Cell Structure of Bacteria

A bacterial cell (Fig. 2.5) shows a typical prokaryotic structure. The cytoplasm is enclosed by three layers, the outermost slime or capsule, the middle cell wall and inner cell membrane. The major cytoplasmic contents are nucleoid, plasmid, ribosome, mesosome etc., and the cell is devoid of endoplasmic reticulum, mitochondria, centrosome and golgi bodies.
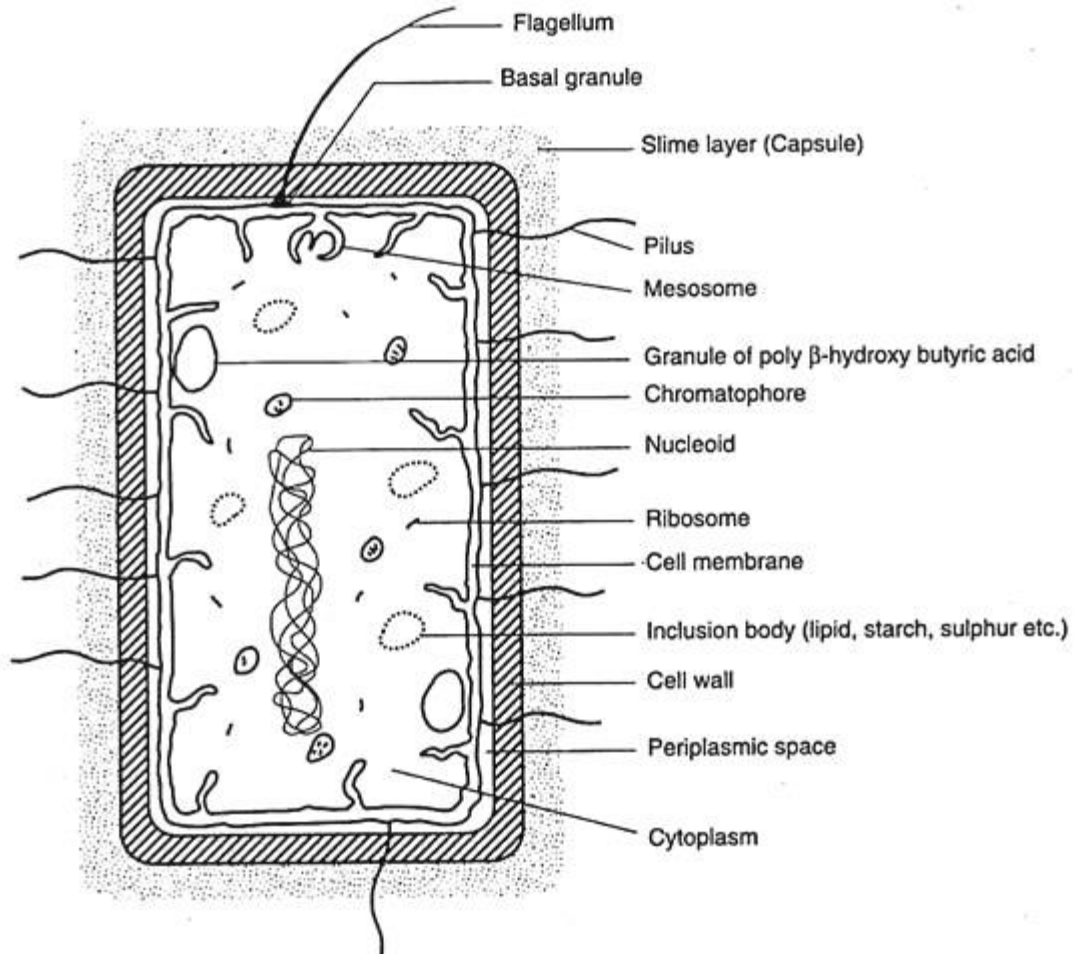

Flagellum
Basal granule
Slime layer (Capsule)
Pilus
Mesosome
Granule of poly β-hydroxy butyric acid
Chromatophore
Nucleoid
Ribosome
Cell membrane
Inclusion body (lipid, starch, sulphur etc.)
Cell wall
Periplasmic space
Cytoplasm

