ULTRASTRUCTURE OF BACTERIA

Bacteria are prokaryotic organisms. All bacteria are unicellular organisms that reproduce by binary fission. Most bacteria are capable of independent metabolic existence and growth, but species of Chlamydia and Rickettsia are obligately intracellular organisms.

SIZE

Bacteria are extremely small in size. Typically, bacteria range from about 1–5 µm. They range in size from large cells such as *Bacillus anthracis* (1.0 to 1.3 µm x 3 to 10 µm) to very small cells such as *Pasteurella tularensis* (0.2 x 0.2 to 0.7 µm). Some bacteria (Cyanobacteria) may be long as 70µm. The largest prokaryote discovered is *Thiomargarita namibiensis* (750µm long) which is a sulfur–metabolizing marine bacterium from coastal sediments of Nambia. Previously the largest bacterium was *Epulopiscium fishelsoni* (600 µm long) which is found in fish gut.

SHAPE AND ARRANGEMENT OF CELLS

Bacteria have varied shapes. The rod shaped bacteria are called bacilli (sing. bacillus), spherical ones are called cocci (sing. coccus) and spiral bacteria are known as spirilla (sing. spirillum). Some bacteria are comma shaped e.g., *Vibrio cholerae* while some others have filamentous–branched cells e.g., *Actinomyces* spp.

The arrangement of cells is also typical in various species of bacteria. The cells may occur in pairs (diplococci and diplobacilli), in chains (streptococci and streptobacilli), in groups of four forming tetrads, in irregular groups (staphylococci) or in cubical arrangement of eight or more (sarcinae). The shape and arrangement of bacterial cells is shown in fig. 3.1.

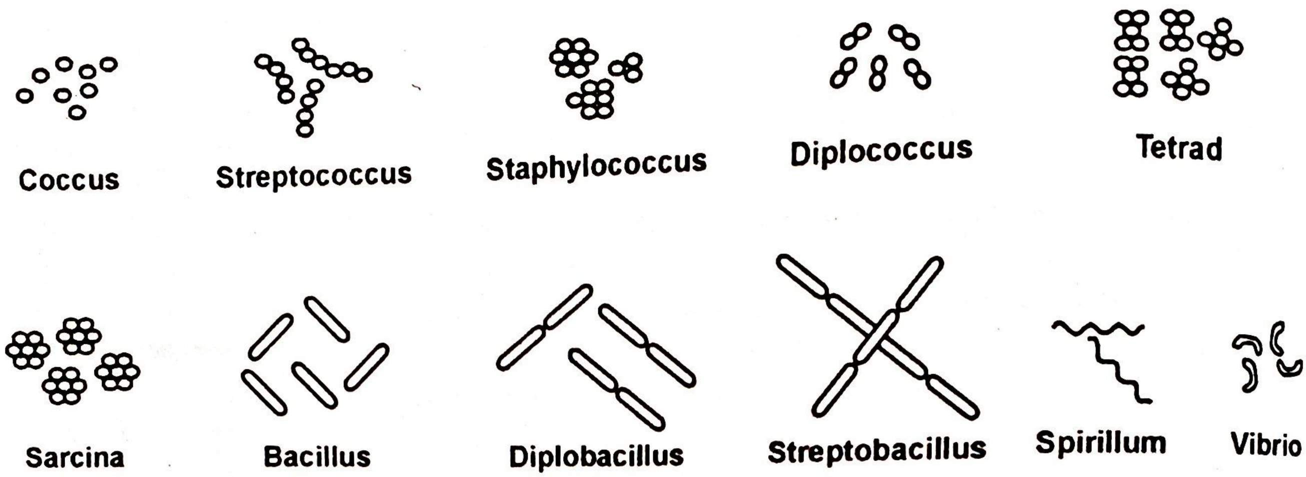

Fig. 3.1 : Bacterial shapes and cell arrangement

MORPHOLOGY OF BACTERIA

A bacterial cell (Fig. 3.2) has three architectural regions– 1) appendages (attachments to the cell surface) in the form of flagella and pili (or fimbriae), 2) a cell envelope consisting of a capsule, cell wall and plasma membrane and 3) a cytoplasmic region that contains the cell genome (DNA), ribosomes and various inclusions.

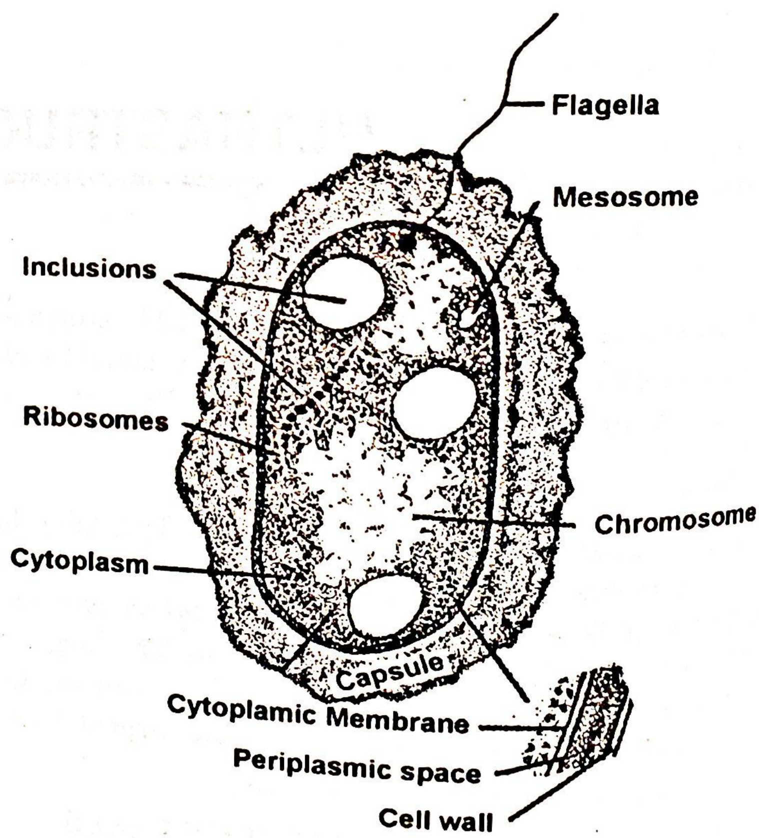

Fig. 3.2 A bacterial cell

SURFACE APPENDAGES

Two types of surface appendages are present on bacterial cell surface, the flagella, which are organs of locomotion, and pili or fimbriae. Flagella occur on both gram–positive and gram–negative bacteria. In contrast, pili found on gram–negative bacteria and only a few gram–positive bacteria.

Flagella

The flagella (sing. flagellum) are long, filamentous surface appendages that provide the swimming movement to the organism. The diameter of a bacterial flagellum is about 20 nm. The bacterial flagella differs from eukaryotic flagella in various ways. The difference between the two is shown in Table 3.1. About half of the bacilli and all of the spiral and curved bacteria are motile by means of flagella. Very few cocci are motile.

Table 3.1 : Differences between bacterial and eukaryotic flagellum

Feature	Bacterial flagellum	Eukaryotic flagellum
Size	15 to 21 μm long	140–300 μm long
Number of fibrils and arrangement	Composed of a single fibril and not surrounded by a membrane	Multifibrilled, microtubule arrangement 9+2
Composition	Consists of only protein (flagellin)	Contains proteins (tubulins), lipids and polysaccharides
Antigenic property	Present, protein are grouped as H antigens	No antigenic property
Amino acids	Some amino acids are not present e.g., tryptophan, cysteine	All usual amino acids are present
Mechanism of action	Driven directly by proton motive force	Driven by ATP hydrolysis

Flagella may be variously distributed over the surface of bacterial cells. Basically flagella are either polar (one or more flagella arising from one or both poles of the cell) or peritrichous (lateral flagella distributed over the entire cell surface).

(1) Monotrichous – A single polar flagellum
(2) Lophotrichous – A cluster of flagella at the pole
(3) Amphitrichous – A cluster of flagella at both the poles
(4) Peritichous – Flagella distributed all over the surface

Arrangement of various types of flagella is shown in fig 3.3.

