Chapter II: The bacterial cell (continued)

The bacterial chromosome

Morphology

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

The chromosomal DNA consists of a double-stranded circular DNA helix. This supercoiled DNA is tightly packed in the cytoplasm through the action of topoisomerases.

The bacterial DNA is organized into 50 to 100 supercoiled loops, 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. Instead, it is attached to the cytoplasmic membrane.

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.

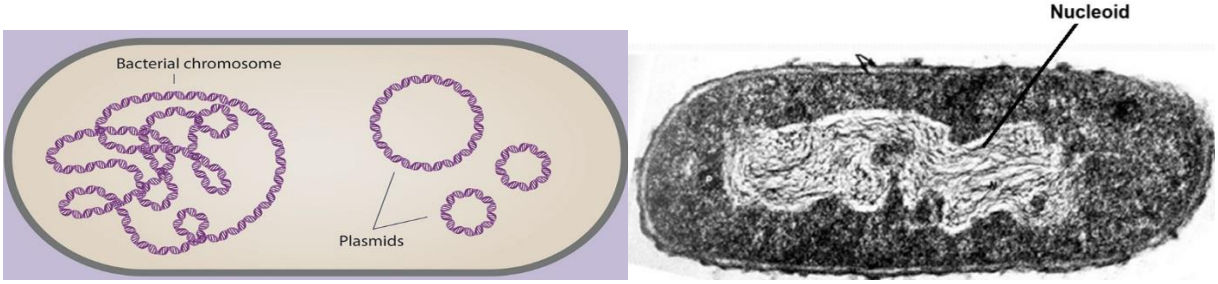


Figure 14: The bacterial chromosome

Some prokaryotes contain up to 4 chromosomes of unequal size. For example, the genomes of *Vibrio cholerae* and *Brucella melitensis* species contain two circular chromosomes.

Chemical Composition

Chemical analysis of the nuclear apparatus indicates that it is composed of:

- 60% DNA (the chromosome),
- 30% ribonucleic acid RNA (structural role),
- 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 three components:

- A purine base (Adenine A and Guanine G) or a pyrimidine base (Cytosine C and Thymine T),
- A sugar (deoxyribose),
- A phosphate group: a diester phosphate at the 3′ and 5′ positions of the deoxyribose.

The DNA molecule is highly rich in negative electrical charges (due to phosphate residues), but it is complexed with Mg^{2+} and Ca^{2+} cations and basic proteins (polyamines or P proteins, similar to histones in eukaryotic DNA), ensuring electrical neutrality and DNA stability.

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

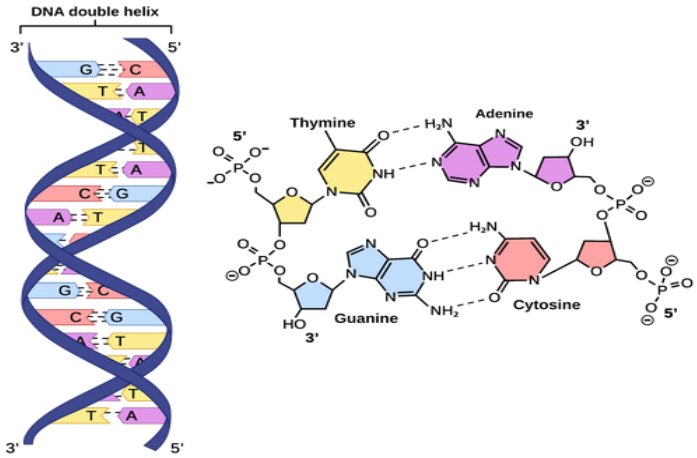


Figure 15 : Structure of DNA

Functions

The bacterial chromosome carries all the genetic information necessary for metabolism, structural integrity, and cellular functions. It consists of **coding genes** (e.g., ~4,300 genes in *Escherichia coli* for protein synthesis) and **non-coding sequences** (~12% of the genome). Gene expression involves:

- Transcription: A specific DNA strand is copied into mRNA, which carries genetic instructions.
- Translation: Ribosomes read the mRNA sequence, while tRNA brings the correct amino acids to form proteins.

Unlike eukaryotes, bacterial genes are often organized into **operons**—clusters of functionally related genes controlled by a single promoter. For example, an operon may be responsible for the degradation of a carbohydrate or the synthesis of an amino acid.

