Chapter II: Bacterial cell

1. General information

The bacterial cell is a unicellular prokaryotic structure with a diversity of shapes and structures. Despite this apparent simplicity, bacterial cells are remarkably versatile and efficient, capable of performing all the vital functions necessary for their survival, growth, and reproduction.

2. Techniques for observing bacteria

The study of bacteria requires specific techniques and a structured process:

2.1. Bacterial culture

Bacterial growth can be achieved in the laboratory using culture media composed of nutrients that provide all the substances necessary for growth (carbon, energy, nitrogen, trace elements, and others).

2.2. Observation

2.2.1. Macroscopic observation (colony description)

Performed with the naked eye and involves describing and counting bacterial **colonies**. A proper description can only be made from well-isolated colonies. The description should include: size, shape, surface, opacity, consistency and pigmentation

2.2.2. Microscopic observation

It allows the characterization of morphology and certain cellular structures.

- **Direct Observation (wet mount microscopy)**: It is a microscopic examination of living microorganisms between a slide and coverslip, without any sample preparation. This main objective of this method is to determine whether or not a micro-organism is motile.
- **Observation with staining**: Bacteria are observed after being fixed on a slide (bacterial smear). Staining is classified into:
  - ✓ **Simple staining**: Uses a single dye to provide information about shape, size, and cellular arrangement.
  - ✓ **Differential staining**: Separates bacteria into distinct groups based on specific staining properties or uses specialized stains to observe particular cellular structures.

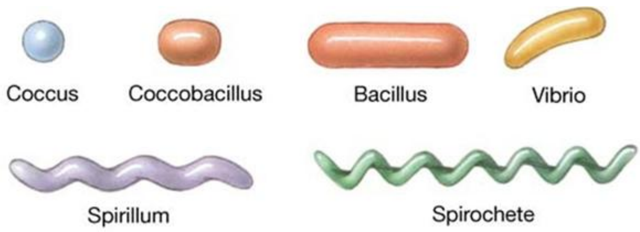
3. Cell morphology

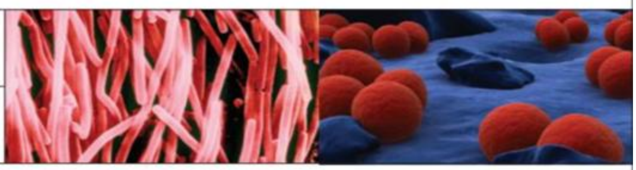
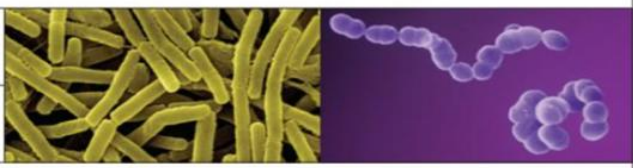
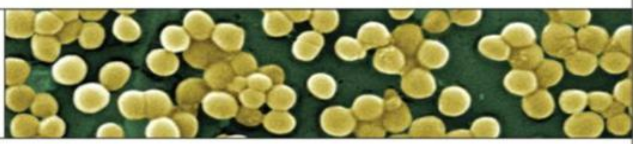
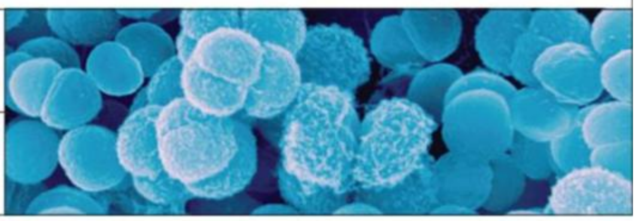
The term morphology refers to the shape of the bacterial cell itself, while arrangement describes the simple organization that bacterial cells form with one another.

3.1. Size: Generally, it ranges from a few micrometres to several hundred micrometres. On average, their size is between 1 and 10 µm.

3.2. Shape: There are numerous combinations of shapes and arrangements among bacteria. Bacteria of the same species exhibit uniform shape and arrangement.

