



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

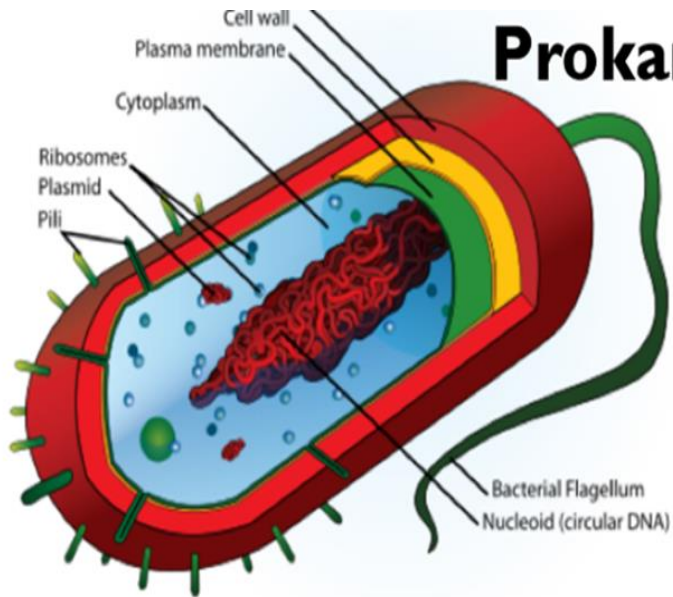
Bacteria : Is a heterogenous group of unicellular organisms , about 1-8 μm in diameter

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

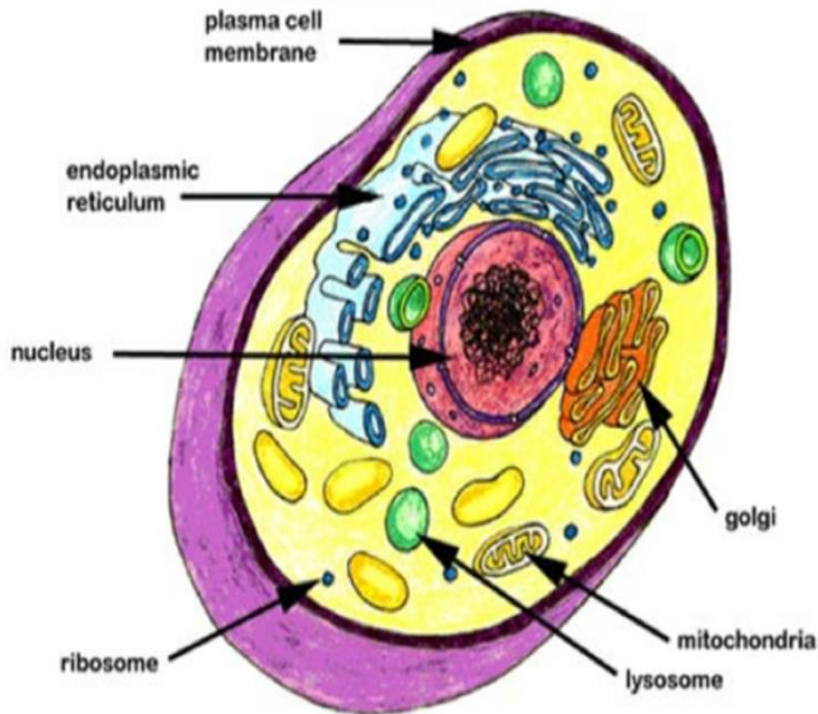
- ~ one chromosome
- ~ no nuclear membrane
- ~ no mitochondria
- ~ no sterols

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

Prokaryotic Cell (Bacteria)



Eukaryotic Cell (Plant)



Eukaryotic Cell (Animal)

Shapes & Types of Bacteria

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

Arrangements of Bacteria

Arrangements among Cocci :

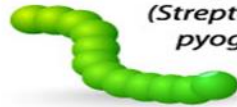
- Pairs.....Diplococci
- Chains.....Streptococci
- Clusters.....Staphylococci
- In four.....Tetrad
- Palisades.....*Corynebacterium*

BACTERIA SHAPES

SPHERES (COCCI)

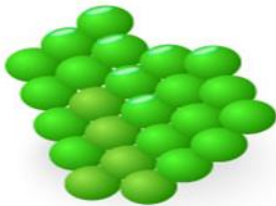


Diplococci
(*Streptococcus pneumoniae*)



Streptococci
(*Streptococcus pyogenes*)

Tetrad



Staphylococci
(*Staphylococcus aureus*)



Sarcina
(*Sarcina ventriculi*)

RODS (BACILLI)



Chain of bacilli
(*Bacillus anthracis*)



Flagellate rods
(*Salmonella typhi*)



Spore-former
(*Clostridium botulinum*)

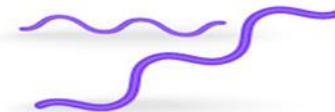
SPIRALS



Vibrios
(*Vibrio cholerae*)



Spirilla
(*Helicobacter pylori*)



Spirochaetes
(*Treponema pallidum*)