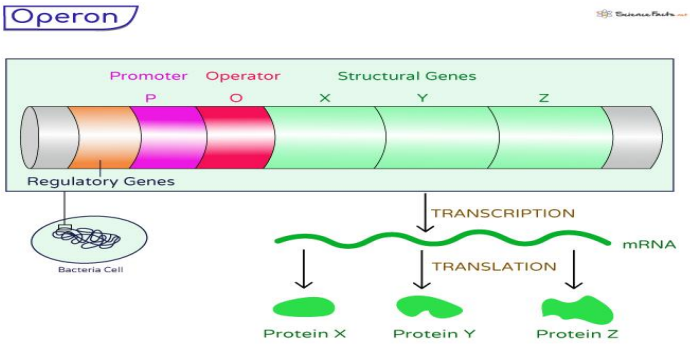


Figure 16: The operon

Replication

In bacteria, chromosome replication must occur once per cell cycle. Under optimal growth conditions, *Escherichia coli* divides every 20 minutes; the genetic material must be duplicated before cell division. Replication is **semi-conservative**. The replication of bacterial DNA follows this mechanism:

a. Initiation point "origin of replication"

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" immediately coat the exposed strands to prevent reannealing. A **topoisomerase** relieves torsional stress ahead of the fork. This step enables helicase activity by preventing DNA supercoiling and breakage during replication bubble formation.

b. Elongation

A **DNA Primase** which is a DNA-dependent **RNA Polymerase** synthesizes a short RNA fragment called **primer**, needed to initiate DNA synthesis. **DNA polymerase III** then extends these primers by adding complementary nucleotides to the 3'OH end of a nucleic acid.

DNA replication is **continuous** on the **leading strand** (synthesized 5′→3′ toward the fork) but **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. This causes a slight delay, justifying the "**lagging**" designation.

Then, **DNA polymerase I** removes the RNA primers through its 5'→3' exonuclease activity and replaces them with DNA using its polymerase function.

Afterward, **DNA ligase** joins the Okazaki fragments by forming phosphodiester bonds, completing lagging strand synthesis.

c. Termination

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

The completed circular chromosomes remain temporarily interlinked as catenanes. **Topoisomerase IV** resolves these interlocked circles by introducing transient double-strand breaks, allowing separation of the daughter chromosomes.

