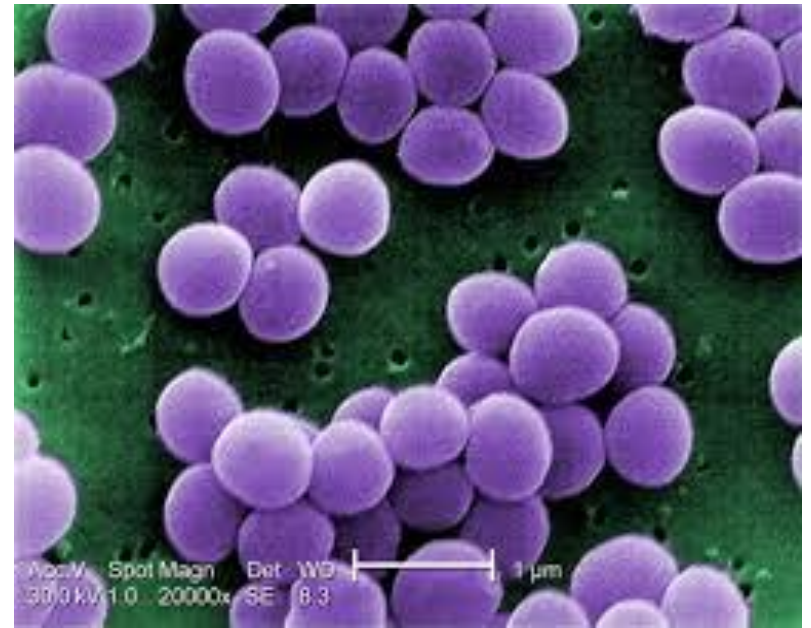


# Bacterial structure





- It is important to understand the basic structural properties and physiology of micro-organisms to establish our approach to infections
- Our understanding of microbial cytology aided by developments in genetic manipulation combined with advances in fluorescence and electron microscopy

# Structure of Bacteria



- **Essential components such as**
  - \* Cell wall
  - \* Cytoplasmic membrane
  - \* Ribosome
  - \* Nucleoid
  
- **Accessory components (not every bacteria has):**
  - \* Capsule, Pilus or fimbria, Flagella
  - \* Spores, Plasmid, Transposons

# Structure of Bacteria

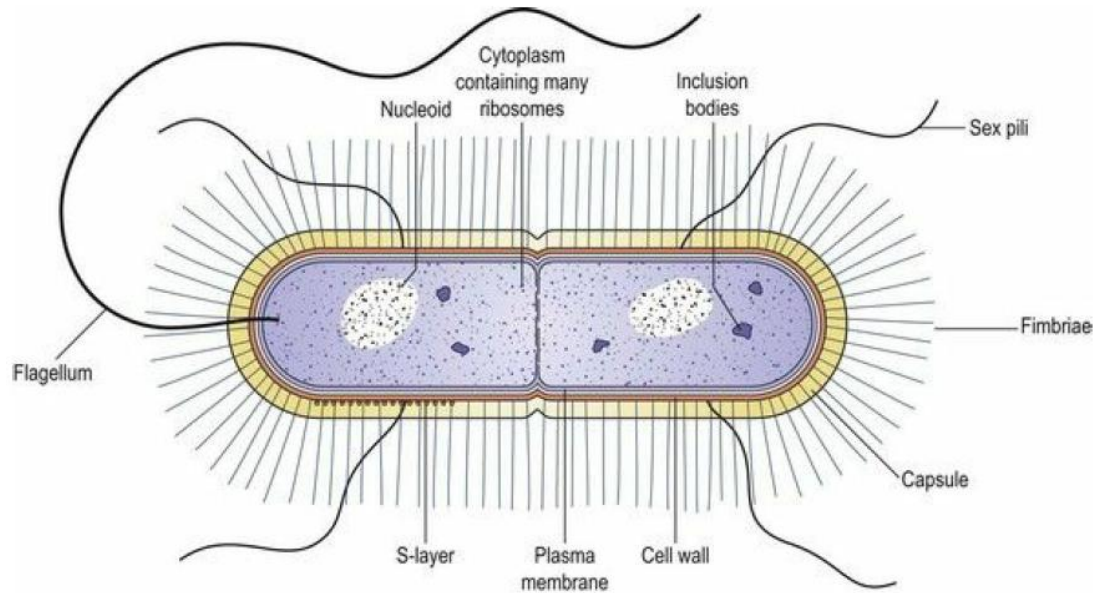


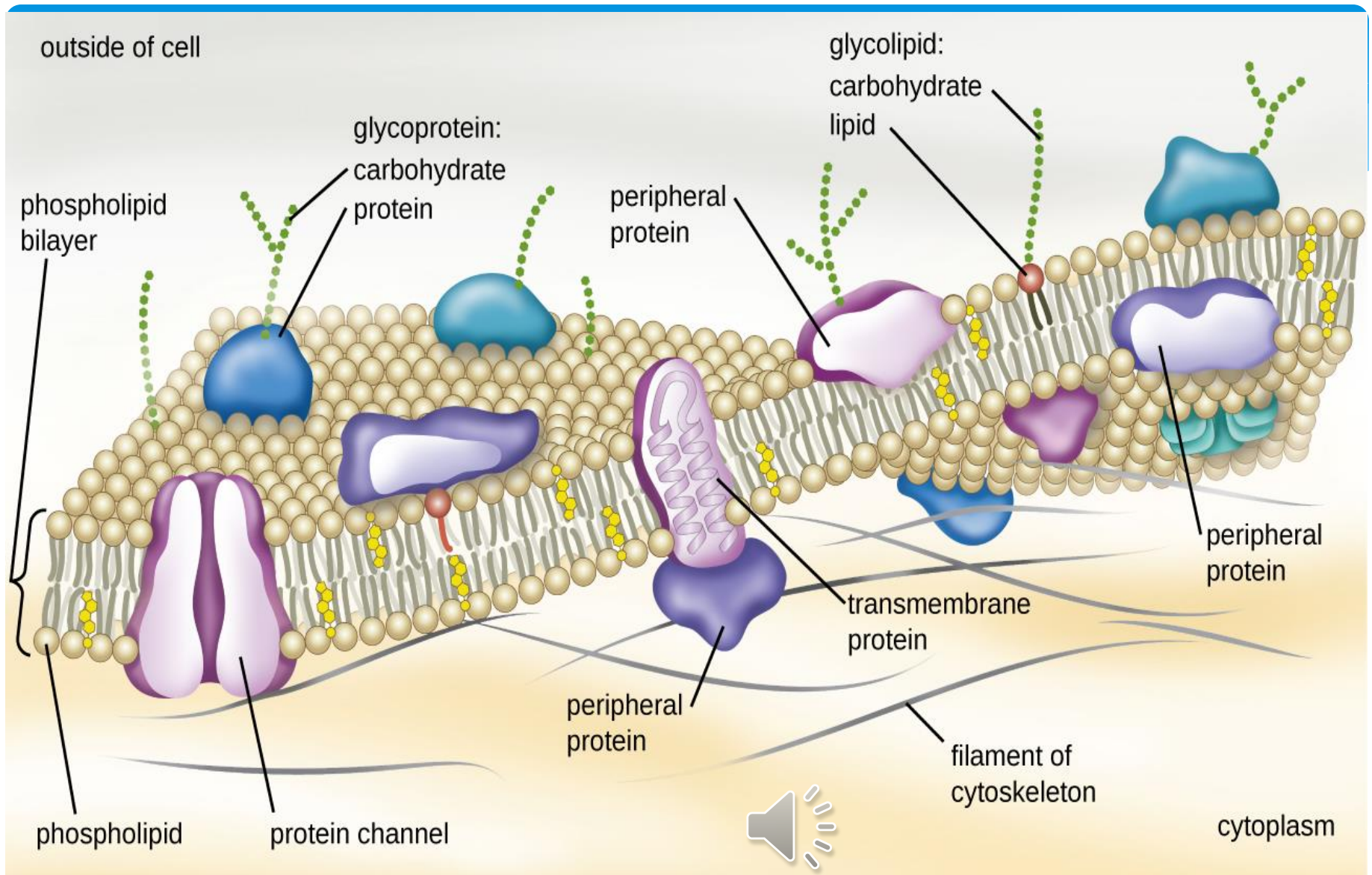
Fig. 2.2 Diagram illustrating the key features of bacterial cells. The S-layer is a variably demonstrated ordered protein layer.

