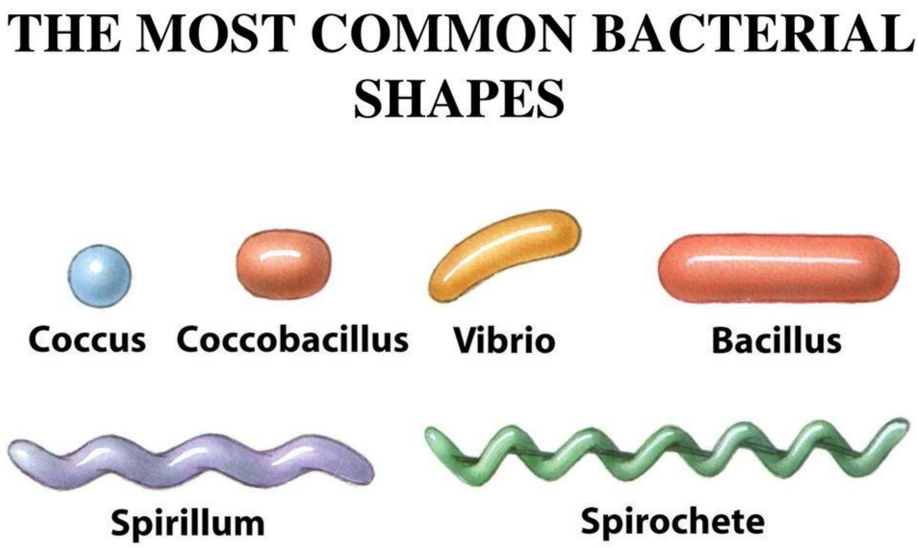
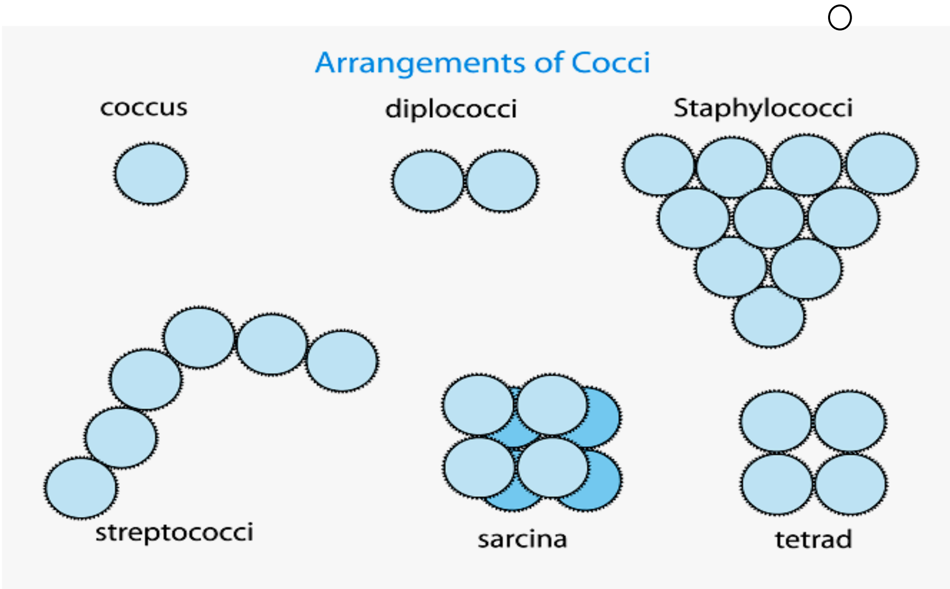
Unit – I   Bacterial morphology and outer ultrastructure of cell