Fig. 2.5 : Bacterial cell (a composite diagram-showing various structures)
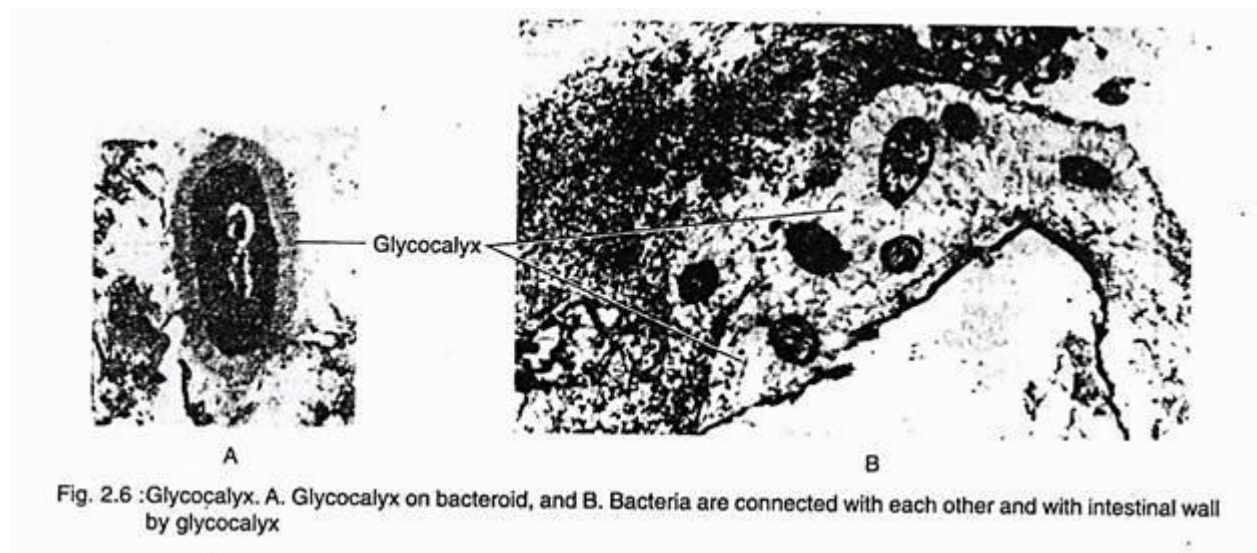
A. Slime layer, Capsule and Glycocalyx:

An amorphous viscid secretion of bacterial cell is present as a loose undemarcated region outside the cell, called slime layer (e.g., Leuconostoc). But, when it originates as a sharply defined structure outside the cell wall, it is called capsule (e.g., Pneumococcus). The capsule is about 0.2 μm in width.

The capsules those are much narrower than true capsule, called microcapsule (e.g., Haemophilus influenzae). Both the layers usually consist of polysaccharide and occasionally polypeptide. The capsule contains 2% solid and 98% water. The solid portion is comprised of

complex polysaccharide (e.g., Pneumococcus, Enterobacter) or polype) ide (e.g., anthrax bacillus) or hyaluronic acid (e.g., Streptococcus).

Both the layers serve as a protective covering and protect the cells from antibacterial substances like bacteriophage, enzymes etc. They enhance the virulence of bacteria.

The glycocalyx (Fig. 2.6) is an extracellular, hygroscopic network of mostly polysaccharides and occasionally of polypeptide or of both. The chemical components of glycocalx are synthesized by the cell and transported through the ceil membrane and cell wall, and finally deposited outside the cell, to form extracellular covering.

Fig. 2.6 :Glycocalyx. A. Glycocalyx on bacteroid, and B. Bacteria are connected with each other and with intestinal wall by glycocalyx

The polysaccharides of glycocalyx contain many different sugars such as glucose, galactose, rhamnose and sugar acids like glucuronic acid etc.

Function:
1. It gives protection to the cell from desiccation under natural condition.

2. It helps the bacteria to attach the surface of solid objects, in aqueous medium or to tissue surface of animals and plants.

Streptococcus mutans, a bacterium associated with the caries of teeth, is attached to the dental surface with the help of glycocalyx, consists of water-insoluble polymer, glucan.

B. Cell Wall:

The bacterial cell wall is tough and rigid due to the presence of strong fibres composed of heteropolymers called mucopeptides, peptido- glycans, mucocomplex, murein etc. The peptidoglycan is composed of alternate units of N-acetyl muramic acid and N-acetyl glucosamine residues, cross-linked with tetra-peptide subunits (Fig. 2.7).
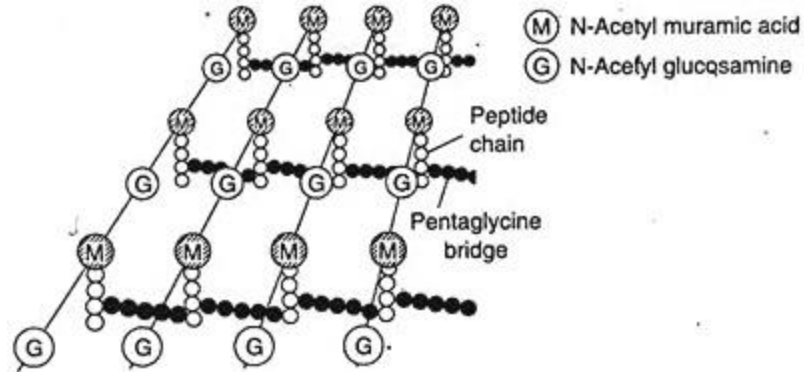

