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Microbiology

Doctor 2017 | Medicine | JU

Sheet Slides

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Cell structure

Major differences between prokaryotic and eukaryotic cells:

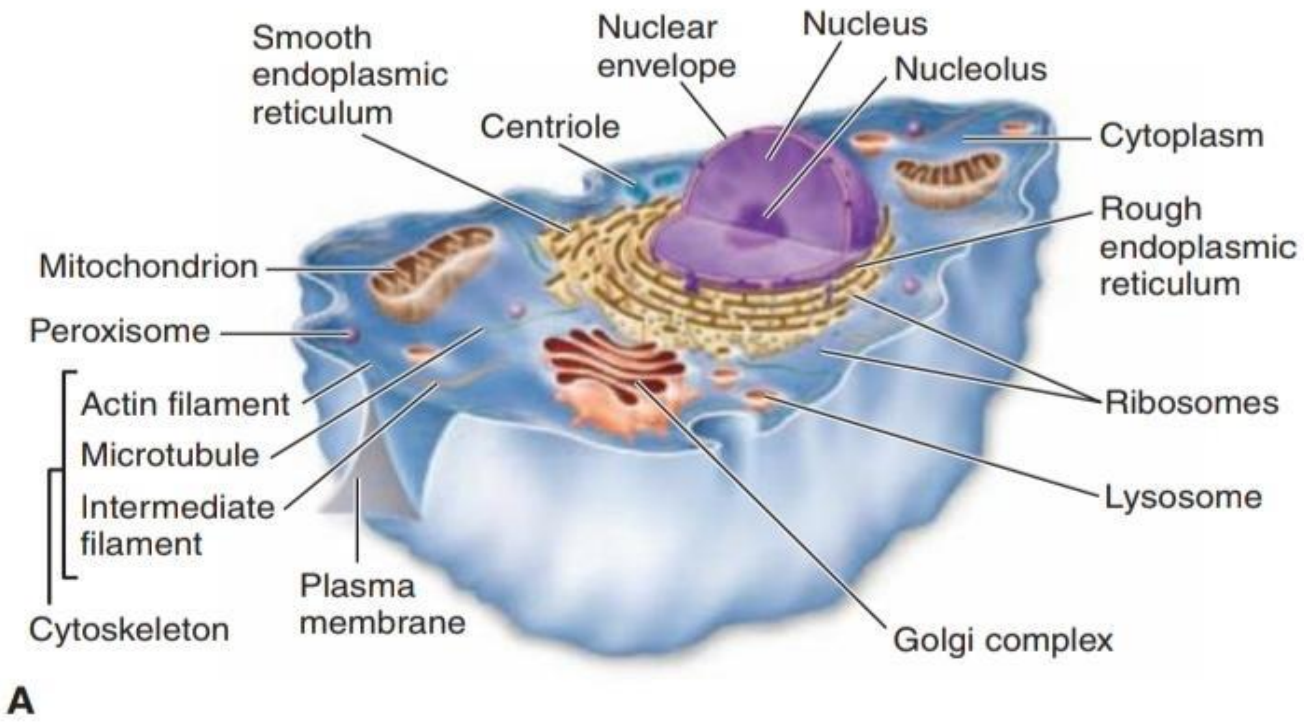
- The presence of nuclear membrane in a eukaryotic cell, so most of the DNA is in an organelle called the nucleus, which is bounded by a double membrane (nuclear membrane). In a prokaryotic cell, the DNA is concentrated in a region that is not membrane-enclosed, called the nucleoid. Also, prokaryotes have no true nuclei.
- The cell membrane differs between them in structure and function.

Other differences:

- The absence of organelles in the prokaryotic cells: For example, some prokaryotes contain regions surrounded by proteins (not membranes), within which specific reactions take place, while eukaryotic cells have extensive and elaborately arranged internal membranes that divide the cell into compartments called organelles.
- The size: Eukaryotic cells are generally much larger than prokaryotic cells.
- The type of ribosomes.
- Prokaryotes are haploid while eukaryotes are diploid.

