Biotechnology: Principles and Processes

INTRODUCTION

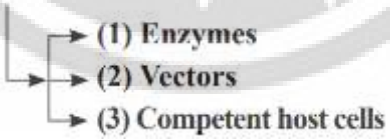
- Biotechnology deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans.
- Principles of Biotechnology/Core Techniques Involved in Modern Biotechnology

Parameters	Genetic engineering	Bioprocess engineering
Definition	Techniques to alter the chemistry of genetic material to introduce these into host organisms and thus change the phenotype of host organism	Maintenance of sterile ambience in chemical engineering processes to enable growth of only the desired microbe/ eukaryotic cell in large quantities
Include	Creation of rDNA Gene cloning Gene transfer	Manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.
The ability to multiply copies of antibiotic resistance gene in E. coli was called cloning of antibiotic resistance gene in E. coli.		

Three Basic Steps in Genetically Modifying Organisms (GMO)

- 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.

KEY TOOLS OF RECOMBINANT DNA TECHNOLOGY



ENZYMES

Restriction Endonuclease

- More than 900 restriction enzymes have been isolated from over 230 strains of bacteria each of which recognise different recognition sequences.

> First restriction endonuclease-HindII: Isolated and characterised in 1968 later, recognises sequence of 6 bp. The first recombinant DNA was constructed by Stanley Cohen and Herbert Boyer, 1972.

Functions:

- Cuts the two strands of dsDNA at specific points in their sugar phosphate backbones and leaves single stranded portions at the ends.

Ligase

- When source DNA and vector DNA are cut by the same restriction enzyme, the resultant DNA fragments have the same kind of sticky-ends.
- Sticky ends are named so because they form hydrogen bonds with their complementary cut counterparts.

- Stickiness facilitates the action of the enzyme DNA ligase.

CLONING VECTORS

Vectors are vehicles for delivering foreign DNA into recipient cells.

Features of cloning vectors:

- Origin of Replication (ori)
- Selectable Marker
- Cloning Sites/Restriction Sites

Transformation: Procedure through which piece of foreign DNA is introduced in a host bacterium.

- Insertional inactivation: Insertion of gene of interest within antibiotic resistance gene/selectable marker results in inactivation.

All transformants are not recombinants but all recombinants are transformants.

- Non-Transformants: Hosts that do not take up the vector DNA (Non-recombinant).
- Transformants: Hosts that take up the vector DNA (Recombinant or Non-recombinant).
- Recombinants: Transformant hosts that take up the recombinant DNA (Vector DNA with desired DNA).
- Non-Recombinants: Transformant hosts that take up the nonrecombinant DNA (Vector DNA without desired DNA)
- rop → Codes for the proteins involved in the replication of the plasmid.

Plasmids as vectors:

- Extra-chromosomal, circular, double-stranded DNA.
- Replicate independent of the control of chromosomal DNA (autonomously).
- They may have 1 or 2 copies per cell or even 15-100 copies per cell.

OTHER CLONING VECTORS

Ti-plasmid of Agrobacterium tumefaciens

- Agrobacterium tumefaciens, a pathogen of several dicot plants is able to deliver a piece of DNA known as 'T-DNA' to transform normal plant cells into a tumor and direct the tumor cells to produce the chemicals required by the pathogen.
- Disarmed tumour inducing (Ti) plasmid is used which is no more pathogenic to the plants but is still able to use the mechanism to deliver the genes of our interest into varieties of plants.

Bacteriophages

- High copy number than plasmid.

Retroviruses

- Retroviruses in animals have the ability to transform normal cells into cancerous cells.
- Disarmed retroviruses are used to deliver desirable genes into animal cells.

Methods of Transformation

1. Micro-injection
    - Recombinant DNA is directly injected into the nucleus of an animal cell.
2. Biolistic/Gene gun
    - Plant cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA.
3. Heat-shock method
4. Disarmed pathogen vectors

Competent Host for Transformation with recombinant DNA

- ❖ DNA is hydrophilic, so it can not pass through cell membranes.
- ❖ In order to force cell to take up alien DNA/rDNA, it must first be made 'competent' by treating with ice cold calcium chloride ($CaCl_2$).
- ❖ Entry of rDNA in host cell is due to transient pores created by heat shock (42°C) and not due to Ca^{2+} ions.
- ❖ Divalent cations increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