Coccus



Coccobacillus



Vibrio



Bacillus

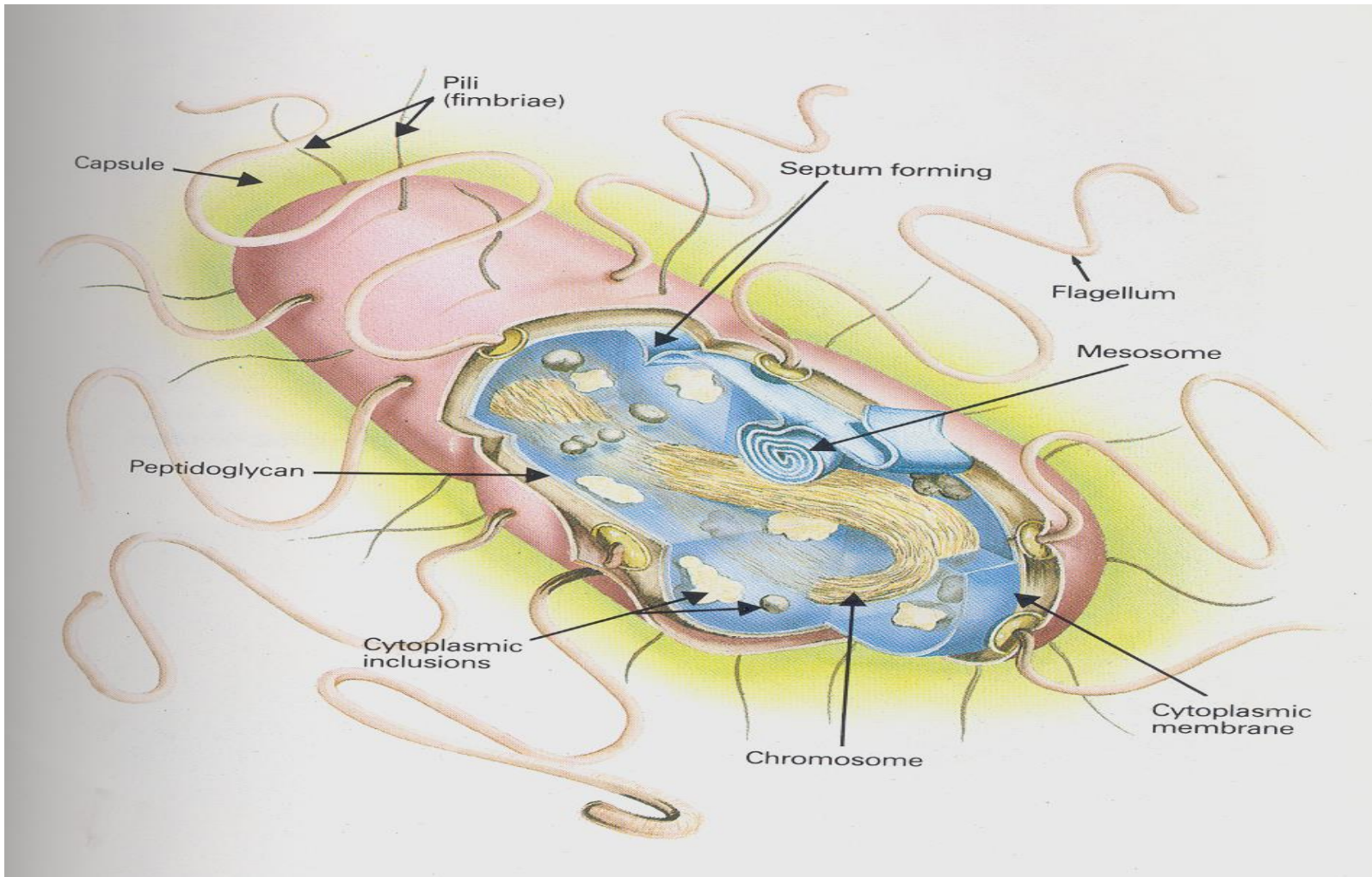


Spirillum



Spirochete

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 BACTERIA: stain **blue/purple** by Gram stain

GRAM NEGATIVE BACTERIA: stain **red** by Gram stain

Note : *Mycoplasma* is naturally have no cell wall.

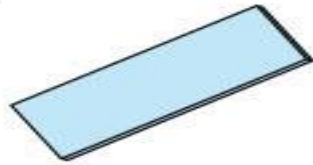
Chemical structure of bacterial cell wall:

Peptidoglycan :

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

GRAM STAINING

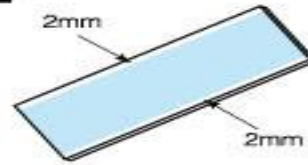
1



Flow Through Procedure

Wipe bottom of biofilm slide clean

2



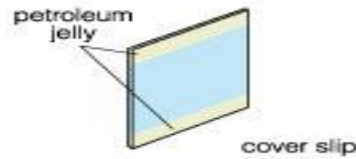
Clean top edges of slide about 2mm

3



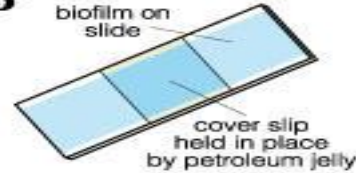
Build up a ridge of petroleum jelly on the top and bottom of a cover slip

4



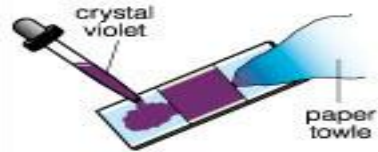
Cover slip with petroleum jelly

5



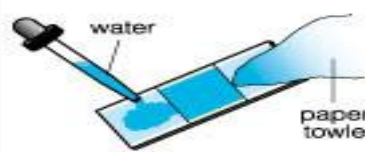
Biofilm on slide with cover slip

6



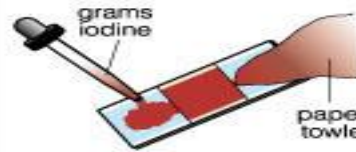
Add crystal violet-wait 30 sec.

7



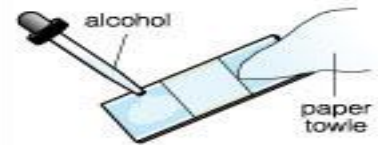
Wash with water

8



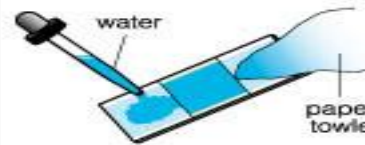
Add Grams Iodine -wait 1.5 min.

9



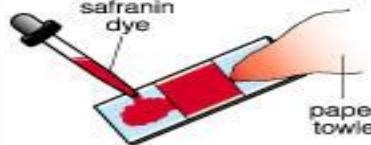
Decolorize with alcohol

10



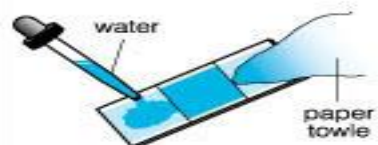
Wash with water

11



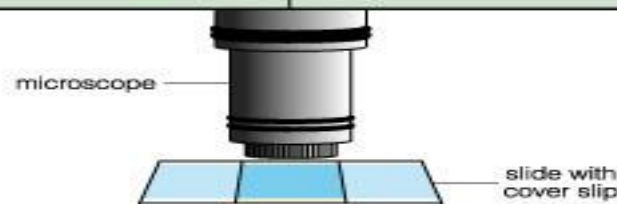
Stain with Safranin dye-wait 30 sec.

12



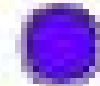
Wash with water

13



Examine under oil immersion through the cover slip

GRAM +



Fixation



Crystal Violet



Iodine Fixative



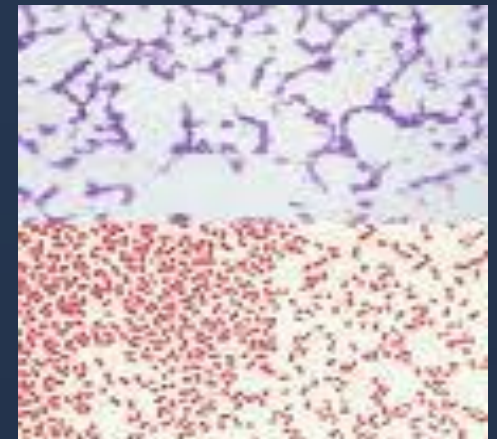
Decolorization



Counter stain (safranin)



GRAM -



GRAM-POSITIVE



GRAM-NEGATIVE



Fixation



Crystal Violet



Iodine Treatment



Decolorisation



Counter stain with
Safranin

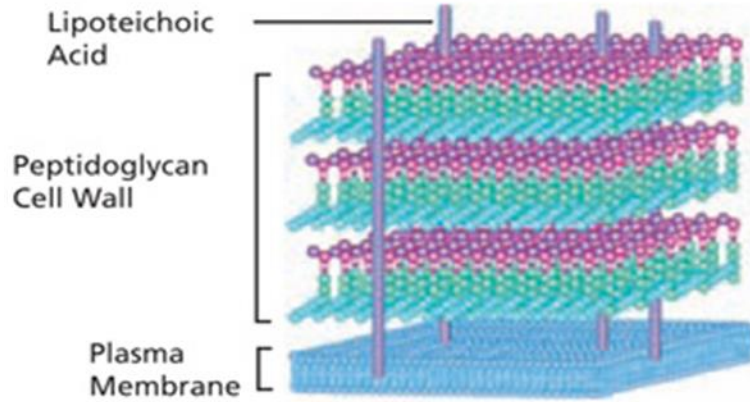
Cell Wall of Gram Positive Bacteria

- 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)

