BACTERIAL ANATOMY

The outer layer or cell envelope of a bacterial cell consists of two components—(a) a rigid *cell wall proper,* and (b) underlying *cytoplasmic membrane* or plasma membrane (Fig. 2.6). The cell wall encloses the protoplasm comprising of *cytoplasm* (ribosomes, inclusion granules, mesosomes) and a single circular chromosome of DNA. Some bacteria, in addition, may possess additional structures, such as protective gelatinous covering outside the cell wall known as *capsule,* and, when it is too thin, it is known as *microcapsule.* Apart from this, some bacwall takes part in cell division by forming an ingrowth from the cell wall. The mucopeptide component of cell wall possesses target site for antibiotics, lysozymes and bacteriophages.

Structure of cell wall

The chemical structure of cell wall of Gram-positive and Gram-negative bacteria differ considerably. It is comparatively simpler in Gram-positive bacteria than in Gram-negative bacteria (Fig. 2.7).

The rigid component of the cell wall is made of peptidoglycan. Peptidoglycan is absent in cell-wall-deficient mycoplas-

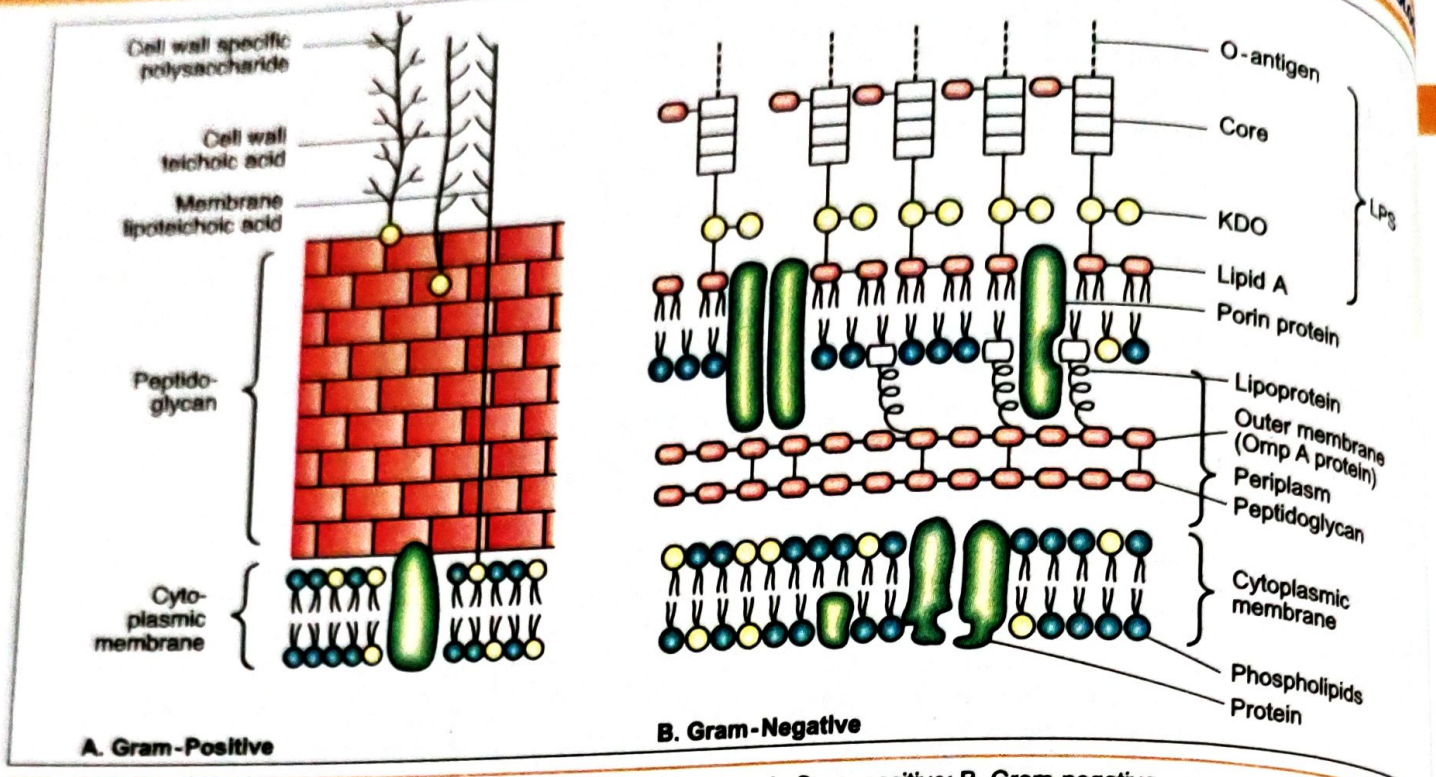

Fig. 2.7 Section of a bacterial cell wall: A. Gram-positive; B. Gram-negative

mas and ureaplasmas. Synthesis of peptidoglycan is disrupted by penicillin and cephalosporin antibiotics. It is present in the cell wall of both Gram-positive and Gram-negative bacteria, although it is more abundant in Gram-positive bacteria.

Chemically, **peptidoglycan** (mucopeptide or murein) is composed of a backbone of alternating carbohydrate moieties of *N-acetyl glucosamine* and *N acetyl* muramic acid molecules, which are cross-linked by short peptide chains (generally D and L amino acids) (Fig. 2.8).