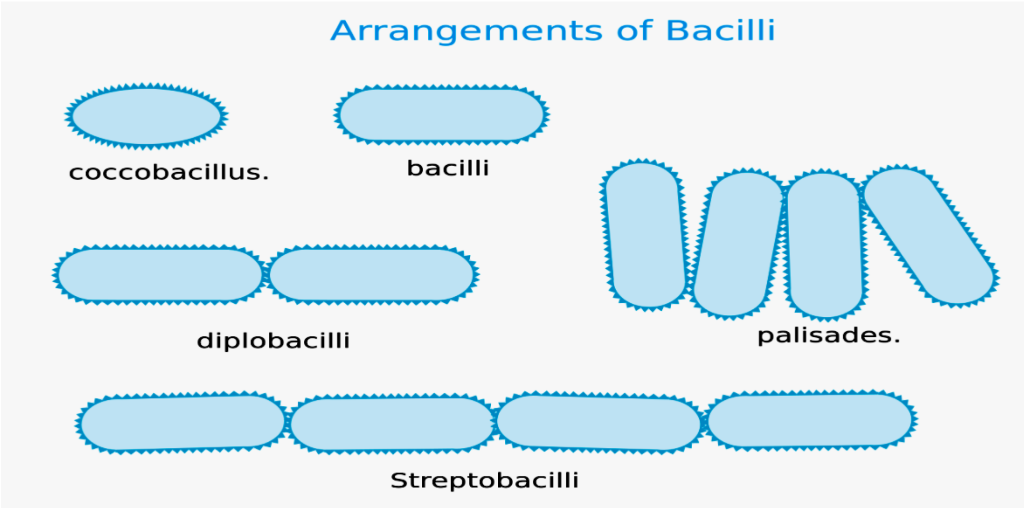
1.1 Cytology of typical bacterial cell

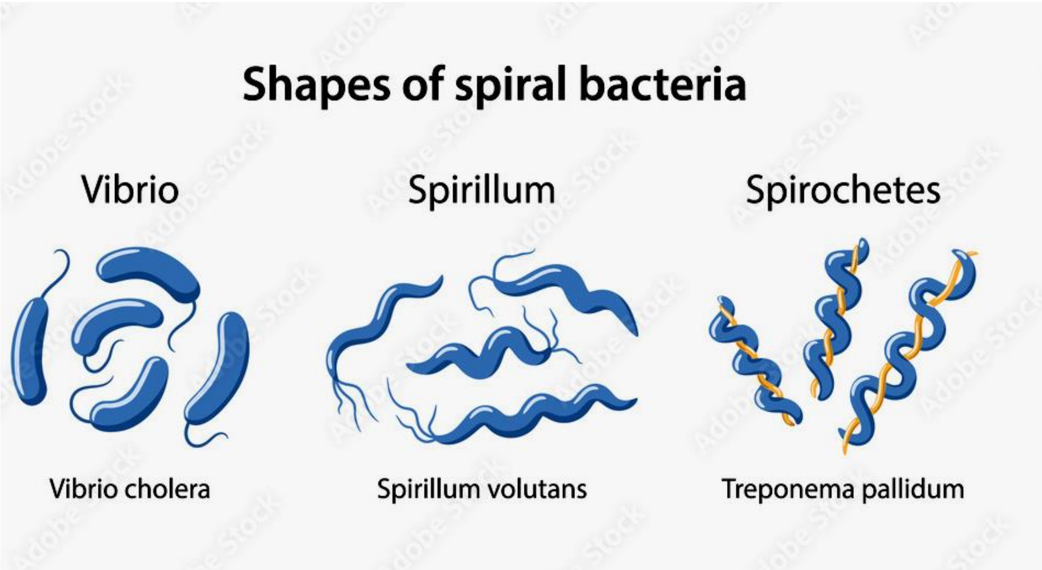
1. Morphology - Size and arrangement of bacterial cells

Bacteria have size from 1 to 4 micrometer in length and 0.5 to 1 micrometer in width in case of rod shaped bacteria, and 0.5 to 1 micrometer in diameter in case of cocci shaped bacteria. Various cell shapes occur in prokaryotes, the sphere (coccus), the rod (bacillus), curved rod (comma shaped, vibrio) or spiral shaped. They occur as single, in pairs, in chains or in clusters.






Arrangements of Bacilli
coccobacillus.
bacilli
diplobacilli
palisades.
Streptobacilli


Shapes of spiral bacteria
Vibrio
Spirillum
Spirochetes
Vibrio cholera
Spirillum volutans
Treponema pallidum

2. Ultra structure of prokaryotic cell:

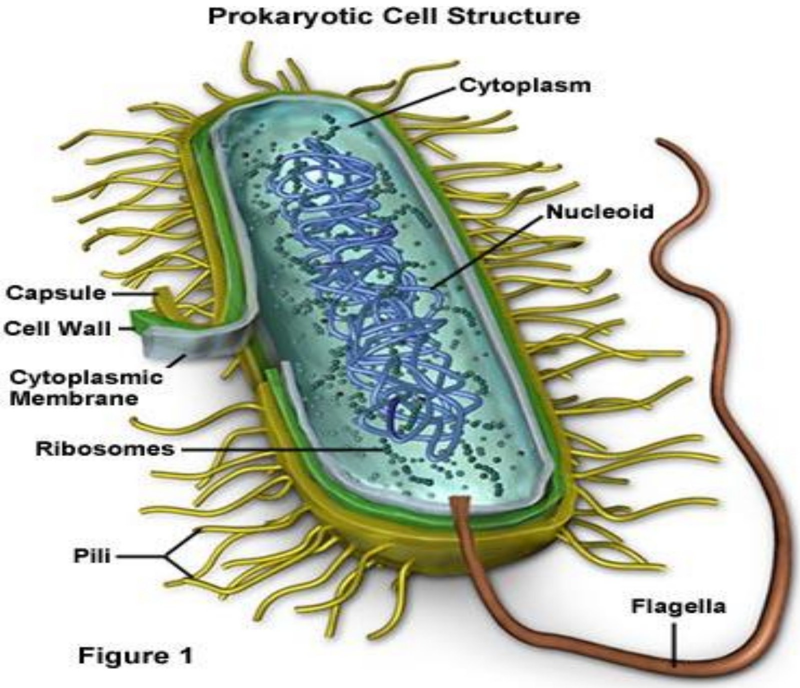


Figure 1

Chemical composition and function of various organelles:

1) Capsule and Slime layer

Many bacteria synthesize organic polymers, which are deposited outside the cell wall as a loose, more or less amorphous layer called as capsule or slime layer. The term capsule, which is usually used to a layer, which remains attached to the cell wall, while slime layer is used to a layer, which is not attached to the cell wall but is dispersed in medium.

These exo-polymers are not essential for cell functions because many bacteria do not produce them. Capsule serves as receptors for adherence to the surface, to tissues. It is protective layer against attack by phagocytes and by viruses; it may also help to prevent too rapid and lethal loss or gain of water in the recurrent dehydration and hydration that occurs in many habitats. Capsule usually has an ion exchange capacity, which may aid in the concentration and uptake of essential cations.

