GENE TRANSFER IN BACTERIA

INTRODUCTION- Gene transfer is defined simply as a technique to efficiently and stably introduce foreign genes into the genome of target cells.

The directed desirable gene transfer from one organism to another and the subsequent stable integration & expression of foreign gene into the genome is referred as genetic transformation.

• Stable transformation occur when DNA is integrated into host genome and is inherited in subsequent generations. •

The transferred gene is known as transgene and the organism that develop after a successful gene transfer is known as transgenic.

METHODS OF GENE TRANSFER-

1. Conjugation
2. Transformation
3. Transduction

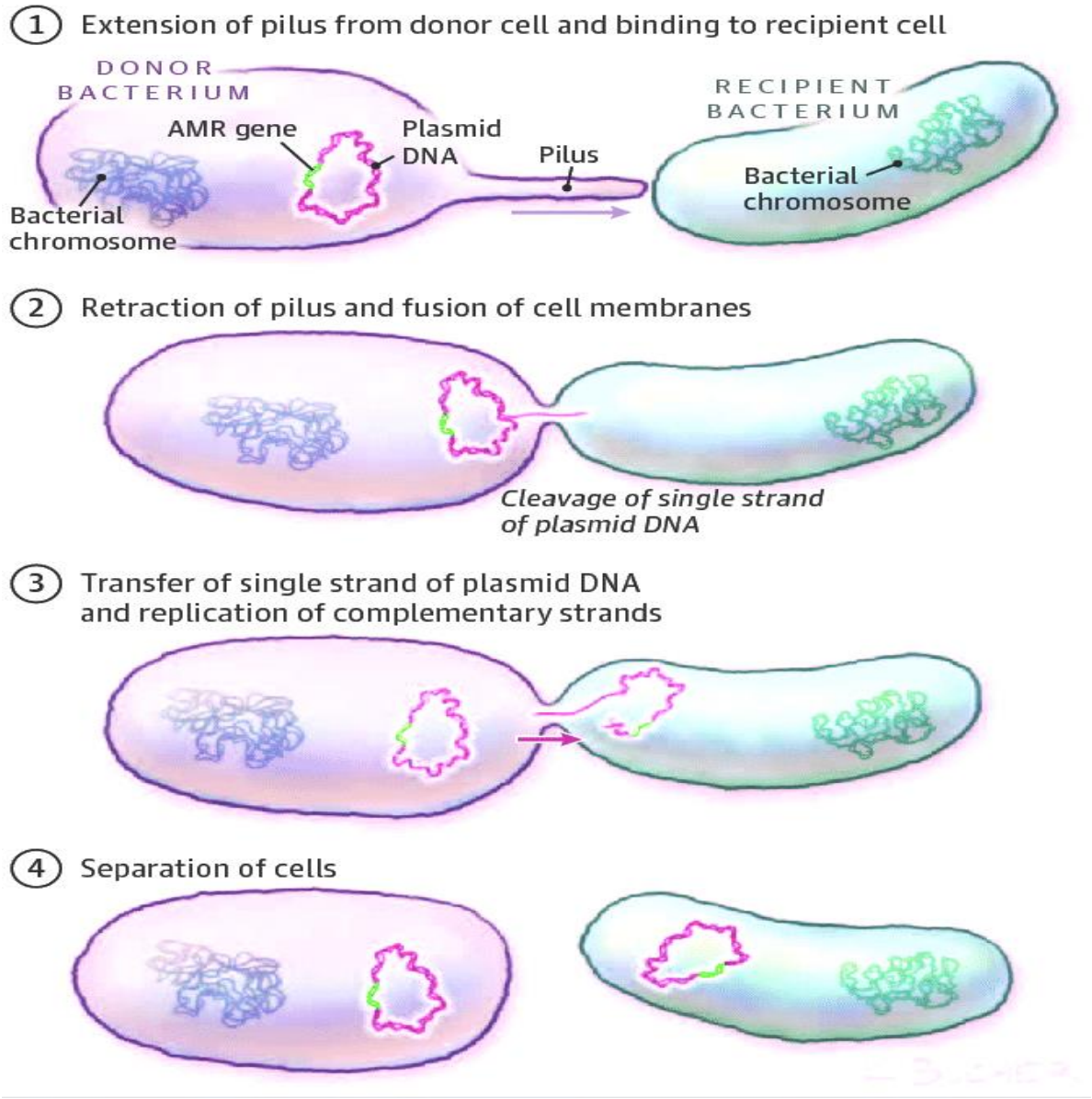
Definition:- It is the process of gene transfer via sex pili between cells of opposite mating types that are in physical contact (cell-to-cell) with each other.

