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

Two types of observation are distinguished:

2.2.1. Macroscopic observation (colony description)

This is performed with the naked eye and involves describing and counting bacterial colonies. The appearance of colonies depends on the medium, duration, and incubation temperature.

A proper description can only be made from well-isolated colonies. The description should include the following elements:

- **Size**: punctiform, small, medium, or large
- **Shape**: raised, convex, flat, umbilicated, umbonate
- **Surface appearance**: smooth, rough
- **Opacity**: opaque, translucent, transparent
- **Consistency**: greasy, creamy, dry, mucoid
- **Pigmentation** : color

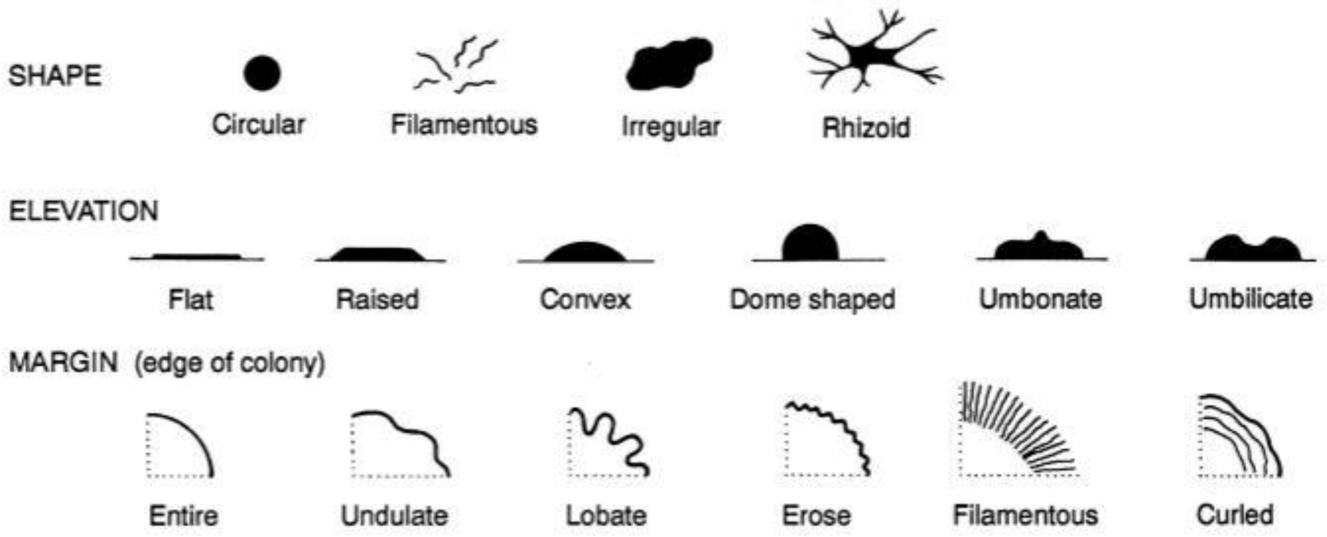


Figure 1: Colony morphology of bacteria

2.2.2. Microscopic observation

Given their size (on the order of microns), bacterial cells are observed under a microscope, either without staining or with staining. Microscopic observation 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) and subjected to one or more stains. Staining, performed on dried and fixed smears, is classified into:
  - ✓ **Simple staining**: Uses a single dye (e.g., methylene blue) to provide information about shape, size, and cellular arrangement.
  - ✓ **Differential staining**: Separates bacteria into distinct groups based on specific staining properties (e.g., Gram staining, Ziehl-Neelsen staining) or uses specialized stains to observe particular cellular structures (e.g., endospore staining with malachite green).

These observations are primarily conducted using optical microscopy. However, due to its resolution limits, revealing elements as small as 5 to 10 nm requires the use of electron microscopy, which exploits the ability of different structures to either retain or allow the passage of an electron beam.

3. Cell morphology

Bacteria are unicellular living organisms, and their structure continues to be a subject of research. Despite their small size, bacterial cells exhibit remarkable complexity, comprising several components and organelles that enable them to perform essential functions.

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. Bacterial cell morphology can vary significantly between species.

It is important to know the morphological structure of bacteria and microbes, as it provides us with a better understanding of microbial physiology, pathogenic mechanisms, antigenic features, and allows us to identify them by species. In addition, knowledge of microbial morphology can be helpful in diagnosing disease and in preventing microbial infections.

3.1. Size

Bacteria are among the smallest autonomous living organisms, capable of self-sustaining life functions. As many species of bacteria exist, they vary widely in size. Generally, it ranges from a few micrometres to several hundred micrometres. On average, their size is between 1 and 10 µm.

- The smallest bacteria, such as *Chlamydia*, measure around 0.2 µm.
- The largest bacteria, including some spirochetes, can reach lengths of up to 250 µm.

