

Bacterial structure

Blue → doctor's notes





- 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

CAHARCTERISTICS



Bacteria vary in size from 0.2 microns, but usually about 2 micron, it is visible with the light microscope (resolving power 0.2 microns).

“A red blood cell measure 7 microns in diameter for comparison.”

Bacteria are prokaryote cells i.e. they have no nucleus, no organelles (endoplasmic reticulum, mitochondria, Golgi apparatus, lysosomes).

They possess a cell wall which characteristically contains peptidoglycan.

They have different ribosomes from eukaryotic cells with a sedimentation value 70S.

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

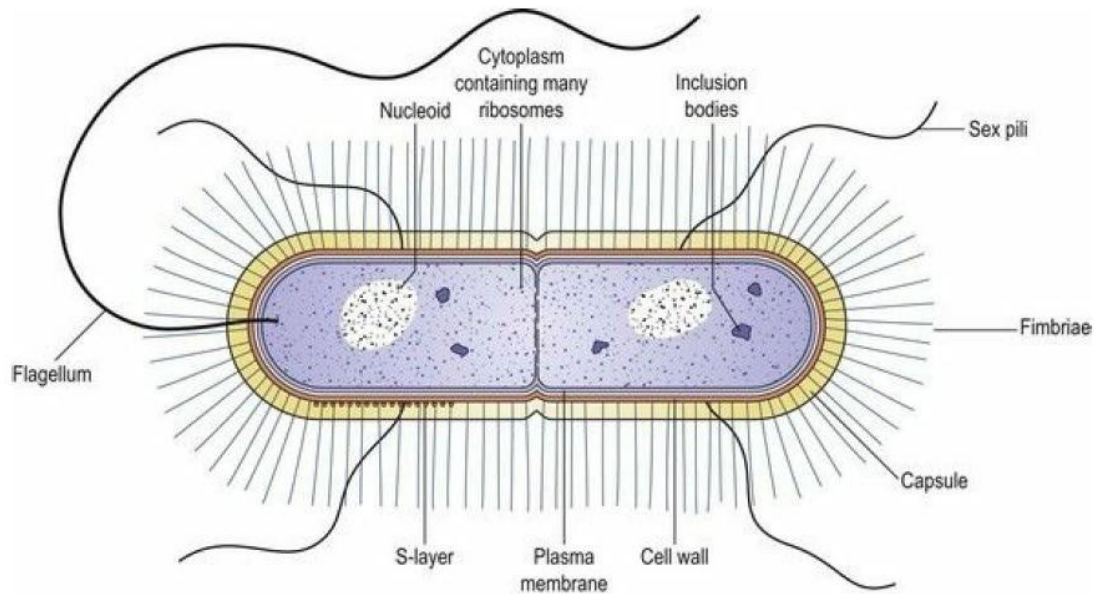
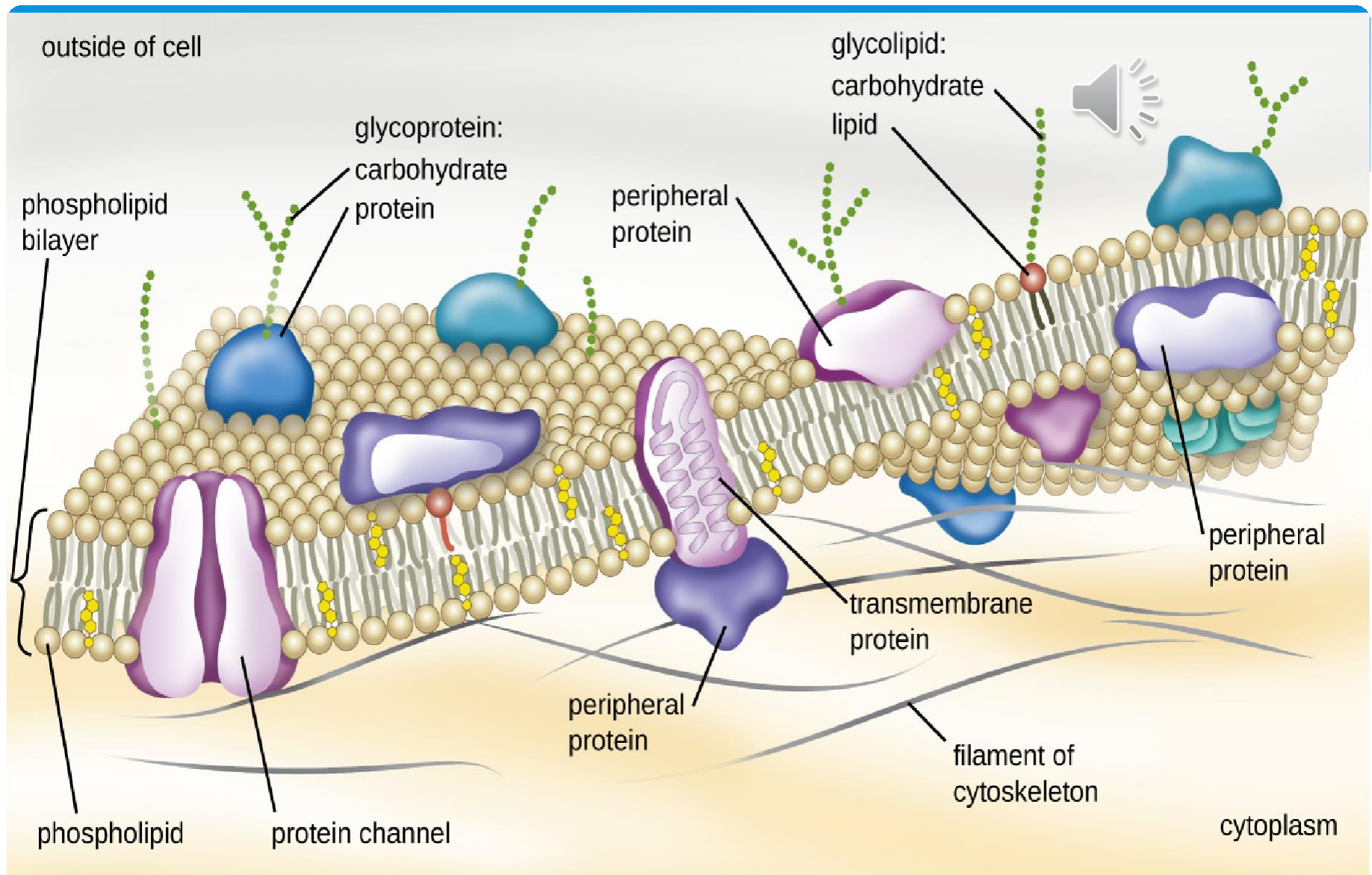