a) **Method by following need**
b) **Pilus formation**
c) **Physical contact**
d) **Transfer of F plsmid**
e) **Complimentory strand synthesis**

Procedure

1. Requires the presence of a special plasmid called the F plasmid.
2. Bacteria that have F plasmid are referred to as F+ or male. Those that do not have an F plasmid are F- or female.
3. The F plasmid consists of 25 genes that mostly code for production of sex pilli.
4. A conjugation event occurs when the male cell extends his sex pilli and one attaches to the female. This attached pilus is a temporary cytoplasmic bridge through which a replicating F plasmid is transferred from the male to the female.
5. When transfer is complete, the result is two male cells.

6. When the F+ plasmid is integrated within the bacterial¬ chromosome, the cell is called an Hfr cell (high frequency of recombination cell).



TRANSFORMATION

Definition:- Transformation refers to the transfer of relatively small segment of naked DNA from donor cell(male) to competent recipient(female) cell

HISTORY- Bacterial transformation was first discovered by GRIFFITH in 1928 between the two strains of Streptococcus (Diplococcus) pneumonia which causes pneumonia in humans and mice

Griffith's experiment,

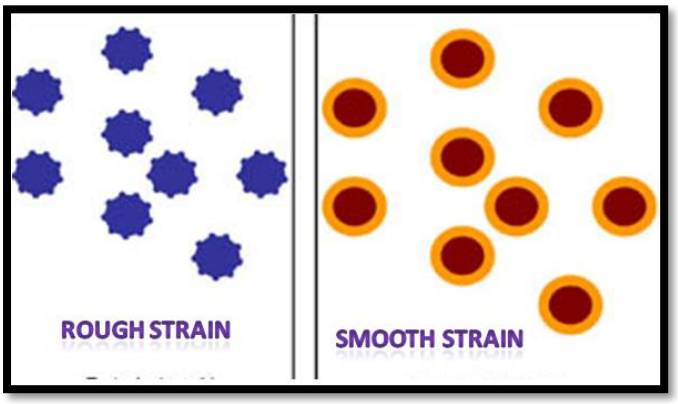
Griffith's experiment, reported in 1928 by Frederick Griffith was the first experiment suggesting that bacteria are capable of transferring genetic information through a process known as transformation.

Griffith's findings were followed by research in the late 1930s and early 40s that isolated DNA as the material that communicated this genetic information.

Pneumonia was a serious cause of death in the wake of the post-WWI Spanish influenza pandemic, and Griffith was studying the possibility of creating a vaccine. Griffith used two strains of pneumococcus (*Streptococcus pneumoniae*) bacteria which infect mice –

a type III-S (smooth) which was virulent, and a type II-R (rough) strain which was non-virulent. The III-S strain synthesized a polysaccharide capsule that protected itself from the host's immune system, resulting in the death of the host. while the II-R strain did not have that protective capsule and was defeated by the host's immune system.

A German bacteriologist, Fred Neufeld, had discovered the three pneumococcal types (Types I, II, and III) and discovered the quellung reaction to identify them *in vitro*. Until Griffith's experiment, bacteriologists believed that the types were fixed and unchangeable, from one generation to another