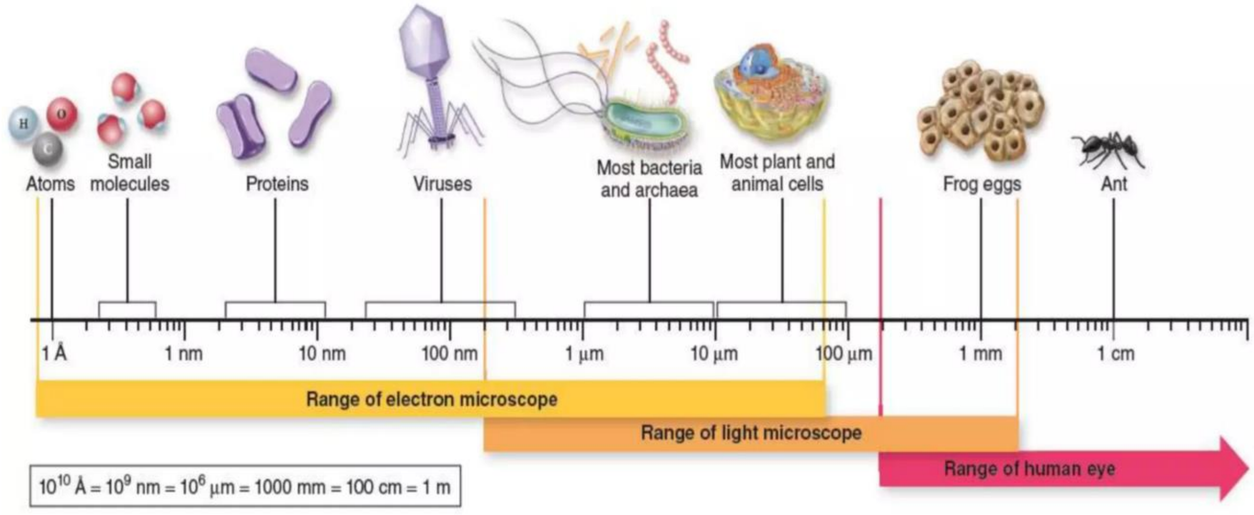


Figure 2: Relative sizes of bacteria

3.2. Shape

The name "bacteria" derives from the Greek word *bakteria*, meaning "little rods," but bacterial shapes are complex and highly diverse. There are numerous combinations of shapes and arrangements among bacteria. Typically, bacteria of the same species exhibit uniform shape and arrangement.

Bacteria tend to display the most representative cell morphologies, with the most common examples listed here:

- **Coccus (pl. cocci)**

They are spherical bacteria that can present various arrangements:

  - **Single cells**: *Coccus*
  - **Pairs** : *Diplococci*
  - **Chains**: *Streptococci*
  - **Clusters** (grape-like): *Staphylococci*

- **Bacillus (pl. bacilli)**: These are elongated, rod-shaped bacteria. They can form pairs (diplobacilli), chains (streptobacilli) or palisades (palisade bacilli). Examples include *E. coli*, *Salmonella*, *Bacillus*, etc.
- **Coccobacilli**: These are bacteria whose shape is intermediate between that of a coccus (spherical) and that of a bacillus (elongated). Examples: *Yersinia, Pasteurella, Brucella, Acinetobacter, Moraxella.*
- **Spirilla /spirochetes:** These are bacteria with a spiral or corkscrew shape. They are generally larger than cocci or bacilli and can be rigid or flexible. Some spirilla may also have a helical shape.

Spiral-shaped bacteria exhibit one or more curves; they are never straight.

✓ Those with a curved rod shape resembling a comma are called vibrio (*Vibrio cholerae*).
✓ Others, known as spirilla (*Campylobacter jejuni*), have a helical shape and a relatively rigid body.
✓ A third group is characterized by a longer, flexible helical shape; these are the spirochetes (*Treponema pallidum*).

- **Pleomorphic bacteria:** Some bacteria can adopt different shapes depending on environmental conditions. They can transition from a spherical shape to an elongated form in response to stress factors or changes in their surroundings.