Autolysins: All bacterial cell walls possess associated enzymes called **autolysins** (such as lysozyme) which can hydrolyse their own cell wall substance. Under normal circumstances, their action is confined to remodelling of the cell wall in the course of their growth. This capacity of autolysins to dissolve the peptidoglycan is essential for cell growth, cell septation, sporulation, and in transformation.

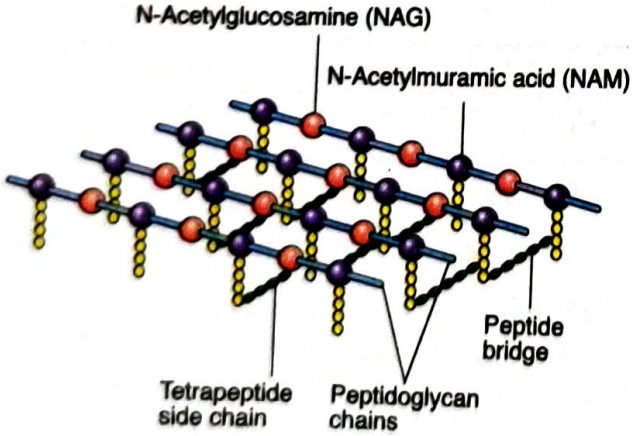

Fig. 2.8 Chemical structure of cross-linking (peptide linkage) in peptidoglycan of cell wall

Gram-positive versus Gram-negative bacteria

Gram-positive bacteria have a simple and thicker cell wall than that of a Gram-negative cell wall which is complex and thinner. Their differences are shown in Table 2.3.

Table 2.3 Differences between Gram-positive and Gram-negative cell wall

Characteristic	Gram-positive	Gram-negative
Thickness	Thicker	Thinner
Peptidoglycan	++++(thick)	+(thin)
Periplasmic space	Absent or small	Present
Outer membrane	Absent	Present (Phospholipids with saturated fatty acids)
Teichoic acid	Present	Absent

(a) **Gram-positive cell wall:** It consists of:

(i) Gram-positive bacteria possess thick, multilayered peptidoglycan cell walls that lie exterior to the cytoplasmic membrane. The *peptidoglycan* layer of Gram-positive bacteria is much thicker (15-50 nm) than that in Gram-negative bacteria (2-6 nm) (Fig. 2.7) and consists of multiple layers. Approximately 40-80% of the dry weight of some Gram-positive cell walls is peptidoglycan. The periplasmic space is absent.

(ii) *Special components:* The peptidoglycan in most Gram-positive bacteria is covalently linked to *teichoic* and *lipoteichoic acids*—as much as 50% of the dry-weight of the cell wall and

10% of the total cell. The teichoic acids constitute major surface antigens of Gram-positive bacteria.

(iii) *Other components*: Certain Gram-positive cell walls also contain antigens such as the polysaccharide and protein.

(b) **Gram-negative cell wall**: Gram-negative bacteria possess two membranes—an inner cytoplasmic membrane and an outer membrane. The peptidoglycan layer is located between the two membranes. Gram-negative cell wall is a complex structure and thinner than that of Gram-positive cell.

(i) *Periplasmic space*: There is a periplasmic space immediately outside the cytoplasmic membrane that contains degradative enzymes (alkaline phosphatases, proteases, nucleosidases etc.) and specific binding and transport proteins for amino acids, vitamins, and ions.

(ii) A thin single-unit-thick *peptidoglycan* layer (2-6 nm) consisting of 5-10% of the cell wall by weight forms the outer border of the periplasmic space (Fig. 2.7). Compared to Gram-positive cell walls, the peptidoglycan of Gram-negative cell wall is thin, hence the Gram-negative cells are more susceptible to physical damage.

(iii) Outside the peptidoglycan layer is the *outer membrane* (Fig. 2.7). It is found only in Gram-negative bacteria. It is a basic structure similar to the cytoplasmic membrane, i.e., a phospholipid bilayer that contains various embedded **lipopolysaccharides** (LPS) and various proteins known as outer membrane proteins (OMP).

Lipopolysaccharide (LPS): LPS is structural component unique to the outer membrane of Gram-negative bacteria and accounts for their endotoxic activity and *somatic* or O antigen specificity. The LPS molecules are high molecular weight complex glycolipids consisting of three regions. Region I is the polysaccharide portion projecting from the outer membrane that determines O antigen specificity. Region II is the core polysaccharide and is generally similar in structure within a given bacterial genus or species. Region III is the glycolipid portion (called lipid A) toxic to humans and animals. Since lipid A is an integral part of the outer membrane, it is called an endotoxin. Lipid A is responsible for the endotoxic activities—i.e., pyrogenicity, tissue necrosis, lethal effect, anticomplementary activity, B cell mitogenicity, immunoadjuvant property and antitumour activity. LPS molecules are found in the outer leaflet of the outer membrane. Attached to the lipid A portion of the LPS is the core polysaccharide.

Lipooligosaccharides (LOS): Some Gram-negative bacteria (e.g., *Neisseria gonorrhoeae, Haemophilus influenzae*, and *Bartonella pertussis*) contain lipooligosaccharides (LOS) rather than LPS in their cell walls. These organisms shed large amounts of LOS resulting in fever and severe symptoms. LOS contains lipid and an oligosaccharide core containing KDO, but does not contain a long-chain as polysaccharide n-antigen as seen in LPS of enteric bacteria.