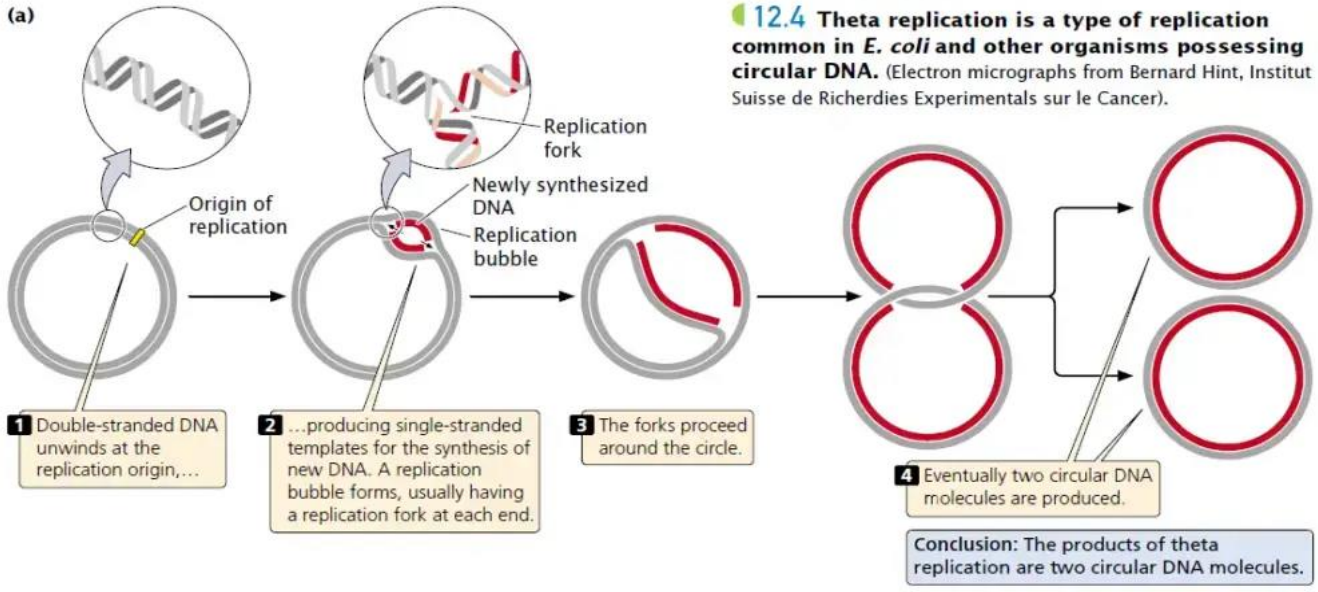


Figure 17: Theta (θ) replication in circular bacterial DNA

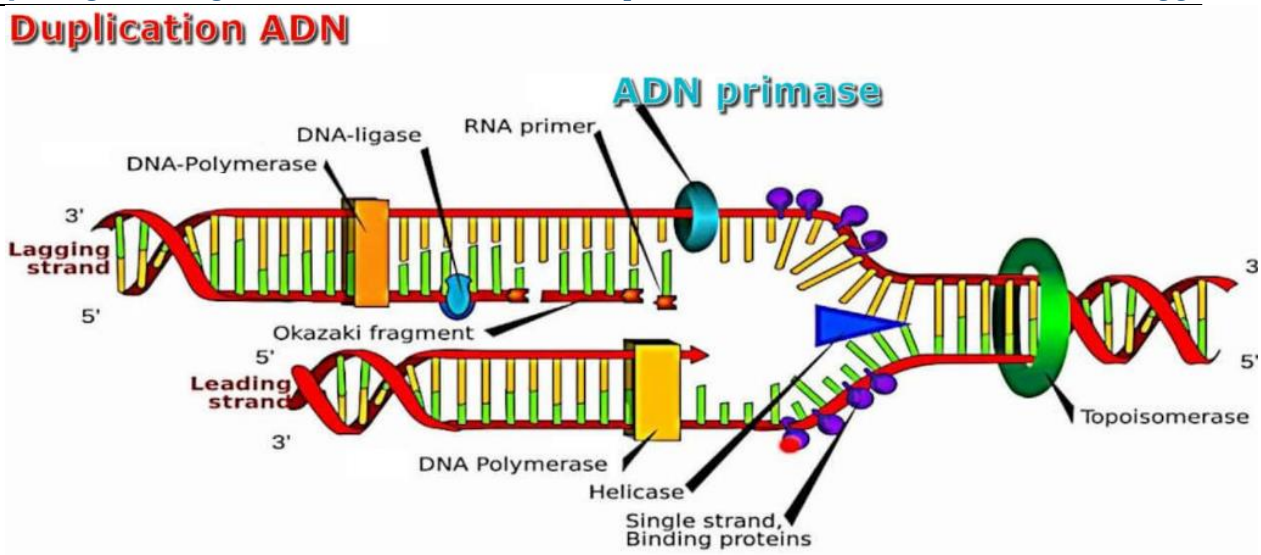


Figure 18: DNA Replication process -Enzymatic machinery

Plasmid DNA

In addition to the genetic material present in the nucleoid, many bacteria possess extra-chromosomal DNA molecules called **plasmids**.

Plasmids are not essential for a host cell's survival or normal metabolism, as bacteria can live without them. However, the genes they carry 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. The size of plasmids varies greatly. The **F plasmid** of *Escherichia coli* is relatively average in this regard, representing about 1% of the size of the *E. coli* chromosome.

Plasmids have relatively few genes, typically fewer than 30.

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

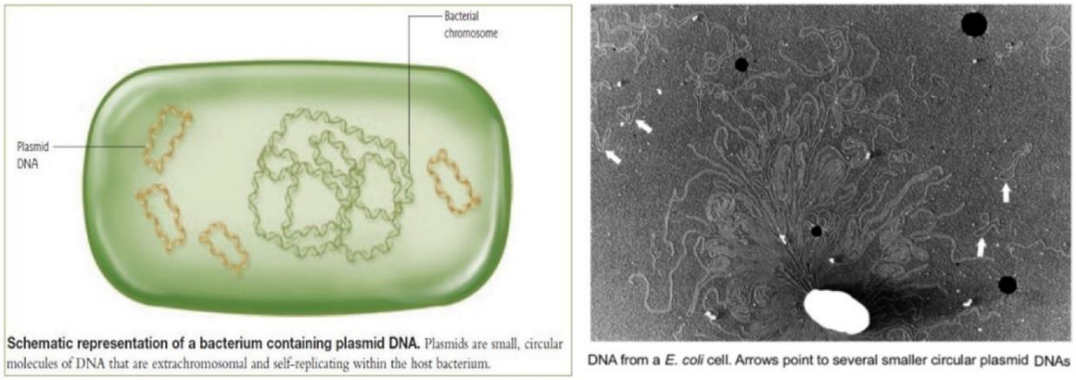


Figure 19: Plasmids

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 process similar to that used to copy the bacterial chromosome (**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 then 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).

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

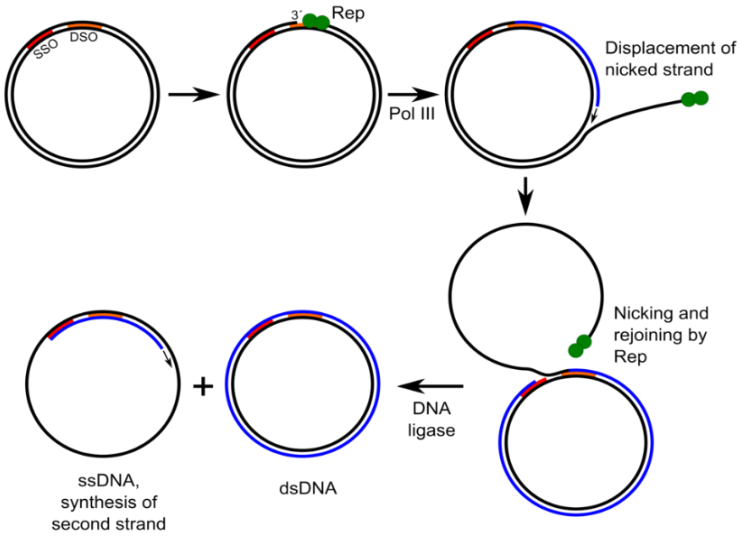


Figure 20: Rolling circle replication.

