Chapter 2: Bacterial Cell

Bacteria are the smallest known organisms, endowed with metabolism and capable of growing and dividing at the expense of nutrients. Their diameter is usually around 1 µm. The bacterial cell is surrounded by a rigid envelope called the cell wall, which gives it its shape and resistance. This wall encloses a second, much thinner and more delicate envelope, the cytoplasmic membrane. The cytoplasm is generally very homogeneous, containing mainly ribonucleic acid granules called ribosomes, and sometimes also reserve substances that give it a coarser structure. It does not contain any of the organelles described in eukaryotic cells (endoplasmic reticulum, mitochondria, etc.).

In the cytoplasm, the nuclear apparatus is distinguished by its fibrillar, finely reticulated appearance and is not enclosed within a membrane. The cell wall, the membrane, the cytoplasm, and the nuclear apparatus represent the essential structures of the cell, and they are always present. Other organelles may occasionally be present: the capsule, an external envelope that can develop considerably; flagella, protein structures that confer motility to the bacterium; and finally, pili or fimbriae, which are thinner than flagella, rigid, and brittle. Some are called sex pili and play a role in bacterial conjugation **(Fig. 1)**.

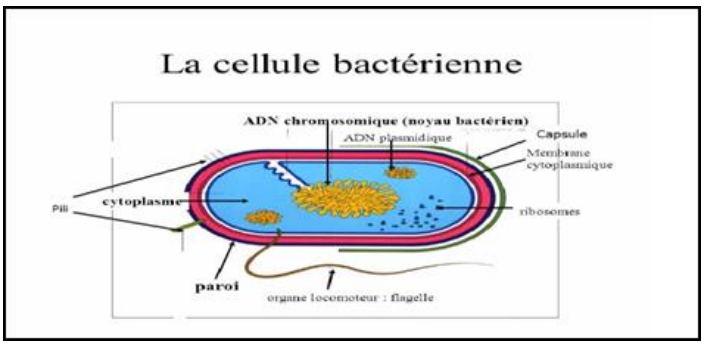


Figure 1: The bacterial cell.

1- Main Bacterial Shapes:

When observing bacteria under an optical microscope from a pathological sample or a culture medium, the shape of the cells, their dimensions, and the arrangements or groupings they form can be quickly recognized. The dimensions of bacteria vary by species, and their shapes are extremely diverse. We will focus on three main shapes: spherical or coccoid, bacillary or cylindrical, and spiral or helical **(Fig. 2)**.

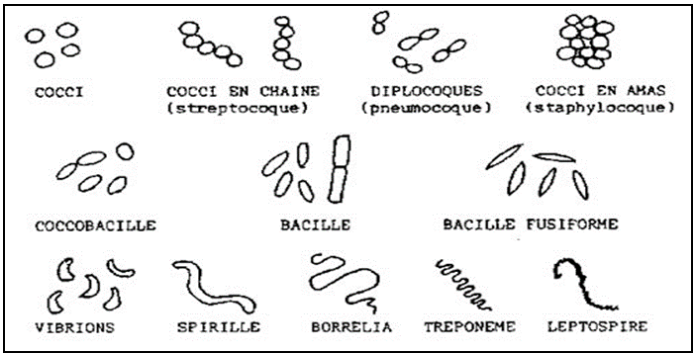


Figure 2: Bacterial shapes.

2- Cell Morphology

Certain structures are present in all bacteria; these are the "constant" elements. Others are found only in some bacteria: these are the "inconstant" or "facultative" elements.

Constant Elements → **Cell Wall - Plasma Membrane - Periplasm - Cytoplasm - Chromosome - Ribosomes - Polysomes**

Inconstant Elements → **Capsule - Pili and Fimbriae - Flagellum - Mesosome - Plasmid - Gas Vacuole - Storage Inclusions - Spore**

2.1- The Cell Wall

Despite the high osmotic pressure (5 to 20 atmospheres) inside the bacterial cytoplasm, the bacterium does not burst due to the presence of a rigid structure called the cell wall, which is polymeric in nature. The polymers and their bonding vary among bacterial species. However, a basic substance, *specific to bacteria*, is universally present: this is murein, also called peptidoglycan. It plays an important role in cell division and is the basis for distinguishing between Gram-positive and Gram-negative bacteria.

2.1.1- Chemical Composition :

-In Gram-positive bacteria:

The cell wall is thick, ranging from 20 to 80 nm, and appears homogeneous under an electron microscope. The main structural element is peptidoglycan, which is a glycosaminopeptide consisting of a molecule of N-acetylglucosamine and a molecule of N-acetylmuramic acid, linked by a β-glycosidic bond. The muramic acid is also associated with a short peptide chain of four amino acids called a tetrapeptide: two alanines, one glutamic acid, and one lysine.

-In Gram-negative bacteria:

The cell wall is much thinner (10 to 15 nm) and has a more complex stratified structure. In addition to the basic peptidoglycan, it includes three other polymeric structures external to or linked to the peptidoglycan:

- A phospholipid layer called the outer membrane, to differentiate it from the cytoplasmic membrane, which contains important proteins.
- A lipopolysaccharide (LPS)
- A lipoprotein that links the outer membrane to the peptidoglycan, providing some rigidity to the structure.

The bacterial cell wall carries a large number of antigens specific to each type of bacteria, called wall antigens or O antigens.