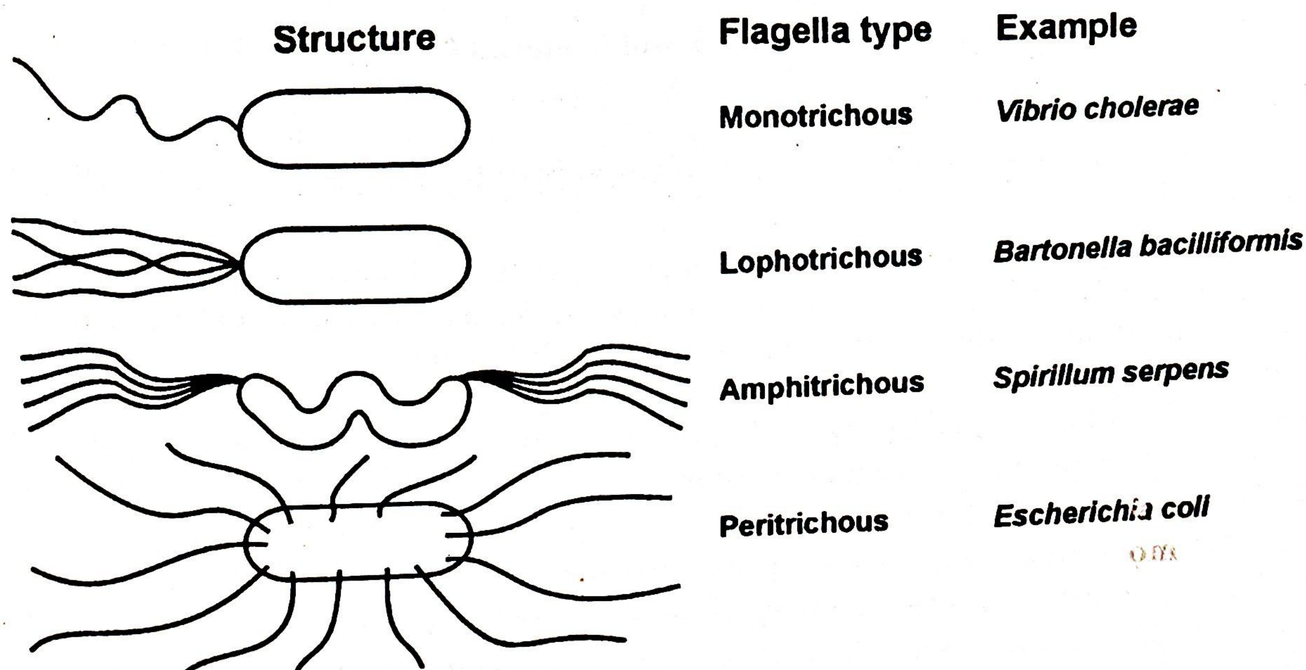

Fig. 3.3 : Different arrangements of bacterial flagella

The bacterial flagellum has three main parts– basal body, hook and filament or shaft (Fig 3.4)– **Basal body :** In gram–negative bacteria the basal body consists of four rings. They are named as M ring (attached to plasma membrane), S ring (located in periplasmic space), P ring (attached to peptidoglyan) and L ring (attached to lipopolysaccharides). The rings are connected by the rod. In gram–positive bacteria the outer two rings are absent. The M ring is located in plasma membrane and the S ring is attached to peptidoglyan layer.

Hook : It connects the basal body with the filament. The hook of gram–positive bacterial flagellum is longer than that of gram–negative flagellum.

Filament : It consists of a class of proteins called flagellin. The molecular weight of flagellin ranges from 30,000 to 60,000. The amino acids cysteine and tryptophan are absent and tyrosine and histidine are present in small amounts. Flagellins are immunogenic and constitute a group of protein antigens called the H antigens.

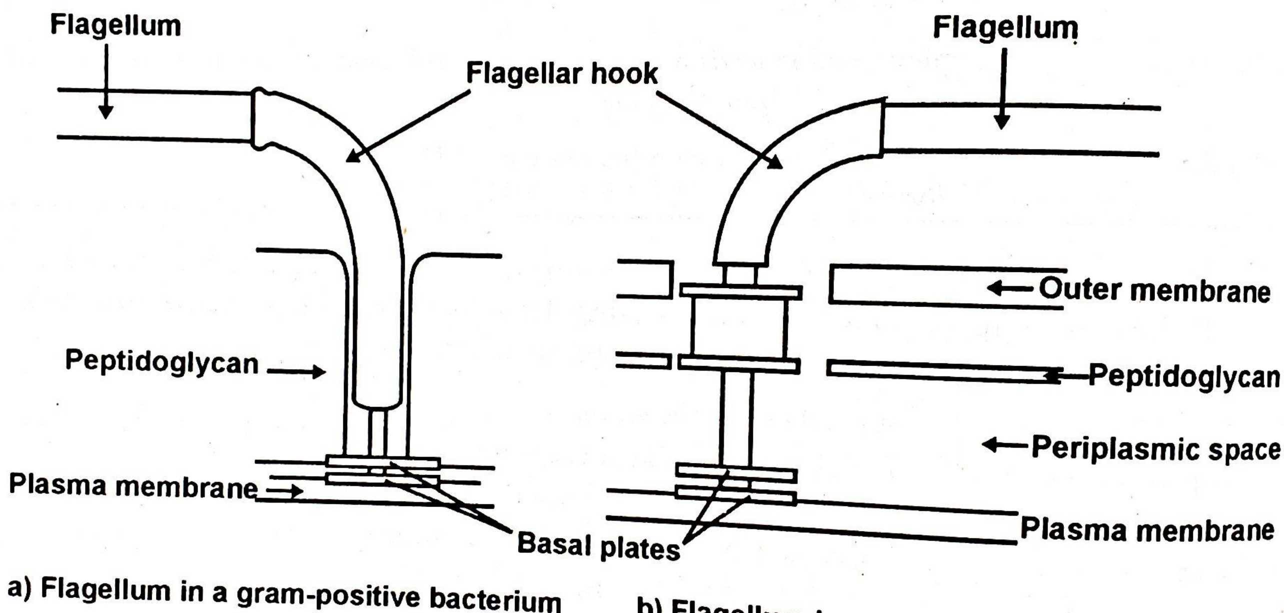

Fig. 3.4. The structure of bacterial flagellum

Bacterial motility

Bacteria move by one of the four mechanisms–adjustable buoyancy, flagella, gliding, or spirochaetal movement.

Adjustable buoyancy : Many aquatic bacteria move up and down in the water column by tuning their buoyancy using gas vacuoles. Gas vacuoles usually contain CO_2 generated by metabolism. Maintaining a particular position in the water column requires continuous adjustment of buoyancy, because as the cell rises, the water pressure decreases and the vacuole will expand, making the cell more buoyant and therefore causing it to rise. Likewise, if a cell sinks, the vacuole is compressed by the increase in pressure, making it less buoyant and causing it to sink further.

Flagellar movement : Flagella are proton–gradient–driven helical propellers. The rotor transports protons across the membrane, and is turned in the process. The rotor speed reaches to 200 to 1000 rpm. The thrust that propels the bacterial cell is provided by counterclockwise rotation. The direction of the movement is controlled by the cell surface sensors. Cells 'run' (move in a straight line) when the flagella turn one direction, and 'twiddle' (tumble) when they turn the other direction (Fig. 3.5). Some bacteria show ability to quickly reverse the directions.

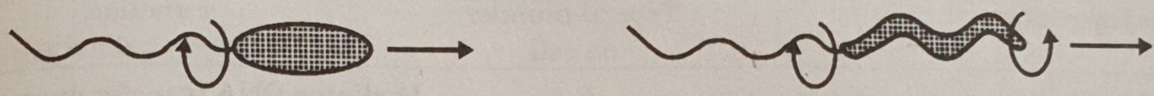

Fig. 3.5 Locomotion in flagellate bacteria

Gliding movement (Fig 3.6) : In this movement pores on one face of the cell secrete polysaccharide; hydration of the polysaccharide causes it to expand dramatically and provides a reactive force like a rocket engine.

Fig. 3.6 Gliding movement of bacteria

Spirochaetal movement (Fig. 3.7) : Spirochetes move by rotation of the axial fiber present inside the cell. This rotation turns the cell and drives it forward.

