Chapter II: The bacterial cell (continued)

The bacterial chromosome

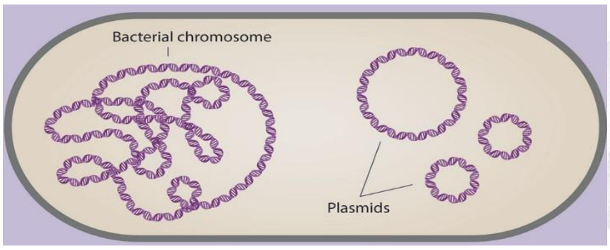
Morphology

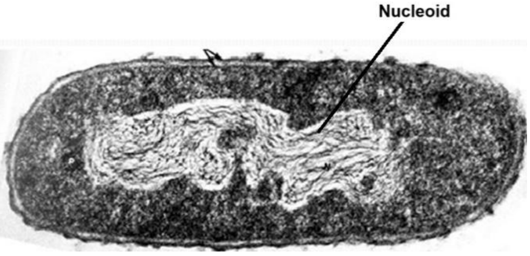
Every bacterium possesses at least one chromosome which, with associated proteins, forms the nuclear apparatus or bacterial genome.

The chromosomal DNA consists of a double-stranded circular DNA helix organized into 50 to 100 supercoiled loops and stabilized by proteins that condense it into a visible structure under a microscope called the nucleoid. Unlike eukaryotic DNA, bacterial DNA is not enclosed within a nuclear membrane and is not associated with histones.

The size of the bacterial chromosome varies depending on the genus, species, or strain. It is approximately 1 mm in length (1,000 times the length of the bacterium) and 3 to 5 nanometres wide.

Some prokaryotes contain up to 4 chromosomes of unequal size.



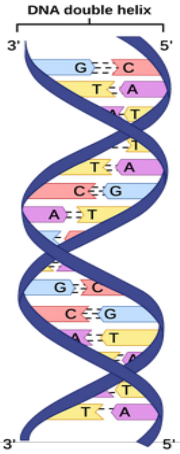


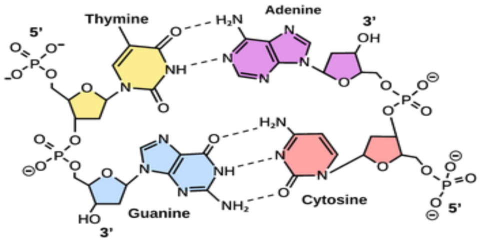
Chemical Composition

Chemical analysis of the nuclear apparatus indicates that it is composed of 60% DNA (the chromosome), 30% ribonucleic acid RNA (structural role) and 10% proteins. These proteins are represented in particular by DNA polymerases, topoisomerases, and RNA polymerases.

DNA or Deoxyribonucleic Acid: It is a high molecular weight polymer composed of units called nucleotides. A nucleotide is formed by the combination of a purine base (A or G) or a pyrimidine base (C or T), a sugar (deoxyribose) and a phosphate group.

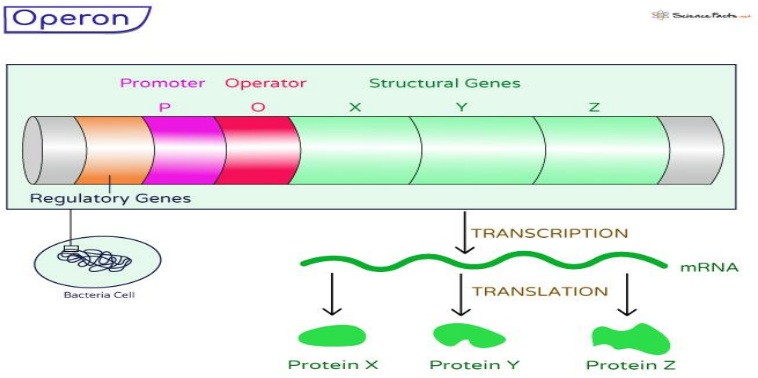
The two antiparallel DNA strands are held together in a double helix by hydrogen bonds between the nitrogenous bases in a specific manner (A=T and C≡G).





Functions

The bacterial chromosome carries all the genetic information necessary for metabolism, structural integrity, and cellular functions. It consists of **coding genes** and **non-coding sequences**. Gene expression involves transcription and translation. Unlike eukaryotes, bacterial genes are often organized into **operons** which are clusters of functionally related genes controlled by a single promoter.