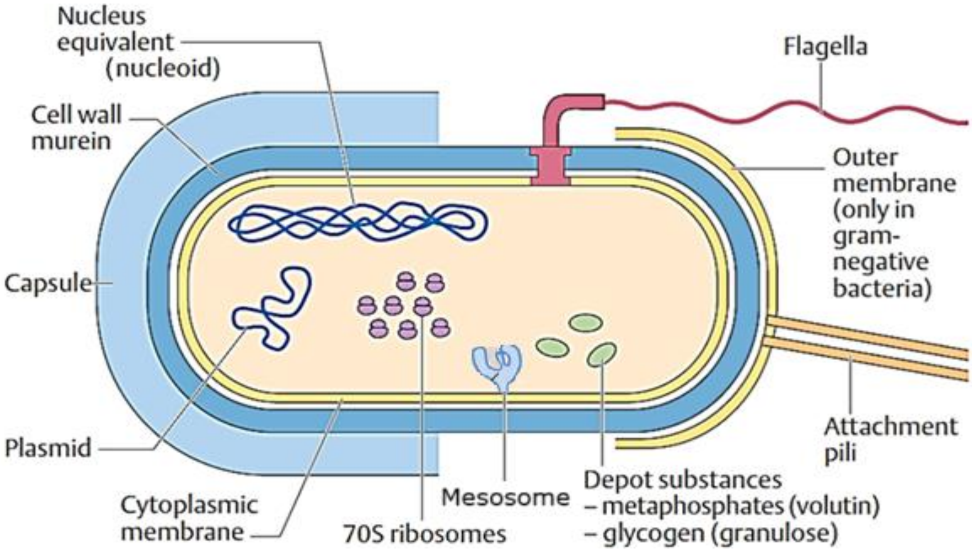
- **Coccus (pl. cocci) :** They are spherical bacteria that can present various arrangements: **single cells** (*Coccus*), **pairs** : (*Diplococci*), **chains** (*Streptococci*) or **clusters** (*Staphylococci*).
- **Bacillus (pl. bacilli)**: These are elongated, rod-shaped bacteria. They can form pairs (diplobacilli), chains (streptobacilli) or palisades (palisade bacilli).
- **Coccobacilli**: These are bacteria whose shape is intermediate between that of a coccus and that of a bacillus.
- **Spiral-shaped bacteria**
  - ✓ Vibrio-shaped bacteria have less than one complete curve or twist.
  - ✓ Helical-shaped bacteria have one or more complete turns. Spiral bacteria can also be classified according to their rigidity:
  - **Spirilla** (singular: *spirillum*) are rigid, spiral-shaped bacteria with a wavy appearance.
  - **Spirochetes** are thin, long, and highly flexible bacteria with a corkscrew-like shape
- **Pleomorphic bacteria:** Some bacteria can adopt different shapes depending on environmental conditions.



Common bacterial arrangements		
Arrangement is the simple organization that characterizes a group of bacteria of the same species. This arrangement is often dependent on the mode of replication of the bacteria.		
Paired arrangements (Diplo-)		
Diplobacillus		
Diplococcus		
Chain arrangements (Strepto-)		
Streptobacillus		
Streptococcus		
Cluster arrangements (Staphylo-)		
Staphylococcus		
Tetrad or cube arrangements		
Tetrad		
Sarcina		

3.3. Structural characteristics of bacteria

In bacteria, we distinguish between **basic** structures, present in all bacteria, and special (**optional**) structures that are characteristic of certain bacterial groups.



Schematic representation of the bacterial cell

The cell wall

The cell wall is a structure that surrounds the cell, characteristic of all bacterial species except for *Mycoplasma*. It acts as a true "exoskeleton," providing the cell with its shape and rigidity.

Chemical composition of the cell wall

Based on the chemical composition of the cell wall, bacteria can be classified into two major groups: Gram-positive bacteria and Gram-negative bacteria.

- **Amino sugars:** They are linked by glycosidic β(1-4) bonds to form peptidoglycan, which consists of two types:
  - NAG = N-acetylglucosamine;
  - NAM = N-acetylmuramic acid.
- **Amino acids**: Four common amino acids include D-alanine, L-alanine, glutamic acid, and lysine or diaminopimelic acid.
- **Teichoic acids**: These are incorporated into the peptidoglycan of Gram-positive bacteria.
- **Lipids**: These are almost absent in Gram positive bacteria. However, lipopolysaccharides are present in significant quantities in Gram negative bacteria.

Molecular structure of the cell wall

Under electron microscopy, a noticeable difference in the structure of Gram positive and Gram negative bacterial cell walls is observed.

- **The cell wall of Gram positive bacteria**

It is generally thicker (20 to 80 nm) and appears more homogeneous. It is primarily composed of peptidoglycan, which constitutes the major structural component.

Additionally, the wall contains polysaccharides, specifically teichoic acids and lipoteichoic acids, which span the peptidoglycan layer and are associated with the plasma membrane through bonds with glycolipids.

Teichoic Acids have many functions:

- ✓ Anchoring: They help attach the peptidoglycan layer to the underlying plasma membrane.
- ✓ Stabilization: They contribute to the stabilization of the cell wall structure.
- ✓ Teichoic acids bind Ca2+ and Mg2+ for their eventual transport into the cell.