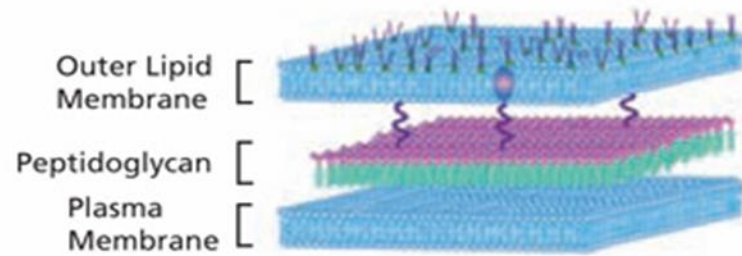
Cell Wall of Gram Negative Bacteria

- **Thin Peptidoglycan**
- Outer membrane that contains :
 - specific proteins (porins) important in the transport of hydrophilic molecules
 - lipopolysaccharide (**ENDOTOXIN**)

Gram-Positive Bacterial Cell Wall

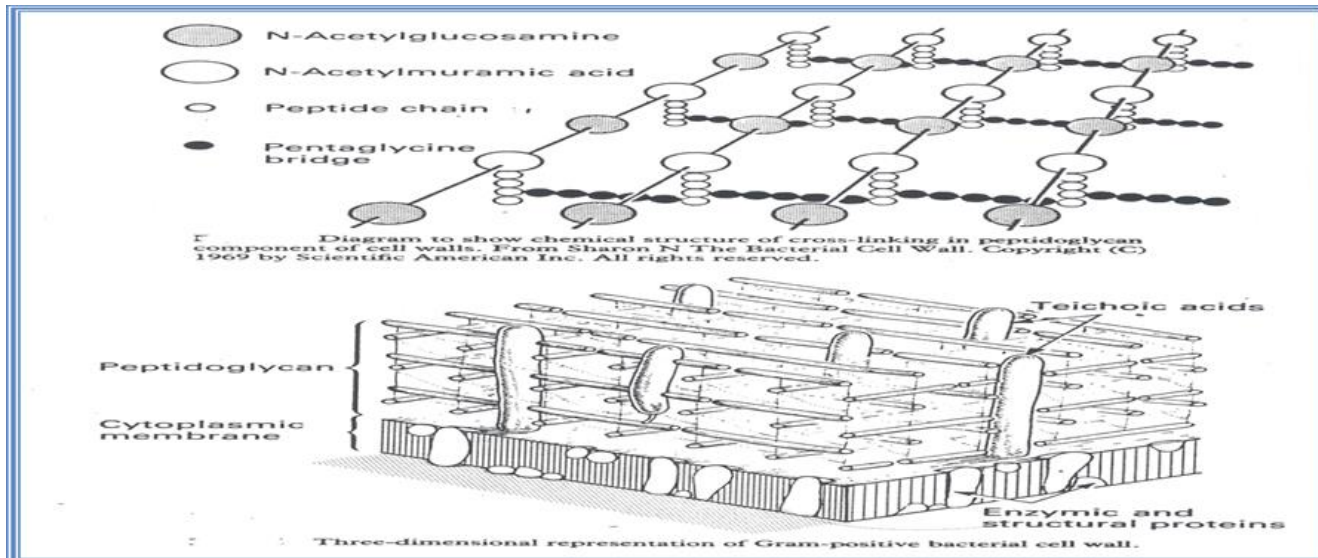
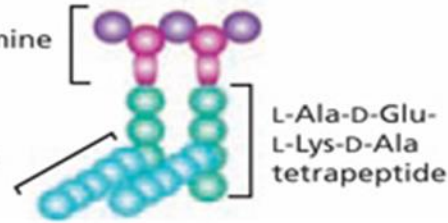


Gram-Negative Bacterial Cell Wall



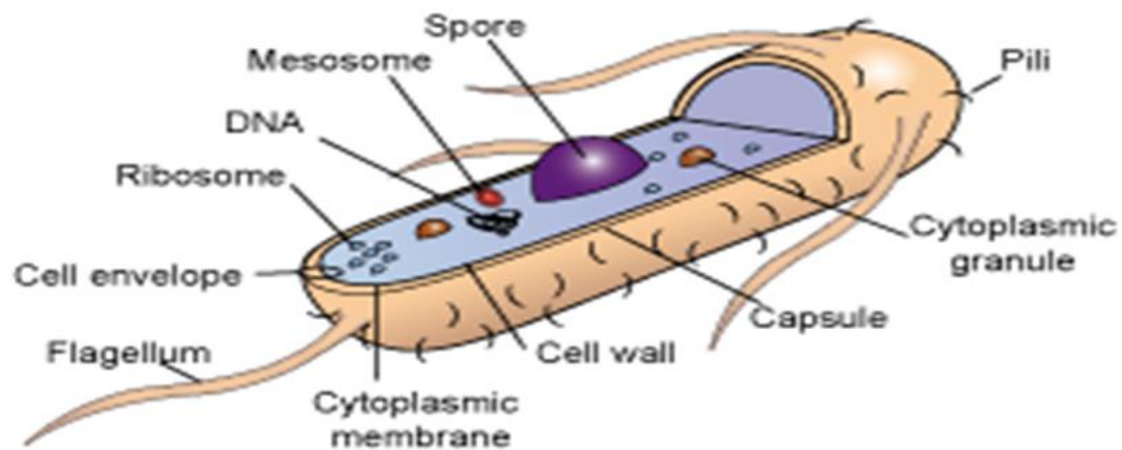
Alternating copolymer of $\beta(1\rightarrow4)$ -N-acetyl-D-glucosamine and N-acetylmuramic acid