Fig. 2.7 : Chemical structure of cell wall of Gram-positive bacteria

These fibres form a three-dimensional, tough meshwork rather than a solid one (Fig. 2.8), thereby inward movement of minerals, amino acids, water etc. and outward movement of wastes take place normally. The peptide linked with muramic acid varies in their compo- sition, but everywhere it contains a minimum of three amino acids viz., glutamic acid, alanine and either lysine or diaminopimelic acid.
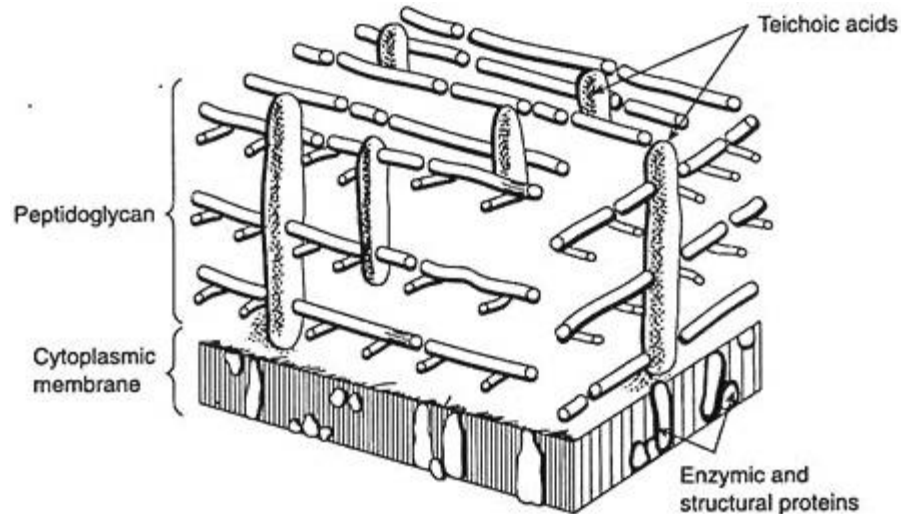

Fig. 2.8 : Three-dimensional representation of Gram-positive bacterial cell wall

The structural constituents of wall vary in Gram- negative and Gram-positive bacteria (Fig. 2.9).

(a) Gram-negative cell wall (Salmonella, Escherichia):
It is a complex structure with three components outside the peptidoglycan layer:
(i) Lipopolysaccharide (LPS):
It consists of a complex lipid with attached polysaccharide. LPS is the endotoxin, whose toxicity is associated with lipid region and polysaccharide confers antigenic specificity.

(ii) Phospholipid:
This layer consists of phospholipid bilayer and specific proteins. The specific proteins form porins and hydrophilic molecules are transported through it. Other proteins are target sites for antibiotic and phages.

(iii) Periplasmic space:
It is situated in between peptidoglycan and phospholipid layer. This space contains a number of important proteins (as enzyme and binding protein for specific substrate) and oligosaccharides (help in osmo-regulation).

(b) Gram-positive cell wall (Staphylococcus, Bacillus):
The peptidoglycan layer present just outside the cell membrane is about 16-80 nm thick. Special components like teichoic acid and teichuronic acid (as much as 50% of dry weight of cell wall) are present. They maintain the level of divalent cations outside the cell membrane. Teichoic acids constitute the major surface antigens. The periplasmic space is absent in Gram- positive cell wall.
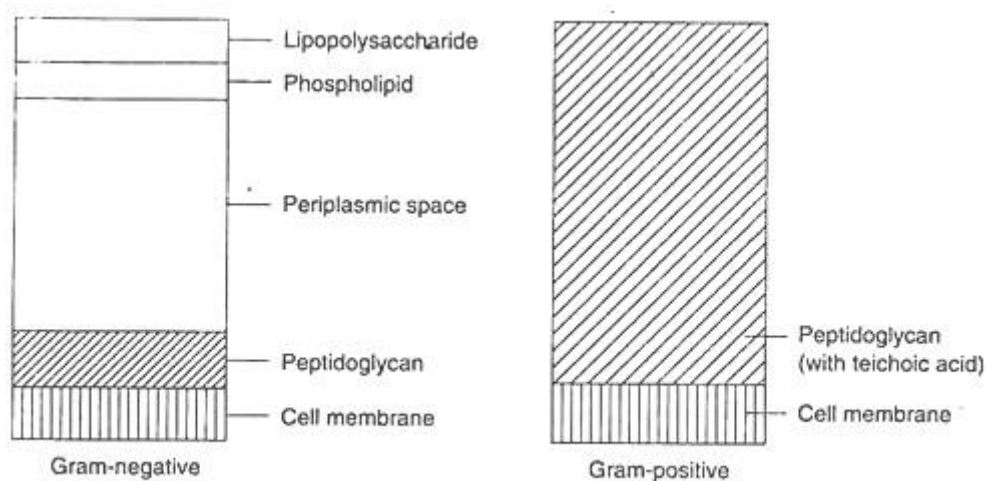

Fig. 2.9 : Comparison of cell walls of bacteria

Staining of Bacteria:
Gram Staining:

This technique was developed by a Danish physician, Hans Christian Gram, in 1884; which is very useful in taxonomic grouping of bacteria. The basic distinction between Gram(+ve) and Gram(-ve) bacteria is in the difference of cell wall structure, along with some other characteristics (Table 2.3).
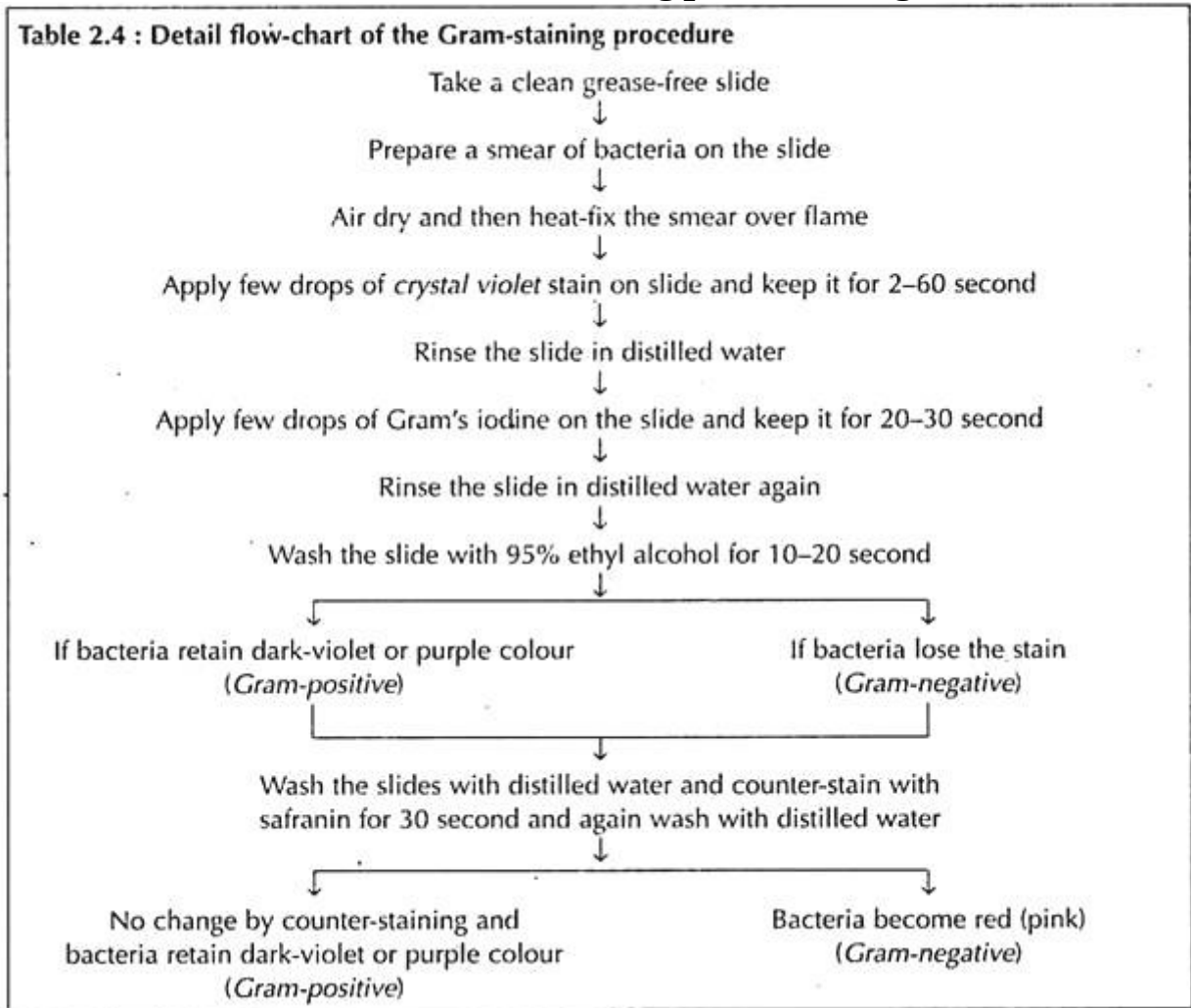
The crystal violet, iodine, ethyl alcohol (95%) and safranin are used for staining. First, the cells are stained with crystal violet, then with Gram's iodine and finally washed in 95% ethanol which differentiate two groups: one which retains stain and becomes dark-violet or purple coloured i.e., Gram-positive and the other one which loses stain i.e., Gram-negative.

The slides are washed with distilled water, followed by counter-stain with safranin for 30 sec. and finally the slides are again washed with distilled water. Gram-positive bacteria remain dark-violet or purple coloured, but Gram-negative bacteria become red (pink) (Fig. 2.10).

Colour of Cells	Treatment	Effect
	Cells fixed by heating	Cells shrink slightly in size.
	Crystal violet dye applied	All cells stain dark violet or purple.
	Gram's iodine applied	All cells remain dark violet or purple.
	Wash with 95% ethyl alcohol	Gram-positive cells remain dark violet or purple; Gram-negative cells become colourless.
	Safranin (red dye) applied	Gram-positive cells remain dark violet or purple; Gram-negative cells appear red.

Purple colour
Red colour

This grouping does not depend on the shape of bacteria, e.g., the bacillus-shaped bacteria like Bacillus subtilis is Gram-positive, while the other rod-shaped bacteria like Coxiella burnetii is Gram-negative. The coccus-shaped bacteria such as Escherichia coli is Gram- negative, but Sporosarcina is Gram-positive.

