GENERAL BACTERIOLOGY
CHAPTER (2)
BACTERIA: THEIR STRUCTURE & ORGANIZATION

- Bacteria were first **discovered by Leeuwenhoek 1674**
- They are among the most **widely distributed forms of life** → they are **found in air, water** & **soil** → they are also found in or on the **human body, animals & plants**

Bacterial Morphology

Bacteria are differentiated into major categories, based on their morphological features such as:

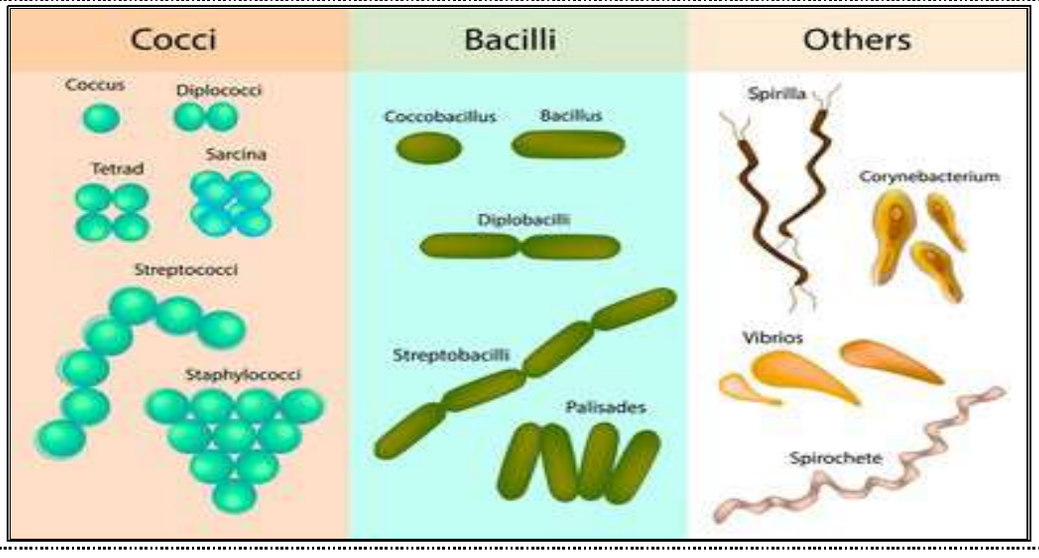
❶ Shape ❷ Size ❸ Arrangement ❹ Staining characteristics

① Bacterial Size

Most bacteria range in size from 0.2-1.2 µm in width and 0.4-14 µm in length

②,③ Bacterial Shape & Arrangement

① Cocci (singular: coccus) → Spherical organisms	② Bacilli (singular: bacillus-stick) → Rod-shaped organisms	③ Spiral bacteria
Diplococci Pairs of cells → e.g. *Neisseria*	These cells may occur Singly, in Pairs or in Chains	Spirilla Rigid
Streptococci Chains of four or more	Some bacilli are short (Coccobacilli)	Spirochaetes Flexible
Staphylococci Irregular grape-like clusters	Others are curved (Vibrios)	



The **arrangement of cells** is **determined by the planes of division**
→ For example, cocci that divide **along a single plane produce diplococci or chains**, e.g. *streptococci*
→ While those that **divide on many planes produce clusters**, e.g. *staphylococci*

