Bacterial Structure / Function & Bacterial Genetics

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Objectives-Bacterial Structure & Function

- Define the cellular organization of bacteria and know the differences between Eukaryotes and Prokaryotes.
- Know major structures of bacteria and its functions.
- Know the structure of cell wall of bacteria including the differences between Gram positive and Gram negative bacteria and main functions.

Objectives, cont.,

- Know the external structures of bacteria with and functions .
- Know the cytosol and internal structures of bacteria .
- Describe bacterial spores and its application in the practice of medicine.

Objectives- Bacterial Genetics

- Know basic information about bacterial genetics and replication of bacteria .
- Describe plasmids ,its origin , types and its importance in clinical practice.
- Recalls genetics variations, including ; mutation and mechanisms of gene transfer and its implication on bacterial resistance to antimicrobial agents.

Definition

Bacteria : **Is a heterogenous** group of uni-cellular organisms , about 1-8 μm in diameter

Bacteria is a Prokaryote (has a primative nucleus):

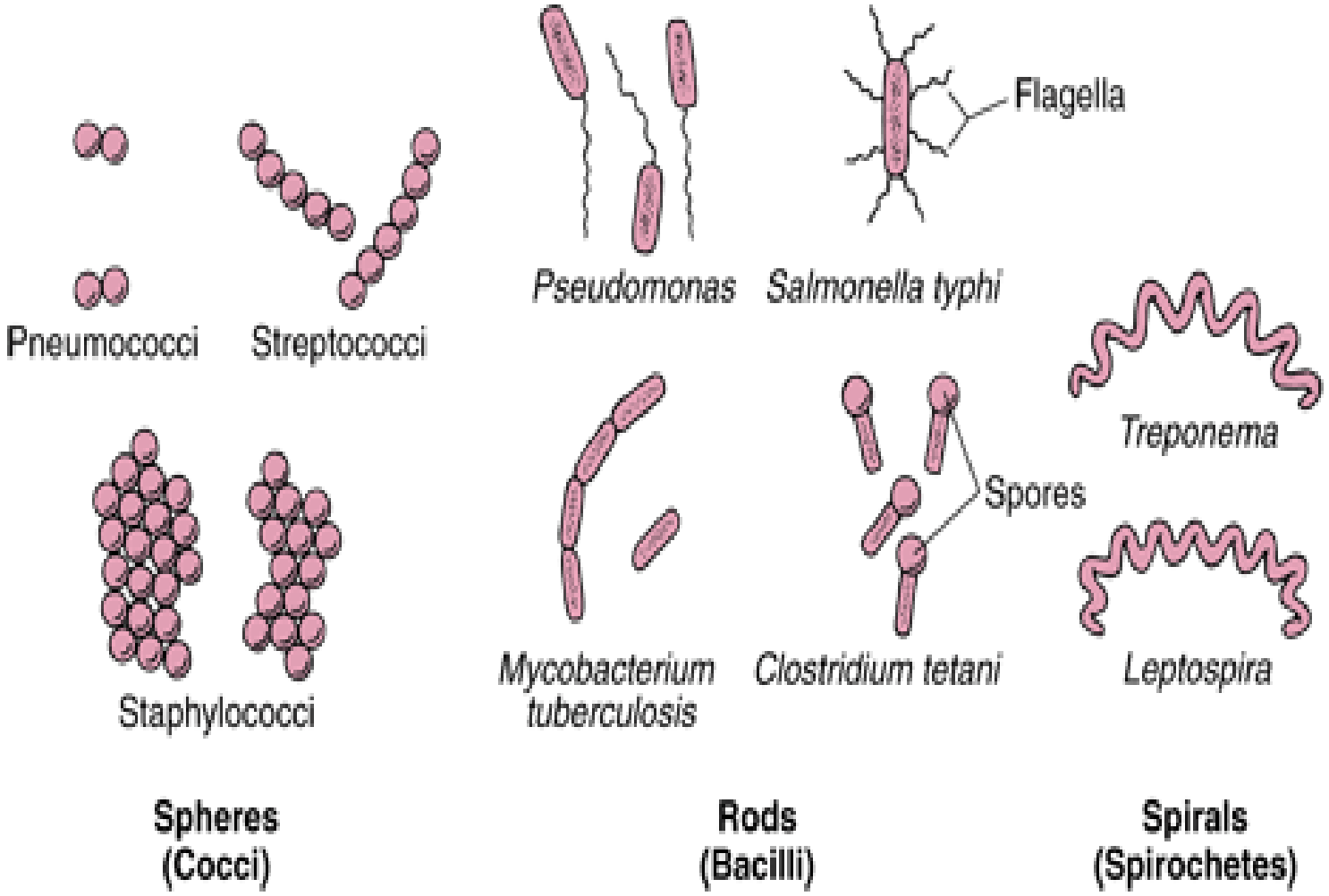
- one chromosome
- no nuclear membrane
- no mitochondria
- no sterols

Bacteria contain Plasmids: an extra piece of **DNA.**

Shapes of Bacteria

- Spherical / Oval…….Cocci
- Rods……..Bacilli
- Very short Bacilli…Coccobacilli
- Tapered end ……Fusiform
- Club-shaped / Curved….Vibrio
- Helical / Spiral… ..Spirochaetes

Shapes of Bacteria

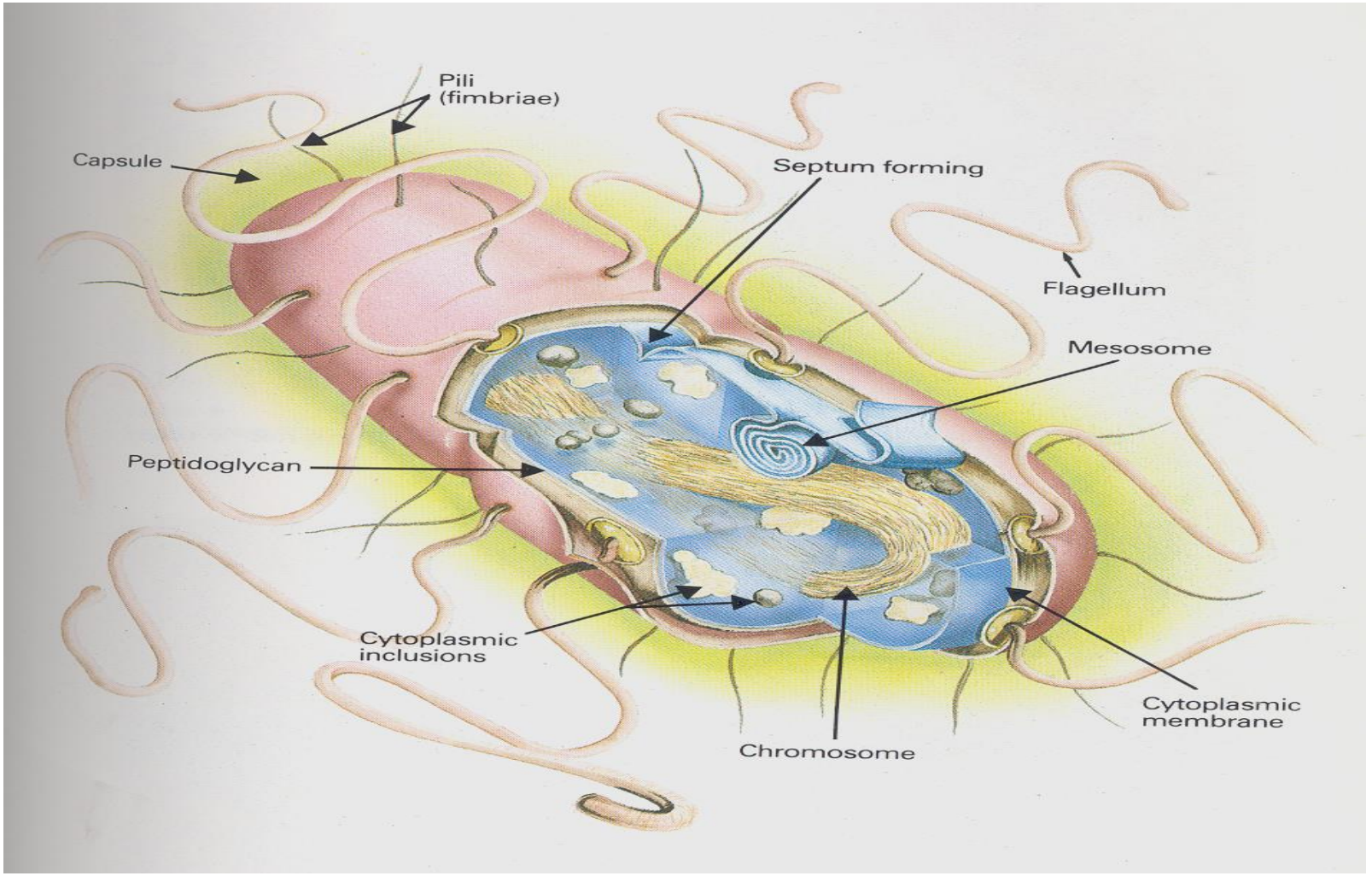


Arrangements of Bacteria

Arrangements among Cocci :

- Pairs……Diplococci
- Chains……Streptococci
- Clusters……Staphylococci
- In four……Tetrad
- Palisades…..*Corynebacterium*

Major Structures of Bacteria



Cell Wall of Bacteria

- Bacteria are cells with rigid cell wall surround cytoplasmic membrane and internal structures.

Functions of cell wall:

- Rigidity
- Shapes bacteria
- Protection
- Porous / permeable to low molecular weight molecules
- Cell division
- Antigenic determinants

Cell Wall of Bacteria

- Two groups of bacteria depending on reaction to **GRAM STAIN:**

GRAM POSITIVE BACTERIA: stain blue/purple by Gram stain

GRAM NEGATIVE BACTERIA: stain red by Gram stain

Note : *Mycoplasma* is a bacteria that is naturally have no cell wall.