Chemical composition:

Exo-polymers are composed of either

→ Proteins called as exopolypeptides
→ Carbohydrates called as exopolysaccharides

Exopolypeptides: -

Some bacillus species produce exopolypeptides made up of only one amino acid D – Glutamic Acid. These amino acids are linked to one another.

Exopolysaccharides: -

These are made up of carbohydrates. These are classified into two types.

i. Exopolysaccharides synthesized from different types of sugars.
ii. Exopolysaccharides synthesized from sucrose sugars.

i. Exopolysaccharides synthesized from different types of sugars.

These are synthesized via sugar nucleotide precursors. E.g. Glucose, Galactose, Mannose etc. The polysaccharide chain is synthesized from sugar molecules by joining together to each other.

ii. Exopolysaccharides synthesized from sucrose sugars.

These are of two types –

i) Dextrans and ii) Levans

i) **Dextrans: -** To the acceptor molecule sucrose, glucose molecules are joined.

ii) **Levans: -** To the acceptor molecule sucrose, fructose molecules are joined.

e. g. *Bacillus, Leuconostoc, Pseudomonas and Streptococcus* species.

Bacteria producing capsule produce mucoid colonies on agar medium with a stringy consistency. Capsules are demonstrated by Negative staining with Nigrosin or India ink. Capsular material is antigenic. Capsules protect bacteria from degrading enzymes. Capsule is essential for virulence of bacteria. In Blue-green algae capsules consists of pectic substances.

Slime layer:

It is exopolypeptide or exopolysaccharide, slimy or gelatinous layer secreted by some bacteria and is not adhered to bacteria, it is dispersed into the medium. These are amorphous and not having definite shape and can be removed easily from bacterial cell surface.

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2. Cell wall

It is the outermost layer in the non-capsulated bacteria. Cell wall gives shape to bacteria. It gives rigidity and ductility to bacteria.

Demonstration of cell wall: -

It is done

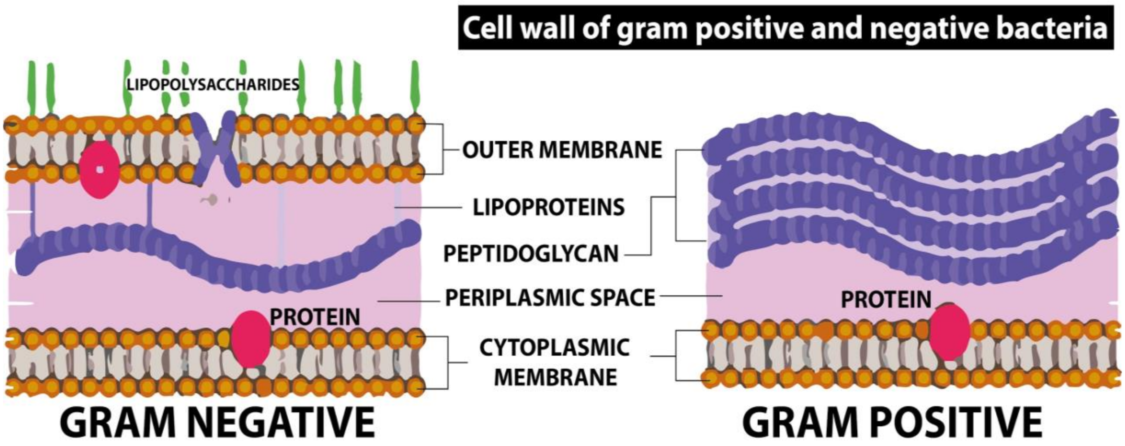
- ➔ By special staining
- ➔ By microdisection
- ➔ By plasmolysis
- ➔ By reaction with specific antibody
- ➔ By mechanical rupture of cell
- ➔ By electron microscopy

Morphology and composition: -

Bacterial cell walls are about 10 to 30 nm in thickness. In Gram-positive bacteria, thickness of cell wall is 25 to 30 nm. In Gram-negative bacteria, thickness of cell wall is 10 to 15 nm. The cell wall of Gram-positive bacteria is thicker and constitutes 20 to 40 % total dry weight of the cell, while cell wall of Gram-negative bacteria is thiner and constitutes 10 to 20 % total dry weight of the cell.

General composition of cell wall: -

Component	% in Gram positive bacteria	% in inner layer of Gram negative bacteria	% in outer layer of Gram negative bacteria
1. Peptidoglycan	40 to 90	8 to 10	Absent
2. Teichoic acid	1 to 10	Absent	Absent
3. Proteins	+ or -	Absent	40
4. Lipoproteins	Absent	Absent	15
5. Lipopolysaccharides	Absent	Absent	10
6. Lipids	Absent	Absent	+



