# 2 Morphology and Physiology of Bacteria



## INTRODUCTION

Microorganisms are a heterogeneous group of several distinct classes of living beings. The original classification under the Plant and Animal kingdoms proved unsatisfactory; they were then classified under a third kingdom, **Protista**. Based on differences in cellular organisation and biochemistry, this kingdom has been divided into two groups: **prokaryotes** and **eukaryotes** (Table 2.1). Bacteria and blue-green algae are prokaryotes, while fungi, other algae, slime moulds and protozoa are eukaryotes (Fig. 2.1).

Bacteria are prokaryotic microorganisms that do not contain chlorophyll. They are unicellular and do not show true branching, except in the so-called 'higher bacteria' (actinomycetales).

## MORPHOLOGY OF BACTERIA

### SIZE OF BACTERIA

The unit of measurement used in bacteriology is the micron (micrometre, μm).

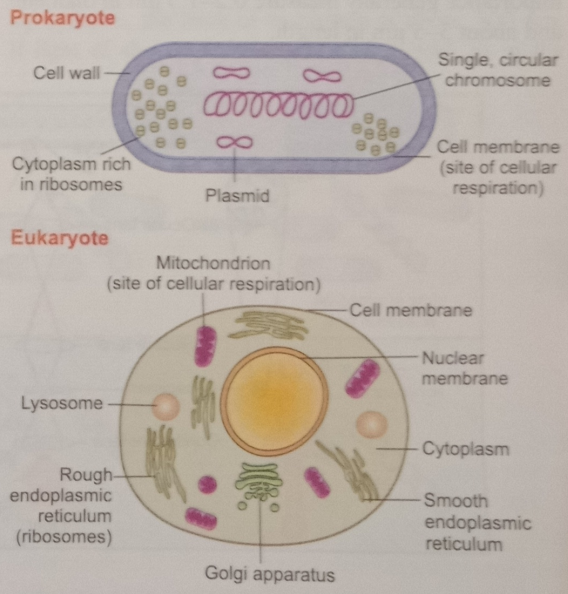



Fig. 2.1 Prokaryote and eukaryote cells

Table 2.1 *Some differences between prokaryotic and eukaryotic cells*

| *Character* | *Prokaryotes* | *Eukaryotes* |
|---|---|---|
| **Nucleus** | | |
| Nuclear membrane | Absent | Present |
| Nucleolus | Absent | Present |
| Deoxyribonucleoprotein | Absent | Present |
| Chromosome | One (circular) | More than one (linear) |
| Mitotic division | Absent | Present |
| **Cytoplasm** | | |
| Cytoplasmic streaming | Absent | Present |
| Pinocytosis | Absent | Present |
| Mitochondria | Absent | Present |
| Lysosomes | Absent | Present |
| Golgi apparatus | Absent | Present |
| Endoplasmic reticulum | Absent | Present |
| **Chemical composition** | | |
| Sterols | Absent | Present |
| Muramic acid | Present | Absent |

1 micron (μ) or micrometre (μm) = one thousandth of a millimetre

1 millimicron (mμ) or nanometre (nm) = one thousandth of a micron or one millionth of a millimetre

1 Angstrom unit (Å) = one tenth of a nanometre

The limit of resolution with the unaided eye is about 200 microns. Bacteria, being much smaller, can be visualised only under magnification. Bacteria of medical importance generally measure 0.2–1.5 μm in diameter and about 3–5 μm in length.

## MICROSCOPY

The morphological study of bacteria requires the use of microscopes. Microscopy has come a long way since Leeuwenhoek first observed bacteria over three hundred years ago using hand-ground lenses. The following **types** of microscopes are in use today:

**Optical or light microscope:** Bacteria may be examined under the compound microscope, either in the living state or after fixation and staining. Examination of wet films or 'hanging drops' indicates the shape, arrangement, motility and approximate size of the cells. But due to lack of contrast, details cannot be appreciated (Fig. 2.2a).

**Phase contrast microscopy:** This improves the contrast and makes evident the structures within cells that differ in thickness or refractive index. Also, the differences in refractive index between bacterial cells and the surrounding medium make them clearly visible. Retardation, by a fraction of a wavelength, of the rays of light that pass through the object, compared to the rays passing through the surrounding medium, produces 'phase' differences between the two types of rays. In the phase contrast microscope, 'phase' differences are converted into differences in intensity of light, producing light and dark contrast in the image.