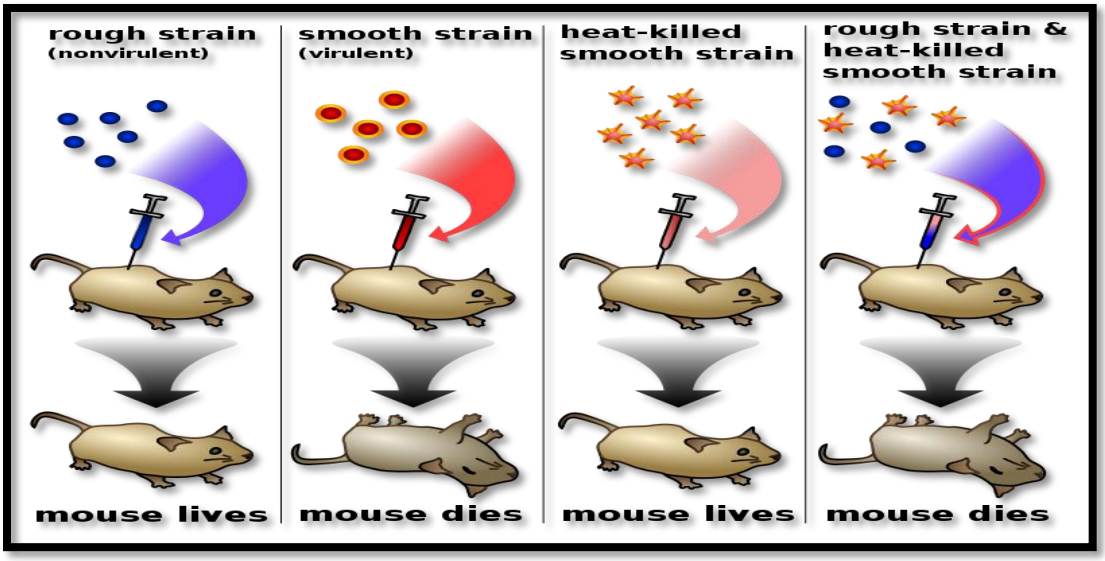
Griffith was able to isolate both live II-R and live III-S strains of pneumococcus from the blood of these dead mice

Griffith took 4 mice and injected them with different solutions.

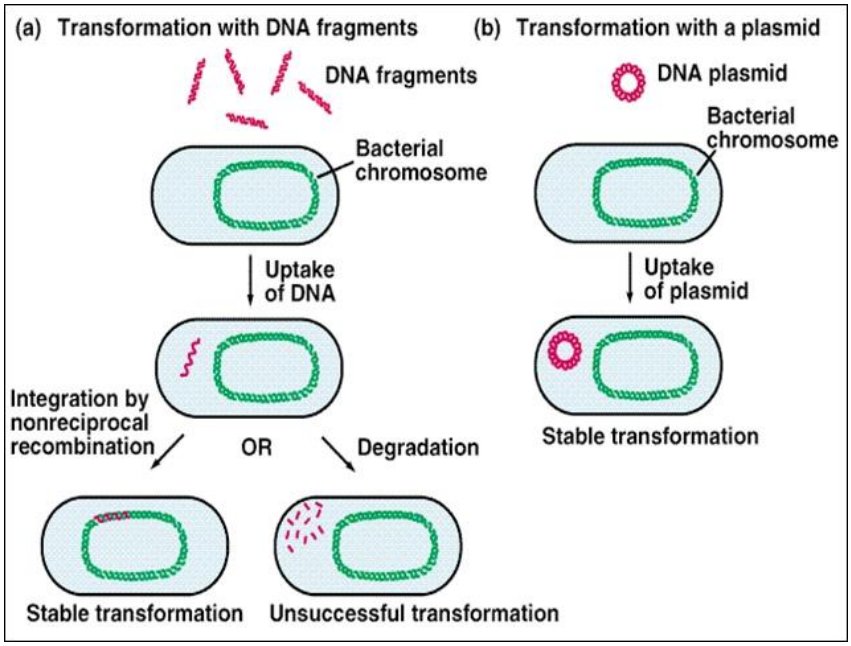
a) The first one was injected with the S strain organisms
b) The second one was injected with the R strain organisms
c) The third mouse was injected with heat-killed S strain organisms;
d) The last one was injected with a mixture of heat-killed S strain and live R strain organisms.

