SHEET NO. 2

MICROBIOLOGY (Bacteriology)

DOCTOR 2019 | MEDICINE | JU

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"In the name of God, the compassionate the merciful"

** Please note that this is the second **bacteriology** lecture**.**

Bacterial structure

- It is important to understand the basic structural properties and the physiology of micro-organisms to establish our approach to infections.

Q: So, why is it important to know this structure and how can we benefit from that?

A: Because we will know how this micro-organism causes an infection so we can protect ourselves from it.

- Our understanding of microbial cytology aided by developments in genetic manipulation combined with advances in **fluorescence** and **electron microscopy.**

And now let's take some general characteristics of the bacteria:

- 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), and to know how much it is small "A red blood cell measure **7 microns** in diameter for comparison."

- **Bacteria are prokaryote cells** i.e. they have:
  1**-** No nucleus
  2- 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.**

Let's move on to the main topic of this sheet "Bacterial structure":

** The components of the bacteria are divided into:

- **Essential components (every bacterium has), such as:**
  1. **Cell wall**
  2. **Cytoplasmic membrane**
  3. **Ribosome**
  4. **Nucleoid**

- **Accessory components (not every bacterium has), such as:**

1. **Capsule, Pilus or fimbria, Flagella**
2. **Spores, Plasmid, Transposons**

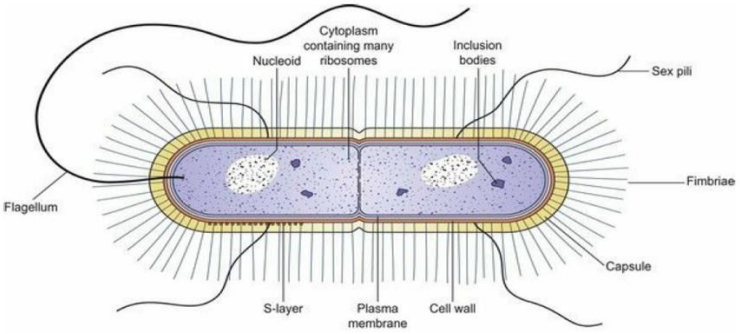


Fig. 2.2 Diagram illustrating the key features of bacterial cells. The S-layer is a variably demonstrated ordered protein layer.

Take a general idea and look at the cell wall, plasma membrane, cytoplasm containing ribosomes, inclusion bodies, nucleoids, fimbriae, flagellum, capsule.

And now let's take some details about each component ….

1. Plasma membrane:

- 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
  - cholesterol
- Functions of plasma membrane:
  - 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.

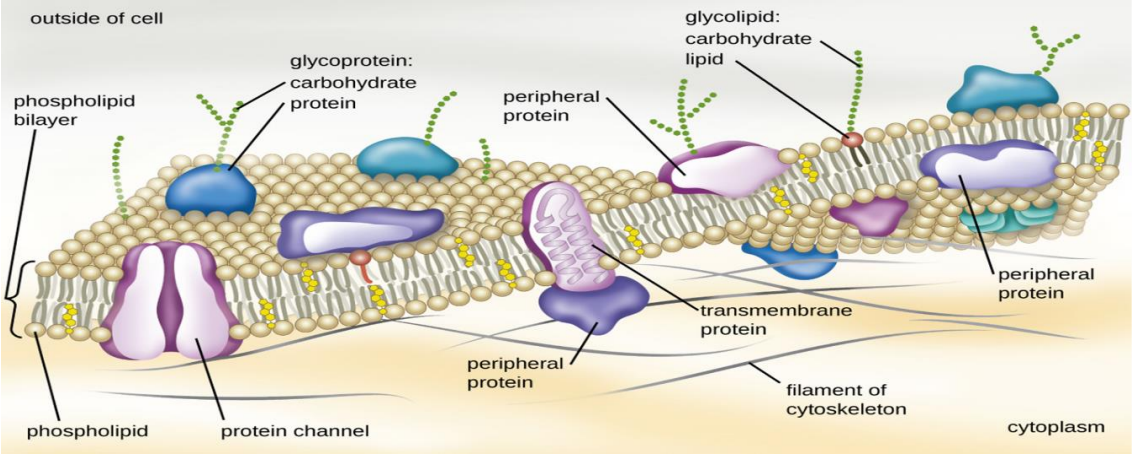
In the below figure we can see:

**Transmembrane proteins: Porins proteins for selective permeability

**Integral proteins that help in attachment

** Phospholipid bilayer

** Carbohydrates and cholesterol



2. Cytoplasm:

- Is a predominantly aqueous environment.
- Contains **<u>nucleoid</u>**, **<u>ribosomes</u>** and **<u>numerous other protein</u>** and **<u>nucleotide–protein complexes</u>**
- Bacterial cytoplasm has **<u>cytoskeletal</u>** structures (filamentous proteins and filament systems), and the importance of these cytoskeletal structures is:
  - Determining cell shape, division and spore formation.
  - Antimicrobials targeting.

3.Nucleoid:

- Area of cytoplasm where bacterial DNA is located.

4. DNA:

- Its features are:
  1. Single chromosome
  2. double stranded
  3. circular present in the cytoplasm with no nuclear membrane
- But **some** (not all) bacteria also have (an extra circular-chromosomal DNA) called **PLASMIDS,** and they are important at the gene transfer.

5. Ribosomes:

- Sites of protein synthesis
- They have a <u>sedimentation coefficient</u> of **70S**, being composed of a **30S** and a **50S** subunit (**80s** in eukaryotes).
- The ribosomes are the target of some antibiotics.

6. 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.**

7. 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.

  **(Cell wall + cytoplasmic membrane = cell envelope)

- Importance of cell wall:
  1. Bacterial rigidity and shape.
  2. protection against osmotic changes.
  3. Porous to allow nutrients passage.
  4. Structure differs in gram positive & negative bacteria.

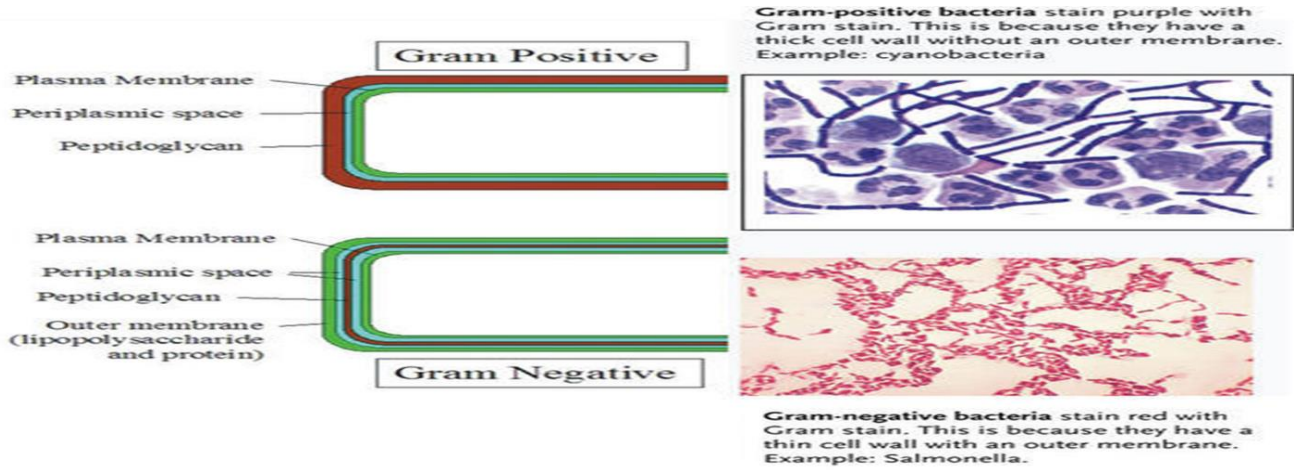
**Now let's talk in more details about the "Cell wall":

- 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), and these factors determine the intensity of the microbe "the weapons of the bacterium ☺", and when it has more adherence agents it will cause more infection for the human cells so it will become more dangerous.**
- Scientists found two major types of bacterial cell walls, and this discovery is the base of bacterial science, the bacterium either be a gram positive or gram negative.
- In **gram positive bacteria**, the peptidoglycan layer consists of about 70 layers while in **gram negative bacteria** there is only one layer of peptidoglycan.