**Fluorescent microscope:** This uses light of a high intensity source which excites a fluorescent agent, which in turn emits a low energy light of a longer wave-

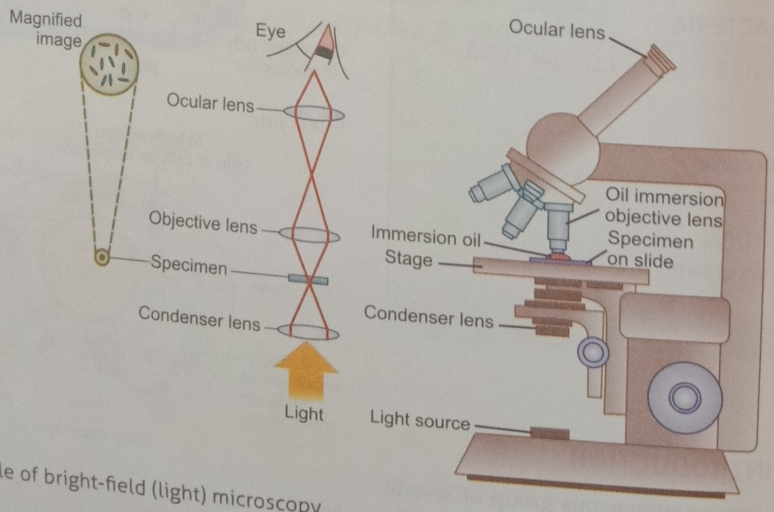
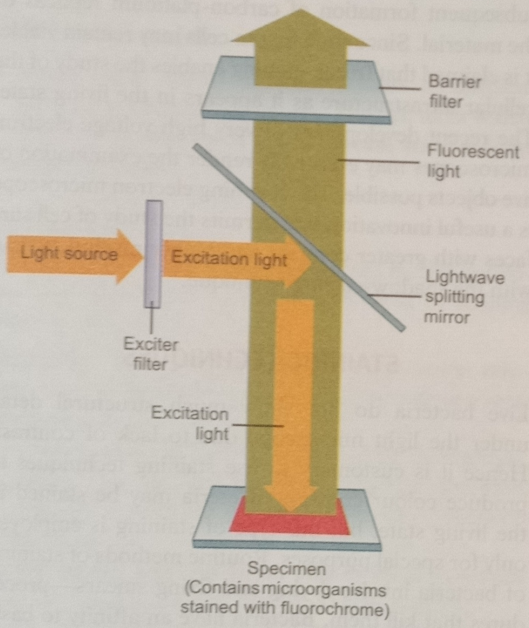



Fig. 2.2 (a) Principle of bright-field (light) microscopy

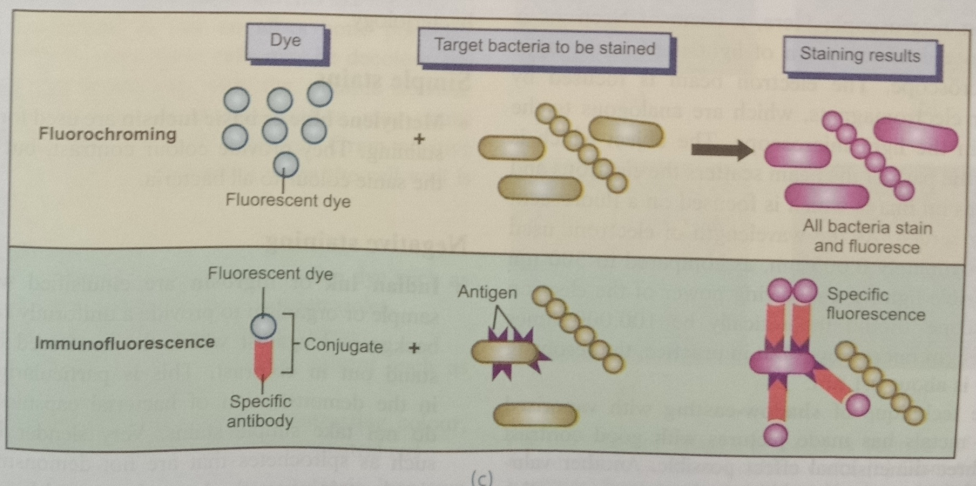
length that produces the image. The fluorescent light can be separated from the surrounding radiation using filters designed for that specific wavelength, allowing the viewer to see only that which shows fluorescence. Microorganisms in a specimen can be stained with a fluorescent dye. On exposure to excitation light, organisms are visually detected by the emission of fluorescent light by the dye with which they have been stained (Fig. 2.2b). This can be of two types: **fluorochroming** and **immunofluorescence**. Fluorochroming involves the non-specific staining of any bacterial cell with a fluorescent dye. Immunofluorescence uses antibodies labelled with fluorescent dye (a conjugate) to specifically stain a particular bacterial species (Fig. 2.2c).