Properties encoded by plasmids

- **Conjugative plasmid (F plasmid or Fertility factor)**

This plasmid 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.

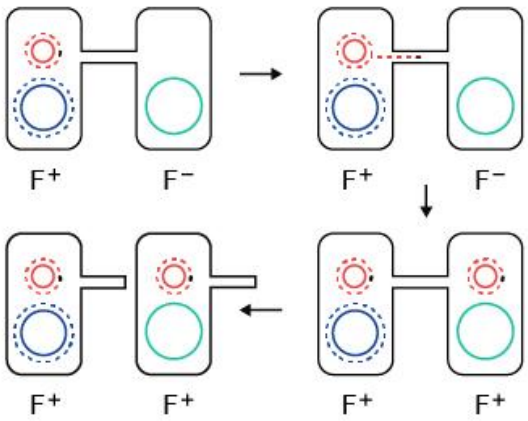


Figure 21: The transfer and replication of the F plasmid during conjugation.

- **Resistance plasmids (R factors)**

They carry genes providing resistance to antibiotics and heavy metals. First identified in *Shigella dysenteriae* strains from Japan in 1959, these plasmids can spread resistance through multiple mechanisms including target modification, enzymatic inactivation, and membrane permeability alterations. Critically, these resistance genes were later found in *Escherichia coli.*

- **Metabolic plasmids**

Metabolic plasmids expand bacterial metabolic capabilities by encoding enzymes for specialized functions. Examples include citrate utilization in E. coli, lactose degradation in *Salmonella*, and urea hydrolysis. These plasmids allow bacteria to exploit alternative nutrient sources in their environment.

- **Virulence plasmids**

Virulence plasmids enhance pathogenicity by encoding virulence factors. For example:

- Enterotoxigenic *E. coli* (ETEC) carries plasmids for colonization factors and toxins
- *Shigella's* invasiveness determinants are plasmid-encoded (*pInv*).
- *Salmonella* virulence plasmids encode adhesins that facilitate attachment to host cells.

- **Bacteriocin and biocide/pollutant resistance plasmids**

Bacteriocin plasmids produce toxic proteins (e.g., colicins in *E. coli*) 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.

During cell division, plasmids are distributed randomly into daughter cells (unlike chromosomes). Plasmids can be eliminated from host cells. This **curing** occurs spontaneously or can be induced by treatments that inhibit plasmid replication without affecting the reproduction of the host cell.

- **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.