Fig. 2.2 Diagram illustrating the key features of bacterial cells. The S-layer is a variably demonstrated ordered protein layer.

Structure of Bacteria



- Cytoplasm is 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.



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

****Integral proteins that help in attachment**

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.
 - * Cytoskeletal structures could be a target for some antibiotics.

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



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.

- Not all bacteria have inclusion bodies.
- Viruses can infect both humans and bacteria, when a virus attacks a bacterium, the viral DNA multiplies. Consequently, we will end up with aggregation of proteins, they're called inclusion bodies.
- * 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

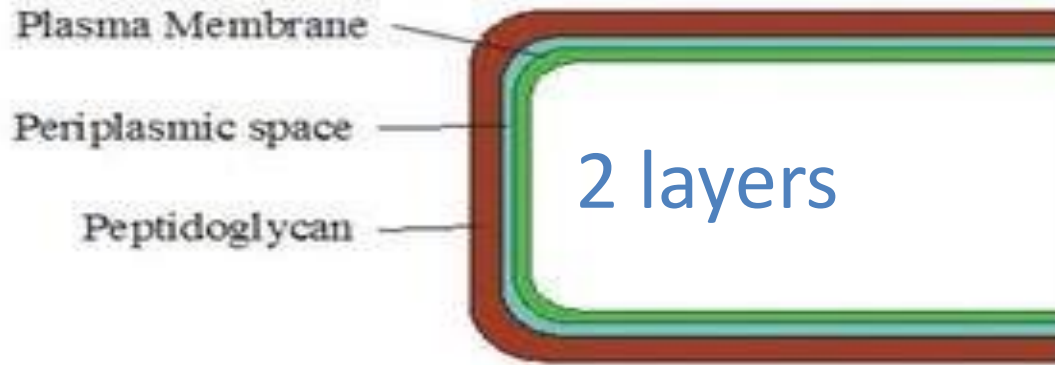
Cell wall:



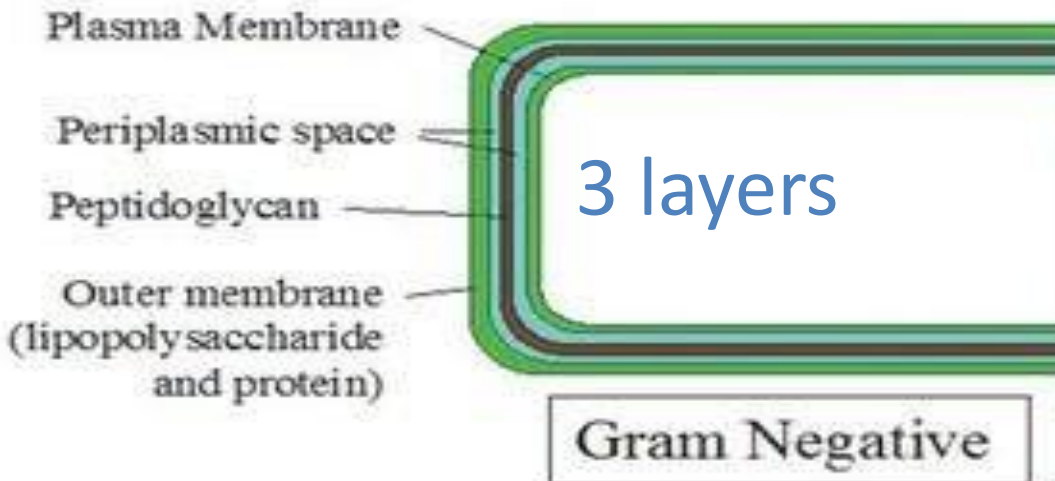
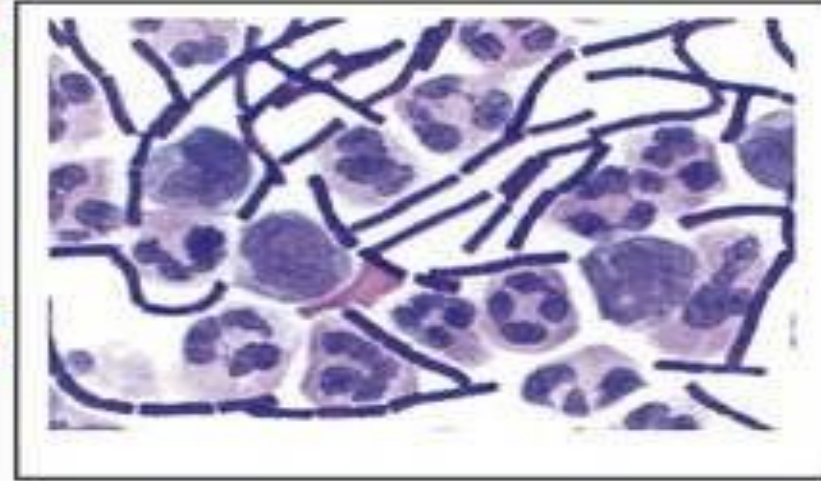
- It has peptidoglycan layer (the main component of cell walls) outside the cytoplasmic membrane made of long polysaccharide chains cross-linked by peptide bridges, it provides rigidity and protection.
- Loss of cell wall leads to death (this can be affected by some antibiotics and by lysozyme).
- The cell wall may also contain proteins that serve as adherence agents (virulence factors)
 - The more virulence factors bacteria have, the more severe they are.
 - Bacterial adhesion is a process in which bacteria attach to other cells.
- 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