Replication

In bacteria, chromosome replication must occur once per cell cycle. Replication is semi-conservative. The replication of bacterial DNA follows this mechanism:

a. Initiation

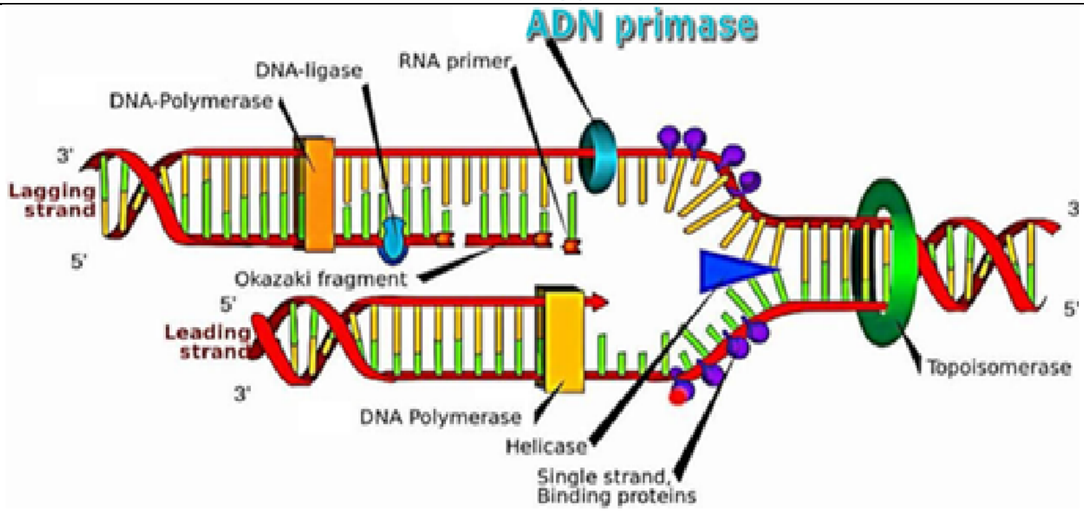
In prokaryotes (bacteria), replication is bidirectional according to the Theta (θ) model. It begins at a specific and single point on the chromosome called the initiation point or origin of replication (***oriC***). At this site, helicase breaks hydrogen bonds between base pairs, separating the DNA strands to form a replication bubble with two forks. Single-strand binding proteins (SSBs) also called "helix-destabilizing proteins" prevent reannealing. A topoisomerase relieves torsional stress ahead of the fork.

b. Elongation

DNA Primase (DNA-dependent RNA Polymerase) synthesizes a short RNA fragment called primer, needed to initiate DNA synthesis. DNA polymerase III extends these primers by adding complementary nucleotides to the 3'OH end.

DNA replication is continuous on the leading strand (synthesized 5’→3’) and discontinuous on the lagging strand due to the 5’→3’ synthesis constraint. The lagging strand is produced as short Okazaki fragments synthesized backward relative to fork movement but still 5’→3’ on their template.

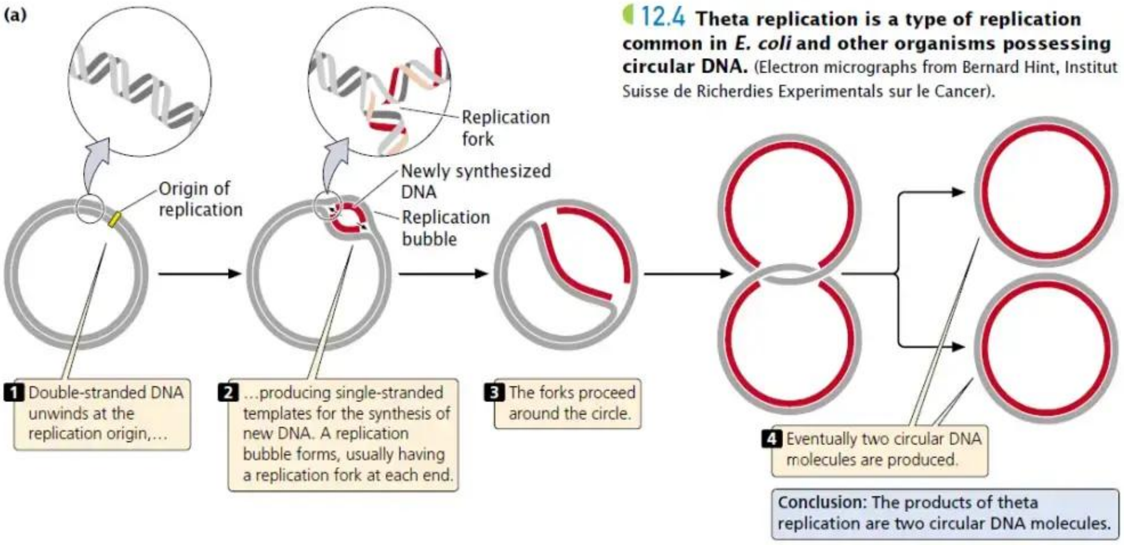
DNA polymerase I removes the RNA primers and replaces them with DNA. Afterward, DNA ligase joins the Okazaki fragments



c. Termination

Replication concludes when the two replication forks meet at termination sites (*ter*) recognized by the Tus protein. The Ter-Tus complex blocks the forks, ending replication.

The completed circular chromosomes remain temporarily interlinked. Topoisomerase IV resolves the interlocked circles allowing separation of the daughter chromosomes.