# Structure of Bacteria



- \* ***Cytoplasm:***

- *Is a predominantly aqueous environment*
- *Contains nucleoid, ribosomes and numerous other protein and nucleotide–protein complexes*
- *Bacterial cytoplasm have cytoskeletal structures ( filamentous proteins and filament systems)*
- *The importance of these cytoskeletal structures:*
  - \* *determining cell shape, division and spore formation*
  - \* *antimicrobials targeting.*



\*\*Transmembrane proteins: Porins proteins for selective permeability

\*\*Integral proteins that help in attachment

# Structure of Bacteria



**Nucleoid:** Area of cytoplasm where bacterial DNA is located

**DNA:** Single chromosome, double stranded, circular present in the cytoplasm with no nuclear membrane.

**Plasmids** (an extra-chromosomal DNA) may be present.

**Ribosomes:** Sites of protein synthesis ,They have a sedimentation coefficient of 70S, being composed of a 30S and a 50S subunit (80s in eukaryotes)

# Structure of Bacteria



- Cytoplasm is bounded peripherally by a very thin, elastic and semi-permeable cytoplasmic or ( plasma) membrane.
- Components of plasma membrane: lipid bilayer, integral and peripheral proteins, carbohydrates and cholesterol.
- Functions:
  - Synthesis of precursors of cell wall polymers and membrane lipids.
  - Selective permeability and active transport of molecules into cells.
  - Energy generation by oxidative phosphorylation.
  - Excretion of toxins.



# Structure of Bacteria



**Inclusion bodies ( granules):** Are nuclear or cytoplasmic aggregates of proteins, they typically represent sites of viral multiplication in a bacterium or a eukaryotic cell and usually consist of viral capsid protein.

- \* These granules function as Food and energy storage e.g. glycogen and starch

# Structure of Bacteria



**Cell wall:** is a layer located outside the cell membrane which is rigid, porous and relatively permeable.

- Cell wall and cytoplasmic membrane called collectively the cell envelope.
- \* Importance of cell wall:
  - Bacterial rigidity and shape
  - protection against osmotic changes
  - Porous to allow nutrients passage.
  - Structure differs in gram positive & negative bacteria.

# Cell wall:



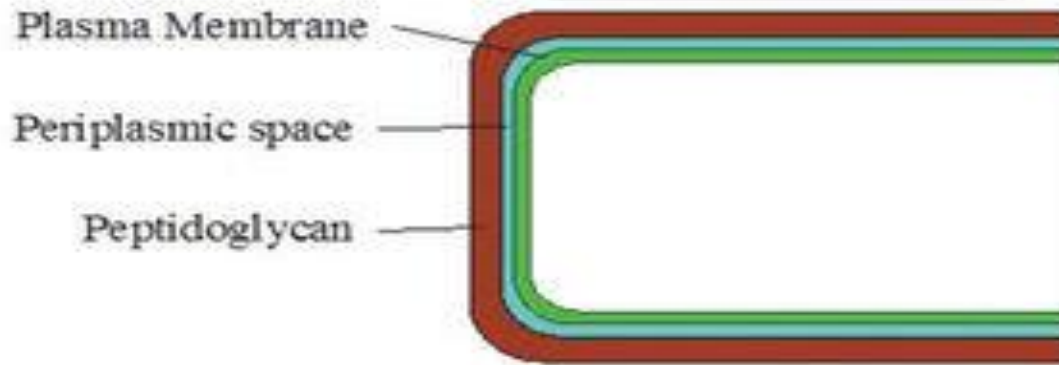
It has peptidoglycan layer outside the cytoplasmic membrane made of long polysaccharide chains cross-linked by peptide bridges, it provides rigidity and protection.

Loss of cell wall leads to death (this can be affected by some antibiotics and by lysozyme).