The detailed flow-chart of the Gram-staining procedure is given in Table 2.4:

Table 2.4 : Detail flow-chart of the Gram-staining procedure

Take a clean grease-free slide
↓
Prepare a smear of bacteria on the slide
↓
Air dry and then heat-fix the smear over flame
↓
Apply few drops of *crystal violet* stain on slide and keep it for 2–60 second
↓
Rinse the slide in distilled water
↓
Apply few drops of Gram's iodine on the slide and keep it for 20–30 second
↓
Rinse the slide in distilled water again
↓
Wash the slide with 95% ethyl alcohol for 10–20 second
↓

| If bacteria retain dark-violet or purple colour (*Gram-positive*) | If bacteria lose the stain (*Gram-negative*) |

↓
Wash the slides with distilled water and counter-stain with safranin for 30 second and again wash with distilled water
↓

| No change by counter-staining and bacteria retain dark-violet or purple colour (*Gram-positive*) | Bacteria become red (pink) (*Gram-negative*) |

Acid-fast Staining:

This technique was developed by Ehrlich and later modified by Ziehl and Neelsen.

The Mycobacteria can be stained by acid- fast stain (Ziehl-Neelsen technique) or by auromine-rhodamine stain. Though the latter is more sensitive, it requires fluorescence microscopy.

Ziehl-Neelsen staining:
Procedure:
1. Prepare the smear on a clean grease- free slide.

2. Heat-fix the smear and stain by hot car- bol fuchsin for about 10 minutes and wash in tap water.

3. The smear is then decolourised by 20% H_2SO_4 (sulphuric acid) or 1% HCI (hydrochloric acid) in 70% alcohol for 1 minute.

4. The decolourisatfon is continued with 70% neutral alcohol for 2-3 minutes, until the smear becomes pinkish in colour. (Decolourisation of M. leprae should be done with 5% H_2SO_4 only).

5. The smear is then counterstained with 2% methylene blue or with malachite green for 2 minutes and then washed with water.

Observation:

The Bacilli after staining appear red in blue background of epithelial cells or pus cells.

1. Mycobacteria like M. leprae (c.o. of Leprosy), M. tuberculosis (c.o. of Tuberculosis) can be stained by this method (M. tuberculosis is a acid fast as well as Gram-positive bacterium).

2. Others like Staphylococcus aureus, Ascospores of some yeast, bacterial spores etc., can be stained following the above procedure.

Stains used for Gram-staining:
1. Crystal violet:
Solution A

i. Crystal violet (90% dry content)… 2 gm

ii. Ethyl alcohol (95%) …20 ml

Solution B

i. Ammonium oxalate …1gm

ii. Distilled water …80ml

Mix solution A and B.

2. Iodine solution:
i. Iodine …1gm

ii. Potassium iodite …2gm

iii. Distilled water ...300ml

3. Safranin:

i. Safranin

(2.5% soln. in 95% ethanol)

ii. Distilled water Stains used for acid fast staining:

1. Ziehl's carbol fuchsin

Solution A

1. Basic fuchsin

(90% dry content) ...0.3gm

ii. Ethyl alcohol (95%) ...10ml

Solution B

i. Phenol ...5gm

during and after Gram-staining

ii. Distilled water ...95 ml

Mix solution A and B.

2. Loeffler's methylene blue.

Solution A

i. Methylene blue

(90% dry content) ... 0.3 gm

ii. Ethyl alcohol (95%) ...30 ml

Solution B

i. Dilute KOH (0.01 by wt.) ...100 ml

Mix solution A and B.

C. Cytoplasmic Membrane of Bacteria:

The cytoplasmic layer is the boundary layer of the protoplast, situated beneath the cell wall (Fig. 2.5). It is thin (5-10 nm), elastic and semipermeable layer (Fig. 2.11). In section, it appears as a triple-layered structure consisting of a bilayer region of phospholipid molecules, with polar heads on the surface and fatty-acyl chains towards the inner side. The proteins are found embedded in the lipid bilayer.
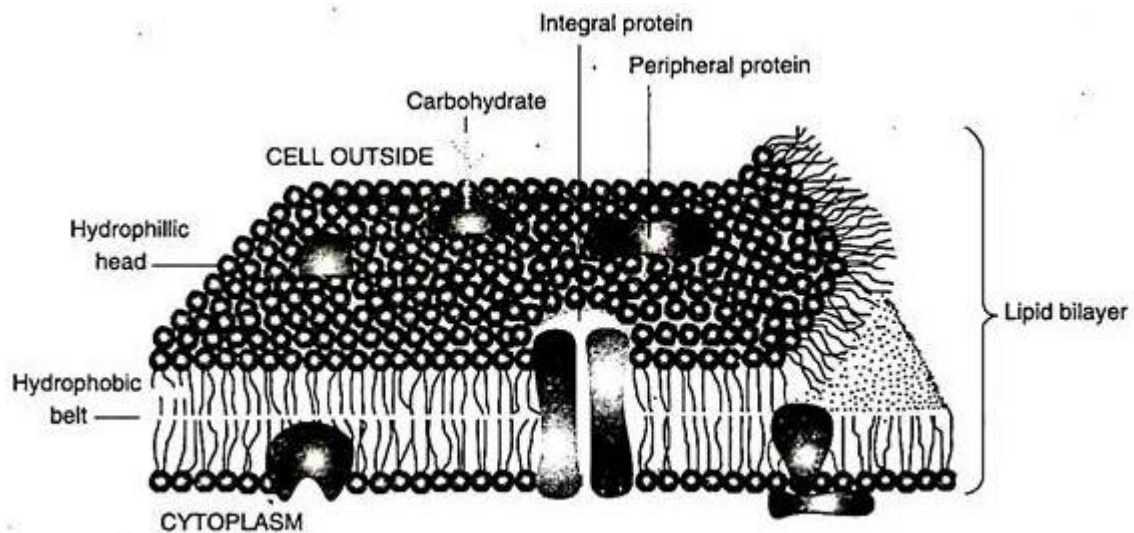