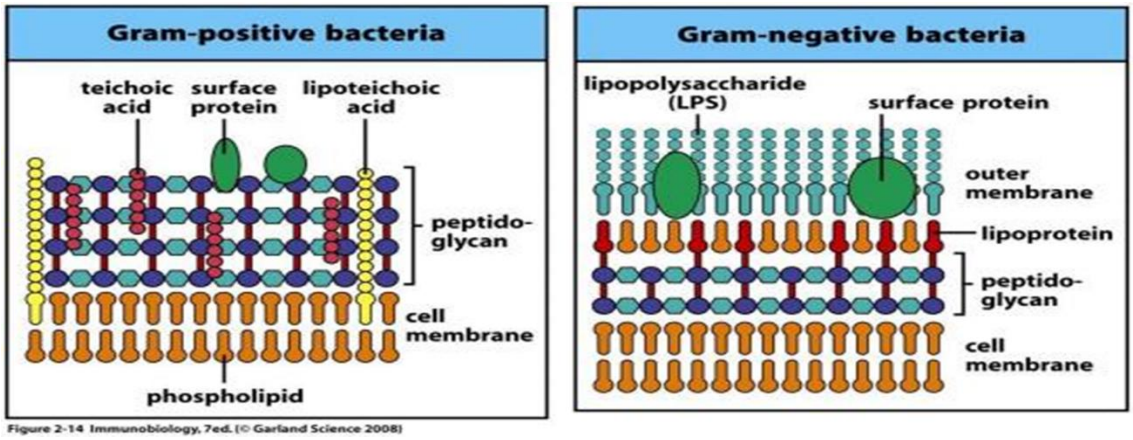
In the below figure we can see the composition of the cell wall of gram positive "the above purple one" and gram negative "the below red and pink one" bacteria.

- The gram-positive bacterial cell wall from left to right: thick layer of peptidoglycan, plasma membrane.
- The gram-negative bacterial cell wall from left to right: Outer membrane " made of lipopolysaccharide and protein", thin layer of peptidoglycan, plasma membrane.

** So the gram positive = 2 layers, gram negative = 3 layers



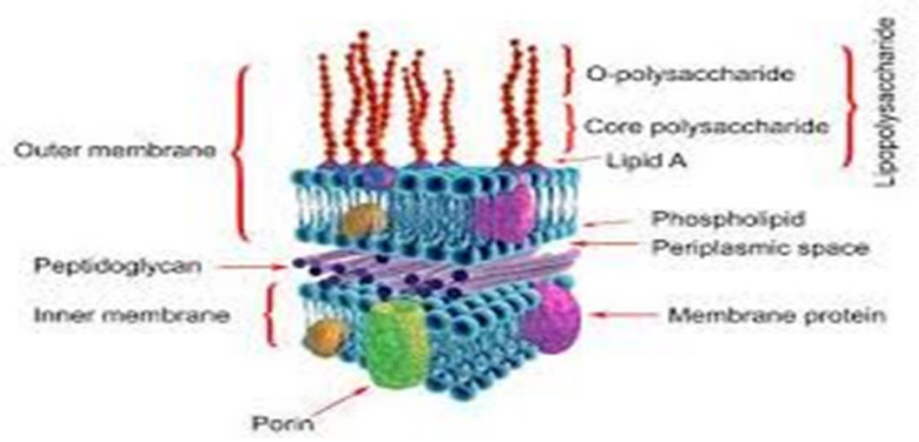
** Another figure to illustrate the topic:



- Notice that the gram-positive cell wall has a large amount of **"teichoic acid" that binds to peptidoglycan** and **"lipoteichoic acid" that binds to plasma membrane.**

Additional explanation for the gram-negative bacteria as we see in the below figure:

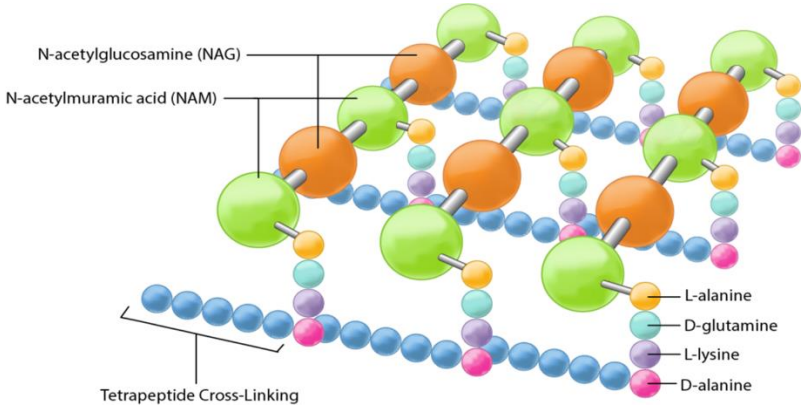
- They have a thin monolayer of peptidoglycan, the outer membrane which is unique to gram negative bacteria.
- The outer membrane (which protects the bacteria) differs from the cytoplasmic membrane in that it contains a special different lipid called **lipopolysaccharide (LPS)** in the **outer** leaflet.
- LPS is also known as **endotoxin "we called them endo- because they are parts of the cell wall"** 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**.