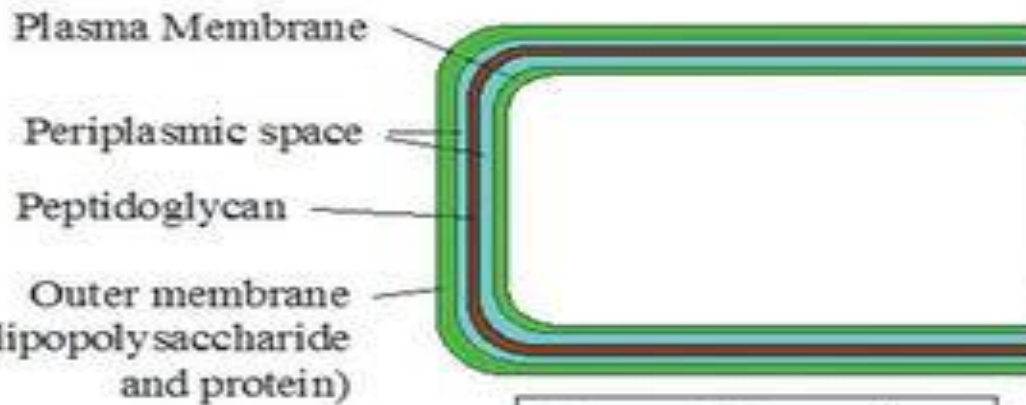
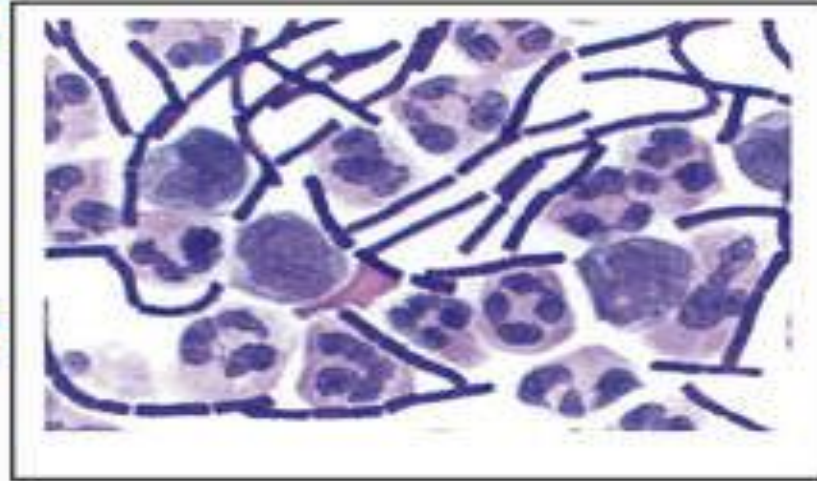
\*\*The cell wall may also contain proteins that serve as adherence agents (virulence factors)

\*\*In gram positive bacteria, the peptidoglycan layer consists of about 70 layers while in gram negative bacteria there is only one layer of peptidoglycan.

## Gram Positive



**Gram-positive bacteria** stain purple with Gram stain. This is because they have a thick cell wall without an outer membrane. Example: cyanobacteria



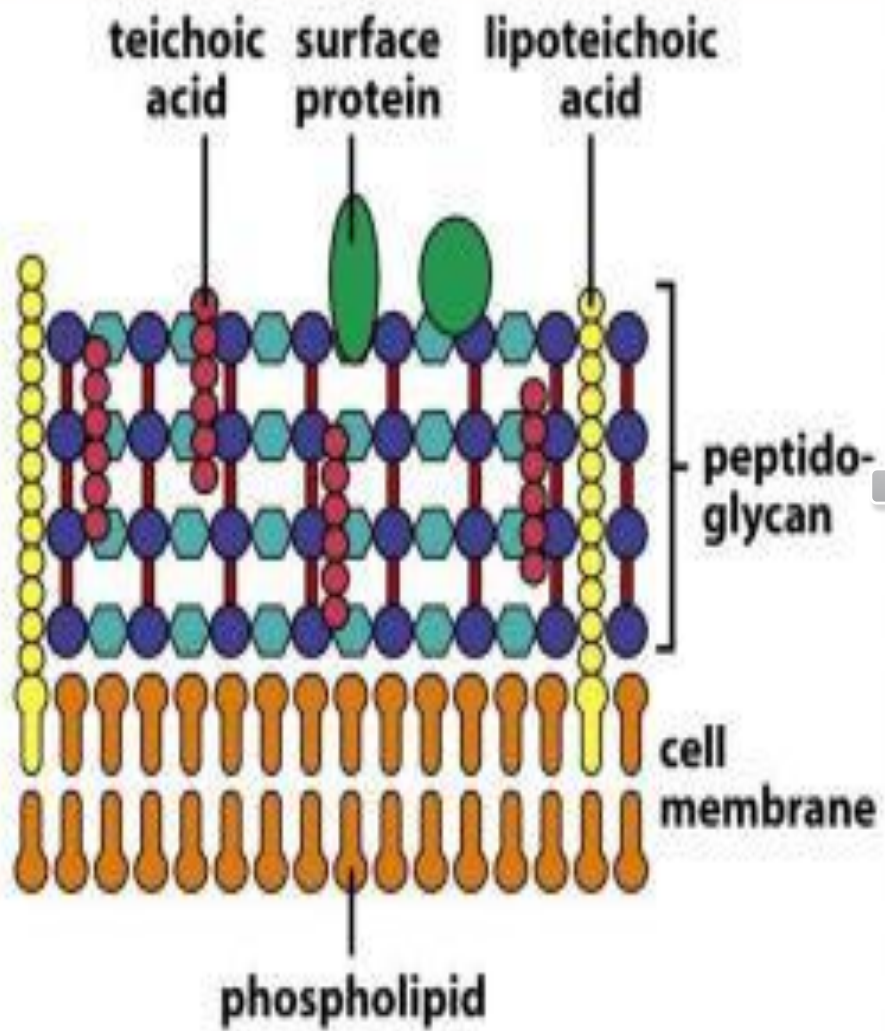
## Gram Negative



**Gram-negative bacteria** stain red with Gram stain. This is because they have a thin cell wall with an outer membrane. Example: Salmonella.