Chemical structure of bacterial cell wall:

- **Peptidoglycan :**

  Rigid part , mucopeptide composed of alternating strands of *N- acetyl muramic acid* and *N- acetyle glucosamine* linked with peptide sub units.

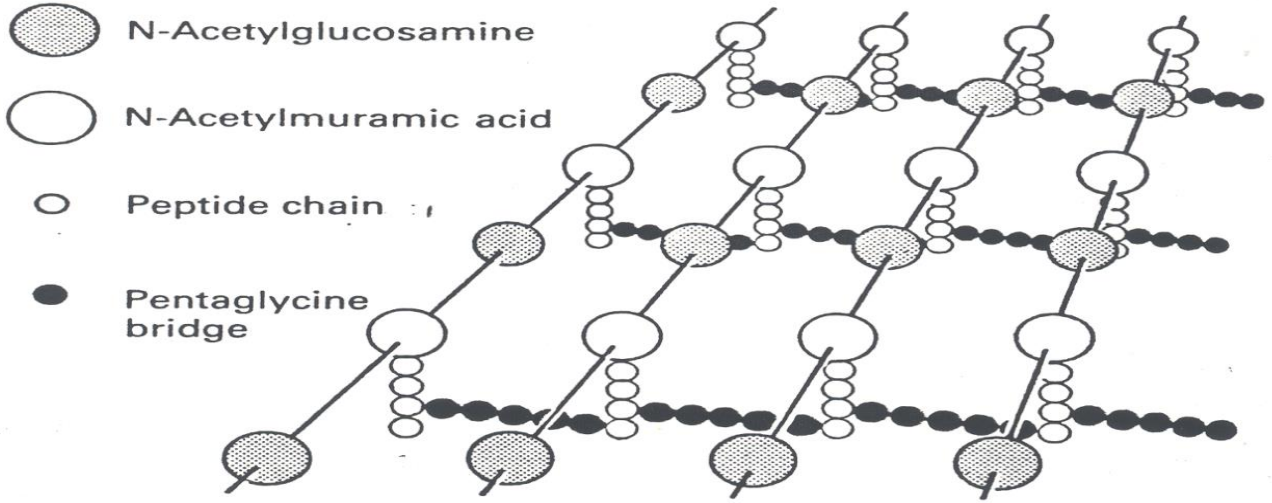
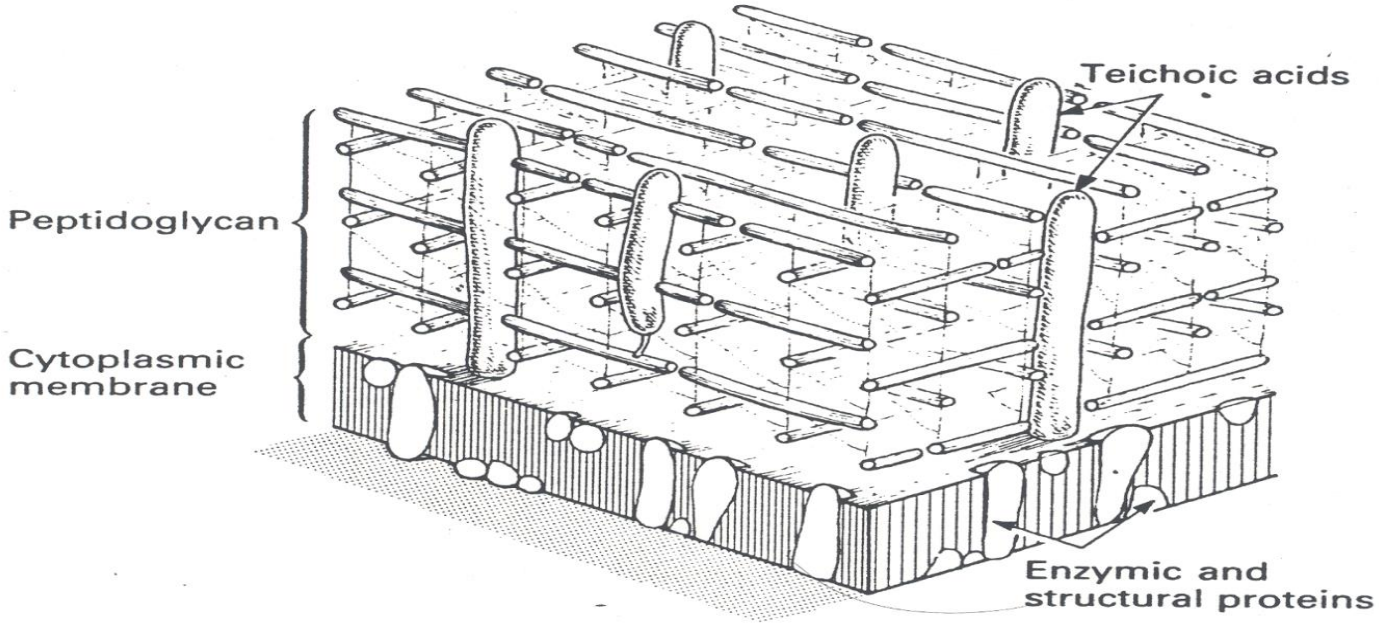


Diagram to show chemical structure of cross-linking in peptidoglycan component of cell walls. From Sharon N The Bacterial Cell Wall. Copyright (C) 1969 by Scientific American Inc. All rights reserved.

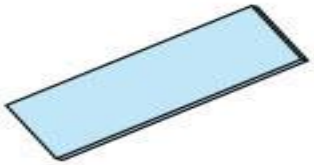


Three-dimensional representation of Gram-positive bacterial cell wall.

GRAM STAINING

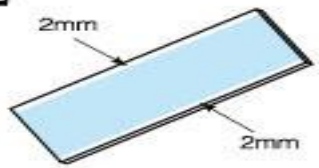
Flow Through Procedure

1



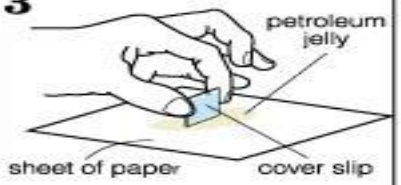
Wipe bottom of biofilm slide clean

2



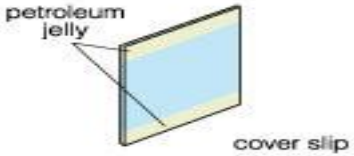
Clean top edges of slide about 2mm

3



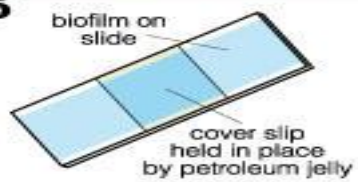
Build up a ridge of petroleum jelly on the top and bottom of a cover slip

4



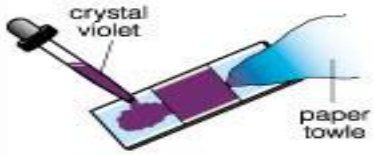
Cover slip with petroleum jelly

5



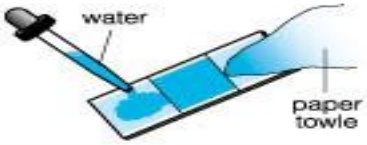
Biofilm on slide with cover slip

6



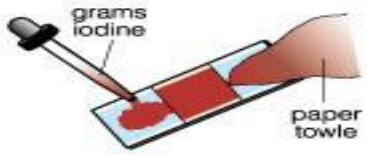
Add crystal violet-wait 30 sec.

7



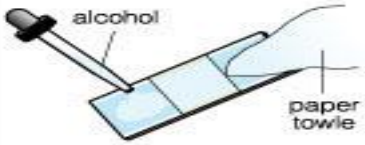
Wash with water

8



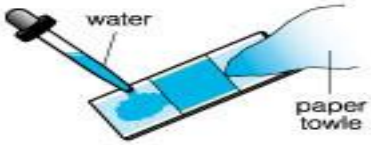
Add Grams Iodine -wait 1.5 min.

9



Decolorize with alcohol

10

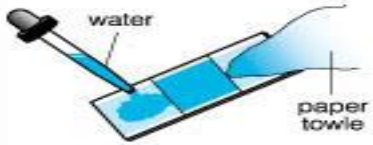


Wash with water

11

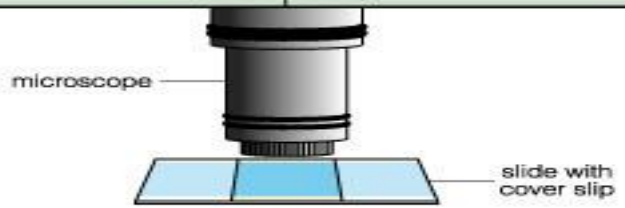
Stain with Safranin dye-wait 30 sec.

