Bacterial Structure , Function & Genetics

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Objectives

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

Objectives, cont.,

- Describe the external and internal structures of bacteria and their functions.

- Describe bacterial spores and its application in the practice of medicine.

- Recall basic information about bacterial genetics and replication of bacteria .

Objectives, cont.

- Describe plasmids , its origin , types and its importance in clinical practice.

- Recall genetics variations, including ; mutation and mechanisms of gene transfer and its implication on bacterial resistance to antimicrobial agents.

Definition

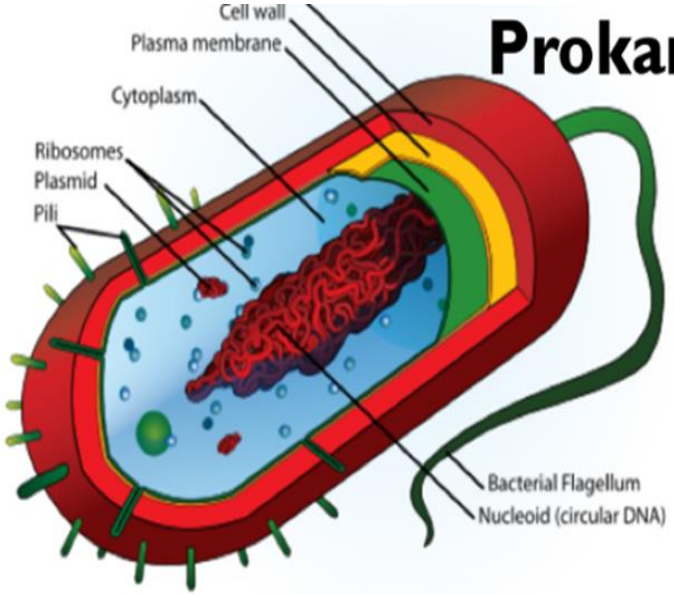
Bacteria : a heterogenous group of unicellular organisms , about 1-8 μm in diameter

Bacteria is a **Prokaryote** (has a primative nucleus):

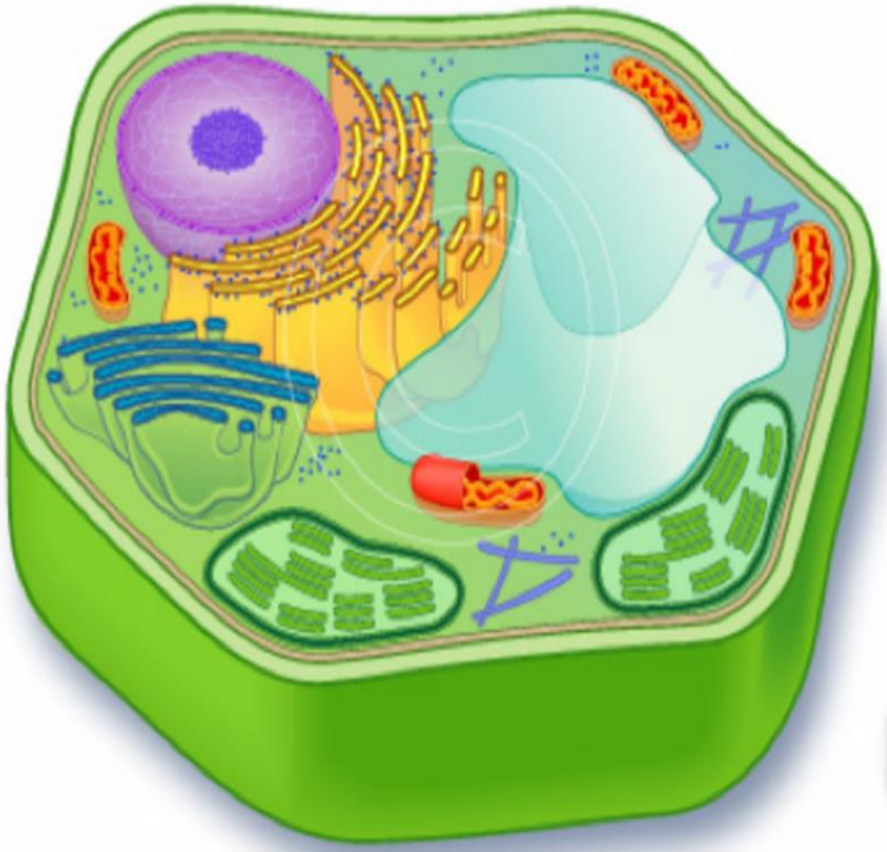
- one chromosome
- no nuclear membrane
- no mitochondria
- no sterols

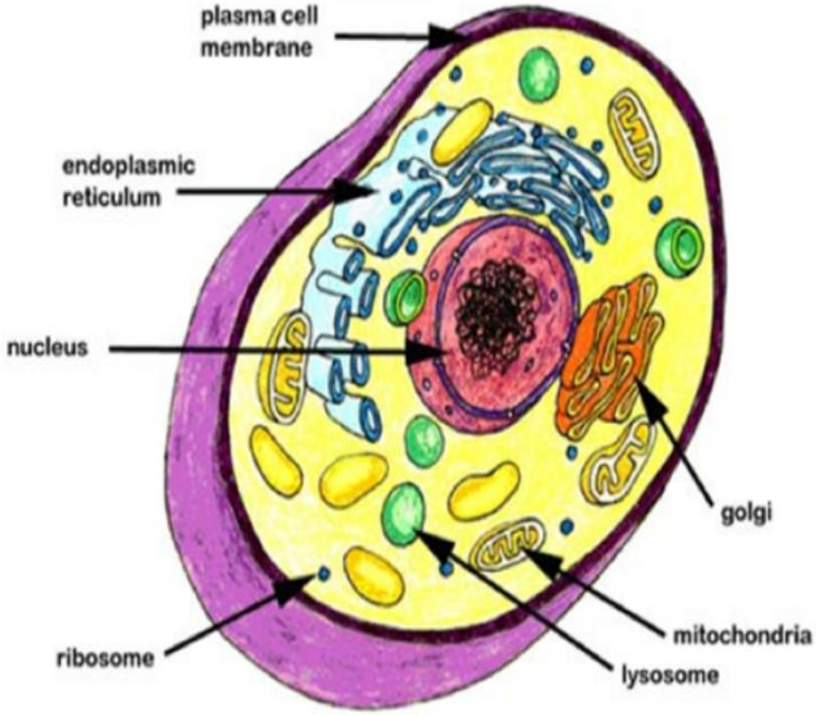
Bacteria contain Plasmids: an extra piece of **DNA.**

Prokaryotic Cell (Bacteria)



Eukaryotic Cell (Plant)





Eukaryotic Cell (Animal)

Shapes of Bacteria

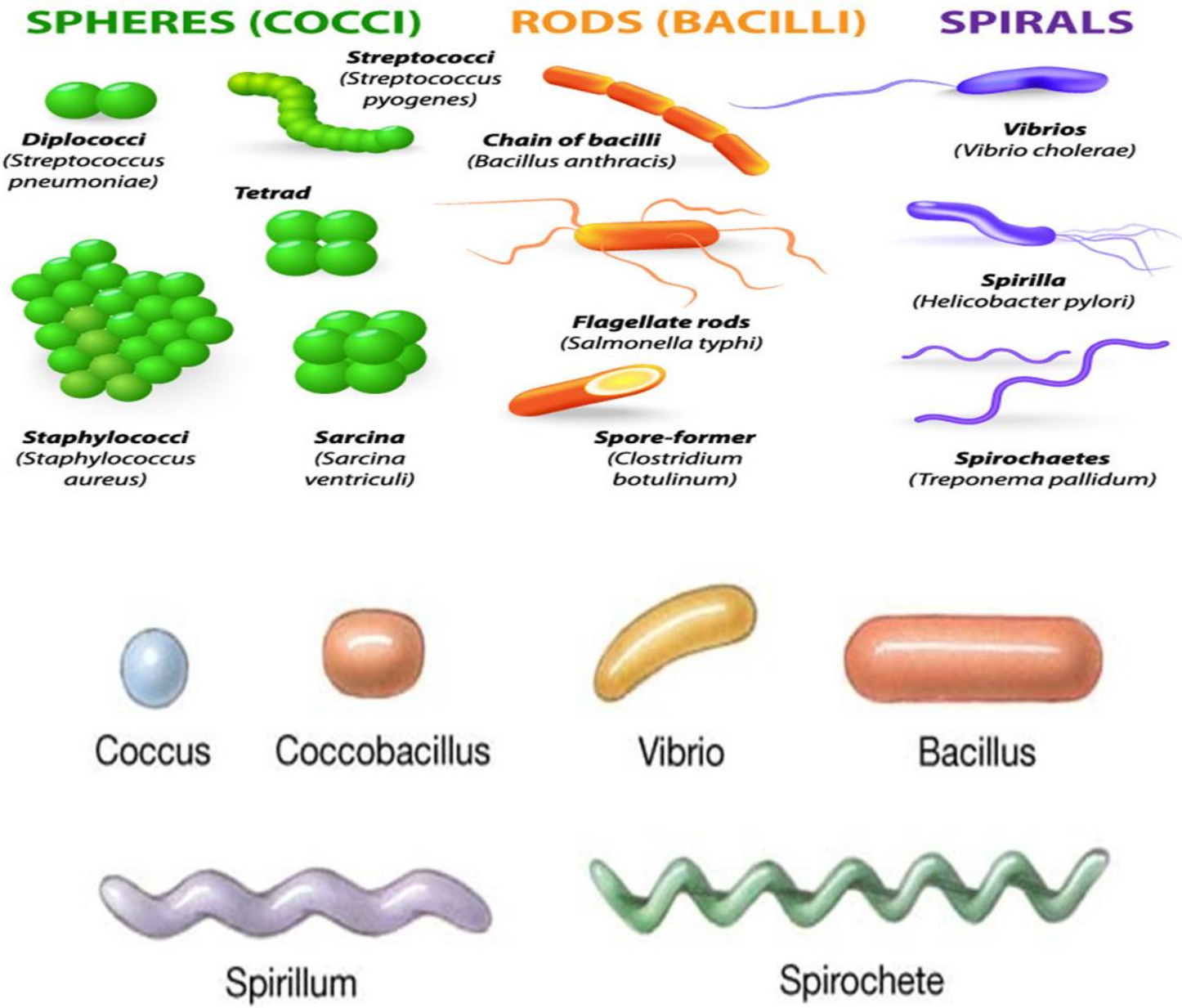
- Spherical / Oval……Cocci
- Rods……Bacilli
- Very short Bacilli……Coccobacilli
- Tapered end ……Fusiform
- Club-shaped / Curved……Vibrio
- Helical / Spiral… ……Spirochaetes