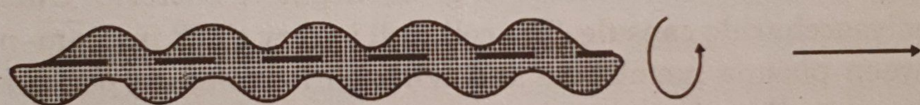

Fig. 3.7 Spirochaetal movement

Pili or Fimbriae

The pili are thin, hair like appendages on the surface of many gram–negative bacteria (Enterobacteriaceae, Pseudomonadaceae and Caulobacter) and on only a few gram–positive bacteria (e.g., *Corynebacterium renale*). Pili are made up of protein referred to as pilins. Pili are shorter and stiffer than flagella, and slightly smaller in diameter. Pili are more rigid in appearance than flagella. In some bacteria, such as *Shigella* sps. and *E. coli*, pili are distributed profusely over the cell surface, with as many as 200 per cell. As is easily recognized in strains of *E. coli*, pili can be of two types:

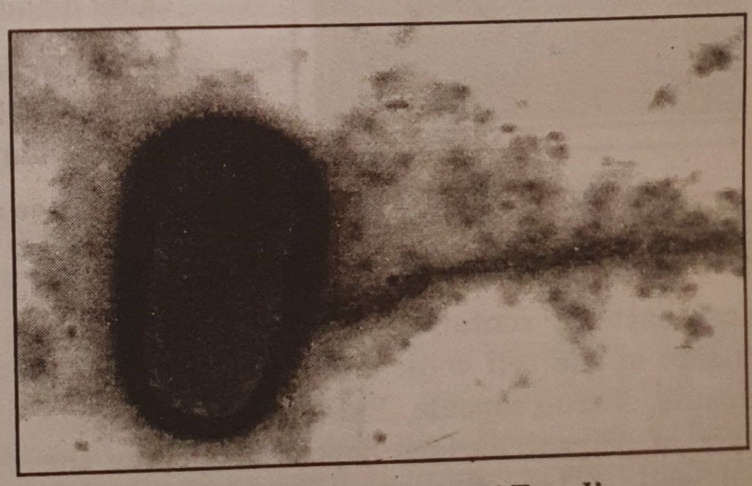

Fig. 3.8 The sex pilus of *E. coli*

a. **Common pili :** These are short and abundant.

b. **Sex pili :** These are long, present in small number (one to six). The sex pilus acts as a specific receptor for male–specific bacteriophages. (Fig. 3.8). The sex pili attach male to female bacteria during conjugation.

The pili or fimbrae of bacteria serve multiple functions. Some important characteristics and functions of pili or fimbrae are presented in table 3.2.

Table 3.2 : Some properties of pili or fimbriae.

Bacterial species	Typical number on cell	Function
Escherichia coli (F or sex pilus)	1–4	Mediates DNA transfer during conjugation
Escherichia coli (common pili or Type 1 fimbriae)	100–200	Surface adherence to epithelial cells of the GI tract
Neisseria gonorrhoeae	100–200	Surface adherence to epithelial cells of the urogenital tract
Streptococcus pyogenes (fimbriae plus the M–protein)	Not known	Adherence, resistance to phagocytosis, antigenic variability
Pseudomonas aeruginosa	10–20	Surface adherence

The Cell Envelope

The cell envelope of bacteria (Fig. 3.9) consists of several layers that enclose the cytoplasm of the cell. The cytoplasm is surrounded by the plasma membrane, a cell wall and a capsule. The cell wall itself is a layered structure in gram–negative bacteria. Outside the cell wall there may be polysaccharide capsule. The cell wall is very thick in gram–positive bacteria. The space between plasma membrane and cell wall is called as periplasmic space. The periplasmic space is thin in gram–positive bacteria as compared to gram–negative bacteria.

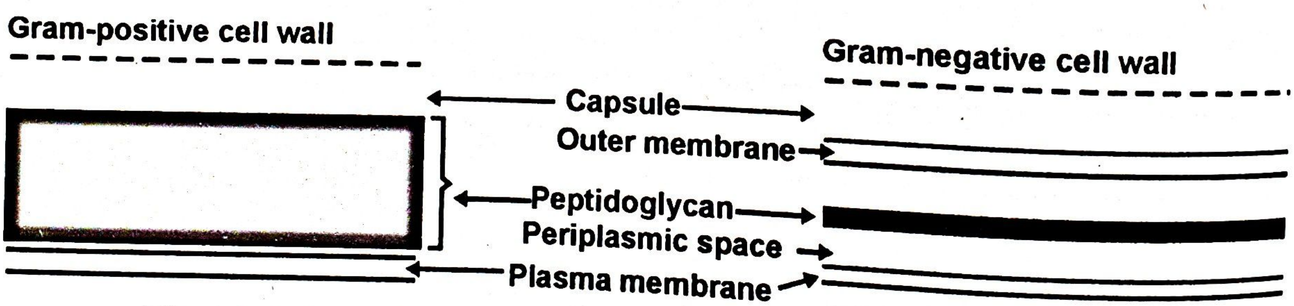

Fig. 3.9 Cell envelope of gram–positive and gram–negative bacteria

Capsules

Most bacteria contain a polysaccharide layer outside of the cell wall. This layer is called a capsule. A true capsule (Fig. 3.10) is a discrete layer of polysaccharides deposited outside the cell wall. A less discrete structure or matrix which embeds the cells is called a slime layer. A type of capsule found in bacteria called a glycocalyx is a thin layer of tangled polysaccharide fibers which is almost always observed on the surface of cells growing in nature.

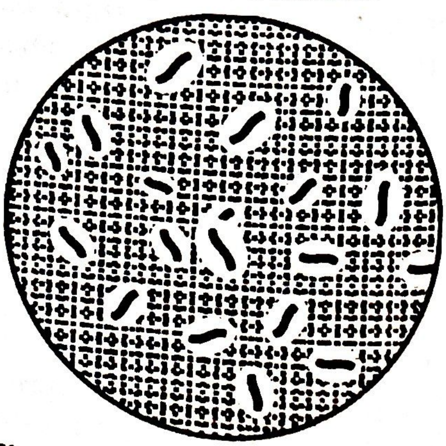

Figure 3.10. Bacterial capsules

The capsules are generally made up of polypeptides. Table 3.3 represents the various capsular substances formed by some of gram–positive and gram–negative bacteria.

Table 3.3 : Chemical composition of some bacterial capsules.

Bacterium	Capsule composition	Structural subunits
Gram–positive Bacteria		
Bacillus anthracis	Polypeptide (polyglutamic acid)	D–glutamic acid
Streptococcus pneumoniae	Polysaccharides	Sugars
Streptococcus pyogenes	Polysaccharide (hyaluronic acid)	N–acetyl–glucosamine and glucuronic acid
Gram–negative Bacteria		
Escherichia coli	Polysaccharide (colonic acid)	Glucose
Pseudomonas aeruginosa	Polysaccharide	Mannuronic acid
Agrobacterium tumefaciens	Polysaccharide	(glucan) Glucose

Capsules have several functions. Like fimbriae, capsules, slime layers and glycocalyx often mediate adherence of cells to surfaces. Capsules also protect bacterial cells from engulfment by predatory protozoa or white blood cells (phagocytes), or from attack by antimicrobial agents of plant or animal origin. Capsules in certain soil bacteria protect cells from perennial effects of drying or desiccation. Capsular materials (e.g. dextrans) may be overproduced when bacteria are fed sugars to become reserves of carbohydrate for subsequent metabolism.

Cell wall

The bacteria have a rigid cell wall. The cell wall is an essential structure that protects the cell protoplast from mechanical damage and from osmotic rupture or lysis. The cell wall of gram–positive bacteria is relatively thick (20 to 80 nm) as compared to the cell wall of gram–negative bacteria (5 to 10 nm thick).

Peptidoglycan

The cell wall of bacteria is made up of peptidoglycan also called as murein. Peptidoglycan is a polymer of disaccharides (a glycan) cross–linked by short chains of amino acids (peptides).