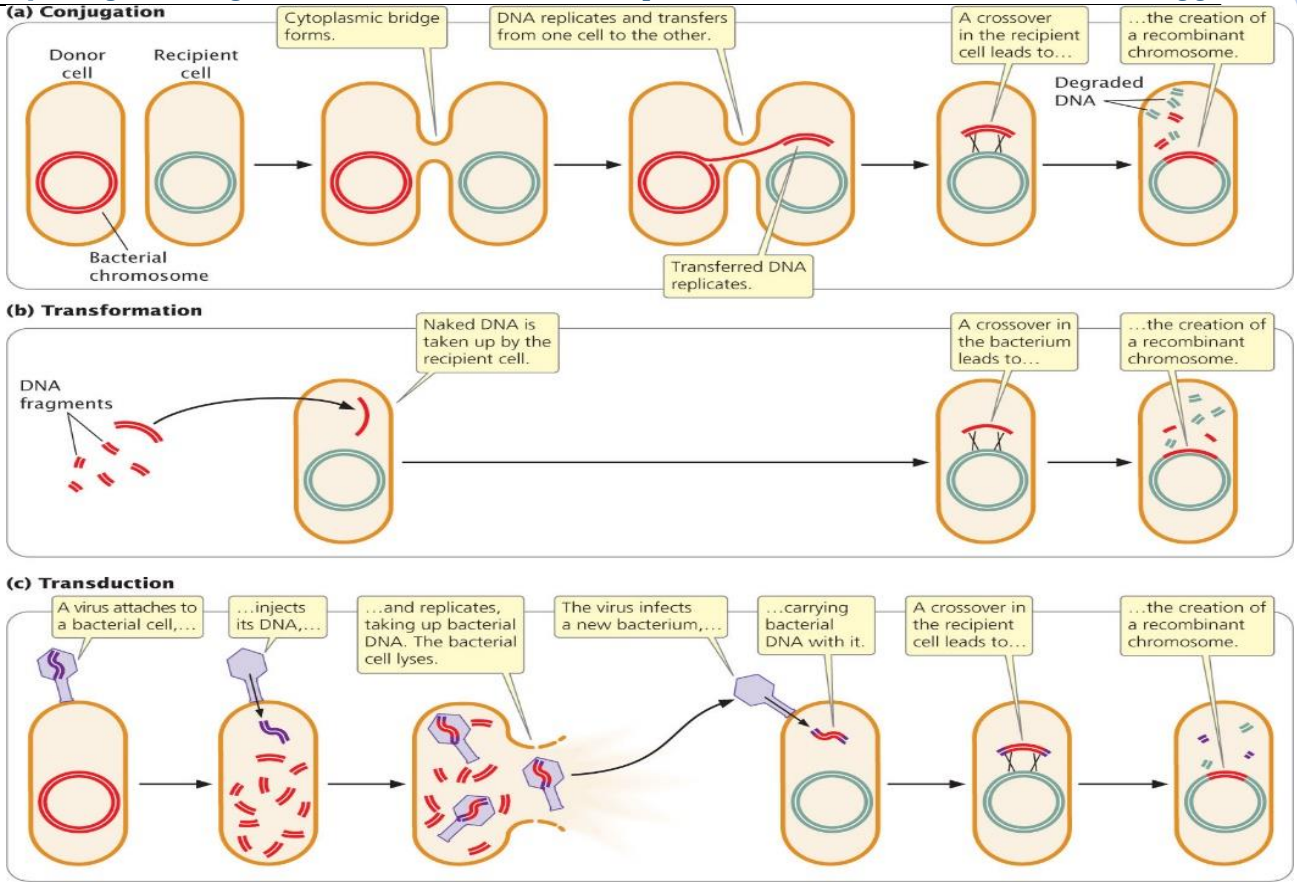


Figure 22: Mechanisms of horizontal gene transfer in bacteria

Pili

Structure

Many Gram-negative bacteria (and rarely some Gram-positive 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 hollow protein filaments that protrude from the outer surface of bacteria. 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 regularly on the bacterial surface. They are formed by the polymerization of a single polypeptide subunit, **pilin**, assembled with minor polypeptides including **adhesin**. Pili enable some bacteria to attach to mucous membranes, which is essential for their pathogenicity.

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. These sex pili also serve as receptors for specific bacteriophages.

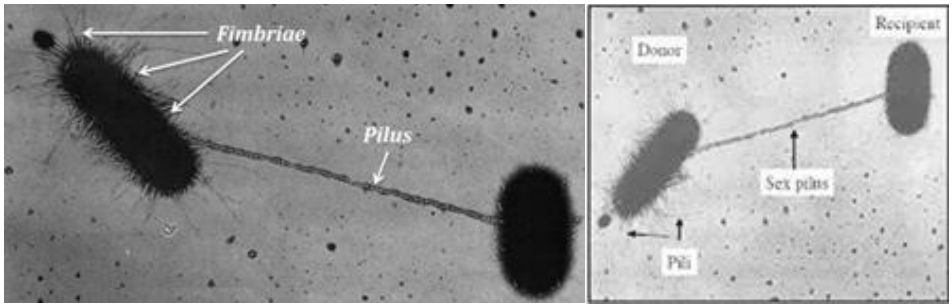


Figure 23: Pili and fimbriae in bacteria

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 (e.g., *Klebsiella pneumoniae*). 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.

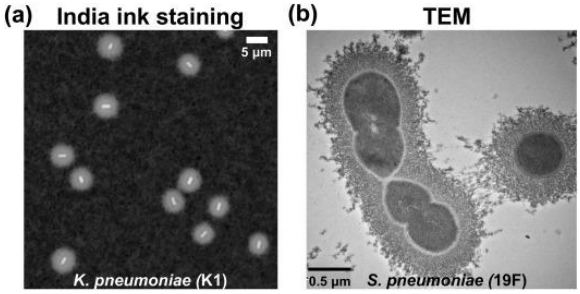


Figure 24: Bacterial capsule

Composition

Most capsules are composed of polysaccharides, although some are made of polypeptides. Capsular polysaccharides are highly hydrated (over 95% water) and bind through covalent bonds to phospholipids or lipid A of the outer membrane in Gram-negative and to the peptidoglycan in Gram-positive bacteria.

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 (e.g., encapsulated *Streptococcus pneumoniae* causes fatal septicemia in mice, unlike non-encapsulated strains).

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

✓ Resists proteolytic enzymes.