(b)

**Dark field/Dark ground microscope:** Another method of improving the contrast is the dark field (dark ground) microscope in which reflected light is used instead of the transmitted light used in the ordinary microscope. The essential part of the dark field microscope is the dark field condenser with a central circular stop, which illuminates the object with a cone of light, without letting any ray of light fall directly on the objective lens. Light rays falling on the object are reflected or scattered on to the objective lens, with the result that the object appears self-luminous against a dark background. The contrast gives an illusion of increased resolution, so that very slender organisms such as spirochetes, not visible under ordinary illumination, can be clearly seen under the dark field microscope (Fig. 2.2d).

The **resolving power** of the light microscope is limited by the wavelength of light. In order to be seen and delineated (resolved), an object has to have a size of approximately half the wavelength of the light used. With visible light, using the best optical systems, the limit of resolution is about 300 nm. If light of shorter wavelength is employed, as in the




(c)

Fig. 2.2 (b) Principle of fluorescent microscopy; (c) Principles of fluorochroming and immunofluoresence

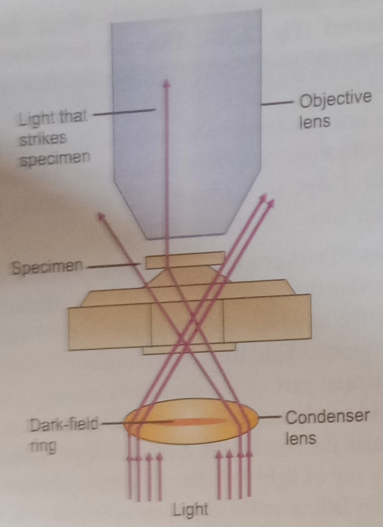



Fig. 2.2 (d) Dark field microscopy

ultraviolet microscope, the resolving power can be proportionately extended.

Two specialised types of microscopes are the **interference microscope** which not only reveals cell organelles but also enables quantitative measurement of the chemical constituents of cells such as lipids, proteins and nucleic acids, and the **polarisation microscope** which enables the study of intracellular structures using differences in birefringence.

**Electron microscope:** Here, a beam of electrons is used instead of the beam of light used in the optical microscope. The electron beam is focused by circular electromagnets, which are analogous to the lenses in the light microscope. The object which is held in the path of the beam scatters the electrons and produces an image which is focused on a fluorescent viewing screen. As the wavelength of electrons used is approximately 0.005 nm, as compared to 500 nm with visible light, the resolving power of the electron microscope should theoretically be 100,000 times that of light microscopes but in practice, the resolving power is about 0.1 nm.

The technique of **shadow-casting** with vaporised heavy metals has made pictures with good contrast and three-dimensional effect possible. Another valuable technique in studying fine structure is **negative staining** with phosphotungstic acid.

Gas molecules scatter electrons, and it is therefore necessary to examine the object in a vacuum. Hence, only dead and dried objects can be examined in the electron microscope. This may lead to considerable distortion in cell morphology. A method introduced to overcome this disadvantage is **freeze-etching**, involving the deep-freezing of specimens in a liquid gas and the subsequent formation of carbon-platinum replicas of the material. Since such frozen cells may remain viable, it is claimed that freeze-etching enables the study of the cellular ultrastructure as it appears in the living state. The recent development of very high voltage electron microscopes may eventually render the examination of live objects possible. The scanning electron microscope is a useful innovation that permits the study of cell surfaces with greater contrast and higher resolution than with the shadow-casting technique.

## STAINING TECHNIQUES

Live bacteria do not show much structural detail under the light microscope due to lack of contrast. Hence it is customary to use staining techniques to produce colour contrast. Bacteria may be stained in the living state, but this type of staining is employed only for special purposes. Routine methods of staining of bacteria involve drying and fixing smears—procedures that kill them. Bacteria have an affinity to basic dyes due to the acidic nature of their protoplasm. The following are staining techniques commonly used in bacteriology.