Eukaryotes

The structure of the animal cell



1. Nucleus

- ✓ It contains the cell's genome.
- ✓ It has a nuclear membrane with many pores that gives it a selective permeability to certain substances like proteins and RNAs which can traffic in and out of the nucleus.
- ✓ Its membrane is separated by a space into ● outer membrane. ● inner membrane.
    - ➢ Our linear DNA is arranged as an extremely long double helix. So, it needs an efficient packaging system to reduce its length and determine the way replication and transcription occur.
    - ➢ This double helix is coiled around globular positively charged proteins called histones, that bind to the DNA by ionic interactions as written in the book, to form nucleosomes .Nucleosomes are then packaged together and folded around each other to form chromatin fibers. Chromatin fibers located in nucleoplasm appear like a stained diffuse (spread out) mass under microscope.
    - ➢ As the cell prepares to divide (Mitosis or Miosis), chromatin fibers are rearranged into separated chromosomes which are more coiled (condensed), thick and distinguishable structures.

2. Nucleolus

A visible structure in the nucleus which is important in ribosomes biogenesis. This is because it's rich in RNA and it synthesizes ribosomal RNA. Later, it combines rRNA with ribosomal proteins, which were synthesized in the cytoplasm , to form the small and large subunits of ribosomes . The nucleus can have more than one nucleoli.

3. Ribosome (its type is 80S)

Its function is synthesis and modifying proteins. Also, it can be free in the cytosol or bounded to the Rough Endoplasmic Reticulum.

4. Vesicle

An organelle that forms a compartment separated from the cytoplasm by a spherical lipid bilayer. It has several types with many functions like:

- **Lysosomes:** contain digestive enzymes with a pH different from the cytosol to degrade macromolecules like proteins, carbohydrates and lipids.
- **Vacuoles:** store nutrients, water or wastes.
- **Transport vesicles:** transport proteins to /from different sites inside the cell.
- **Secretory vesicles:** secrete substances outside the cell through fusing with the cell membrane then releasing their contents in a way that resembles the secretion of hormones.

5. Endoplasmic Reticulum

A network of membrane-bounded channels continuous with the nuclear membrane, and has two types:

- **Rough**: its function is related to protein synthesis because it has bounded ribosomes (80 s) in the outer layer of its membrane.
- **Smooth**: its function is related to lipid synthesis and carbohydrates metabolism.

6. Golgi apparatus (or "Golgi body")

It consists of a stack of membranes that modify and sort the products of Endoplasmic Reticulum. For example, if the RER has synthesized a certain protein to be secreted, Golgi apparatus should modify this protein and pack it into secretory vesicles.

7. Cytoskeleton

A Network of fibers that fill the cytoplasm, hold the cell structure and facilitates transport , motility and migration. It has two major types of fibers:

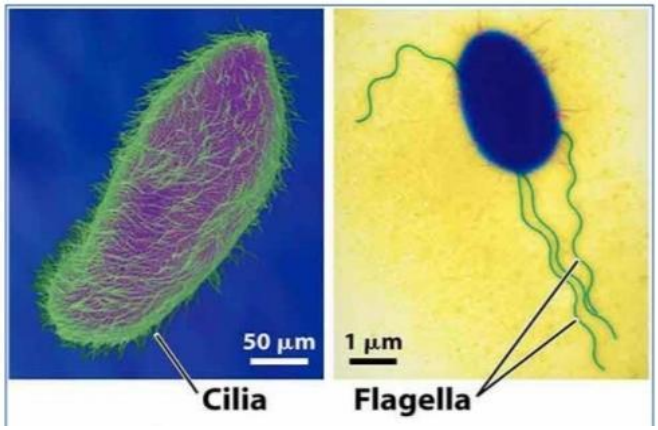
- **Microfilaments:** scaffold of actin polymers.
- **Microtubules:** scaffold of tubulin polymers. They also form cilia and flagella.

8. Mitochondrion

It's responsible of ATP production through oxidative phosphorylation.