12

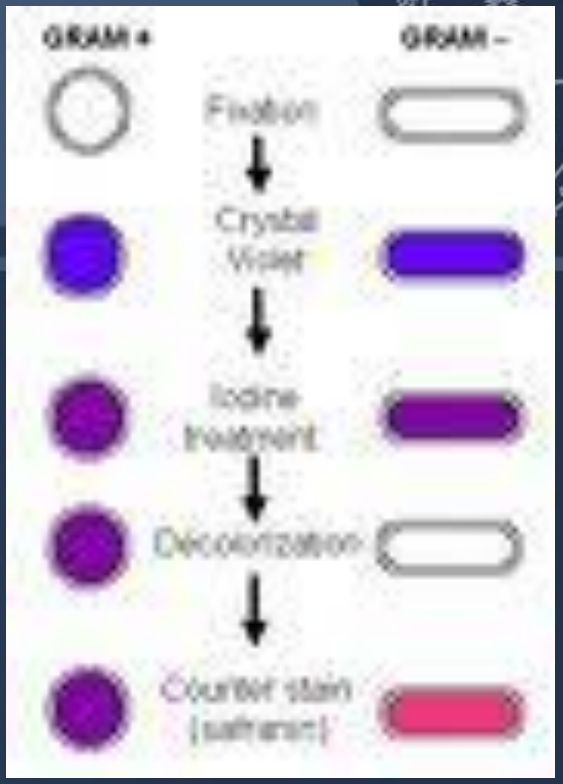


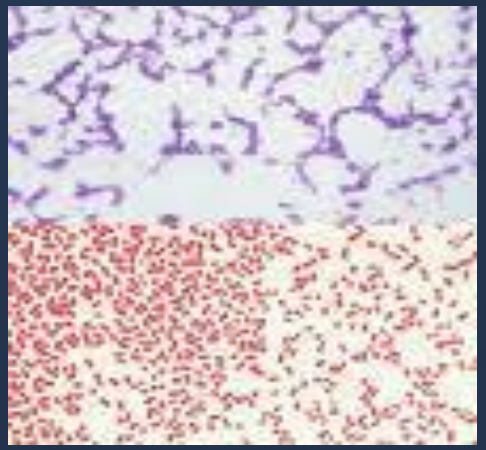
Wash with water

13



Examine under oil immersion through the cover slip





Cell Wall of Gram Negative Bacteria

- **Thin** Peptidoglycan
- Outer membrane that contains :

  - specific proteins (porins) important in the transport of hydrophilic molecules

  - lipopolysaccharide & lipid **(ENDOTOXIN)**

Cell Wall of Gram Positive Bacteria

- Peptidoglycan **thicker** than Gram negative bacteria.
- Closely associated with cytoplasmic membrane.
- **Teichoic acid** : anchors cell wall to cell membrane , epithelial cell adhesion.
- **Antigens** : ~ polysaccharides (Lancefield)
  ~ protein (Griffith)

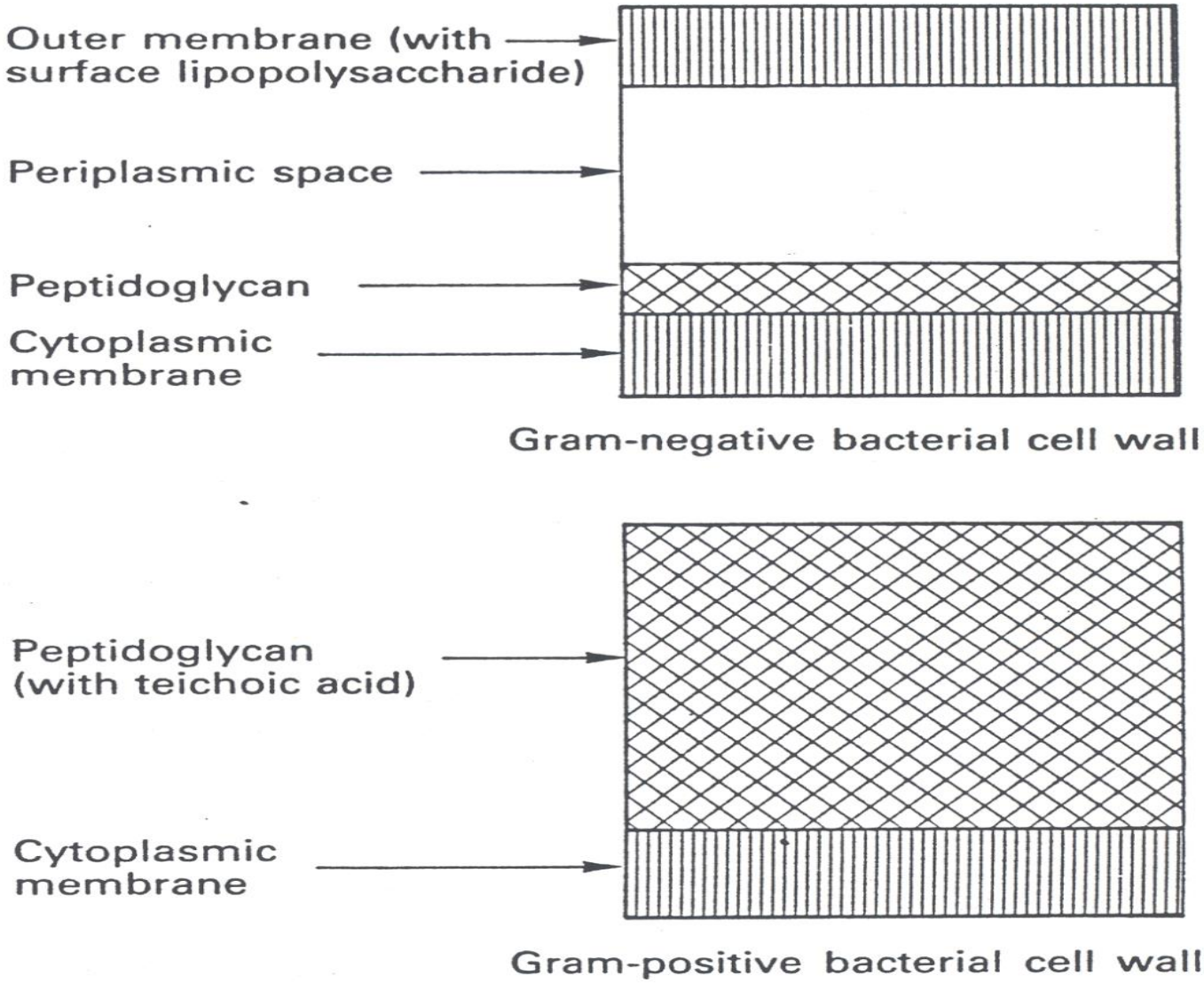


Diagram showing the structure of Gram-negative and Gram-positive bacterial cell walls.

External Structures of Bacteria

External protrude from the cell into the environment.:

- Flagella
- Pili
- Capsule

Flagella

- Composed of protein **FLAGELLIN.**
- Helical filaments
- Found in Gram positive & Gram negative bacteria.

Distribution:

- Peritrichous
- Monotrichous
- Lophotrichous

Structure of Flagella

Basal Body : a protein arranged as rings on central rod (4 ring in Gram negative, 2 ring in Gram positive).

~outer pair of rings: only in Gram negative, pushed through outer membrane.

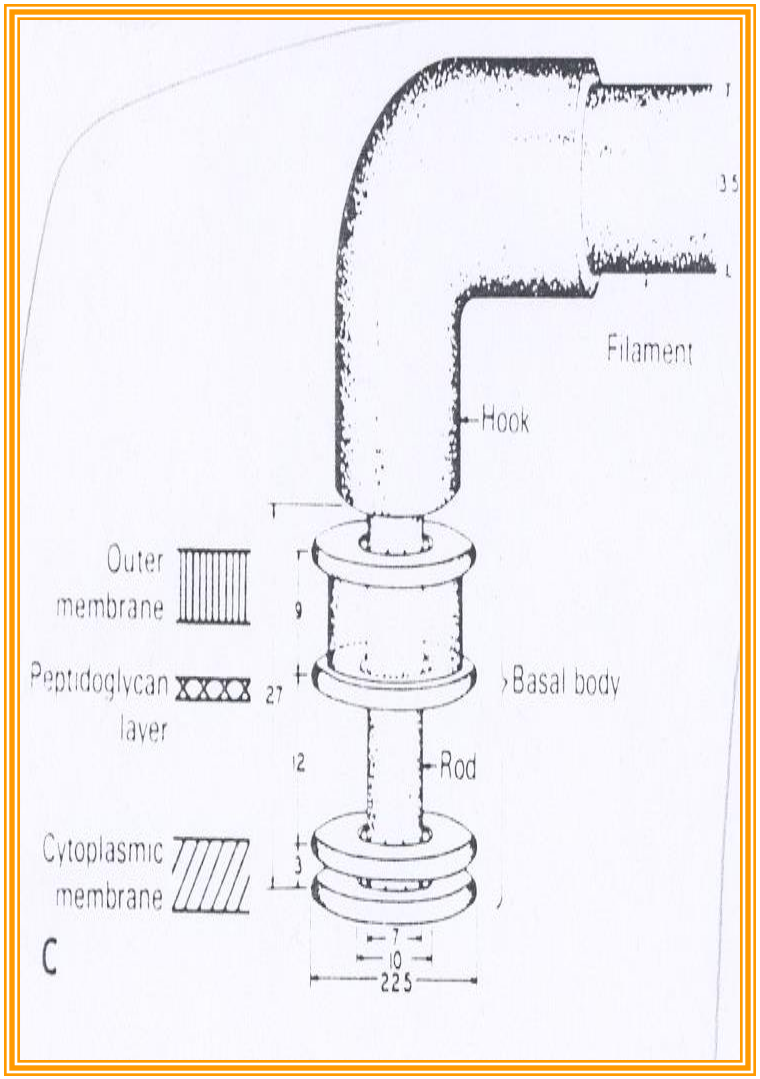
~ inner pair of rings : inserted into peptidoglycan & cytoplasmic membrane.