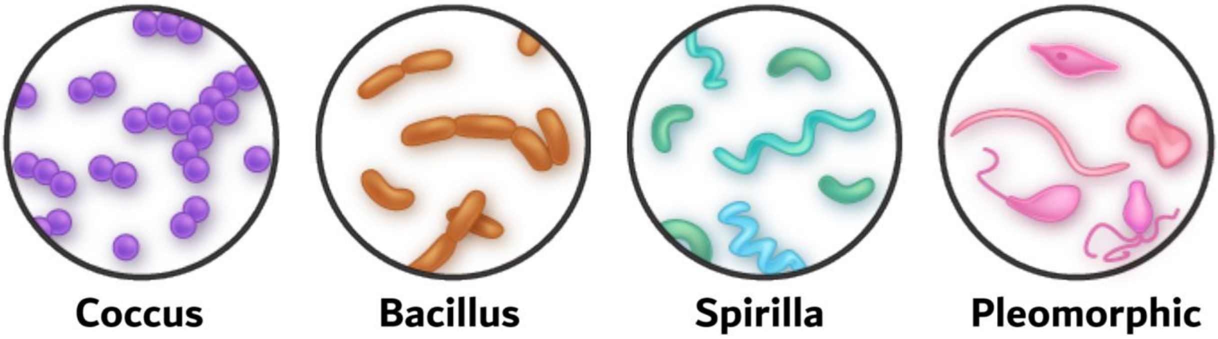
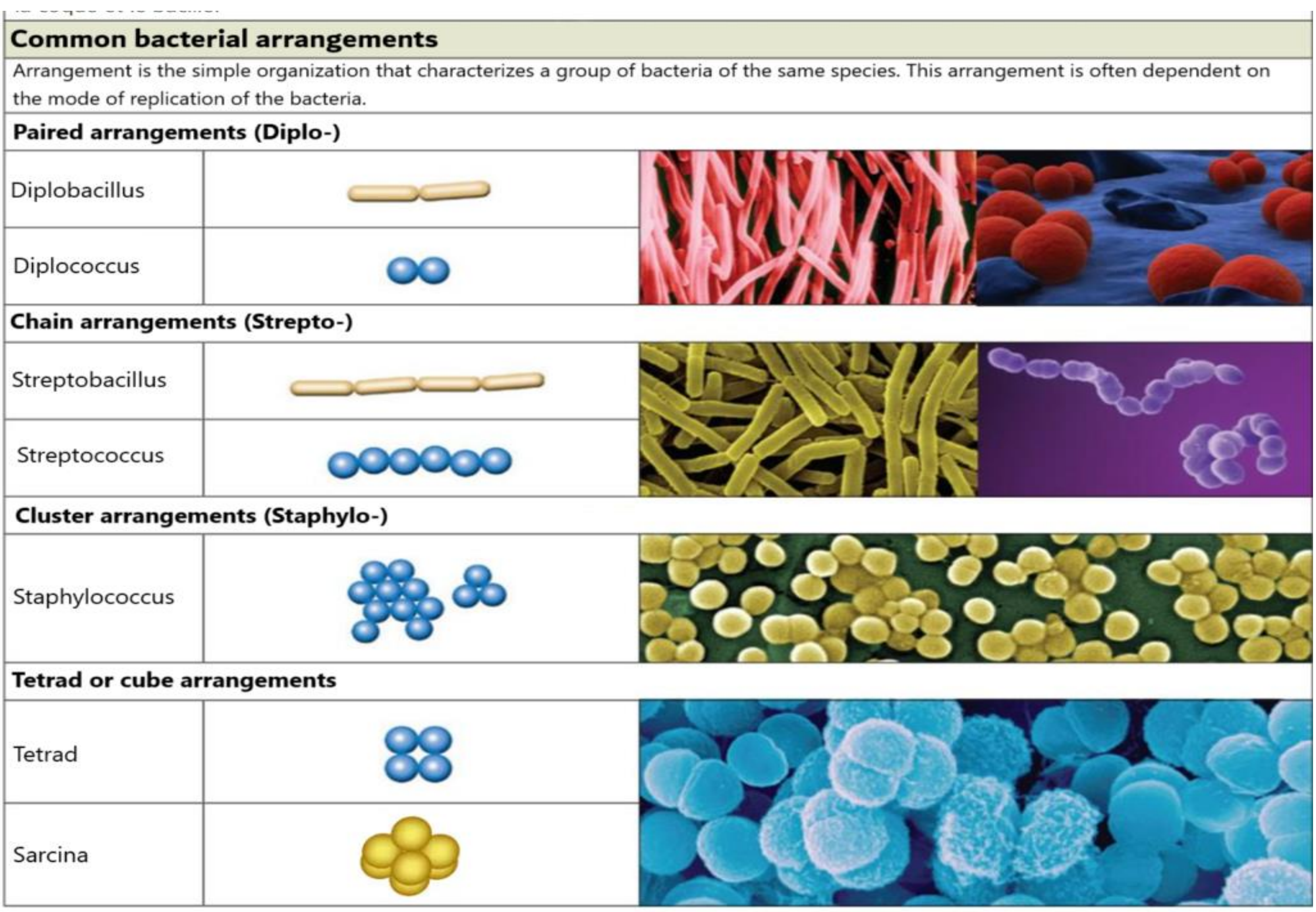


Figure 3: Common bacterial shapes



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		

Figure 4: Common bacterial arrangements

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.