## Gram-positive bacteria



## Gram-negative bacteria

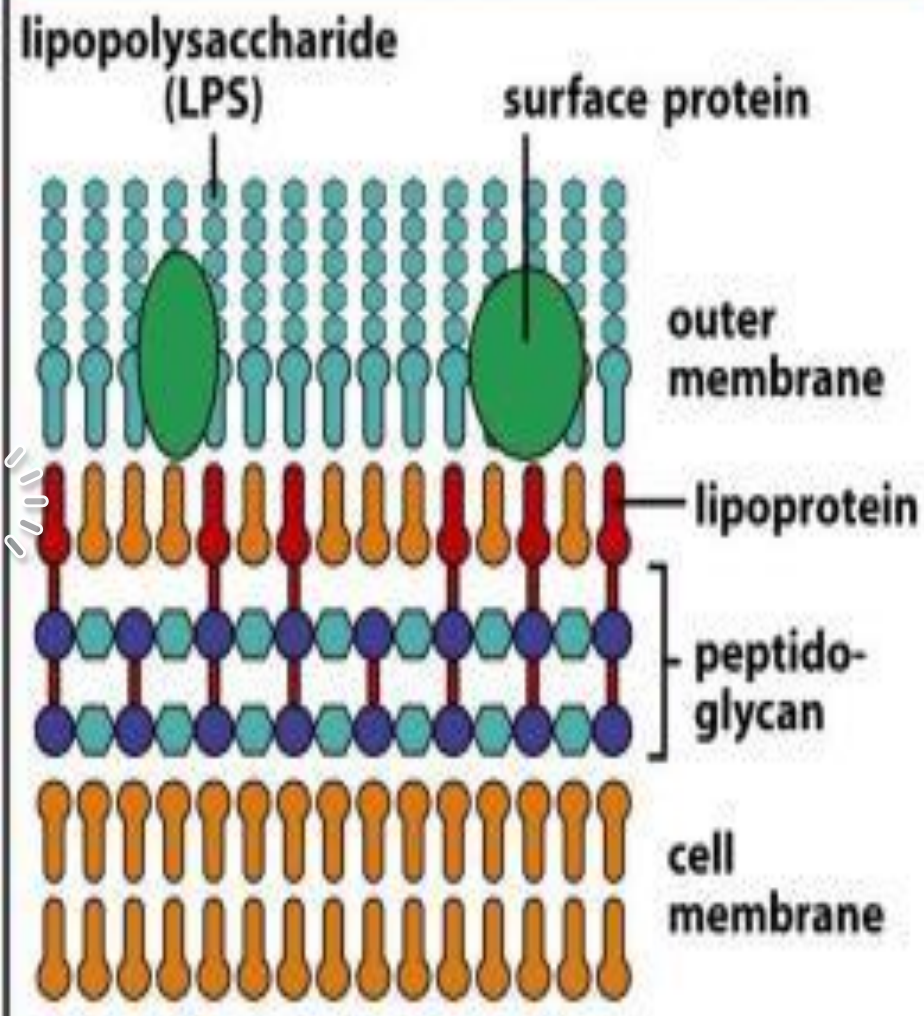


Figure 2-14 Immunobiology, 7ed. [© Garland Science 2008]

# Gram positive bacteria

- \*\* The cell wall of G+ve usually contains a large amount of
- 1- **Teichoic Acid** which connected to peptidoglycan.
  - 2- **Lipoteichoic Acid** which connected to plasma membrane.





# Gram negative bacteria

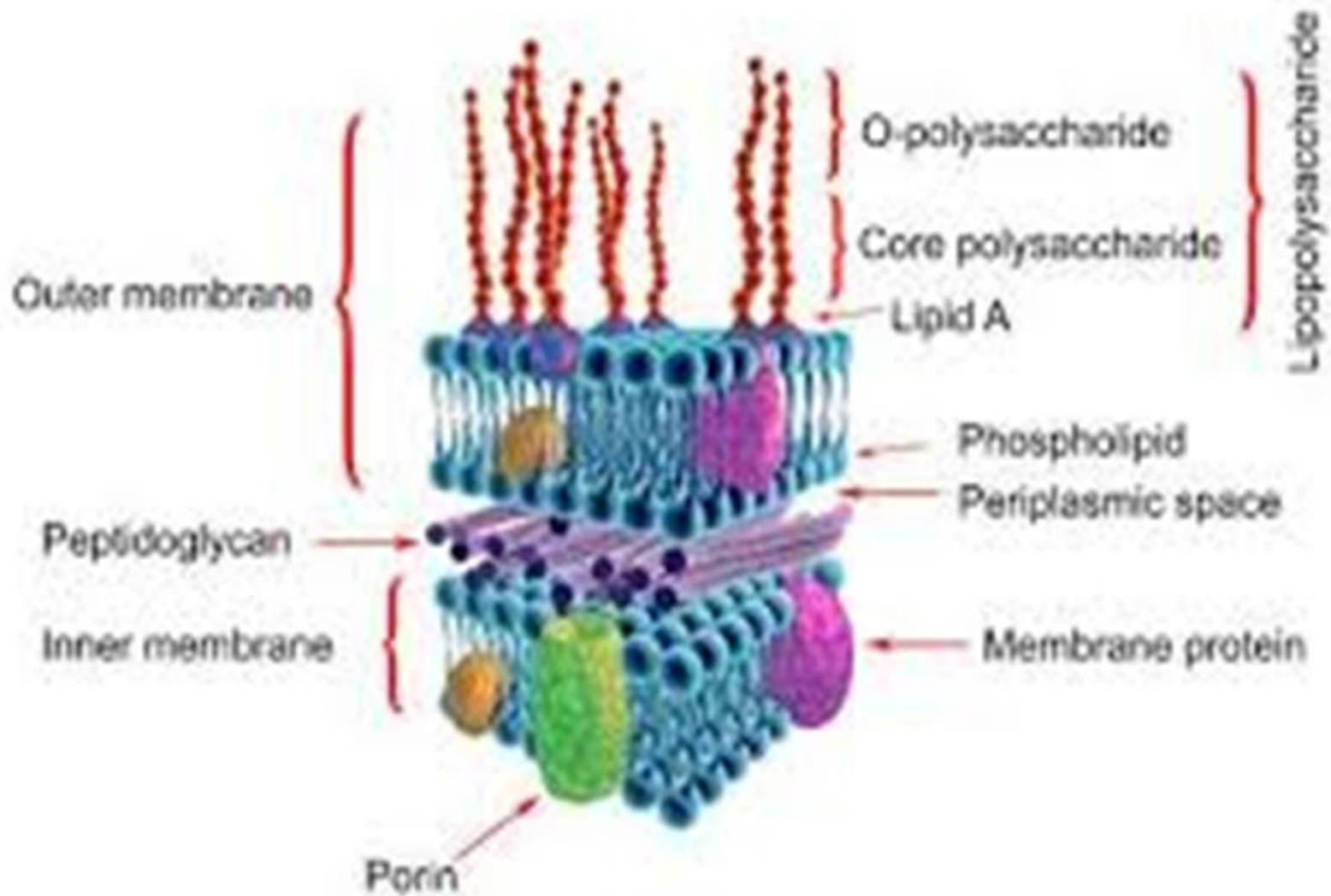
1- They have a thin monolayer of peptidoglycan, the outer membrane which is unique to gram negative bacteria.

The outer membrane (which protects the bacteria) differs from the cytoplasmic membrane in that it contains a special different lipid called lipopolysaccharide (LPS) in the outer leaflet.

LPS is also known as endotoxin which is only found in gram negative bacteria and is responsible for producing toxic shock.