The first and fourth mice died due to the infection, while the second and third mice survived. When he extracted the infectious agent from the dead mice, in both cases, he found S strain organisms. The first 2 mice showed that S strain is the virulent strain, while the R strain is a virulent. The third mouse

proved that heat-killed S strain organisms cannot cause an infection. Now here is where it gets interesting. The death of the 4th mouse, and the retrieval of live S strain organisms showed that, somehow, the heat-killed S strain organisms had caused the transformation of live R strain organisms to live S strain organisms. This was called the *transformation experiment*…not particularly creative in the naming department.



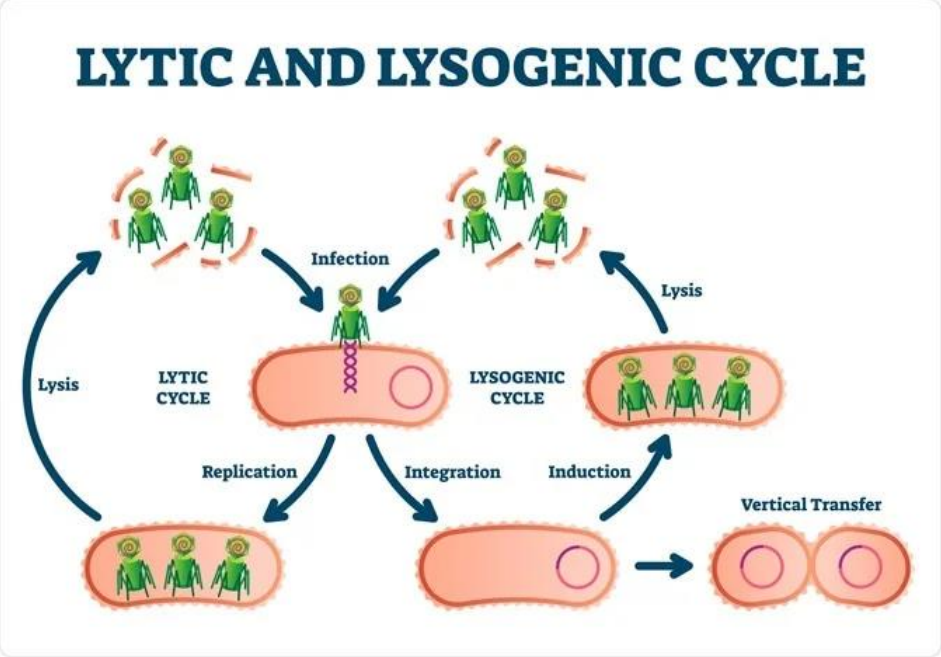
Griffith Experiment



Transduction

DEFINITION:- - Unlike transformation in which the naked DNA is transferred in transduction DNA is carried by a bacteriophage. or In transduction, DNA is transferred from cell to cell through the agency of viruses NOTE :- All phages can be transducer and not all bacteria are transduciblem.

ExamplesDesulfovibrio, Escherichia, Pseudomonas, Rhodococcus, Rhodobacter, Salmonella, Staphylococcus, and Xanthobacter.



PHASES OF TRANSDUCTION

Transduction happens through either the lytic cycle or the lysogenic cycle

1. Lytic cycle-Bacteriophages attached to the body of bacterium and insert their gentic material and start replication in side the body and after replication it form their own individuals and comes out from the body of bacterium through lysis.
2. **Lysogenic cycle** is adopted, the phage chromosome is integrated (by covalent bonds) into the bacterial chromosome, where it can remain dormant for thousands of generations. If the lysogen is induced (by UV light for example), the phage genome is excised from the bacterial chromosome and initiates the lytic cycle, which culminates in lysis of the cell and the release of phage particlesThere are two types of transduction: –

A. Generalized transduction: A DNA fragment is transferred from one bacterium to another by a lytic bacteriophage that is now carrying donor bacterial DNA due to an error in maturation during the lytic life cycle.