Pentaglycine cross-link

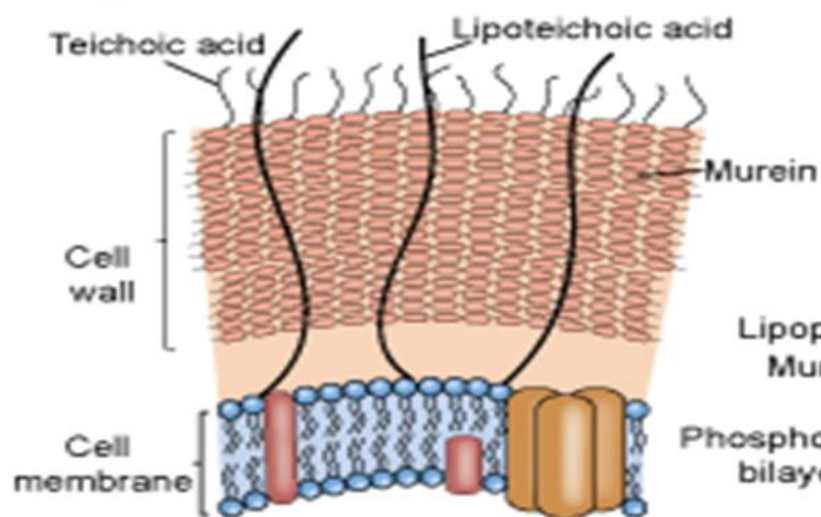


a

Bacterial Cell Structure

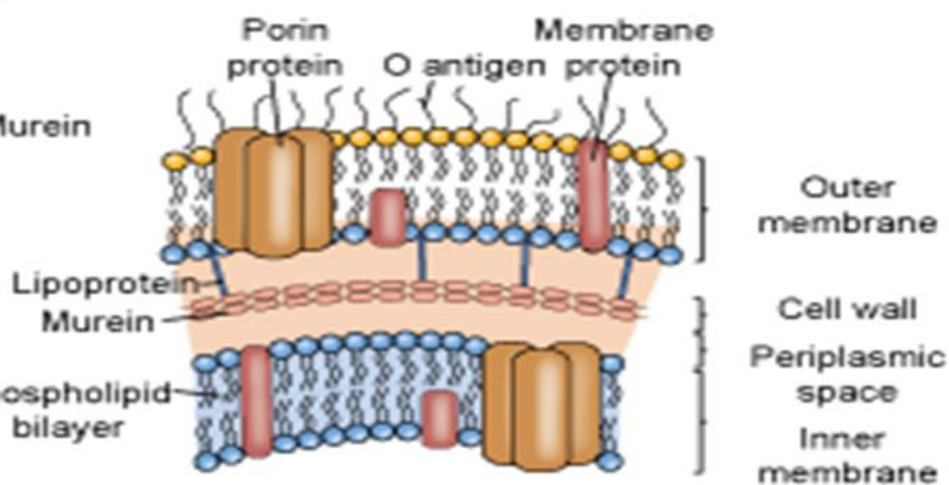


b



Gram+

c



Gram-

External Structures of Bacteria

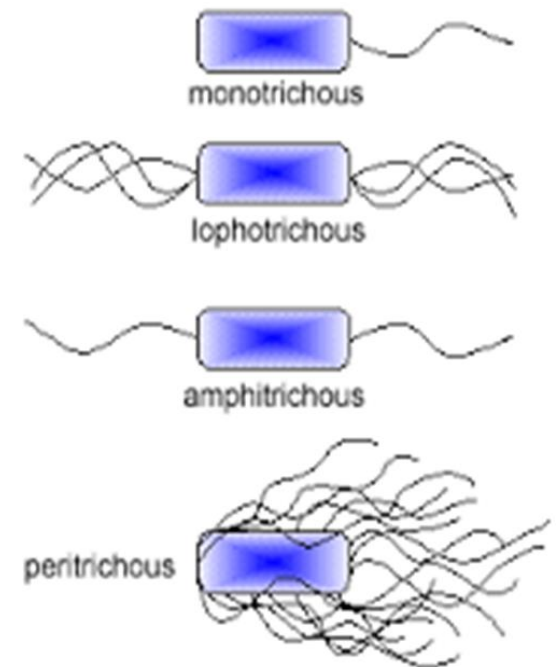
External protrude from the cell into the environment:

- **Flagella**
- **Pili**
- **Capsule**

Flagella



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



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:

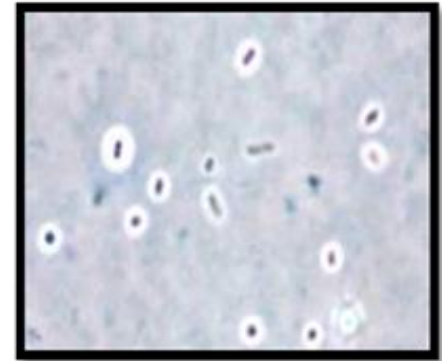
- 1~ **Common pili** (*fimbriae*): covers the surface—
responsible for: adhesion & colonization
- 2~ **Sex pili** : in some bacteria only, responsible for
conjugation.

Capsules and Slime layer

- These are the structures surrounding the outside of cell envelop.
- 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 :

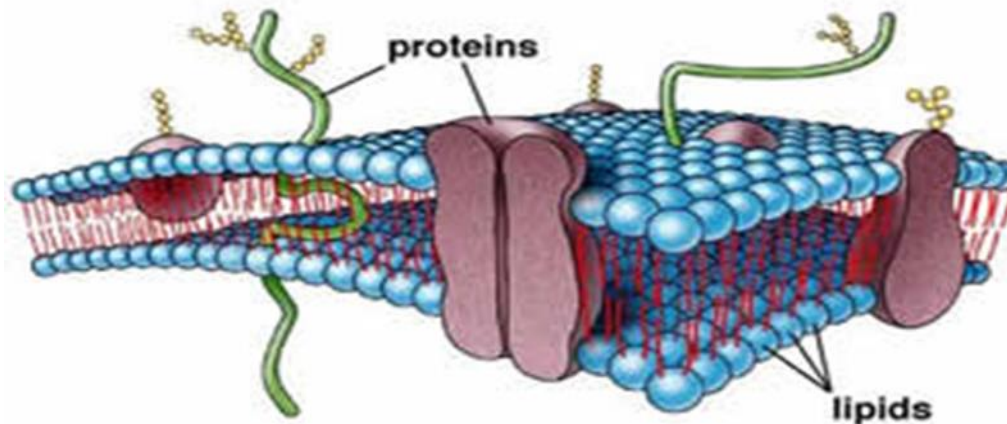
- Attachment
- Protection from phagocytic engulfment
- Resistant to dryness
- Reservoir for certain nutrient



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

Small Portion of a Plasma Membrane



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

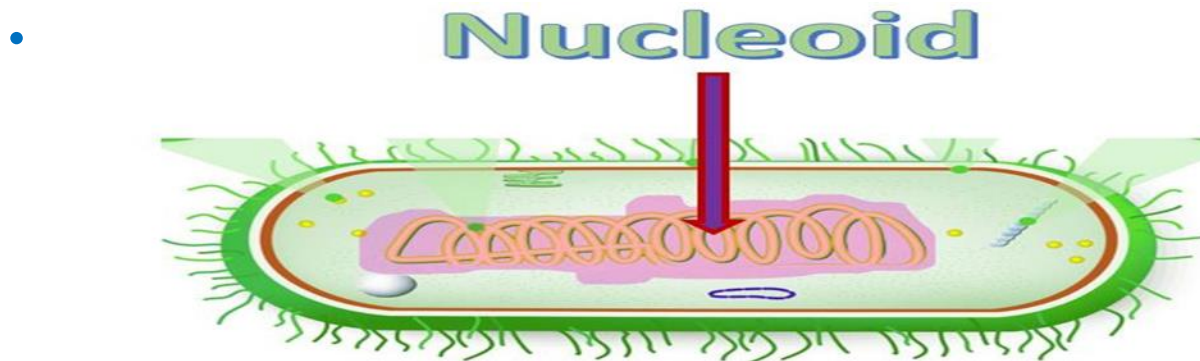
Cytoplasmic inclusions:

Are nutritional storage granules , examples:

- ~ Volutin
- ~ Lipid
- ~ Starch / or Glycogen

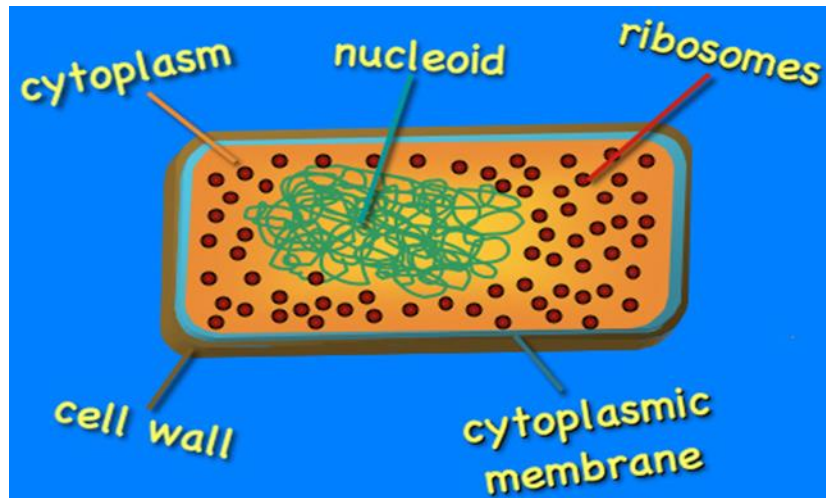
Nucleoid (Nuclear Body)