12.4 **Theta replication is a type of replication common in *E. coli* and other organisms possessing circular DNA.** (Electron micrographs from Bernard Hint, Institut Suisse de Richerdies Experimentals sur le Cancer).

1 Double-stranded DNA unwinds at the replication origin,…

2 …producing single-stranded templates for the synthesis of new DNA. A replication bubble forms, usually having a replication fork at each end.

3 The forks proceed around the circle.

4 Eventually two circular DNA molecules are produced.

Conclusion: The products of theta replication are two circular DNA molecules.

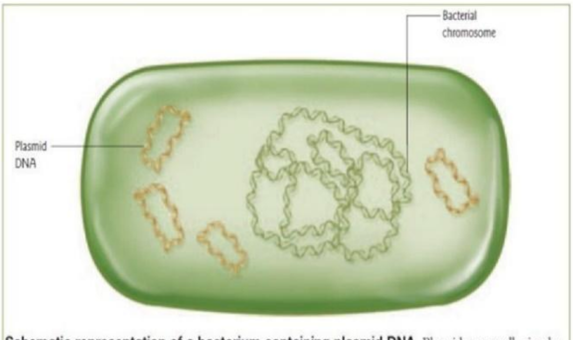
Plasmid DNA

In addition to the genetic material present in the nucleoid, many bacteria possess extra-chromosomal DNA molecules called plasmids, which are not essential for a host cell's survival or normal metabolism. However, they can offer a selective advantage in specific environments, enhancing the cell's adaptability.

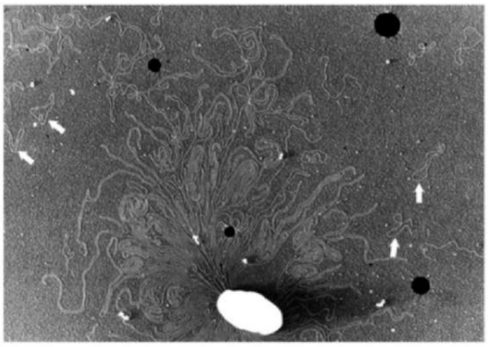
Structure and characteristics

Plasmids are small, circular, double-stranded DNA molecules independent of the bacterial genome, with fewer than 10 kb nucleotides. They have relatively few genes (fewer than 30).

Some plasmids are capable of integrating into chromosomes; these plasmids are called episomes.



Schematic representation of a bacterium containing plasmid DNA. Plasmids are small, circular molecules of DNA that are extrachromosomal and self-replicating within the host bacterium.



DNA from a *E. coli* cell. Arrows point to several smaller circular plasmid DNAs

Replication

Plasmids are autonomous replication units. They possess their own origin of replication and generally replicate independently of the bacterial chromosome. However, it is the cell's machinery that ensures their replication.

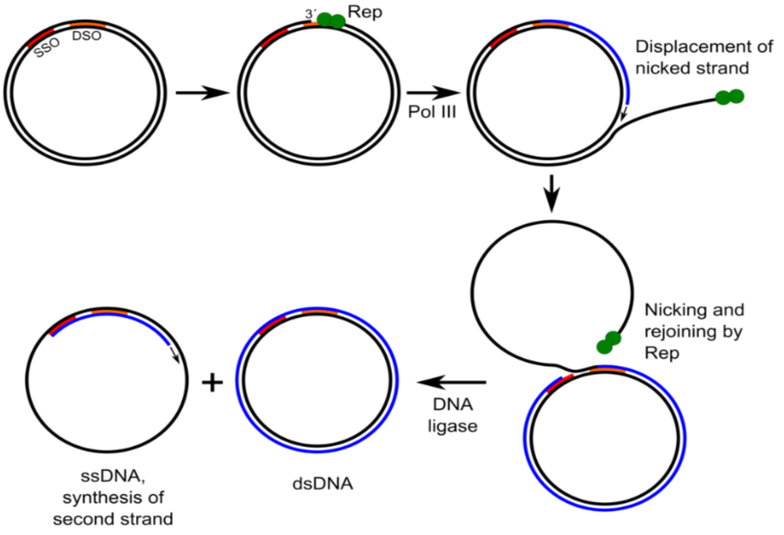
Replication models

While many bacterial plasmids replicate using a θ model, other plasmids use rolling circle replication. This mechanism begins when the *Rep* endonuclease recognizes and cleaves one strand of the double-stranded plasmid DNA at the *double-strand origin (**dso**)*. This cleavage:

- Generates a free 3'-OH end that serves as a primer for DNA polymerase
- Leaves a 5'-phosphate end covalently bound to ***Rep***

