BIOTECHNOLOGY :

- **"Biotechnology is the controlled use of biological agents such as microorganisms or certain cellular components for beneficial purposes."** (Definition by U.S National Foundation).
- Any technique in which a living organism is used to produce a useful product or desirable chemical change is an example of biotechnology.
- The industrial use of microorganisms is one aspect of biotechnology. To day with the development of modern technologies, the scope of biotechnology has been considerably expanded.
- One of them is the recombinant DNA technology. Recombinant DNA technology involves a complex sequence of redactions that allows manipulation of the genetic nature of the gene or organism and is popularly referred to as genetic engineering.

RECOMBINANT DNA (r DNA) TECHNOLOGY :

- Recombinant DNA technology involves breakage of a DNA molecule at desired places, isolation and insertion into another DNA molecule.
- The product thus obtained is called r DNA. It refers to the creation of new combinations of DNA segments that are not found naturally.
- Although crossing over results naturally in a type of recombinant DNA, the term rDNA is used only for the DNA segment produced by joining segments derived from different biological sources.

I. Recombinant DNA technology involves the following basic steps:

- **1. Isolation of donor DNA for cloning:** Restriction endonucleases cut double strand DNA at specific places. Thus restriction fragments of DNA are produced. These fragments can be separated by **gel electrophoresis**.
- A restriction fragment containing desired base sequence (desired gene) can be identified by southern blotting technique. This DNA to be cloned is called **donor DNA**.
- Some times gene to be cloned is synthesized artificially in the laboratory from nucleotides.
- **2. Isolation of vector:** Find a suitable cloning vector, which is a self replicating genetic material and can carry the donor DNA.
- Commonly used vectors are **plasmids of bacteria**. Bacterial cells are treated with enzyme lysozyme and EDTA solution (ethylene diamine tetra acetic acid) to dissolve cell walls. Later these cells are treated with sodium lauryl sarcosinate solution.
- Other vectors are **Bacterial artificial chromosome** (BAC) & **Yeast artificial chromosome** (YAC)
- **BAC & YAC are used for much longer fragment manipulation**
- Plasmids are purified by gel electrophoresis method, or ultra centrifugation. Cloning vector serves as the carrier for the donor DNA.
- **3. Formation of chimeric DNA:** Vector DNA is cleaved at specific sites with the restriction endonuclease with which donor DNA is cut. It cuts the vector DNA in such a way that it forms complementary single stranded ends (stickyends).
- These ends are capable of joining the fragments of donor DNA that has similar complementary sticky ends. Complementary sticky ends of donor DNA and vector DNA are joined by using an enzyme **ligase** to form a hybrid DNA called r **DNA** or **chimeric DNA.**
- **4. Uptake of chimeric DNA:** The hybrid DNA is then added to a suitable recipient bacterial suspension, which takes up the rDNA.
- Chimeric DNA can be introduce directly by using calcium chloride, or by a process called **phage-mediated genetic transfer called transudation.**
- **5. Selection of the cells containing chimeric DNA:** The bacteria containing rDNA are identified by using **genetic markers,** or by **colony hybridisation technique.**
- **6.** The genetically engineered bacteria are propagated in large quantities and the product generated by the transplanted gene is recovered from the culture.

i) Enzymes used in r DNA technology :

- Three categories of enzymes, endonucleases, exonucleases and ligases are used in rDNA technology.

a) Endonucleases:

- The success of rDNA technology is due to the discovery of **restriction endonucleases.**
- These endonucleases are the enzymes that make sequence specific cuts in DNA and hence are known as **molecular scissors.**
- Some of the endonucleases clave the DNA at a nucleotide sequence called **restriction site**.
- Hence they are known as **restriction endonucleases.** They are widely used in rDNA research.
- Restriction endonucleases recognise a specific base sequence in DNA molecule and make 2 cuts, one in each strand.
- An important feature of restriction endonucleases is that they produce limited number of cuts in a DNA molecule, as a particular enzyme recognises unique sequences.
- There are 2 distinct arrangements of these breaks.
  1) If both strands break at the centre of symmetry, it generates DNA strands with blunt **ends (flush ends).**

2) If the cuts are symmetrically placed around the line of symmetry, it generates DNA strands with **sticky (cohesive)** ends.