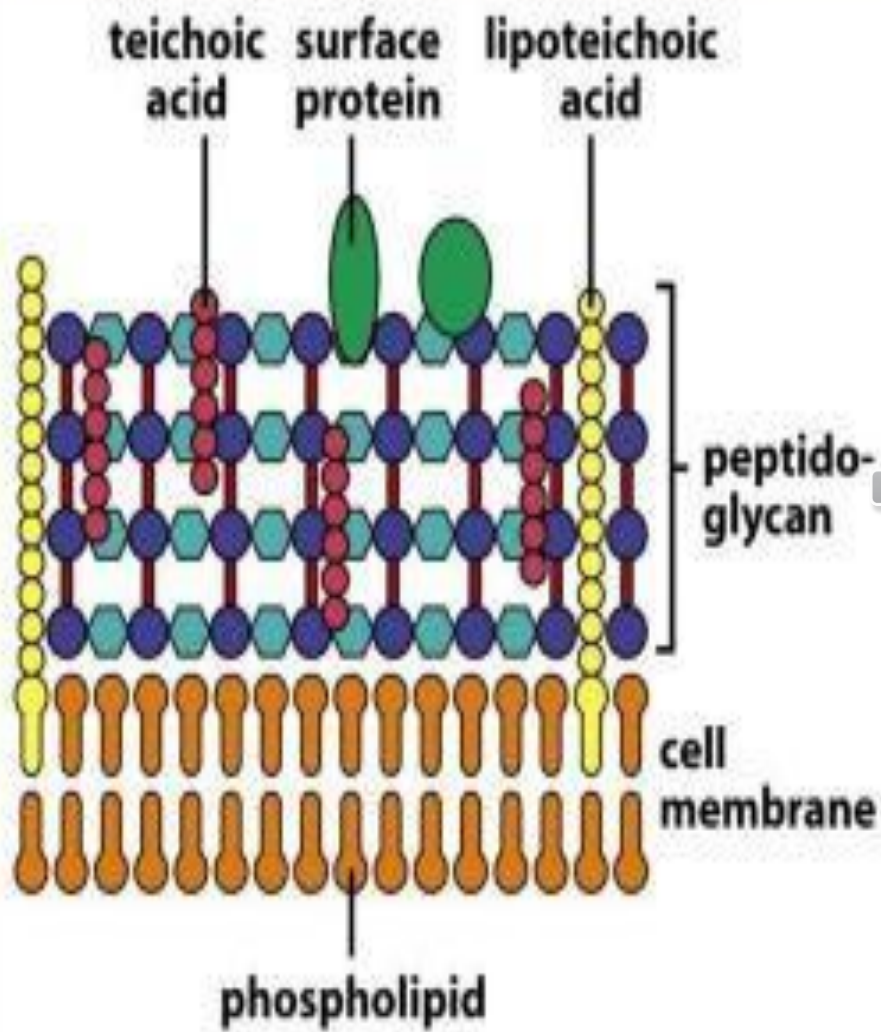
Gram-positive bacteria stain purple with Gram stain. This is because they have a thick cell wall without an outer membrane. Example: cyanobacteria



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

	Gram positive	Gram negative
Layers	2 layers	3 layers
Peptidoglycan thickness	Thick	Thin