Other molecules may also be associated with the cell wall:

- Polysaccharides
- Proteins

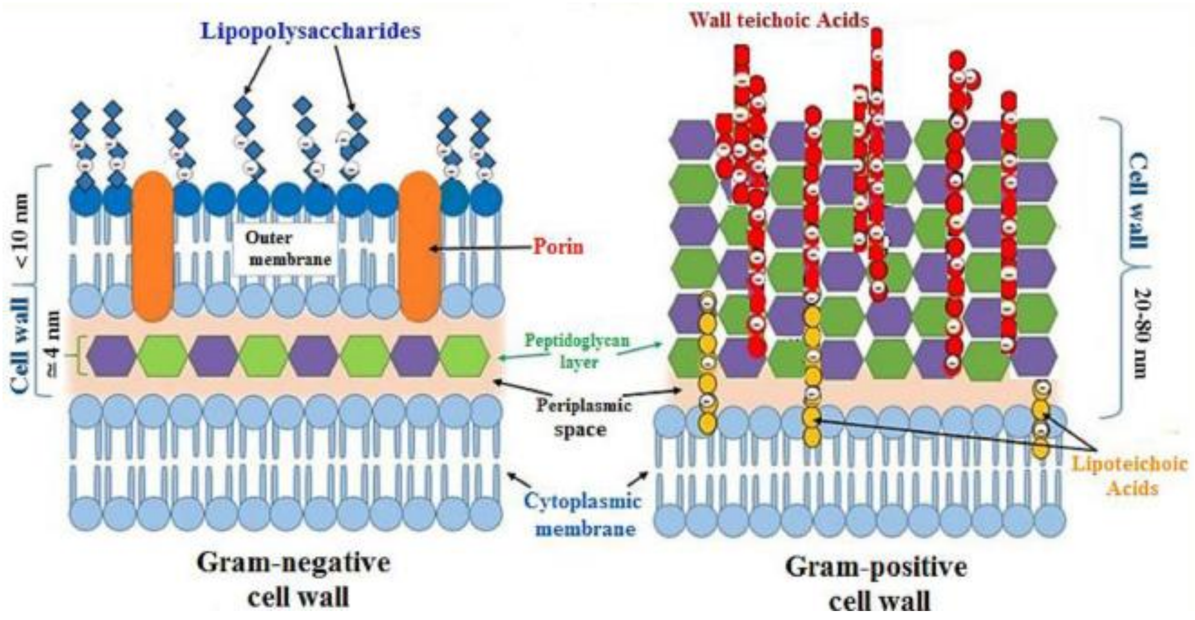
- **The cell wall of Gram-negative bacteria**

It is heterogeneous, complex, and thinner (6 to 15 nm) and consists of:

- The periplasmic space: Located between the outer membrane and the cytoplasmic membrane. It is composed of a gel-like matrix containing a wide range of proteins involved in various cellular functions.
- A thin layer of peptidoglycan, situated within the periplasmic space.
- An outer membrane composed of a phospholipid bilayer. The outer membrane contains:
- ✓ Porins: allow the passive diffusion of hydrophilic molecules
- ✓ Lipopolysaccharides (LPS) corresponding to the endotoxin of Gram-negative bacteria.

LPS is composed of three parts:

- ▪ The O-side chain: Determines antigenic specificity (O antigen).
- ▪ The core polysaccharide: Common to all Gram-negative bacteria.
- ▪ Lipid A: Responsible for the molecule's toxicity.