i. Peptidoglycan: -

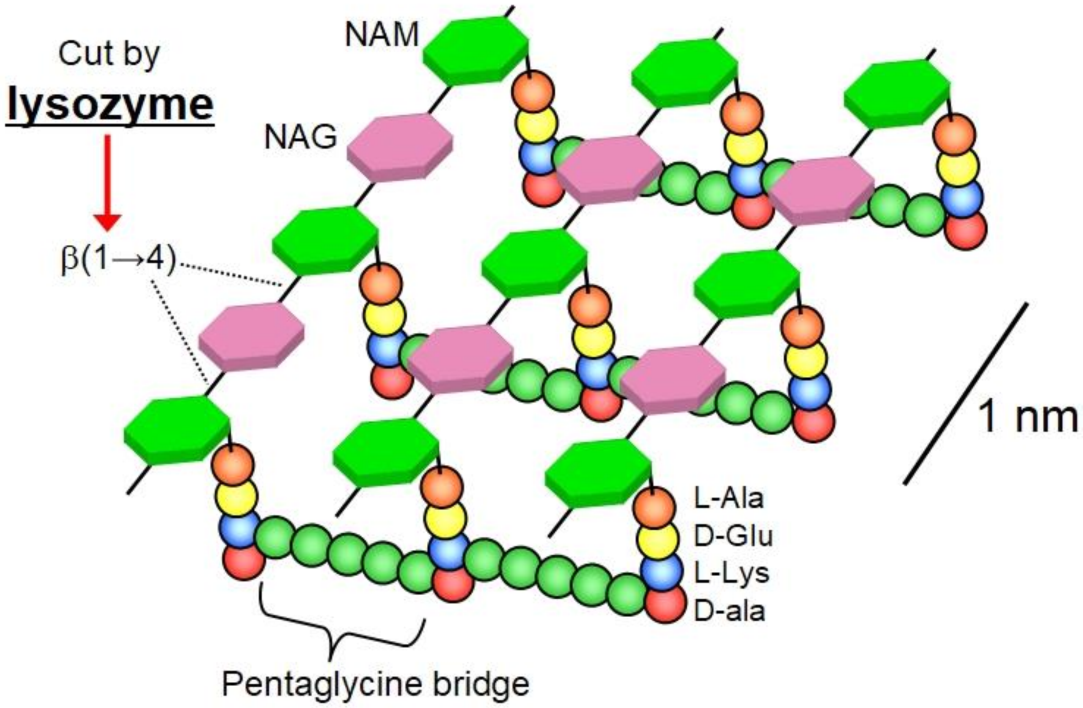
These are hetropolymers of sugars and amino acids. It is also called as **'Murein'.** It is composed of two acetylated amino sugars.

- N Acetyl Glucosamine (NAG)
- N Acetyl Muramic Acid (NAM)
- A small number of amino acid chain is attached to NAM

NAG and NAM form glycan strands composed of alternating residues of NAG and NAM in beta 1-4 linkage. Each strand contains about 10 to 65 sugars. To the NAM, peptide chain is attached. The common sequence of amino acids in this peptide chain is shown in figure i.e. L-Alanine, D-Glutamic acid, Diaminopimelic acid and D-Alanine.

The 4 amino acid chains may be cross linked by formation of peptide bond between the carboxyl group of 4th D-Alanine in one chain and the free amino group of 3rdDiaminopimelic acid in another chain. Due to cross linkage fabric is formed. In Gram-positive bacteria, there are many layers of peptidoglycan.

General structure of peptidoglycan showing structure of NAG and NAM and tetrapeptide side chain.



Cell wall of Gram-positive bacteria

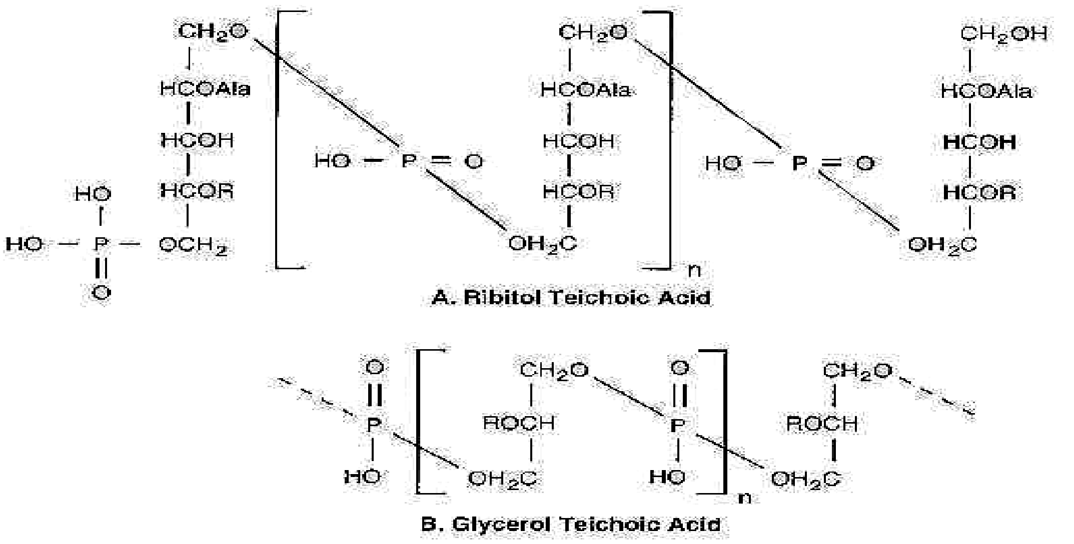
Sr. No.	Components	%
1	Thickness	25 to 30 nm
2	Peptidoglycan	40 to 90 % of dry weight of cell wall
3	Teichoic acid	5
4	Protein	1 to 10
5	Lipopolysaccharides	Absent
6	Lipoproteins	Absent
7	Lipids	Absent

In Gram-positive bacteria, peptidoglycan is about 40 to 90 % i.e. it is major part of cell wall. Cell wall of Gram-positive bacteria is thicker than cell wall of Gram-negative bacteria.