- **Adhesion factor:**

✓ Enables attachment to surfaces (e.g., *Streptococcus mutans* adheres to teeth to form biofilms).
✓ Facilitates colonization of specific niches or inert surfaces.

- **Protection against desiccation:** it acts as a water reservoir to prevent drying out.

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, such as *Candidatus Ovobacter propellens*, have more than 400 flagella. The position of flagella on the bacterium can also vary. The following types of flagellar arrangements are distinguished:

- Monotrichous bacteria have a single flagellum (e.g., *Vibrio cholerae*).
- Lophotrichous bacteria have multiple flagella located at the same spot on the bacteria's surfaces which act in concert to drive the bacteria in a single direction.
- Amphitrichous bacteria have flagella on each of two opposite ends.
- Peritrichous bacteria have flagella projecting in all directions (e.g., *E. coli*).

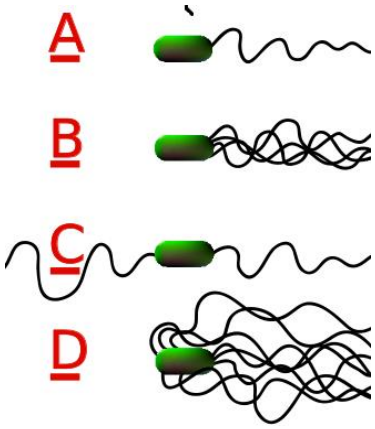
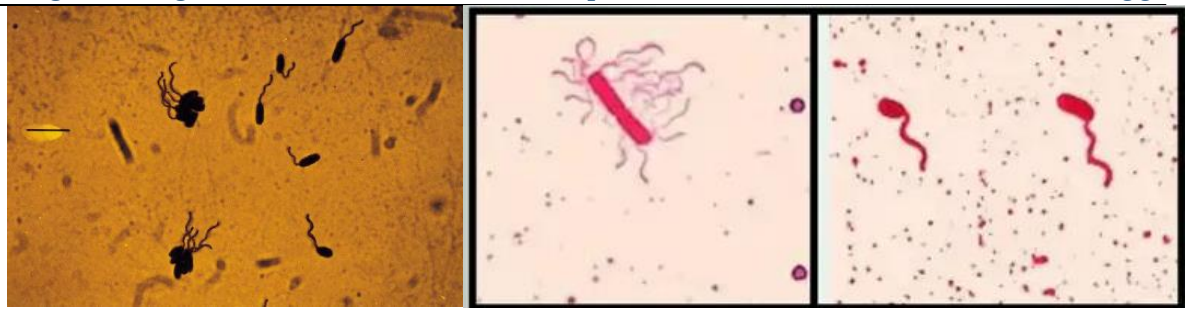


Figure 25: Examples of bacterial flagella arrangement schemes.
A-Monotrichous; B-Lophotrichous; C-Amphitrichous; D-Peritrichous.

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**

Figure 26 : Flagella staining techniques

Structure

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

1. **The helical 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 at speeds of up to 300 rotations per second in *Escherichia coli*. 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** is inserted into the outer membrane.
  - **P ring** is embedded in the peptidoglycan layer.
  - **MS ring** is located in the cytoplasmic membrane.
  - **C ring** is found in the cytoplasm.

– **In Gram-positive bacteria** (which lack an outer membrane): only **the MS and C rings** are present.