Arrangements of Bacteria

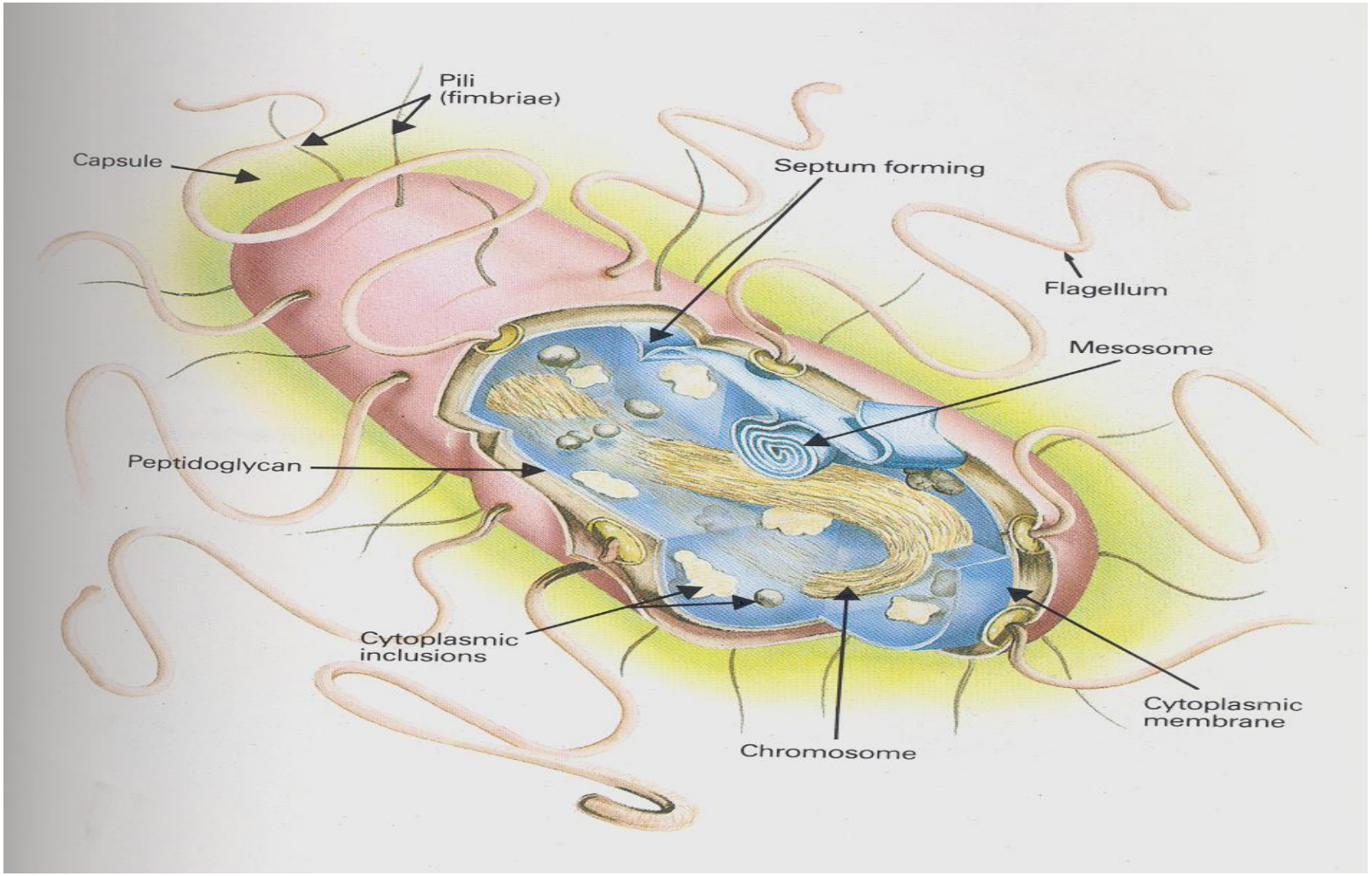
Arrangements among cocci :

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

BACTERIA SHAPES



Structure 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: stain blue/purple .

Gram negative: stain red .

Note : *Mycoplasma* naturally have no cell wall.

Chemical structure of bacterial cell wall:

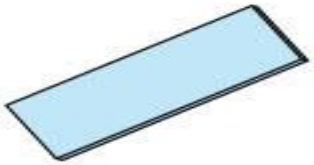
Peptidoglycan :

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

GRAM STAINING

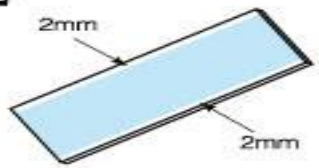
Flow Through Procedure

1



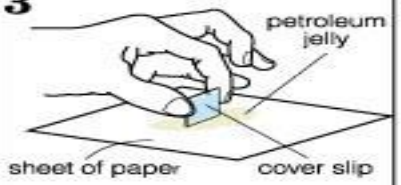
Wipe bottom of biofilm slide clean

2



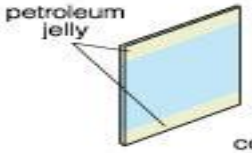
Clean top edges of slide about 2mm

3



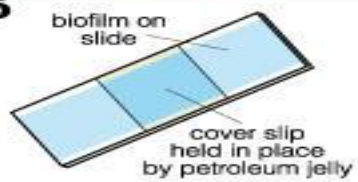
Build up a ridge of petroleum jelly on the top and bottom of a cover slip

4



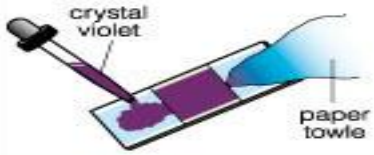
Cover slip with petroleum jelly

5



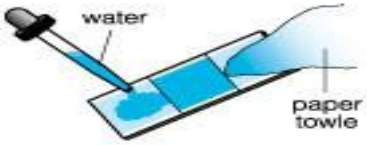
Biofilm on slide with cover slip

6



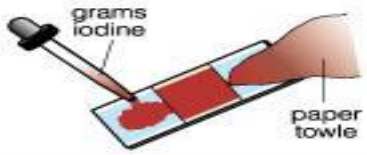
Add crystal violet-wait 30 sec.

7



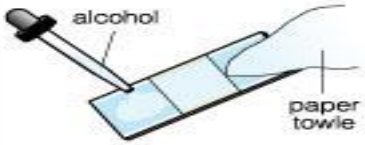
Wash with water

8



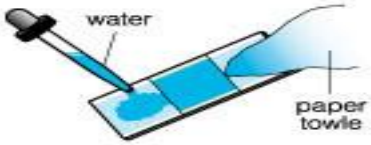
Add Grams Iodine -wait 1.5 min.

9



Decolorize with alcohol

10

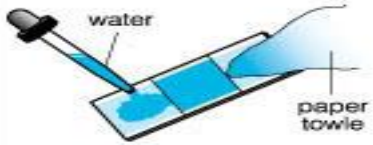


Wash with water

11

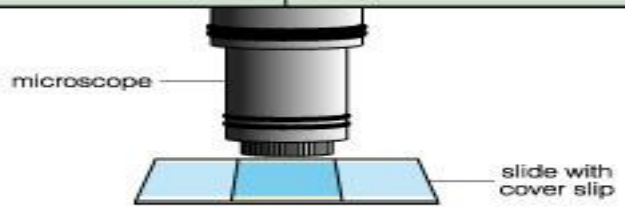
Stain with Safranin dye-wait 30 sec.

