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PHARMACEUTICAL BIOTECHNOLOGY

UNIT 4

TOPIC :

- c) Microbial genetics including transformation, transduction, conjugation, plasmids and transposons.



Microbial Genetics

- Microbial genetics is the branch of biology that studies genes, heredity, genetic variation, and gene transfer in microorganisms such as bacteria, viruses, fungi, and protozoa.
- In bacteria, genes are usually present on a single circular chromosome and sometimes on extra-chromosomal DNA called plasmids.
- Microbial genes can be transferred from one microorganism to another through:
 1. Transformation
 2. Transduction
 3. Conjugation

1. Transformation

- Transformation is a method of genetic transfer in which a bacterium takes up free or naked DNA from its surroundings and incorporates it into its own genetic material.
- The bacterium receiving DNA is called the recipient cell, while the DNA released from another bacterium is called donor DNA.

Steps of Transformation

1. Release of DNA from donor bacterial cell.
2. Uptake of free DNA by recipient cell.
3. Integration of DNA into bacterial chromosome.
4. Expression of new genetic character.

Griffith's Transformation Experiment (1928)

- Transformation was first discovered by Frederick Griffith in 1928 while studying *Streptococcus pneumoniae*.

Types of Bacteria Used

1. S-Strain (Smooth Strain)

- Has polysaccharide capsule.
- Virulent (causes disease).
- Forms smooth colonies.

2. R-Strain (Rough Strain)

- Lacks capsule.
- Non-virulent.
- Forms rough colonies.

Experimental Procedure

Experiment	Result
Live S-strain injected into mice	Mouse died
Live R-strain injected into mice	Mouse survived
Heat-killed S-strain injected	Mouse survived
Live R-strain + Heat-killed S-strain	Mouse died

Conclusion

- DNA from dead S-strain bacteria entered living R-strain bacteria and transformed them into virulent S-strain bacteria.
- This demonstrated that genetic information can be transferred between bacteria.

Importance of Transformation

- Produces genetic variation.
- Transfers antibiotic resistance genes.
- Important in biotechnology.
- Used in genetic engineering.

2. Transduction

- Transduction is a method of genetic transfer in bacteria in which bacterial DNA is transferred from one bacterium to another through a virus called a bacteriophage.
- The bacteriophage acts as a carrier of genetic material.

Components

1. Donor bacterium
2. Recipient bacterium
3. Bacteriophage

Types of Transduction

1. Generalized Transduction
2. Specialized Transduction

1. Generalized Transduction

- Generalized transduction is a type of transduction in which any bacterial gene can be transferred from one bacterium to another by a bacteriophage.
- It usually occurs during the lytic cycle.

Steps

1. Bacteriophage infects donor bacterium.
2. Phage multiplies inside the cell.
3. Bacterial chromosome breaks into fragments.
4. Some bacterial DNA is accidentally packed into phage particles.
5. Donor bacterium bursts and releases phages.
6. Phage infects recipient bacterium.
7. Bacterial DNA enters recipient cell.
8. DNA recombines with recipient chromosome.
9. Recipient acquires new characteristics.

Importance

- Produces genetic variation.
- Transfers useful genes.
- Helps bacterial evolution.
- Used in genetic research.

2. Specialized Transduction

- Specialized transduction is a process in which only specific bacterial genes are transferred by a bacteriophage.
- It occurs during the lysogenic cycle.

Principle

- A prophage integrates into the bacterial chromosome. During excision, it may carry nearby bacterial genes along with its own DNA.

Steps

1. Temperate phage infects bacterium.
2. Phage DNA integrates into chromosome.
3. Prophage is formed.
4. During induction, prophage excises incorrectly.
5. Adjacent bacterial genes are removed with phage DNA.
6. New phage particles are formed.
7. Phages infect recipient bacterium.
8. Bacterial genes integrate into recipient chromosome.