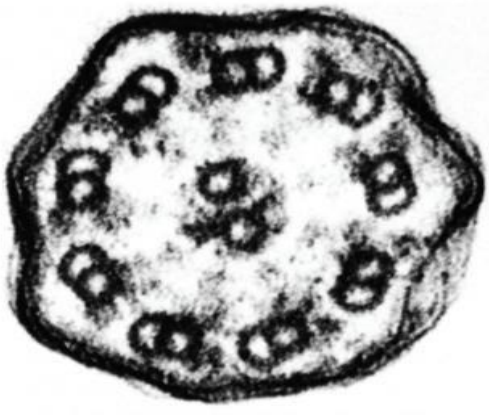
9. Cytosol

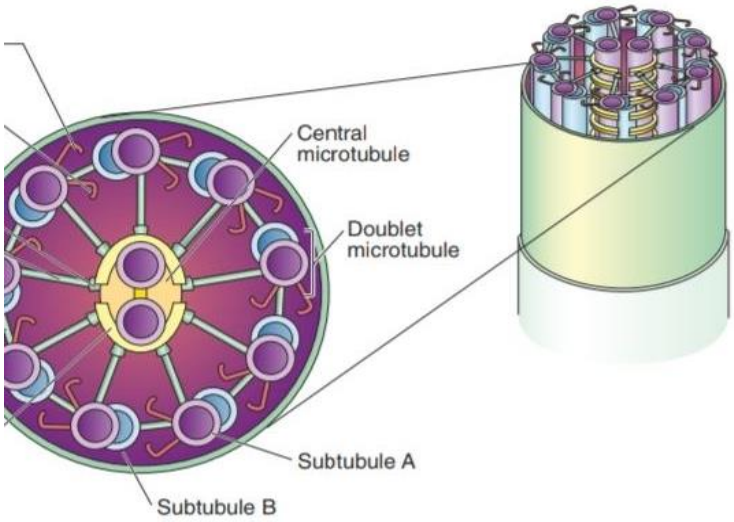
The liquid where organelles are suspended.



10. Motility organelles :

- ✓ Cilia and flagella are composed of microtubules, they move the cell.
- ✓ Cilia are the smaller one while flagella are longer.
- ✓ Microtubules are arranged in 9+2 system : nine duplicates of microtubules are arranged in a cylinder and two microtubules are found in the middle of the cylinder. This structure is the same in both cilia and flagella.



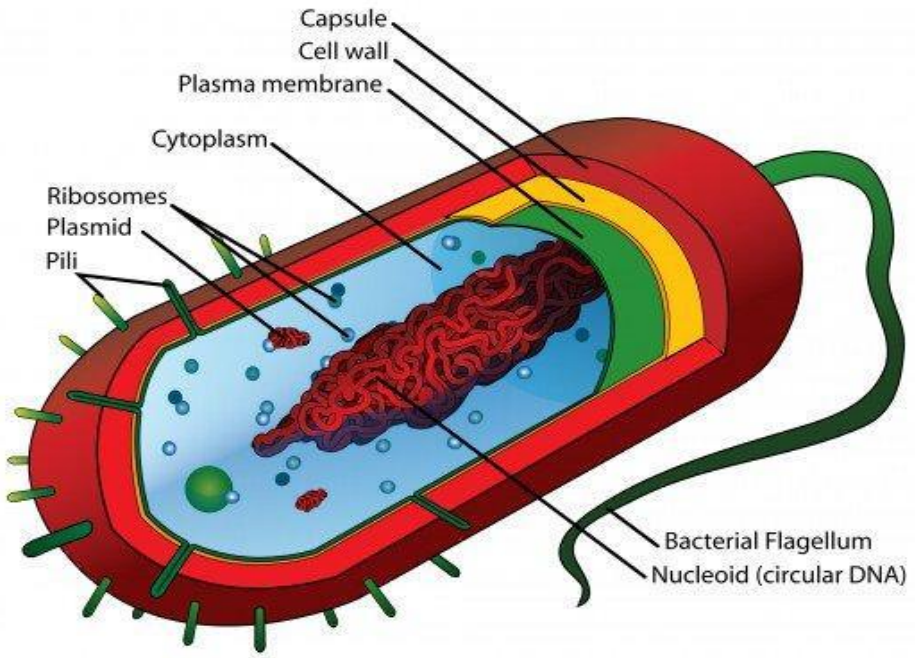


Prokaryotes

- ✓ Has One Circular Continuous DNA molecule that is packaged in nucleoid. More than one nucleoid can be found especially when dividing, but all are similar (so prokaryotic cells are haploid).
- ✓ Its Cytoplasm contains :

A. *Smaller ribosomes (70S).*

B. *Inclusion bodies* which are insoluble granules function in the storage of energy or as a reservoir of structural building blocks (e.g. Glycogen, PHB, polyphosphate).