④ Staining Characteristics

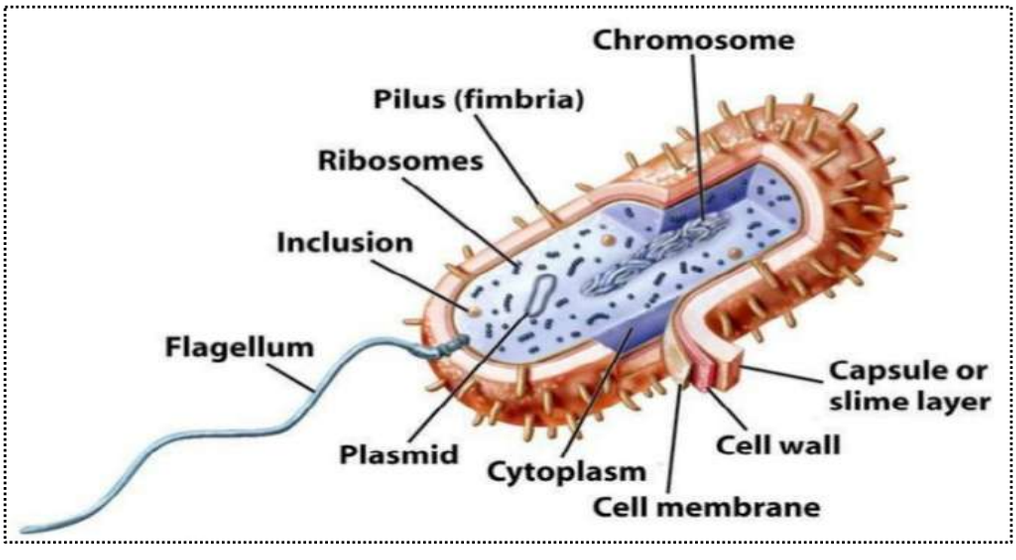
There are two kinds of stains → **simple & differential**

① Simple Stains	② Differential stains
Employ a **single dye** like methylene blue, crystal violet or fuchsin	Require **more than one dye**
Cells & structures stained with them give the **same color**	**Distinguish between different types of bacteria** by giving them **different colors**
Therefore, they only reveal the characteristics of **size, shape & arrangement**	**Gram's stain** **The most important differential stain in clinical microbiology** → it divides bacteria into: ❶ **Gram-positive** (**violet**-staining) ❷ **Gram-negative** (**red** staining) The second important stain is **Ziehl-Neelsen stain** → it is used to identify the **acid-fast bacilli of the genus *Mycobacterium***

Bacterial Ultra-Structures & their Functions

(**Final details** of subcellular structures are **best revealed by electron microscopy**)

- **All bacteria:** have **nucleoid**, **ribosomes** & **cytoplasmic membrane**
- **Most bacteria:** also have **cell wall**
- **Some bacteria:**
  ❶ Are further enveloped by **capsule** or **slime layer**
  ❷ Have also **cytoplasmic inclusions** & **various appendages as flagella** & **pili**



① Cytoplasm

Few morphologically distinct components can be found within the cytoplasm:

① Nucleoid:

- Genetic information of bacterial cell is contained in **single circular molecule of double-stranded DNA**, → which constitutes the **bacterial chromosome**
- It is **1 mm long** and is **packed into supercoiled state** inside the cell

② Plasmids:

In many bacteria, **additional genetic information** is contained on **plasmids** → which are **small circular extra-chromosomal DNA molecules** that can **replicate independently of the chromosome**

③ Ribosomes:

- They are the **site of protein synthesis** in the cell
- Ribosomes consist of **protein** & **RNA**
- **Prokaryotic ribosomes** have **sedimentation constant of 70S → smaller** than the **80S ribosomes of eukaryotes** → this **difference** makes **bacterial ribosomes a selective target for antibiotic action**

④ Inclusion granules:

- These are **granules of nutrient materials** → usually phosphates, sulphur, carbohydrates & lipids
- **Energy reserves** are usually **stored as glycogen**, **starch** or **poly-β-hydroxybutyrate**
- **Phosphate** is stored in **metachromatic or volutin granules** → which are **used for synthesis of ATP**

⑤ Mesosomes:

- These are **complex invagination of the cytoplasmic membrane**
- They are **involved in cell division** & **sporulation**
- They also have **function analogous to the mitochondria in eukaryotes** → providing a **membranous support for respiratory enzymes**

② Cytoplasmic Membrane

- In bacteria, as in other cells, the protoplast is **limited externally** by **thin elastic cytoplasmic membrane**
- It is **phospholipid protein bilayer similar to** that of **eukaryotic cells** → except that, **in bacteria, it lacks sterols**
- **It has the following functions:**