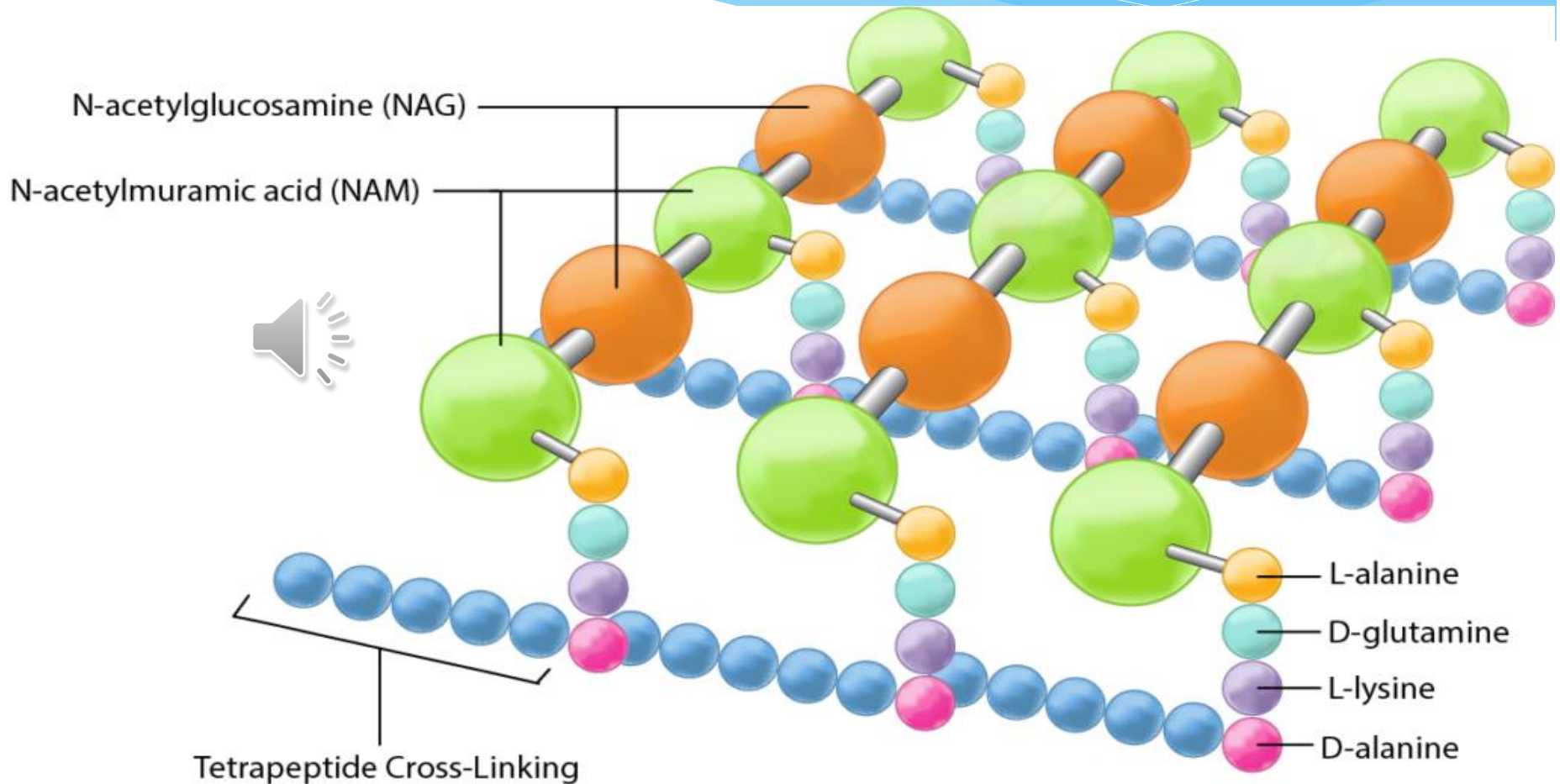
LPS contains lipid A which is embedded in the outer cytoplasmic membrane, then core antigen (polysaccharide), then O-specific polysaccharide chain.

**\*\*Presence of Lipid A can lead to endotoxin shock.**





# Structure of peptidoglycan in bacteria cell wall



# Structure of peptidoglycan in bacteria cell wall



- \* The peptidoglycan layer in the bacterial cell wall is a crystal lattice structure formed from linear chains of two alternating amino sugars, namely **N-acetylglucosamine** (GlcNAc or NAGA) and **N-acetylmuramic acid** (MurNAc or NAMA). The alternating sugars are connected by a  $\beta$ -(1,4)- glycosidic bond.

# The gram negative cell wall



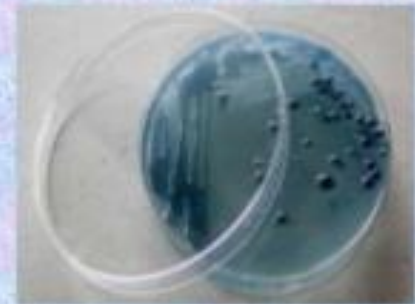
- \* Advantages of outer membrane:

- It protects the peptidoglycan from the effects of lysozyme (a natural body defense substance that cleaves the link between *N-acetylglucosamine and N-acetylmuramic acid*).
- It impedes the entry of many antibiotics.

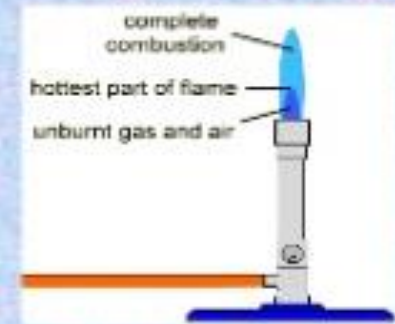
# Equipment for culturing bacteria

## EQUIPMENT :

- 1. Petri dish :