Gram-positive bacteria



Gram-negative bacteria

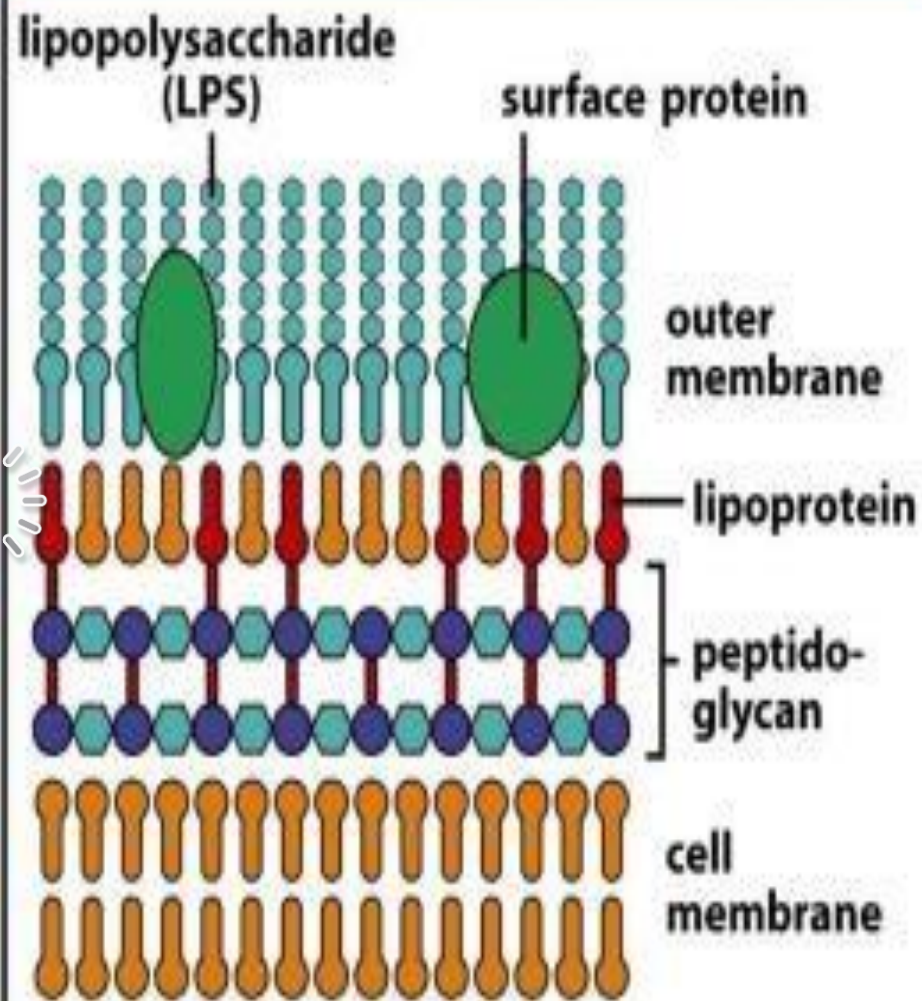


Figure 2-14 Immunobiology, 7ed. (© Garland Science 2008)

Gram positive bacteria

****** The cell wall of G+ve usually contains a large amount of

1 Teichoic Acid which is connected to peptidoglycan.

2 Lipoteichoic Acid which is connected to plasma membrane.

* Note from us: only exist in germ positive cells



Gram negative bacteria



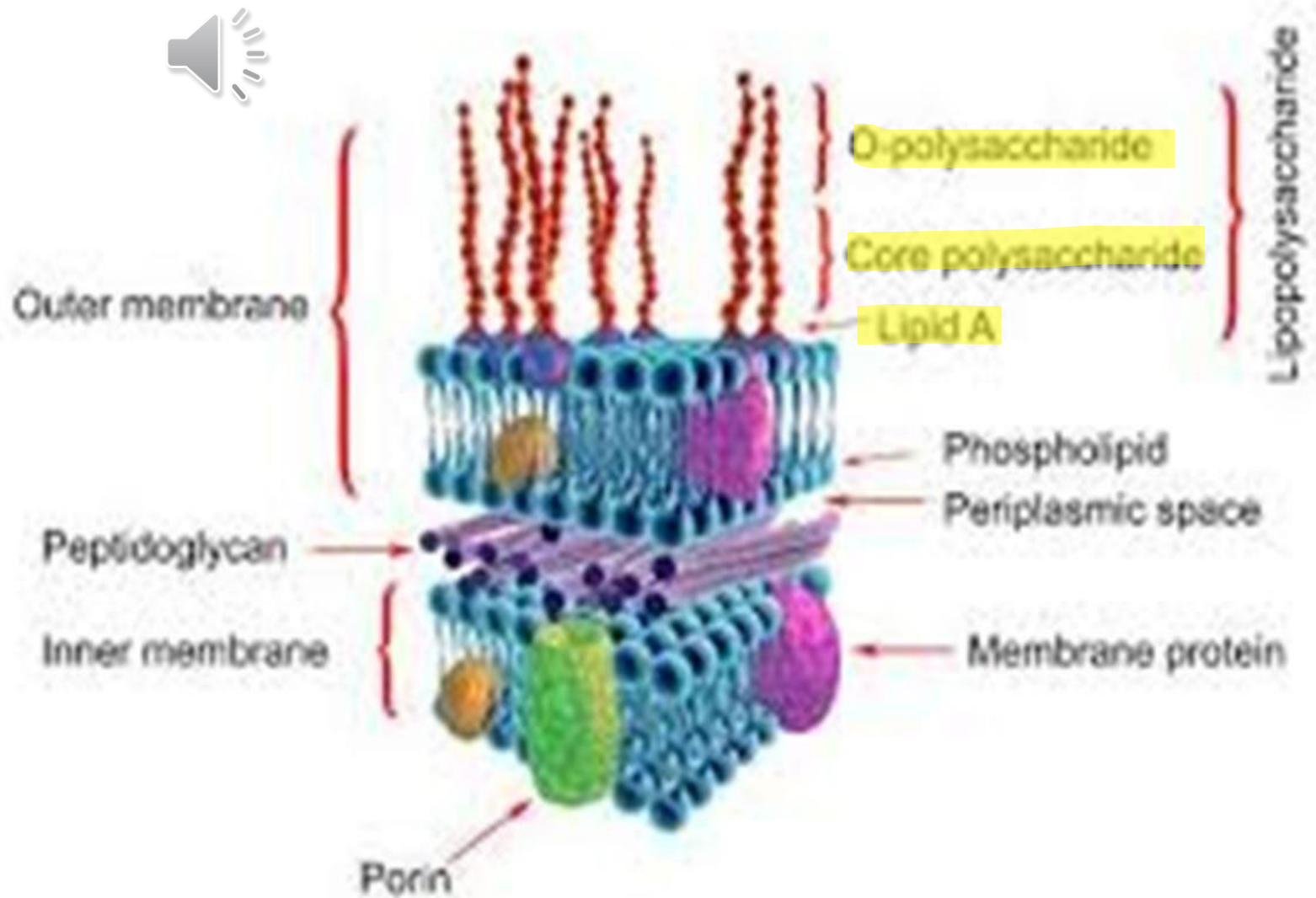
1- They have a thin monolayer of peptidoglycan, the outer membrane which is unique to gram negative bacteria.