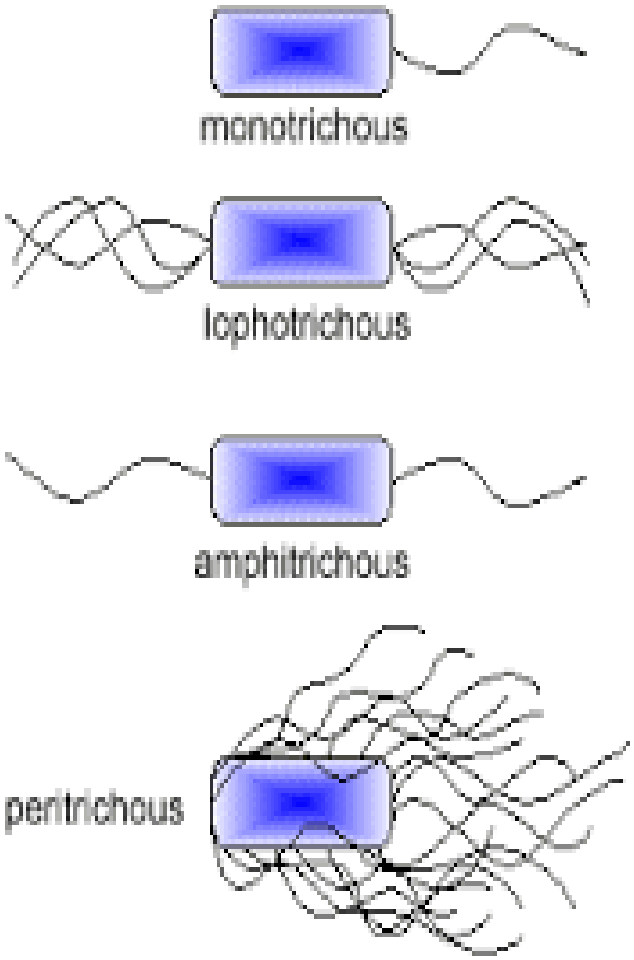
Hook : bent structure ~act as joint

Long Filament : Flagellin protein

Function of Flagella : motility & chemotaxis

Structure & Distribution of Flagella





Pili

Fine short filaments extruding from cytoplasmic membrane.

Found on the surface of many Gram negative & Gram positive bacteria.

Composed of protein Pilin.

Two classes:

1- **Common pili** (*fimbriae*): covers the surface— responsible for: adhesion & colonization

2- **Sex pili** : in some bacteria only, responsible for conjugation.

Capsule

- Amorphous material surrounds bacteria.
- Usually polysaccharide
- Occasionally protein
- **Function** : ~ Inhibits phagocytosis

  ~ Acts as *Virulence factor* in some bacteria by assessing attachment to the surfaces.

Cytoplasmic Membrane

- Cytoplasmic membrane (cell membrane)
  - Double layered structure composed of phospholipid & protein
  - Act as semi- permeable membrane (passive diffusion)
  - Site of numerous enzymes involved in active transport of nutrients and various metabolic processes

Internal Structures of Bacteria

Mesosomes : convoluted invaginations of cytoplasmic membrane.

Function:

» Involved in DNA segregation during cell division & respiratory activity

» Contain receptors involved in chemotaxis

» Permeability barrier (active transport of solutes).

Core of Bacteria

- **Composed of :** Cytoplasmic inclusions
  Nucleoid (nuclear body)
  Ribosome

1. **Cytoplasmic inclusions:**

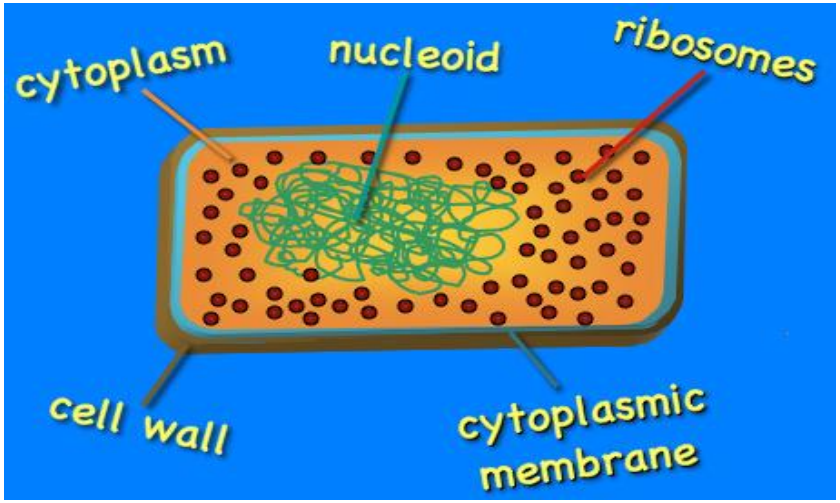
- Nutritional storage granules , examples:
  - **Volutin**
  - **Lipid**
  - **Starch / or Glycogen**

Nucleoid (Nuclear Body)

- Circular single stranded chromosome (bacteria genome or DNA)
- No nuclear membrane
- DNA undergoes semi-conservative replication , bidirectional from a fixed point
-

Ribosomes of Bacteria

- Distributed throughout the cytoplasm
- Site of protein synthesis
- Composed of RNA and protein



Spores of Bacteria

- Small ,dense, metabolically inactive , non-reproductive structures produced by ***Bacillus & Clostridium***
- Enables the bacteria to survive adverse environmental conditions.
- Contain high concentration of Calcium dipicolonate.
- Resistant to heat, dissecation & disinfectants
- Often remain associated with the cell wall

Spores of Bacteria-cont.

- **Spores are described as :**

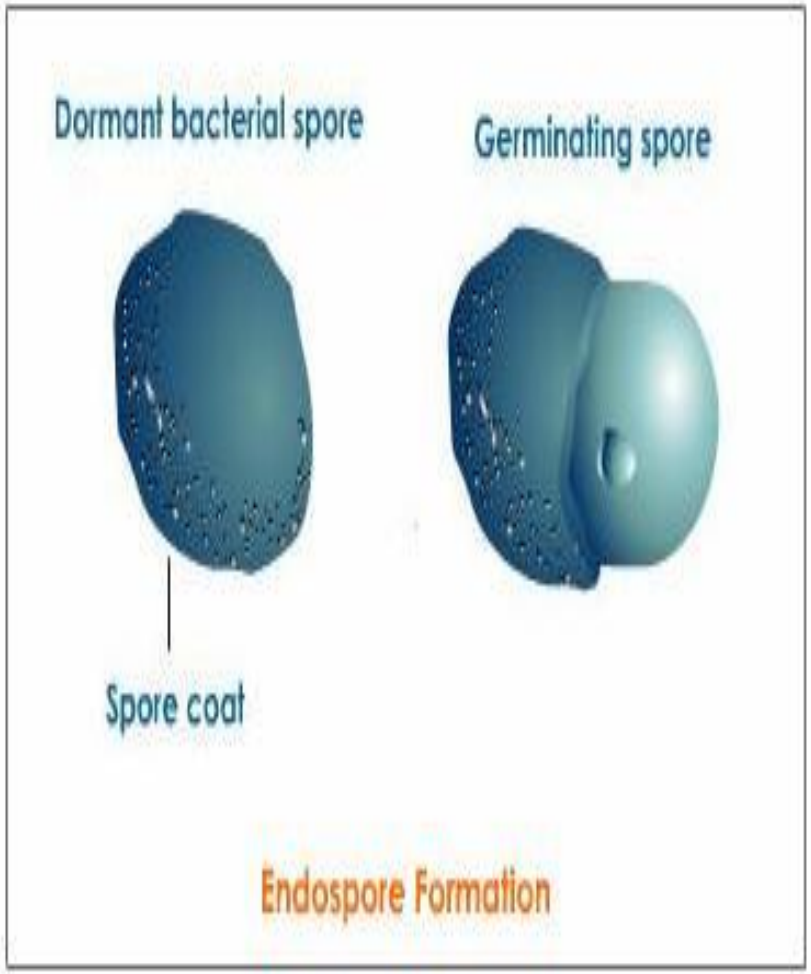
1- **Terminal** spores

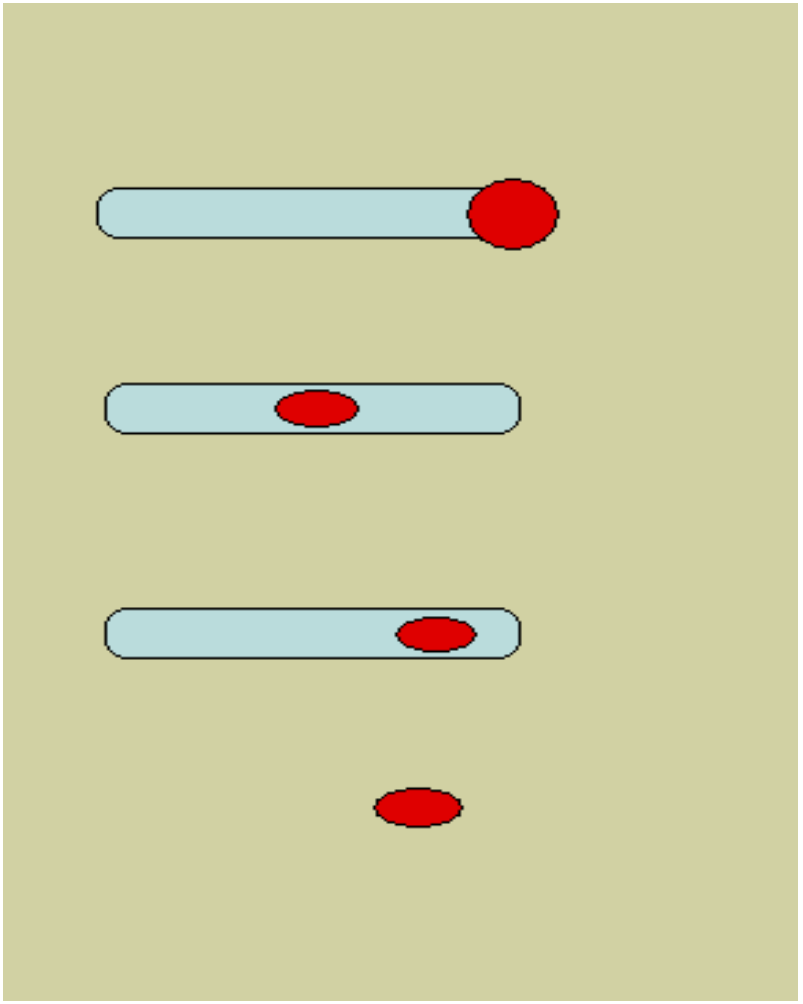
2- **Sub-terminal** spores

3- **Central** spores

- Spores germinate when growth conditions become favorable to produce vegetative cells.
- Application in medical practice :spore preparations used for checking the efficacy of **Autoclaves**, eg. *Bacillus subtilis & Bacillus sterothermophilus.*

Spores of Bacteria





BACTERIAL GENETICS

Bacterial Genetics- definitions

- **Genetics** is the study of inheritance and variation.
- Genetic information encoded in DNA.