12

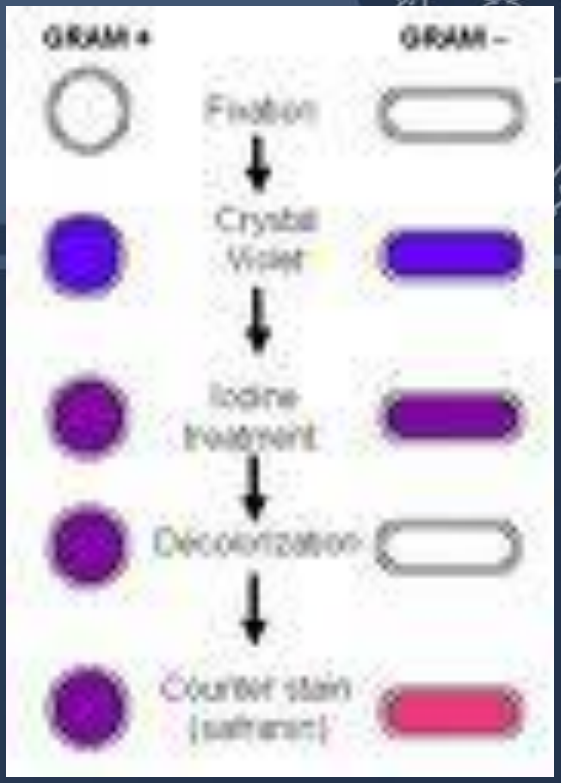


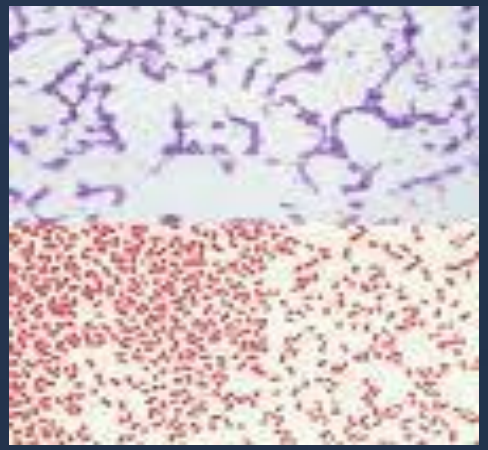
Wash with water

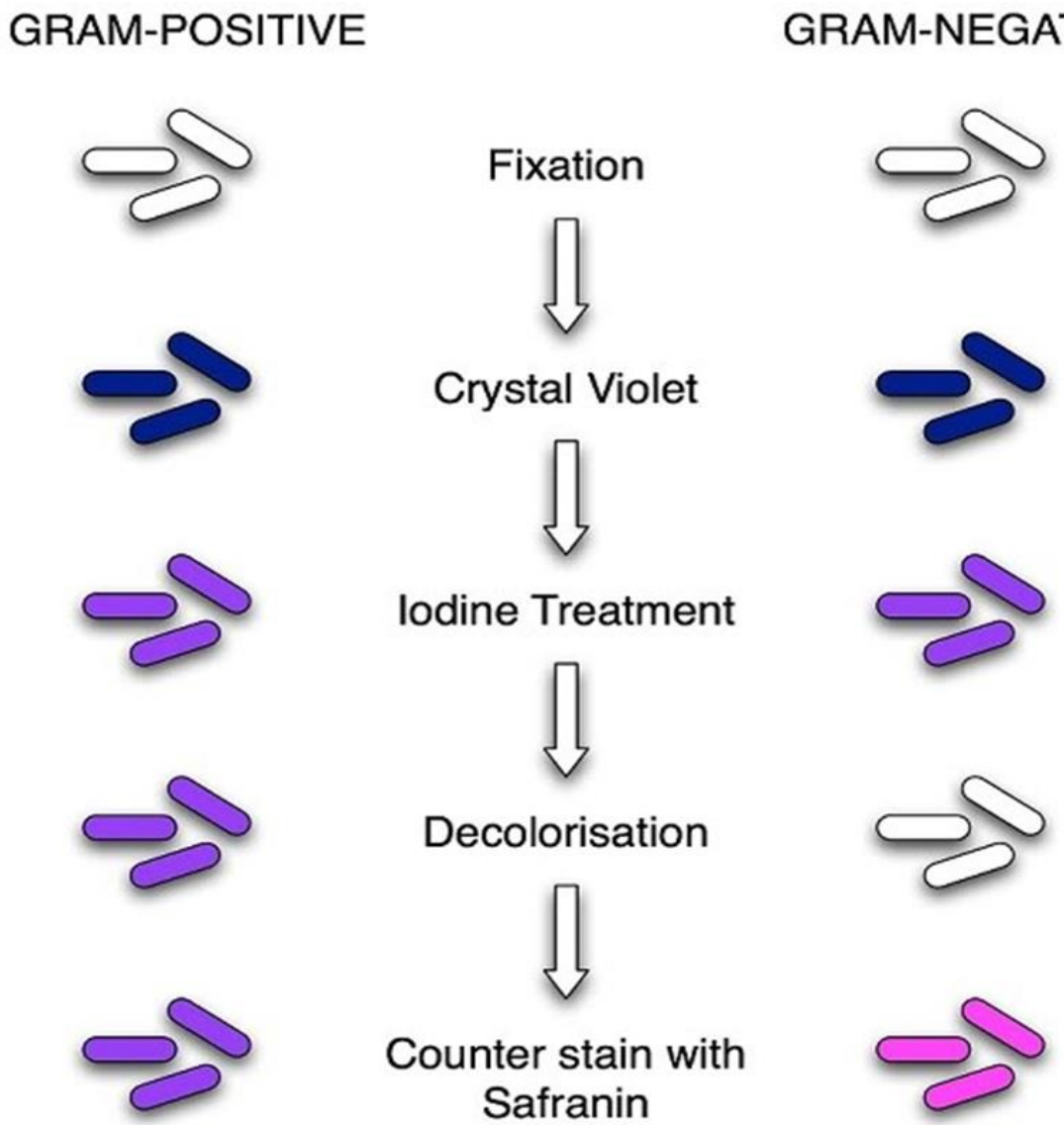
13



Examine under oil immersion through the cover slip






GRAM-POSITIVE
GRAM-NEGATIVE
Fixation
Crystal Violet
Iodine Treatment
Decolorisation
Counter stain with Safranin

Cell Wall of Gram Positive Bacteria

- Peptidoglycan is **thick**
- Closely associated with cytoplasmic membrane.
- Contain :

Teichoic acid : anchors cell wall to cell membrane , epithelial cell adhesion.

Antigens : ~ polysaccharides (Lancefield)

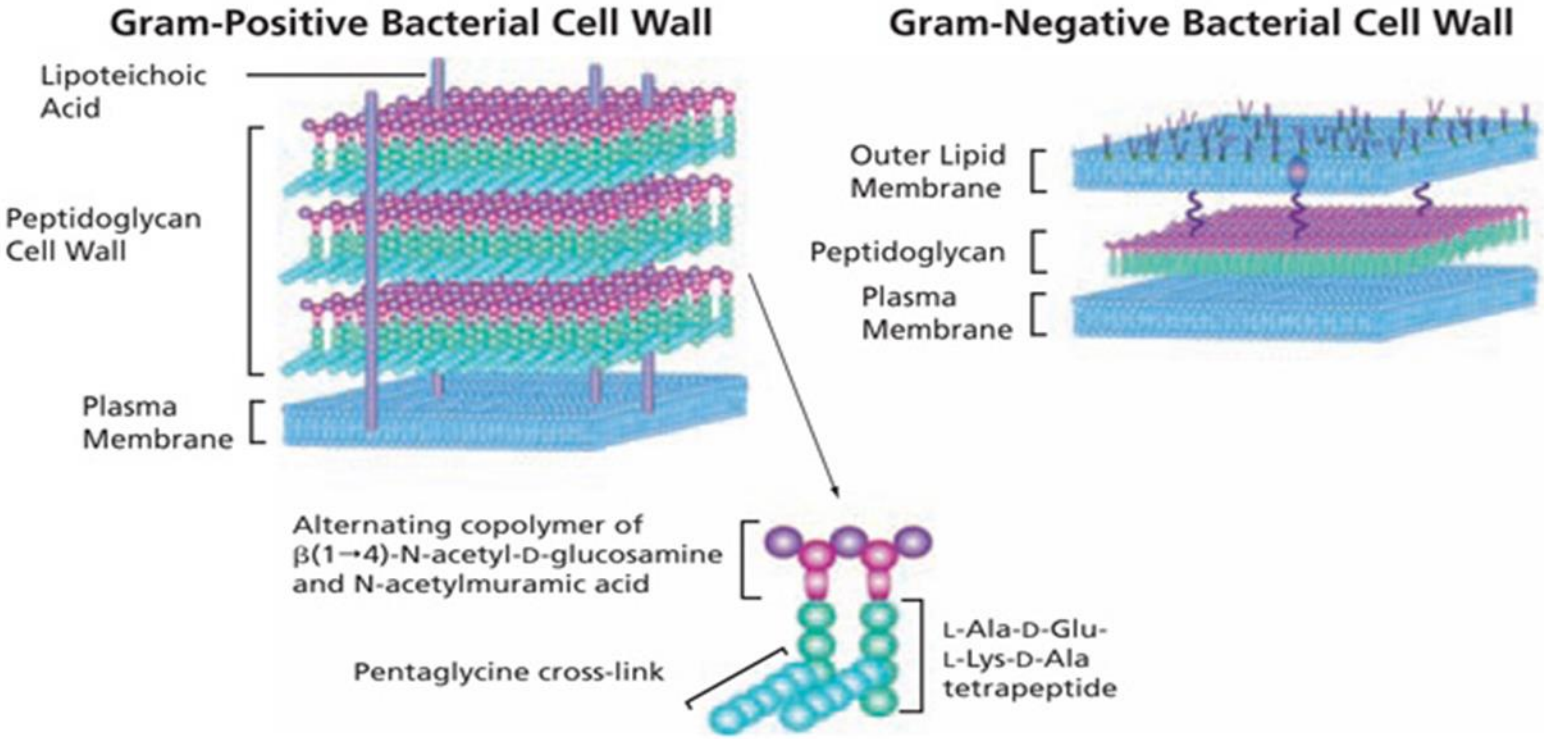
~ protein (Griffith)