① Selective transport: in bacteria, molecules move across the cytoplasmic membrane by:

❶ Simple diffusion ❷ Facilitated diffusion ❸ Active transport

② Excretion of extracellular enzymes:

① Hydrolytic enzymes: → which **digest large food molecules into subunits small** enough to penetrate the cytoplasmic membrane

② Enzymes used to destroy harmful chemicals: → such as **antibiotics**, e.g. **penicillin-degrading enzymes**

③ Respiration: → The **respiratory enzymes are located in the cytoplasmic membrane**
→ Which is thus **functional analogue of the mitochondria** in eukaryotes

④ Cell wall biosynthesis: → the cytoplasmic membrane is **the site of:**
❶ The **enzymes of cell wall biosynthesis**
❷ The **carrier lipids** on which the **subunits of the cell wall are assembled**

⑤ Reproduction:
❶ **Specific protein** in the membrane **attaches to DNA** & **separates duplicated chromosomes from each other**
❷ **Septum forms** by cytoplasmic membrane to **separate the cytoplasm of the two daughter cells**

⑥ Chemotactic system:
Attractants & repellants bind to **specific receptors** in the cytoplasmic membrane → and **send signals to the cell's interior** → cell then **responds to the surface message**

③ Cell Wall

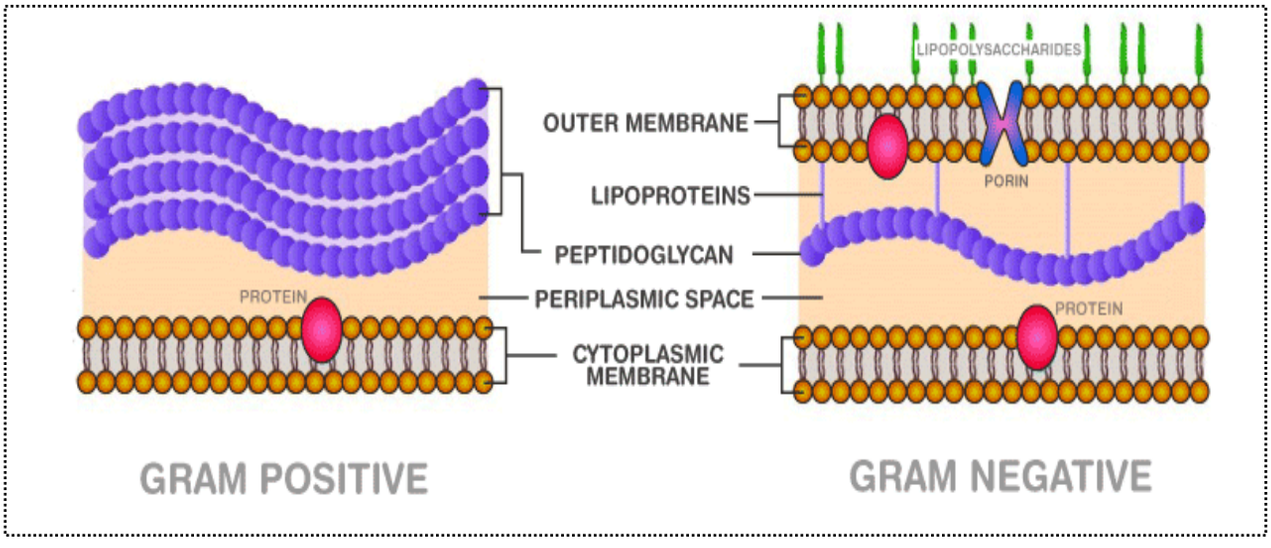
- The bacterial cell wall is the **structure** that **immediately surrounds the cytoplasmic membrane**
- It is **10-25 nm** thick
- **Strong** & relatively **rigid** → though having **some elasticity**

Structure of the cell wall:

∗ The cell wall of bacteria is a **complex structure**
∗ Its **impressive strength** is primarily due to **peptidoglycan** (PG), (**murein** or **mucopeptide**)

∗ **Peptidoglycan** is complex **polymer** consisting of **N-acetylglucosamine (NAG)** & **N-acetylmuramic acid (NAM)** unique to bacteria → set of **identical tetra-peptide side chains** are **attached to NAM**

∗ **Besides PG, additional components in the cell wall divide bacteria into Gram +ve & Gram -ve:**



	① Gram-positive cell wall	② Gram-negative cell wall
Peptidoglycan	• There are as many as **40 sheets of peptidoglycan** → comprising up to **50% of the cell wall material** • Despite the thickness of peptidoglycan, **chemicals can readily pass through**	• It is **much thinner** • Composed of only **one or two sheets** → comprising **5-10% of the cell wall material**
Teichoic acids	• Polymer of **ribitol or glycerol phosphate** • They are found in **cell wall of most Gram-positive bacteria** • Teichoic acids & cell wall associated proteins are **major surface antigens of Gram +ve bacteria**	Not present
Outer membrane	Not present	• **Phospholipid protein bilayer** present **external to the peptidoglycan layer** • **Outer surface** of lipid bilayer is composed of molecules of **lipopolysaccharide (LPS)** → which consists of **complex lipid called lipid A** chemically **linked to polysaccharides** ❶ **Lipid A** of the LPS → forms the **endotoxin of the Gram-negative bacteria** ❷ **Polysaccharides** are the outermost molecules of the cell wall & are major **surface antigens of Gram -ve bacterial cell (somatic or O antigen)**
Periplasmic space	Not present	• **Space between** cytoplasmic & outer membranes • **It contains:** ❶ Peptidoglycan layer ❷ Gel-like solution of proteins

Functions of the cell wall:

❶ Maintains the **characteristic shape** of the bacterium
❷ **Supports the weak cytoplasmic membrane against high internal osmotic pressure** of protoplasm (5-25 atm.)
❸ Plays an important role in **cell division**
❹ Responsible for the **staining affinity of the organism**

Wall deficient variants:

① Mycoplasma

• The **only** group of **bacteria** that **exists naturally without cell wall**

• **Because they lack a rigid cell wall:**
❶ Mycoplasmas do **not assume a defined recognizable shape**
❷ These organisms are **naturally resistant to cell wall inhibitors** (as penicillins & cephalosporins)

② L-Forms

- They are **wall defective** or **wall deficient bacteria**
- "L" stands for **Lister Institute in London** → where they **were first discovered**
- L-forms may **develop from cells that normally possess cell wall** → when they are exposed to **hydrolysis by lysozyme** or by blocking peptidoglycan biosynthesis with **antibiotics (such as penicillin)** → provided that they are **present in isotonic medium**
- Some **L. forms resynthesize their walls** once the **inducing stimulus is removed** → their **reversion** to the **walled state** can produce **relapses of overt infection**
- Others, however, **permanently lose the capacity to produce cell wall**
- L. forms may **survive antibiotic therapy**

④ Capsule & Related Structures

- Many bacteria synthesize large amount of **extracellular polymer** that **collects outside the cell wall** to form **additional surface layer** → this layer is **formed only inside the host** (**in-vivo**)
- With **one known exception** (the **polypeptide capsule of *Bacillus anthracis***), the **extracellular material** is made of **polysaccharides**