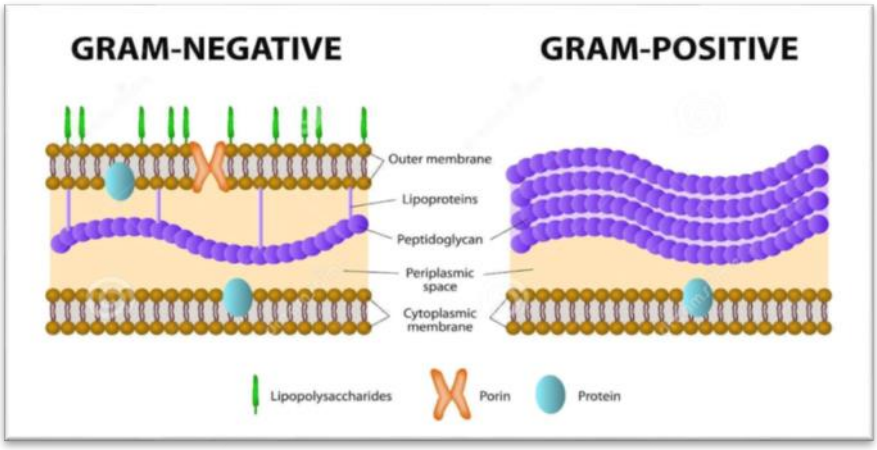


Figure 4: Molecular structure of the cell wall.

2.1.3- Functions of the Cell Wall

- The cell wall gives the bacterium its true morphology. It constitutes the external skeleton of the bacterium and represents 25 to 35% of the total weight of the bacterium.
- The cell wall contains the internal osmotic pressure. Without a cell wall, bacteria take on a spherical shape called *protoplast* if it is a Gram-positive bacterium, or *spheroplast* if it is a Gram-negative bacterium. Bacteria can survive without a cell wall and even multiply (they are then called L-forms) provided they are placed in a medium where the osmotic pressure is balanced with the internal osmotic pressure of the bacterium.
- It plays a decisive role in Gram staining. In Gram-positive bacteria, the cell wall blocks the extraction of gentian violet and iodine by alcohol, whereas it does not block this extraction in Gram-negative bacteria.
- It plays a decisive role in the antigenic specificity of bacteria.
- It is the site of action of certain exogenous enzymes (lysozyme) or endogenous enzymes (autolysins) and certain antibiotics, particularly β-lactams (penicillins), which inhibit the synthesis of peptidoglycan.
- Lipopolysaccharide (LPS) and peptidoglycan can activate the complement via the alternative pathway, releasing, among others, the C3a and C5a fractions (chemotactic effect) and C3b (opsonizing effect by phagocyte receptors for C3b), which play an important role in non-specific defense against infection.

2.1.4- Gram Staining

The differences in the constitution and chemical structure of Gram (+) and Gram (-) cell walls allow the establishment of the principle of **Gram staining**, developed by Christian GRAM (1884).

2.1.4.1- Gram Staining Procedure

After fixing the smear, it is stained with **gentian violet**. It is then rinsed with water. A fixative, **Lugol's iodine**, is added. It is rinsed with distilled water. A decolorization step is then performed using a mixture of alcohol and acetone. The latter penetrates Gram-negative bacteria but not Gram-positive bacteria, whose pores have closed due to dehydration by

alcohol. It is rinsed, and a counterstain with safranin is applied. Gram-positive bacteria will appear **purple**, and Gram-negative bacteria will appear **pink (Fig. 5)**.

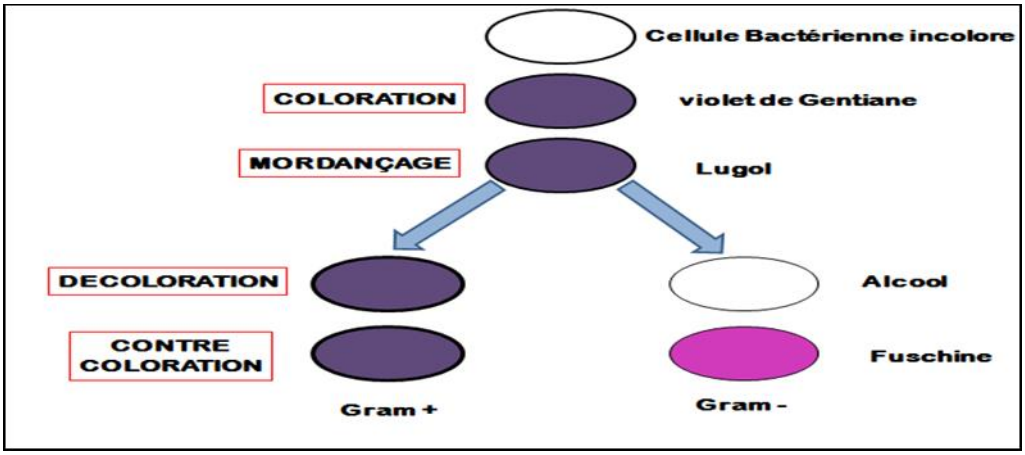


Figure 5: Steps of Gram staining.

2.2- The Cytoplasmic Membrane

2.2.1- Chemical Composition:

It has the same type of structure as that of a eukaryotic cell (phospholipid bilayer) but with much fewer carbohydrates and no sterols (except in mycoplasmas). It is composed of 60 to 70% proteins and 30 to 40% lipids. The plasma membrane contains the enzymes of the respiratory chain, dehydrogenases, and associated enzymes: NAD+, FAD+, cytochromes, cytochrome oxidase. Other enzymes involved in lipid synthesis and DNA replication are also located here.

2.2.2- Structure of the Cytoplasmic Membrane

Isolated cytoplasmic membranes observed under a phase-contrast microscope appear homogeneous and of low density. They are about 7.5 nm thick and consist of an inner transparent lipid sheet sandwiched between two dense, electron-opaque protein sheets **(Fig. 6)**. Chemical analysis of these membranes reveals three types of substances: lipids, proteins, and carbohydrates. Lipid molecules are by far the most abundant (phospholipids), particularly phosphatidylglycerol and/or phosphatidylethanolamine. The membranes of Gram-positive bacteria contain one or both of these components along with several other similar substances. Gram-negative bacteria contain only one or two types of lipid molecules.