### Simple stains

- **Methylene blue** or **basic fuchsin** are used for simple staining. They provide colour contrast, but impart the same colour to all bacteria.

### Negative staining

- **Indian ink** or **nigrosin** are emulsified with the sample or organism to provide a uniformly coloured background against which the unstained bacteria stand out in contrast. This is particularly useful in the demonstration of bacterial capsules which do not take simple stains. Very slender bacteria such as spirochetes that are not demonstrable by simple staining methods can be viewed by negative staining.

### Impregnation methods

- **Silver impregnation** cells and structures too thin to be seen under the ordinary microscope may be rendered visible if they are thickened by impregnation of silver on the surface. Such methods are used for the demonstration of spirochetes and bacterial flagella.

### Differential stains

- These stains impart different colours to different bacteria or bacterial structures. The two most widely used differential stains are the **Gram stain** and the **acid fast stain**.

### Gram stain

The **Gram stain** was originally devised by the histologist Christian Gram (1884) as a method of staining bacteria in tissues.

**Principle:** The exact mechanism of the Gram reaction is not understood. Various theories that have been suggested are as follows:

- Gram-positive cells have a more acidic protoplasm, which may account for their retaining the basic primary dye more strongly than Gram-negative bacteria.
- The peptidoglycan of Gram-positive bacteria is thick and thus able to retain the dye-iodine complex.
- The high lipid content of Gram-negative bacteria makes them permeable to secondary dye after decolourisation with organic solvents like acetone.

Decolourisation is not an all-or-none phenomenon. Even Gram-positive cells may be decolourised by prolonged treatment with the organic solvent. Conversely, inadequate decolourisation may cause all cells to appear Gram positive. Gram-positive bacteria become Gram negative when the cell wall is damaged.

**Procedure:**

1. Primary staining with a pararosaniline dye such as crystal violet, methyl violet or gentian violet
2. Application of a dilute solution of iodine
3. Decolourisation with an organic solvent such as ethanol, acetone or aniline
4. Counterstaining with a dye of contrasting colour, such as carbol fuchsin, safranine or neutral red

The Gram stain differentiates bacteria into two broad groups. Gram-positive bacteria are those that resist decolourisation and retain the primary stain, appearing violet. Gram-negative bacteria are decolourised by organic solvents and, therefore, take the counterstain, appearing red (Figs 2.3a and 2.3b).

**Application:** Gram staining is an essential procedure used in the identification of bacteria and is frequently the only method required for studying their morphology. Gram reactivity is of considerable importance as Gram-positive and -negative bacteria differ not merely in staining characteristics and in structure but also in several other properties such as growth requirements, susceptibility to antibiotics and pathogenicity.

### Acid fast stain

This was discovered by Ehrlich, who found that after staining with aniline dyes, tubercle bacilli resist decolourisation with acids. The method, as modified by Ziehl and Neelsen, is in common use today (Figs 2.3c and 2.3d).

**Principle:** Acid fastness has been ascribed to the high content and variety of lipids, fatty acids and higher alcohols found in tubercle bacilli.

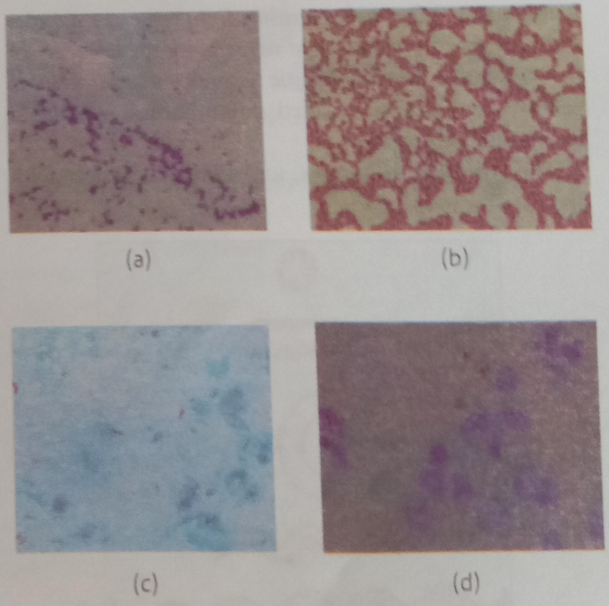



Fig. 2.3 Colour images of bacteria stained using different stains; (a) Gram-positive cocci; (b) Gram-negative bacilli; (c) Ziehl–Neelsen acid fast *M.tuberculosis*; (d) Acid fast *M.leprae*

- A lipid peculiar to acid fast bacilli, a high molecular weight hydroxy acid wax containing carboxyl groups (mycolic acid), is acid fast in the free state.
- Acid fastness is not a property of lipids alone but depends also on the integrity of the cell wall.