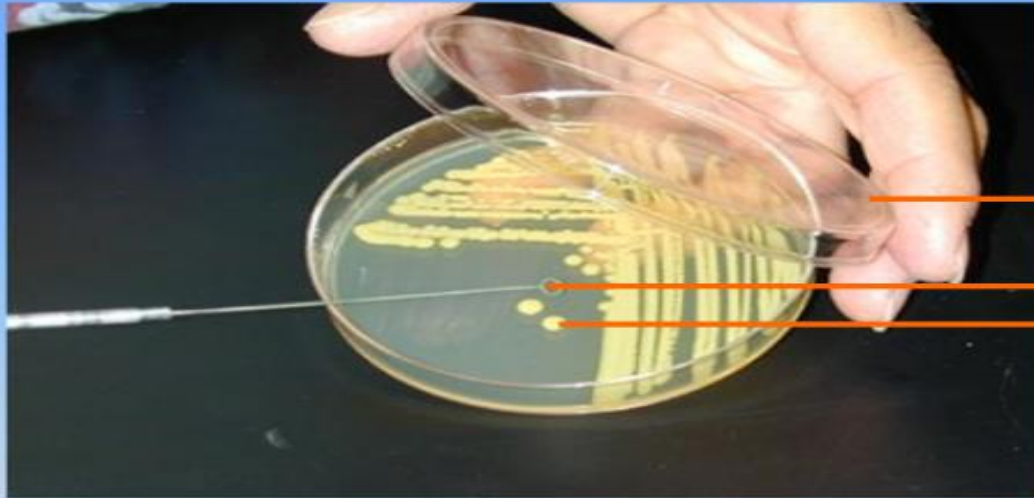
- 2. Bunsen Burner :



- 3. Inoculating loop :



## Streaking



*Dish*

*Wire loop*

*Colony  
(pure culture)*

By spreading a large amount of bacteria over the surface of a plate, the amount of bacteria is **diluted** and individual cells are **spread**. From these individual cells a single **colony** arises.

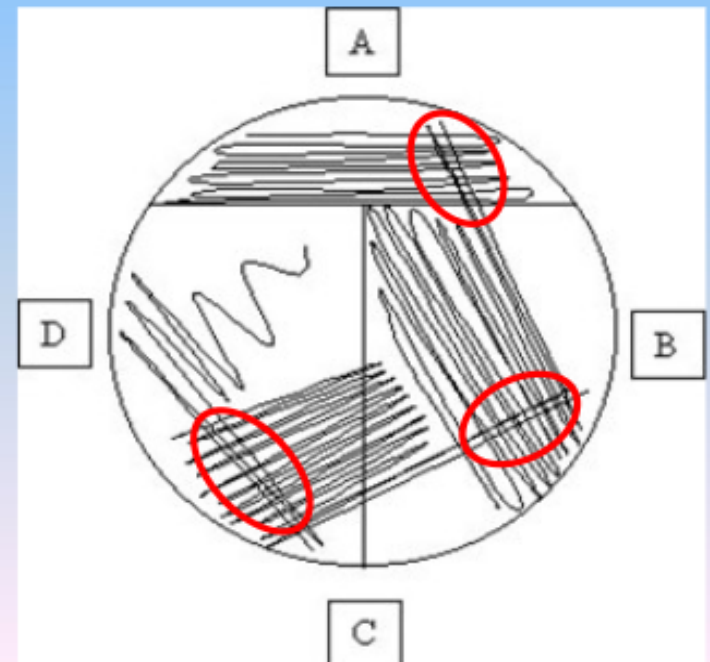
A **pure culture** is a **culture** in which only one strain of bacteria is present. A colony is **considered a pure culture** because it is known as several individual organisms, especially of the same species, living together in close association.



# Streaking

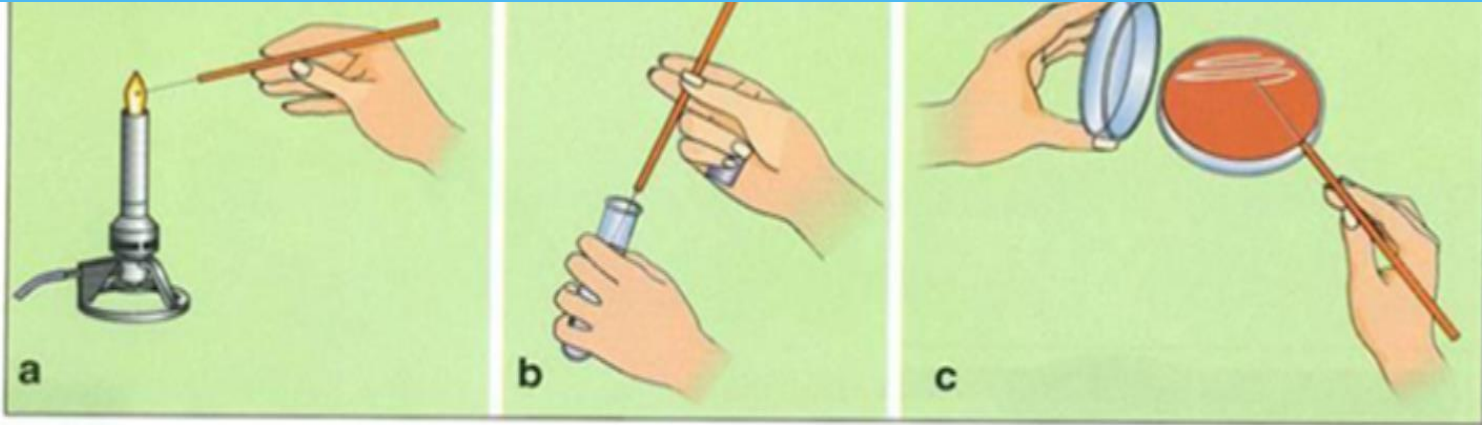
## Procedure:

1. Flame the loop and streak a loopful of broth culture as at **A** in the diagram.
2. Reflame the loop and cool it.
3. Streak as at **B** to spread the original inoculum over more of the agar.
4. Reflame the loop and cool it.
5. Streak as at **C**.
6. Reflame the loop and cool it.
7. Streak as at **D**.
8. Incubate the plate inverted.





# Streaking method



initial inoculum



second set of streaks



third set of streaks



fourth set of streaks



Courtesy Becton Dickinson

# Gram stain

We use four stains in the staining process

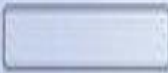
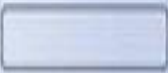





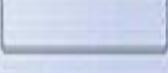


- 1- Crystal Violet or methylene blue : primary stain
- 2- Iodine : mordant stain
- 3- 95% of alcohol : decolorizing
- 4- Safranin ( red-pink) : contrast stain





# Gram stain: GO TO LAB

## Outline of Gram stain

	Gram-positive	Gram-negative
1. Unstained		
2. Crystal violet		
3. Iodine		
4. Decolorize		
5. Red/pink dye		

The dye is non-covalently bound to negatively charged molecules (particularly nucleic acids) in the cell