Function of genetic material:

1- Replication of the genome

2- Expression of DNA to mRNA then to protein.

Definitions-cont.

- **Genotype**: the complete set of genetic determinants of an organism.
- **Phenotype:** expression of specific genetic material .
- **Wild type:** reference (parent) strain~ active.

Mutant: progeny with mutation.

<u>2 types of DNA in bacteria</u>

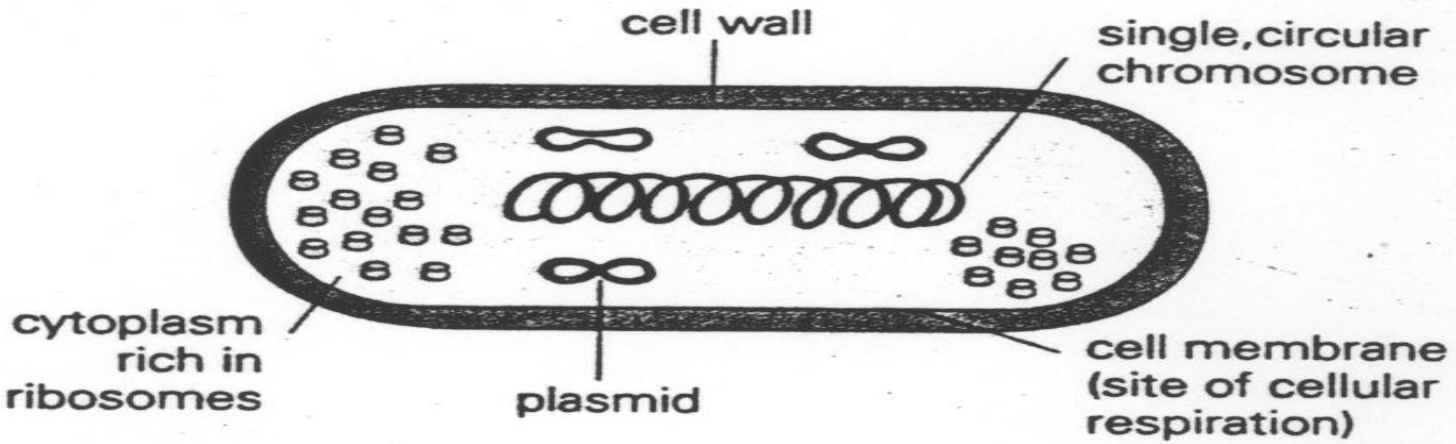
- ~ Chromosomal
- ~ Extra-chromosomal (Plasmid).

Bacterial Chromosomes

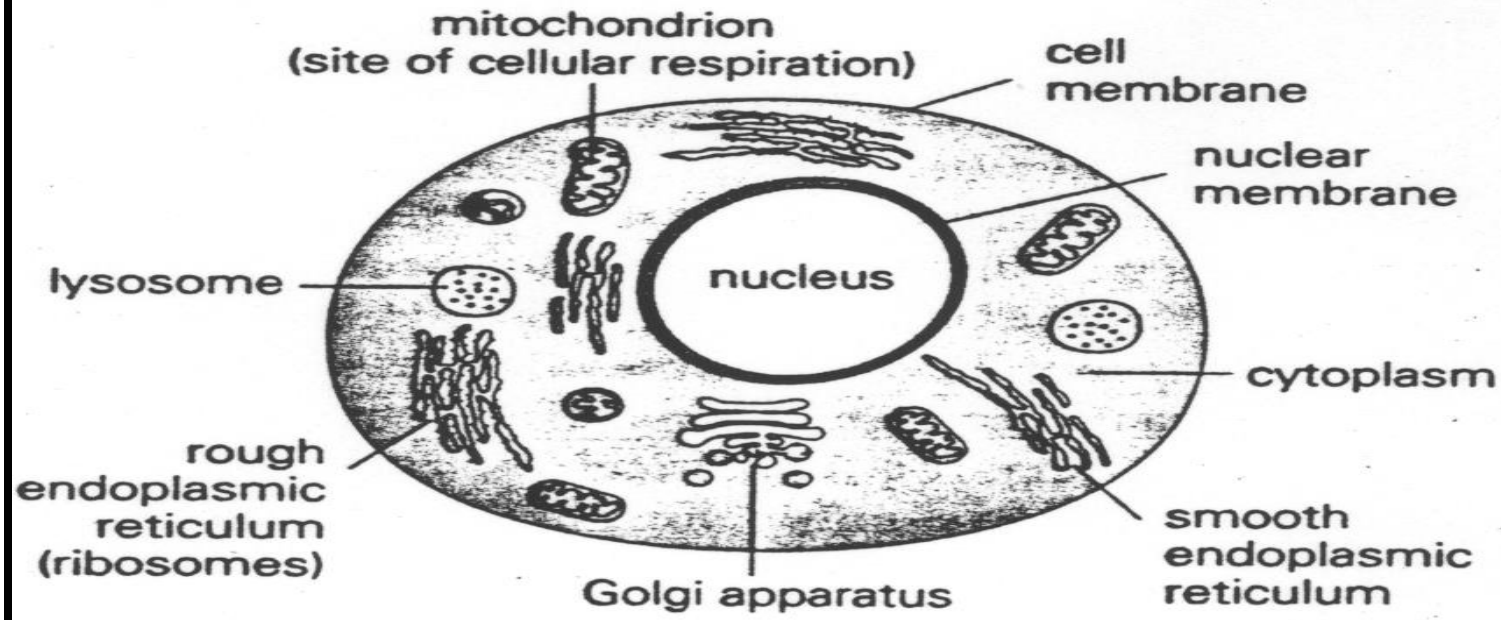
- Haploid, circular molecule of double stranded- DNA attached to cell membrane. No nuclear membrane **(prokaryotes).**
- DNA a double helical structure, genetic code in Purine and Pyrimidine bases of nucleotides that makes DNA strand.
- 3 bases comprise one code, each triplet codon codes for one amino acid.
- **Replication is semi-conservative.**