**Procedure:**

1. The smear is stained by a strong solution of carbol fuchsin with the application of heat.
2. It is then decolourised with 20% sulphuric acid and counterstained with a contrasting dye such as methylene blue.
3. The acid fast bacteria retain the fuchsin (red) colour, while the others take the counterstain.

*Mycobacterium leprae* resists decolourisation with 5% sulphuric acid.

### Albert's stain

**Principle:** On staining with Albert's stain the granules of *Corynebacterium diphtheriae* take up a bluish purple colour and hence they are called metachromatic granules.

**Procedure:**

1. The smear is stained with Albert I stain, drained and washed.
2. Albert II is poured to cover the smear and drained.

**Application:** *Corynebacterium diphtheria* are slender bacilli with an arrangement resembling the letters V or L. They have metachromatic granules at the poles of bacilli which are also called **polar bodies**, volutin or **Babes–Ernst granules**.

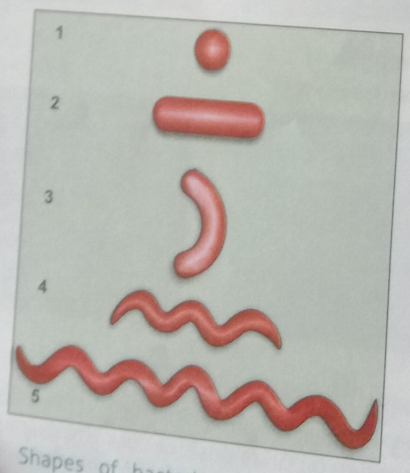
The **Albert's**, **Neisser's**, and **Ponder's** stains demonstrate these granules.

## SHAPE OF BACTERIA

Depending on their shape, bacteria are classified into several types (Fig. 2.4):

- **Cocci** (from *kokkos* meaning berry) are spherical or oval cells.
- **Bacilli** (from *baculus* meaning rod) are rod-shaped cells.
- **Vibrios** are comma-shaped, curved rods and derive their name from their characteristic vibratory motility.
- **Spirilla** are rigid spiral forms.
- **Spirochetes** (from *speira* meaning coil and *chaite* meaning hair) are flexuous spiral forms (Fig. 2.5).
- **Actinomycetes** are branching filamentous bacteria, so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from *actis* meaning ray and *mykes* meaning fungus).
- **Mycoplasmas** are bacteria that are cell wall deficient and hence do not possess stable morphology. They occur as round or oval bodies and as interlacing filaments. When cell wall synthesis becomes defective, either spontaneously or as a result of drugs like penicillin, bacteria lose their distinctive shape. Such cells are called protoplasts, spheroplasts or L forms.Bacteria sometimes show characteristic cellular arrangement or grouping. Thus, cocci may be arranged in pairs (diplococci), chains (streptococci), groups of four (tetrads) or eight (sarcina), or as grape-like clusters (staphylococci).

Some bacilli too may be arranged in chains (streptobacilli). Others are arranged at angles to each other,




2.4 Shapes of bacteria: 1. coccus; 2. bacillus; vibrio; 4. spirillum; 5. spirochete

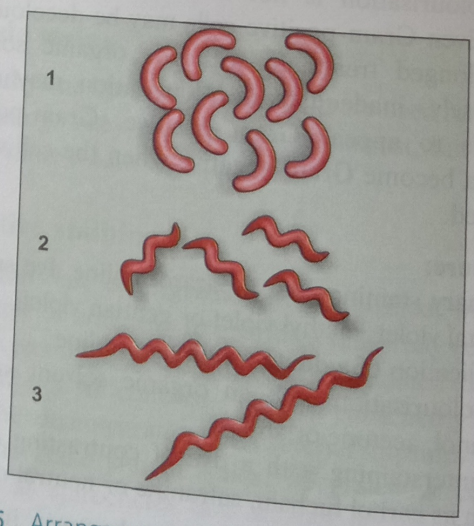



Fig. 2.5 Arrangement of curved bacteria: 1. vibrio; 2. spirilla; 3. spirochetes

presenting a cuneiform or Chinese letter pattern (corynebacteria). The type of cellular arrangement is determined by the plane through which binary fission takes place and by the tendency of the daughter cells to remain attached even after division.