Importance

- Causes genetic variation.
- Transfers bacterial genes.
- Important in biotechnology.
- Used in microbial genetics research.

3. Conjugation

- Conjugation is a method of genetic transfer in bacteria involving direct cell-to-cell contact.
- DNA transfer occurs through a tube-like structure called the sex pilus or conjugation pilus.

Cells Involved

- **F⁺ Cell (Donor Cell)** – Contains F plasmid.
- **F⁻ Cell (Recipient Cell)** – Lacks F plasmid.

Steps of Conjugation

1. F⁺ cell forms sex pilus.
2. Sex pilus attaches to F⁻ cell.
3. Conjugation bridge develops.
4. Plasmid DNA replicates.
5. One DNA strand transfers to recipient.
6. Complementary strands are synthesized.
7. Recipient becomes F⁺.

Types of Conjugation

1. F⁺ × F⁻ Conjugation

- Transfer of F plasmid only.
- Recipient becomes F⁺.

2. Hfr Conjugation

- Hfr = High Frequency Recombination.
- Transfer of chromosomal genes along with F factor.

3. F' Conjugation

- Transfer of F plasmid carrying bacterial genes.
- Recipient acquires additional genes.

Importance of Conjugation

- Produces genetic variation.
- Transfers antibiotic resistance genes.
- Used in recombinant DNA technology.
- Important in biotechnology.

Plasmids

- Plasmids are small, circular, double-stranded DNA molecules found mainly in bacteria.
- They are separate from chromosomal DNA and replicate independently.

Features of Plasmids

- Circular DNA structure.
- Double-stranded DNA.
- Self-replicating.
- Small size (few kilobases).
- Provide survival advantages.

Types of Plasmids

1. Fertility (F) Plasmids

- Help in bacterial conjugation.

2. Resistance (R) Plasmids

- Provide antibiotic resistance.

3. Col Plasmids

- Produce bacteriocins that kill other bacteria.

4. Degradative Plasmids

- Help metabolism of unusual compounds.

5. Virulence Plasmids

- Increase pathogenicity.