Bacteria with defective cell wall

Cell wall protects the delicate underlying cytoplasmic membrane and maintains the high intracellular levels of metabolites. Removal of cell wall results in lysis of bacteria. Synthesis of cell wall may be **inhibited** by lysozyme of tears, bacterial autolysin, antibiotics and bacteriophages.

(i) *Gram-positive bacteria*: Complete removal of the cell wall of a Gram-positive bacteria results in the formation of **protoplasts**, which lyses unless the growth medium is osmotically stabilised. The protoplast is constituted by the cytoplasmic membrane and the bacterial contents. In order to maintain a spherical configuration, protoplasts require an isotonic medium. They cannot maintain integrity in a hypo or hypertonic medium. Protoplasts do not multiply (see L forms) and cannot revert to normal bacterial morphology by forming their cell walls.

(ii) *Gram-negative bacteria*: Gram-negative bacteria with damaged cell walls become **spheroplasts** (i.e., they assume a spherical shape) even in a nonisotonic medium because of their resistance to differences in osmotic pressure between the extracellular and intracellular compartments.

Spheroplast differs from protoplast in that some cell wall material is retained by spheroplast. In the laboratory, spheroplasts are produced by growth with penicillin. When placed in suitable environment, spheroplasts may multiply by binary fission or budding and reproduce through many serial subcultures.

(iii) *L-forms* (wall-less organisms): These cell wall deficient forms of bacteria may emerge spontaneously (e.g., in *Streptobacillus moniliformis* and *Bacteroides* spp) or by inhibition of cell wall synthesis in bacteria of normal morphology (e.g., during antibiotic therapy). These aberrant organisms could account for bacterial persistence during therapy with certain antibiotics. Because of lack of rigid cell wall, L-forms do not have any regular size and shape, but, unlike protoplasts, they are capable of growing and multiplying in a suitable culture medium. Colonies of L-forms on agar medium have a characteristic 'fried egg' appearance, like that of mycoplasmas (naturally lack peptidoglycan). Kleinberger-Nobel observed the aberrant forms of *Streptobacillus moniliformis* in the Lister Institute, London, and proposed the term L-forms after Lister Institute.

Pleomorphism and involution forms: Some bacterial species exhibit great variation in size and shape of individual cells which is called pleomorphism. Certain species of bacteria like plague bacillus and gonococcus show swollen and aberrant forms in ageing laboratory cultures which are known as **involution forms**. Some of these are nonviable. Defective cell wall synthesis is often responsible for development of pleomorphism and involution forms.

Demonstration of cell wall

(a) *Plasmolysis*: When an intact bacteria is placed in a solution with high solute concentration there is shrinkage of protoplast and retraction of the cytoplasmic membrane from the

cell wall. This process, known as plasmolysis, is useful for cell wall demonstration.

(b) Other methods of demonstration of cell wall include: microdissection, antigen-antibody reaction, differential staining of different parts of bacterial cell, and electron microscopy.

Cytoplasmic membrane

The cytoplasmic (plasma) membrane (also known as cell membrane) is a thin (5-10 nm thick), elastic semipermeable layer and lies beneath the cell wall separating it from the cell cytoplasm (Fig. 2.8). Chemically, the membrane consists of a phospholipid bilayer structure with small amount of carbohydrates. In electron microscopy of the cell membrane, lipid molecules are arranged in a double layer with their hydrophilic polar regions externally aligned and in contact with a layer of protein on each surface (Fig. 2.9). Cell membranes of most bacteria contain phosphatidyl glycerol, phosphatidyl ethanolamine and diphosphatidyl glycerol. The membranes of prokaryotic cells differ from the membranes of eukaryotic cells by the absence of sterols, except in mycoplasma that contains cholesterol.

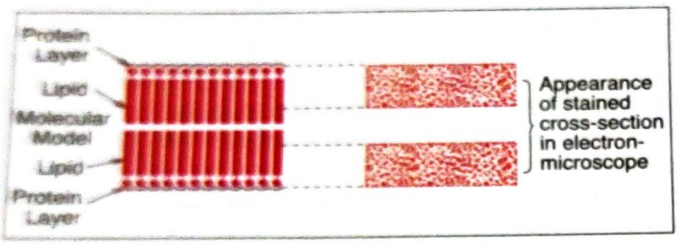

Fig. 2.9 Cytoplasmic (unit) membrane. A lipid bilayer with hydrophilic polar regions lying externally (electron microscopic view)

Functions

It is the physical and metabolic barrier between the interior and exterior of the bacterial cell:

1. **Transport:** (a) It is the site of numerous enzymes (*oxidase, polymerase, permease*) involved in the *active transport* of selective nutrients. It is impermeable to macromolecules and ionised substances. (b) It acts as a *semipermeable membrane* through inward and outward passage of water, and *passive transport* of small molecular lipid soluble solutes take place by diffusion.
2. **Concentration:** It also concentrates sugar, amino acids and phosphates so that a 300-400 fold gradient exists across osmotic barrier.
3. It also contains cytochrome oxidase, enzymes of tricarboxylic acid cycle, and polymerising enzymes necessary for *synthesis of cell wall*.

Cytoplasm

The bacterial cytoplasm is amorphous gel of a variety of organic and inorganic solutes in a viscous watery solution, many of which represent food and energy reserves. It differs from eukaryotic cytoplasm in lacking internal mobility (*protoplasmic streaming*) and subcellular structures (e.g., mitochondria, endoplasmic reticula, etc.). When young cultures are stained with basic dyes, the cytoplasm stains uniformly but becomes increasingly granular with age. The cytoplasm contains cytoplasmic inclusions and vacuoles, ribosomes and mesosomes. Some bacteria also contain extrachromosomal DNA (*plasmids*), while some others may possess transposons and insertion sequences.