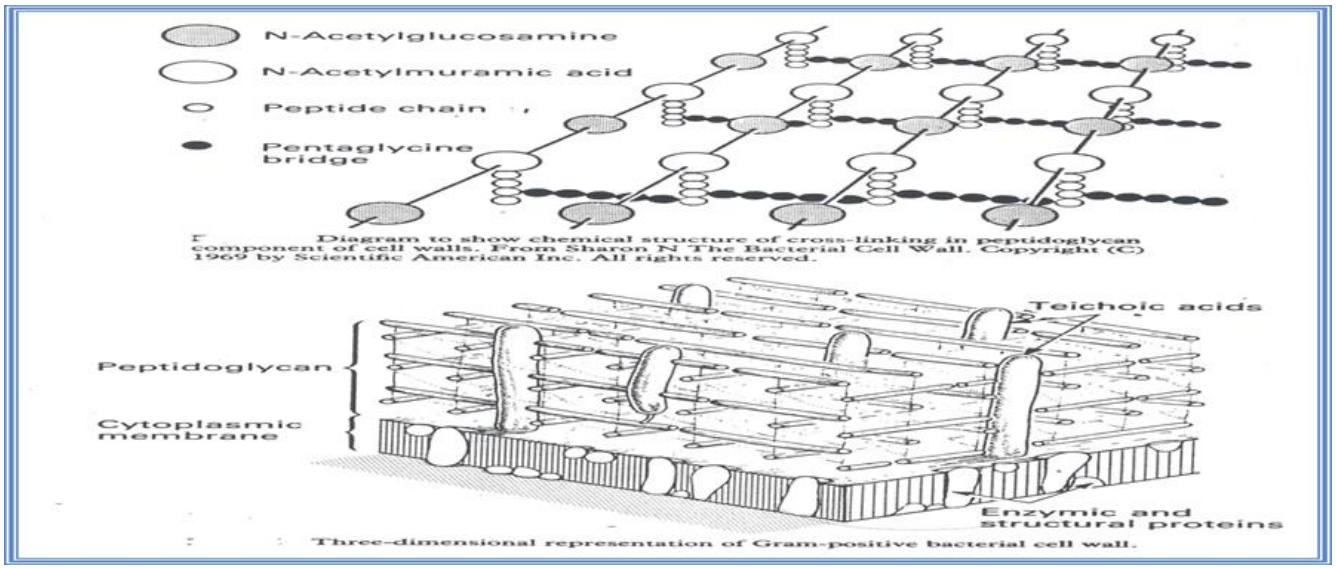
Cell Wall of Gram Negative Bacteria

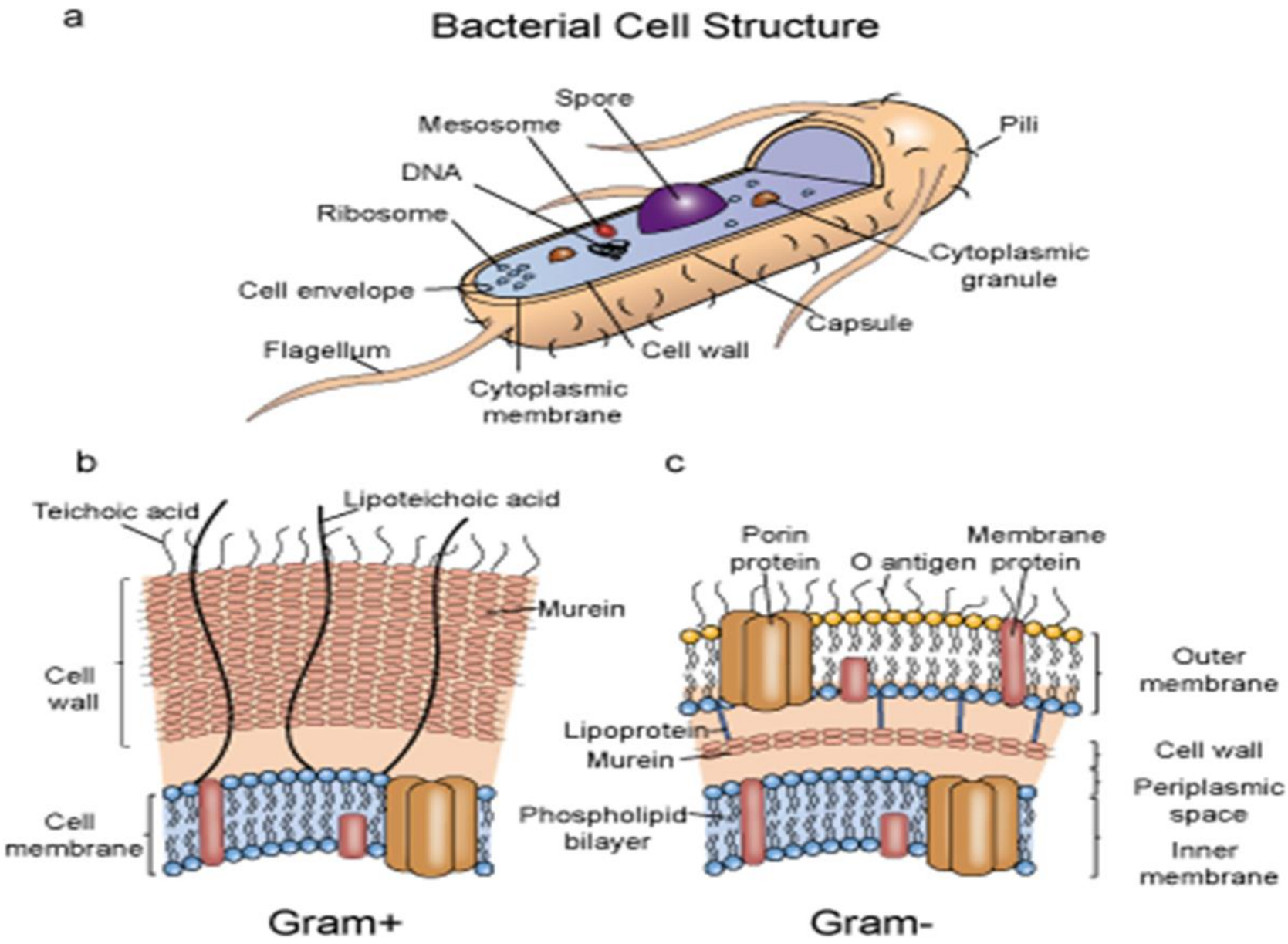
- Thin Peptidoglycan
- Has an outer membrane that contains :

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

  - lipopolysaccharide (Endotoxin)


Gram-Positive Bacterial Cell Wall
Lipoteichoic Acid
Peptidoglycan Cell Wall
Plasma Membrane
Gram-Negative Bacterial Cell Wall
Outer Lipid Membrane
Peptidoglycan
Plasma Membrane
Alternating copolymer of β(1→4)-N-acetyl-D-glucosamine and N-acetylmuramic acid
Pentaglycine cross-link
L-Ala-D-Glu-L-Lys-D-Ala tetrapeptide


N-Acetylglucosamine
N-Acetylmuramic acid
Peptide chain
Pentaglycine bridge
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.
Teichoic acids
Peptidoglycan
Cytoplasmic membrane
Enzymic and structural proteins
Three-dimensional representation of Gram-positive bacterial cell wall.


a
Bacterial Cell Structure
Spore
Mesosome
Pili
DNA
Ribosome
Cytoplasmic granule
Cell envelope
Capsule
Flagellum
Cell wall
Cytoplasmic membrane
b
Teichoic acid
Lipoteichoic acid
Murein
Cell wall
Cell membrane
Gram+
c
Porin protein
O antigen
Membrane protein
Outer membrane
Lipoprotein
Murein
Cell wall
Periplasmic space
Phospholipid bilayer
Inner membrane
Gram-

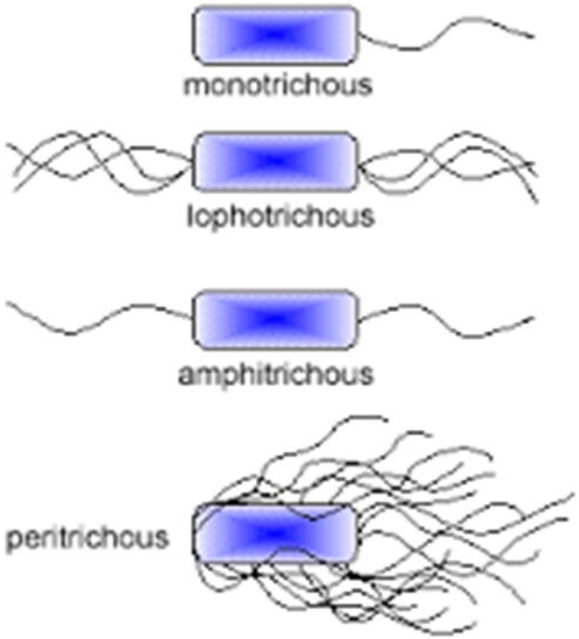
External Structures of Bacteria

External protrude from the cell into the environment:

- Flagella
- Pili
- Capsule

Flagella

- Helical filaments
- Composed of protein **FLAGELLIN.**
- Found in Gram positive & Gram negative bacteria.
- **Function** : motility& chemotaxis
- **Distribution:**
  - ~ Peritrichous
  - ~ Monotrichous
  - ~ Lophotrichous
  - ~Amphitricous



Pili

Fine short filaments extruding from cytoplasmic membrane.

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

Composed of protein Pilin.

Two types:

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

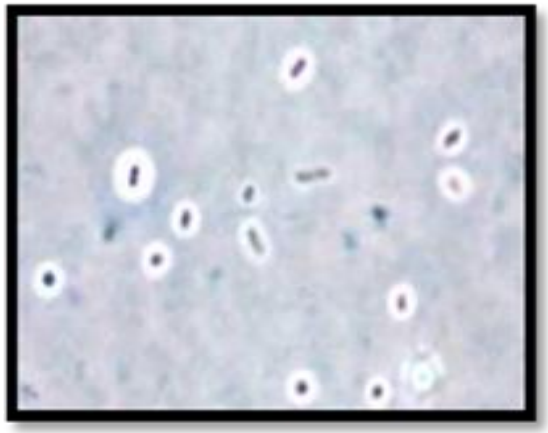
2- **Sex pili** : in some bacteria only, responsible for conjugation.

Capsules and Slime layer

- These are the structures surrounding the outside of cell envelop. Can be seen by India ink or special stains
- Usually consist of **polysaccharide,** however ;in some bacteria consist of polypeptide(**protein**).
- They are not essential for cell viability, some strains within species produce capsule while others do not .