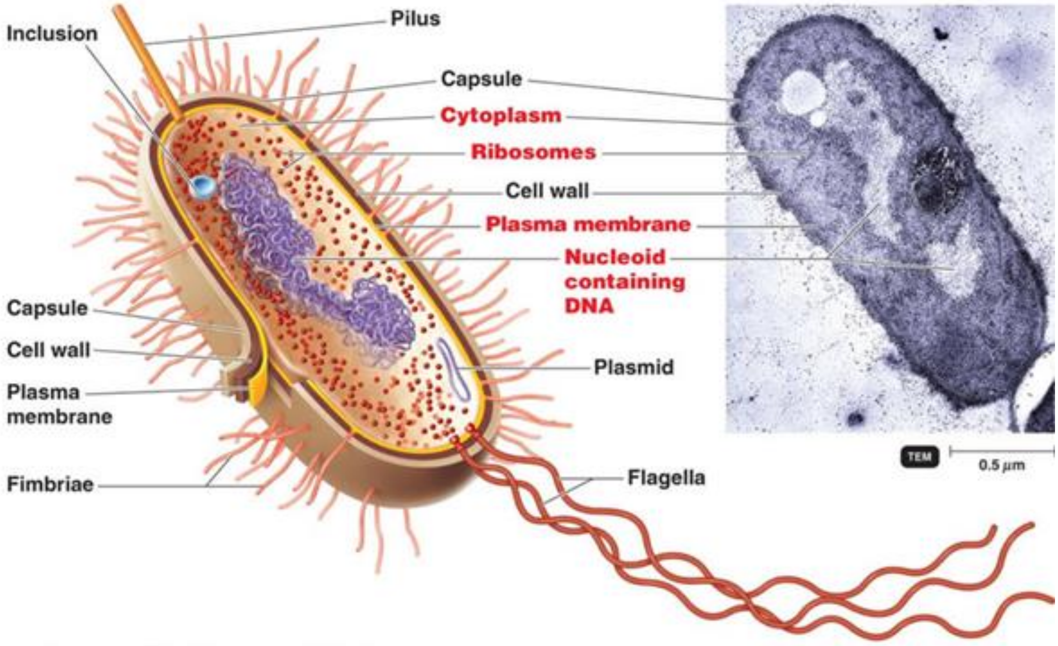


Figure 4: The Fine structure of bacteria

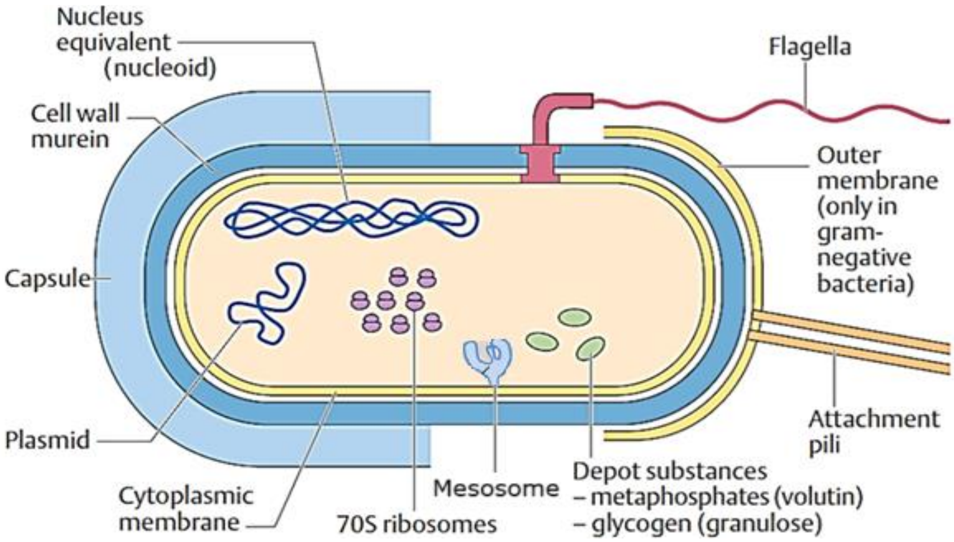


Figure 5 : Schematic representation of the bacterial cell

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. This rigid consistency is due to the presence of a layer composed of **peptidoglycan** (also called murein), which is a heteropolymer of protein and sugar molecules that gives the cell its shape and structural support.

- **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. The Gram staining method easily highlights these differences.

- **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.

- **Simple sugars:** They are numerous, including glucose, galactose, and mannose.
- **Amino acids**: Four common amino acids include D-alanine, L-alanine, glutamic acid, and lysine or diaminopimelic acid (a structure analogous to lysine but with a -COOH group on the last carbon).
- **Teichoic acids**: These are incorporated into the peptidoglycan of Gram-positive bacteria via carbon 6 of NAM. Teichoic acids are composed of phosphates associated with either glycerol or ribitol. They can be categorized as: Polyglycerol phosphate and Polyribitol phosphate.
- **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.

Other molecules may also be associated with the cell wall:

- **Polysaccharides**
- **Proteins**

These additional components enhance the functionality and interactions of the bacterial cell wall, influencing its structural integrity and interaction with the host immune system.

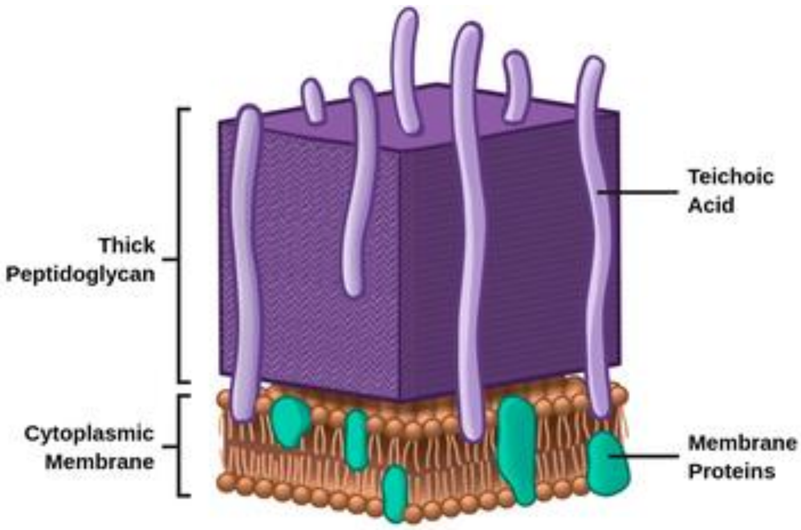


Figure 6: Structure of the Gram-Positive bacterial cell wall

- **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** made up of a phospholipid bilayer. It protects the bacterium and acts as a barrier against certain antibiotics. Within the outer membrane, we find:

✓ **Porins**: Proteins that span the outer membrane of Gram-negative bacteria and form pores, allowing the passive diffusion of hydrophilic molecules between the bacterium and its external environment.

