the Host

- The recombinant DNA obtained in the above step is brought nearer to a suitable host bacterium in the presence of dilute $\mathbf{CaCl_2}$ solution. It renders the bacterial cell to absorb the DNA and changes into *Transformed cell*.
- The recombinant DNA divides in the host cell and carried to successive generations of bacterial cells. In this way Transformed bacterial *clones* are formed.

4. Selection of Transformed cells

- Selection method depends on the nature of cloned gene.

Without using probes:

- If the cell with genes for *antibiotic resistance* has to be selected, the cells first should be incubated for **an hour** in medium without antibiotic to make genes for antibiotic resistance expressed. The cells are then placed in a medium containing antibiotic for selection of colonies containing recombinant DNA.
- **Colony hybridization** method is commonly used to identify the cells with desired genes from number different varieties of cells or colonies. In this method, labeled probes for a specific gene in the form of single **stranded DNA** or **RNA** segments are used. These probes search, and make hydrogen bonds with the desired gene with complementary sequence of transformed cells.

cDNA

- It is **complementary DNA** synthesized on **mRNA** in the presence of enzyme *reverse transcriptase*. It can be used as probe.
- It is cloned upon inserting into a vector or directly used to detect the gene of interest.
- These are used in the diagnosis of *infectious diseases, identification of food contaminants* and a variety of *microbiological tests*.
- **Forensic tests** are conducted by DNA finger printing technology.

Transgenic Plants

- These are plants into which the genes of unrelated organisms are inserted through genetic engineering methods.
- Transgenic plants formation utilises *genetic engineering technique* and *Tissue culture*.
- $\mathbf{T_i}$ (Tumour inducing) **plasmid** of *Agrobacterium tumefaciens* is the most commonly used vector in plant genetic engineering.
- Though initially genetic engineering was carried out in dicots, now it is extended to even monocots like wheat, maize, rice, oats etc.
- Till now about **50** transgenic plants have been produced. But field tests are conducted only in **25** plants.

Uses

- Transgenic plants show *higher crop production* because they are resistant to herbicides, insects and viruses.
- These plants are suitable for **food processing**. *Delayed ripening* and *Bruise resistant* tomato plants are produced by plant transgenics.
- It produced **male sterile lines** of *Brassica napus* (Rape seed). Such plants can be directly used as female parents in hybridization experiments. It reduces the cost for emasculation for hybrid seed production.
- These are used as **bioreactors** for large-scale production of *commercially valuable products*, *specialized medicines*, *chemicals* and *antibodies*. Such usage is known as **Molecular farming**.
- These plants are used for **identification** of *regulatory sequences* for many genes.