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.**



The gram negative cell wall



- * Advantages of outer membrane:

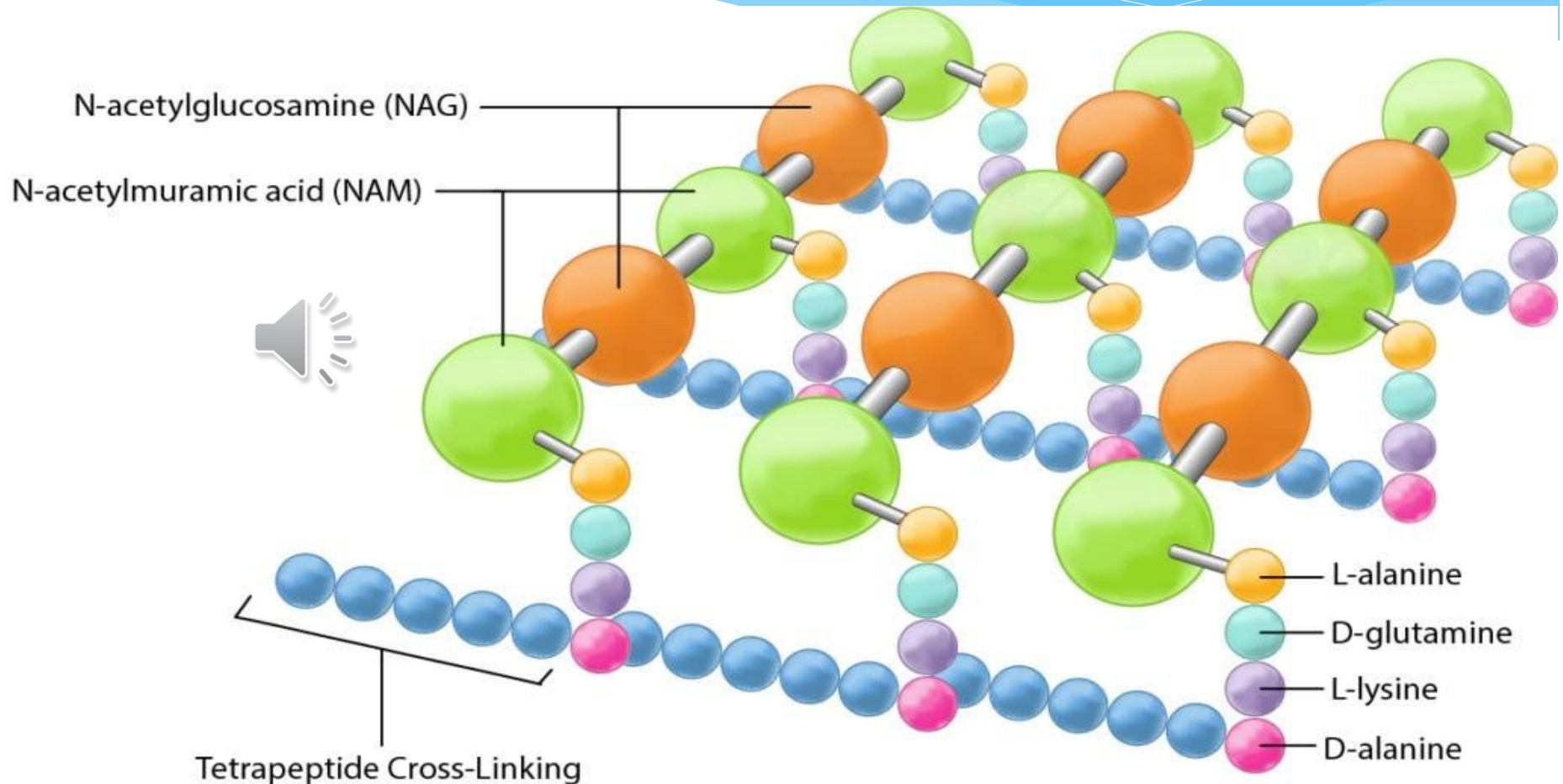
- It protects the peptidoglycan from the effects of lysozyme (a natural body defense substance that cleaves the link between *N-acetylglucosamine* and *N-acetylmuramic acid*).
- It impedes the entry of many antibiotics.

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 β -(1,4)- glycosidic bond.

Structure of peptidoglycan in bacteria cell wall



Culturing helps us know what type of microorganism there and thus what antibiotic to use in case of inflammation

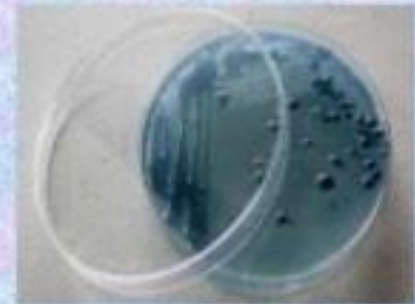
Equipment for culturing bacteria



EQUIPMENT :

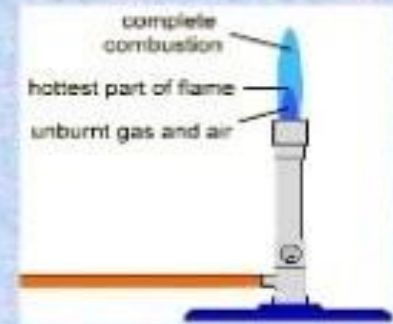
• 1. Petri dish :

Petri dishes contain media to keep bacteria alive.