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



Ribosomes of Bacteria

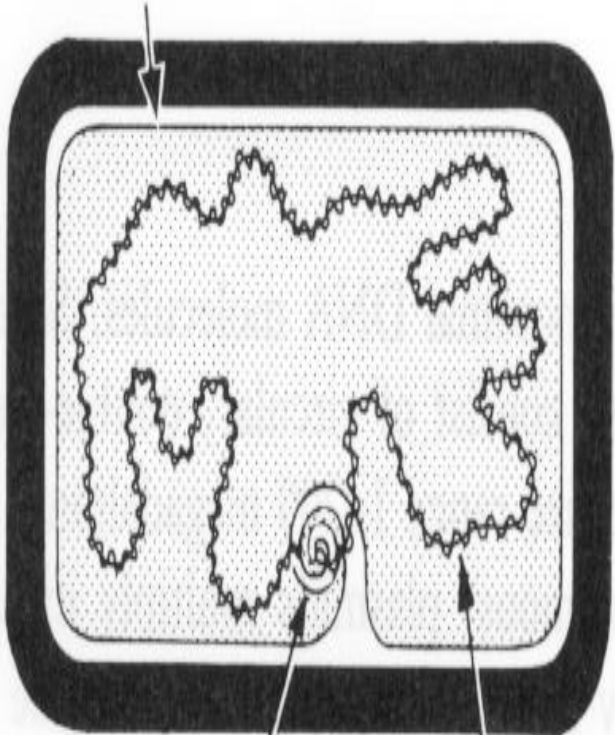
- Distributed throughout the cytoplasm
- Site of protein synthesis
- Composed of RNA and protein



Bacterial Chromosomes

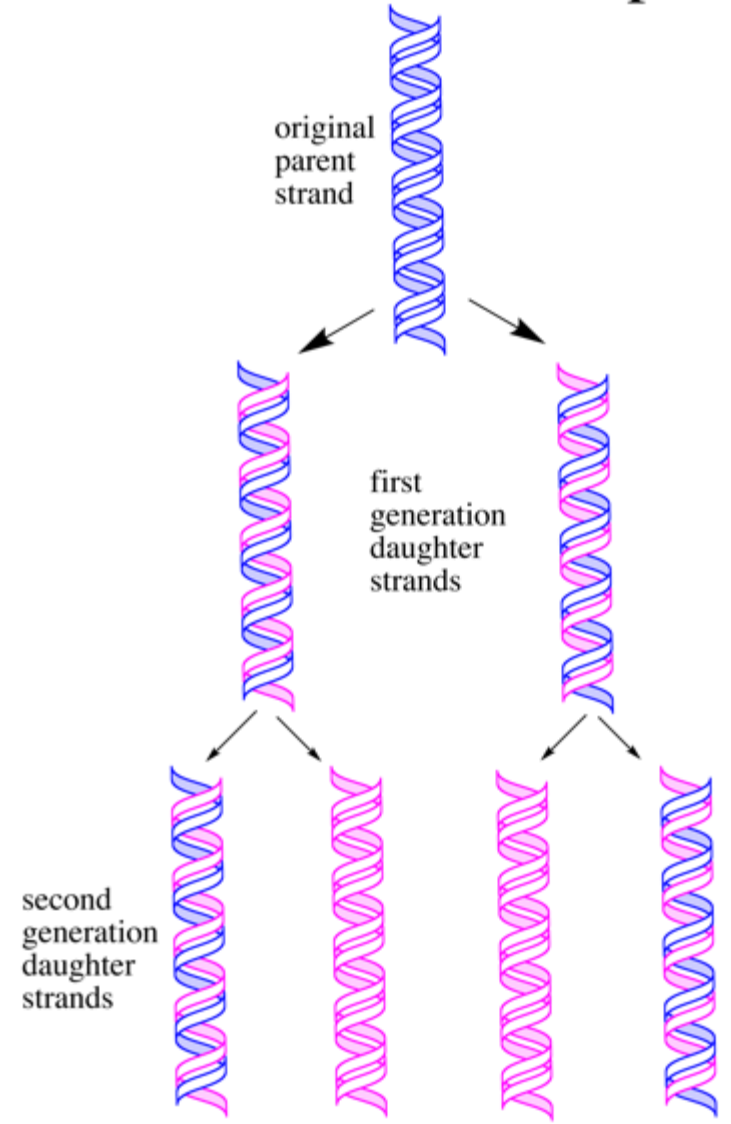
- Haploid, circular molecule of double stranded~ DNA attached to cell membrane.
- Genetic code in Purine and Pyrimidine bases of nucleotides that makes DNA strand.
- 3 bases comprise one code, each triplet codon codes for one amino acid.
- Replication is semiconservative ,takes place by **binary fission** .