- The recognition sequence of Type II enzymes is symmetrical i.e. sequence in 5' to 3' directions on one strand is same as the sequence in 5' to 3' direction on the complemental strand **These sequences that read the same on both strands are known as palindromes.**
- The sticky ends bind to the complementary nitrogen bases of the other DNA fragments by hydrogen bonds.

b) Exonucleases and Ligases:

- Exonucleases cleave nucleotides from the ends of polynucleotides molecule. They hydrolyse phospodiester bonds of terminal nucleotides.
- Ligases help in joining two nucleotide fragments, using energy derived from the simultaneous hydrolysis of nucleotide triphosphate.

ii) Vectors :

- Vectors arc **cloning vehicles.** They carry DNA molecules. To be useful, a vector must have three properties.
- 1) It must be able to replicate
- 2) It should be possible to introduce vector DNA in to the cell.
- 3) It must be possible to grow and identify them easily.
- There are three most common types of vectors: **Plasmids**, **Bacteriophages** and **cosmids.**

1. Plasmids: Many bacteria have several copies of circular DNA in addition to a chromosome. They are called plasmids.

- They contain genes and replicate independently. Therefore they are carried from generation to generation. pBR322 is one of the earliest genetically engineered plasmids.

2. Bacteriophagcs: These are small particles capable of growing only in bacteria. They contain protein molecules that form a coat or Phage head.

- One nucleic acid molecule is present in the head.e.g Lambda and **M 13 bacteriophages (Virus)**.

3. Cosmids: These are the hybrid vectors, which had the combined features of plasmid and bacteriophages. They are constructed using the *cos* sequences of the phage lambda and plasmid DNA of bacteria.

- *'Cos'* sequence facilitates the incorporation of long foreign DNA fragments and plasmid sequence is useful in replication.

II. Application of rDNA technology:

- Recombination DNA technology is used in the basic research and in the production of drugs, hormones, synthetic vaccines, genetically engineered and modified plants and animals.

Polymerase chain reaction (PCR):

Huge number of copies of gene can be made by PCR
PCR is done on automated cycler
Protocol of PCR
-Denaturation of 94^0C
-Annealing at 54^0C
-Extension at 72^0C
Denaturation, the double stranded DNA melts by breaking down of hydrogen bonds
Primers of reaction mixture moving around is caused by Brownian movement
Ideal working temperature of DNA polymerase of reaction mixture – 72^0C

INDUSTRIAL USE OF MICROORGANISMS AND rDNA TECHNOLOGY:

- As per Microbiology, microorganisms are considered as 'miniature chemical factories'. They have the capacity to convert a raw material in to useful and valuable end product.
- Industrial microbiology has experienced a dramatic expansion after the invention of recombinant DNA technology, which helped in producing new genetically engineered microorganisms.
- The important microbial products are :
  1) Microbial cells that are used as immunising agents (vaccine) and food supplements.
  2) The large molecules like enzymes.
  3) **Primary metabolic products** f products that are used by microorganisms) like vitamins.
  4) **Secondary metabolic products** (products that arc not used by microbes) like antibiotics.
- The two major industrial processes of microbial technology are fermentation and distillation.
- **Fermentation** is the reduction of organic substrates by anaerobic methods,
- **Distillation** is the reduction of organic substrates by aerobic methods using submerged microbial cultures.