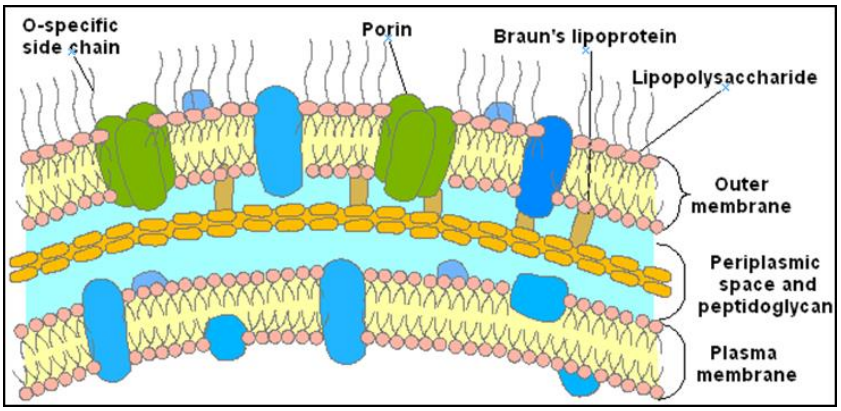


Figure 6: Structure of the bacterial membrane.

Lipids form the basis of the structure. Each lipid molecule is amphiphilic, characterized by a hydrophobic part soluble in oil and insoluble in water, and a hydrophilic part with opposite properties, carrying a negatively charged phosphate group. These molecules spontaneously organize into two leaflets where the hydrophobic parts face each other and are protected from the aqueous environment, while the external hydrophilic heads are exposed. Thus, each membrane is made of two hydrophilic layers separated by a hydrophobic layer.

Membrane proteins are composed of amino acids linked end-to-end in a linear sequence, forming a highly folded chain. Extrinsic proteins, or peripheral proteins, are weakly bound to the membrane and appear on one of the two faces of the bilayer, with no groups inserted into the hydrophobic zone. Intrinsic or internal proteins span the entire bilayer, appearing on both the inner and outer faces of the membrane.

2.2.3- Functions of the Cytoplasmic Membrane

- Selective permeability and transport of soluble substances into the bacterium: the membrane acts as both an osmotic barrier and a site of active transport via permeases.
- Respiratory function through electron transport and oxidative phosphorylation in aerobic bacterial species (equivalent to the role of mitochondria in eukaryotes).
- Excretion of hydrolytic enzymes, which degrade polymers into subunits small enough to cross the cytoplasmic membrane and be imported into the bacterium.
- Support for enzymes and transporters of molecules involved in the biosynthesis of DNA, cell wall polymers, and membrane lipids.

2.3- Cytoplasmic Elements

2.3.1- The Cytoplasm

The cytoplasm is a colloidal hydrogel with a pH ranging from 7 to 7.2. It consists of a dispersing phase made up of a solution of mineral salts and soluble lipoproteic compounds, and a dispersed phase formed of nucleoproteins and lipids.

2.3.2- RNA and Ribosomes

Bacterial ribosomes are small spherical granules, 10 to 30 nm in diameter, dispersed throughout the cytoplasm except in the nuclear regions. They are the site of protein biosynthesis. Ribosome particles are often associated by a thin filament of mRNA.

Bacterial ribosomes (sedimentation constant 70S) dissociate into two subunits **(Fig. 7)** :

-The small subunit with a sedimentation constant of 30S.

-The large subunit, with a sedimentation constant of 50S.

Ribosomes involved in **protein synthesis** are associated in chains on mRNA in the form of **polysomes(Fig. 8)**.

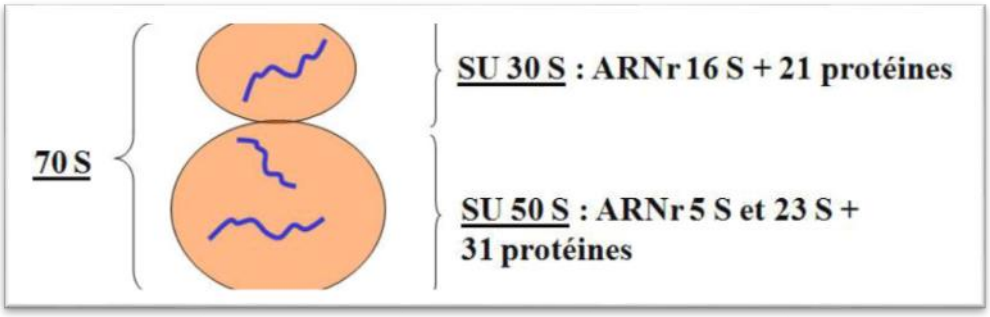


Figure 7 : Bacterial ribosome.

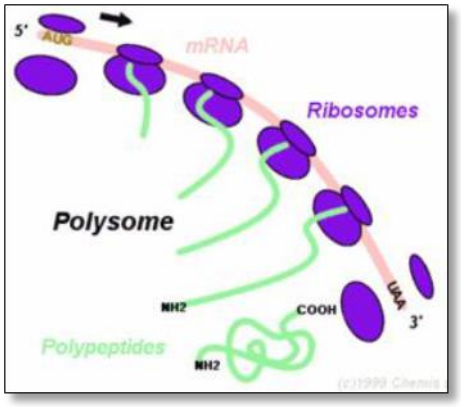


Figure 8 : Polysomes.

2.3.3- Granules and Reserve Substances

Bacteria can accumulate organic and inorganic materials, generally constituting energy reserves. When these substances reach a sufficiently large size, they form reserve granules. Generally, each bacterial group synthesizes only one category of substance. Bacteria can store reserves in the form of glycogen or starch. Some bacteria, such as *Pseudomonas, Vibrio*, or *Micrococcus,* can synthesize and store poly-B-hydroxybutyric acid.

2.3.3.1- Chromatophores

In algae and higher plants, photosynthesis occurs in chloroplasts, the mechanism that converts light energy from the sun into chemical energy. In photosynthetic bacteria, the specialized organelles that perform this activity are called chromatophores due to their different structure from chloroplasts and the nature of their photosynthetic pigments.

2.3.3.2- Gas Vacuoles

The cytoplasm of the three groups of photosynthetic prokaryotes contains gas vacuoles (blue-green algae, purple bacteria, green bacteria). These allow these aquatic microorganisms to float and rise to the water's surface. They have an irregular contour. Under an electron microscope, they have a cylindrical shape.