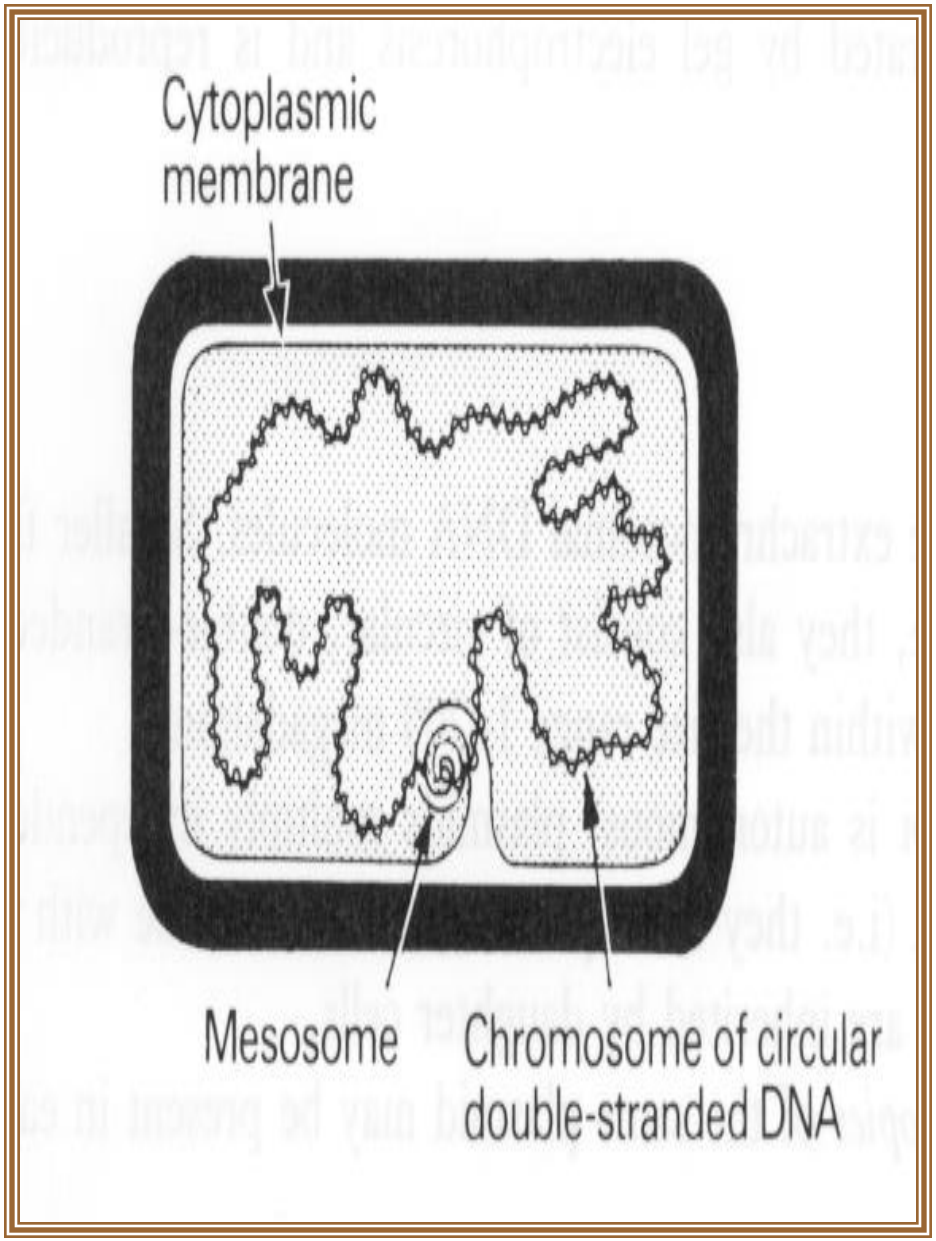
PROCARYOTES AND EUCARYOTES

procaryote

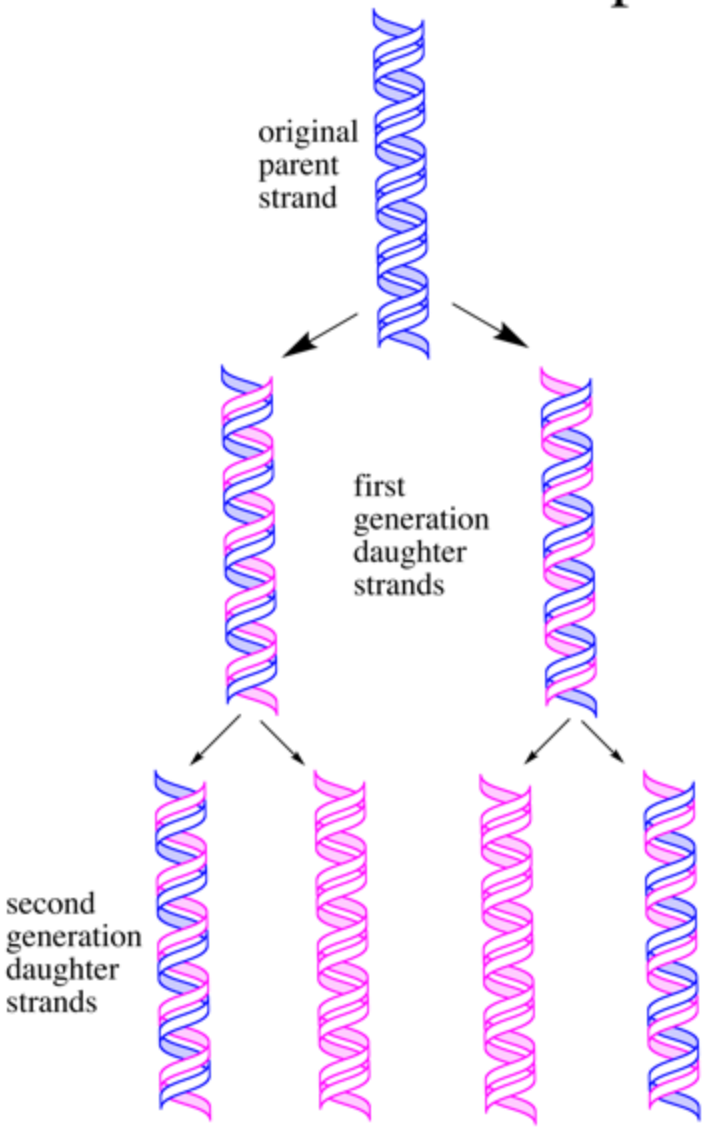


eucaryote





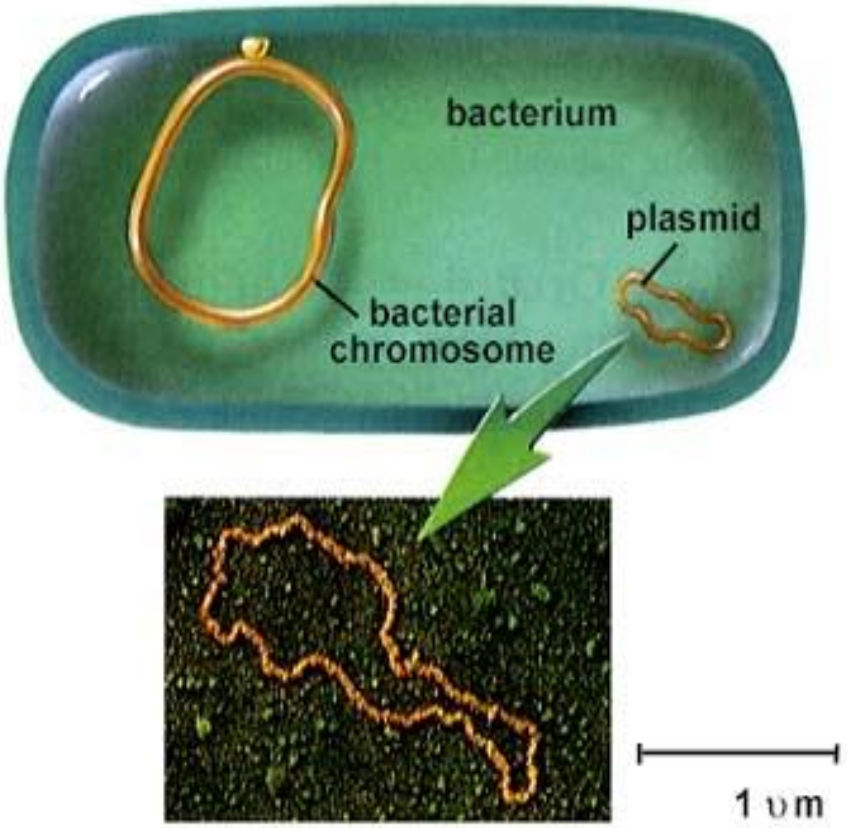
Semiconservative Replication

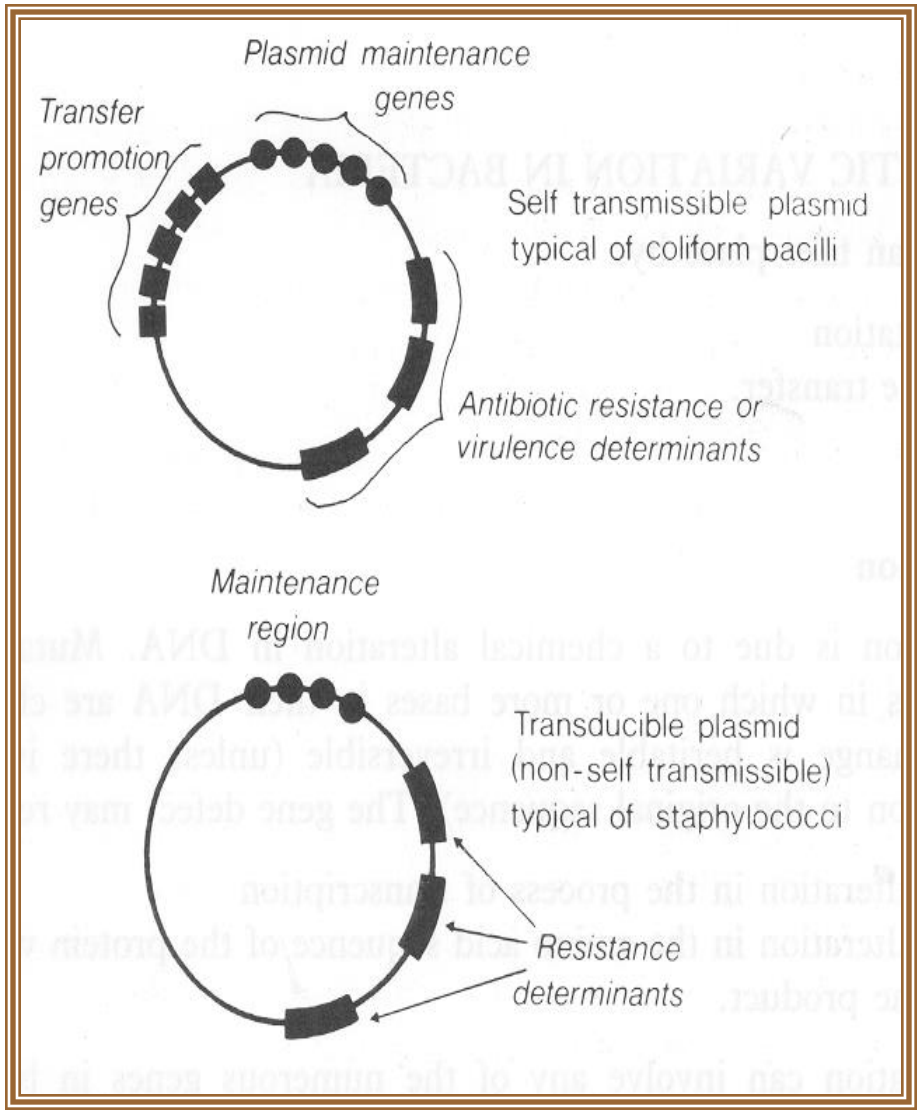


Plasmids

- Extra chromosomal DNA composed of double stranded-DNA.
- Found in most species of bacteria.
- Origin?
- Govern their own replication
- **Application** :Genetic exchange, amplify genes
- Transfer by conjugation
- Unrelated plasmids coexist together only

Plasmids


bacterium
plasmid
bacterial chromosome
1 υm


Plasmid maintenance genes
Transfer promotion genes
Self transmissible plasmid typical of coliform bacilli
Antibiotic resistance or virulence determinants
Maintenance region
Transducible plasmid (non-self transmissible) typical of staphylococci
Resistance determinants

Types of Plasmids

1~ **R~plasmids**: genes code for antibiotic resistance particularly Gram negative bacteria.