The peptidoglycan is linked with polysaccharides and with Teichoic acids.

ii. Teichoic Acids: -

These are water-soluble polyphosphate polymers containing Ribitol and Glycerol residues joined through phosphodiester linkages.



The function of teichoic acid is not known but is helpful for the passage of ions through it. The cell wall of Gram-positive bacteria generally does not contain lipid and proteins. If proteins are present they occur as separate layer.

Cell Wall of Gram-negative bacteria

Sr. No.	Components	% in inner layer	% in outer layer
1	Thickness	10 to 15 nm	--
2	Peptidoglycan	5 to 10 %	Absent
3	Phospholipids	Absent	15 %
4	Protein	Absent	40 %
5	Lipopolysaccharide	Absent	10 %
6	Lipoproteins	Absent	15 %

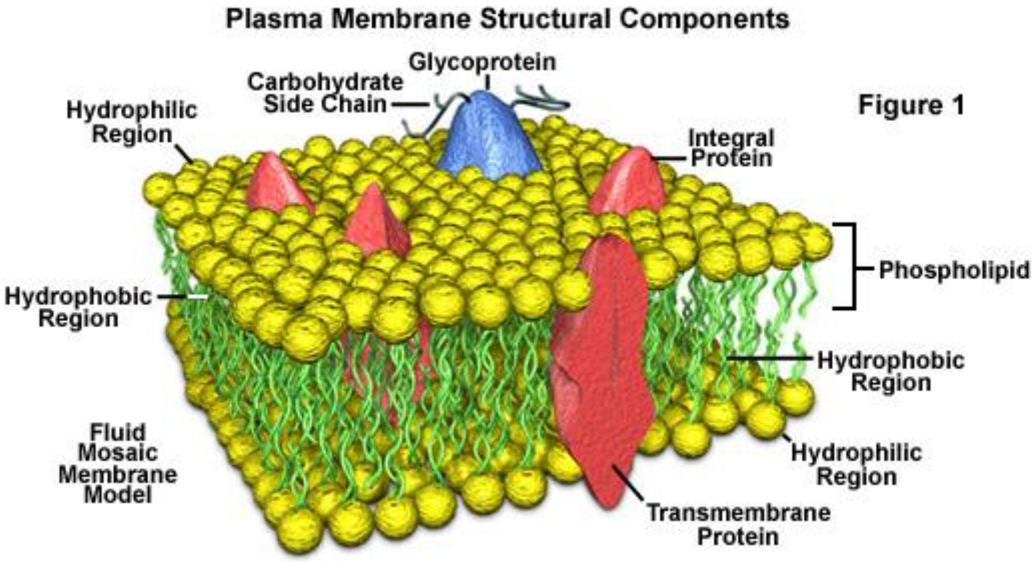
In Gram-negative bacteria, peptidoglycan is present in inner layer and is about 5 to 10 % and outer layer consists of phospholipids, proteins, lipo-polysaccharides and lipoproteins in the percentage as shown in the table.

3) Cell membrane / Unit Membrane / Fluid Mosaic model

Below the cell wall, cell membrane is present. It bounds to cytoplasm. It is main osmotic barrier. It is about 5 to 10 nm thick. It has a typical fine structure called as **'Unit Membrane system' or 'Fluid Mosaic model'.**

Unit Membrane system or Fluid Mosaic model:

The structure of cell membrane is like unit membrane or fluid mosaic model proposed by scientists S.J. Singer and Nicholson.



According to this model, cell membrane is made up of bilayer of phospholipids. In phospholipid bilayer, proteins are embedded.

Phospholipids: -

These are about 20 to 30 % of dry weight of cell membrane. These consist of Head and Tail. The head is polar and is located at the two outer surfaces of the bilayer. These are called as hydrophilic (water loving) region. The tail is made up of fatty acids; it extends into a centre of the membrane, and is called as hydrophobic region (water hating).

Proteins: -

These are about 50 % of dry weight of cell membrane and are intercalated (embedded) into the phospholipid bilayer. The proteins are embedded like ice crystals floating in a sea of phospholipids.

Functions of cell membrane: -

1. It is an important centre of metabolic activities and bound to cytoplasm and main osmotic barrier.
2. It contains different types of proteins having different functions.

a) Permeases: - These proteins transport many organic and inorganic nutrients inside the cell.
b) Biosynthetic enzymes (cell wall synthesizing).
c) Proteins, which synthesize ATP.
d) Photosynthetic machinery is present in the cell membrane in photosynthetic bacteria.
e) Cell membrane contains infoldings called as mesosomes responsible for transverse septum formation.

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4) Flagella

These are extremely thin hair like structures, which penetrate through cell wall and are originated from glandular structure (basal body), located within cell wall and cell membrane. Function of flagella is to provide motility or movement or displacement to bacteria.

All motile bacteria except spirochetes (spiral shaped bacteria) possess flagella. Flagellum is 3 to 12 micrometer in length and about 0.02 micrometer (20 nm) in thickness hence they are not seen with the help of ordinary light microscope. They can be seen by special staining technique in which thickness of flagella is increased by using mordant during staining. Motility of bacteria can also be observed by hanging drop preparation by using ordinary light microscope.