2.4- The Bacterial Chromosome

2.4.1- Morphology of the Bacterial Chromosome

The nuclear elements observed in bacteria are often referred to as the "bacterial chromosome." The gross morphological aspects revealed by cytological techniques tell us nothing about their intimate structure. However, the observation of ultrathin sections under an electron microscope of cells fixed, dehydrated, and embedded in a resinous solution (Kellenberger) has revealed highly interesting facts about the supramolecular architecture of this chromosome :

- The nuclear apparatus is not surrounded by a membrane, unlike the nucleus of eukaryotic cells, and there is no evidence of more than one chromosome per nucleus.
- The chromatin body has a fibrillar structure. The fibrils have a diameter of 20 to 60 Å and are composed mainly, if not exclusively, of DNA. They are coiled into parallel bundles, forming a true rope. They would retain the same structure during the division process.

2.4.2- The Circular Chromosome

Genetic transfer experiments, especially conjugation, have allowed a better understanding of the functions of the bacterial genetic material. The analysis of these functions suggests that the hereditary traits of the bacterium are located on a single linkage group, in other words, on the chromosome, which would have neither a beginning nor an end. The bacterial chromosome, which carries genes, would therefore be circular. These observations are also confirmed by the results obtained by Cairns using other methods, such as the "autoradiography technique," which shows that the bacterial chromosome is formed by a long and unique DNA molecule forming a continuous structure.

These two types of experiments, based on different principles and leading to the same conclusions, have more or less shown the structure of the bacterial chromosome, which is nothing more than a thin, unique, continuous, and circular filament formed by a double strand of DNA. Its molecular weight is 3x10^9, and the number of base pairs is about 5x10^6, spaced along the double helix. Similar to eukaryotic DNA, which is coupled to basic proteins such as histones, it is possible that bacterial DNA is neutralized, not by histones (which have never been isolated in bacteria), but by polyamines such as spermine and spermidine.

Double-stranded and circular DNA, carefully isolated in its native form from bacteria, viruses, or plasmids, presents a crossed or twisted structure called a **supercoil**. It therefore contains more base pairs per unit length than in the case of the double helix. The breaking of a strand followed by a weld, the insertion of certain chemical agents, or the binding of proteins releases the contained forces and gives rise to a so-called relaxed DNA. Finally, after denaturation, the double helix becomes linear.

2.4.3- Chemical Replication "The Jacob and Brenner Model"

The speed of replication fork progression is constant under fairly broad experimental conditions. With *E. coli*, for a generation time of 40 minutes at 37°C, the time required for the doubling of the DNA molecule is 40 minutes. If, on the other hand, more favorable conditions allow a shorter generation time, for example, 20 minutes, new replication forks are formed before the first duplication cycle is completed. It is easy to deduce that the initiation of the replication cycle is subject to control. This initiation indeed requires a specific protein, the initiator. It is likely that the programming of this synthesis occurs during the final phase of the division cycle. The initiation site of the synthesis, in other words, the point where the separation of the double DNA strand (fork) begins, is called the **replicator**. According to the **Jacob and Brenner model**, the replicator site and the gene controlling the synthesis of the initiator would form an autonomous replication unit called the **replicon**.

- The Jacob and Brenner model also accounts for the separation of daughter chromosomes and their migration into the cell before division. It allows us to understand, on the one hand, the unwinding of the duplex at the same time as the synthesis of the new strands, and on the other hand, their separation and localization in the two daughter cells.
- At the beginning of the replication cycle, the duplex is attached to the cytoplasmic membrane at the replicator site. The enzymatic system is also localized at the chromosome-membrane attachment point. One strand is closed but can unwind from the replicator site, which serves as a pivot. The other is cut, releasing a 5' phosphate at one end and a 3' hydroxyl at the other.
- One of the free ends (5'P) attaches to a new attachment site near the previous one.
- At the replication fork thus formed, the synthesis of the two new strands occurs according to a well-defined enzymatic mechanism. The DNA polymerase remains fixed at the membrane level while the parental duplex moves. This movement of the duplex is caused by the synthesis of the cell membrane in the region separating the two attachment sites, old and new.
- Membrane growth continues until the complete unwinding of the parental duplex and, consequently, until the formation of the two daughter duplexes. The two daughter chromosomes are then completely separated from each other while other syntheses have continued in the bacterium, which has progressively elongated. One of the two chromosomes is completely open : the free ends of one of the strands must then weld to reproduce the initial closed circle **(Fig. 9)**.

The completion of the chromosome replication cycle is immediately followed by the formation of a transverse division septum. Similarly, the complete synthesis of the division septum is the starting point for a new chromosome replication cycle.

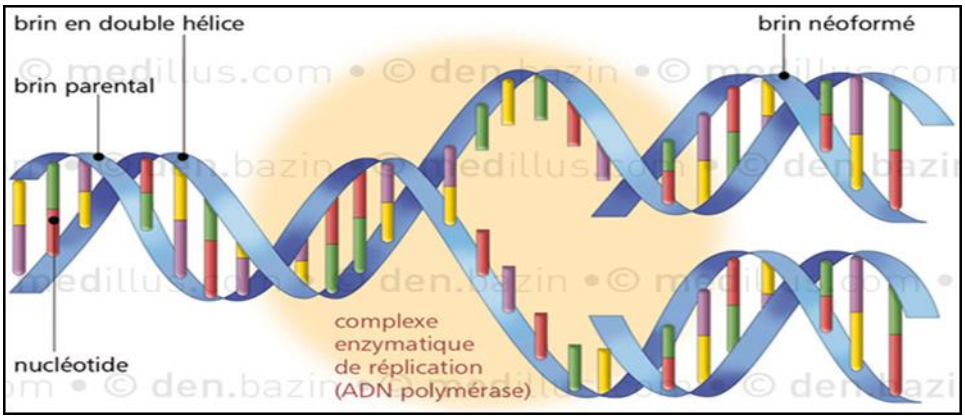


Figure 9: Semi-conservative replication.