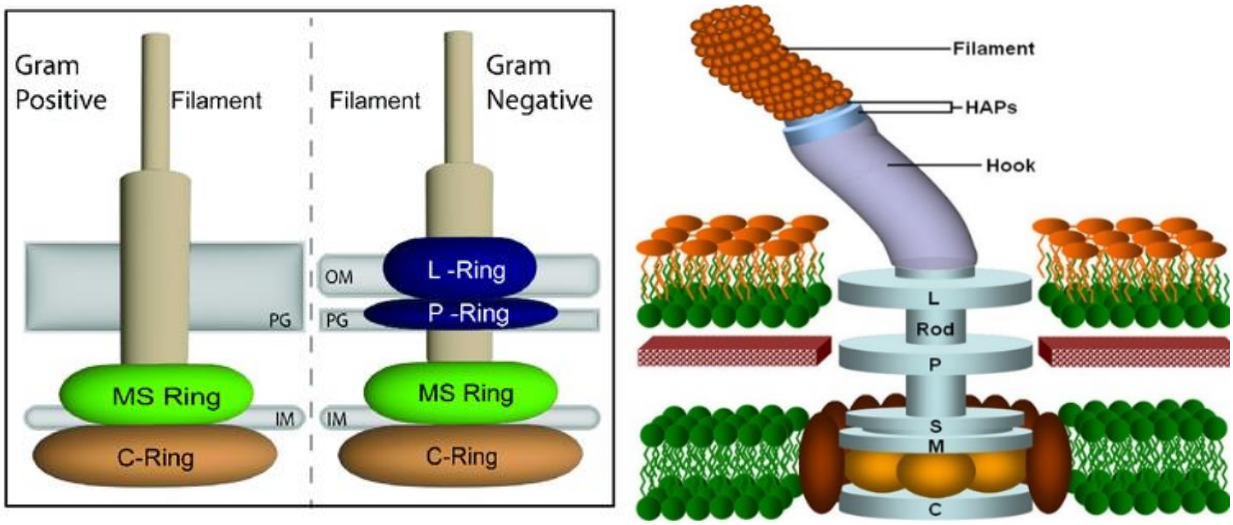


Figure 27: Structure of bacterial flagellum

Functions of the flagellum

- **Locomotion**: Bacteria move actively in different environments due to flagellar rotation.
- In liquid media, they exhibit swimming motility, propelling themselves through the medium.
- In semi-solid media (*soft agar*), they spread outward as they move through the gel-like structure.
- On solid media, some species, such as *Proteus*, display swarming motility, forming concentric waves as they expand across the surface.

- **Chemotaxis**: Motile bacteria detect and respond to chemical signals, moving toward attractants such as nutrients and away from repellents like toxins or harmful substances.

- **Antigenic role**: Flagellar antigens (*H antigens*) are used to distinguish bacterial serotypes, as in *Salmonella* typing.

Endospore (Spore)

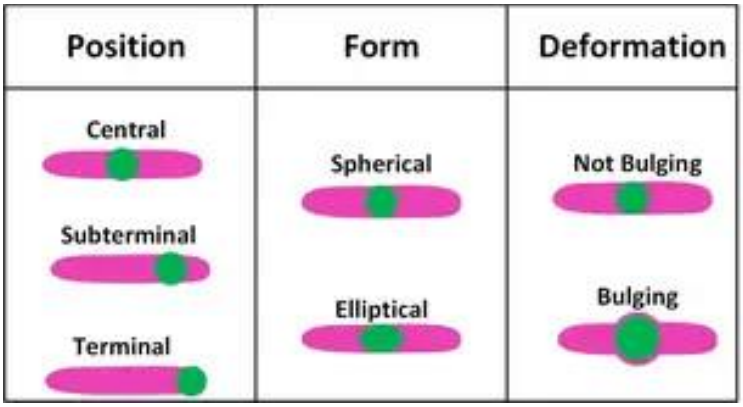
The endospore is an **optional, resistant structure** formed by some Gram-positive bacteria (e.g., *Bacillus*, *Clostridium*, *Sporosarcina*) under unfavorable conditions. It enables bacteria to survive extreme environments while preserving their genetic material. Endospores confer resistance against:

- Nutrient deprivation
- High temperatures (e.g., heat shock)
- Chemical/physical agents (antibiotics, disinfectants)
- Cellular aging
- 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: central, terminal, or subterminal. The spore may be free or attached. These characteristics are studied for taxonomic purposes (helping to identify the species).



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 cortex**: Composed of peptidoglycan with a distinct composition from that of the vegetative cell. The cortex contains nearly all of a specific spore component, dipicolinic acid in the form of calcium dipicolinate.
- **The outer membrane**: it plays a critical role during spore formation.
- **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 surface membrane may serve as a protective barrier against antibodies, as observed in *Bacillus anthracis*.