Arrangement of flagella on cell: -

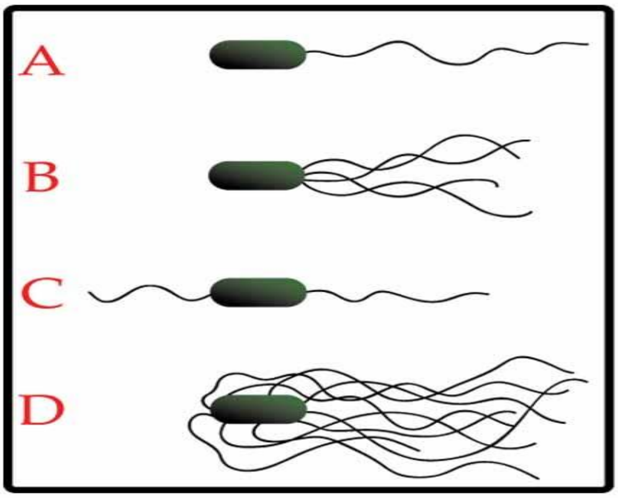
According to arrangement of flagella on cells, these are classified as follows—

A) Monotrichous: - Single flagellum present at one end

B) Lophotrichous: - More than one flagellum present at one end

C) Amphitrichous: - Flagella are present at both ends.

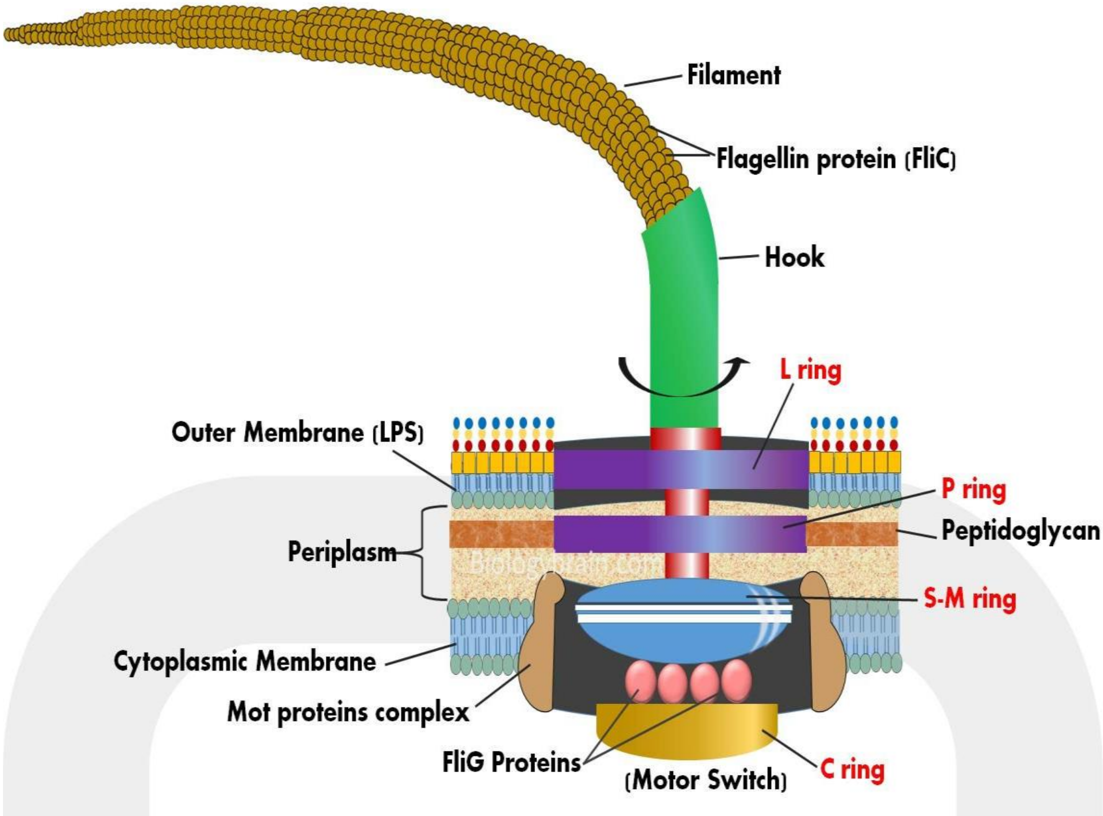
D) Peritrichous: - Flagella are present all over the body of cell.



Structure of flagellum

Entire flagellum structure is made up of 3 main regions.

i) Filament
ii) Hook
iii) System of rings (Basal Body)



i) Filament: -

It is very thin and long with constant width. It is made up of protein called as **'Flagellin'.**

ii) Hook: -

It is slightly thicker than filament, about 45 nm long. It is made up of flagellin and different types of proteins. Near the outer surface of the hook, filament is attached and this entire hook with filament is attached to basal body (glandular structure), which is located within cell wall and cell membrane.

iii) System of rings (Basal Body): -

Basal body consists of small central rod inserted into a system of rings. In Gram-negative bacteria, 4 rings are present which are named as L, P, S and M rings. In Gram-positive bacteria, only 2 rings are present i.e. only S and M rings are present, it indicates that only S and M rings are essential for flagellar movement.