## BACTERIAL ANATOMY

The structure of an idealised bacterial cell shows:

- The **outer layer** or **cell envelope** consists of two components:
  - A **rigid cell wall**
  - A **cytoplasmic** or **plasma membrane** (beneath the cell wall)
- Components of the **cell interior**
  The cell envelope encloses the protoplasm, comprising the cytoplasm, cytoplasmic inclusions such as ribosomes and mesosomes, granules, vacuoles and the nuclear body.
- **Additional structures**
  The cell may be enclosed in a viscid layer, which may be a loose slime layer, or organised as a capsule. Some bacteria carry filamentous appendages protruding from the cell surface—the flagella which are organs of locomotion and the fimbriae which appear to be organs for adhesion (Fig. 2.6).

### Cell wall

The cell wall accounts for the shape of the bacterial cell and confers on it rigidity and ductility. The cell wall cannot be seen by direct light microscopy and does not stain with simple stains.

**Demonstration:**

- It may be demonstrated by plasmolysis. When placed in a hypertonic solution, the cytoplasm loses water by osmosis and shrinks, while the cell wall retains its original shape and size (bacterial ghost).
- The cell wall may also be demonstrated by:
  - microdissection,
  - reaction with specific antibodies,
  - mechanical rupture of the cell,
  - differential staining procedures,
  - electron microscopy.

**Structure:** Bacterial cell walls are about 10–25 nm thick and account for about 20–30 per cent of the dry weight of the cell. Chemically the cell wall is composed of mucopeptide (**peptidoglycan** or **murein**) scaffolding