✓ **Lipopolysaccharides (LPS)**: Major components of the outer membrane of Gram-negative bacteria, corresponding to the endotoxin of Gram-negative bacteria.

LPS is composed of three parts:

- **The O-side chain**: Contains many repeating sugar units and determines antigenic specificity (O antigen).
- **The core polysaccharide**: Common to all Gram-negative bacteria.
- **Lipid A**: Embedded in the outer membrane and responsible for the molecule's toxicity.

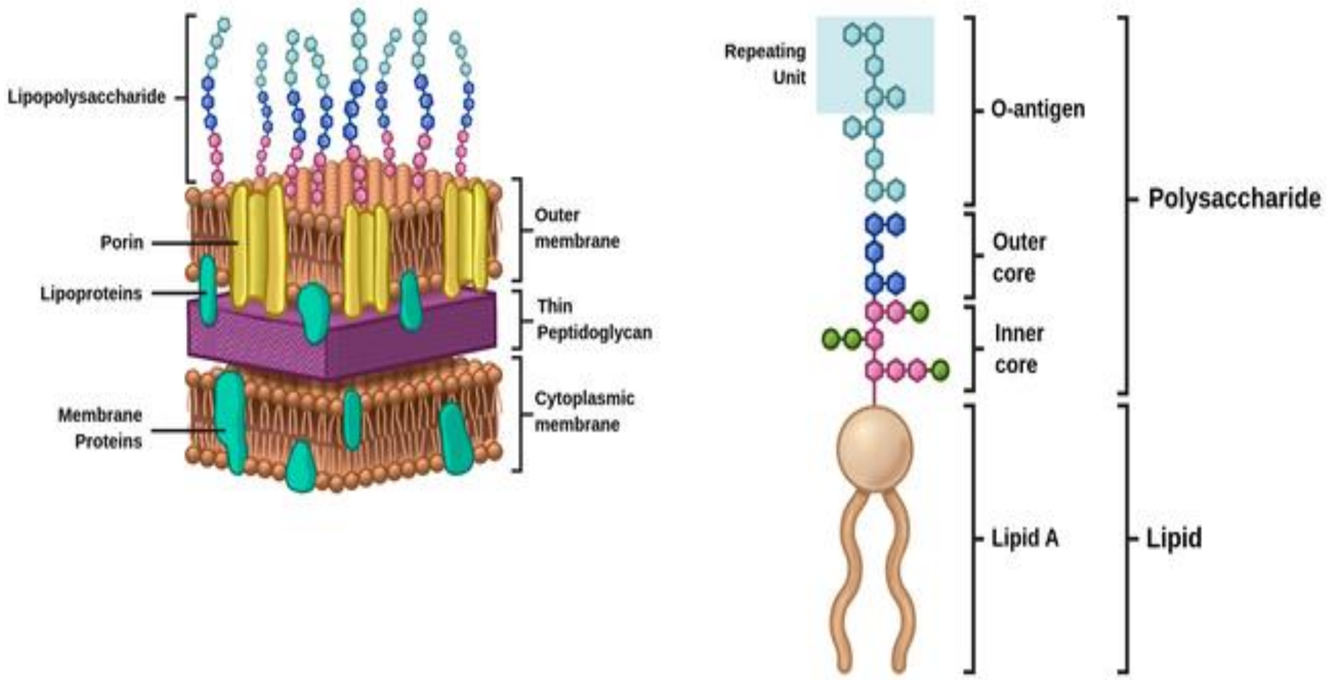


Figure 7: Structure of the Gram-Negative bacterial cell wall

Peptidoglycan

The common constituent of all eubacterial cell walls is peptidoglycan, which is a macromolecule composed of:

1. <u>Parallel Polysaccharide chains:</u> Each chain is an alternating polymer of **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)** linked by **beta-1,4** glycosidic bonds.

NAG is an acetylated glucose molecule, while NAM is an acetylated glucose molecule bound to a lactic acid moiety.

2. <u>Tetrapeptide bridges</u>: These bridges link the polysaccharide chains together. Each tetrapeptide substitutes the carboxyl group of NAM and consists of four amino acids, which can include:
    - L-alanine,
    - D-glutamic acid,
    - L-lysine or diaminopimelic acid (a derivative of lysine),
    - D-alanine.
3. <u>Interpeptide bridges</u>

- 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 not directly linked but are connected by a peptide chain, such as the pentaglycine bridge in *Staphylococcus aureus*. These bridges connect the tetrapeptides of different polysaccharide chains, ensuring the cohesion of the peptidoglycan network.

The entire structure forms a three-dimensional (3D) network that envelops the bacterial cell.