Ribosomes: Ribosomes are the centres of protein synthesis. In prokaryotic organisms, the ribosome consists of 30S-50S subunits, forming a 70S ribosome (sedimentation constant—S for Svedberg unit), smaller than that of eukaryotic cell (80S). The ribosomes in prokaryotes are integrated in linear strands of mRNA, and these ribosome-mRNA are called *polyribosomes* or *polysomes*. The polysomes contain all components of protein-synthesising system. These are also major target for antibacterial drugs.

Mesosomes: Mesosomes are coiled invaginations of the cytoplasmic membrane that extend into the cytoplasm. These are vesicular, convoluted or multilaminated structures. In Gram-positive bacteria, the mesosomes are more prominent. Mesosomes are the main sites of respiratory enzymes in bacteria and are analogous to mitochondria of eukaryotic cells. They are often found in relation to the nuclear body and the site of synthesis of cross-wall septa, suggesting that they coordinate nuclear and cytoplasmic division during binary fission.

Intracytoplasmic inclusions: Intracytoplasmic inclusions or granules may be of various types and represent accumulation of food reserves such as polysaccharides, lipids, or polyphosphates. Most of the cellular inclusions are bound by a thin nonunit membrane consisting of lipid and that separates the inclusion from the cytoplasm proper. These storage granules are characteristic for different species and their numbers and types depend on the culture medium and functional state of the bacterial cells. High molecular weight polymers of polyphosphate known as *volutin granules (metachromatic or Babes-Ernst granules)* are characteristic features of the *Corynebacterium* species. The granules appear reddish pink when stained with polychrome methylene blue or toludine blue (metachromasia). They can be demonstrated more clearly by special staining techniques such as Albert's or Neisser's staining. These granules can be degraded and utilised as sources of phosphate in the form of granules of polyphosphate. *Bacillus* and *Pseudomonas* species store high-molecular lipid known as *poly-β-hydroxybutyrate*. *Starch* constitutes the principal storage product among the *Neisseria* and *Clostridium* species. *Gas vesicles* are found almost exclusively in microorganisms from aquatic habitats, where they maintain buoyancy granules of polysaccharide. This can be demonstrated by staining with iodine, while lipid inclusions are stained by lipid soluble dyes such as Sudan black. Vacuoles, fluid-containing cavities, are found in the cytoplasm which are separated from the cytoplasm by a membrane. Role of these vacuoles are not clear.

Plasmid: Some bacteria may contain extranuclear genetic material in the cytoplasm consisting of DNA. These cytoplasmic carriers of genetic information are called **plasmids**. Plasmids are circular but much smaller than bacterial chromosomes (vide Chapter 9). Plasmids are mostly found in Gram-negative bacteria.

Nucleus

Bacteria, like other prokaryotes, do not have true nuclei, instead they package their chromosomal DNA inside the cytoplasm in a structure called nucleoid. The nucleoid or genome of most bacterial cells consists of a single molecule of double-stranded DNA arranged in the form of a circle. When straightened the nucleus measures approximately 1 mm in length. In some organisms, linear DNA is present, e.g., *Borrelia burgdorferi* and *Streptomyces*. A few bacteria possess two or more or dissimilar chromosomes due to asynchrony between nuclear and protoplasmic divisions. Bacterial nuclei do not possess any nuclear membrane or nucleolus. The nuclear DNA is not associated with basic protein. The double stranded DNA is *haploid*. It undergoes semiconservative replication by simple fission and maintains bacterial genetic characteristics. The nucleus represents 2-3% of the cell's dry weight which is greater than 10% of the cell volume. The nucleus of *E. coli* codes for approximately 400 different proteins.

By ordinary staining under light microscope, the nucleus cannot be differentiated from the bacterial cytoplasm but, under electron microscope, the nucleus appears as a central oval or elongated area in the cytoplasm which is of lower electron density than the rest of the cell. The nucleioid can be seen with the light microscope in specially stained preparation. It is Feulgen-positive, which indicates the presence of DNA.

Capsule and glycocalyx

1. **Definition**: It is the amorphous viscid bacterial secretion which surrounds some bacteria as their outermost layer (Fig. 2.10). Some bacteria secrete a sticky, viscous material that diffuses into the surrounding medium and forms an extracellular coating around the cell. The material is usually a complex polysaccharide, however, in the case of pathogenic *Bacillus anthracis*, it is polypeptide. If the material is tightly bound to the bacterial cell and possesses an organised structure, it is called capsule. If the material is loosely bound and amorphous—it is referred to as a slime layer or glycocalyx.

Capsules which are much narrower than true capsule and cannot be demonstrated by light microscope are called *microcapsules*, e.g., *N. meningitidis*, *S. pyogenes*, and *H. influenzae*.

2. **Functions**: Important functions include: (i) Capsule serves as a *protective covering* against antibacterial substances such as bacteriophage, phagocytes, and enzymes. (ii) It enhances *bacterial virulence*. (iii) Capsular antigen is *hapten* in nature and specific for the bacteria.

3. **Capsulated organisms**: *S. pneumoniae*, *Bacillus anthracis*, *C. perfringens*, *Friedlander's pneumobacillus*, *H. influenzae*.