1- Cell membrane :

- ❖ Has high protein density, proteins can be integral or peripheral.
- ❖ Lacks sterols which are found in eukaryotes membranes, that's why cell membrane is rigid in prokaryotes. Also, it has a metabolic activity.
- ❖ Integral proteins should have hydrophobic and hydrophilic regions.
- ❖ Sterols are steroid alcohols like cholesterol. Cholesterol is important in signaling between cells in Eukaryotes .
- ❖ Its functions are :

A. Permeability and transport of solutes (active and passive).

B. Electron transport and oxidative phosphorylation:

C. The bacterial cell membrane is thus a functional analog of the mitochondrial membrane—a relationship which has been taken by many biologists to support the theory that mitochondria have evolved from symbiotic bacteria → Endosymbiosis.

D. Excretion of hydrolytic exoenzymes that degrade polymers to small subunits to penetrate the cell membrane. Also, Excretion of pathogenicity proteins.

E. Biosynthetic functions: since it has enzymes and carriers that are involved in synthesis of membrane lipids, cell wall polymers and DNA.

F. Chemotactic systems because it has receptors and proteins to transfer signals from the environment to the cell to have a response which can be a change in protein synthesis, DNA transcription, etc.

** Active transport needs ions exchange or ATP. Passive transport doesn't need that.

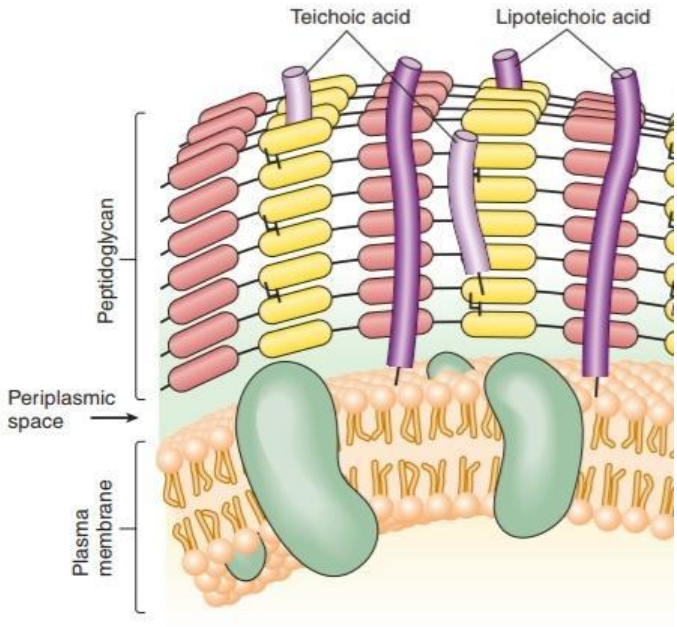
2-The cell wall

- Most bacteria are classified as gram positive or gram negative according to their response to the Gram-staining procedure, the staining reflects essential differences in the cell wall composition.
- The cell wall protects the bacteria cell from bursting due to the very high atmospheric pressure.
- According to the cell wall components prokaryotes can be classified into :