Cytoplasmic membrane



Mesosome
Chromosome of circular double-stranded DNA

Semiconservative Replication



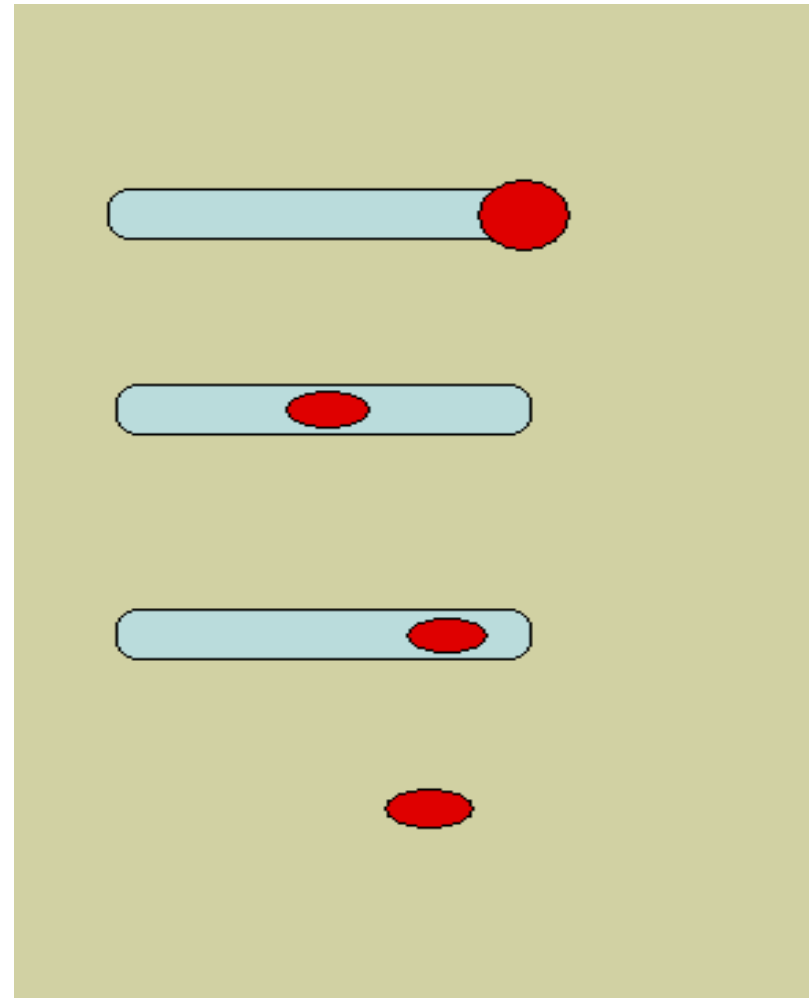
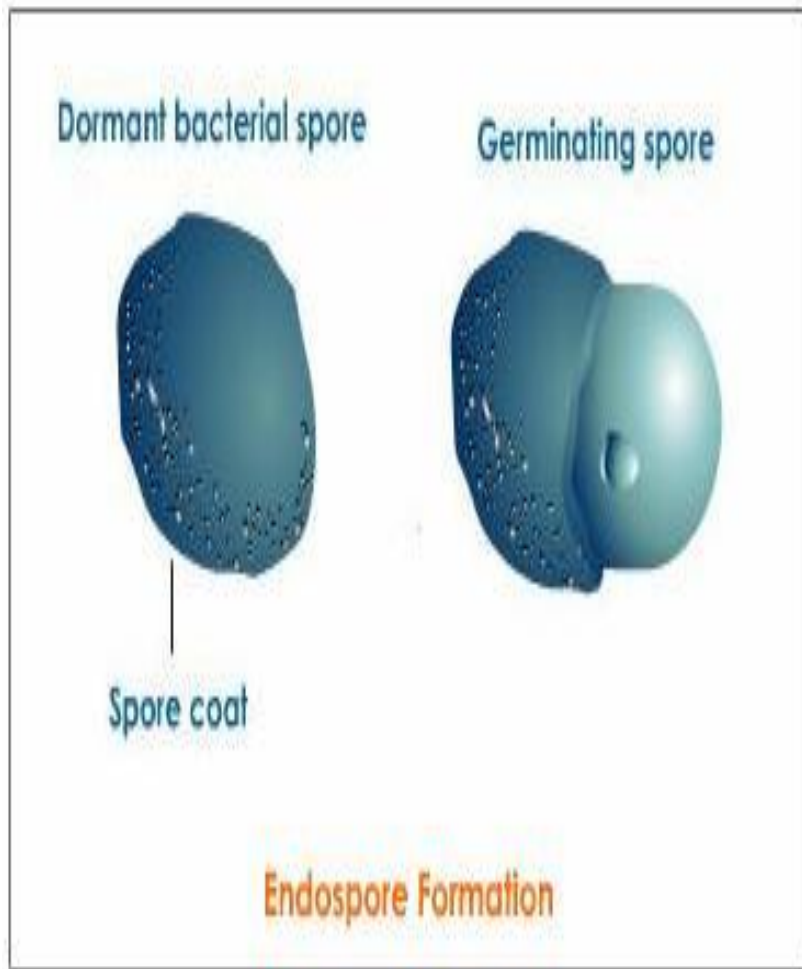
Spores of Bacteria

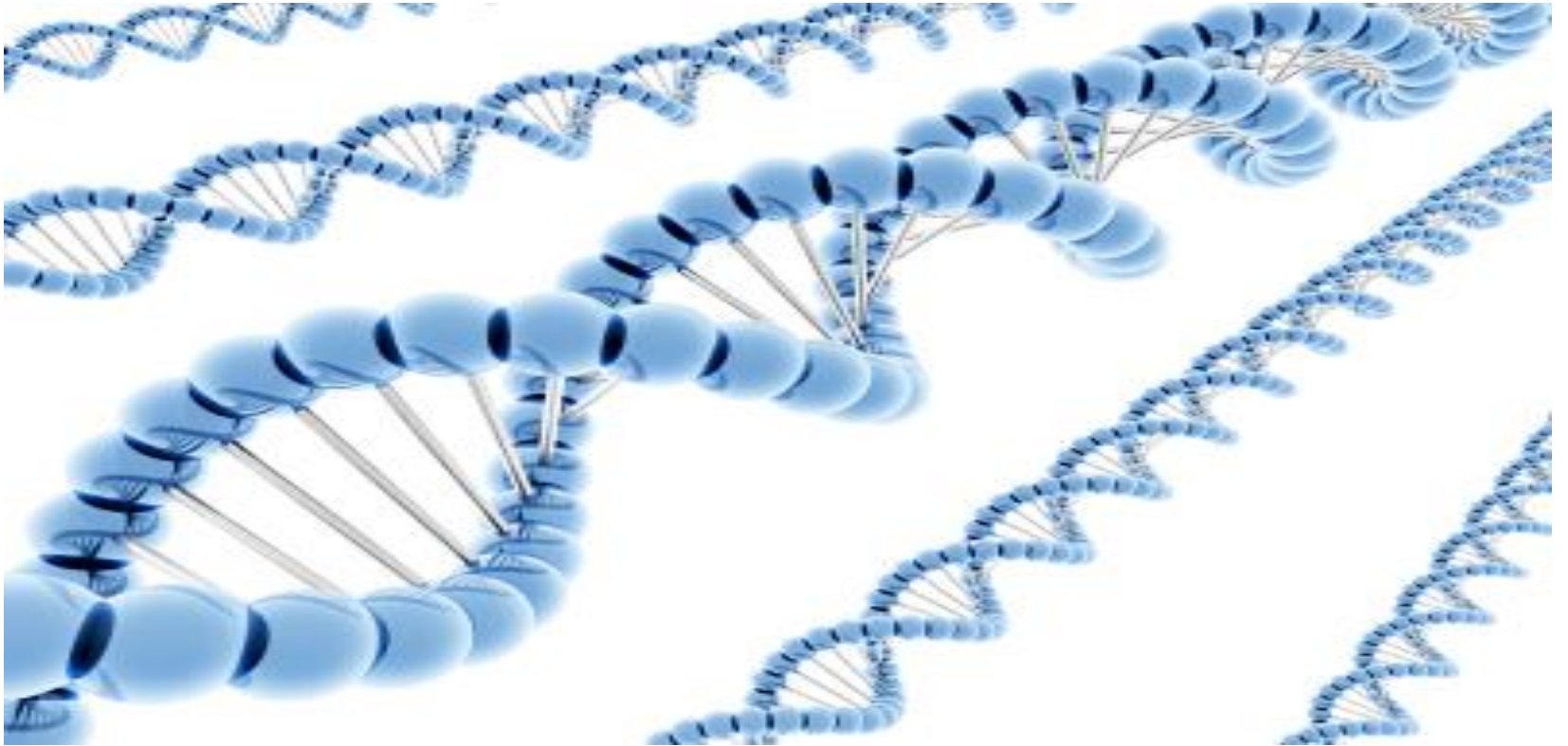
- Small ,dense, metabolically inactive , non-reproductive structures produced by *Bacillus* & *Clostridium*
- Enables the bacteria to survive adverse environmental conditions.
- Contain high concentration of Calcium dipicolonate.
- Resistant to heat, dissection & disinfectants
- Often remain associated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