- Advantages of outer membrane:
  1. 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).** ← The definition of the lysozyme.
  2. It impedes the entry of many antibiotics.

And think that now is a good time to talk about the structure of peptidoglycan in bacterial cell wall, so let's move on:

- 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, and this bond is the target of lysozyme. "see the figure".



Now, lets talk about bacteria culturing in the laboratory:

Equipment:

**Don't forget that we should work under sterilization conditions, you should wear lap-coats, wash your hands and wear gloves, you should make sure that your dish is clean to prevent the contamination of the sample with some types of bacteria that we don't want.

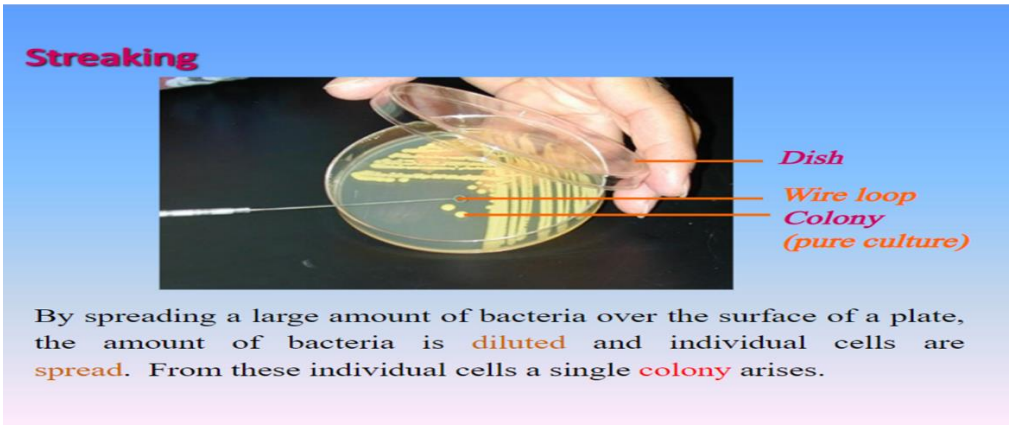
1.petri dish: that contains the nutrition that the bacteria need to grow.

2. Bunsen Burner: to sterilize the equipment.

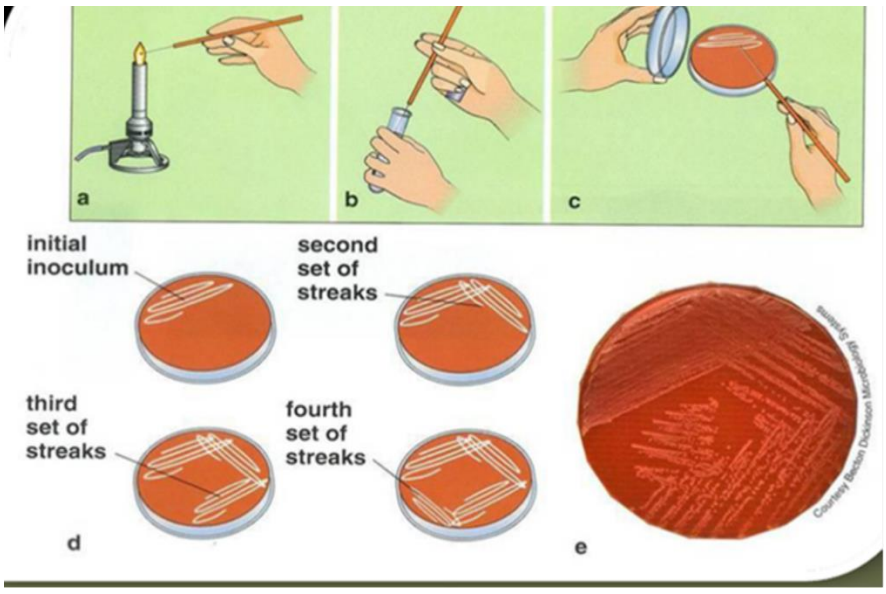
3. Inoculating loop

- So, we use this technique to see whether there is a bacterial growth or not, and if there is to choose the correct antibiotic for the patient.