Peptidoglycan structure and arrangement (Fig. 3.11) of gram–negative bacteria (*E. coli*) is different from gram–positive bacteria (*Staphylococcus aureus*). The peptidoglycan back-bone is made up of alternating molecules of N–acetylglucosamine (NAG) and N–acetyl-muramic acid (NAM) connected by a beta 1,4–glycoside bond. In gram–negative bacteria a peptide side chain that contains L–alanine, (L–ala), D–glutamate (D–glu), Diaminopimelic acid (DAP), and D–alanine (D–ala) is attached to N–acetylmuramic acid. The adjacent peptidoglycan subunits are crosslinked by intrapeptide bonds (Fig. 3.12) between a free amino group on DAP and a free carboxyl group on a nearby D–ala.

In gram–positive bacteria tetrapeptide chain contains L–lysine (L–lys) in place of DAP (in *E. coli*).In place of the intrapeptide bond (in gram–negatives) an interpeptide bridge of amino acids is present in gram –positive bacteria. The interpeptide bridge connects a free amino group on lysine to a free carboxy group on D–ala of a nearby tetrapeptide side chain. In *S. aureus*, the interpeptide bridge is a peptide consisting of 5 glycine (Gly) molecules called as pentaglycine bridge (Fig. 3.13).

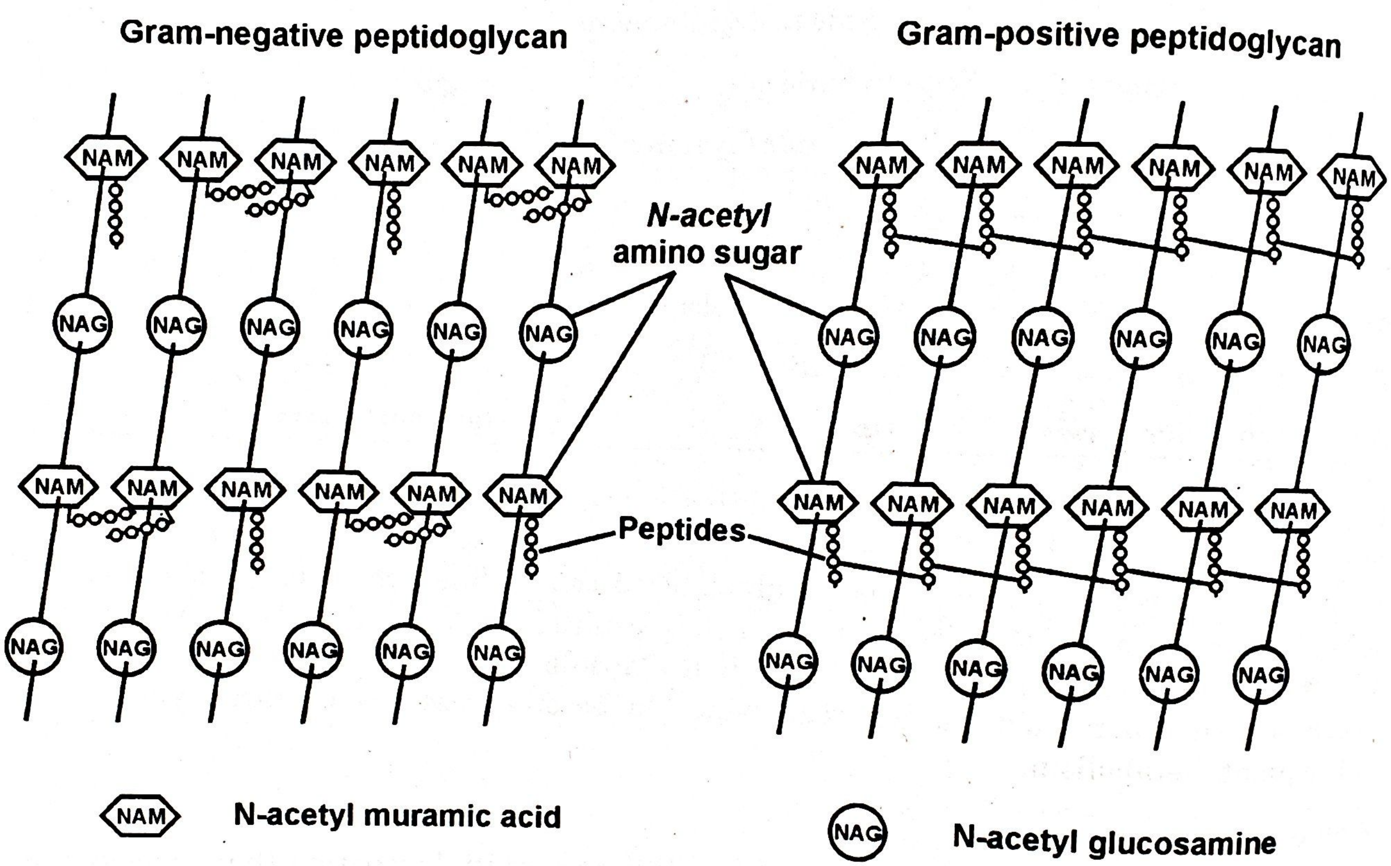

Fig. 3.11 The structure of peptidoglycan

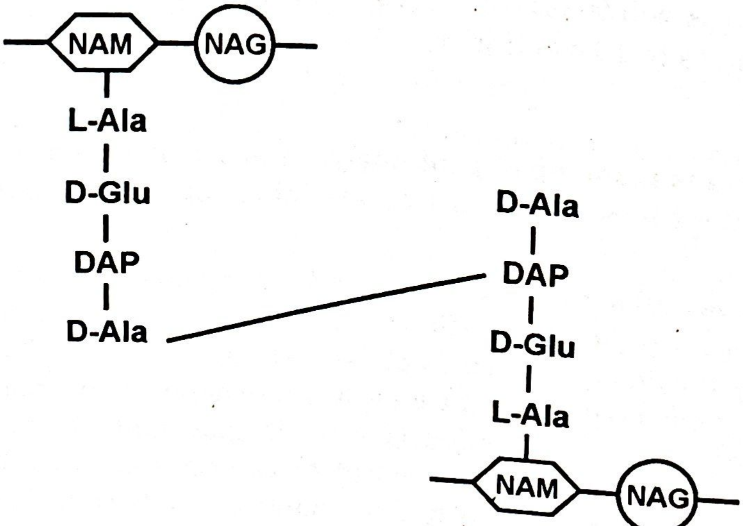

Fig. 3.12 : Peptidoglycan subunits–cross–linked by intrapeptide bond in gram–negative bacteria

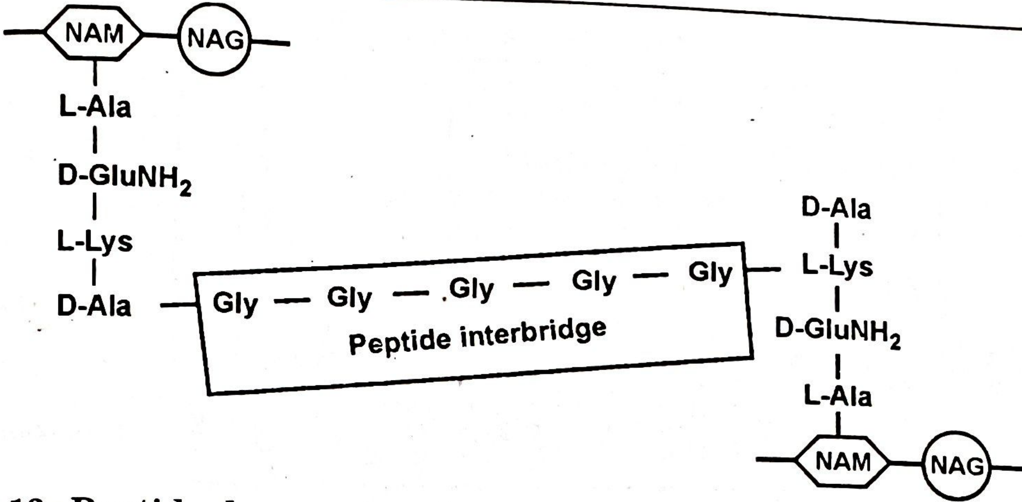

Fig. 3.13 : Peptidoglycan subunits cross-linked by pentaglycine bridge in gram-positive bacteria

Gram-positive cell wall

The gram-positive bacteria have greater amount of peptidoglycan in their cell wall than gram-negative bacteria. The gram-positive bacteria contain 50 per cent peptidoglycan of their cell content. The gram-positive bacterial cell wall (Fig.3.14) is made up of several layers of peptidoglycan subunits. Teichoic and Lipoteichoic acids run throughout the layers of the peptidoglycan. Teichoic acid is a polymer made from ribitol-phosphate and/or glycerol-phosphate. Lipoteichoic acid is a teichoic acid attached to a lipid, which is seated in the cell membrane.