2~**Col~plasmids:** in Enterobacteria, codes for extracellular toxins.

3~ **F~plasmids:** (fertility) factor, transfer of chromosome during mating .

Genetic variation in bacteria :

takes place by :

1~ Mutations

2~Gene transfer

Mutation

- Inheritable changes in the structure of genes (DNA).
- Chemical changes in one or more bases of DNA.

Mutation /gene defect leads to alteration in:

- Transcription,
- Amino acid sequence,
- Function eg. **Bacteria resistant to antibiotic.**

Classification of Mutation

Depends on biological sequencing:

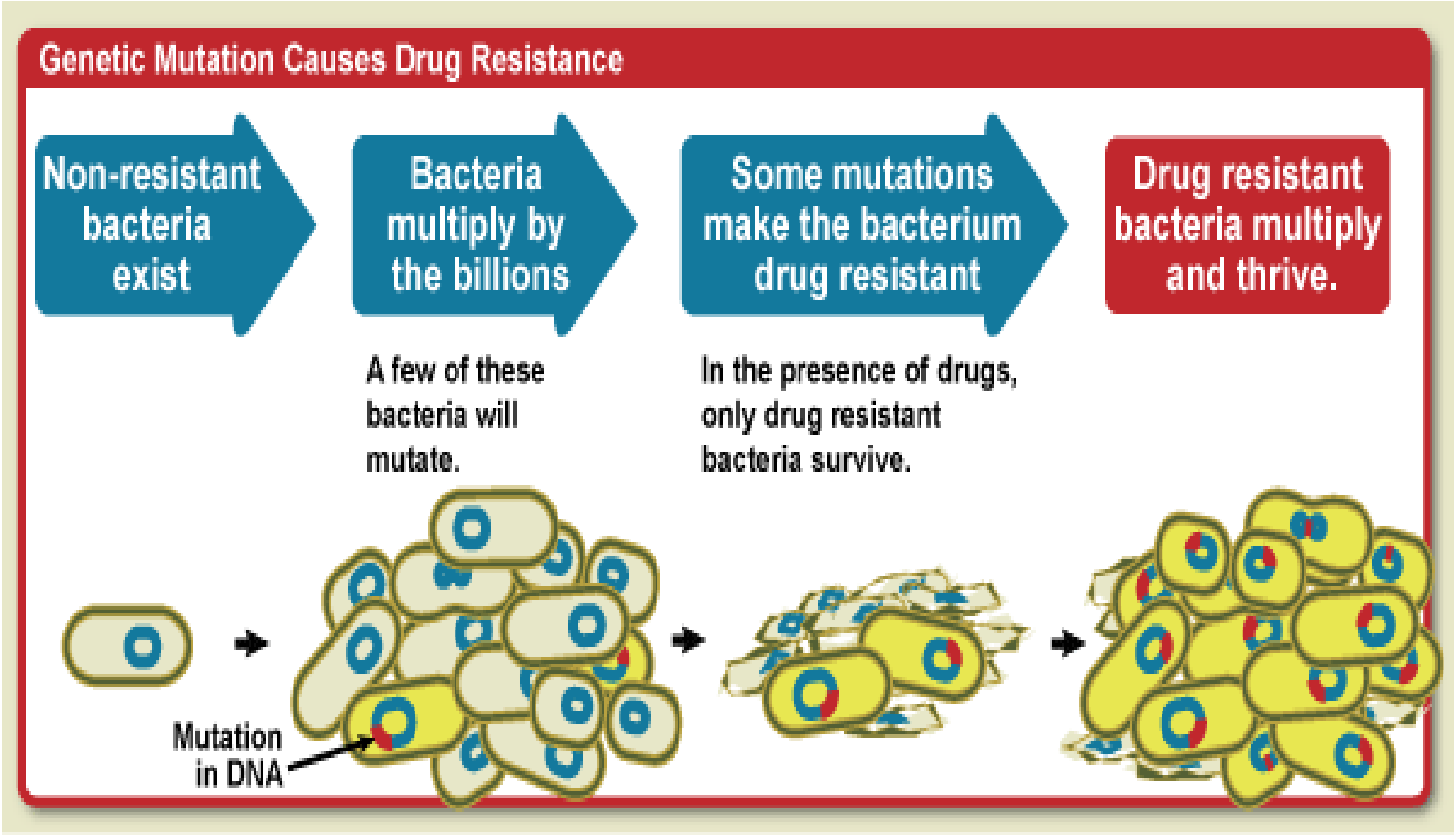
1- **Resistance mutation**: affect structure of cell protein. **Main application in medical practice.**

Bacteria become resistant to antibiotics

2- **Auxotrophic mutation**: affect biosynthetic enzyme resulting in a nutritional requirement of mutant cell.

3- **Lethal mutation.**

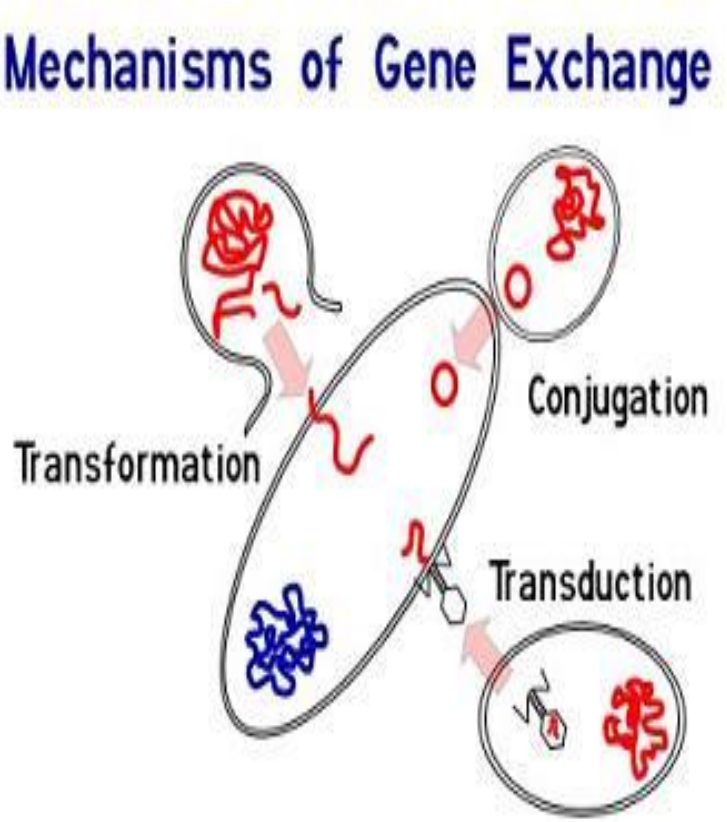
Mutation Causes Antimicrobial Resistance



Gene Transfer Among Bacteria

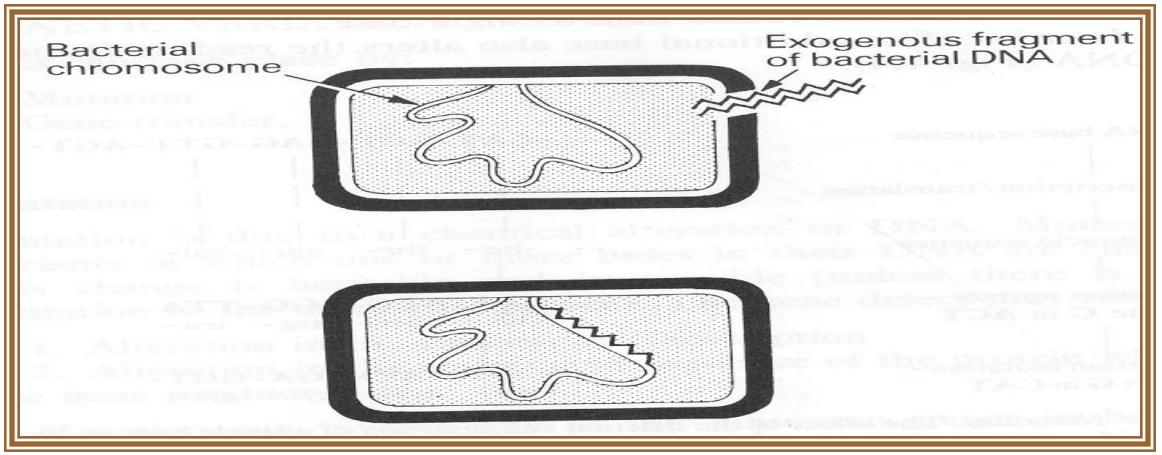
Three mechanisms:

- 1- Transformation
- 2- Transduction
- 3- Conjugation.