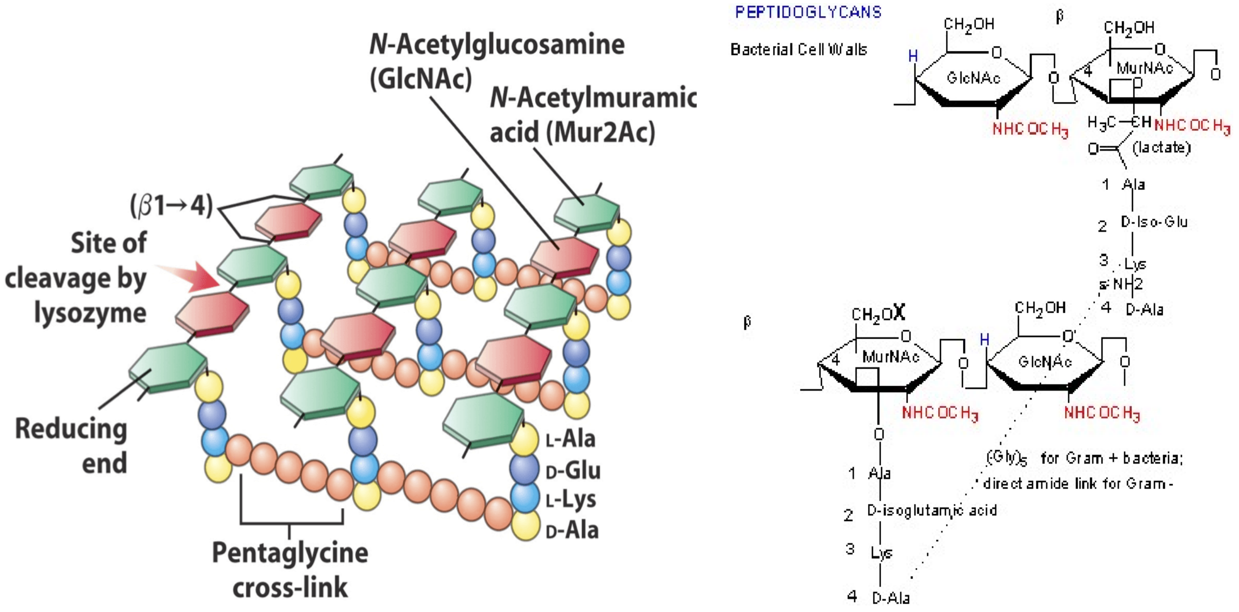


Peptidoglycan

It is the common constituent of all bacterial cell walls. It is a macromolecule composed of:

1. Parallel Polysaccharide chains: Each chain is an alternating polymer of **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)** linked by **beta-1,4** glycosidic bonds.
2. Tetra-peptide bridges: link the polysaccharide chains together. Liked to the NAM and consists of four amino acids: L-alanine, D-glutamic acid, L-lysine or diaminopimelic acid and D-alanine.
3. Interpeptide bridges

- In Gram negative bacteria, the diaminopimelic acid of one tetrapeptide is linked directly to the D-alanine of another.
- In Gram positive species, the chains are connected by a penta-peptide bridge



Structure and composition of Peptidoglycan

Functions of the bacterial cell wall

- Providing structural integrity and shape
- Essential for identification
- Formation of the division septum
- Antigenic properties: (LPS) of Gram-negative bacteria
- Hosting bacteriophage and antibiotic receptors
- Protection against external aggressions
- Maintaining osmotic pressure: To highlight this function, lysozyme is used. It cleaves the β (1-4) bonds between NAM and NAG. This results in:

✓ Complete destruction of the peptidoglycan in Gram-positive bacteria.

✓ Fragmentation of peptidoglycan in Gram-negative bacteria, as it is less accessible due to the outer membrane.

- Experiment with Gram-positive bacilli
  - When placed in a hypotonic medium, the bacteria behave normally.
  - If lysozyme is added to this suspension, the bacteria burst due to osmotic lysis.
  - Experiment in an isotonic medium: In the presence of lysozyme, the bacteria do not burst but instead take on a spherical shape, called a **protoplast**.
- Experiment with Gram-negative bacilli

In an isotonic medium supplemented with lysozyme and EDTA, the bacteria take on a spherical shape, called a **spheroplast** which can restore their cell wall, regain their original shape, and recover their protection against osmotic lysis.

Gram staining

Gram staining is a differential staining method developed by the Danish scientist Hans Christian Gram in 1884 to classify bacteria into two distinct categories: Gram-positive and Gram-negative. This classification is achieved by staining a bacterial smear as follows:

1. Staining the bacteria with Crystal Violet.
2. Adding a Lugol's solution (iodine-iodide solution, as a mordant).
3. Decolorizing with alcohol or an alcohol-acetone mixture.

After this step, some bacteria remain stained violet and are referred to as Gram-positive, while others lose their colour and are referred to as Gram-negative.

To better observe the decolorized bacteria, fuchsin (counterstain) is used. Gram-negative bacteria take on a pink coloration.