2.5- Plasmid

The bacterial cell can contain extrachromosomal genetic elements capable of self-replication, which Lederberg in 1952 proposed to call Plasmids. To mark their independent character relative to the genes carried by the chromosome. This definition at the time applied only to the F sex factors that Lederberg had discovered a few years earlier. But it was only in 1959 that the scientific community understood the true nature of these elements and their biological role by discovering in Shigella a multiresistance to antibiotics.

2.5.1- Structure and Physical Properties

Like any structure carrying genetic information, the plasmid is a DNA molecule. This DNA can be separated from chromosomal DNA by centrifugation in a cesium chloride gradient from a bacterial lysate. Overall, plasmid DNA molecules are small in size, about 1/100th that of the chromosome. Their tight coiling (supercoiling) probably ensures minimal bulk and confers great resistance. Their molecular weight ranges from about 1 megadalton (MW = 1 million) to over 100 megadaltons, that is, comparable to that of viral DNA. The structure of viral DNA. The structure of plasmid DNA can be analyzed using recent molecular genetics techniques **(Fig. 10)**.

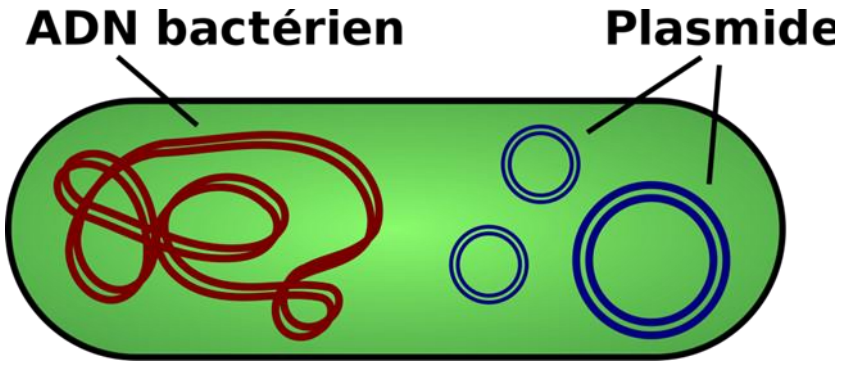


Figure 10: Diagram of bacterial DNA and plasmid.

2.5.2- Replication

Plasmid replication is similar to that of the chromosome or the F factor, tightly regulated at membrane sites. The model proposed by Jacob and Brenner is perfectly applicable. It depends on a large number of genes. Plasmids from which long fragments of DNA have been excised remain viable. It is possible to remove two-thirds of the *Staphylococcus* plasmid without affecting its replication functions. However, a deletion in a small region, called the "forbidden region," eliminates the plasmid's viability. This region, which carries the genes involved in replication, is called the "replication control unit."

The replication of a plasmid and its regulation could proceed according to the following scheme (Fig.11). It is triggered by a protein called an initiator, which depends on a replication gene. This gene is controlled by another protein, the repressor, encoded by a gene. In the first step, a DNA polymerase traverses the origin sequence at the promoter site, and the RNA formed serves as a primer for DNA synthesis. In a second step, it dissociates the two DNA strands, which form loops through intrastrand base pairing. These loops, recognized by the replication machinery, lead to the synthesis of plasmid DNA. This replication is independent of the replication of the chromosome; the larger the plasmid, the lower the number of copies. The number of plasmid copies varies between 1 and 100, depending on the size of the plasmid; the larger the plasmid, the fewer the copies. The transmission of plasmids from generation to generation naturally requires a mechanism ensuring the equal distribution (bipartition) of copies into daughter cells.

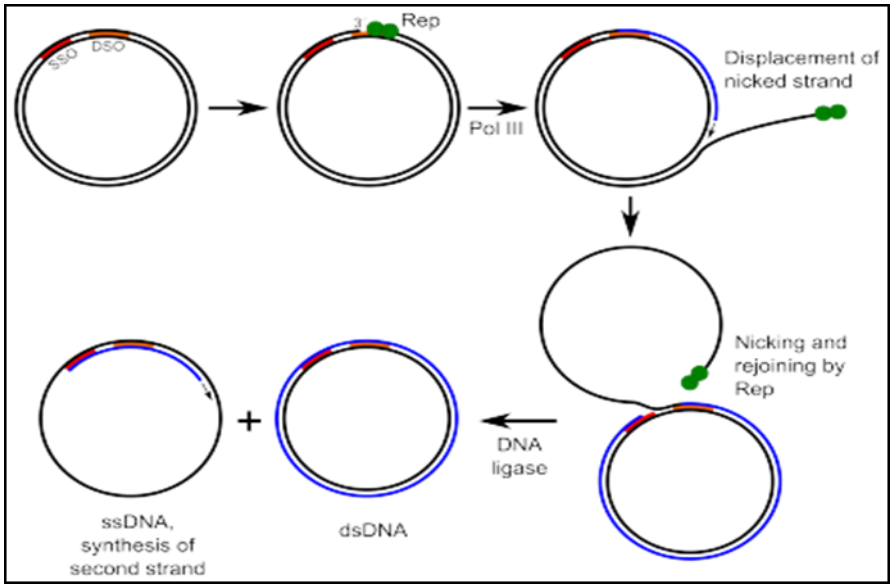


Figure 11: Plasmid replication.

2.5.3- Properties of Plasmids

The discovery of plasmids has led biologists to completely reconsider traditional genetic concepts. The notion of a constant and stable genetic potential over time should be questioned. Many genetic traits of bacterial cells are indeed variable, unstable as they undergo losses or gains, and mobile as they can be transferred from one cell to another or transposed to different sites within the same cell. These properties are due to their plasmid insertion.