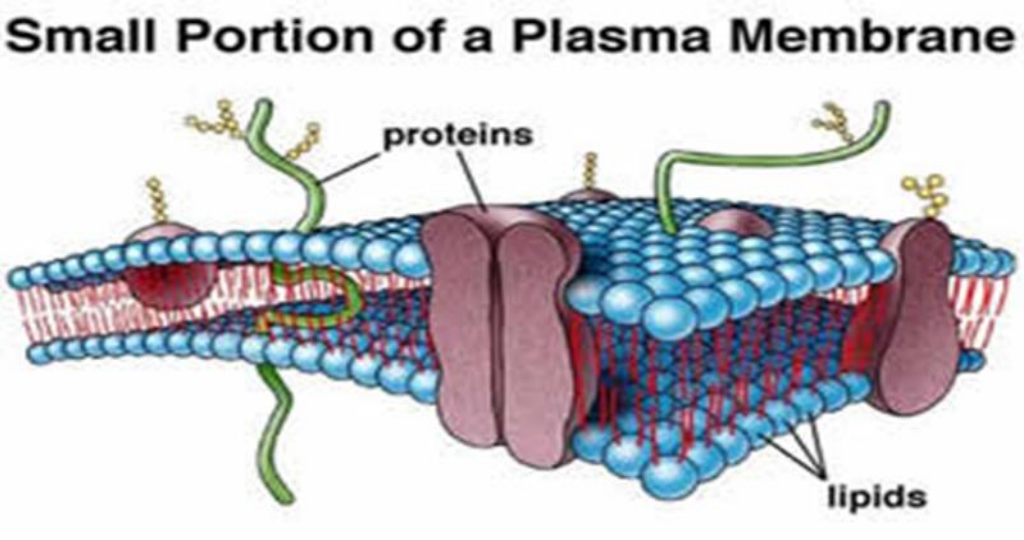
Functions, include :

- Attachment
- Protection from phagocytic engulfment
- Resistant to dryness
- Reservoir for certain nutrient



Cytoplasmic Membrane (plasma 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 :convolutes invaginations of cytoplasmic membrane .

Function:

1. Involved in DNA segregation during cell division and respiratory activity
2. Contain receptors involved in chemotaxis
3. Permeability barrier (active transport of solutes).

Core of Bacteria

Core composed of : Cytoplasmic inclusions
Nucleoid (nuclear body)
Ribosomes

Cytoplasmic inclusions:

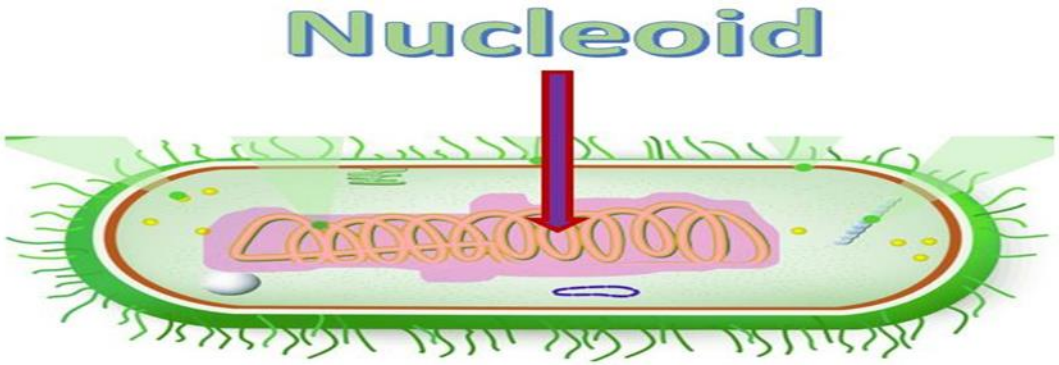
Are nutritional storage granules , examples:

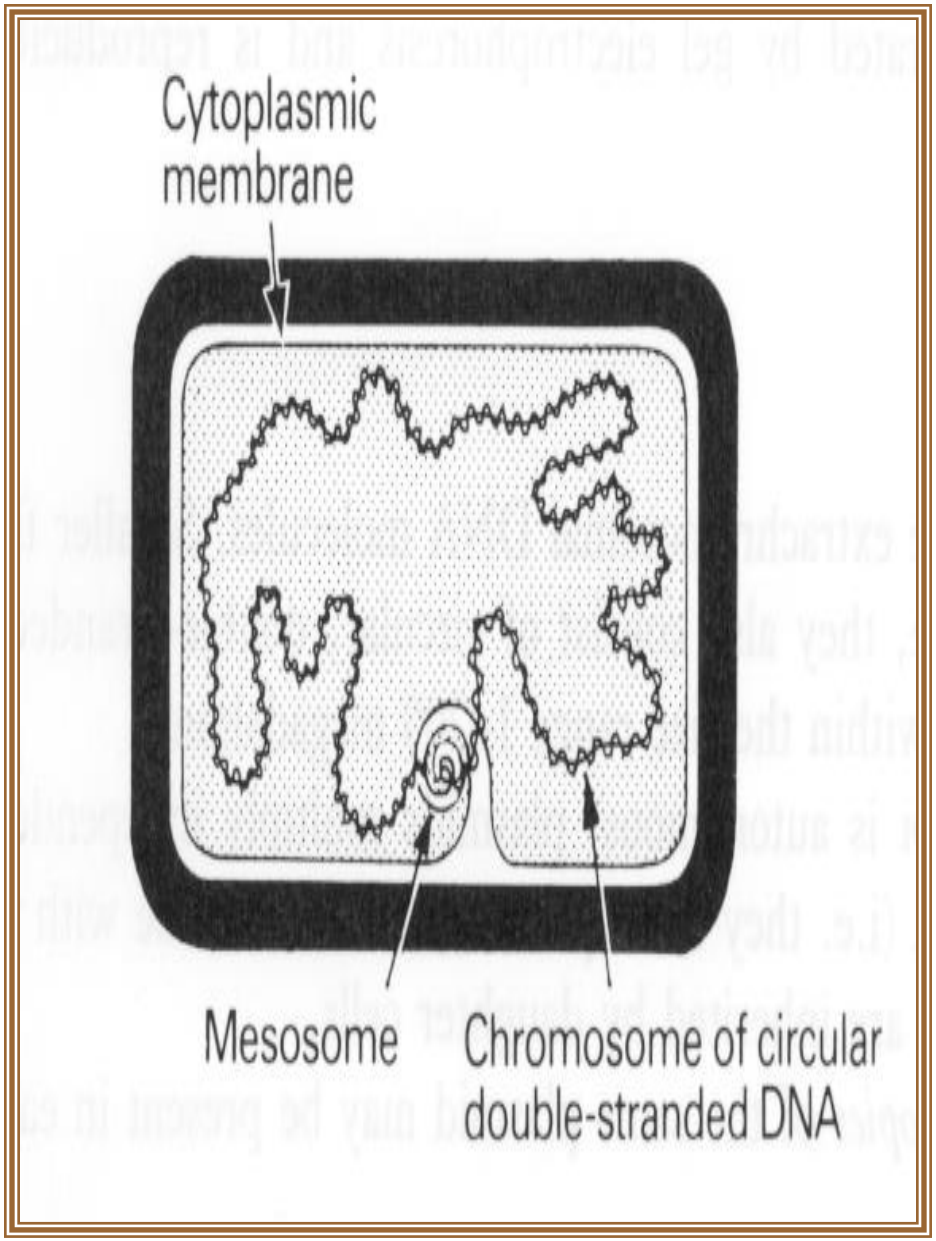
- Volutin
- Lipid
- Starch / or Glycogen

Bacterial Chromosomes

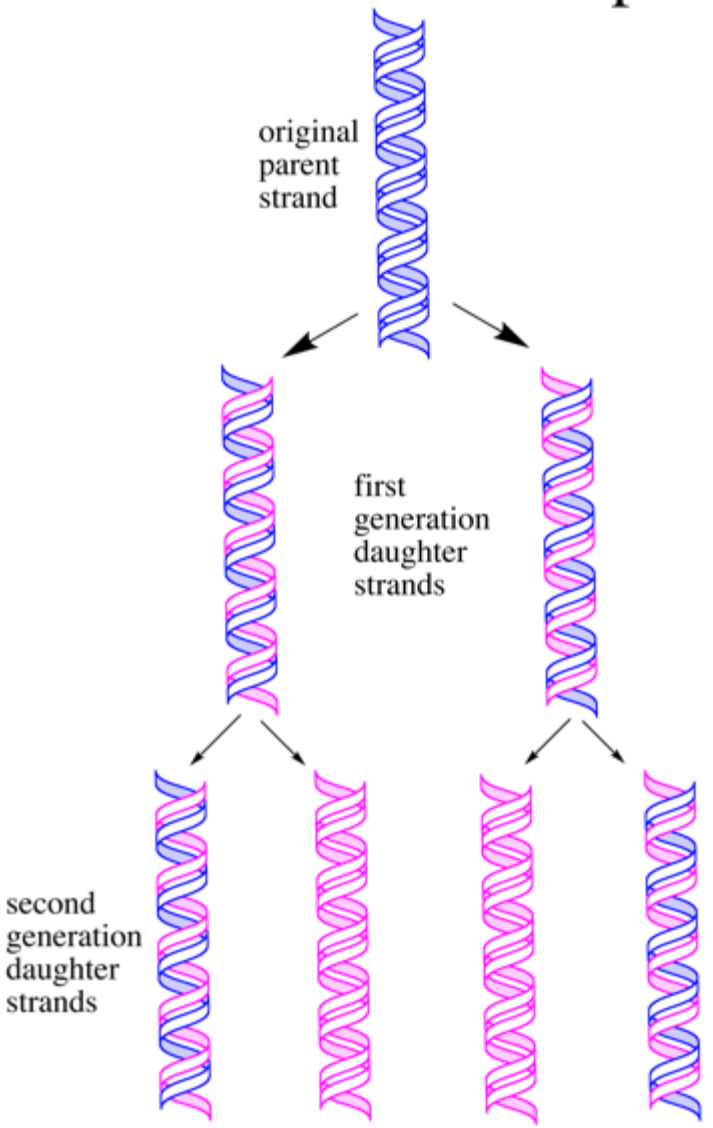
- A **circular** molecule of double stranded- DNA located in the cytoplasm.
- It is packed with RNA molecules and proteins to form irregular shaped structure the nucleoid.
- Genetic code in Purine and Pyrimidine bases of nucleotides that makes DNA strand.
- Replication is semiconservative ,takes place by binary fission .