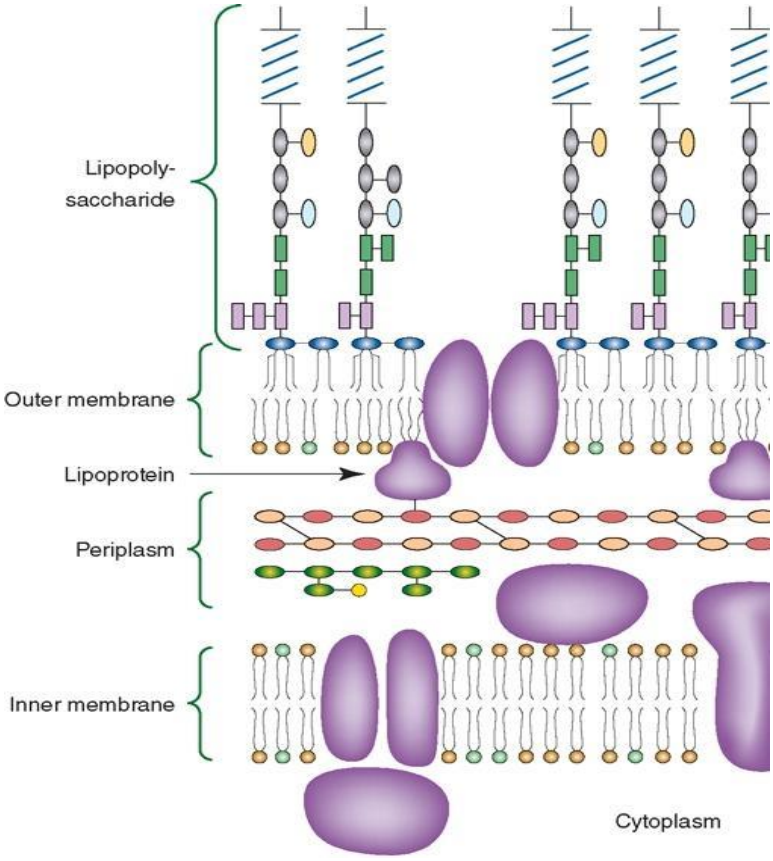
a) Gram-positive Cell wall

- High tensile strength owing to thick layer of Peptidoglycans.
- Peptidoglycans are polymers arranged in sheets and composed of alternating N-acetylglucosamine and N-acetylmuramic acid connected by β1→4 linkages. Teichoic acid connects the sheets together and increases the tensile.

- Peptidoglycans are the major antigenic determinants in bacteria. Through million years of interactions between the animal host and bacteria cell, the animal host starts to recognize certain parts of bacteria. That helps our body to know the pathogens and attacks them.
- Peptidoglycans work as patterns to recognize the pathogen
- Plays a role in cell division and its own synthesis.
- Teichoic acid is found in Gram(+) bacteria and contributes to tensile strength, porosity and antigenicity.