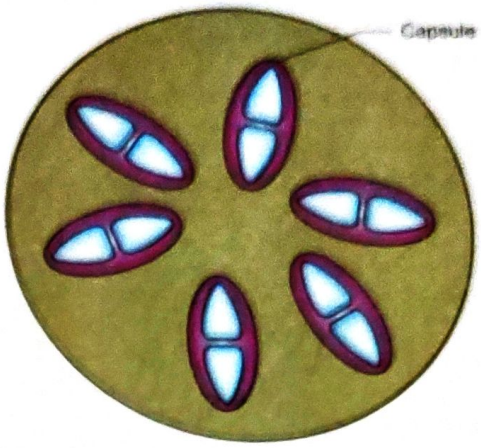

Fig. 2.10 Diagram showing pneumococcal capsule (negatively stained with India ink)

4. **Demonstration**: The capsule is best seen in pathological specimens like pus, blood, sputum and exudate. In artificial culture medium, the size of the capsule is reduced, and, finally, in the later stages of growth, the capsule may disappear due to accumulation of capsule degrading enzymes (hyaluronidase in *S. pyogenes*), or due to carbon or energy starvation:

(i) By ordinary (Gram's or acid-fast) stain, capsule cannot be stained, it appears as an unstained halo around the stained bacterial body.

(ii) In negative staining (India ink preparation), the capsule appears as a clear halo around the bacterium as the ink cannot penetrate the capsule (Fig. 2.10). The slime layer or glycocalyx is loosely associated with bacterial cell and the ink can penetrate it.

(iii) Immunological method: Capsular substance being antigenic, it can be stained by serological method. After mixing a suspension of capsulated bacteria with specific antiserum, the capsule becomes delineated and refractile, and appears swollen when examined under the microscope, e.g., the so-called capsular swelling reaction of pneumococcus (**Quellung phenomenon**) employed for typing the bacteria.

Flagella

Flagella are filamentous, cytoplasmic appendages protruding through cell wall. These are unbranched, long, semirigid, helical, hollow tubular structures composed entirely of protein (*flagellin*), 12-30 nm in diameter and 5-16 μm in length. They are the organs of locomotion.

1. **Types** (Fig. 2.12): Flagella have characteristic patterns of distribution in the bacterial cell (Fig. 2.12). There are 4 types of flagellar distribution on bacteria:

(i) *Monotrichous*—Single polar flagellum, e.g., cholera vibrios.

(ii) *Amphitrichous*—Single flagellum attached to each end, e.g., *Alkaligenes faecalis*.

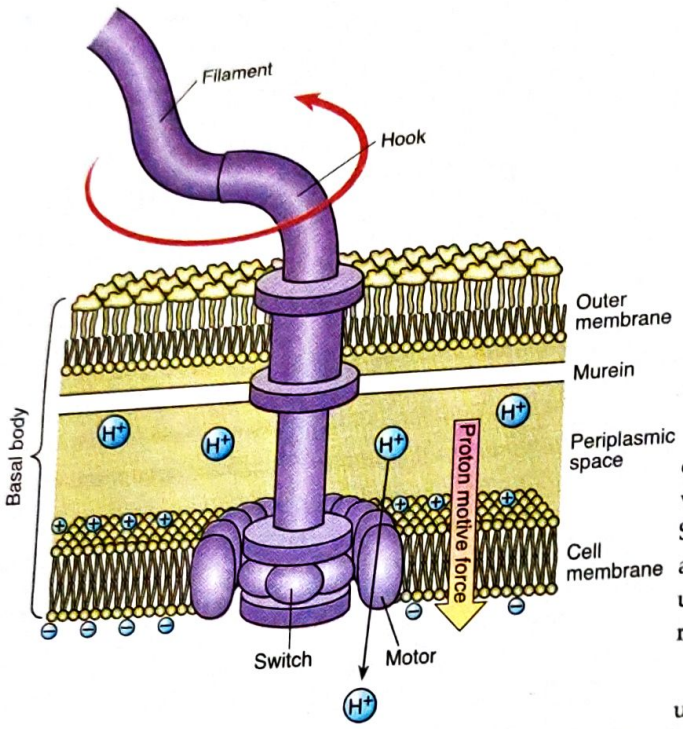

Fig. 2.11 Diagram showing structure of bacterial flagellum (rotator machine)

(iii) *Lophotrichous*—Tufts of flagella at one or both ends, e.g., spirilla.

(iv) *Peritrichous*—Numerous flagella all over the bacterial body, e.g., typhoid bacilli.

2. **Parts**: Flagellum is attached to the cell wall, cell membrane, or both, by a basal body. The basal body is a complex molecular machine which rotates the flagellum like the screw propeller of a ship. Each flagellum consists of three distinct parts—*filament, hook,* and *basal body* (Fig. 2.11). The filament lies external to the cell and the hook-basal body portion is embedded in the cell envelope. Antigenically, the hook and basal body are different. Mechanical detachment of the filament does not impair the viability of the bacterial cell. The basal body is attached to the cytoplasmic membrane by ring-like structures. The flagella are driven by the rotatory action of the swivel-like basal-hook as a part of an energy-dependent reaction. Energy for this reaction is derived by the passage of protons from the outside into the cytoplasm of the bacterial cell via the basal body.