Process of Recombinant DNA Technology

1. Isolation of the Genetic Material (DNA)
2. Fragmentation by restriction endonucleases
3. Separation and isolation of DNA fragments
    - Gel electrophoresis:
    - Separation of negatively charged DNA molecules under an electric field through a medium/matrix.
    - Most commonly used matrix for DNA separation is agarose.
4. PCR-Polymerase Chain Reaction
    - In vitro amplification of DNA (gene of interest)
    - The amplified fragment if desired can now be used to ligate with a vector for further cloning.
5. Ligation of the DNA fragment into a vector by DNA ligase
6. Insertion of recombinant DNA into the host cell
    - Transformed host cells are selected with the help of selectable marker genes.
7. Culturing of recombinant host cells (Biosynthetic stage)
    - The cells harbouring cloned genes of interest may be grown in laboratory/ bioreactors.
    - Bioreactors: Vessels in which raw materials are biologically converted into specific products using microbial plant, animal or human cells and provide optimal growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).
8. Downstream processing
    - Separation and purification of the desired product/ recombinant protein from heterologous host (non native host).
    - Product has to be formulated with suitable preservatives.
    - Strict quality control testing is done for each product.
    - The downstream processing and quality control testing vary from product to product.
9. Product is subjected for marketing as a finished product

In Open Culture System/Continuous Culture System

- ❖ Used medium is drained out from one side.
- ❖ Fresh medium is added from the other to maintain the cells in their physiologically most active log/exponential phase.
- ❖ Larger biomass → Higher yields of desired protein.

Biotechnology and Its Applications

- ❖ **Biotechnology:** Deals with industrial scale production of biopharmaceuticals and biologicals using GM microbes, fungi, plants and animals.
- ❖ Applications of biotechnology include:
    - Therapeutics processed food
    - Diagnostics bioremediation

- Genetically modified organisms
- Crops for agriculture
- Waste treatment
- Energy production

BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE

- ❖ Made crops more tolerant to abiotic stresses (cold, drought, salt, heat).
- ❖ Reduced reliance on chemical pesticides (pest-resistant crops).
- ❖ Helped to reduce post harvest losses.
- ❖ Increased efficiency of mineral usage by plants (prevents early exhaustion of fertility of soil).
- ❖ Enhanced nutritional value of food, e.g., golden rice, i.e., Vitamin 'A' enriched rice.
- ❖ Insect resistant plants-Bt Cotton
- ❖ Pest resistant plants-Tobacco plant (By RNAi)

BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE

30 recombinant therapeutics have been approve for human use the world over. In India, 12 of these are presently being markted.

- ❖ Genetically Engineered Human Insulin (humulin) → manufactured by Eli Lilly, an American company in 1983
- ❖ Gene Therapy → First clinical gene therapy was conducted in 1990 in a 4 year old girl to treat adenosine deaminase (ADA) deficiency

MOLECULAR DIAGNOSIS METHODS

Parameters	Conventional	Modern
Early detection	Not possible	Possible
Examples	Serum and urine analysis	RDT, PCR, ELISA

TRANSGENIC ANIMALS

- ❖ Possess manipulated DNA and express foreign gene
- ❖ Transgenic rats, rabbits, pigs, sheep, cows
- ❖ 95% of transgenic animals are mice.

Uses of Transgenic Animals

- ❖ Transgenic models exist for study of diseases like cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's
- ❖ Biological products
  - a-1 antitrypsin - Treat emphysema
  - Similar attempts are made for treatment of PKU (Phenylketonuria) and cystic fibrosis.
  - First transgenic cow: Rosie developed in 1997 producing human protein enriched milk (2.4 grams per litre)
- ❖ Vaccine Safety
  Transgenic mice are being used to test the safety of polio vaccine to replace the use of monkey.
- ❖ Chemical safety testing
  Transgenic animals are made more sensitive to toxic substances to obtain results in less time.

ETHICAL ISSUES

- ❖ GEAC (Genetic Engineering Approval Committee): Makes decisions regarding the validity of introducing GMO for public services.
- ❖ Biopiracy refers to the use of bio-resources by multinational companies and other organisations without proper authorization from the countries and people concerned without compensatory payment.
- ❖ The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill.

CONTROVERSIES REGARDING PATENTS AND BIOPIRACY

- Basmatic rice:
  - 2,00,000 varieties of rice in India, 27 documented varieties of Basmati rice in India.
  - In 1997, an American company got patent rights on Basmatic rice through the US patent Trademark office.
- Turmeric and Neem
  - Though Indian were using turmeric for hundred of years, in 1995, the patent for the use of turmeric in wound healing is given to university of Mississippi medical centre.
  - Several traditionally herbal based medicinal products made up of turmeric and neem were also got patent.

PW Web/App - https://smart.link/7wwosivoicgd4

Library- https://smart.link/sdfez8ejd80if