The functions of teichoic acid are not known. They are essential to viability of gram-positive bacteria in the wild. They provide a channel of negative charges for transporting positively charged substances through the peptidoglycan layer. In some bacteria it is thought to be useful for adherence of the bacteria to tissue surfaces.

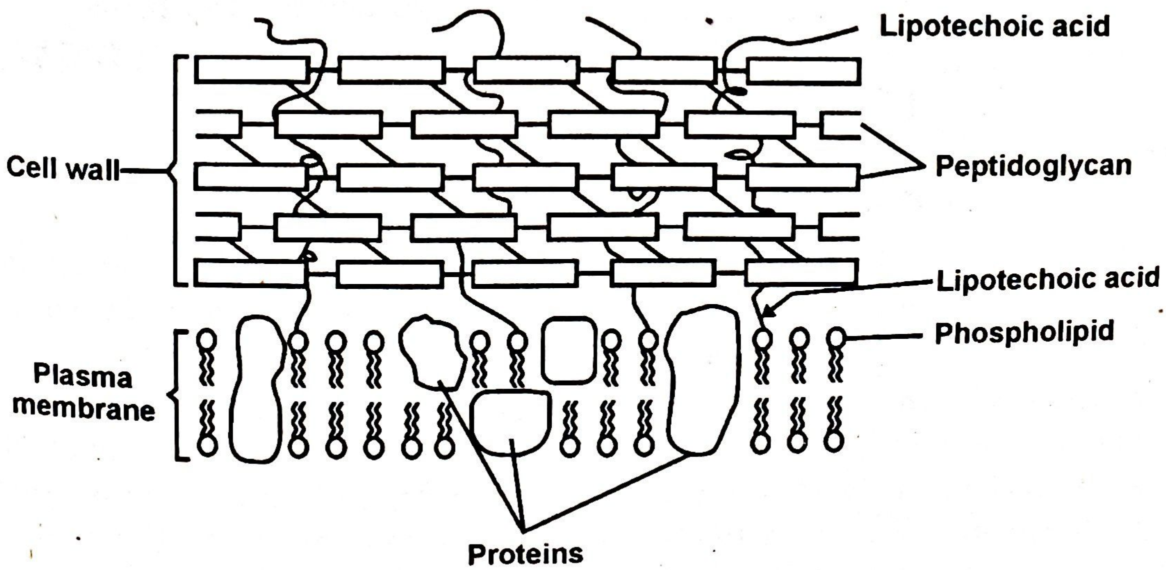

Fig. 3.14 : The structure of gram-positive cell wall

Gram-negative cell wall

Gram-negative cell walls (Fig. 3.15) are composed of two parts– the inner peptidoglycan layer and an outer membrane. The layer of peptidoglycan is thinner in gram-negative cell walls. The outer layer is composed of lipoproteins, lipopolysaccharides and proteins.

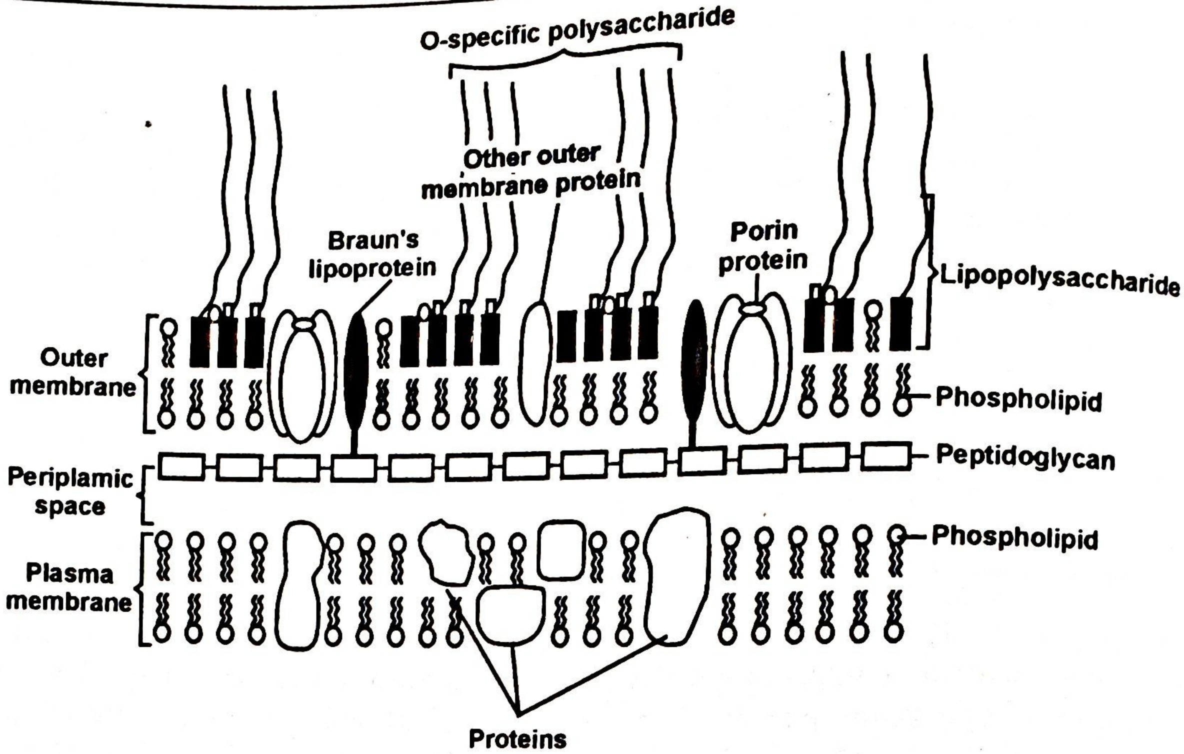

Fig. 3.15 : The structure of gram-positive cell wall

a. **Lipoproteins :** The most abundant lipoprotein is Braun's lipoprotein. The outer membrane is anchored to the peptidoglycan by Braun's lipoprotein.

b. **Lipopolysaccharides :** These molecules (Fig. 3.16) are composed of a hydrophobic region called Lipid A, the core polysaccharide and the O–specific polysaccharide (O antigen).

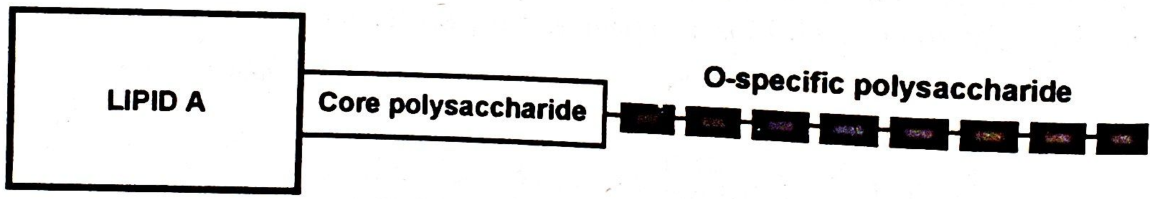

Fig. 3.16: The lipopolysaccharide molecule

These lipopolysaccharides are important for a number of reasons: their lipid portion, lipid A, is a highly reactive toxic molecule also, every lipopolysaccharide has a side chain, the O side chain, that is important for antigen identification by the immune system.

Bacterial lipopolysaccharides are toxic to human beings. The toxic component of endotoxin (LPS) is Lipid A. This highly reactive molecule is responsible for endotoxic shock if the human body is exposed to it. The O–specific polysaccharides facilitate bacterial attachment to the host cell surface. Variation in the exact sugar content of the O polysaccharide prevents gram–negative organisms to be phagocytosed by host cells.

c. **Proteins :** In addition to these components, the outer membrane possesses several major outer membrane proteins; the most abundant is called porin. The assembled subunits of porin form a channel that limits the passage of hydrophilic molecules across the outer membrane. Porins act as channels for smaller molecules to pass through. Various other proteins are also present in the outer membrane.