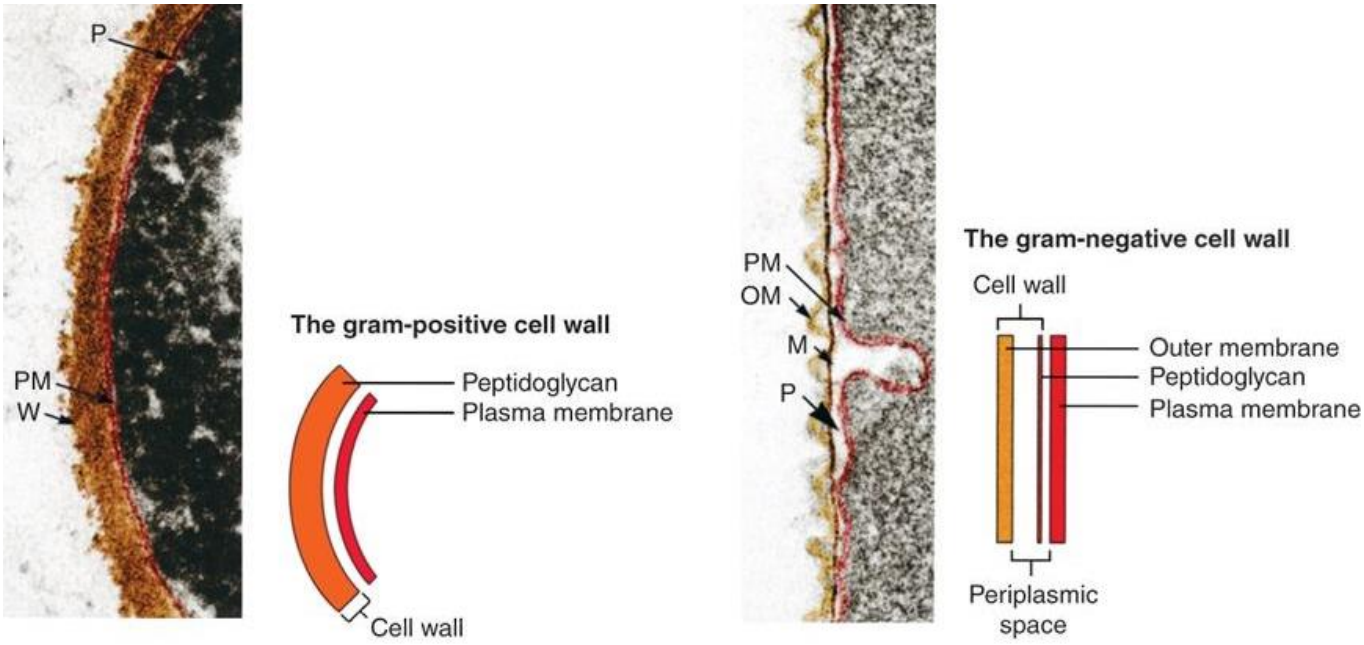


b) Gram-negative Cell wall

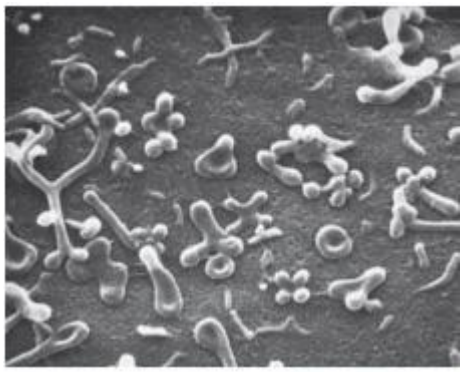


- Smaller peptidoglycan layer, but an extra outer asymmetrical membrane with Lipopolysaccharide (LPS) outer leaflet.
- The outer membrane of lipopolysaccharides is an antigenic determinant which is specific to Gram negative bacteria. LPSs are extremely toxic for animal host since they stimulate a strong immune response in our bodies.
- Antibiotics pass slowly through the outer membrane contributing to the high antibiotic resistance of Gram (-) s.

- Periplasm contains transporters and detoxifiers (e.g. the enzyme β-lactamase, important in antibiotic resistance because it breaks down the antibiotic).

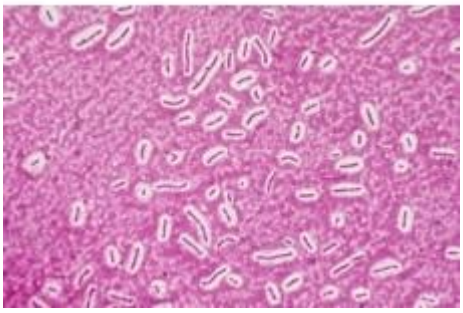


c) Lacking the cell wall!



- Mycoplasmas lack a cell wall, leading to variability in shape. They lack a target for cell wall–inhibiting antimicrobial agents (e.g. penicillin) and are therefore resistant to these drugs.

3. The capsule (or the slime layer) :



- Some bacteria form an external layer (outside the cell wall) of polysaccharides which contributes to invasiveness and resistance to phagocytosis. It is called: A capsule if it's rigid, or A slime layer if it's loose.
- This layer of polysaccharides contributes to the invasiveness of pathogenic bacteria—encapsulated cells are protected from phagocytosis unless they are coated with anticapsular antibody.
- The bacteria can have a capsule or a slime layer even they have cell wall!

- Because capsule and slime layer are composed of polysaccharides they are also known as **glycocalyx**.