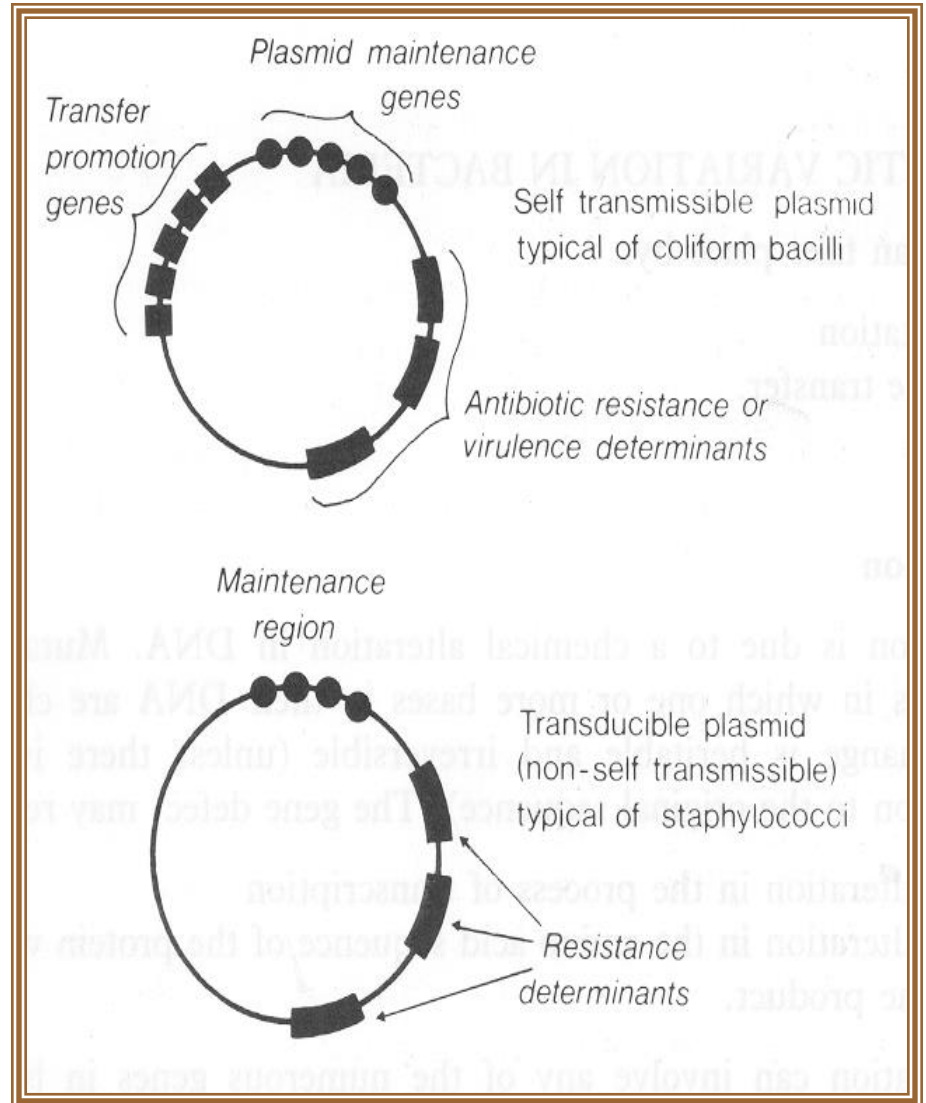
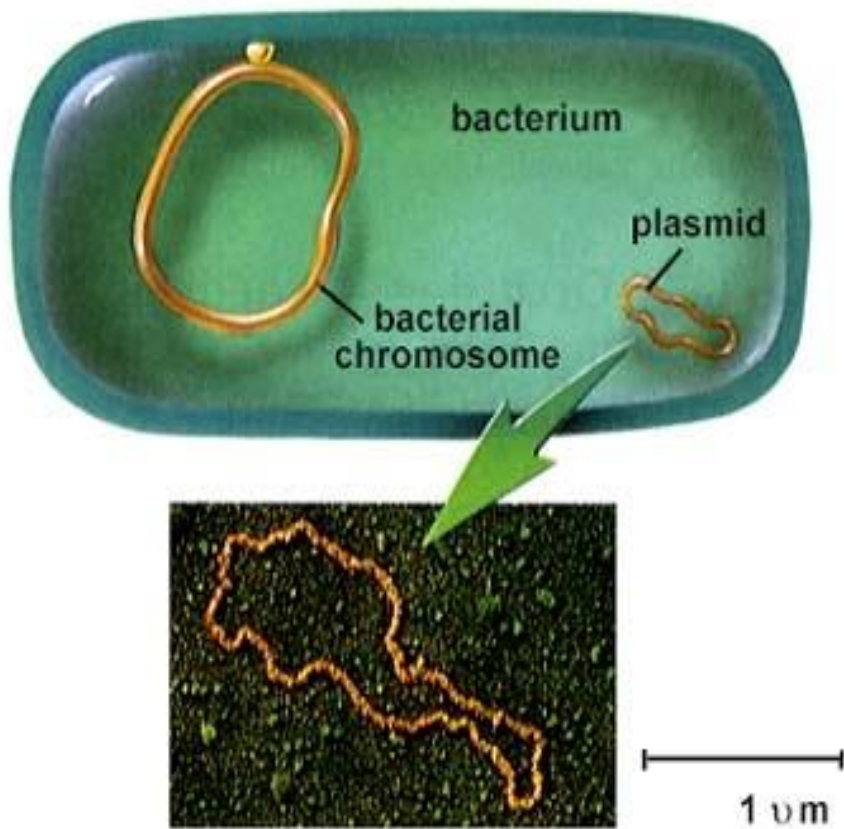
2 types of DNA in bacteria

- 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** :Genetic exchange, amplify genes
- Transfer by **conjugation**

Plasmids



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 sequences,
- Function eg. **Bacteria 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

Genetic Mutation Causes Drug Resistance

Non-resistant bacteria exist

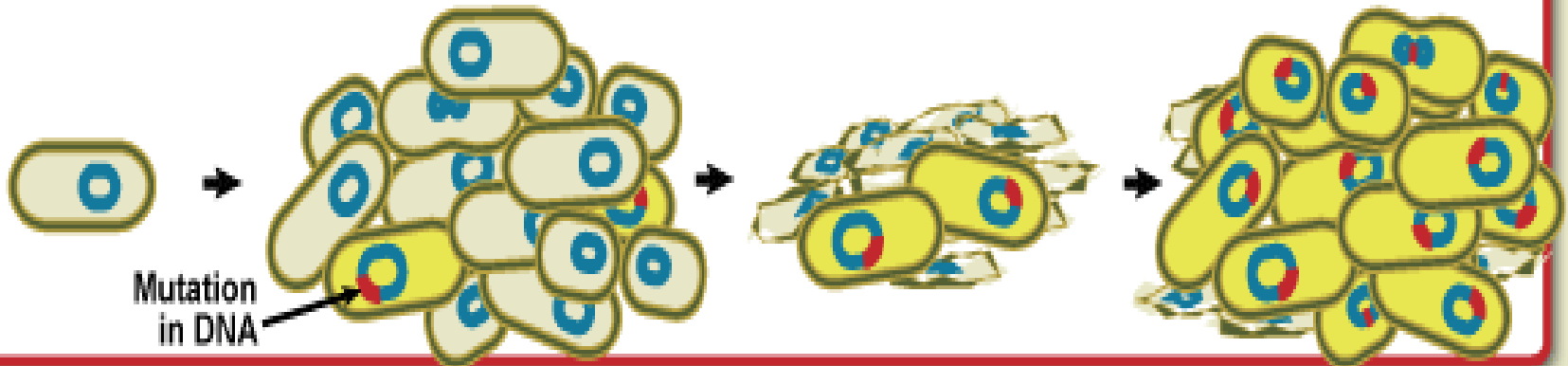
Bacteria multiply by the billions

Some mutations make the bacterium drug resistant

Drug resistant bacteria multiply and thrive.