Fig. 2.11 : Cytoplasmic membrane (fluid mosaic model)

Functions
(i) Transport:
(a) Active:

Being the site of many enzymes like oxidase, polymerase etc., it is involved in the active transport of selective nutrients. It is impermeable to ionised substances and macromolecules.

(b) Passive:

The passive transport of fat soluble micromolecular solutes takes place by diffusion.

(ii) Energy production:

It is the site of electron flow in both respiration and photosynthesis leading to phosphorylation and, therefore, the membrane is the site of carriers and enzymes in these reactions.

(iii) Polymer production:
Cell membrane is the site of polymerising enzymes necessary for synthesis of cell wall.

D. Cytoplasm of Bacteria:
The cytoplasm is a colloidal system containing both organic and inorganic substances. It lacks mitochondria, endoplasmic reticulum, centrosome and golgi bodies. It contains many ribosomes, few mesosomes, soma inclusions and vacuoles (Fig. 2.5).

Ribosome:
It is a complex substance of 10-20 nm size and of 70S (S = sedimentation coefficient) type having two subunits, 50S and 30S. The larger one comprises of molecules of RNA and 35 amino acids and the smaller one has one molecule of RNA and 21 amino acids. They are the sites of protein synthesis. The ribosomes are held together on m-RNA (messenger RNA) strands, known as polysomes or polyribosomes.

Mesosomes (Chondroids):
These are convoluted multi-laminated localised infoldings of the cytoplasmic membrane into the cytoplasm (Fig. 2.12). Their number is usually 2-4, but often found to be more in cells with high respiratory activity, e.g., Nitrosomonas.
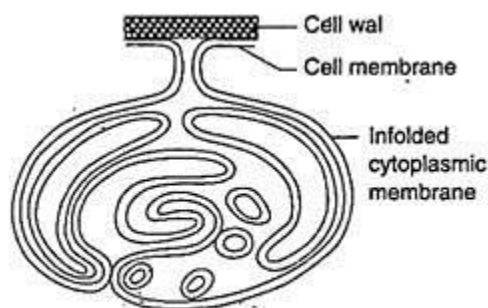

Fig. 2.12 : Single mesosome

Perhaps it serves to accommodate more spaces for respiration. In photosynthetic bacterial (Rhodopseudomonas), they are the site of photosynthetic pigments. The mesosomes are of two types — septal mesosome and lateral mesosome. The septal mesosomes are involved in DNA segregation and in the formation of transverse septum during cell division.

Chromatophores:

These are the pigment- bearing structures, found in photosynthetic bacteria. They are found in all members of Chromatiaceae and Rhodospirillaceae in different forms such as membranes, vesicles, tubes, bundle tubes etc. or as thylakoids, as found in Cyanobacteria.

Cytoplasmic inclusion:

These are the sources of stored energy, characteristic of different species of bacteria such as lipid (poly (3 hydroxybutyrate), volutin (polymetaphosphate), starch or glycogen (polysaccharide) and granules of sulphur.

E. Genetic Material of Bacteria:

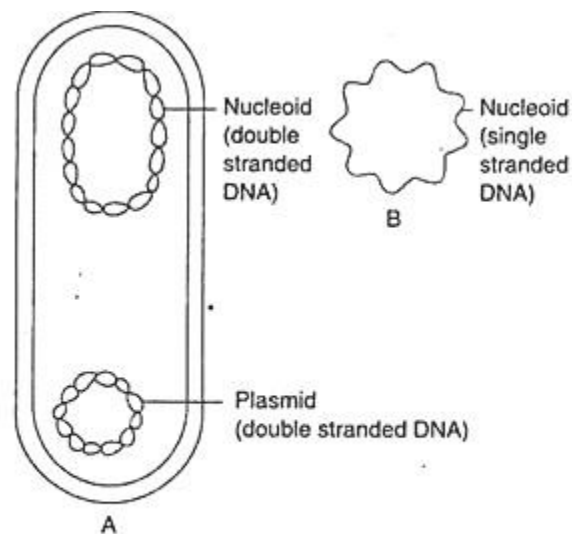The genetic material is present both in nucleoid and plasmid (Fig. 2.13).