2.5.3.1. Resistance to Antibiotics and Heavy Metals

Several mechanisms explain the resistance to toxic substances induced by plasmid genes. In the simplest case (penicillin, aminoglycosides, chloramphenicol), the antibiotic is destroyed by an enzyme. In other cases, the toxic agent cannot access the cellular target, etc. Generally, it is estimated that plasmid-mediated resistance accounts for more than 90% of cases observed in clinical settings, with the remaining 10% due to chromosomal resistance. Plasmid resistance is also increasingly observed with heavy metals. Plasmids are involved in resistance to mercury compound, cadmium salts, and lead in *Staphylococci* and Gram-negative bacilli.

2.5.3.2. Production of Pathogenic Substances

One of the most remarkable and well-studied examples is found in *E. coli*, where three groups responsible for diarrhea are defined: enteropathogenic *E. coli*, enterotoxigenic *E. coli*, and enteroinvasive *E. coli*. The pathogenicity of these three bacteria is dependent on plasmid determinants responsible for the synthesis of enterotoxins and certain substances called colonization factors, which ensure the adhesion of bacteria to the intestinal epithelium and subsequent invasion.

2.5.3.3. Production of Bacteriocins

In 1915, Gratia described a specific antagonistic action between two strains of *E. coli*. The substances responsible are called bacteriocins. These are proteins whose biosynthesis is lethal and whose adsorption is conditioned by the presence of a specific receptor. The names of bacteriocins are always derived from the bacterial species or genus on which they act: colicins (*E. coli*), pyocins (*Pseudomonas*), vibriocins (*Vibrio cholerae*).

2.5.3.4. Metabolic Traits

A large number of biochemical traits in bacteria are plasmid-encoded. These have been particularly observed in *Enterobacteriaceae*, such as citrate utilization, hydrogen sulfide production, urea hydrolysis, sucrose and lactose degradation, etc. Plasmids that encode these unusual properties in wild strains are called metabolic plasmids. Their presence constitutes a significant source of error in the identification of microbial strains. Other functions also have a plasmid basis, including nitrogen fixation in *Enterobacteriaceae*, degradation of chemical products by *Pseudomonadaceae*, pigment production, and the synthesis of fibrinolysin, hemolysin, and coagulase in *Staphylococci*.

2.6- Pili or Fimbriae

2.6.1- Structure of Pili

The existence of filamentous appendages different from flagella was revealed by electron microscopy. They are common in Gram-negative bacilli and rare in Gram-positive forms. They are named Pili (fimbriae) **(Fig.12)**. Two categories are distinguished, differing in morphology and function: **common Pili** (also called fimbriae or type I, III, and IV Pili) and **sex Pili** (also called type II Pili).

-Common Pili: These are distributed in large numbers around the bacterium. They are thin, short, rigid, and therefore brittle. Their presence is related to the hemagglutinating activities of the bacterium.

-Sex Pili: These are longer and end with swellings. Their number is low, ranging between 1 and 4. They appear to play a definitive role. In some bacteria, such as streptococci, they carry antigenic M protein.

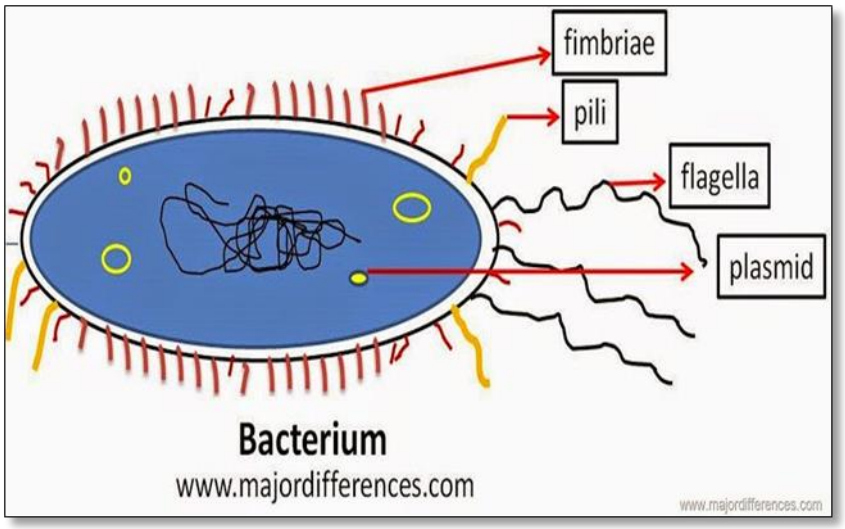


Figure 12: Diagram representing fimbriae and Pili.

2.6.2- Function of Pili

-Type I and III fimbriae : play a role in the adhesion of bacteria to various living or non-living surfaces. They promote biofilm formation.

-Type IV fimbriae : found for example in *Pseudomonas aeruginosa*, in addition to attachment, are involved in another mode of mobility. They are found at the poles of bacterial cells. Type IV fimbriae contract and retract like a spring, allowing bacterial movement.

-Sex Pili or type II Pili: They play a role in bacterial conjugation (one of the three modes of genetic material transfer from one bacterium to another). They lead to the creation of a cytoplasmic bridge between the two bacteria, allowing the passage of a plasmid molecule.

2.7- The Capsule

2.7.1- Morphology of the Capsule

Many bacteria produce viscous organic substances that surround their cell wall with a more or less compact layer. This layer, of a gelatinous-mucous nature, is called the Capsule. Not all bacteria produce a capsule, and in the same strain, capsule formation is largely influenced by the constituents of the medium.

Capsulated bacteria, after development on agar medium, produce smooth colonies (called "S" for "Smooth") or mucous colonies, while non-capsulated bacteria produce rough colonies (called "R" for "Rough"); in the latter case, these are bacteria that have lost the ability to synthesize the capsule due to a mutation.

2.7.2- Chemical Composition

The capsule is usually polysaccharide in nature (sugars in the form of uronic acids such as galacturonic acid, glucuronic acid, but also in the form of phosphorylated sugars), although in the case of *Bacillus anthracis* (the anthrax bacillus), it consists of a polypeptide of D-glutamic acid.

2.7.3- Function of the Capsule

The capsule plays an important role not only in bacterial attachment but also in their **virulence** by protecting them against phagocytosis. Non-capsulated cells are **avirulent**.