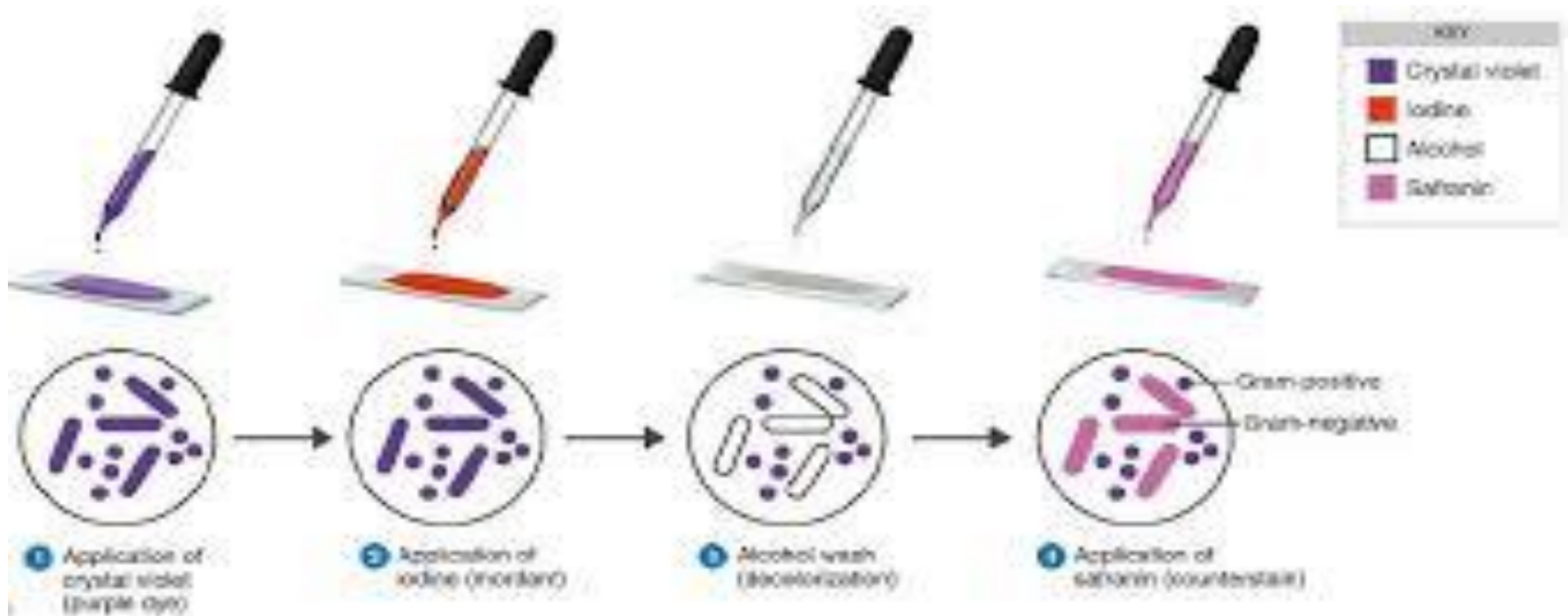
This forms macromolecular complexes with Crystal violet

The complexes are extracted through the Gram negative wall by solvents such as acetone but retained by Gram positives  
A further dye is needed to colourise the unstained Gram negatives





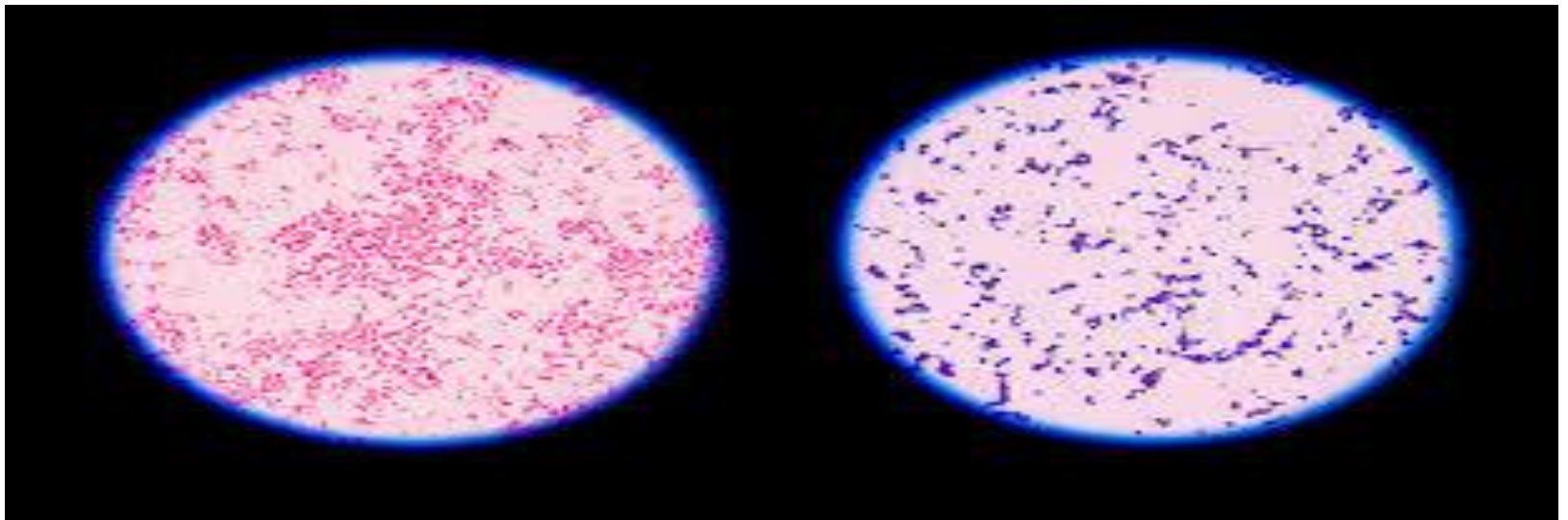
## STEPS OF THE PROCESS





## THE RESULT

Gram positive appear violet/blue while gram negative appear pink.

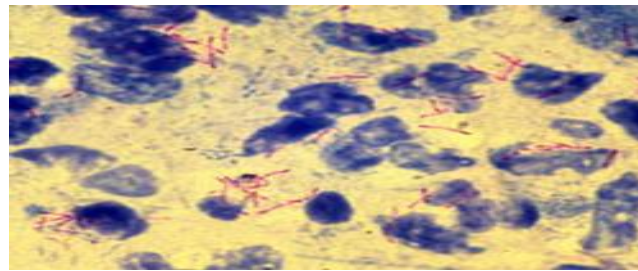




- Some bacteria are classified as Gram positive but stain poorly because the cell wall complex contains peptidoglycan, but otherwise it is composed of complex lipids.