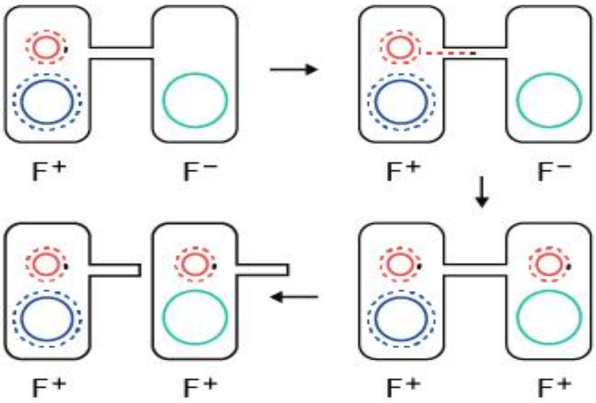
DNA polymerase III extends the 3'-OH primer unidirectionally along the intact template strand, displacing the nicked strand. Upon completing one full round of replication, the displaced strand circularizes into single-stranded DNA (ssDNA).

The RNA primase synthesizes a short primer at the *single-strand origin (**sso**)*. DNA polymerase uses this primer to convert the ssDNA into double-stranded DNA (dsDNA), yielding two identical circular plasmids.



Properties encoded by plasmids

- Conjugative plasmid (F plasmid or Fertility factor): enables bacterial conjugation by encoding sex pili that mediate DNA transfer between F^+ donor and F^- recipient cells. These plasmids contain their own origin of replication and all necessary genes for pili synthesis and plasmid transfer.

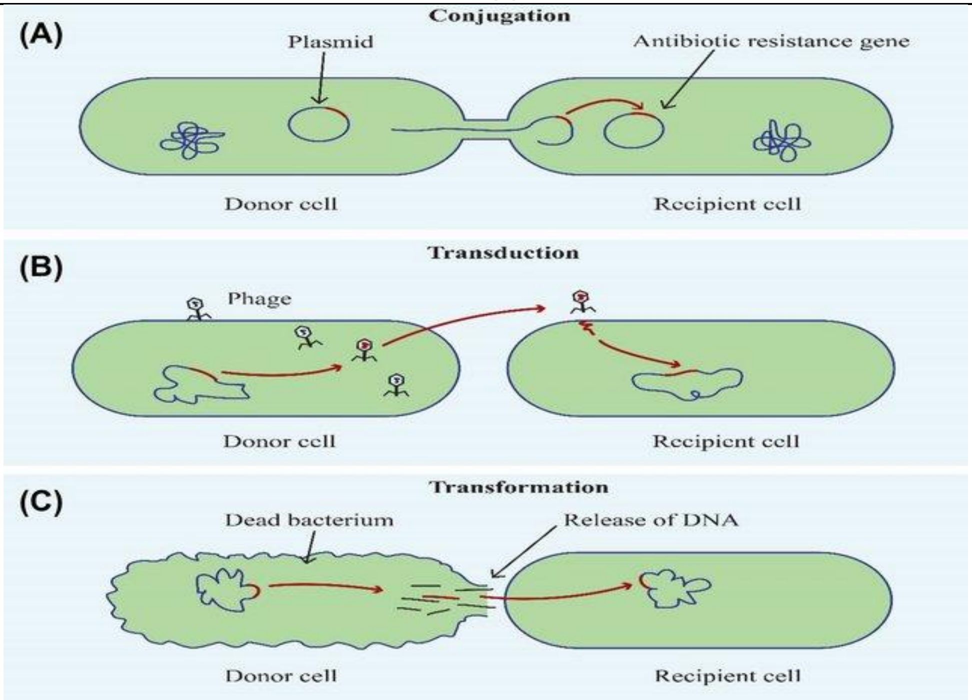


- Resistance plasmids (R factors): They carry genes providing resistance to antibiotics and heavy metals. These plasmids can spread resistance through multiple mechanisms.
- Metabolic plasmids: expand bacterial metabolic capabilities by encoding enzymes for specialized functions. These plasmids allow bacteria to exploit alternative nutrient sources in their environment.
- Virulence plasmids: enhance pathogenicity by encoding virulence factors (colonization factors and toxins, invasiveness and attachment to host cells).
- Bacteriocin and biocide/pollutant resistance plasmids: produce toxic proteins that kill competing bacteria. These toxins target essential cellular components, such as DNA (*colE*1 endonuclease) or ribosomes (*colE*3 ribonuclease), providing a competitive ecological advantage.

Plasmid transfer

Plasmids can be transferred between bacteria through two main pathways:

- **Vertical transfer** occurs during cell division, where plasmids are passed from the parent cell to the daughter cells. Plasmids are distributed randomly into daughter cells. Plasmids can be eliminated from host cells. This curing occurs spontaneously or can be induced by treatments that inhibit plasmid replication.
- **Horizontal transfer** allows bacteria to exchange genetic material with one another, even without direct lineage. This process can occur through three primary mechanisms:
  a) Transformation: Some bacteria can take up free DNA from their environment, including plasmids, and incorporate it into their own genome.
  b) Transduction: In this case, bacterial viruses (bacteriophages) act as vectors, accidentally transferring plasmids from one bacterium to another during their infectious cycle.
  c) Conjugation: This mechanism involves direct contact between two bacteria via a structure called a pilus. A donor bacterium transfers a copy of its plasmid to a recipient bacterium.