Microbial technology is useful in the production of the following :

I. Alcohols, Acids and vitamins

1. Ethanol (ethyl alcohol) is a common solvent used in producing **gasohol** (alcohol and petrol mixture used as fuel).

- Corn starch, molasses, sugar beets, potatoes and grapes are the raw materials from which **ethanol is produced industrially by using the yeast *Saccharomyces cerevisiae,* by fermentation.**

2. **Beverages** like whisky, brandy and rum are produced in **distillation** using microbes.
- Beer, wine and sake (rice wine) are the **fermented beverages.**

3. **Organic acids** like vinegar, citric acid, lactic acid, amino acids and vitamins are also produced industrially. Commercial **vinegar** (3 to 5% acetic acid) is produced by using yeast *Saccharomyces cerevisiae* (for fermentation) and bacterium *Acetobacter (*for oxidation of ethanol) from corn starch, molasses etc.
- *A spergillus niger* is used to produce **citric acid** by distillation from corn starch. *Lactobacillus bulgaricus* is used to produce **lactic acid** by distillation from molasses and dairy whey.

4. *Micrococcus glutamicus* is used to produce **amino acid** glutamic acid from alpha ketoglutaric acid of citric acid cycle.
5. **Vitamin** B_2 and **vitamin** $\mathbf{B_{12}}$ are industrially produced by using microbes like *Ashbya gossypii* (vitamin B_2) and *Streptomyces olivaceus* (Vitamin B_{12}).

Antibiotics :
- **An antibiotic** (wonder **drug) is a substance that destroys or inhibits the** growth **of** the **microorganism. It is often produced by one microorganism.**
- **Alexander Fleming,** 1929 observed the antibiotic activity **of penicillin** produced by the mould *Penicillium notatum,* while working with bacterium *Staphylococcus aureus.*
- In 1945 **Fleming, E. Chain, W. Florey** developed industrial production of penicillin using *P.chrysogenum.* Other antibiotics were produced later. Waksman discovered **streptomycin** from *Streptomyces griseus.*
- These antibiotics prevent cell wall synthesis or damage the cytoplasmic membrane or inhibit nucleic acid synthesis and protein synthesis in microorganisms.
- Penicillin, cephalosporin, gentamycin, kanamycin, erythromycin, chloramphenicol, bacitracin, colistrin are antibacterial. **Nystatin, griseofulvin are antifungal**.
- At present **probiotics** are used instead of antibiotics. The term probiotics is used describe either the healthy flora of the gut or supplements of beneficial intestinal bacteria.
- They are some times called "friendly germs/bacteria". They are nonpathogenic and have a positive influence on the health and physiology of the host.
- They are also used as food supplements. Two groups like **Lactobacilli** and **Bifidobacteria** are mainly used as probiotics. Now-a-days probiotics are also used to remove the harmful competitors from the body.

III. Vaccines :
- **Vaccines are used in the prevention of diseases.**
- They induce produce artificial active immunity in the body of host. First generation vaccines are live vaccines (attenuated), inactivated vaccines (contain killed microbes) and toxoid vaccines (contain toxoids of microbes).
- Attenuated whole agent of vaccines are against yellow fever, measles, rubella, mumps etc
- Inactivated whole agent vaccines are against Flu, cholera, Bubonic plaque, Heaptitis A
- Toxoid vaccines are against Teatnus, diphtheria etc.
- Vaccines contain the antigen protein without genomes. Such vaccines without genome are called **sub unit vaccines. (Used against Haemophylus influenza B)**
- These are produced by rDNA technology. These vaccines do not represent entire organisms, but contain only the protein antigens that induce immunity.
- rDNA vaccines are used to induce immunity against disease that have complex infection process.
- These vaccines produced by using biotechnology are called **recombinant vaccines.**

IV. Enzymes :
- **Enzymes are the biological** catalysts. Biological sources of enzymes are animals, plants and microorganisms.
- Enzymes of micorbes are produced commercially.
- **Enzymes of microbial origin :**

  a) Enzymes **streptokinase** (used in dissolving the blocks of coronary and cerebral blood vessels to prevent attacks or strokes) is produced by bacterium *Streptococcus pyogenes.*

  b) DNA polymerase produced by *Thermus aquaticus* is used in Polymerase Chain Reactions (PCR).