We put a large amount of the bacteria in the dish that contains a good nutrition medium of the bacteria, and at each time we make a dilution to the number of this bacteria until we reach the pure colony" culture".



- 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.



sterilizing

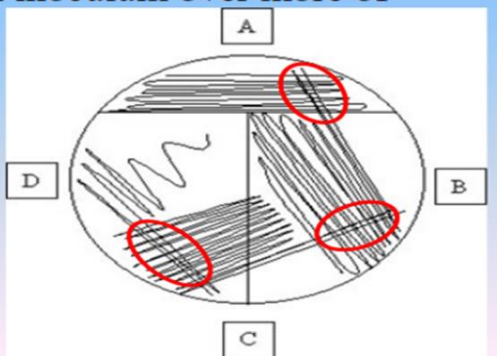
To keep the bacteria alive

Or initial

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.

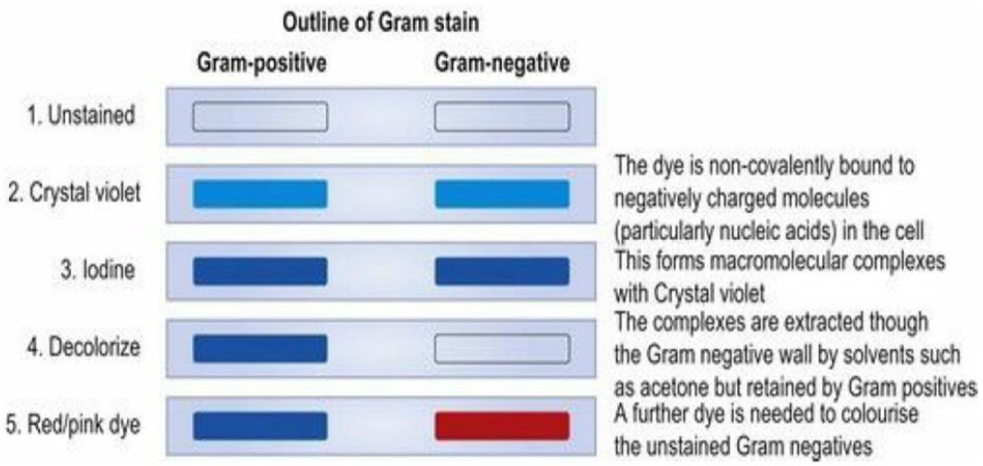


*Notice that each time we make a dilution to the microorganisms.

* We have more than one colony but all are the same type.

- I know that you get tired, the remaining material is so easy and we will now talk about the GRAM STAIN, "we call them gram because the scientist who discovered them is Hans Gram", and according to the final color we decide if it is a gram positive or negative, and there are four stains:
  a) Crystal Violet or methylene blue: primary stain
  b) Iodine: mordant stain
  c) 95% of alcohol: decolorizing
  d) Safranin (red-pink): contrast stain

  **Now we will make an experiment, we have two samples one of them is gram positive and the other one is gram negative:



** In each step, we will put the samples under microscope to observe them, but actually the scientists put them only when they finish the experiment. يعني احنا بس هالمرة بنتطلع عالمايكروسكوب بكل خطوة لأننا مبتدئين، بسم الله نبدأ بكلام طويل بس سهل