In Gram-negative bacteria, the outer pair of rings (L and P) is situated at the level of outer and inner cell wall layers respectively. Function of L and P rings is to support the rod. The inner pair or inner two rings (S and M) are located at the level of cell membrane. The L ring associates with the lipopolysaccharides, the P ring associates with peptidoglycan layer, the S ring is directly attached to the plasma membrane and M ring is embedded in the plasma membrane.

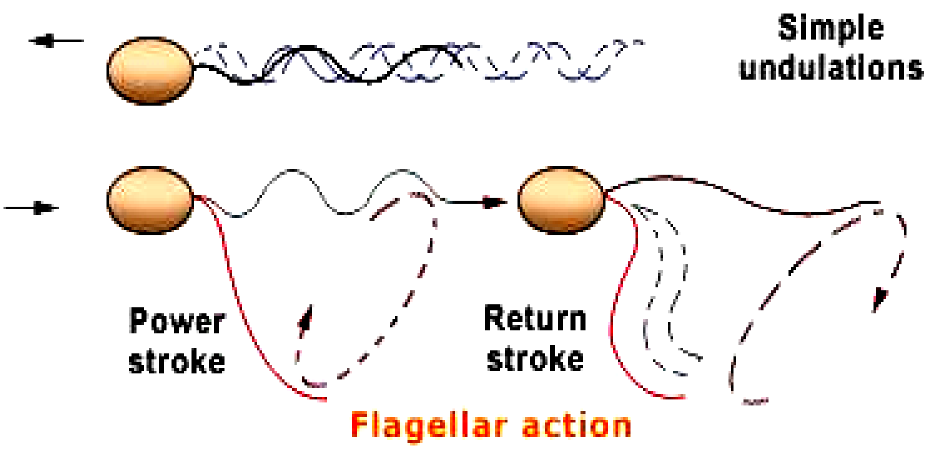
In Gram-positive bacteria, only S and M rings are present. S ring is present in the peptidoglycan layer and M ring in the plasma membrane. They do not require L and P rings for support to rod because rod passes through the relatively thick and homogeneous cell wall than Gram-negative cell wall. Thus in both Gram-positive and Gram-negative bacteria possessing flagella movement is possible.

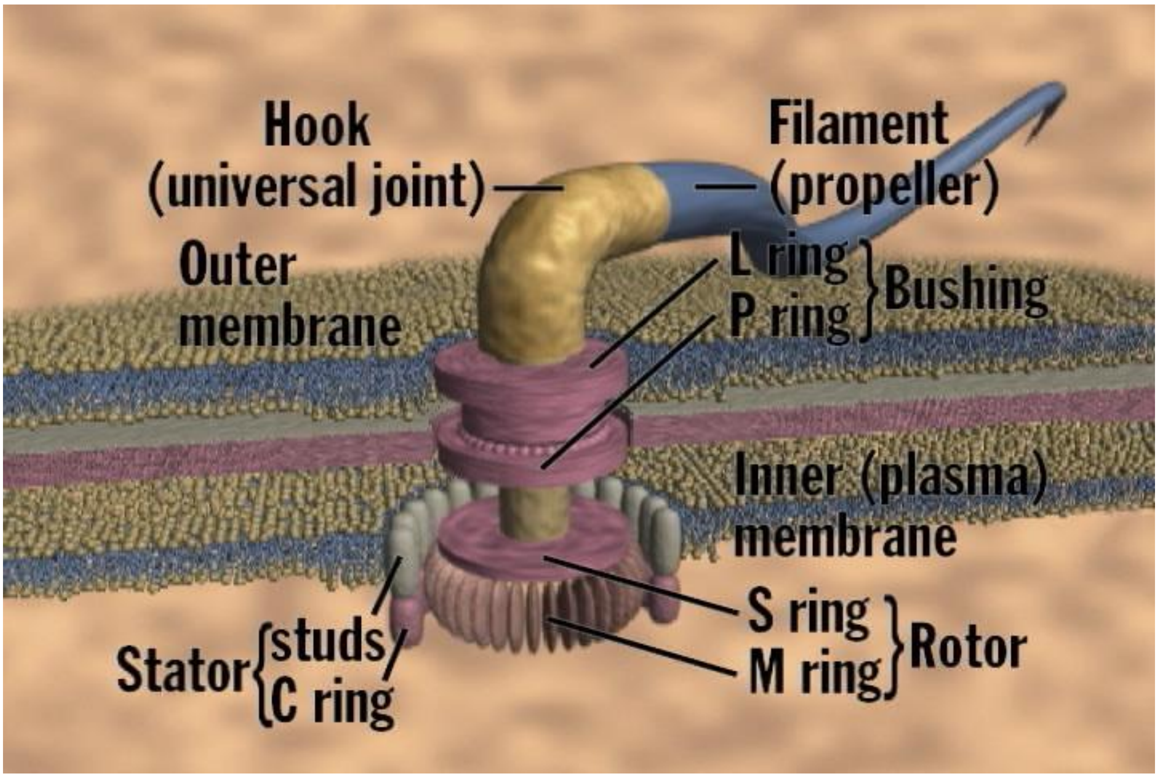
In Gram-positive bacteria only S and M rings are present and are motile, it indicates that only S and M rings are essential for flagellar movement.