<table>
<tr><th></th><th>① Capsule</th><th>② Glycocalyx</th><th>③ Slime layer</th></tr>
<tr><td>Structure</td><td>Layer that adheres to cell surface & forms well-defined halo when differentially stained, to be resolved with L/M</td><td>Loose meshwork of polysaccharide-fibrils extending outwards from the cell</td><td>Surface layer that is loosely distributed around the cell</td></tr>
<tr><td rowspan="3">Functions</td><td>❶ Protects bacterial cell from phagocytosis → hence, the capsule is considered important virulence factor</td><td></td><td></td></tr>
<tr><td colspan="2">❷ Some bacteria attach to target surface by using their capsules or glycocalyx in order to establish infection → For instance, Streptococcus mutans form glycocalyx, with which the bacteria stick to the tooth enamel</td><td></td></tr>
<tr><td colspan="3">❸ Protects the cell wall against various kinds of anti-bacterial agents → e.g. bacteriophages, colicins, complement & lysozymes</td></tr>
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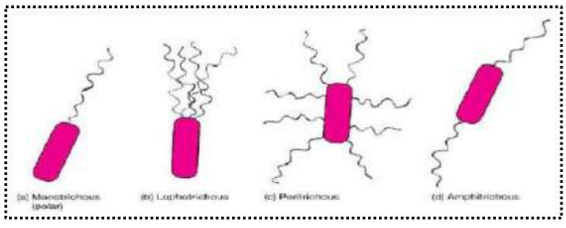
⑤ Appendages

- Several structures project through the cell wall of bacteria to **form surface appendages**
- The most commonly observed are **flagella & pili**

	① Flagella	② Pill or fimbriae (singular: pilus)
Diameter	**Only 20 nm in diameter** → **too small to** be detected by **light microscope** → they **can be demonstrated clearly** with the **electron microscope**	**Shorter & thinner** than flagella → can be **observed only** by the **electron microscope**
Structure	Flagella **consist of protein** called **flagellin** → which **differs in different bacterial species** → the **flagellins are highly antigenic** (**H antigen**)	**Protein tubes** that extend from the cells → they are composed of **protein subunits** termed **pilins**
Location & number	**Location & number of flagella on a cell vary according to bacterial species → Organisms may be:** ❶ **Monotrichous** (single polar flagellum), ❷ **Lophotrichous** (multiple polar flagella), ❸ **Peritrichous** (flagella distributed over the entire cell surface) 	**Short pili (fimbriae)** → distributed over the entire cell surface
Functions	Many bacteria **move** by means of flagella → Motile bacteria **migrate towards** regions where there is **higher concentration** of **nutrients** & solutes (process known as **chemotaxis**) & **away from disinfecting substances** (**negative chemotaxis**) **Axial filaments** • Structurally & chemically, fibres of axial filaments are **similar to flagella** & they are **sometimes called "endoflagella"** • **Spirochaetes move** by means of these axial filaments • These structures are **composed of two groups of fibres** that **originate within the opposite ends** of the cell and **overlap in the middle** → when the cell moves, it rotates around its longitudinal axis & flexes & bends along its length	**❶ Adherence** ∗ It is the function of the **short pili (fimbriae)** that occur in great numbers around the cell ∗ They **enable bacteria to attach** to the surfaces → thus contributing to the **establishment of infection** → **virulence factor** ∗ For example, ***N. gonorrhoeae***, withstands the flushing action of urine by adhering to urethral mucosa **❷ Conjugation** **Special long pilus** called **sex pilus (F pilus)** is involved in **transfer of DNA between bacteria** → process known as **conjugation**

Bacterial Spores (Endospores)

Some bacteria (***Bacillus*** & ***Clostridium***) develop **highly resistant resting phase** or **endospore** that does **not grow or reproduce**, & exhibits **absolute dormancy**

Sporulation	Germination
Single vegetative bacterium forms **single spore** by a **process called sporulation**	Single vegetative bacterium emerges from a spore during germination
• Sporulation is **triggered by unfavourable environmental conditions:** ❶ Depletion of nutrients ❷ Accumulation of metabolites ❸ Changes in the growth requirements (e.g. temperature, pH, or oxygen tension)	Endospores respond quickly to environmental changes **returning to vegetative state within 15 min.**
• **Cytoplasmic membrane invaginates enclosing section of cytoplasm that contains:** ❶ Bacterial chromosome ❷ Some ribosomes ❸ Other cytoplasmic materials that will be needed for germination • It acquires **thick cortex** & **thin but tough outer spore coat**	**In the process of germination:** ❶ Spores absorb water & swell ❷ The protective coat disintegrates → **single vegetative cell emerges**