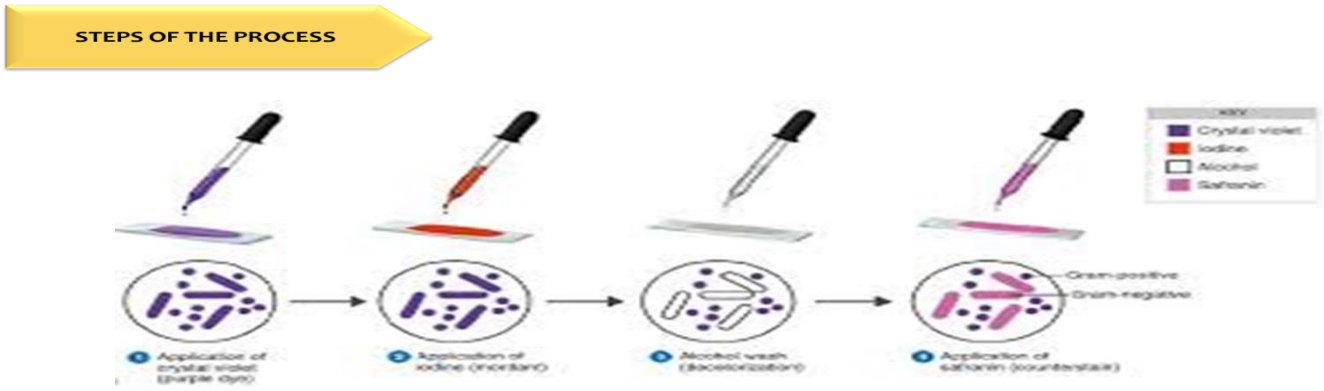
We grab the two slides and add a drop of distilled water ...then we spread a loop full of bacteria on the first slide ...we let it dry then we pass it on a Bunsen burner to fixate it and we repeat these steps on the second slide Now we begin the steps:

1) first, we add the primary stain (**crystal violet**) colored purple so we cover the slide with it and leave it for about 20 seconds then we rinse with water (at this point if we look at either slide under the microscope, we'll see they're both purple

2) then we cover the slides with (**iodine)** and leave it for 1 minute this acts as a mordant مثبت (which forms a complex with crystal violet) then we rinse with water (at this point they're still both purple.

3) the critical step ...we run alcohol till the solution clears شفاف يصير (when we see that we immediately rinse with water to stop the action of alcohol (its critical because if we look at the slides now, we'll see that one is purple (gram positive) and one is colorless (gram negative) Why? Because polysaccharide outer membrane of the negative gram bacteria melts in alcohol and lets the alcohol enter the peptidoglycan to pull the color from it ...while the gram positive one is made of 70 layers which makes pulling the color very difficult .... can be done if we keep adding alcohol but we don't do that

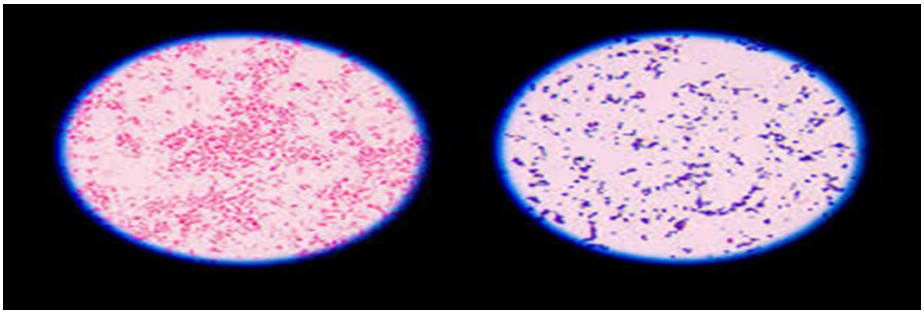
4) then we add the red-pink safranin for 20 seconds and gently rinse with water at this point we're done What happens is that safranin enters both bacteria but it finds the gram negative one colorless so it colors it and it finds the gram positive one colored with the dominant purple color of crystal violet that it can't overcome visually بتدخل عليها بس ما بتتغلب عاللون البنفسجي(so it remains purple while the gram negative becomes pink.)



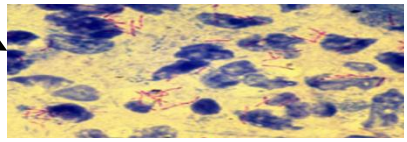
Crystal violet: All the samples are purple	Iodine: as a mordant and the samples still purple	Critical step using alcohol for decolorizing: Positive=purple Negative= the color disappeared	Safranin: enters the gram-negative and gives it a pink color

THE RESULT

Gram positive appears violet/blue while gram negative appears 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 cannot be stained because they are intracellular as **Chlamydiae & Rickettsiae**

Lets back to the structural components:

8- 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 and is referred to as **slime**

9- 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.