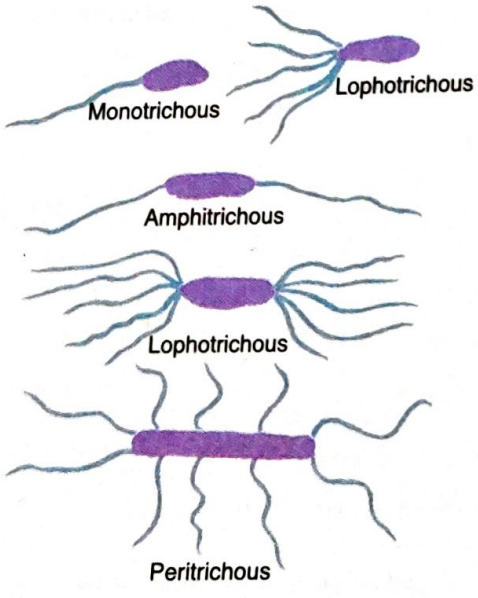

Fig. 2.12 Distribution of flagella on bacteria

The flagella are present in all motile bacteria excepting spirochaetes. Their number varies up to 10-20 per cell—depending on the bacterial species. *E. coli* has a motility of 25-30 μm and cholera vibrios about 55 μm per second, respectively.

3. **Phase variation**: Flagella is immunogenic and strain determinants. Some bacteria develop a system of antigenic variation that enables them to switch between types of flagella. Specific antibodies are produced in high titres in response to antigenic stimulation by flagella. Flagellar antibodies are useful in serological diagnosis but do not have any protective role.

4. **Demonstration**: The flagellum is too small to be seen under ordinary microscope. However, it can be seen well under an electron microscope after staining the bacteria with flagella stain (Fig. 2.13). When stained with phosphotungstic acid, flagella appear as hollow tubes.

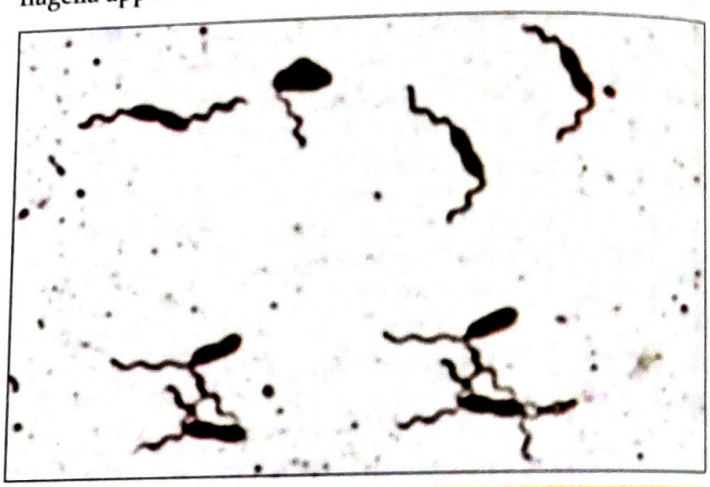

Fig. 2.13 Flagella stain, *Pseudomonas* spp. (wet mount)

Fimbriae

Fimbriae (Latin for 'fringe') (also called pili) are thin hair-like smaller appendages that project from the cell surface as thin filaments. They are 0.5 μm long and less than 8 nm thick. Fimbriae are found on the surface of many Gram-negative bacteria and few Gram-positive bacteria. Each bacterium possesses 100-500 pili peritrichously. They are shorter and thinner than flagella but more numerous than flagella.

The fimbriae are best developed in freshly isolated strains from liquid culture. They tend to disappear when subcultures

are made on solid media. Pimbria is composed of protein subunits called *pilin*. They are antigenic. Pili can be seen only under the electron microscope.

1. **Occurrence**: Fimbriae occur in *Enterobacteriaceae*, e.g., *Proteus, Shigella, Salmonella, Serratia* etc. Only a limited number of Gram-positive bacteria express surface fimbriae, e.g., streptococci, corynebacteria, and *Actinomyces* spp.

2. **Types**: There are 3 main types of fimbriae:

(i) *Common pili*: Common pili are of 6 types based on their morphology, number per cell, adhesive properties, and antigenic nature. The virulence of certain pathogenic bacteria depends both on toxins as well as "*colonization antigens*" (pili) which provide adhesive properties. *N. gonorrhoeae* possesses antigenically distinct fimbrial proteins which undergo both phase variation and antigenic variation.

(ii) Sex or F (fertility pili).

(iii) Col. 1 (colicin pili).

3. **Functions**: Important functions are:

(i) *Adhesion*: Pili are organs of adhesion on cells. The attachment can occur between one bacterium to another, or between the bacterial cell and the host eukaryotic cell.

(ii) *Transfer of genetic material*: Sex pili are specialised fimbriae and fewer in number, possessed by male bacteria. They are longer (18-20 μm) and 1-4 in number and are hollow tubes. They help the male cells to attach with non-male (female) cells in forming hollow "conjugation tubes" through which genetic material is transferred from the donor to the recipient cell.

(iii) *Haemagglutination*: Certain fimbriated bacteria (*Escherichia, Klebsiella*) strongly agglutinate red blood cells of guinea pigs, fowl, horses and pigs; human and sheep red cells are weakly and ox cells are scarcely agglutinated. This property of haemagglutination offers a simple technique to detect such fimbriae.