The cytoplasmic membrane

Structure and chemical composition

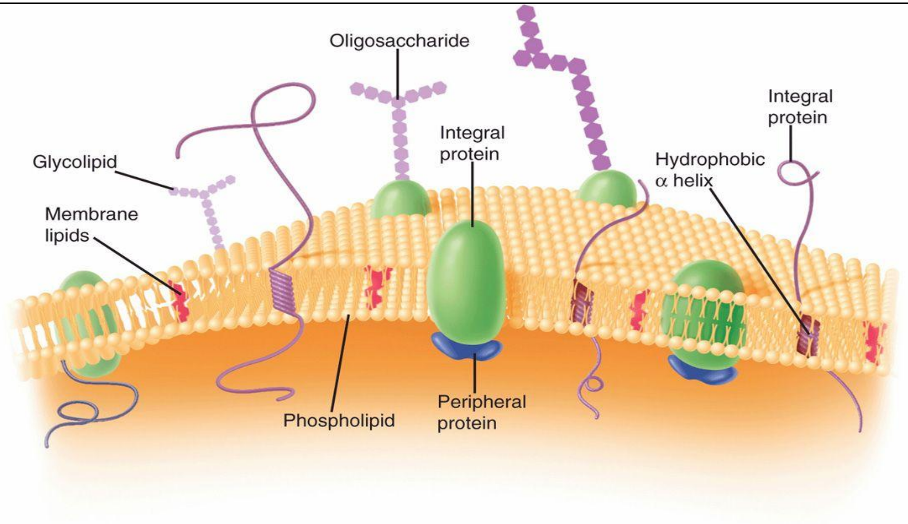
The bacterial plasma membrane shares the same basic structure as that of a eukaryotic cell. It is a thin structure, flexible and resilient, consisting of two dense layers surrounding a transparent inner layer.

Chemical analysis reveals three main components:

- **Lipids**: Approximately 30 to 40% of the membrane's molecular composition consists of lipids (phospholipids). These lipids have an asymmetric structure with two distinct ends: a hydrophilic and a hydrophobic end.

Unlike eukaryotes, prokaryotes lack sterols (such as cholesterol), which stabilize the structure of cytoplasmic membranes. This role is likely fulfilled by lipids called **hopanoids**.

- **Proteins**: Membrane proteins are the majority component of the membrane (60 to 70%). They are divided into two types: **peripheral proteins** and t**ransmembrane (integral) proteins**
- **Carbohydrates**: minor components associated with lipids (**glycolipids**) or proteins (**glycoproteins**).



Structure of the bacterial cytoplasmic membrane

Functions of the cytoplasmic membrane

1. Selective barrier
2. Transport (passive and active)
3. Respiratory function
4. Excretion of hydrolytic enzymes
5. It serves as a platform for enzymes and molecule transporters involved in the biosynthesis of DNA, cell wall polymers, and membrane lipids.
6. It acts as the attachment site for flagella and the initiation point for their movement.

Mesosomes

The cell membrane of some bacteria can form invaginations into the cytoplasm called **mesosomes**. They can be divided into two main types:

- **Septal mesosomes** involved in regulating cell division
- **Lateral mesosomes**: metabolic processes

The cytoplasm

The cytoplasm occupies the entire intracellular space and appears as a colloidal hydrogel, containing 70 to 80% water, along with organic and mineral substances, under considerable internal pressure

The structure of the bacterial cytoplasm is much simpler than that of eukaryotic cells, as it lacks distinct intracellular structures and does not contain mitochondria. It suspends the bacterial genetic material, a large amount of soluble RNA and, ribosomal or particulate RNA, along with a few optional inclusions.

Cytoplasm contains:

a) Ribosomes

In prokaryotic cells, ribosomes account for 90% of the total RNA content. Prokaryotic ribosomes have a sedimentation coefficient of 70S (ribosomal subunits: 30S and 50S).

Bacterial ribosomes are composed of approximately 33% ribosomal proteins and 67% ribosomal RNA (rRNA), including 16S rRNA, 23S rRNA, and 5S rRNA.

- The 30S subunit contains 16S rRNA
- The 50S subunit contains 23S rRNA and 5S rRNA

When cells are metabolically active, ribosomes can form **polysomes** which are chains of ribosomes bound to a single messenger RNA (mRNA) molecule.

b) Inclusion bodies

They serve as the storehouse of the cell, containing organic, inorganic substances, and energy. Their composition depending on the bacterial species and growth conditions. Among the most common are:

- Polyphosphate granules
- Glycogen granules
- Sulphur globules

c) Chromatophores

Chromatophores are vesicles found in photosynthetic bacteria, filled with pigments and surrounded by a membrane that may either be connected to or distinct from the plasma membrane. They play a role similar to that of chloroplasts in higher plants by enabling photosynthesis, although their structure differs.

d) Gas vacuoles

These gas-filled vesicles are found in three major groups of photosynthetic prokaryotes: cyanobacteria, purple bacteria, and green bacteria. They allow these aquatic microorganisms to float and ascend to the water surface for optimal light exposure.