2. Bunsen Burner :

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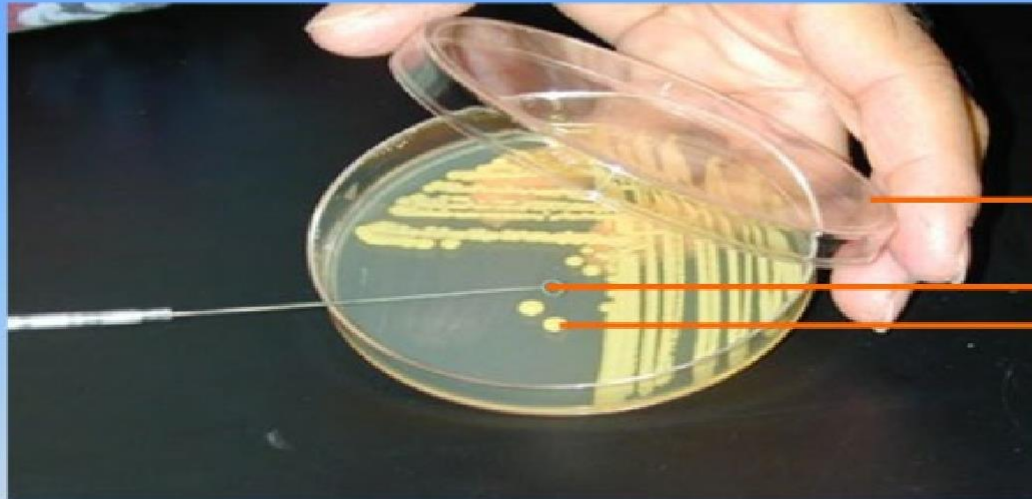


3. Inoculating loop :

A simple loop that's used to pick up a small sample of the dish



Streaking



Dish

Wire loop

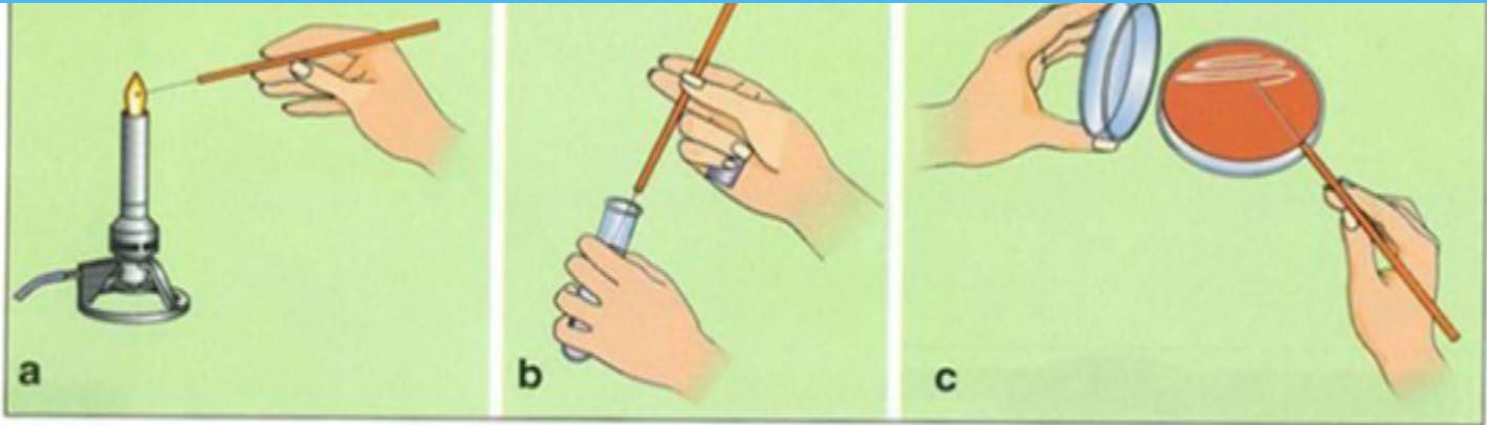
*Colony
(pure culture)*

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.

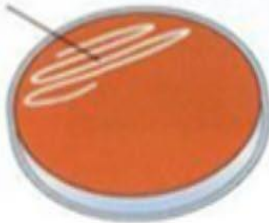
A **pure culture** is a **culture** in which only one strain سلالة of bacteria is present. A colony is **considered a pure culture** because it is known as several individual organisms, especially of the same species, living together in close association.



Streaking method



initial
inoculum



second
set of
streaks



third
set of
streaks



fourth
set of
streaks



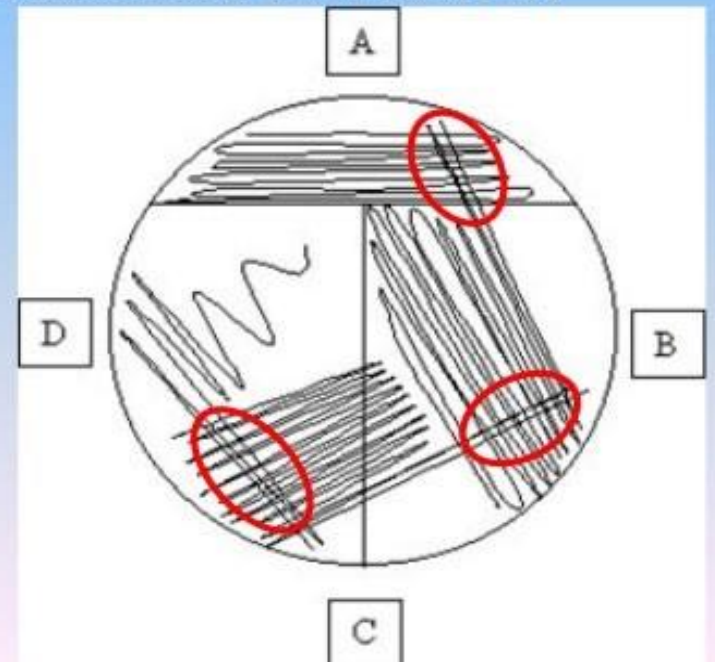
Courtesy Section Dickinson Microbiology

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.