Pili

Structure

Many bacteria possess appendages on their cell surface. These appendages, called pili (from *pilus* = hair), are shorter and thinner than flagella and are not involved in motility. Like flagella, these appendages originate from the plasma membrane. They are composed of protein monomers called pilins.

Function

Bacteria produce two main types of pili with distinct roles:

- **Common Pili** (or **Fimbriae)**

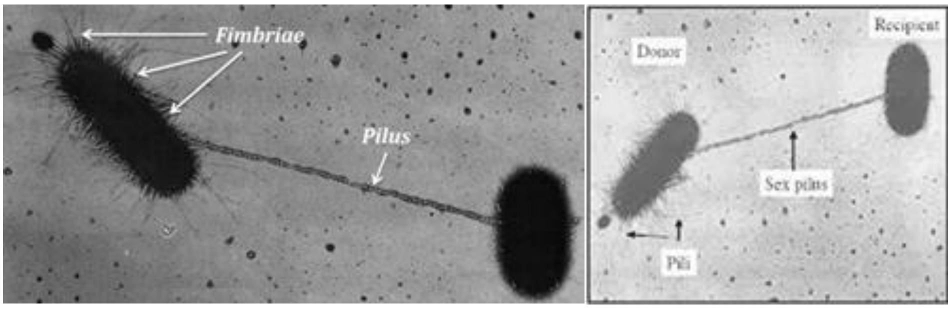
These are the most common and numerous (several hundred around the bacterium). These pili are filamentous protein structures, 2 to 3 µm long, arranged on the bacterial surface. They are formed by the polymerization of a single polypeptide subunit, pilin, assembled with minor polypeptides including adhesin.

Pili enable bacteria to attach to host cells through specific receptor interactions, which is essential for colonization and pathogenicity. They may also contribute to surface attachment during biofilm formation.

These pili are highly antigenic, triggering the production of specific antibodies that, by binding to the pili, prevent their attachment to host cells.

- **Sex Pili**

Longer but much fewer in number (1 to 4) than common pili, sex pili are encoded by plasmids (F factor). They play an essential role in the attachment of bacteria to each other during conjugation.

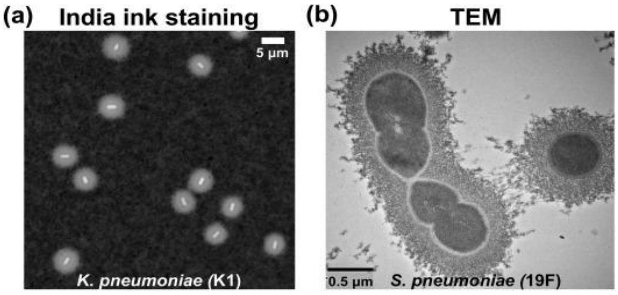


Capsule

Structure

The bacterial capsule is an outer, viscous, and amorphous layer of slime that surrounds the cell wall of some bacteria. It is secreted by bacteria and diffuses into the surrounding environment. The presence of the capsule gives colonies on solid media a characteristic mucoid appearance, known as "M-type" colonies. It can envelop a single bacterium or a chain of bacteria.

When using standard staining techniques, the capsule appears as a clear halo around the bacterium, but special staining methods can differentiate it from the bacterial cell.



Composition

Most capsules are composed of polysaccharides, although some are made of polypeptides. Capsular polysaccharides are highly hydrated (over 95% water).

Function

Although the capsule is not essential for the survival of the bacterium, it provides a competitive advantage in various natural environments.

- **Virulence factor**

✓ Prevents phagocytosis

✓ Makes bacteria "slippery," allowing them to evade immune cells

✓ Resists proteolytic enzymes.

- **Adhesion factor**

✓ Enables attachment to surfaces (biofilms).

✓ Facilitates colonization of specific niches or inert surfaces.

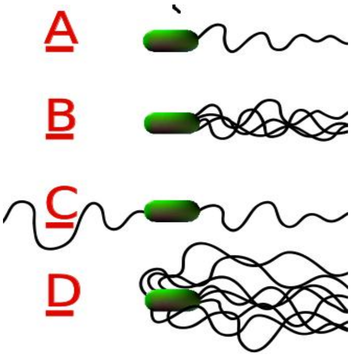
- **Protection against desiccation:** it acts as a water reservoir to prevent drying out.
- **Antigenicity:** responsible for serological specificity of bacteria: capsular antigens (Ag K)

Flagella

Flagella are filamentous appendages composed of proteins called flagellins. These whip-like structures protrude from the cell body and typically measure between 5 and 20 µm in length and 10 to 30 nm in diameter. They are commonly found in bacilli but are rarely observed in cocci.