Viability & resistance:

- Spores are **much more resistant to** disinfectants, drying and heating
- **Moist heat at 121°C for 10-20 minutes** is **needed to kill spores** → while **60°C is sufficient to kill vegetative forms**
- **Marked resistance of the spores has been attributed to several factors:**
  - ❶ **Thermal resistance** is provided by their **high content of Ca^{2+} & dipicolinic acid** (compound **unique to endospores**)
  - ❷ The **impermeability of their cortex** & **outer coat**
  - ❸ Their **low content of water**
  - ❹ Their **very low metabolic** & **enzymatic activity**

Morphology:

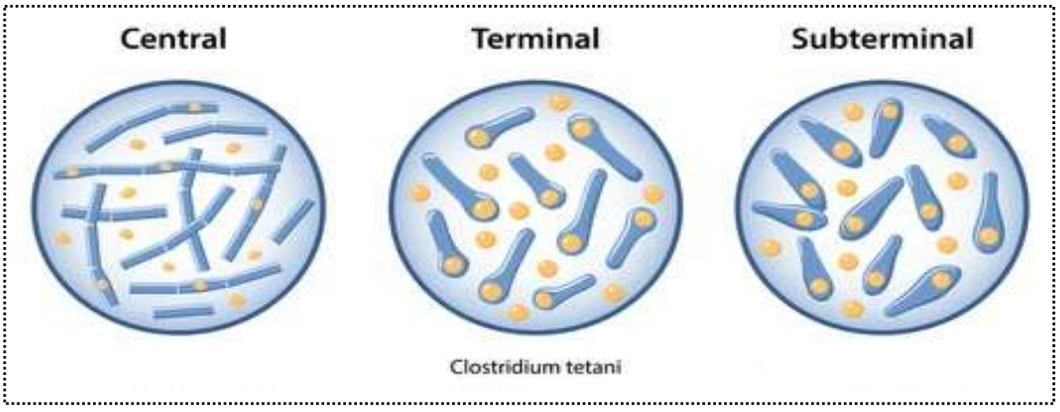
Staining:

- Using Gram's stain, the **spore remains uncoloured** → can be seen as **clear area within stained cell**
- The spores can be **stained using special procedures**

The shape: the spores may be **oval or rounded**

The position: in relation to the body of bacillus → the spore may be **central, terminal or subterminal**

Position & shape of spores are **characteristic of the species** & may help in **microscopic identification of the bacterium**



Test Yourself

1) The following are functions of the cytoplasmic membrane EXCEPT:

a- Respiration b- Cell wall biosynthesis
c- Reproduction d- Staining affinity e- Selective transport

2) Lipid A is a cell wall component of:

a- Gram positive bacteria b- Gram negative bacteria
c- Fungi d- Algae e- Viruses

3) One of the following is a function of the cell wall:

a- Maintaining the characteristic shape of the bacterial cell
b- Selective transport
c- Respiration, since respiratory enzymes are located in it
d- Protein synthesis
e- Excretion of extracellular enzymes

4) All the following are characters of L-forms of bacteria EXCEPT:

a- They are naturally occurring bacteria without cell wall
b- They are resistant to antibiotics which inhibit cell wall synthesis
c- They develop only in isotonic media
d- They can produce relapses of overt infection
e- They may resynthesize the cell wall

5) Bacteria are protected from phagocytosis by:

a- The capsule b- Lipoprotein
c- The mesosome d- The outer membrane e- Peptidoglycan