Fig. 2.13 :A. Bacterial cell having nucleoid (double stranded DNA) and plasmid (extrachromosomal carrier of gene). B. Nucleoid (single stranded DNA)

Nuclear material:

Under light microscope nuclear body cannot be differentiated in the cytoplasm, but it is differentiated only under electron microscope as a central area of lower electron dense region than rest of the cytoplasm.

The bacterial nuclear body is devoid of nuclear membrane, nucleolus and nuclear sap and is known as genophore or nucleoid. The genophore is composed of a single or double stranded circular DNA. When straightened the DNA measures 1000 μm. It has approximately 5×10^6 base pairs and a molecular weight of about 3×10^9. It is devoid of any basic protein. The

DNA is haploid — it undergoes semiconservative replication by simple fission and maintains genetic characteristics.

Plasmid:

Bacterial cytoplasm may contain some genetic material excepting the genophore, called plasmid or episomes. Lederberg (1952) termed as plasmid those extragenophoral genetic materials.

Plasmids are ring-like double stranded DNA molecules which may contain about 100 genes having the molecular weight range from 5 x 10^7 to 7 x 10^7 or less. The replication of plasmid seems self-controlled. They contain different non-essential characters. Based on host properties, the plasmids are classified into different types.

These are:

(i) "F-factor" for fertility

(ii) "Col-factor" — for colicinogeny

(iii) "R-factor"—for resistance

(iv) Tumor inducing plasmid (e.g., Agrobacterium tumit'aciens),

(v) Degradative plasmid (e.g., Pseudomonas),

(vi) Pathogenecity to mammals,

(vii) Penicillase plasmid (e.g., Staphylococcus),

(viii) Mercury resistance, and

(ix) Cryptic plasmids.

F. Flagella of Bacteria:

Most of motile bacteria (e.g., Spirochaetes) possess long (5-20 μm), thin (12-30 nm), helical appendages, called flagella. Electron microscopy shows that the flagellum consists of three distinct regions — filament, hook and basal body (Fig. 2.14). Filament is attached at one end through the cell wall to the cell membrane by the hook, which, in turn, is attached to the basal body. The rings of basal body remain attached to the cell membrane and cell wall. The filament lies external to the cell.

Filament is made up of identical subunits (3 or more), arranged helically along the axis to give a hollow tube (Fig. 2.15). These subunits are made up of protein molecule, called flagellin. Each subunit is about 4.5 nm thick.

The filament ends with a capping protein. Some bacteria have sheath surrounding the flagella (e.g., Vibrio cholerae has a sheath of lipopolysaccharide).

Structurally, the hook and basal body are quite different from the filament (Fig. 2.14). The hook is slightly wider than the filament and made up of protein subunits.

The structure of basal body is quite different in gram (-ve) and gram (+ve) bacteria. Gram (-ve) bacteria like E. coli and others have 4 rings connected to a central rod. The outer L arid P-rings are associated with the lipopolysaccharide and peptidoglycan layers.

The inner two rings, i.e., S and M rings, are associated with plasma membrane (Fig. 2.14). On the other hand, in gram (+ve) bacteria like Clostridium sporogens, Bacillus subtilis and others, have only two rings attached with the plasma membrane. The structural differences of flagella between gram (-ve) and gram (+ve) bacteria are given in Fig. 2.14.
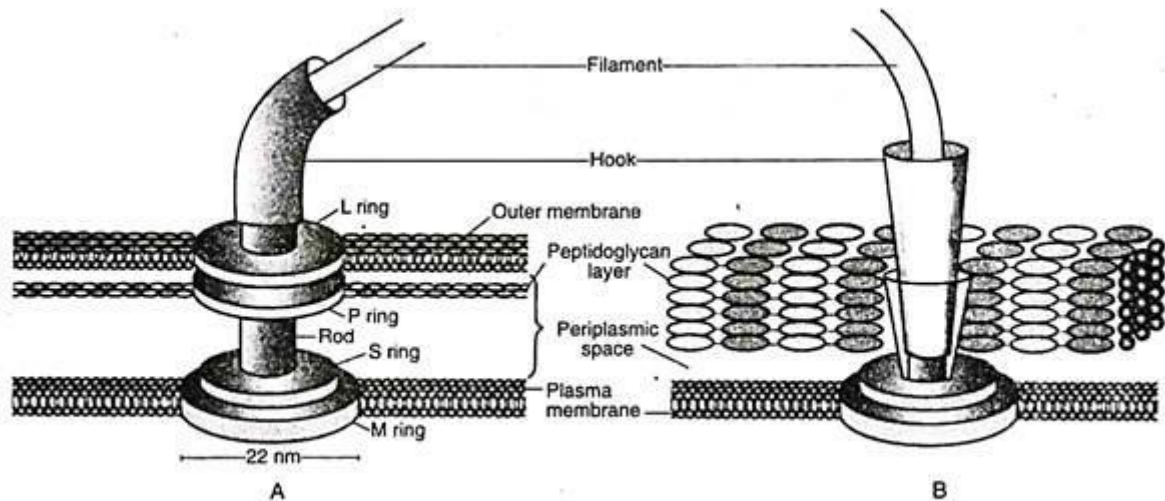

Fig. 2.14 : The ultrastructure of bacterial flagella showing flagellar basal bodies and hooks. A. Gram-negative bacteria and B. Gram-positive bacteria

Some bacteria are devoid of flagella and are non-motile, called atrichous (Fig. 2.16A) e.g., Cocci, Lactobacillus, etc.