Some bacteria possess a single flagellum, while others may have up to ten. Some bacteria have more than 400 flagella. The position of flagella on the bacterium can also vary:

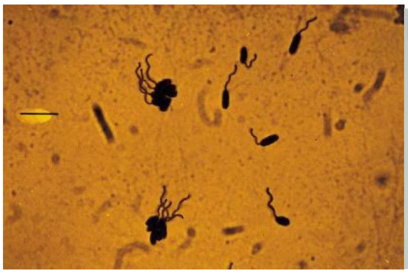
- Monotrichous bacteria have a single flagellum
- Lophotrichous bacteria have multiple flagella located at the same spot
- Amphitrichous bacteria have flagella on each of two opposite ends.
- Peritrichous bacteria have flagella projecting in all directions



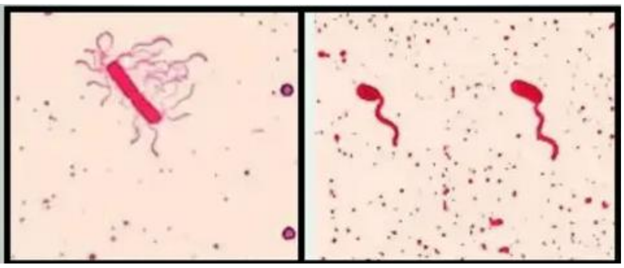
Visualization

Due to their thin and delicate structure, special staining techniques are required to observe flagella under a light microscope. These techniques enhance the visibility of flagella by darkening or increasing their contrast:

- **Leifson staining**: Uses basic fuchsin, staining the flagella red.
- **Rhodes staining (silver staining)**: Uses silver nitrate, which deposits silver on the flagella and bacterial surfaces, making them appear dark and more pronounced under the microscope.



Rhodes staining



Leifson staining

Structure

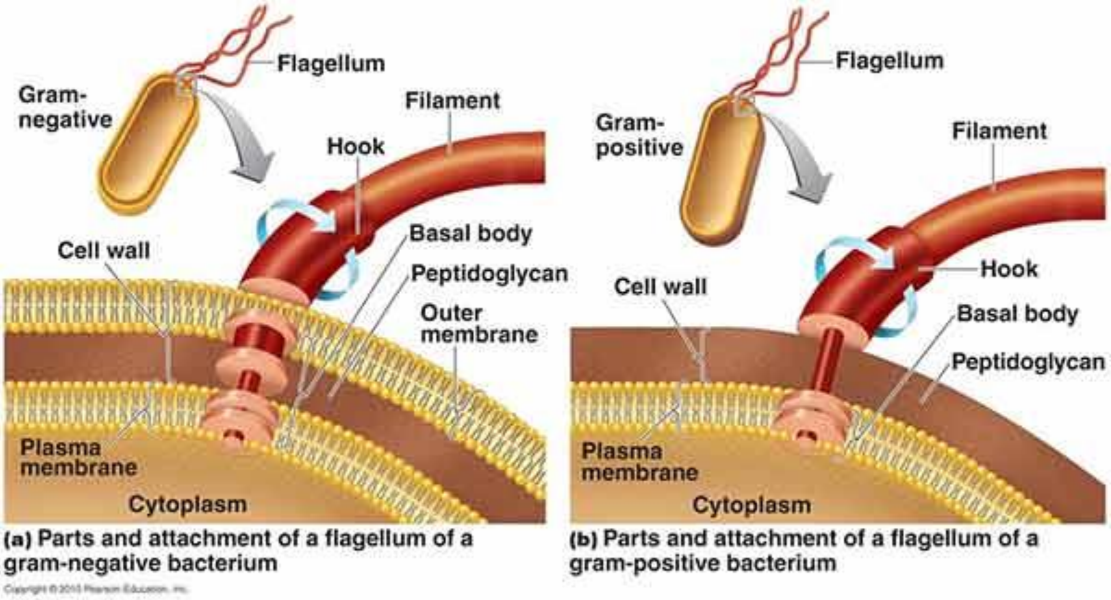
Flagella are anchored in the bacterial cell envelope by a complex structure consisting of three main parts:

1. **The filament**: It is a hollow cylinder composed mainly of a protein called flagellin. The filament adopts a helical structure and rotates like a propeller, allowing the bacterium to move efficiently in its environment.

2. **The hook**: it connects the filament to the basal body. Its composition is similar to that of the filament, but it has a curved and more flexible structure. Its main function is to transmit the rotational force from the basal motor to the filament.

3. **The basal body**: this part acts as a rotary motor, capable of spinning in both directions. It is firmly anchored in the bacterial envelope by a set of rings, whose arrangement varies depending on the type of bacterium:

- **In Gram-negative bacteria** : L ring, P ring, MS ring and C ring
- **In Gram-positive bacteria** only the MS and C rings are present.