The plasma membrane

The plasma membrane is composed of lipids and proteins. The structure of plasma membrane (Fig. 3.17) is described as the fluid mosaic model of Singer and Nicolsan (1972). The membrane is considered to be a quasifluid structure in which lipids and proteins are arranged in mosaic pattern. The lipids and proteins float around, exchanging positions. This gives the membrane flexibility.

Multiple functions are performed by the plasma membranes of both gram–positive and gram–negative bacteria. The membrane allows passage of water and uncharged molecules up to molecular weight of about 100 daltons, but does not allow passage of larger molecules or any charged substances except by means special membrane transport processes and transport systems. Plasma membranes are the site of active transport, respiratory chain components, energy–transducing systems, the H^+ ATPase of the proton and membrane stages in the biosynthesis of phospholipids, peptidoglycan, lipopolysaccharides, and capsular polysaccharides.

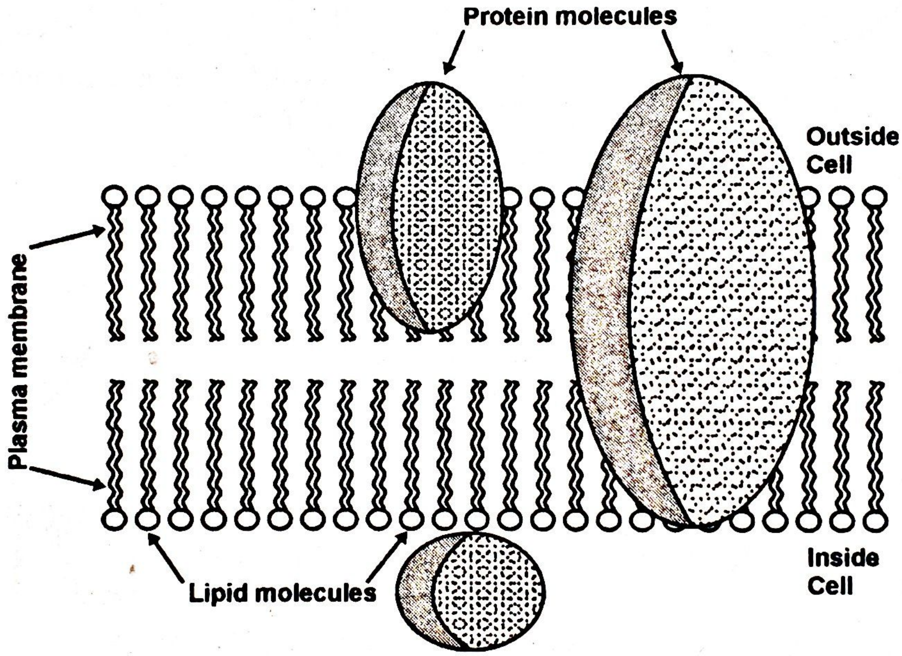

Fig. 3.17 : The structure of plasma membrane

Components of Cytoplasmic Region

Cytoplasm

The substance inside the plasma membrane is referred to as cytoplasm. Cytoplasm contains about 80 per cent of water and various other molecules like proteins (enzymes), carbohydrates, lipid inorganic ions and low molecular weight components. The cytoplasm of bacterial cells includes the chromosome, ribosomes, plasmids, mesosome and various inclusions.

Nucleoid

Prokaryotic and eukaryotic cells were initially distinguished on the basis of nuclear structure. The prokaryotic nucleoid is different from the true eukaryotic nucleus, which has a complex mitotic apparatus and surrounded by a nuclear membrane. The bacterial nucleoid lacks a limiting membrane. The DNA is a single, continuous, giant circular molecule. It can be folded into a number of supercolied loops to be packaged in the cell (Fig. 3.18). The molecular weight of the DNA is 3 x 10^9 daltons. The unfolded nuclear DNA would be about 1 mm long.

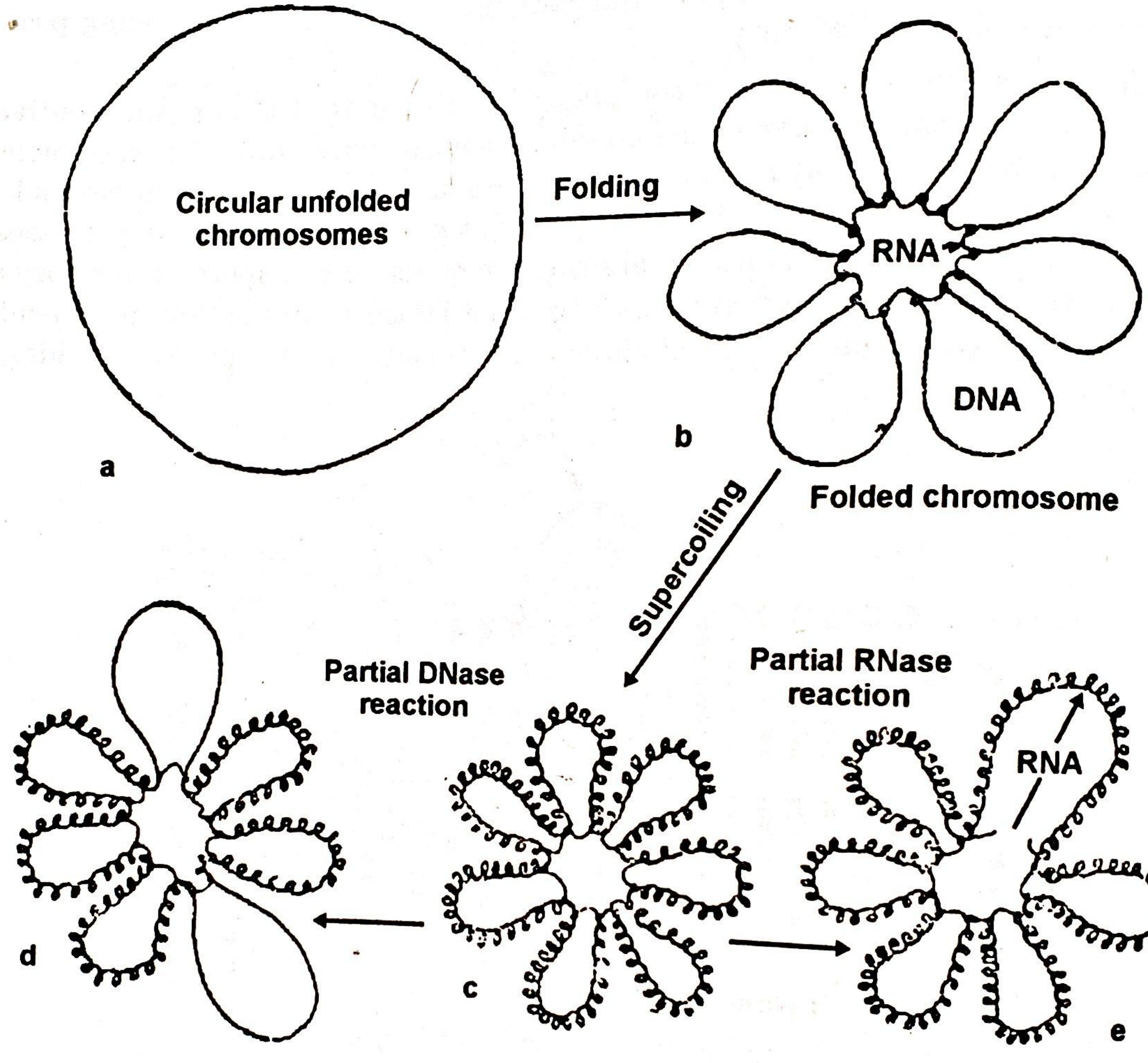

Fig. 3.18 : Model of bacterial chromosome
a) Circular unfolded chromosome b) Folded chromosome c) Folded and supercoiled chromosome d) Supercoiling relaxed into loops by DNase nicks e) RNase cuts one RNA molecule, causing two loops to coalesced without loosing supercoiling

The bacterial nucleoid contains a single chromosome. The number of copies of this chromosome in a cell depends on the stage of the cell cycle (chromosome replication, cell enlargement, chromosome segregation, etc.). The chromosome is permanently attached to the cell membrane throughout the various stages of the cell cycle. Bacterial chromatin does not contain basic histone proteins, but low–molecular–weight polyamines and magnesium ions may fulfill a function similar to that of eukaryotic histones.