Nucleoid (Nuclear Body)

- Circular double stranded chromosome
- No nuclear membrane
- DNA undergoes semi-conservative replication , bidirectional from a fixed point
- 

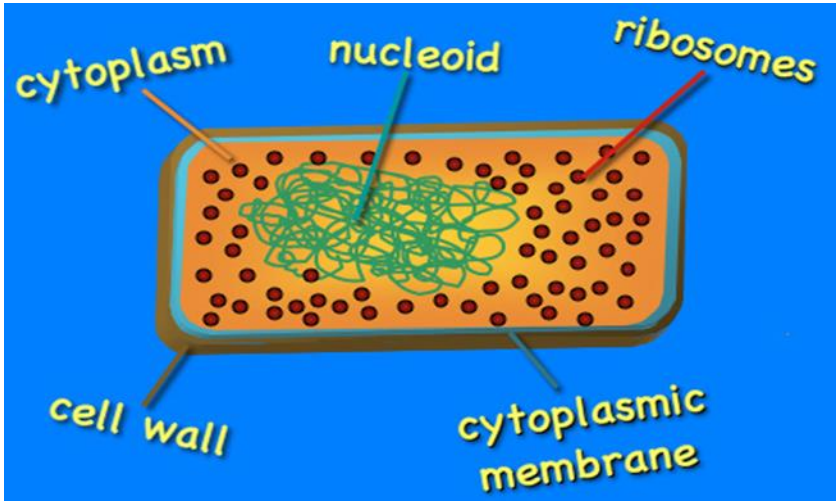


Semiconservative Replication



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 :**

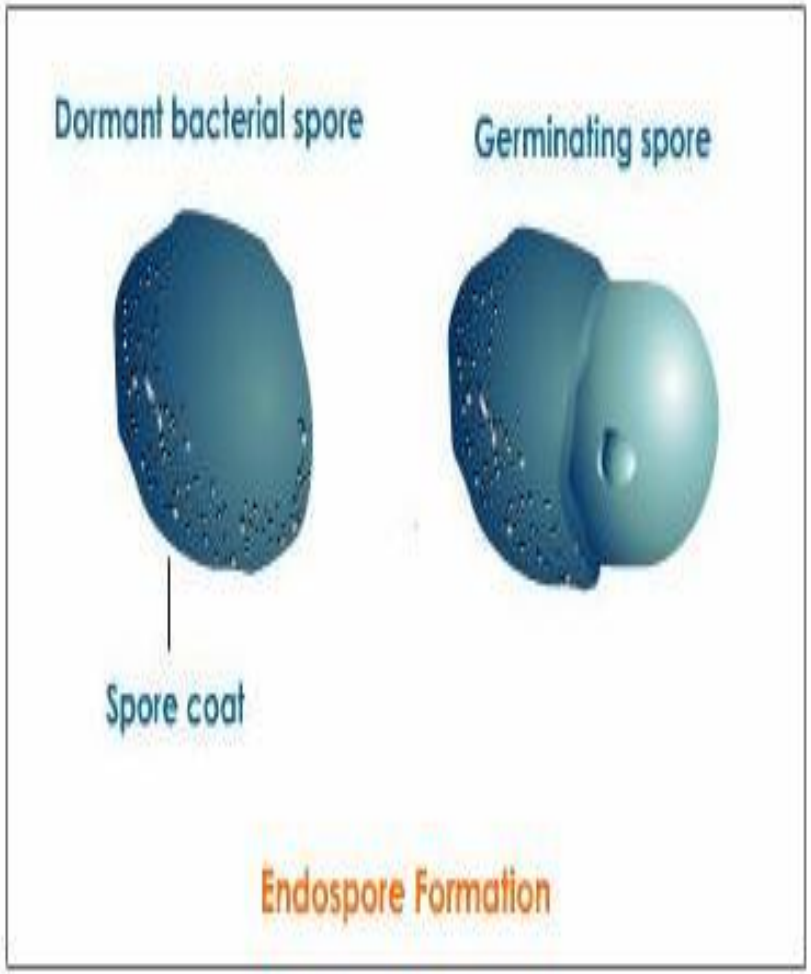
1- **Terminal**

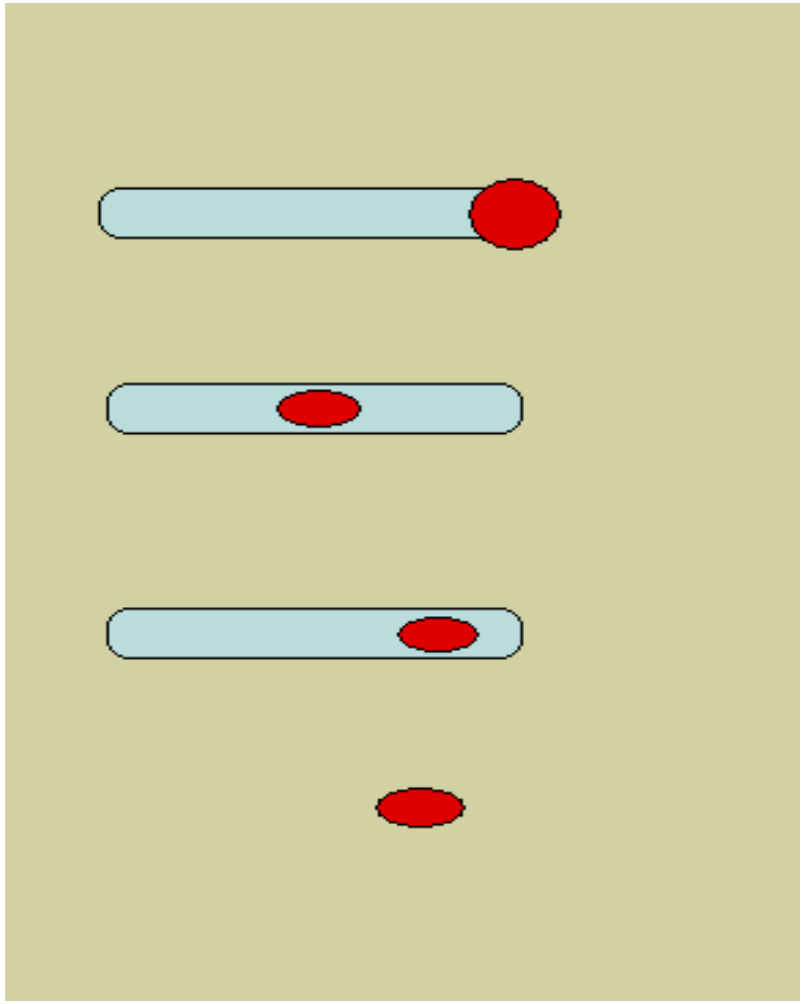
2- **Sub-terminal**

3- **Central**

- 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.

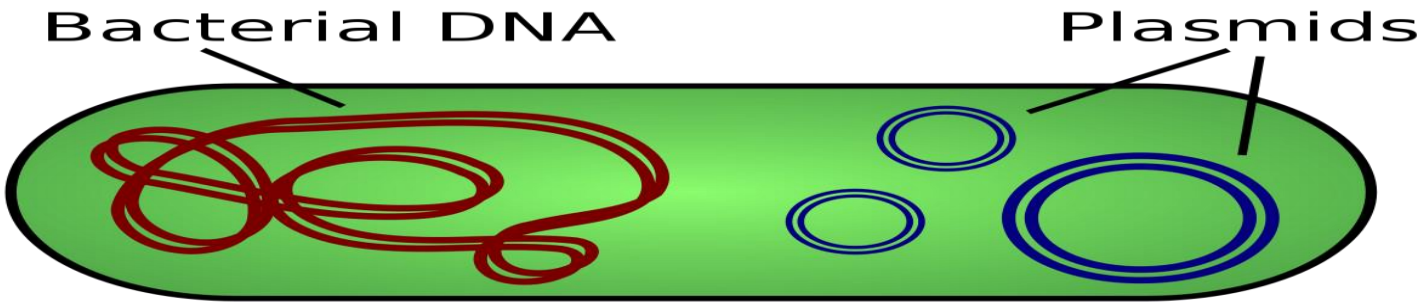
- **Genotype:** the complete set of genetic determinants of an organism.
- **Phenotype:** expression of specific genetic material .
- **Wild type:** reference (parent) strain
  - **Mutant:** progeny with mutation.