Notice that in A the sample is crowded; so, It can't be easily tested under microscope. In D the sample is less crowded.

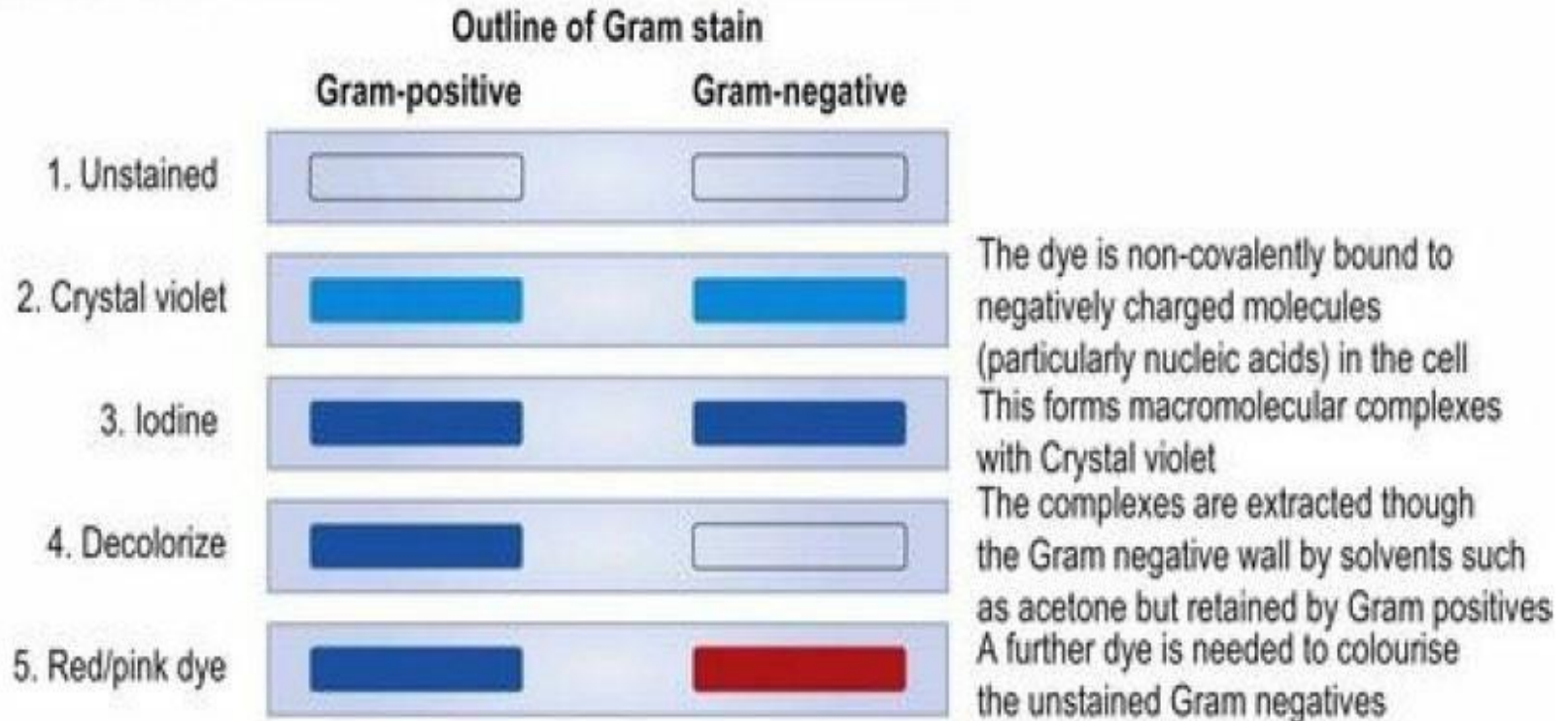
Gram stain

We use four stains in the staining process



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

Gram stain: GO TO LAB



- In case of gram-positive cells, the sample doesn't decolorize because it can't overcome the thick peptidoglycan layer, but gram-negatives' have a thin layer of peptidoglycans. Therefore, we don't use alcohol for a long time, because this will decolorize both types of cell walls at some point.
- Notice the colors in each step.
- So, what we do is adding the four stains and finally the gram negative will have red color.