NB/ The capsule is antigenic, and the capsular antigens are called **K antigens**. Their study allows the distinction of several serotypes within the same bacterial species.

2.8- Cilia and Flagella

Flagella, also called cilia, are optional bacterial structures. They are filamentous organs that allow bacterial locomotion. In enterobacteria, they allow a movement speed of 10 to 20 micrometers per second; on a human scale, this speed would correspond to about sixty km/h.

2.8.1- Detection

Indirect: Fresh state (moving bacteria) or in semi-solid medium. Several factors influence mobility, such as the age of the culture, temperature (*Yersinia sp* is immobile at 37°C and mobile at 22°C).

Direct: In optical microscopy after thickening the flagella with special stains (Rhodes, Leifson: basic fuchsin); or in electron microscopy.

The best method of study is observation under an electron microscope, which alone allows detailing their shape, mode of insertion, and dimensions.

2.8.2- Structure of Cilia and Flagella

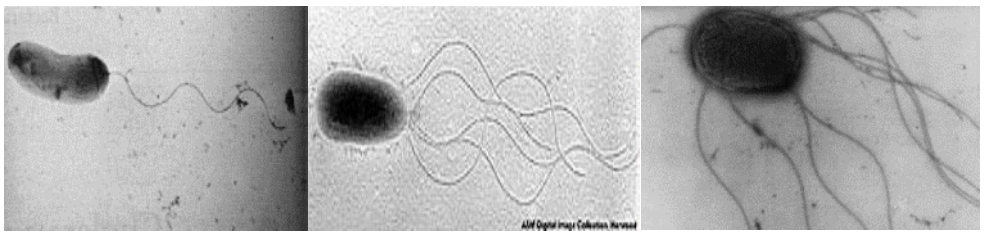
They are about ten μm long and have a diameter ranging from 12 to 30 nanometers. They are composed of proteins (**flagellins**), with a MW of 15 to 70 kDal. Their number varies from 1 to 30 depending on the bacterial species. They are often found in bacilli and rarely in cocci. They play an important role in the antigenic specificity of bacteria (H antigens). Due to their thinness, special staining techniques are used to observe them under a light microscope, which allows thickening of the flagella **(Fig.13)**.

Figure 13: Flagellated cells of *Vibrio cholerae.*

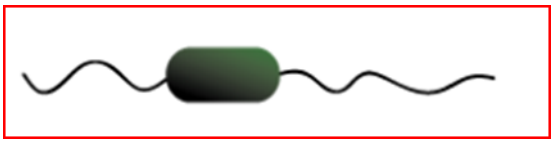
According to the arrangement of the flagella **(Fig.15)**, we distinguish:

- **Monotrichous** bacteria (a single polar flagellum),
- **Amphitrichous** (a flagellum at each pole),
- **Lophotrichous** (a tuft of polar flagella),
- **Peritrichous** (flagella distributed over the entire surface of the bacterium).

Spirochetes have an internal flagellum called an axial filament **(Fig.16).**



Monotrichous ciliation Lophotrichous ciliation Peritrichous ciliation



Amphitrichous ciliation

Figure 15 : Different bacterial ciliary systems.

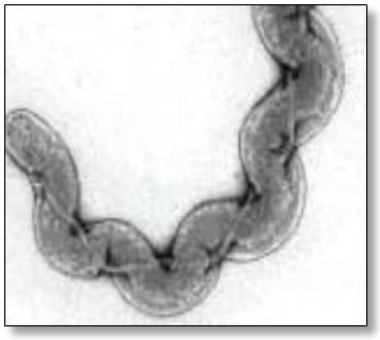


Figure 16: Leptospira with internal flagellum.

2.8.3- Function of Flagella

They have a role :

- In bacterial **mobility** (monotrichous/polar or peritrichous implantation).
- **Antigenic:** Flagellar antigens (H antigens) determine different serotypes (e.g. serotyping of *Salmonella*). Antigenic specificity depends on the number and sequence of amino acids in flagellin.

2.9- The Spore

Some bacteria have the ability to transform into small oval or spherical resistant units when the medium is depleted of nutrients or when external physicochemical conditions change. These are called "spores" or "endospores" since their formation is intracellular.

Endospores characterize three main bacterial genera: *Bacillus*, *Clostridium*, and *Sporosarcina*.

2.9.1- Morphology of the Spore :

Spores are small oval or spherical units. They may or may not deform the bacterial body. Their position in the cell is variable : central, terminal, subterminal **(Tab.1).** They are also used in bacterial identification. The spore can be free or not. The search for all these characteristics is done for taxonomic purposes.

Table 1: Different forms and positions of spores

Forme	Position	Déformation
Ovalaire	Centrale	Non déformante
Sphérique	Subterminale	Déformante
	Terminale	

2.9.2- Structure of the Spore

This cellular form is multilayered, each layer having different properties **(Fig.17).**

- **Spore cell (core)**

The spore cell contains all cellular structures (ribosomes, DNA, etc.). The cell is highly dehydrated (water represents 25 to 55% of the wet weight). It is surrounded by an internal membrane. The DNA is protected by proteins (SAPS, *acid-soluble spore proteins* in English).

Internal Membrane

The (internal) membrane has lipids similar to vegetative cells, but the proteins found are different. These modifications allow reducing the permeability of the membrane.

Cortex

The cortex is composed of peptidoglycan. Its composition is different from that of the peptidoglycan of the vegetative cell.

Outer Membrane (Tunic)

The outer membrane is located between the cortex and the tunic. It plays an important role during spore formation. However, it has no known role in the resistance properties of the spore. It may contain pigments that protect against radiation or serve as a selectivity barrier.

Exosporium

The exosporium is a very thin membrane found on the surface of spores of certain bacteria. Its role is still unknown. This membrane may serve as a barrier against antibodies in *Bacillus anthracis*.