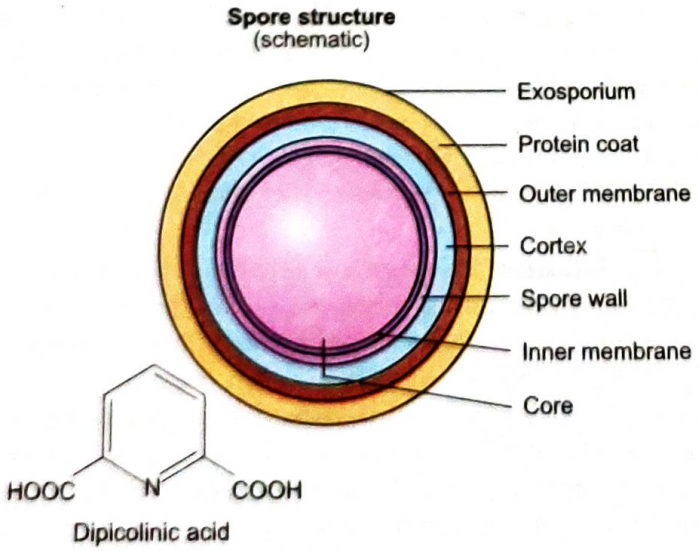

Fig. 2.14 Diagram—bacterial spore

4. **Detection**: (i) These are stained negatively by phosphotungstic acid (PTA), (ii) by electron microscopy, and by (iii) haemagglutination test.

BACTERIAL SPORES

Spores are highly resistant dormant stage of bacteria formed in unfavourable environmental conditions—such as starvation and desiccation. Anatomically, spore is a dehydrated, multishelled structure that protects and enables the bacteria to exist in suspended animation. As the bacterial spores are formed within the parent bacterial cell, they are called *endospores* (Fig. 2.14). *Exospores*, found in fungi (conidia), are formed extracellularly from the ends of the parent cells. Each bacterium forms only one spore and, during germination, each spore gives rise to only one vegetative bacterium. The spore contains a complete copy of the chromosome, minimal concentration of essential proteins and ribosomes, and a high concentration of calcium bound to dipicolinic acid.

1. Spore forming Bacteria

(i) Obligately aerobic—genus *Bacillus*, e.g., *B. anthracis, B. subtilis, B. cereus* (Fig. 2.15).

Fig. 2.15 Spore of *B. cereus*: stained by special stain (heated malachite green)

(ii) Obligately anaerobic genus—genus *Clostridia*, e.g., *C. tetani, C. welchii, C. botulinum*.

2. Sporulation

Spontaneous sporulation occurs in conditions unfavourable to bacterial growth—such as starvation, desiccation, presence of disinfectants, and in extreme temperature. Sporulation can be induced by depleting PO_4, S, C, N and Fe from culture medium.

The chromosome is duplicated. Spore formation is initiated by the appearance of a clear area (Fig. 2.16), usually near one end of the bacterial cell. One copy of the DNA and cytoplasmic contents (core) are surrounded by the cytoplasmic membrane along with peptidoglycan and membrane of the septum. The septum then grows together and the spore *core* becomes surrounded by a double membrane—the *spore wall*. The cell wall of

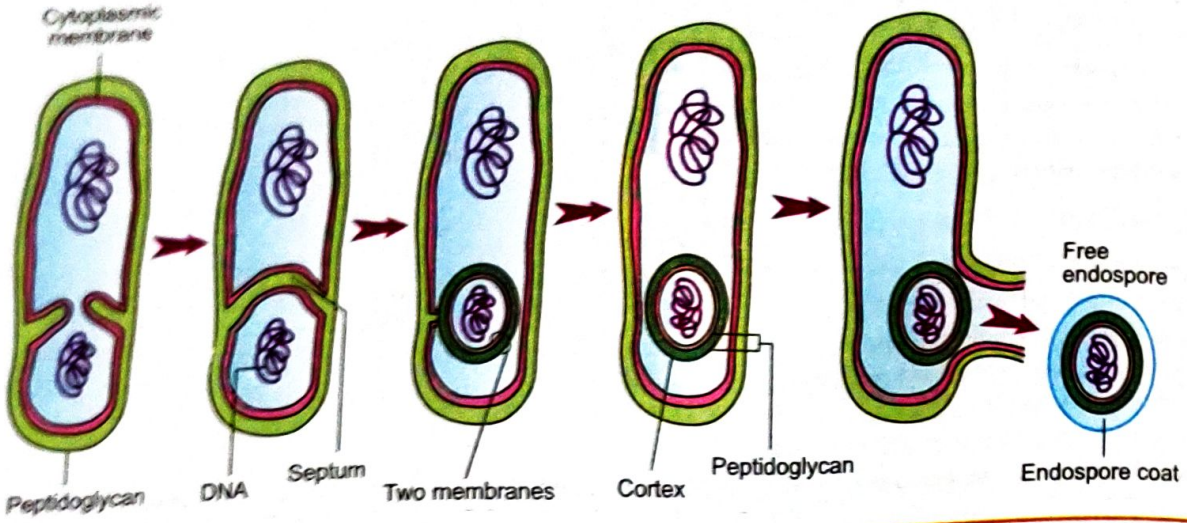

Fig. 2.16 Spore formation

the future vegetative bacterium will develop from the delicate inner membrane of the spore wall. These two layers are surrounded by the *spore cortex* which is composed of a thin inner layer of tightly cross-linked peptidoglycan and a loose outer peptidoglycan layer. The cortex is, in turn, enclosed by a multilayered tough keratin-like protein *spore coat*, which protects the core. Some spores possess an additional covering known as *exosporium*, which may have distinct ridges and grooves. The entire process of sporulation requires 6 to 8 hours.