Transformation

- A fragment of exogenous naked bacterial DNA are taken up and absorbed into recipient cells.
- Common in *Haemophilus influenzae & Streptococcus pneumoniae*. **Bacteria become resistant to Ampicillin.**



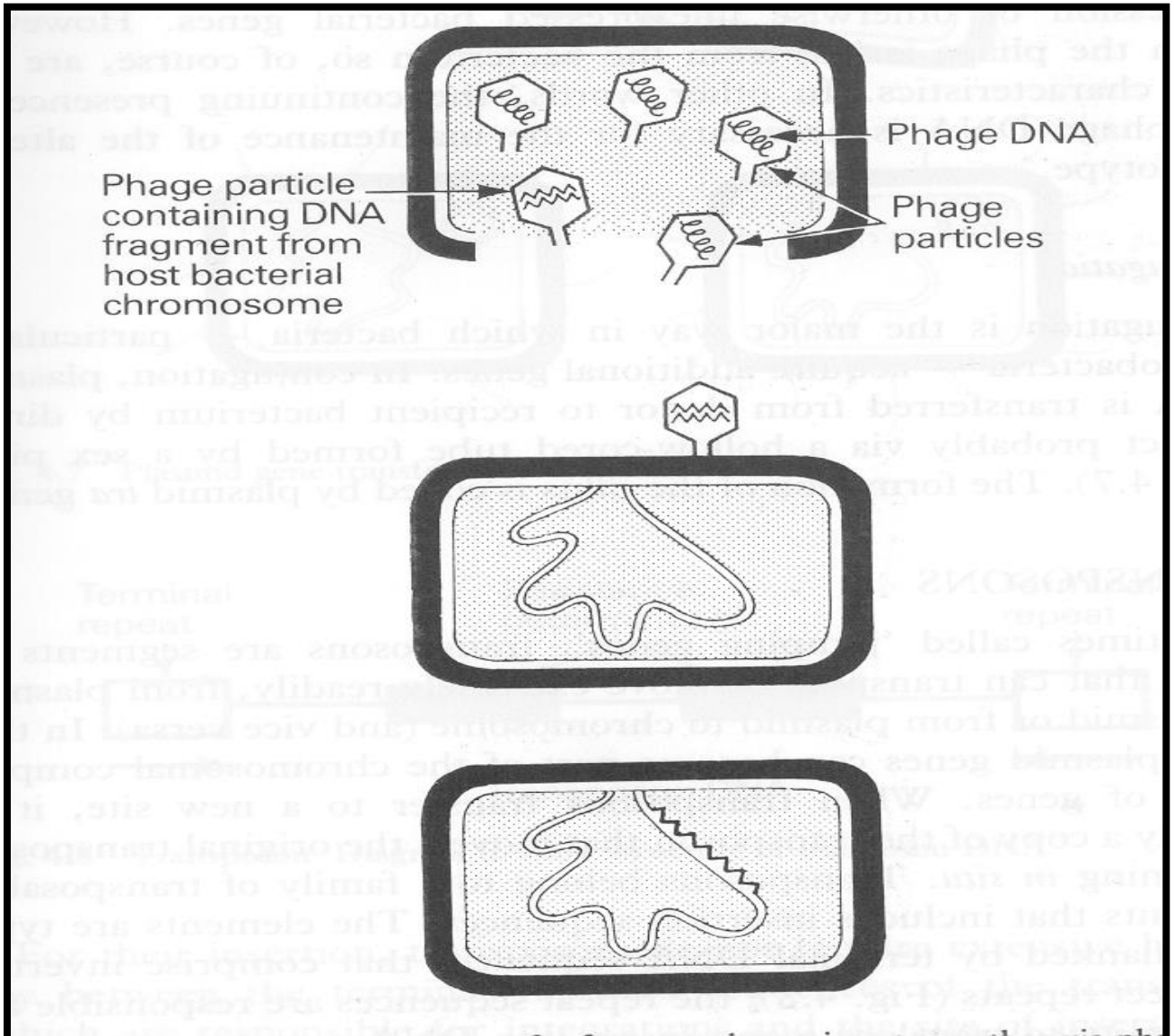
Transduction

- Phage mediated transfer of genetic information from donor to recipient cells.

Example:

- **Beta – Lactamase production in *Staphylococcus aureus* : Bacteria becomes resistant to penicillin.**
- Toxin production in *Corynebacterium diphtheriae.*

Transduction



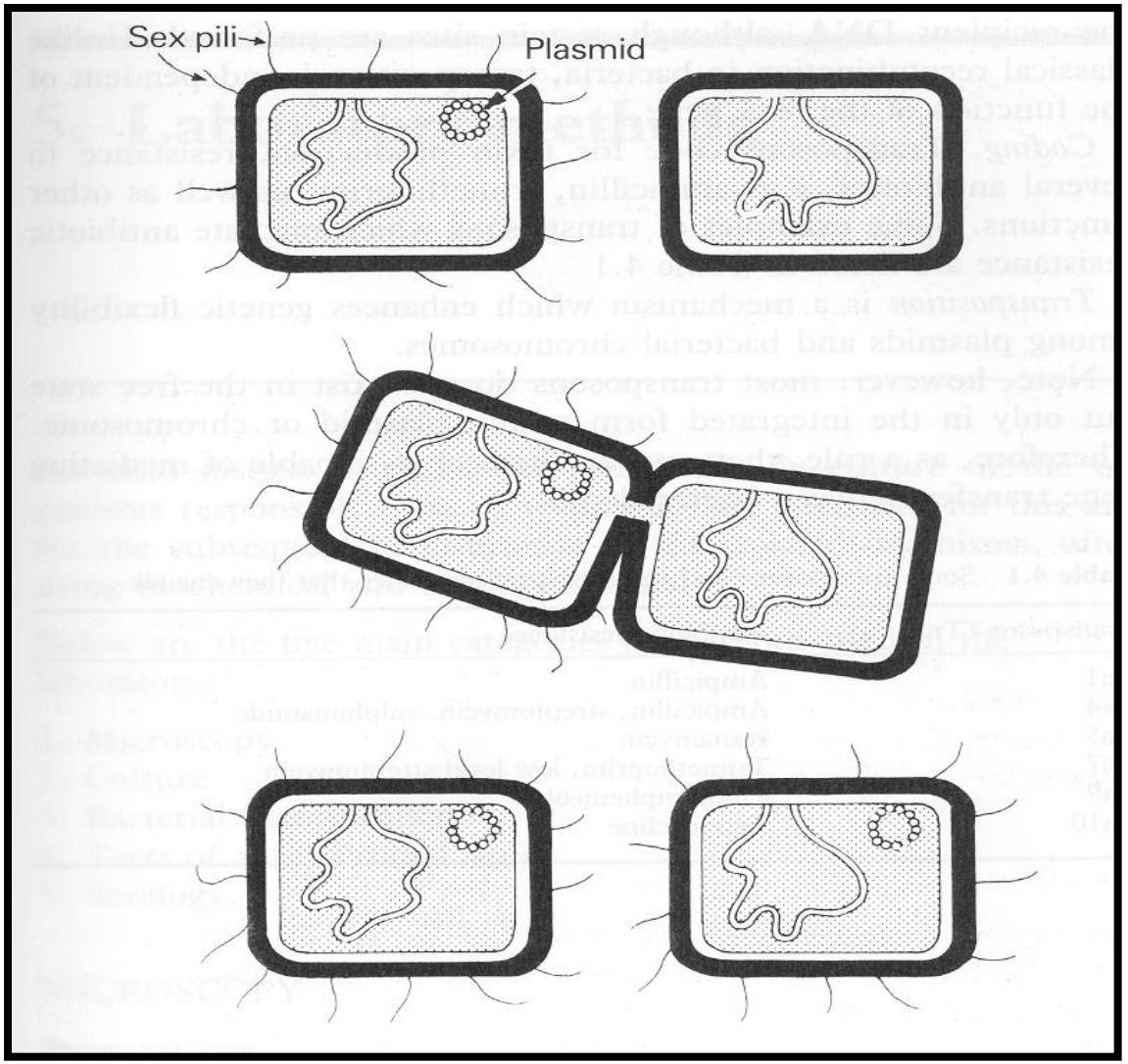
Conjugation

- Major way bacteria acquire additional genes.
- Plasmid mediated
- Cell contact required and genes reside on plasmid resident within donor cells transfer to recipient cell **(mating).**
- **Conjugation is the common way of transfer of genes resistance to antibiotics among bacteria in hospitals.**

Conjugation

- Mediated by plasmid called **F factor** (fertility).
- Gene encode changes in surface by producing a **sex pilus** .this facilitates capture of F^- cells and the formation of a conjugation bridge through which DNA passes from F^+ into F^- cells.

Conjugation in Bacteria



Genetic Recombination

After gene transfer, there are three possible fates:

1~Exogenous DNA degraded by nuclease.

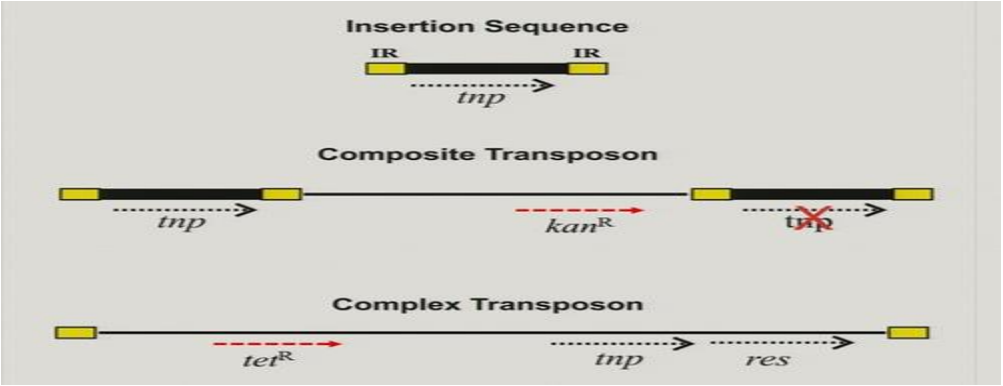
2~Stabilized by circulization and become plasmid.

3~ Form a partially hybrid chromosome with segment derived from each source.

Transposable Elements

- Genetic units capable of mediating own transfer from chromosome to another, from location to other on same chromosome or between plasmid and chromosome or phage DNA.
- Types: 1- Transposons .

  2- Insertion sequence



Reference Book

Sherries Medical Microbiology, an Introduction to Infectious Diseases.

Latest edition, Kenneth Ryan and George Ray.
Publisher : McGraw Hill .

Chapter 2 : page 11-25, **Chapter 4**: page 53-75 .