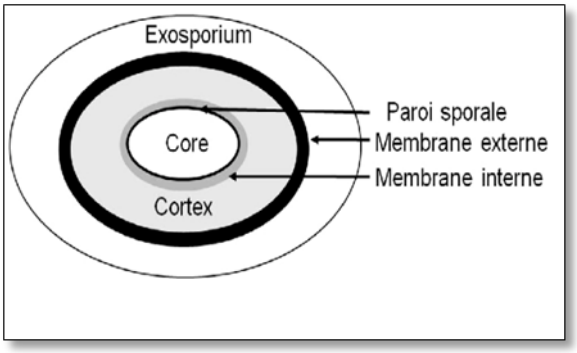


Figure 17 : General structure of a bacterial spore.

2.9.3- Sporulation Process

During sporulation, the vegetative cell undergoes progressive dehydration of the cytoplasm, the appearance of certain compounds (calcium dipicolinate), densification of

nuclear structures, and finally the synthesis of a thick, impermeable spore wall, and therefore highly resistant **(Fig.18).**

The spore that arises in the vegetative cell is an entirely new and different cell in terms of structure, chemical composition, and enzymatic content. Sporulation occurs at the end of the exponential phase in a time of seven hours and takes place in seven stages :

[Stage 1]{.underline}: Sporulation begins with the cessation of DNA, RNA, and protein synthesis. The first change is the transformation of the nucleotide into an axial chromatin filament that extends throughout the cell.

[Stage 2]{.underline}: The nuclear filament condenses at one end and breaks. There is an autonomization of the future spore nucleus and asymmetric cell division favored by a subpolar transverse septum that divides the cell into two unequal parts : the sporangium and the future spore.

[Stage 3]{.underline}: The synthesis of the septum continues and results in the formation of a smooth, transparent, entirely autonomous zone, comprising a nucleus, cytoplasm, and a double membrane, one cytoplasmic, the other prefiguring the future spore wall: this is the forespore.

[Stages 4 to 7]{.underline}: In the sporangium, the forespore will mature progressively by surrounding itself with a certain number of teguments. In the cytoplasm of the mature spore, individualized and released, most of the enzymes of the vegetative cell are degraded and replaced by a set of spore constituents. At this point, the cytoplasm is called the core. It contains a nucleus comparable to that of the cell and all the necessary components for protein synthesis and an energy-producing system. The envelopes surrounding the core are four in number: the spore wall (inner envelope = peptidoglycan). The cortex (outer layer = lipoprotein containing 20% sugar).

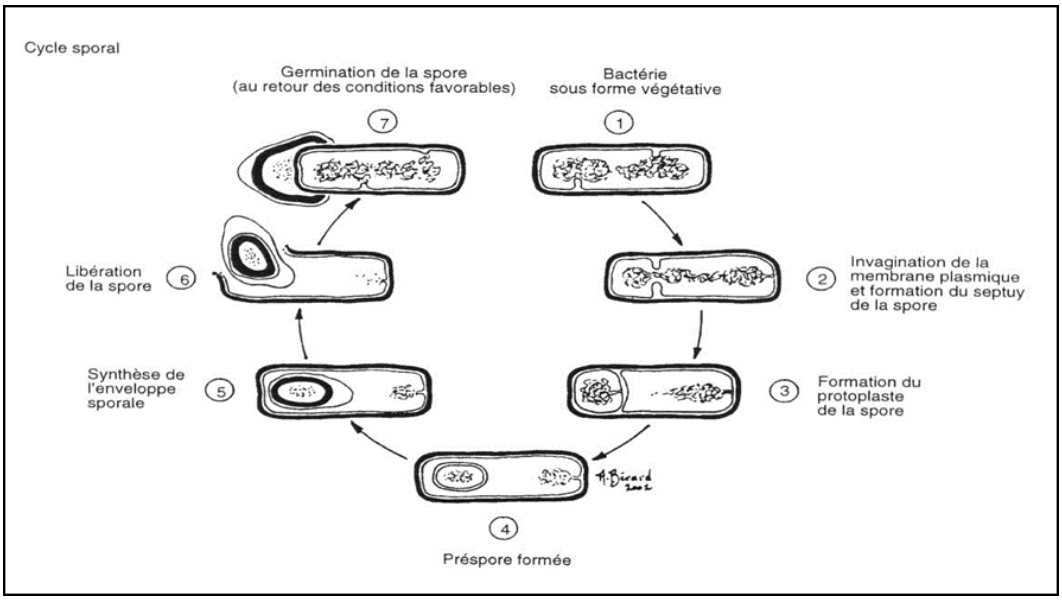


Figure 18: Stages of endosporulation.

2.9.4- Properties of Sporulation :

The spore has new properties compared to the vegetative cell: in nature (natural conditions), the spore allows resistance to lack of water and nutrients. Experimentally, the following properties have been demonstrated:

-Thermal resistance: The spore generally resists temperatures of 70-80°C for 10 minutes, sometimes more. This property is due to the presence of dipicolinic acid, the dehydration of the spore, and the "SASP" proteins (small acid-soluble proteins that can bind to DNA).

-Resistance to physical and chemical agents: The spore resists ultraviolet rays, gamma rays (calcium and SASP). To antiseptics, disinfectants, antibiotics (the tunic).

-Synthesis of antibiotics: Some bacteria synthesize antibiotics at the beginning of the sporulation phase. But also **toxins** (enterotoxin of *Clostridium perfringens*) or substances with biopesticidal activity (toxins that kill insects).

2.9.5- Germination:

When the spore is placed in favorable growth conditions, it undergoes a series of progressive transformations and eventually becomes a new vegetative cell. This process, called germination, comprises three stages:

- **Activation:** Corresponding to damage to the spore envelopes by physical (thermal shock) or chemical (acids, lysozyme) or mechanical (abrasion, shock) agents.
- **Initiation:** Begins in the presence of favorable hydration conditions and effector metabolites (alanine, magnesium, adenosine) that penetrate through the damaged envelopes. Hydrolytic enzymes degrade the spore constituents; calcium dipicolinate is released. The cortex thus destroyed, the spore imbibes water and swells.
- **Emergence of the new vegetative cell,** thanks to the alteration of the envelopes. **(Outgrowth)**