  c) Cellulase produced by *Trichoderma reesi* is used in fabric softening and brightening.

  d) Alkaline serene protease from *Bacillus lichemformis* is used to remove the protein stains.

  e) Rennin is industrially produced by using *Mucor pusilus* and is used in coagulation of milk and of cheese preparation.
- Designer enzymes for specific activities are produced using rDNA technology.
- These enzymes are used in detergents, in manufacturing photographic products and rubber products and in industries like baking, brewing, dairy, fruit juice and paper.

V. Hormones :
- Biotechnology is also used in producing hormones like insulin, human growth hormone, somatostatin, erythropoietin etc. **Insulin** is the first human gene product manufactured using rDNA technology.
- Insulin is produced by beta cells of islets of Langerhans in pancreas. It is a hypoglycemic hormone reducing the blood sugar, glucose. Deficiency of insulin leads to diabetes mellitus. **Human** growth **hormone** (hGH) is called somatotropin.

- This hormone is produced at low cost by using genetically engineered microorganisms. **Somatostatin** is a peptide hormone produced in hypothalamus and delta cells of pancreas. It inhibits secretion of hGH, and of delta cells inhibits the secretion of insulin (and glucogon).
- It is used in the treatment of pancreatitis.
- **Erythropoietin** is secreted by kidneys. It induces the production of RBC, and is used in the treatment of burns.

VI. Interferons :

- **Interferons are small antiviral proteins produced by animal cells as means of defence following infection by viruses.** These were first isolated by **Issac and Lindermann,** 1957.
- α-interferons & β-interferons are CLASS I Interferons.
- α-interferons & β-interferons produced by all viral infeted cells.
- γ-interferons are CLASS II interferons produced by T cells.
- Interferons enhance the expression of MHC molecules
- Interferons activate the **Macrophages and NK cells**
- All these interferons are antiviral in function. Nowadays interferons are produced using genetically engineered bacterial, fungal and mammalian cells.
- Interferons are used in the cancer therapy and in the treatment of viral infections like Hepatitis, Dengue fever and Rabies.

VII. Monoclonal antibodies

- Antibodies produced by a clone of specific lymphocytes are called **monoclonal antibodies.**
- **G.F. Kohler** and **C. Milstein, 1975** developed a method called **hybridoma technology** to produce monoclonal antibodies.
- This technique is based on **somatic cell hybridisation**.
- Antibody producing B lymphocytes are fused with a myeloma cell (cancer cell) to produce a hybridoma cell.
- This hybridoma cell retains the properties of both the parent cells, i.e. antibody secretion property of B lymphocyte and uncontrolled division property of cancer cell.
- The clone of hybridoma cells always produces a particular type of **antibodies that react with specific antigens (MAb).**
- B lymphocytes are obtained from the **spleen of rat**. Myeloma cells are obtained from tumours of rats.
- These cells are mixed in the presence of fusogens like polyethylene giycol (PEG).
- The mixture is then washed and placed on selective medium where only hybridoma cells can grow.
- The medium used for the identification and selection is HAT medium (hypoxanthine, aminopterin and thymidine mixture).
- **Spleen cell and hybrid cells are HAT resistant**, but myeloma cells die due to HAT. Spleen cells can live for one or two days and die.
- Hybridomas are selected and cultured for commercial production of monoclonal antibodies.
- MAbs are used diagnostically to identify Chlamydia (a streptococcus bacterium)
- These antibodies are useful in the **suppression of rejection reaction in the organ transplantation** and are also useful in **several serological tests for the detection of antigens and pathogens in quick and accurate manner.**
- Also used in Neutralizing exotoxins, inhibition of platelet clumping in patients who had undergone angioplasty treatment of auto immune reactions.