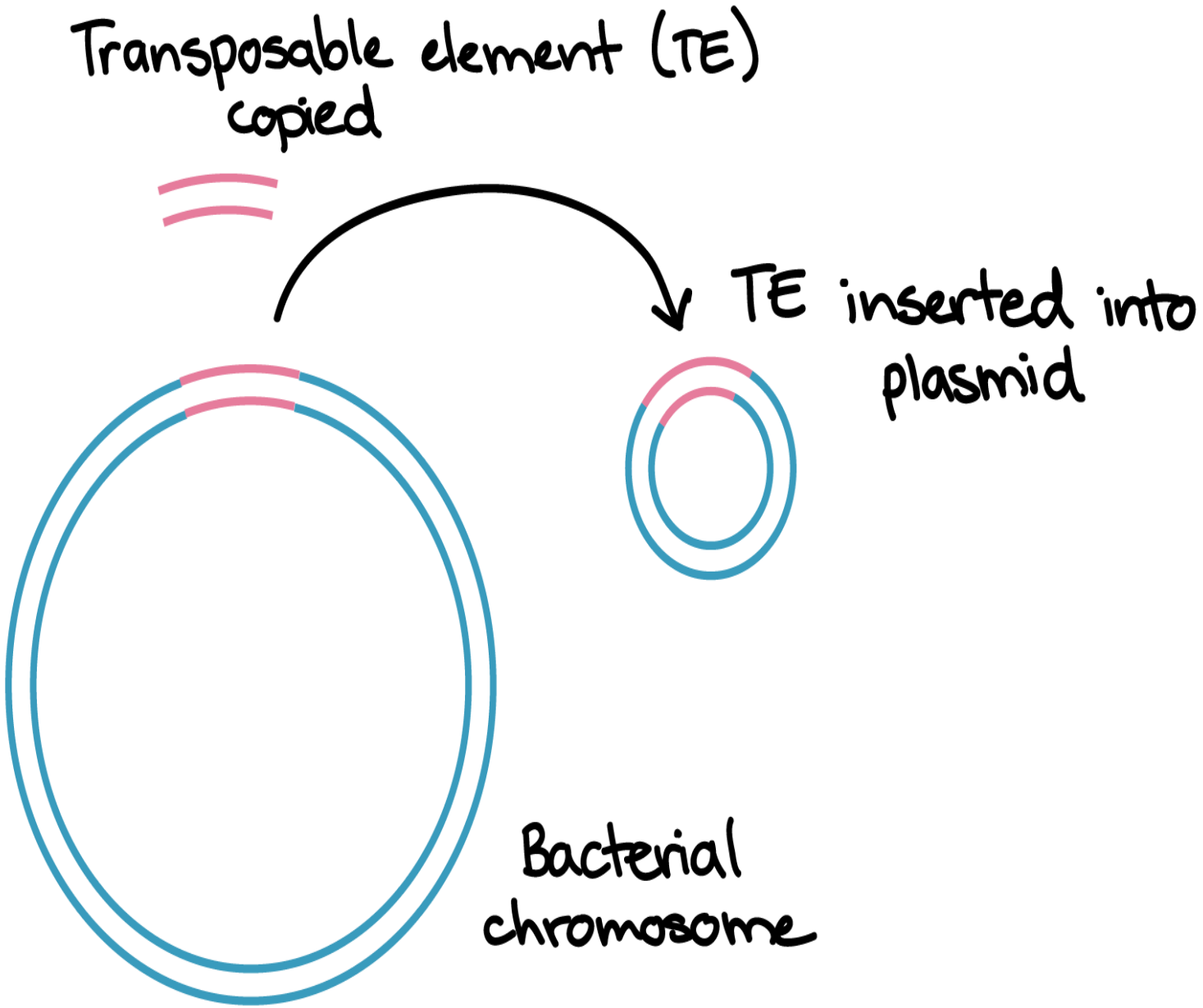
Two types of DNA in bacteria

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

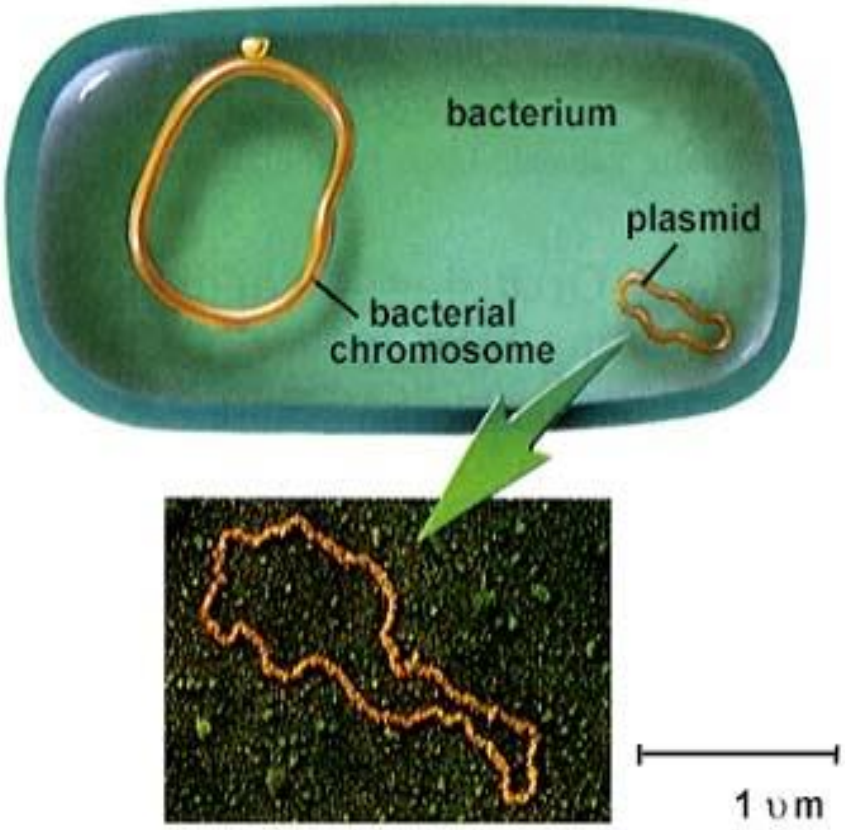
Plasmids

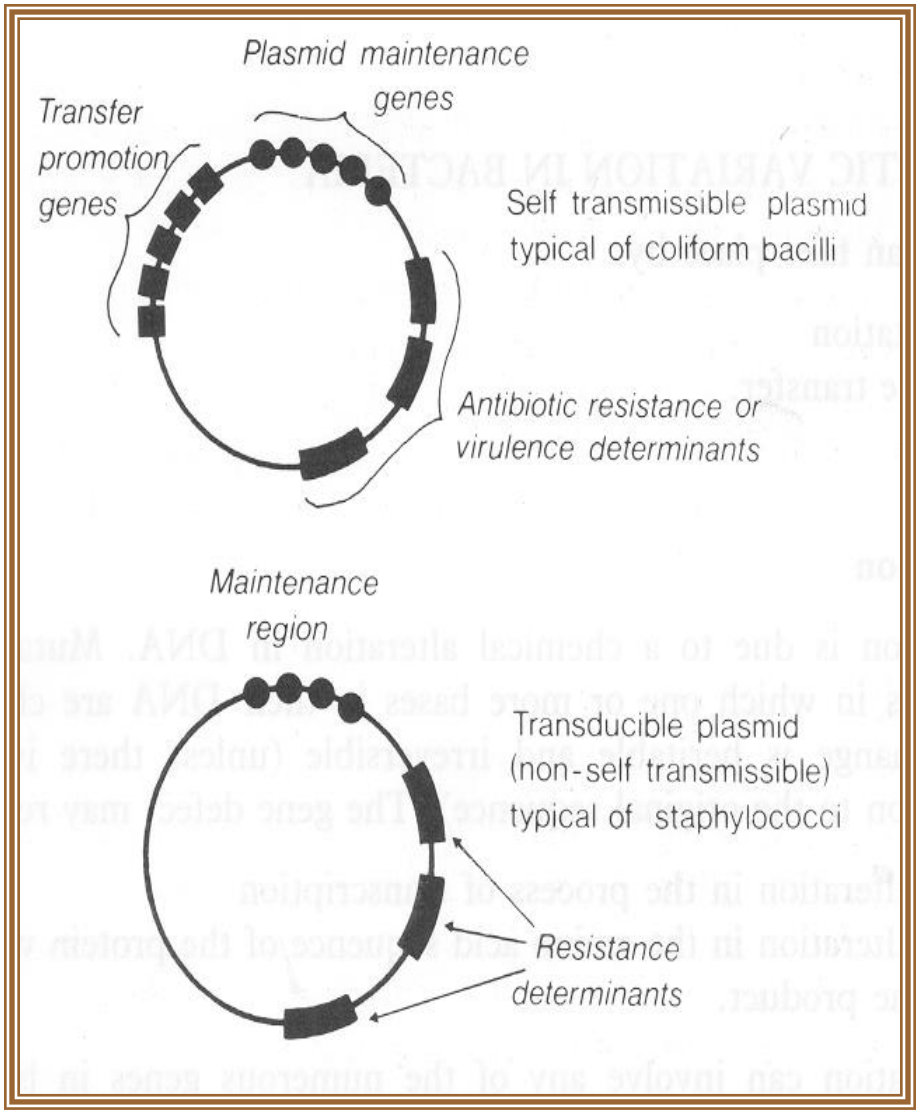
- Extra chromosomal DNA composed of double stranded-DNA.
- Found in most species of bacteria.
- Origin?
- Govern their own replication
- **Application** :in genetic exchange, amplify genes
- Transfer to other bacteria by **conjugation**




Transposable element (TE) copied
TE inserted into plasmid
Bacterial chromosome

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 DNA.
- Chemical changes in one or more bases of DNA.

Mutation /gene defect leads to alteration in:

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

Classification of Mutation

Depends on biological sequencing:

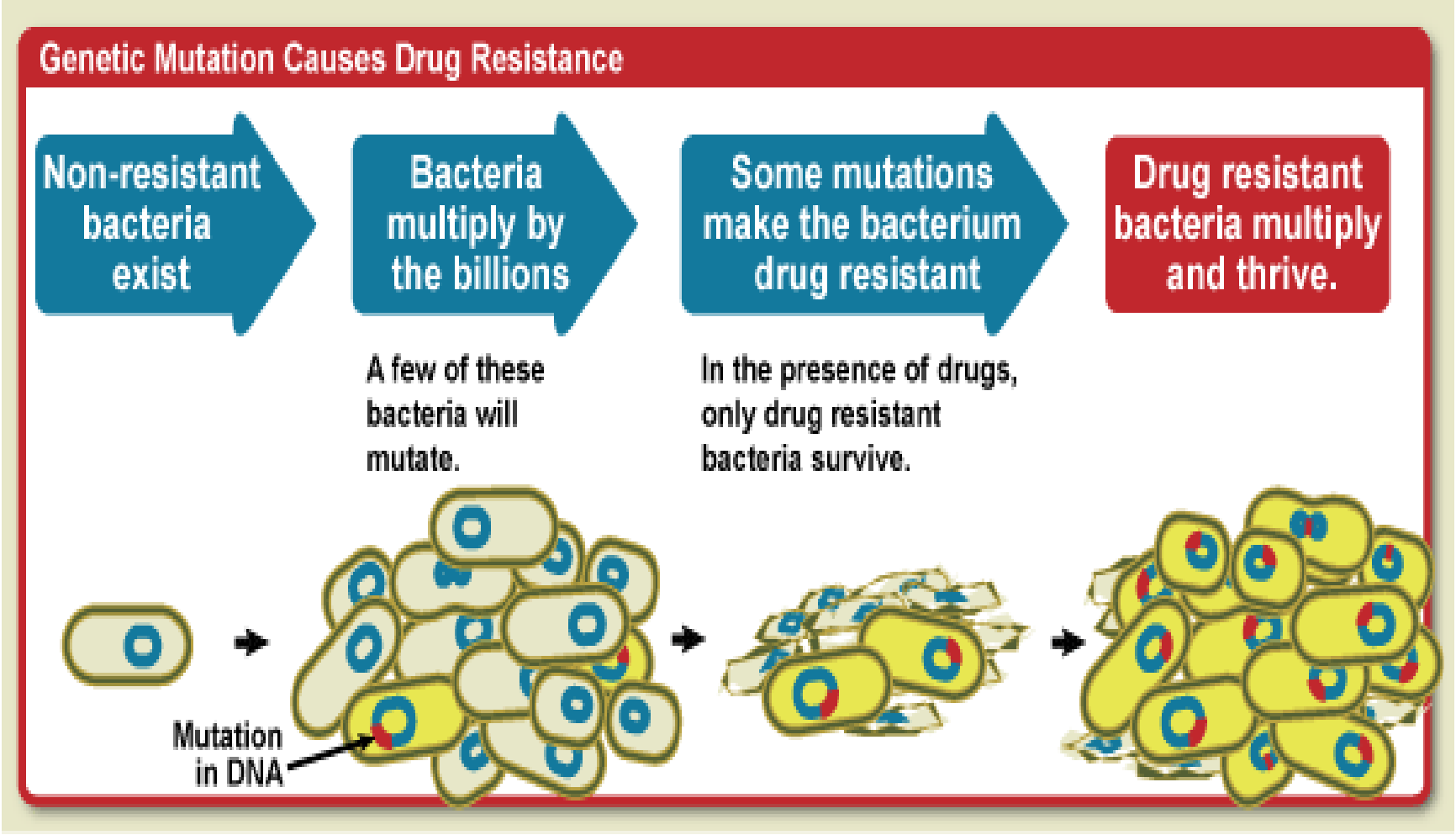
1- **Resistance mutation**: affects structure of cell protein. **Main application in medical practice.**

Bacteria become resistant to antibiotics.

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

3- **Lethal mutation:** leads to death of bacteria.

Mutation Causes Antimicrobial Resistance



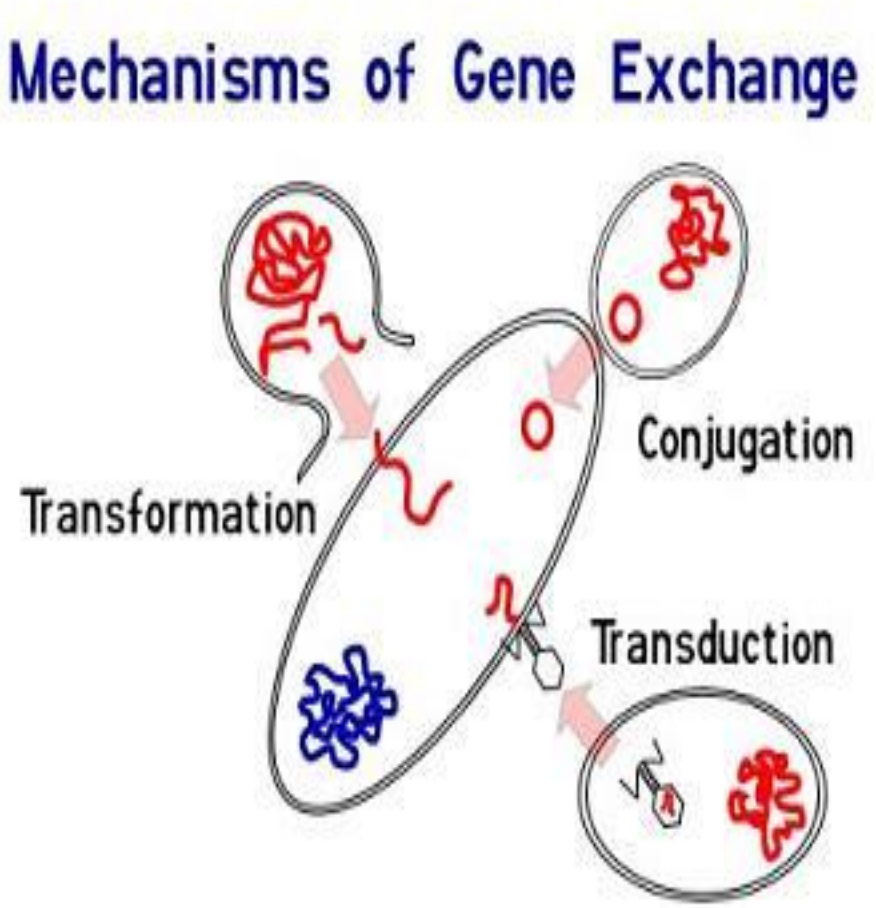
Gene Transfer Among Bacteria

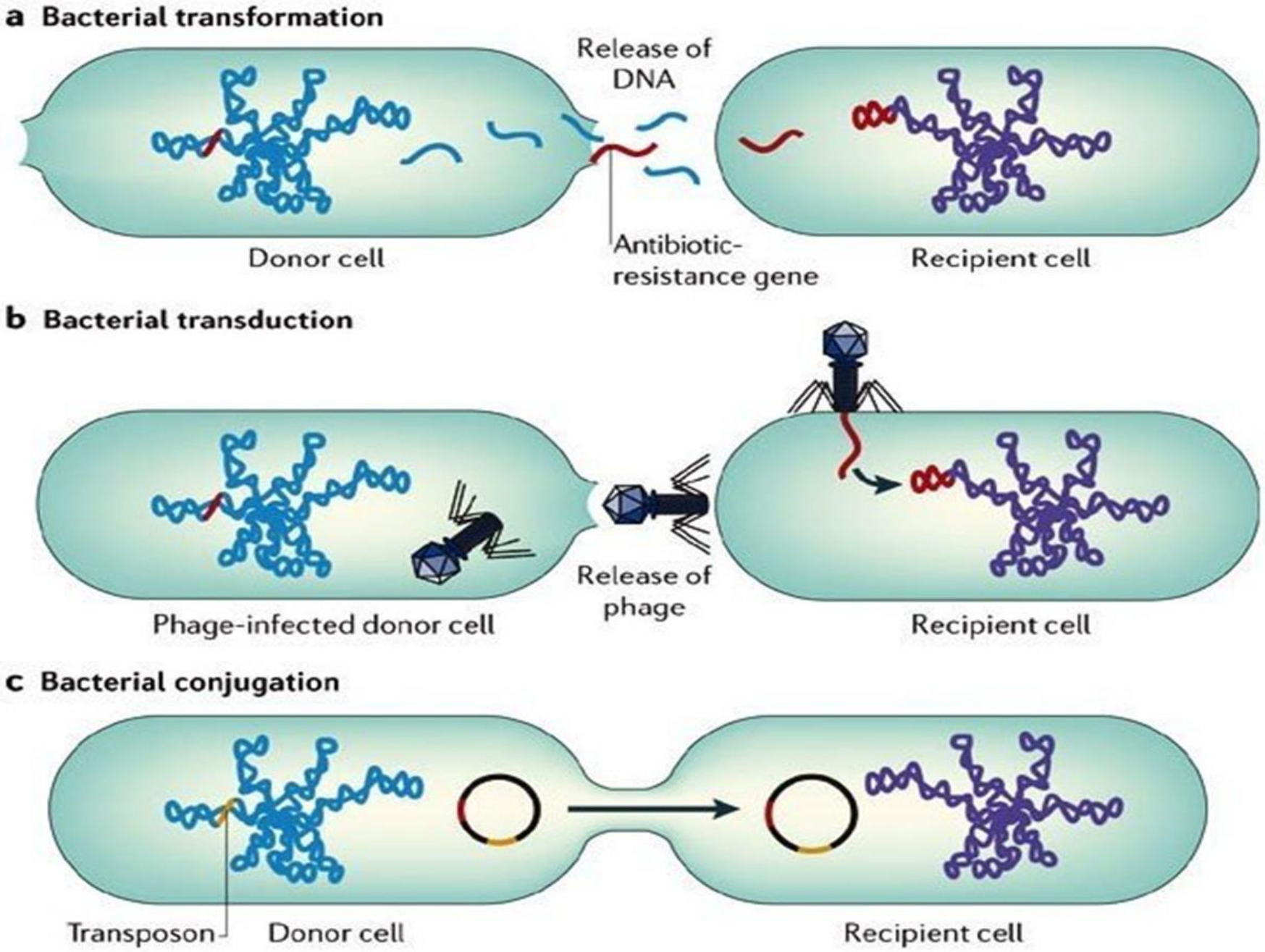
Three mechanisms:

1- Transformation

2- Transduction

3- Conjugation.




a Bacterial transformation
Release of DNA
Donor cell
Antibiotic-resistance gene
Recipient cell
b Bacterial transduction
Phage-infected donor cell
Release of phage
Recipient cell
c Bacterial conjugation
Transposon
Donor cell
Recipient cell

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.

Examples:

- **Beta – Lactamase production in *Staphylococcus aureus* : resistance to penicillin.**
- **Toxin production by *Corynebacterium diphtheriae.***

Conjugation

- Major way bacteria acquire additional genes.
- **Plasmid mediated**(F factor)
- 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.**

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.

Reference Book

Sherris Medical Microbiology, an Introduction to Infectious Diseases.

Latest edition, Kenneth Ryan and George Ray.
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