A few of these bacteria will mutate.

In the presence of drugs, only drug resistant bacteria survive.

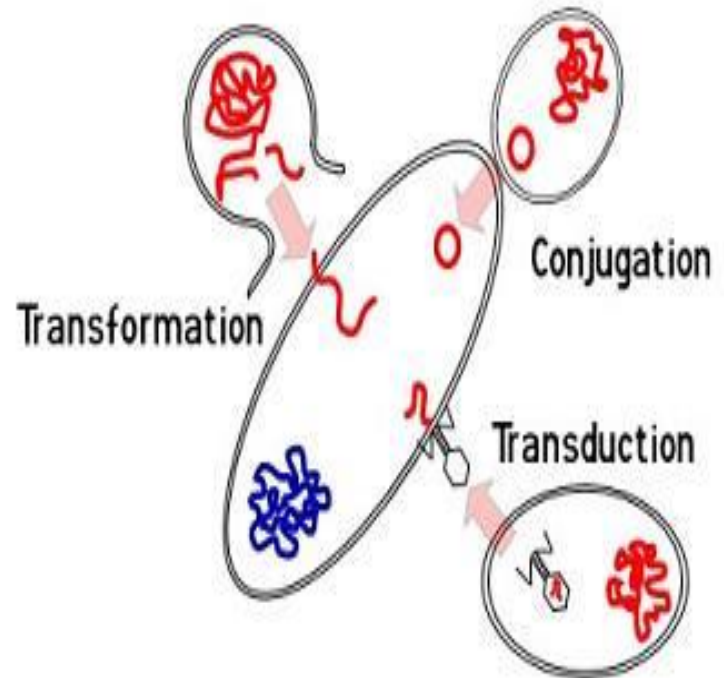


Gene Transfer Among Bacteria

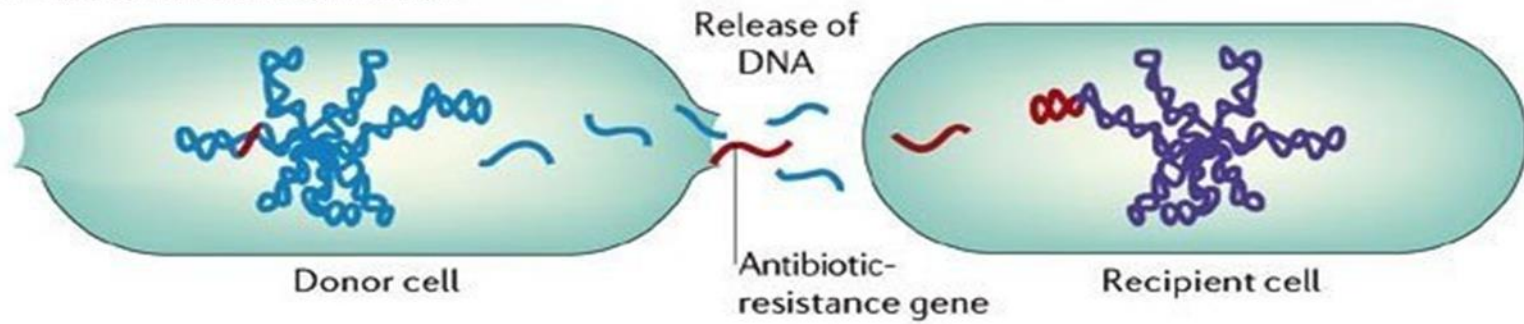
Three mechanisms:

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

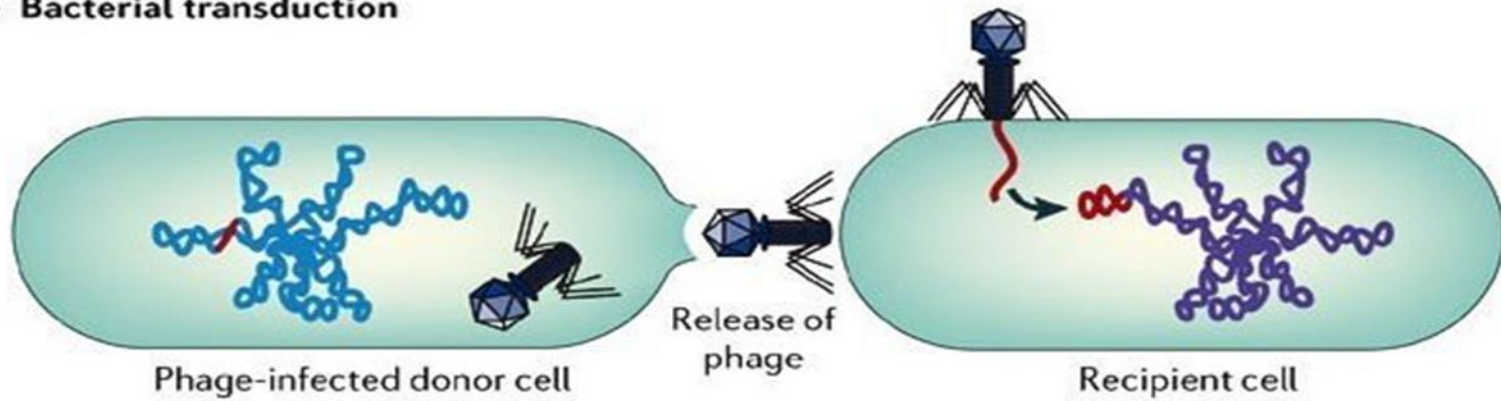
Mechanisms of Gene Exchange



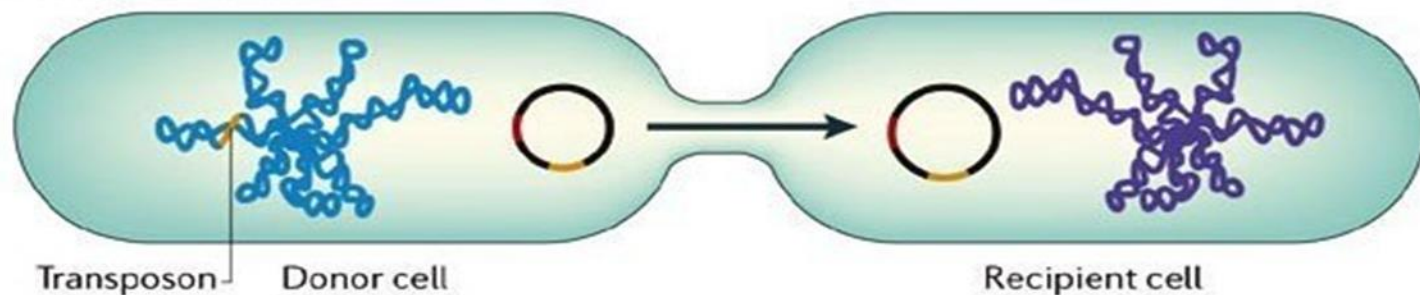
a Bacterial transformation



b Bacterial transduction



c Bacterial 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.

Examples:

- Beta – Lactamase production in *Staphylococcus aureus* : Bacteria becomes resistant 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.
Publisher : McGraw Hill .