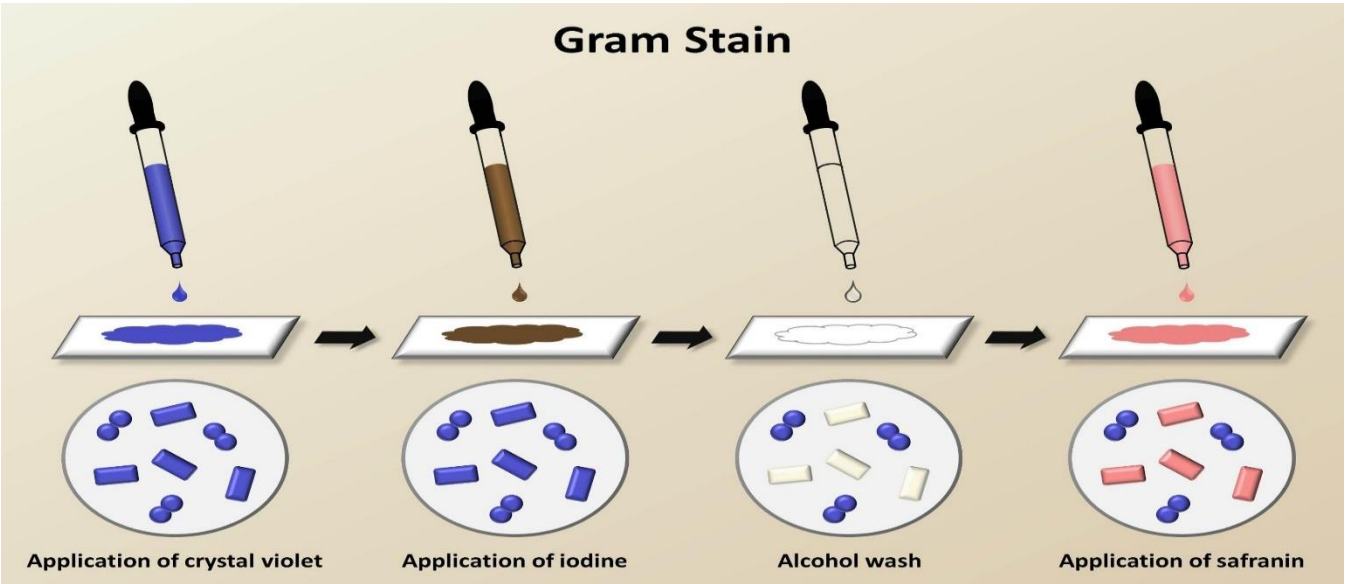
Staining prokaryotes

a. Gram staining:

- this way is simple, common in laboratories and depends on the structure of the cells wall. We use these steps to apply Gram stain:

1) Getting a sample of bacteria on a slide and heat it so that the bacteria stick to it.
2) Adding crystal violet (a purple dye which stains G+ and G- bacteria).
3) Adding iodine (to trap the dye inside the cells). All bacteria will be stained blue at this point in the procedure.
4) Adding alcohol for decolorization (it washes the sample, the G+ bacteria remain blue but G- ones become colorless).
5) Adding safranin (a counterstain makes G- bacteria pink, and G+ bacteria purple).

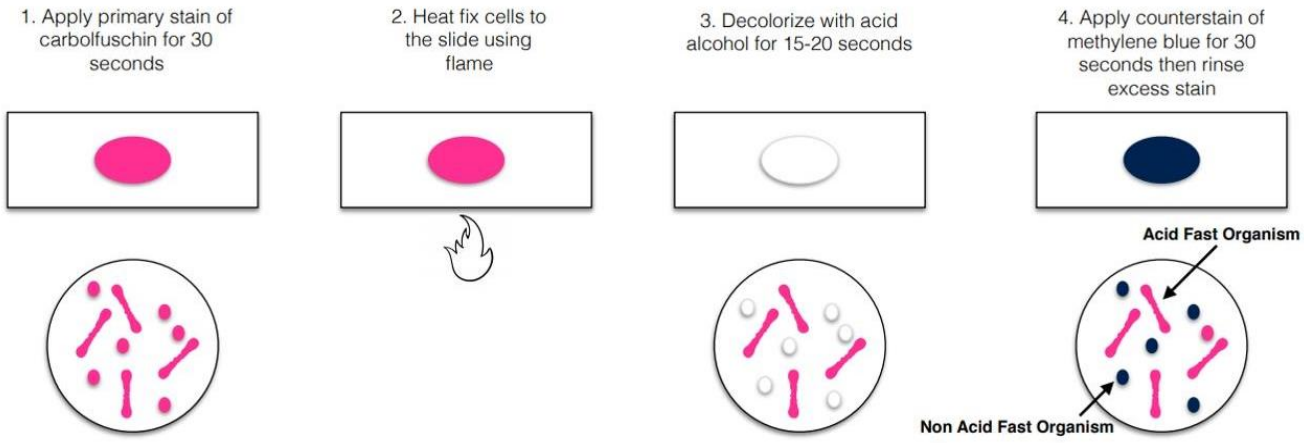
    - The cells with thick layer of peptidoglycans can't be decolorized (G+ bacteria).
    - Gram stain is not efficient for mycoplasmas since they don't have cell wall.



b. Acid-fast staining:

- Some bacteria are resistant to many harsh chemicals, including detergents and strong acids. After dying these bacteria can't be decolorized by diluted hydrochloric acid.
- Mycobacteria and some of the related actinomycetes are acid-fast prokaryotes.
- This name indicates that this type of bacteria is strong against acid decolorization.
- To apply this stain follow these steps:

1) Getting a sample of bacteria on a slide.
2) Applying a primary stain called carbofuschin for 30 seconds (all prokaryotes become red).
3) Heating the slide so that the bacteria stick to it.
4) Adding acid alcohol for 15-20 seconds (the acid-fast prokaryotes remain red).
5) Adding methylene blue (a counterstain that gives its color to the non-acid-fast bacteria. But the acid-fast bacteria remain red).



This is a diagram of the basic steps of a Ziehl-Neelsen (Acid Fast) staining procedure.

ACID-FAST STAIN