(a) Parts and attachment of a flagellum of a gram-negative bacterium

(b) Parts and attachment of a flagellum of a gram-positive bacterium

Functions of the flagellum

- **Locomotion**: The rotation of helical flagella propels bacteria through their environments in a characteristic "run-and-tumble" pattern. This movement is regulated by the chemotaxis signaling network, which adjusts the tumble frequency allowing bacteria to navigate toward favourable environments or away from harmful ones.
- **Antigenic role**: Flagellar antigens (*H antigens*) are used to distinguish bacterial serotypes, as in *Salmonella* typing.
- **Attachment**: Some bacteria use their flagella to adhere to surfaces or host tissues allowing bacteria to establish infections or form biofilms.

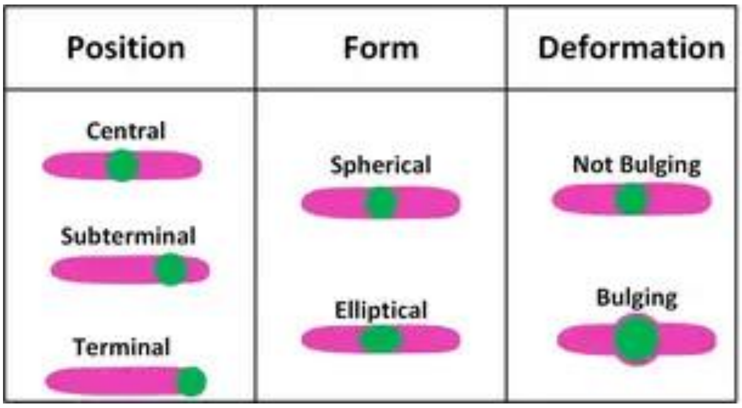
Endospore (Spore)

The endospore is an optional, resistant structure formed by some Gram-positive bacteria (e.g., *Bacillus*, *Clostridium*, *Sporosarcina*) under unfavorable conditions. It enables bacteria to survive extreme environments while preserving their genetic material. Endospores confer resistance against nutrient deprivation, high temperatures, chemical/physical agents and UV/gamma radiation

Endospores appear as unstained voids in Gram staining and as refractile bodies under microscopy. Special stains like malachite green method are required for clear visualization.

Morphology

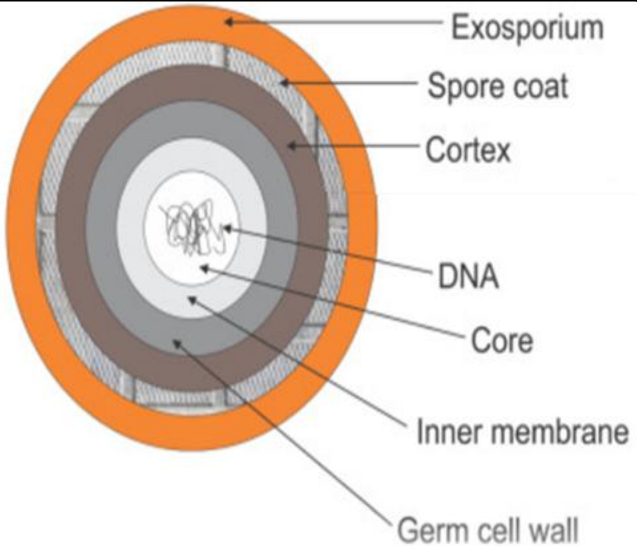
Spores are small, elliptical, or spherical units. They may or may not deform the vegetative cell. Their position within the cell is variable. The spore may be free or attached.



Structure of the spore

Endospore structure differs from the vegetative cell. It has a very hardy and robust structure comprised of many layers:

- **The core:** it has a homogeneous texture and contains all essential cellular structures, including ribosomes and DNA. It is notably poor in RNA, enzymes, and water.
- **The inner membrane**: This membrane contains lipids similar to those of the vegetative cell but features different proteins, resulting in reduced permeability.
- **The germ cell wall:** Its structure is identical to the peptidoglycan found in the cell wall of a vegetative bacterial cell. When the spore germinates, this layer will become the new cell wall of the growing bacterium.
- **The cortex**: it is a thick layer composed of modified peptidoglycan (less cross-linked peptidoglycan which contains almost all of a specific spore component: muramic-δ-lactam)
- **The coat** which consists of proteins arranged in fine concentric layers that act as a barrier, limiting the permeability of high-molecular-weight molecules such as enzymes.
- **The exosporium**: Present in some bacteria, this outermost surface layer may serve several functions, including acting as a protective barrier against antibodies and facilitating surface adhesion.