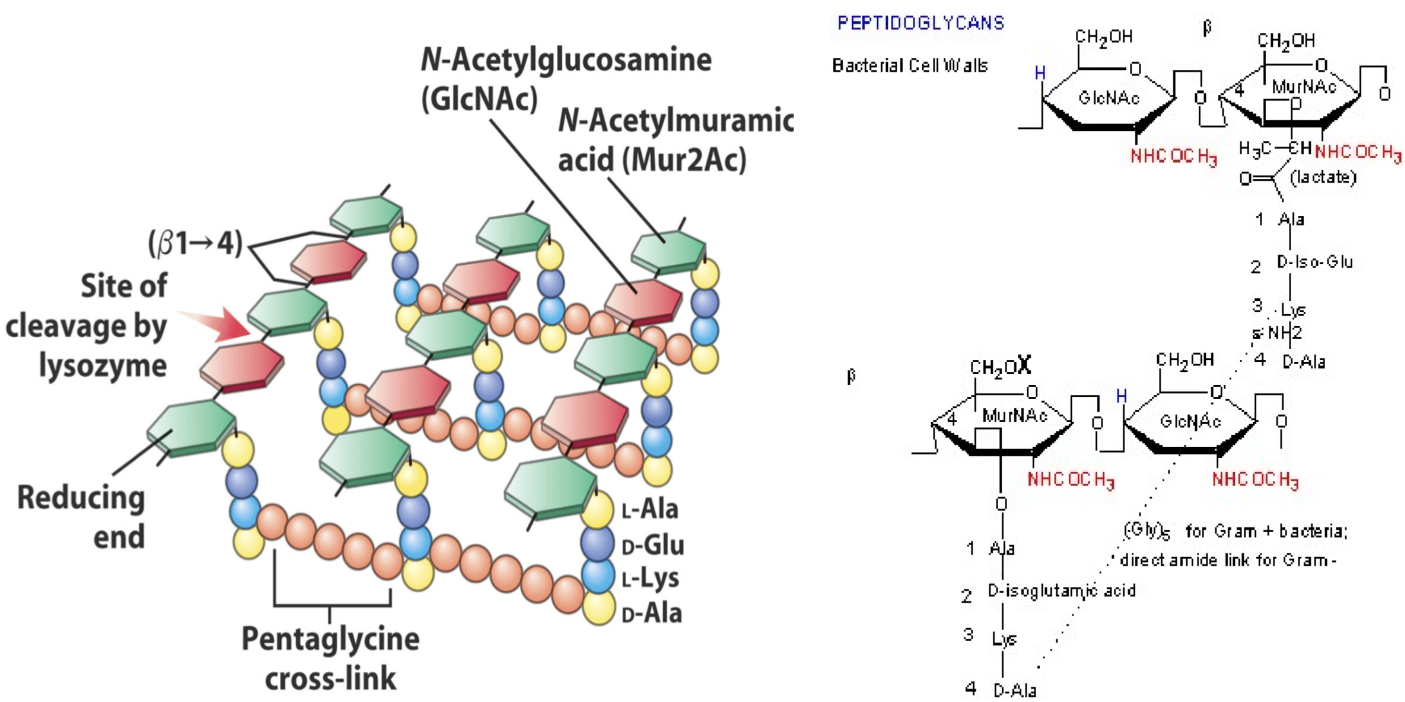


Figure 8: Structure and composition of Peptidoglycan

Functions of the bacterial cell wall

The bacterial cell wall is a dynamic structure that plays a basic role in the survival and functionality of bacteria. Its key roles include:

- Providing structural integrity and shape: The wall defines the shape of bacteria and ensures their mechanical strength.
- Essential for identification: It is a critical determinant in differentiating between Gram-positive and Gram-negative bacteria.
- Formation of the division septum: The cell wall is integral to the formation of the septum during bacterial cell division.
- Antigenic properties: The cell wall contains antigenic structures, such as O-antigen in the lipopolysaccharides (LPS) of Gram-negative bacteria, which can trigger immune responses.

- Hosting bacteriophage and antibiotic receptors:
  - The wall carries receptors for bacteriophages (important in phage typing).
  - It contains targets for antibiotics, such as penicillin, which disrupts peptidoglycan assembly, leading to bacterial lysis.
- Protection against external aggressions: The wall protects the bacterium from environmental stress and chemical threats.
- Maintaining osmotic pressure: It allows the bacterium to withstand high internal **osmotic pressure**, preserving its shape and preventing lysis under hypotonic conditions.

To highlight this function, lysozyme can be used. Lysozyme is a substance commonly found in biological fluids such as secretions (tears, saliva, etc.) or in the cytoplasm of phagocytic cells. It cleaves the β (1-4) **bonds** between NAM and NAG in peptidoglycan. 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 *Bacillus subtilis* (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 (sucrose): In the presence of lysozyme, the bacteria do not burst but instead take on a spherical shape, called a **protoplast**.

Protoplasts lack the following properties:

- ✓ Antigenic properties of the original bacteria.
- ✓ Ability to divide.
- ✓ Ability to attach bacteriophages.
- ✓ Motility.

- Experiment with *Escherichia coli* (Gram-negative bacilli)

In an isotonic medium supplemented with lysozyme and EDTA (to weaken the outer membrane), the bacteria take on a spherical shape, called a **spheroplast**.

Spheroplasts retain the initial properties of the bacteria: They 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 after treatment with alcohol. Gram-positive bacteria retain their violet coloration, whereas Gram-negative bacteria take on a pink coloration.

This indicates structural and/or chemical differences between these two types of bacteria:

- **Gram-negative bacteria** have walls with a high lipid content and a thin layer of peptidoglycan.