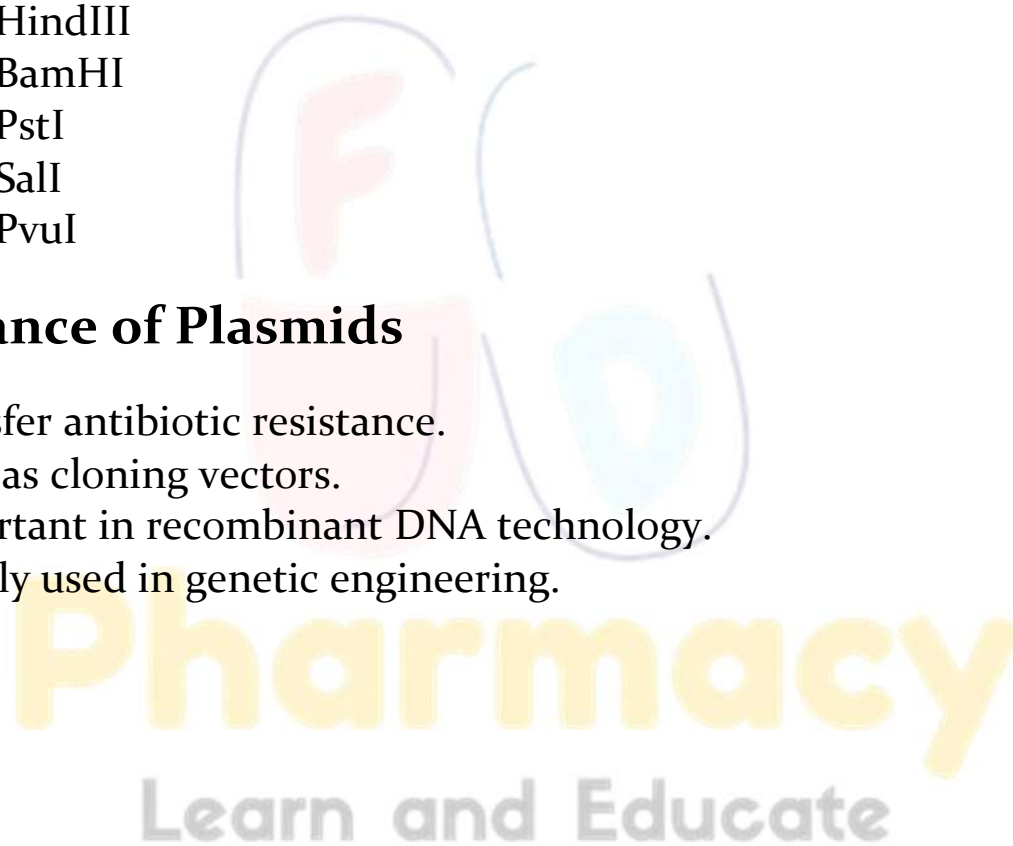
pBR322 Plasmid

Important Features

- **ori** – Origin of replication.
- **ampR** – Ampicillin resistance gene.
- **tetR** – Tetracycline resistance gene.
- Multiple restriction sites such as:
 - EcoRI
 - HindIII
 - BamHI
 - PstI
 - SalI
 - PvuI

Importance of Plasmids

- Transfer antibiotic resistance.
- Used as cloning vectors.
- Important in recombinant DNA technology.
- Widely used in genetic engineering.



Transposons (Jumping Genes)

- Transposons are movable DNA sequences that can change their position within the genome.
- They were first discovered by Barbara McClintock.
- The movement of transposons is called transposition.

Movement of Transposons

They may move:

- Chromosome to chromosome.
- Chromosome to plasmid.
- Plasmid to chromosome.

Characteristics

- Mobile genetic elements.
- Present in prokaryotes and eukaryotes.
- Can insert into new DNA sites.
- Carry antibiotic resistance genes.
- Cause mutations and genetic variation.

Structure of Transposons

1. Transposase Gene

- Encodes transposase enzyme.
- Responsible for movement of transposon.

2. Inverted Repeat (IR) Sequences

- Short repeated sequences at both ends.
- Recognized by transposase enzyme.

3. Additional Genes

- Functional genes.
- Often antibiotic resistance genes.

Types of Transposons

A. Based on Structure

1. Insertion Sequences (IS Elements)

- Simplest transposons.
- Contain only transposase gene and inverted repeats.
- No additional genes.

Examples: IS₁, IS₂

2. Composite Transposons

- Two IS elements at both ends.
- Carry additional genes.

Examples: Tn₅, Tn₁₀

3. Non-Composite Transposons

- Do not contain complete IS elements.
- Carry functional genes.

Example: Tn₃

B. Based on Movement

1. DNA Transposons

- Move directly as DNA.
- Follow cut-and-paste mechanism.

2. Retrotransposons

- Move through RNA intermediate.
- RNA is converted back into DNA by reverse transcriptase.
- Follow copy-and-paste mechanism.
- Increase genome size.

C. Based on Ability to Move

1. Autonomous Transposons

- Move independently.
- Possess transposase gene.

2. Non-Autonomous Transposons

- Cannot move independently.
- Lack transposase gene.
- Depend on autonomous transposons.

Mechanism of Transposition

1. Cut-and-Paste Mechanism

- Transposon removed from original site.
- Inserted into new site.
- Original copy is lost.

2. Copy-and-Paste Mechanism

- New copy is produced.
- One copy remains at original site.
- Second copy inserts at new site.

Importance of Transposons

- Cause genetic variation.
- Produce mutations.
- Transfer antibiotic resistance genes.
- Used in genetic engineering.
- Useful in biotechnology research.
- Help in evolution studies.
- Important tools for gene mapping.