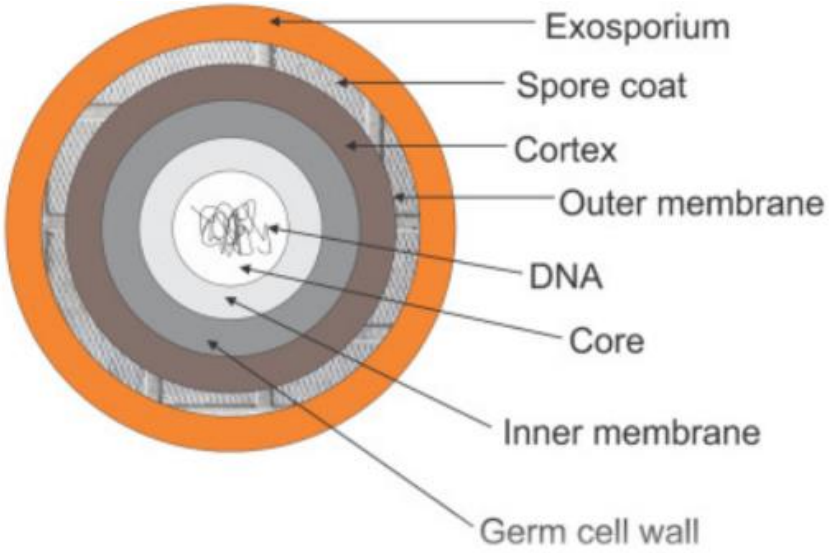


Figure 28: Structure of the endospore

Bacterial sporulation cycle and process

Sporulation is a cellular differentiation mechanism that enables some bacteria (e.g., *Bacillus* and *Clostridium*) to form highly resistant **endospores** in response to environmental stress (e.g., nutrient depletion, oxygen presence or absence depending on the species). This 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 (Axial filament formation): The two copies of bacterial DNA condense into an axial filament without cell division.
2. Stage II (Asymmetric septum): The cytoplasmic membrane invaginates near one pole, creating an asymmetric septum. The cell is divided into:
    - Mother cell (large, metabolically active).
    - Forespore (small, destined to become the spore).
3. Stage III (Engulfment of the forespore): The mother cell engulfs the forespore via endocytosis, surrounding it with a double membrane.
4. Stage IV (Cortex synthesis): A modified peptidoglycan cortex forms between the two membranes. It contains calcium dipicolinate (DPA), which stabilizes DNA and confers heat resistance.

5. Stage V (Coat and exosporium formation): Protein layers (spore coat) assemble around the cortex, protecting against enzymes, UV radiation, and desiccation.

In some species (e.g., *Bacillus anthracis*), an additional exosporium layer (optional) is synthesized.

6. Stage VI (Maturation and dehydration): The spore dehydrates, entering a metabolically dormant state. DPA and small acid-soluble proteins (SASPs) protect the DNA.
7. Stage VII (Spore release): The mother cell is lysed by autolytic enzymes, releasing the mature spore into the environment.

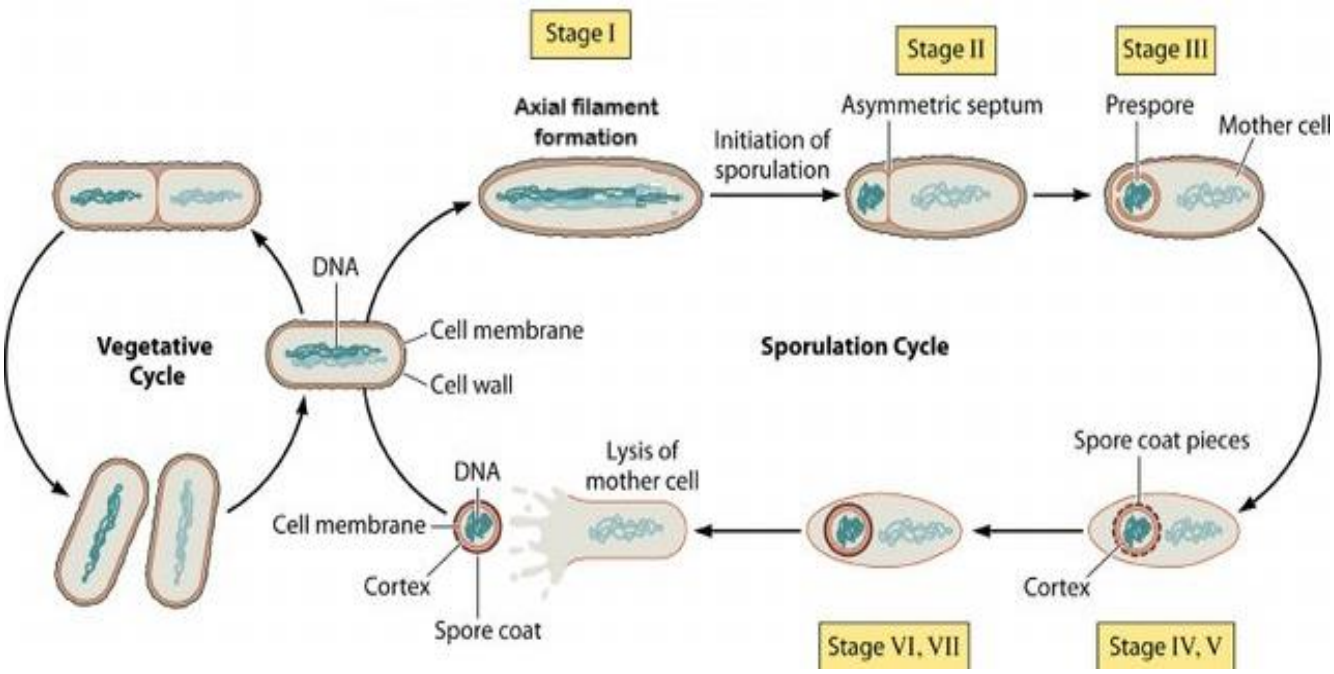


Figure 29: Sporulation cycle

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 (e.g., L-alanine), pH changes, high water content, chemical treatments (acids/lysozyme), or mechanical stress (abrasion). It primes the endospore for germination without visible morphological changes.

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

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