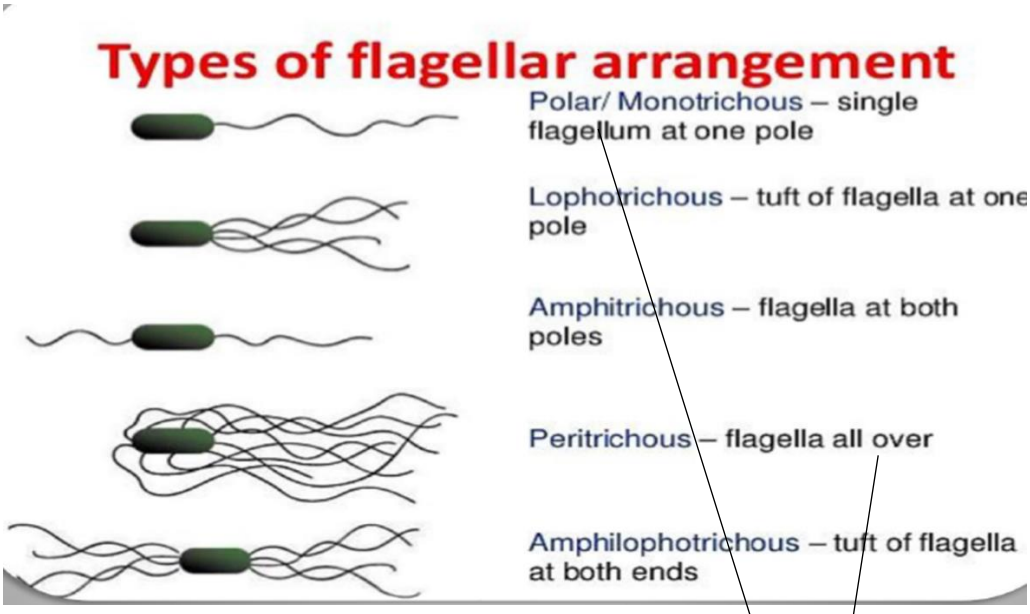
10- Flagella:

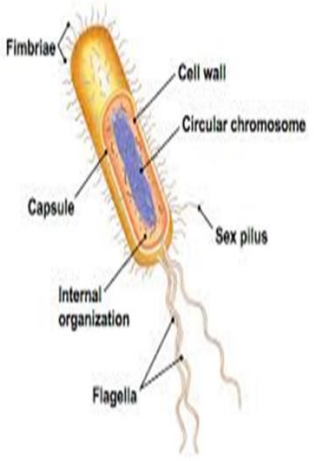
- **Helically** coiled protein subunits called **flagellin**
- anchored to bacterial membranes through **hooks and basal body**
- they are responsible for **motility**.

11- 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.

**Now, these are the types of flagellar arrangement:





Pseudomonas

Salmonella enterica

12. Spores:

- **Some gram-positive bacteria but NEVER gram-negative** ones produce spores under harsh conditions.

- **Endospore "spores" definition:** 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 even if there aren't any nutrition.
- **But what are 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
- Also surrounded by **two** peptidoglycan layers and outer **keratin** like protein which بحموه كلهم protect the DNA from desiccation (drying) and toxic agents, thus some spores may last for centuries**.**
- **This process does not involve multiplication.**

Hmmmm, 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.

All these factors enable spores to remain viable for many years in the dry state.

****Let's talk about a new term about the spores:**

Germination: is the reactivation of spores and occurs under stimulation of external condition that favors growth, and one of these conditions is:

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

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

Q: How can we stain the spores???

A: Spores stained by specific methods; appearance of mature spores varies **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.

So, spores staining enables us to determine the shape and location and then we can identify this type of bacteria.

- Spores are much more resistant than vegetative cell to exposure to disinfectants, so I want an unusual disinfectant to kill it and 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.

NOTE: Heating at 60°C is enough to kill vegetative cells, but cant kill the spores.

****An example on Spore-forming bacteria include Bacillus (aerobic) and Clostridium (anaerobic) species.**

13. 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

14. Transposons:

- **Pieces of DNA** that moves from one site to another either within or between:
  - ✓ The DNAs of bacteria
  - ✓ plasmids
  - ✓ and bacteriophages

  They are called "Jumping genes".

God protect you