- Examples: mycobacterium ( over 60% of the mycobacteria cell wall is lipid) and corynebacterium

Staining method used: Acid fast stain (Ziehl Neelsen stain)



- Some bacteria can not be stained because they are intracellular as *Chlamydiae* & *Rickettsiae*.

# Structure of Bacteria



**Capsule : Made of polysaccharide, hard to visualize under the light microscope, it is consider as a virulence factor, antiphagocytic, and as an adherence factor.**

**E.g. to teeth (S.mutans)**

**Sometimes it is not very well demarcated and is referred to as  
slime**

# Structure of Bacteria



**Free slime / Glycocalyx : Polysaccharide coat similar to capsule but secreted extracellularly**

**Allow firm adherence to structures e.g.: heart valves, skin, catheters, surface of the teeth.**

# Structure of Bacteria



**Flagella:** Helically coiled protein subunits called flagellin, anchored to bacterial membranes through hooks and basal body, they are responsible for motility.

**Fimbriae (pili) :** is a hair-like structures of protein subunits called pilin, arranged uniformly along the whole surface of the bacterium, they cause adherence.

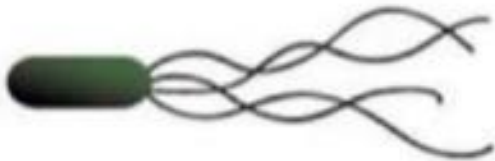
Some pili (F pili) are called sex pili, they promote passage of large DNA from one bacterium amounts to another.



# Types of flagellar arrangement



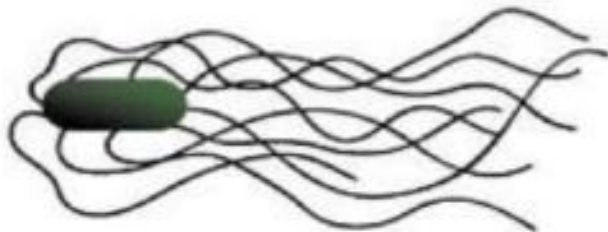
Polar/ Monotrichous – single flagellum at one pole



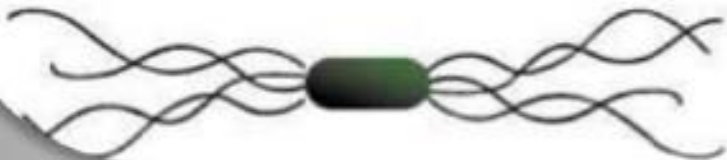
Lophotrichous – tuft of flagella at one pole



Amphitrichous – flagella at both poles

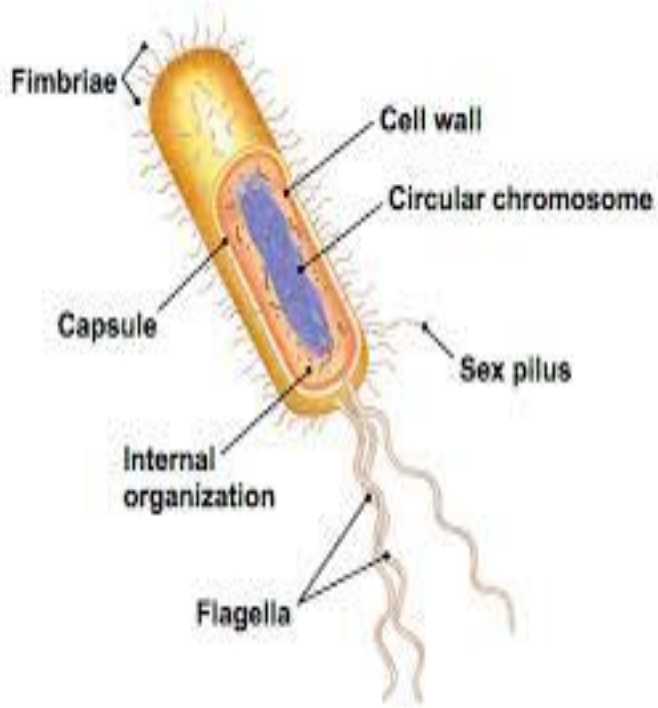


Peritrichous – flagella all over



Amphiloophotrichous – tuft of flagella at both ends





*Pseudomonas*



*Salmonella enterica*

# Structure of Bacteria

- \* **Spores:** Some gram positive bacteria but NEVER gram negative ones produce spores under harsh conditions.
- \* **Endospore:** is a highly resistant phase, whereby the organism can survive in a dormant state through a long period of starvation and under harsh environmental conditions.

## **The stimuli for sporulation:**

Starvation, transition from growth to stationary phase which triggers a program of sequential expression of specific genes morphological distinct structure (the endospore) within the mother cell.

# Formation of spores



The cell duplicates the chromosome, **one DNA portion** becomes surrounded by **an inner membrane, two peptidoglycan layers and outer keratin like protein** which protect the DNA from desiccation (drying) and toxic agents, thus some spores may last for centuries.

\*\* This process does not involve multiplication.

## **Spores resistance due to many factors as:**

- impermeability of their cortex and outer coat.
- their high content of calcium and dipicolinic acid.
- low content of water.

which enable spores to remain viable for many years in the dry state.



Reactivation of spores is termed **germination** and occurs under stimulation of external condition that favors growth.

\*\* Water and nutrients are required for germination which leads to a bacterium identical to the original one.

\*\*In sporulation each vegetative cell form only one spore and in subsequent germination, each spore give rise to a single vegetative cell.





Spores stained by specific methods, appearance of mature spores vary according to species being: spherical ovoid or elongated, occupying a terminal, sub terminal or central position and being narrower than the cell or broader and bulging it.

Spores are much more resistant than vegetative cell to exposure to disinfectants, The only way to kill spores is autoclaving and it is the application of moist heat at 100-121°C or greater for 10-20 min.

\*\*Heating at 60°C is enough to kill vegetative cells.

\* Spore-forming bacteria include **Bacillus** (aerobic) and **Clostridium** (anaerobic) species.

# Structure of Bacteria



**Plasmid:** Extra-chromosomal, circular DNA, double-stranded molecule.

- Replicate independent of bacterial chromosome
- Transmissible or non-transmissible plasmids
- contain genes that confer some properties such as antibiotic resistance, virulence factors (exotoxin), genes for pili.
- Plasmids are not essential for cellular survival.

# Structure of Bacteria



**Transposons:** Pieces of DNA that moves from one site to another either within or between the DNAs of bacteria, plasmids and bacteriophages  
“Jumping genes”.