The number and arrangement of flagella on a cell are useful for identification and classification of bacteria. On the basis of arrangement of flagella, the bacteria are categorised into the following types (Fig. 2.16):
i. Polar flagellation:
(a) Monotrichous (Fig. 2.16B). Single fla- gellum at one pole of the cell, e.g., Vibrio cholerae.

(b) Amphitrichous (Fig. 2.16C). Each single flagellum is attached at both ends, e.g., Alkaligenes faecalis, Nitrosomonas.

(c) Cephalotrichous (Fig. 2.16D). Two or more flagella at one end only, e.g., Pseudomonas fluorescens.

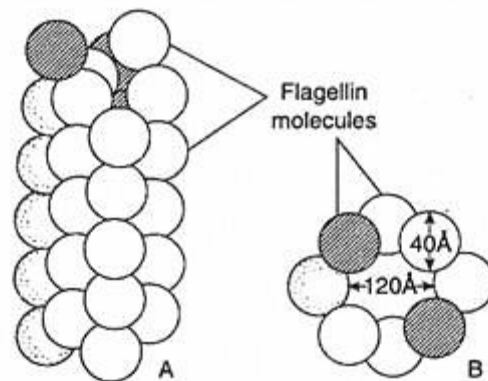(d) Lophotrichous (Fig. 2.16E). A tuft of flagella at both ends, e.g., Spirillum volutans.

Fig. 2.15 : Structure of filament. A. Arrangement of flagellin molecules showing hollow cylinder and B. T.S. of a filament
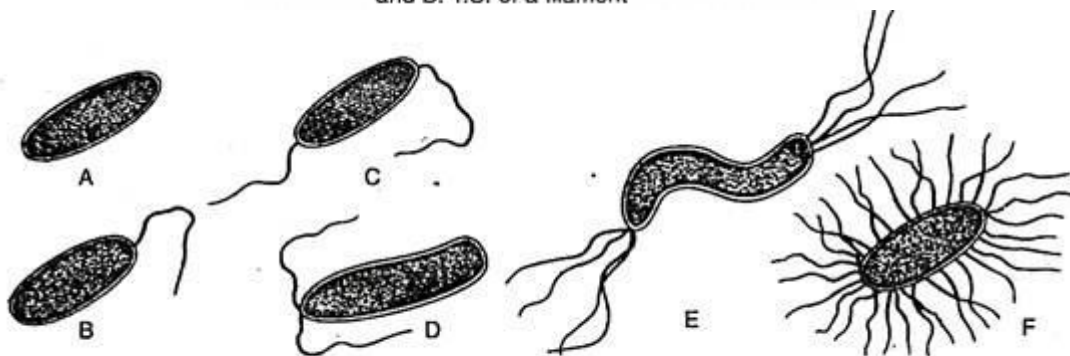

Fig. 2.16 : Different types of flagellation : A. Atrichous, B. Monotrichous, C. Amphitrichous, D. Cephalotrichous, E. Lophotrichous, and F. Peritrichous

ii. Non-polar flagellation:
Peritrichous (Fig. 2.16F). Numerous flagella are distributed all over the surface of the cell e.g., Bacillus typhosus, Clostridium.

G. Fimbriae or Pili:

The term fimbriae (sing, fimbria) was introduced by Duguid et al. (1955) and pili (sing. pilus) by Brinton (1959). Fimbriae (Fig. 2.1 7A) are observed mostly in Gram-negative rods (Salmonella typhi, typhoid fever; Shigella dysen- teriae, bacillary dysentery) and also in cocci (Neisseria gonorrhoeae, gonorrhoea). Gram- positive bacilli like Corynebacterium renale also have fimbriae or pili.

These are extremely thin and short, filamentous, non-flagellar appendages projecting peritrichously from cell surface. Their number is 100-500 per cell and measure 0.5-20 μm in length and 3-25 nm in diameter. They are made up of subunits of protein, the pilin, arranged helically and form hollow filament (Fig. 2.1 7B, C).

They are antigenic. Pili are of 3 types: Common pili, sex pili or F (fertility) pili and col I (colicin) pili (Fig. 2.1 7A). The common pili are responsible for adhesion; the sex pili (1-5 per cell) help in the transfer of genetic material during conjugation and the-col I for colicin or hemolysin production.

H. Spinae:

Spinae are tubular, pericellular rigid appendages. They are made up of single protein moiety, the spinin. They possibly help to acclimatize the cells in different environmental conditions such as salinity, temperature etc. Spinae have been reported in some Gram-positive bacteria.