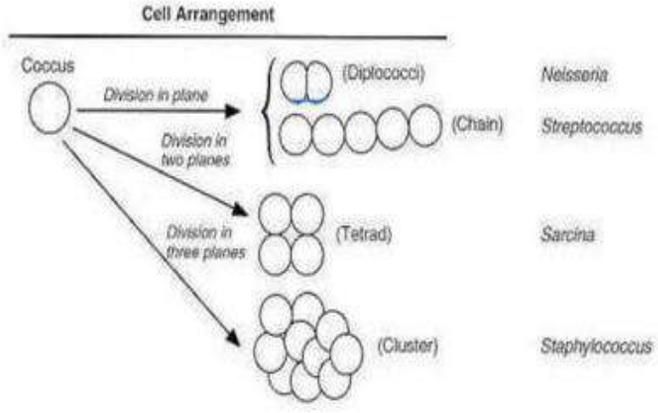
6) All of the following are true concerning pili EXCEPT:

a- They mediate bacterial adherence
b- They may be involved in bacterial conjugation
c- Their antigen is called H antigen
d- They are important virulence factors
e- They are protein in nature

7) The marked resistance of the spores can be attributed to all the following factors EXCEPT:

a- The impermeability of their cortex and outer coat
b- Their ability to resist phagocytosis
c- Their low content of water
d- Their very low metabolic and enzymatic activity
e- Their high content of Ca^{2+} & dipicolinic acid

LECTURE EXTRAS



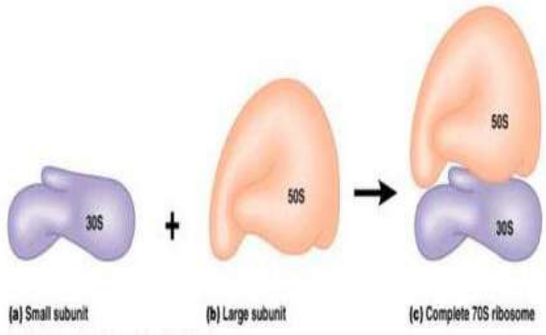
Bacterial Structures

Bacterial Coats	Cytoplasmic structures	External Appendages
Capsule	Nucleoid material	Flagella
Cell wall	Ribosome	Pili (Fimberia)
Cell membrane	Inclusion granules	

2-Ribosomes:

- They are the site of protein synthesis in the cell.
- Ribosomes consist of protein and RNA.
- Prokaryotic ribosomes have sedimentation constant of 70S, smaller than the 80S ribosomes of eukaryotes (S stands for Svedberg unit, a measure of size).
- This difference makes bacterial ribosomes a selective target for antibiotic action.