The alcohol in the decolorizing agent extracts the lipids, making the cell wall of Gram-negative bacteria more porous and unable to retain the violet-Lugol complex, resulting in decolorization.

- **Gram-positive bacteria** have a thicker peptidoglycan layer and a higher degree of cross-linking, which traps the violet-Lugol complex more effectively, making the Gram-positive cell wall less sensitive to decolorization.

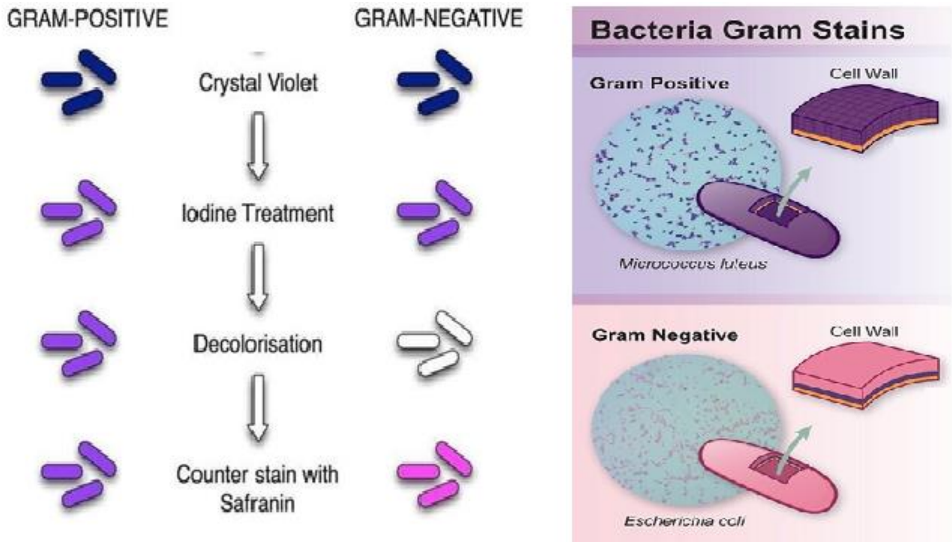


Figure 9: Gram staining method

The cytoplasmic membrane

Also referred to as the **inner membrane**, it is in direct contact with the cytoplasm, which it continuously surrounds. It extends beneath the cell wall in both Gram-positive and Gram-negative bacteria.

Structure and chemical composition

The bacterial plasma membrane shares the same basic structure as that of a eukaryotic cell (a phospholipid bilayer).

It is a thin structure, approximately 8 nm thick, both flexible and resilient, consisting of two dense layers surrounding a transparent inner layer (a double-layered structure).

Chemical analysis reveals three main components:

- **Lipids**: Approximately 30 to 40% of the membrane's molecular composition consists of lipids (phospholipids), which form the lipid bilayer. These lipids have an asymmetric structure with two distinct ends: a polar (hydrophilic) end that interacts with water and a non-polar (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**: These are loosely associated with the membrane through weak bonds and have a polar character (water-soluble).
    - **Transmembrane (integral) proteins**: These exhibit an amphipathic character, meaning they have both hydrophilic and hydrophobic regions.

- **Carbohydrates**: These are quantitatively minor components and are often associated with lipids (forming **glycolipids**) or proteins (forming **glycoproteins**).

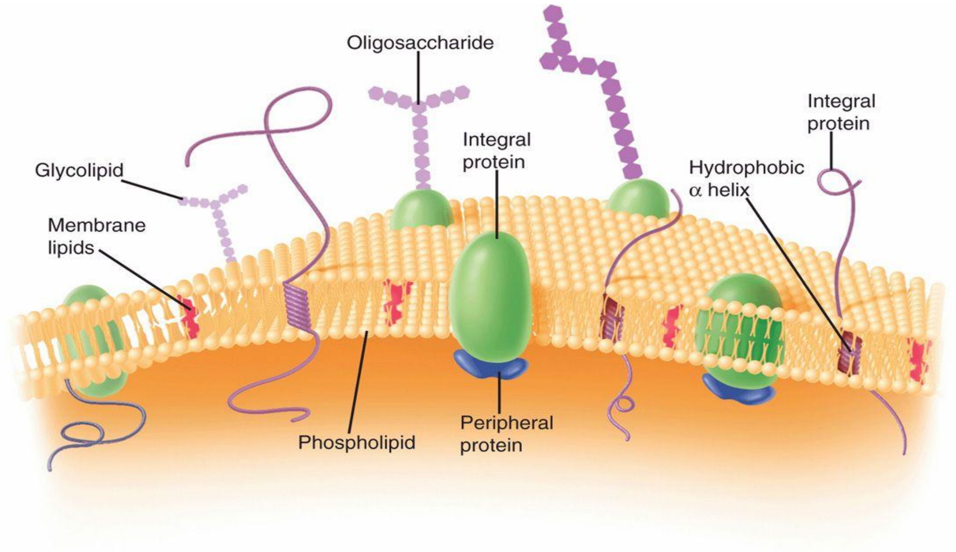


Figure 10: Structure of the bacterial cytoplasmic membrane

Functions of the cytoplasmic membrane

The bacterial cytoplasmic membrane performs several essential roles:

1. **Selective barrier**: It separates the cytoplasm from the external environment, acting as a permeable and selective barrier. It allows the passage of lipophilic molecules while preventing the passage of hydrophilic ones.
2. **Active transport**: The membrane houses specific systems for active transport, which require energy (usually provided in the form of ATP). These systems facilitate the movement of various molecules, including ions and sugars, in both directions.
3. **Respiratory Function**: In aerobic bacterial species, the cytoplasmic membrane plays a role equivalent to that of mitochondria in eukaryotic cells. It is involved in electron transport and oxidative phosphorylation.
4. **Excretion of hydrolytic enzymes**, which break down polymers into subunits small enough to cross the cytoplasmic membrane and be imported into the bacterium.
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**. These structures, which appear as vesicular, tubular, or lamellar formations, are associated with specific cellular functions.