Mechanism of Flagellar movement: -

The bacterial flagellum is driven by a rotary engine (Mot complex) made up of protein, located at the flagellum's anchor point on the inner cell membrane. The engine is powered by proton motive force, i.e., by the flow of protons (hydrogen ions) across the bacterial cell membrane due to a concentration gradient set up by the cell's metabolism. The rotor transports protons across the membrane, and is turned in the process. The rotor alone can operate at 6,000 to 17,000 rpm, but with the flagellar filament attached usually only reaches 200 to 1000 rpm.

Flagella spin either clockwise or anticlockwise direction around its long axis with the help of motor. Motor is operated by causing rotation of S and M rings relative to each other. Energy to motor is provided by ATP molecules.





Specialized movement in bacteria: -

1. Chemotaxis: - This is the mechanism for swimming towards or away from chemical stimuli, a behaviour known as **chemotaxis.** The chemo sensors in the cell envelope called binding proteins detect certain chemicals and signal the flagella to respond.

When the bacteria move, they periodically change direction rather than reaching their destination by swimming in a single straight line. The straight line movement is known as **'run'** and the turn which occurs when bacteria stops are called **'tumble' or 'twiddle'.** The direction of flagella rotation, and hence the length of run is determined by the interactions of chemo sensors in cytoplasmic membranes with attractants or repellents. In increase in concentration of attractant, e.g. interacts with chemo sensors to decrease the frequency of tumbling, whereas decrease in concentration of attractant, interacts with chemo sensors to increase the frequency of tumbling and hence shorter runs. The same is true for reactions with repellents.

2. Magnetotaxis: - Some bacterial cells contain inclusion of iron granules known as **'Magnetosomes'**, that permit them to orient their movement in response to magnetic fields, a phenomenon known as **'Magnetotaxis'**. Bacteria can use those granules to navigate along the Earth's magnetic field. Some bacteria move predominantly north and others to south. Magnetotaxis allows some anaerobic bacteria to orient themselves so that they point downward into the sediment.

3. Phototaxis: -

Some bacteria are able to detect and respond to differences in light intensity, a phenomenon called **'Phototaxis'.** In some bacteria this is similar to chemotaxis. Other bacteria form membrane bound gas vacuoles that enable them to respond to light. Boundary layers of these vacuoles are not true membranes but are composed of exclusively proteins, which are hydrophilic as well as hydrophobic. In aquatic bacteria gas vacuoles provide a mechanism for adjusting the buoyancy of the cell and thus the height of bacterium in water column. Many aquatic Cyanobacteria use their gas vacuoles to move up and down in water column depending upon the light intensity levels, to achieve optimum conditions for photosynthesis.

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5) Fimbriae or Pili

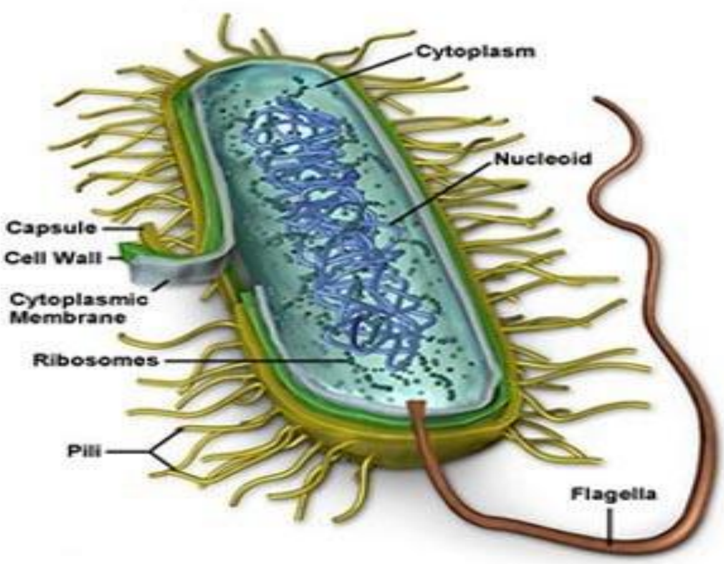
Fimbriae or **pili** are short, hair-like structures on the surfaces of procaryotic cells. Like flagella, they are composed of protein. Pilli are composed of protein called as **'Pillin'** of molecular weight about 70,000 Daltons. These are smaller and thinner than flagella. They are straight and less rigid and shorter than flagella.

Length of pillus is about 0.02 micrometers to 10 micrometer and width about 30 to 150 A^{o}.

Pilli are very common in Gram-negative bacteria, but occur in some archaea and Gram-positive bacteria as well. Generally, pilli are not required for bacterial movement. Pilli are most often involved in adherence of bacteria to surfaces, substrates and other cells or tissues in nature. In *E. coli*, a specialized type of pilus, the **F or sex pilus**, apparently stabilizes mating bacteria during the process of conjugation and provides a tube through which DNA is transferred from one bacterium to other during conjugation.

Pilli are classified into 6 groups.

Group-I: - Pilli which function as in adherence of bacteria to surfaces, substrates and other cells or tissues.
Group-II: - Includes sex pilli which are special pilli for conjugation.
Group-III: - These are thick present in agrobacter.
Group-IV: - These are flexible and rod like present in vibrio.
Group-V: - These are contractile tubules, which help during conjugation.
Group-VI: - These are found in Gram-Positive bacteria.



Functions: -

1. Pilli are mainly associated with adhesive properties hence bacteria attach to substrate (food) and other cells or tissues.
2. Sex pilli provide a tube through which DNA is transferred from one bacterium to other during conjugation.