3. Shape and position of Spores (Fig. 2.17)

The young spores remain attached to the parent cell. The precise position and shape and relative size of spore remain constant within a particular species of bacteria. Spores may be *central*, *subterminal* or *terminal* in position (Fig. 2.17). Its diameter may be same or less than the width of the bacteria (*Bacillus*), or may be wider than the bacterial bodies producing a bulge in the contour of the cell (*Clostridium*). It may be oval or spherical.

4. Resistance

Bacterial spores are highly resistant to ordinary boiling, heating and disinfectants. They can withstand boiling up to 3 hours, dry heat at 150°C for one hour. Spores may remain viable for long periods and even for centuries! However, they are readily destroyed by autoclaving at 121°C for 15-20 minutes and by 1% aqueous solution of iodine in few hours.

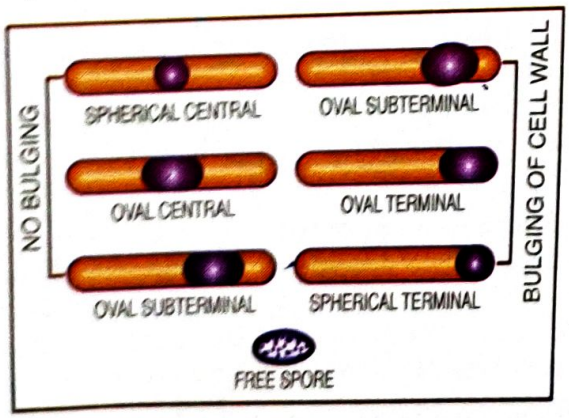

Fig. 2.17 Types of bacterial spores

The highly impervious spore coat, low water content, low metabolic activity and high concentration of calcium dipicolinate of spore make it much resistant to heating and drying.

5. Medical significance

Some of the most notorious organisms are spore-formers, e.g., *Clostridium tetani* (tetanus), *Clostridium botulism* (botulism—food poisoning), *Clostridium perfringens* (gas gangrene), *B. anthracis* (anthrax), and *Bacillus cereus* (gastroenteritis).

6. Germination

The process of conversion of a spore into a vegetative cell under suitable environment is known as *germination*. Germination may occur in less than two hours under optimal conditions and consists of three stages:

(a) **Activation:** The germination of bacterial spores do not occur even when placed in nutritionally rich medium, unless first activated by one or another agent that damages the outer coat of the spore, such as mechanical stress, heat (60°C for one hour), low pH (acidic), abrasion and compounds containing free sulphydryl groups and requires water and a trigerring agent (e.g., alanine).

(b) **Initiation:** The process of initiation is not clear. However, the spore will initiate germination in favourable conditions once it has been activated. Different species of bacteria recognise different effectors as signalling a rich medium, such as L-alanine for one species and adenosine for another species. Binding of the effector substance to the spore coat activates an autolysin that destroys the peptidoglycan of the cortex. Thereafter, water is taken up and calcium dipicolinate is released, and a number of hydrolytic enzymes degrade the spore constituents.

(c) **Outgrowth:** With the swelling of spore wall and disintegration of the cortex, a single germ cell emerges after breaking open the spore coat. The new vegetative cell consists of the spore protoplast with its surrounding wall. This is followed by a period of active biosynthesis producing an outgrowth. Outgrowth is denoted as the stage from germination up to the formation of first vegetative cell, and prior to the first cell division.

7. Demonstration

(a) By *ordinary stain* (Gram) spore appears as an unstained refractile body within the bacterial cell.

(b) By *modified Ziehl-Neelsen stain* with 0.25-0.5% sulphuric acid as decolourising agent, spores appear as acid-fast (red).

8. Laboratory use

Spores of certain species of bacteria are employed as sterilisation control:

(a) *B. stearothermophilus*—destroyed at a temperature of 121°C in 10-20 minutes.

(b) *B. subtilis*—destroyed at 105°C in 5 minutes.

QUESTIONS

1. What is protista?
2. How do eukaryotes differ from prokaryotes?
3. What are the uses of examining unstained wet film microscopically?
4. What are the principles of Gram's and Ziehl-Neelsen techniques of staining?
5. How do bacteria differ from viruses?
6. Draw a bacterial cell and label the different parts.
7. What are the functions of bacterial cell wall?
8. How does cell wall of Gram +ve and Gram –ve bacteria differ?
9. What are the functions of cytoplasmic membrane?
10. Name the intracytoplasmic inclusions.
11. Define spore.
12. Which of the following is found in Gram +ve bacteria but not in Gram –ve bacteria:
    (a) Capsule, (b) Peptidoglycan, (c) Flagella, (d) Teichoic acid, (e) Diaminopimelic acid?
13. Write short notes on: Bacterial cell wall, cytoplasmic membrane, bacterial capsule, fimbriae, flagella, bacterial endospore.

REFERENCES

1. Rogers H: *Bacterial Cell Structure.* American Society for Microbiology, 1983.
2. Davis BD et al (editors): *Microbiology,* 4th ed., Philadelphia: Lippincott, 1990.
3. Dawes IW, Sutherland W: *Microbial Physiology,* 2nd ed., Oxford: Blackwell Scientific, 1991.
4. Talaro K, Talaro A (editors): *Fundamentals in Microbiology,* 4th ed., New York: McGraw Hill, 2002.