Ribosomes

The distinct granular appearance of prokaryotic cytoplasm is due to the presence and distribution of ribosomes. The ribosomes of prokaryotes are 70S in size, being composed of

30S and 50S subunits (Fig. 3.19). S is referred as Svedberg units. The ribosomes are ribonucleoprotein consist of RNA (60 %) and protein (40 %). The diameter of ribosome is around 200 A°. The RNA is called as ribosomal RNA and is of different types 5S, 16S and 23S.

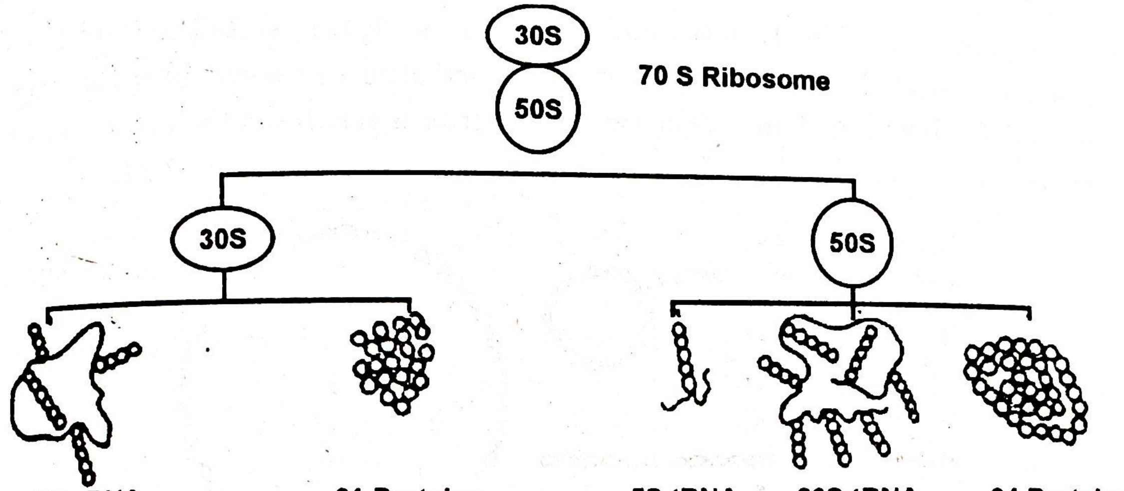

Fig. 3.19 : Components of the 70S ribosome

Ribosomes are involved in the process of protein synthesis. Often, in a cell actively synthesizing protein these ribosomes group together in chains or clusters to form polysomes.

Mesosomes

The mesosomes (Fig. 3.20) are vesicular or tubular membranes which are formed by an invagination of the plasma membrane. These structures are much more prominent in gram–positive than in gram–negative organisms.

Mesosome can be useful for many ways. They increase the surface area for transportation of molecules in and out the cell. Mesosomes are attached to the nucleoid. They are involved in replication and cell division. In the phototrophic bacteria mesosomes are the sites of photosynthetic apparatus of the cell.

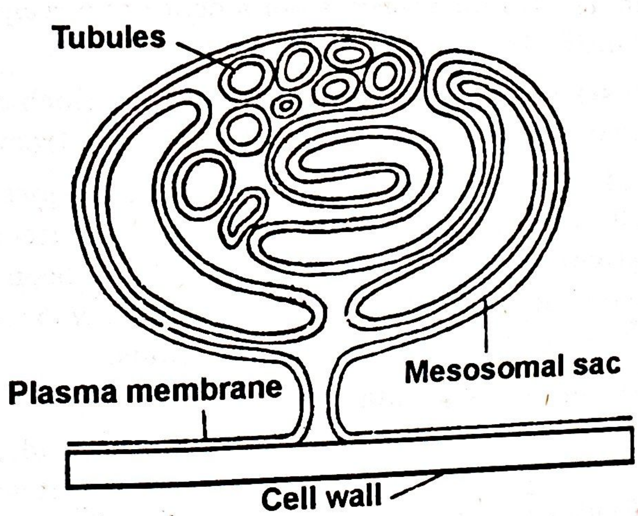

Fig. 3.20 : Diagram of a mesosome

Plasmids

A plasmid is an independent, circular, self–replicating DNA molecule that carries only few genes. They replicate during cell division and are inherited by both the sister cells. Some plasmids are capable of replicating autonomously as well as integrating into bacterial chromosal DNA and are named as episomes. Thus the F sex factor of *E.coli* is called as an episome as it can exist in the F^+ or Hfr state.

Plasmids are circular double stranded molecules. In the resting state the DNA is coiled in a right–handed superhelical coil. The twisted conformation is known as covalently closed circular (ccc) DNA. Nicking of one of the two DNA strands results in the release of twists to form open circular form. The three forms of plasmids are shown in Fig. 3.21.

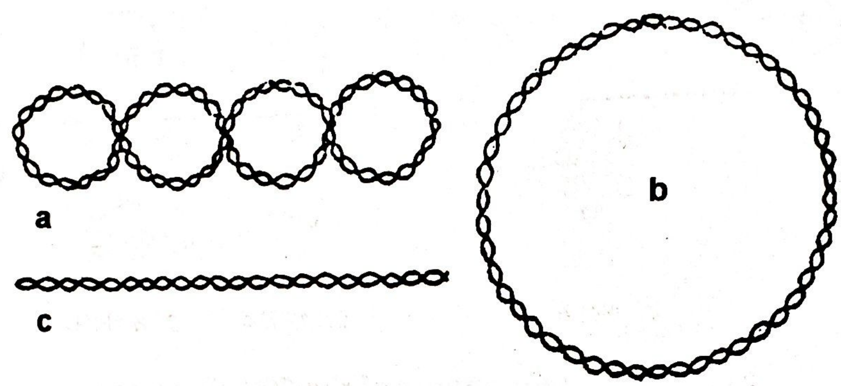

Fig. 3.21 : Plasmid DNA a) supercoiled form b) open nicked circle c) linear duplex

Plasmids can be of various types–

(a) **Fertility (F) plasmid :** Plasmids, which contain tra–genes. They are capable of conjugation. During bacterial conjugation, F plasmid directs transfer of DNA, in single–stranded form, from a donor to a recipient cell. F plasmid of *E. coli* is the first identified among the conjugative plasmids.

(b) **Resistance (R) plasmid :** Plasmids, which contain genes that can build a resistance against antibiotics (such as ampicillin, tetracyclin etc.) or poisons.

(c) **Col plasmid :** Plasmids, which contain genes that code for colicines, proteins that can kill other bacteria. Colicins are bacteriocins(proteins that have antimicobicidal activity). Various other bacteria have been identified to produce bacteriocins like plantaricin, casecin 80 (produced by *Lactobacillus* spp.) Some of these bacteriocins are encoded by genes present on plsamids.

(d) **Degradative plasmids :** These enable the digestion of orgainc substances or unusual substances, e.g., toluole or salicylic acid. Some plasmids in *Pseudomonas* are degradative plasmids they can degrade lactose causing fermentation.

(e) **Virulence plasmids :** These are the plasmids which turn the bacterium into a pathogen, e.g., *Agrobacterium tumefaciens* which bears a plasmid named as Ti plasmid which induces tumor in infected plants. The disease is referred to as crown gall disease.

Inclusions

Inclusions are distinct granules that are a part of the cytoplasm. Inclusion granules are usually reserve materials. Some common inclusions of bacterial cells are presented in table 3.4.