Prokaryotic ribosomes have sedimentation constant of 70S



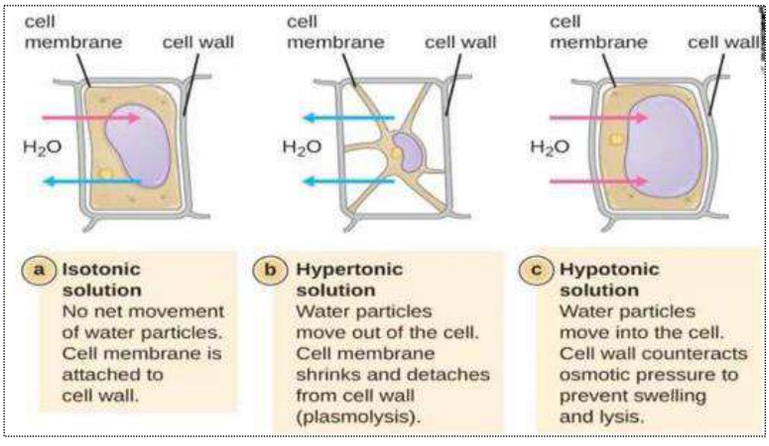
• Functions of the cell wall

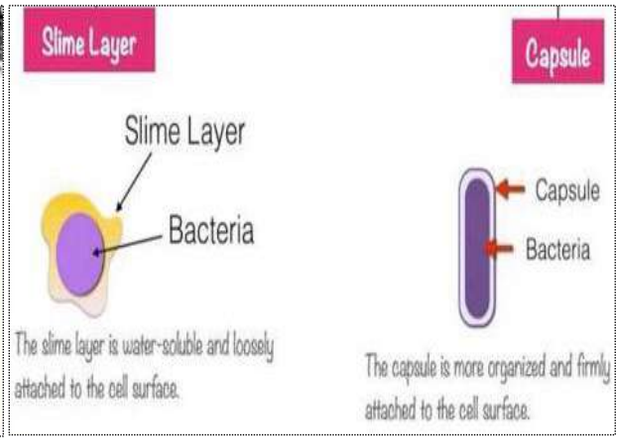
1. It maintains the characteristic **shape** of the bacterium.
2. It **supports** the weak cytoplasmic membrane against the **high internal osmotic pressure** (5-25 atm).
3. It is responsible for the **staining affinity** of the organism.
4. It plays an important role in **cell division**.

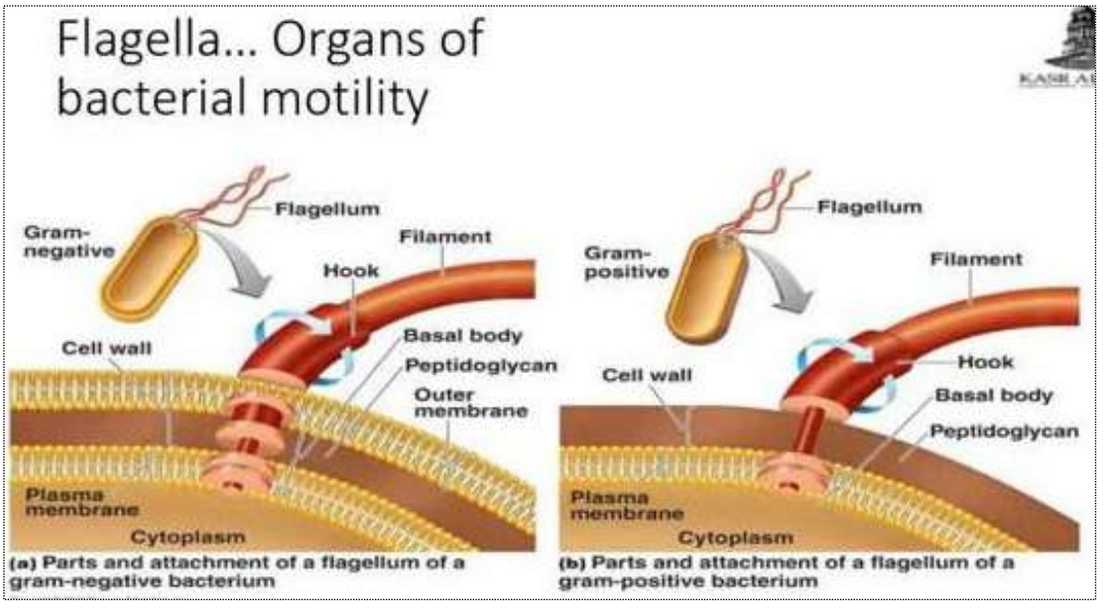
Wall deficient variants (Bacteria without cell wall)

Mycoplasma	"L" Forms
Naturally present	Artificially induced
Can replicate	Can't replicate
Can't reassume a walled form	May (or may not) reassume the walled form upon removal of the causative agents.

- Have no a defined recognizable shape.
- Not stained by gram stain.







Comparison between flagella and pili

	Flagella	Pili
Morphology	Tall, thin & tubular	Short thin & tubular
Composition	Flagellin protein	Pillin protein
Antigenicity	Antigenic	Not antigenic
Functions	Motility of the organism	-Attachment to the host - Gene transfer by conjugation

	Capsule	Spore
Site of formation	Invivo (inside the host)	Invitro (in the environment
Metabolic activity and replication	The organism is active and can replicate	The organism is inactive and can't replicate
Functions	Adhesion to the host, protection and antiphagocytic	Protection from bad conditions of the environment

Bacterial Ultra-structures

Essential structures	Additional structures
Nucleoid material	Flagella
Cytoplasm & cytoplasmic organelles	Pili (Fimberia)
Cytoplasmic membrane	Capsule
Cell wall	Spore