STEPS OF THE PROCESS



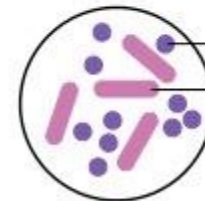
1 Application of crystal violet



2 Application of iodine (mordant)



3 Alcohol wash (decolorisation)



4 Application of safranin (counterstain)

KEY

- Crystal violet
- Iodine
- Alcohol
- Safranin

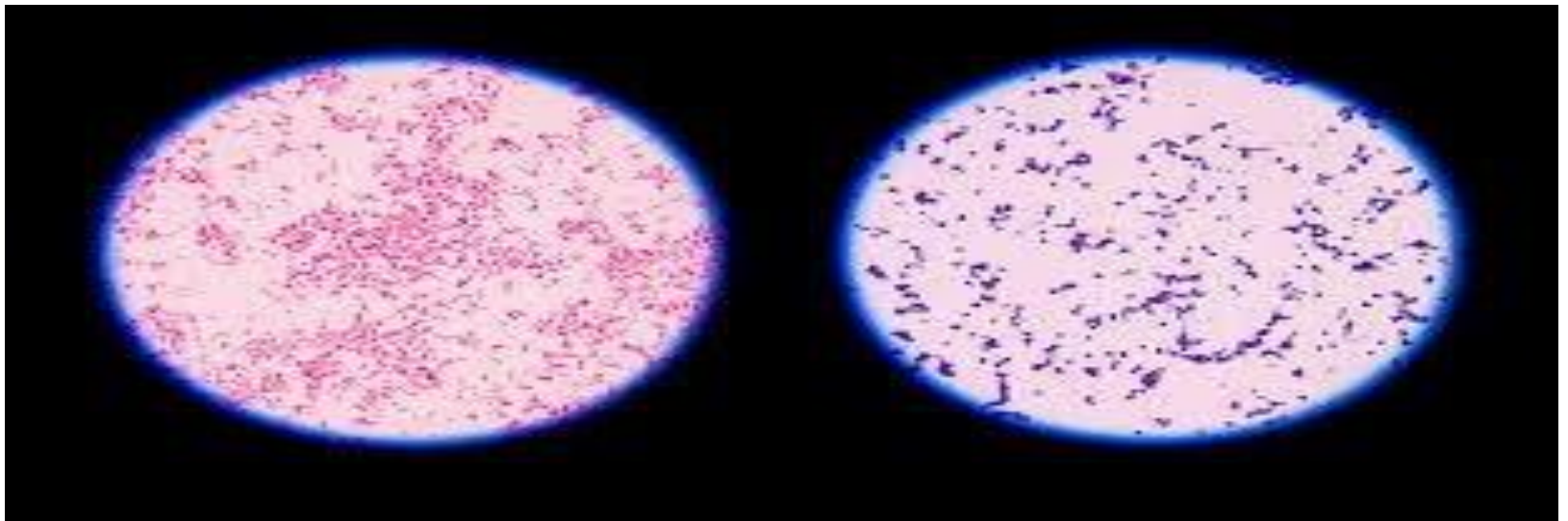
Gram-positive

Gram-negative



THE RESULT

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

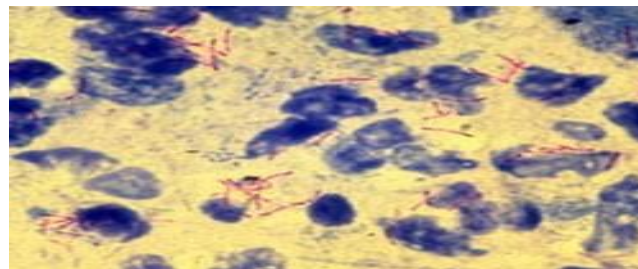




- Some bacteria are classified as Gram positive but stain poorly because the cell wall complex contains peptidoglycan, but otherwise it is composed of complex lipids.

- 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 considered as a virulence factor, antiphagocytic, and as an adherence factor.

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

Sometimes it is not very well demarcated (having a constant shape) 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.

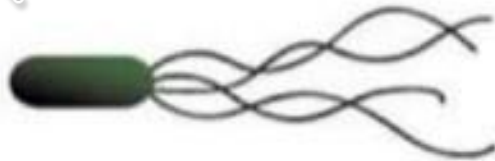
Fimbriae (pili) : is a hair-like structures of protein subunits called pilin, arranged uniformly along the whole surface of the bacterium, they cause adherence.

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

Types of flagellar arrangement



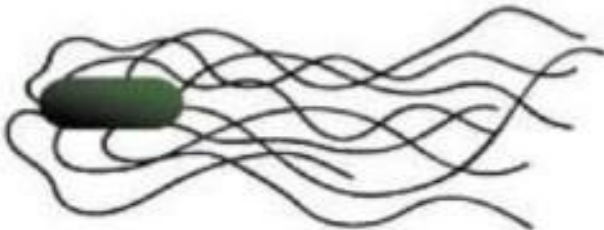
Polar/ Monotrichous – single flagellum at one pole



Lophotrichous – tuft of flagella at one pole



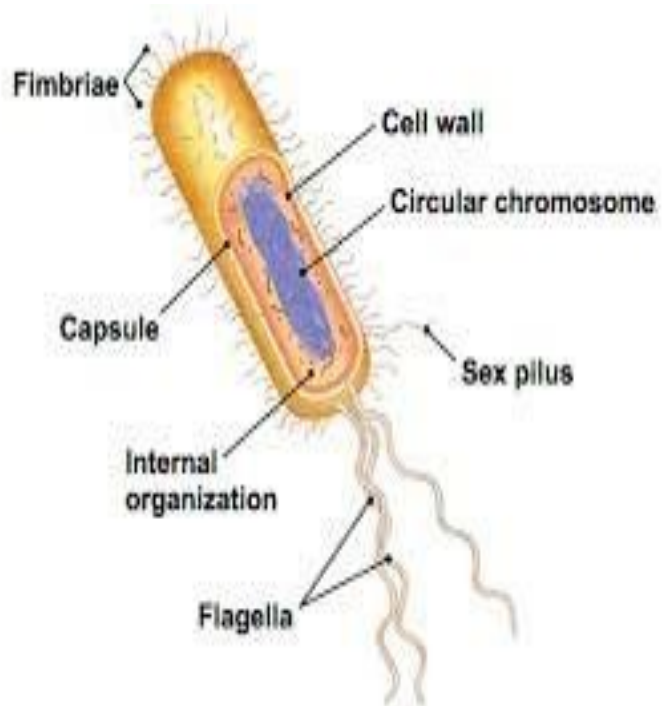
Amphitrichous – flagella at both poles



Peritrichous – flagella all over



Amphilophotrichous – 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 (non-productive spore):** 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.

* These layers cover and protect the spores

** 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 100-120°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”.