1. A lytic bacteriophage adsorbs to a susceptible bacterium.

2. The bacteriophage genome enters the bacterium. The genome directs the bacterium's metabolic machinery to manufacture bacteriophage components and enzymes

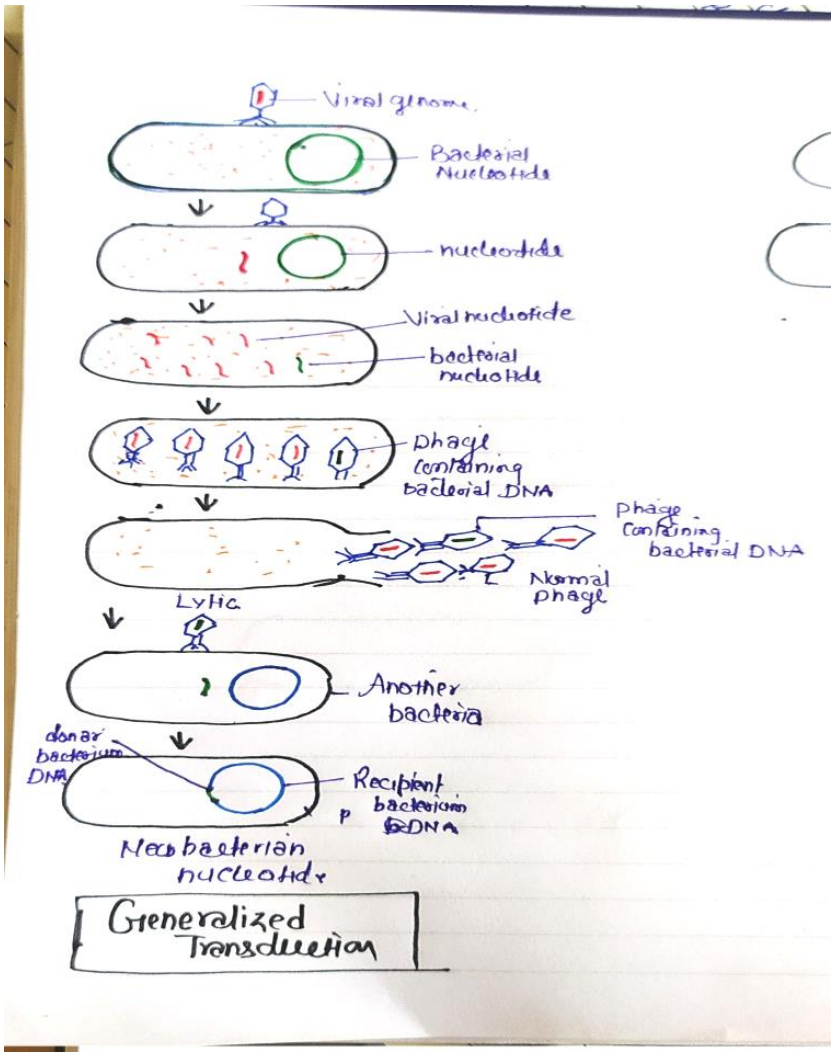
3. Occasionally, a bacteriophage head or capsid assembles around a fragment of donor bacterium's nucleoid instead of a phage genome by mistake. steps in Generalised Transduction (cont'd)

4. The bacteriophages are released.

5. The bacteriophage carrying the donor bacterium's DNA adsorbs to a recipient bacterium

6. The bacteriophage inserts the donor bacterium's DNA it is carrying into the recipient bacterium

7. The donor bacterium's DNA is exchanged for some of the recipient's DNA



B. Specialized transduction:

A DNA fragment is transferred from one bacterium to another by a temperate bacteriophage that is now carrying donor bacterial DNA due to an error in spontaneous induction during the lysogenic life cycle. In specialized transduction the phage inserts its genome at the specific sites

1. A temperate bacteriophage adsorbs to a susceptible bacterium and injects its genome .

2. The bacteriophage inserts its genome into the bacterium's nucleoid to become a prophage.

3. Occasionally during spontaneous induction, a small piece of the donor bacterium's DNA is picked up as part of the phage's genome in place of some of the phage DNA which remains in the bacterium's nucleoid.

4. As the bacteriophage replicates, the segment of bacterial DNA replicates as part of the phage's genome. Every phage now carries that segment of bacterial DNA.

5. The bacteriophage adsorbs to a recipient bacterium and injects its genome.

6. The bacteriophage genome carrying the donor bacterial DNA inserts into the recipient bacterium's nucleoid.