Mesosomes can be divided into two main types:

- **Septal mesosomes** are involved in regulating cell division, particularly in DNA segregation and septum formation.
- **Lateral mesosomes**, are linked to metabolic processes such as electron transport and redox reactions.

While their exact roles remain debated, mesosomes are thought to play a part in organizing key cellular activities.

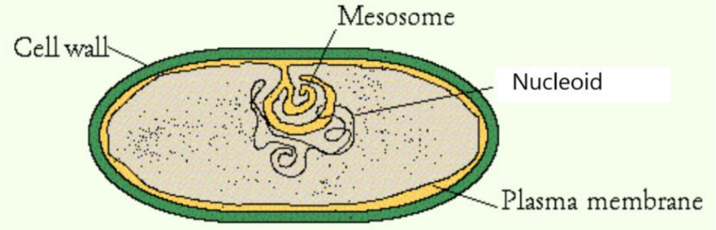


Figure 11: Mesosom

The cytoplasm

The cytoplasm is a constant component present in all bacteria. It 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 (5 to 20 atmospheres).

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 (chromosome and plasmid), a large amount of soluble RNA (messenger RNA and transfer RNA), and, most importantly, ribosomal or particulate RNA, along with a few optional inclusions.

Cytoplasm contains:

a) Ribosomes

All cellular life synthesizes proteins, and organisms in all three domains of life possess ribosomes, structures responsible for protein synthesis. However, ribosomes in each of the three domains are structurally different.

Each bacterium contains approximately 15,000 ribosomes, making them the most abundant and essential organelles in the granular cytoplasm. In prokaryotic cells, ribosomes account for 90% of the total RNA content.

Prokaryotic ribosomes have a sedimentation coefficient of 70S, which is slightly lower than that of eukaryotic ribosomes (80S). This difference is due to the smaller size of prokaryotic ribosomal subunits (30S and 50S) compared to eukaryotic ones (40S and 60S).

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 and is the target of aminoglycosides and tetracyclines.
- The 50S subunit contains 23S rRNA and 5S rRNA and is the target of macrolides and related antibiotics.

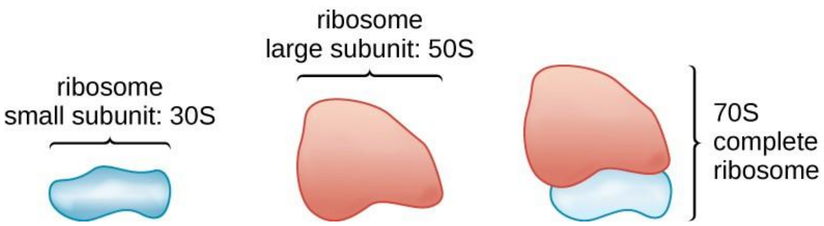


Figure 12: Bacterial ribosome

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

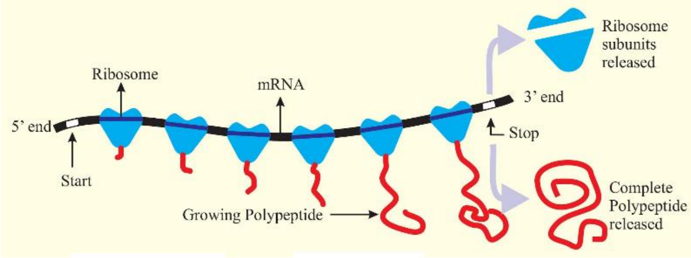


Figure 13: The polysome

b) Inclusion bodies

They serve as the storehouse of the cell, containing organic, inorganic substances, and energy. They can appear in various forms such as globules, crystals, or granules. These structures are visible microscopically in the cytoplasm, with their composition depending on the bacterial species and growth conditions. Among the most common are:

- **Polyphosphate granules**: Provides phosphate for nucleic acid synthesis.
- **Glycogen granules**, which is a polymer of glucose and is found in many intestinal bacteria.
- **Sulphur globules**: Supply reduced sulphur for energy-conserving metabolic processes.

c) Chromatophores

In photosynthetic bacteria, chromatophores vesicles filled with bacterial pigments and are surrounded by a membrane, which can be either a unitary membrane connected to the plasma membrane or a non-unitary membrane distinct from the plasma membrane. They have a similar function to chloroplasts in higher plants, as they both facilitate photosynthesis. However, their structure differs significantly. These chromatophores contain various photosynthetic pigments, such as bacteriochlorophylls, which enable the conversion of light energy into chemical energy. Other pigments include bacteriorhodopsin, Vitamin K2, carotenoids, phycocyanin, and xanthophylls.

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.