Bacterial sporulation cycle and process

Sporulation cycle includes two phases:

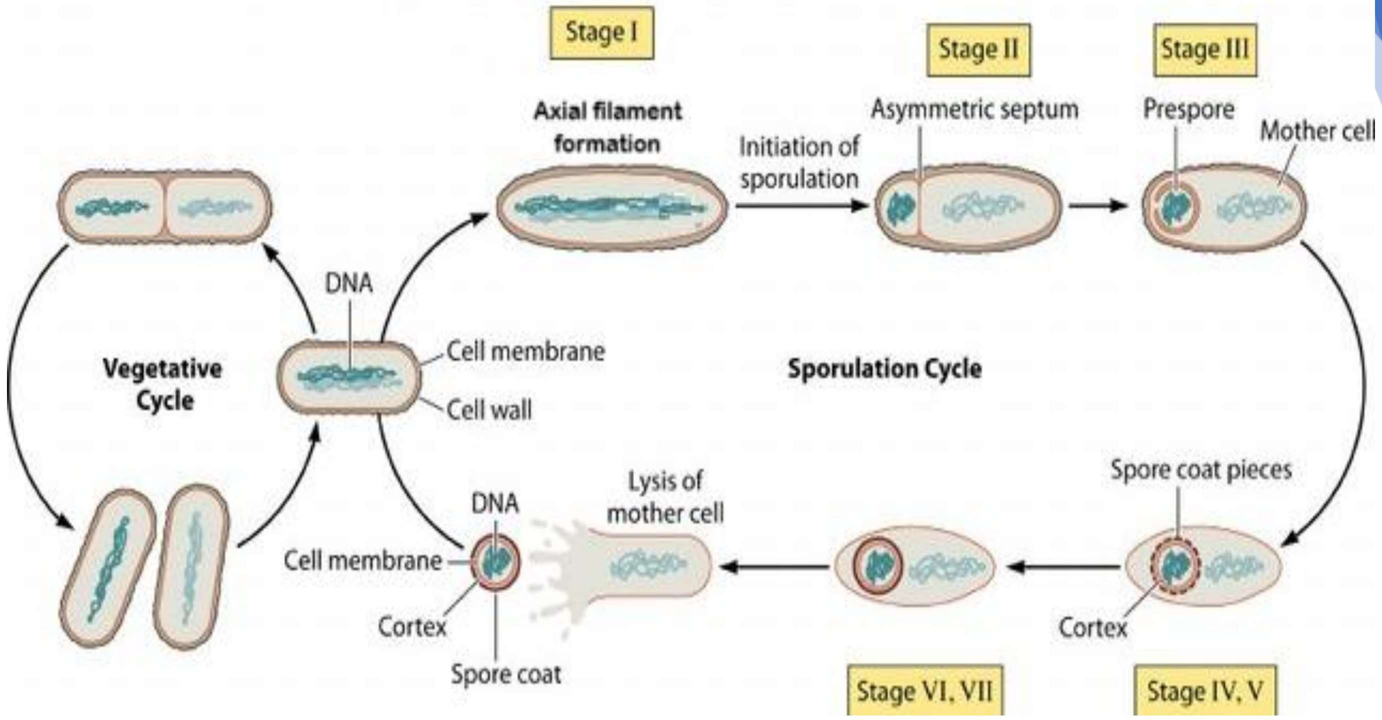
1. **Sporulation**: Transition from the vegetative to the sporulated form.
2. **Germination**: Return to the vegetative form when favourable conditions are restored.

Stages of sporulation

The process typically lasts 7 to 10 hours and follows a strict sequence:

1. Stage I: The two copies of bacterial DNA condense into an axial filament without cell division.
2. Stage II: The cytoplasmic membrane invaginates near one pole, creating an asymmetric septum. The cell is divided into a mother cell and a pre-spore.
3. Stage III: The mother cell engulfs the pre-spore via endocytosis, surrounding it with a double membrane.
4. Stage IV: A modified peptidoglycan cortex forms between the two membranes.
5. Stage V: Protein layers (spore coat) assemble around the cortex, protecting against enzymes, UV radiation, and desiccation.

   In some species, an additional exosporium layer (optional) is synthesized.
6. Stage VI: The spore dehydrates, entering a metabolically dormant state. Dipicolinic acid (DPA) bound to Ca^{2+} and small acid-soluble proteins (SASPs) protect the DNA.
7. Stage VII: The mother cell is lysed by autolytic enzymes, releasing the mature spore into the environment.



Germination

When the spore is placed in favourable growth conditions, it undergoes a series of progressive transformations and becomes a new vegetative cell. This process includes three stages:

1) Activation: This step is triggered by specific stimuli including moderate heat, nutrient availability, pH changes, high water content, chemical treatments (e.g., lysozyme), or mechanical stress (abrasion). It primes the endospore for germination without visible morphological changes.

Note: Thermal activation is utilized in the process of tyndallization.

2) Initiation: An autolytic process occurring only under favourable conditions, characterized by:

- Release of calcium dipicolinate (DPA)
- Water uptake (core rehydration), causing spore swelling.
- Cortical peptidoglycan degradation by lytic enzymes
- Loss of spore refractility and resistance

3) Outgrowth: The germinated spore develops into a vegetative cell through:

- Metabolic reactivation (RNA/protein synthesis, respiration)
- Cell wall regeneration and division capacity restoration