Table 3.4 : Some inclusions in bacterial cells

Cytoplasmic inclusions	Organism where found	Composition	Function
Volutin granules	*Corynebacterium diptheriae*	Polymers of phosphates	Reserve of high energy phosphate
Polysaccharide granules	Many bacteria e.g., *E. coli*	Starch and glycogen	Reserve of carbon and energy source
Polybetahydroxy-butyric acid (PHB)	Many bacteria e.g., *Bacillus*	Polymerized hydroxy butyrate (lipid)	Reserve of carbon and energy source
Gas vacuoles	Aquatic bacteria especially cyanobacteria	Hollow cylinder covered by protein	Buoyancy (floatation) in the vertical water column
Sulfur granules	Phototrophic purple and green sulfur bacteria	Elemental sulfur	Reserve of electrons (reducing source) in phototrophs
Parasporal crystals	Endospore–forming bacilli (genus *Bacillus*)	Protein	Unknown but toxic to certain insects
Magnetosomes	*Aquaspirillum magnetotacticum*	Magnetite (iron oxide) Fe_3O_4	Orienting and migrating along geo– magnetic field lines
Carboxysomes	Nitrifying bacteria	Cyanobacteria and thiobacilli	Ribulose1,5–diphosphate carboxylase
Chycobilisomes	Cyanobacteria	Phycobiliproteins	Light–harvesting pigments
Chlorosomes	Green bacteria	Lipid and protein and bacteriochlorophyll	Light–harvesting pigments

Endospores

A bacterial structure sometimes observed as a dormant cell called an endospore. Endospores are formed by a few groups of bacteria such as *Bacillus*, *Clostridium* and *Thermoactinomycetes* etc.

Endospores (Fig. 3.22) appear as round cells within the vegetative cell wall. Some strains produce autolysins that digest the walls and liberate free endospores. The spore protoplast core contains a com-

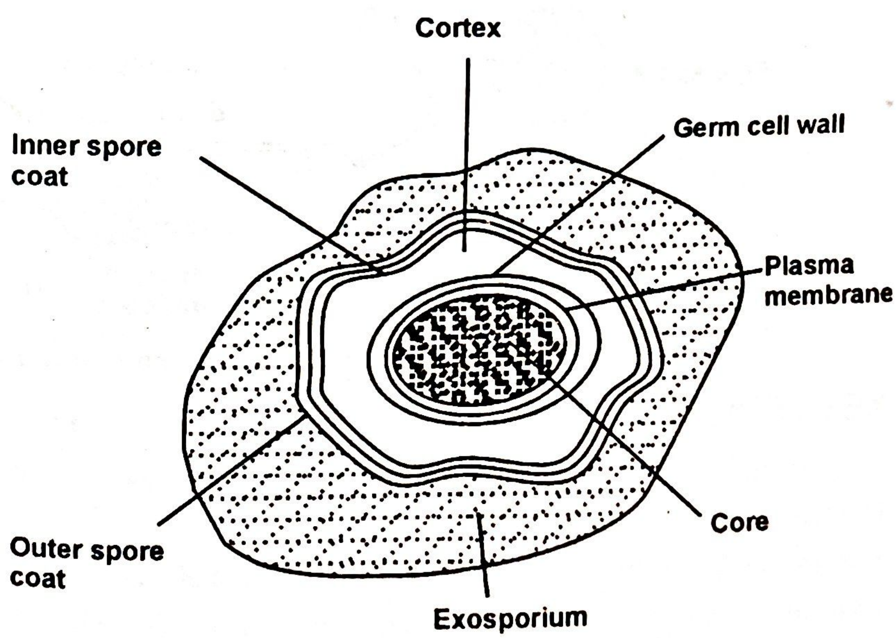

Fig. 3.22 : A bacterial endospore

plete nucleus, ribosomes and energy generating components that are enclosed within a modified cytoplasmic membrane. The germ cell wall surrounds the plasma membrane. On germination, this wall becomes the vegetative cell wall. Surrounding the spore wall is a thick cortex that contains an unusual type of peptidoglycan, which is rapidly released on germination. A spore coat (inner and outer) composed of keratin–like protein covers the cortex.

The process of endospore formation is called as **sporulation**. The endospores are highly resistant to environmental stresses such as high irradiation, strong acids, disinfectants, etc. This is because of presence of cortical cell wall and large amounts of calcium dipicolinate. They are probably the most durable cells produced in nature. The endospores may remain dormant for several years. Under favourable conditions spore is converted into vegetative cell. This process is called as spore germination. The process of sporulation and spore germination is shown in fig. 3.23. Hence, endospore formation is a mechanism of survival rather than a mechanism of reproduction.

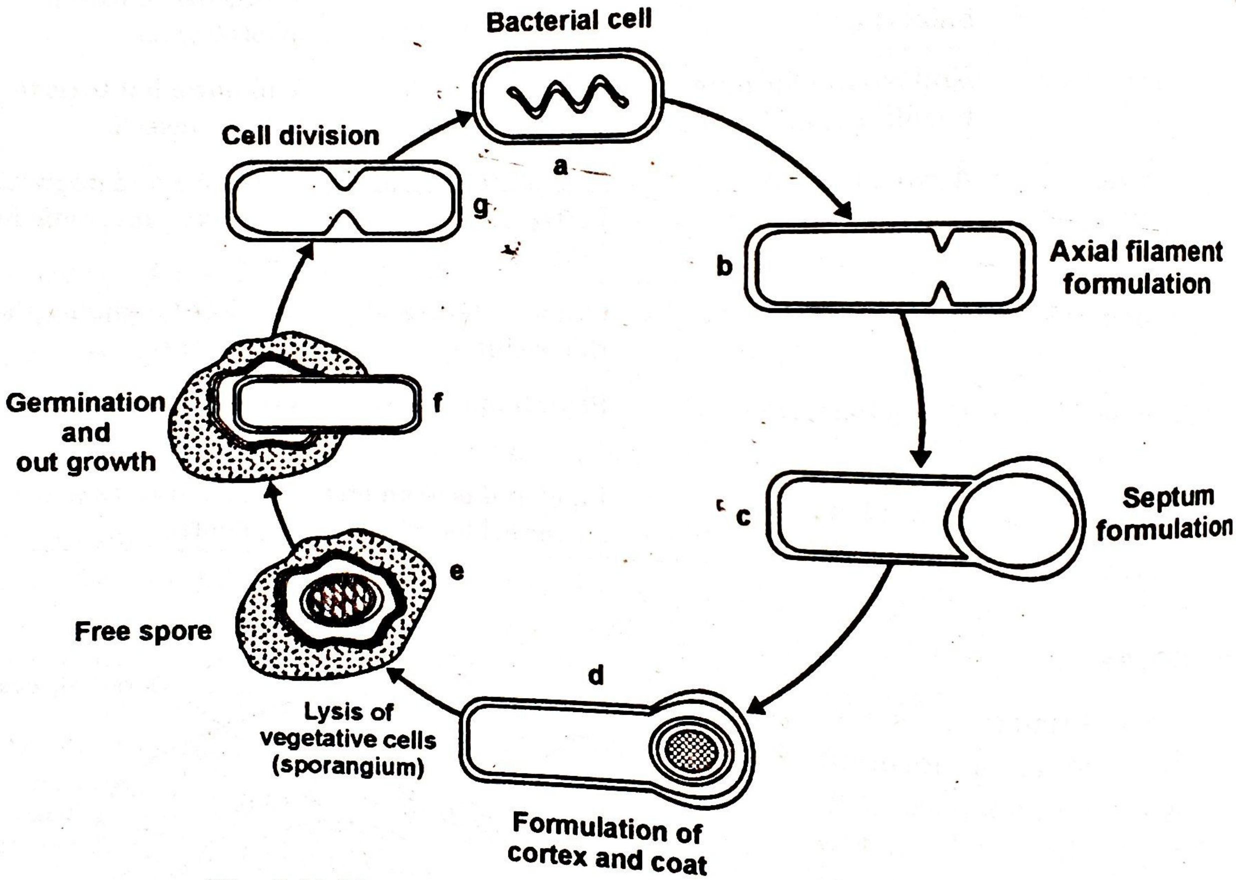

Fig. 3.23 The cycle of spore formation and germination

KEY TERMS

Amphitrichous : A cluster of flagella at both the poles.

Bacilli : A group of rod –shaped bacteria.

Bacteria : All prokaryotes that are not members of the domain Archaea.

Capsule : A colorless, transparent, mucopolysaccharide sheath on the wall of a cell.