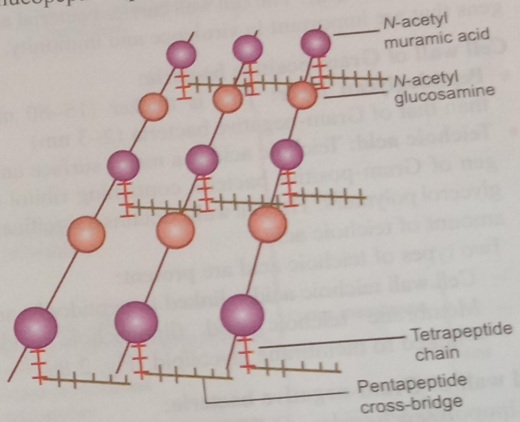



Fig. 2.7 Chemical structure of bacterial cell wall

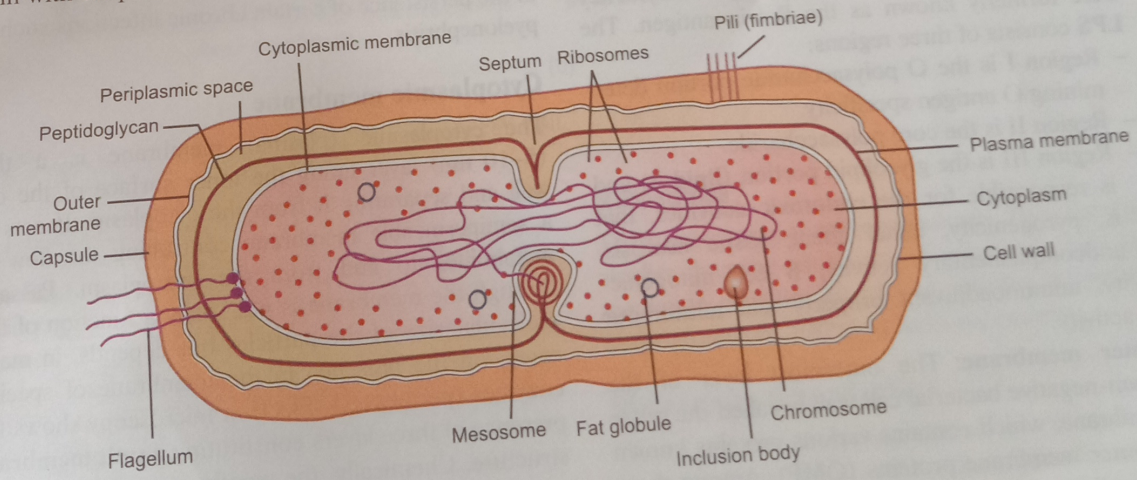



Fig. 2.6 Diagram of an idealised bacterial cell

Table 2.2 *Comparison of cell walls of Gram-positive and Gram-negative bacteria*

| | Gram-positive | Gram-negative |
|---|---|---|
| Thickness | Thicker | Thinner |
| Variety of amino acids | Few | Several |
| Aromatic and sulphur containing amino acids | Absent | Present |
| LPS | Absent or scant | Present |
| Teichoic acid | Present | Absent |

formed by *N*-acetyl glucosamine and *N*-acetyl muramic acid molecules alternating in chains, which are cross-linked by peptide chains (Fig. 2.7). The interstices of this scaffolding contain other chemicals, varying in the different species.

In general, the walls of Gram-positive bacteria have a simpler chemical nature than those of Gram-negative bacteria (Table 2.2). The cell wall carries bacterial antigens that are important in virulence and immunity.

**Cell wall of Gram-positive bacteria:**

- **Peptidoglycan layer:** This is thicker (15–80 nm) than that of Gram-negative bacteria (2–3 nm)
- **Teichoic acid:** Teichoic acid is a major surface antigen of Gram-positive bacteria containing ribitol or glycerol polymers. The cell wall contains a significant amount of teichoic acid.

  Two types of teichoic acid are present:
  - Cell wall teichoic acid – linked to peptidoglycan
  - Membrane teichoic acid (lipoteichoic acid) – linked to membrane glycolipid (Fig. 2.8a).

**Cell wall of Gram-negative bacteria:**

- **Lipopolysaccharides (LPS)** present on the cell walls of Gram-negative bacteria account for their **endotoxic activity** and O antigen specificity. They were formerly known as the Boivin antigen. The **LPS** consists of three regions:
  - Region I is the O polysaccharide portion determining O antigen specificity.
  - Region II is the core polysaccharide.
  - Region III is the glycolipid portion (**lipid A**) and is responsible for the **endotoxic** activities, that is, pyrogenicity, lethal effect, tissue necrosis, anticomplementary activity, B cell mitogenicity, immunoadjuvant property and antitumour activity.
- **Outer membrane:** The outermost layer of the Gram-negative bacterial cell wall is called the outer membrane, which contains various proteins known as outer membrane proteins (OMP). Among these are porins which form transmembrane pores that serve as diffusion channels for small molecules. They also serve as specific receptors for some bacteriophages.
- **Lipoprotein:** Attaches the protein of peptidoglycan to lipid of outer membrane.
- **Peptidoglycan:** Is thin (2–3 nm) and is bound by the lipoprotein and plasma membrane (Fig. 2.8b).

**Inhibition of cell wall synthesis: Lysozyme**, an enzyme normally present in many tissue fluids, lyses susceptible bacteria by splitting the cell wall mucopeptide links.

**Protoplasts and spheroplasts:**

**Protoplasts:** When lysozyme acts on a Gram-positive bacterium in a hypertonic solution, a protoplast is formed, consisting of the cytoplasmic membrane and its contents.

**Spheroplast:** When lysozyme acts on Gram-negative bacteria, the result is a spheroplast which differs from the protoplast in that some cell wall material is retained. Protoplasts and spheroplasts are spherical, regardless of the original shape of the bacterium.

**Cell wall–deficient forms** of bacteria may have a role in the persistence of certain chronic infections such as pyelonephritis.

## Cytoplasmic membrane

The cytoplasmic (plasma) membrane is a thin (5–10 nm) layer lining the inner surface of the cell wall and separating it from the cytoplasm. It acts as a semipermeable membrane controlling the flow of metabolites to and from the protoplasm. Passage through the membrane is not solely a function of the molecular size of the particles but depends, in many cases, on the presence in the membrane of specific enzymes (permeases). Electron microscopy shows the presence of three layers constituting a **unit membrane** structure. Chemically, the membrane consists of lipoproteins with small amounts of carbohydrates. Sterols are absent, except in mycoplasma.

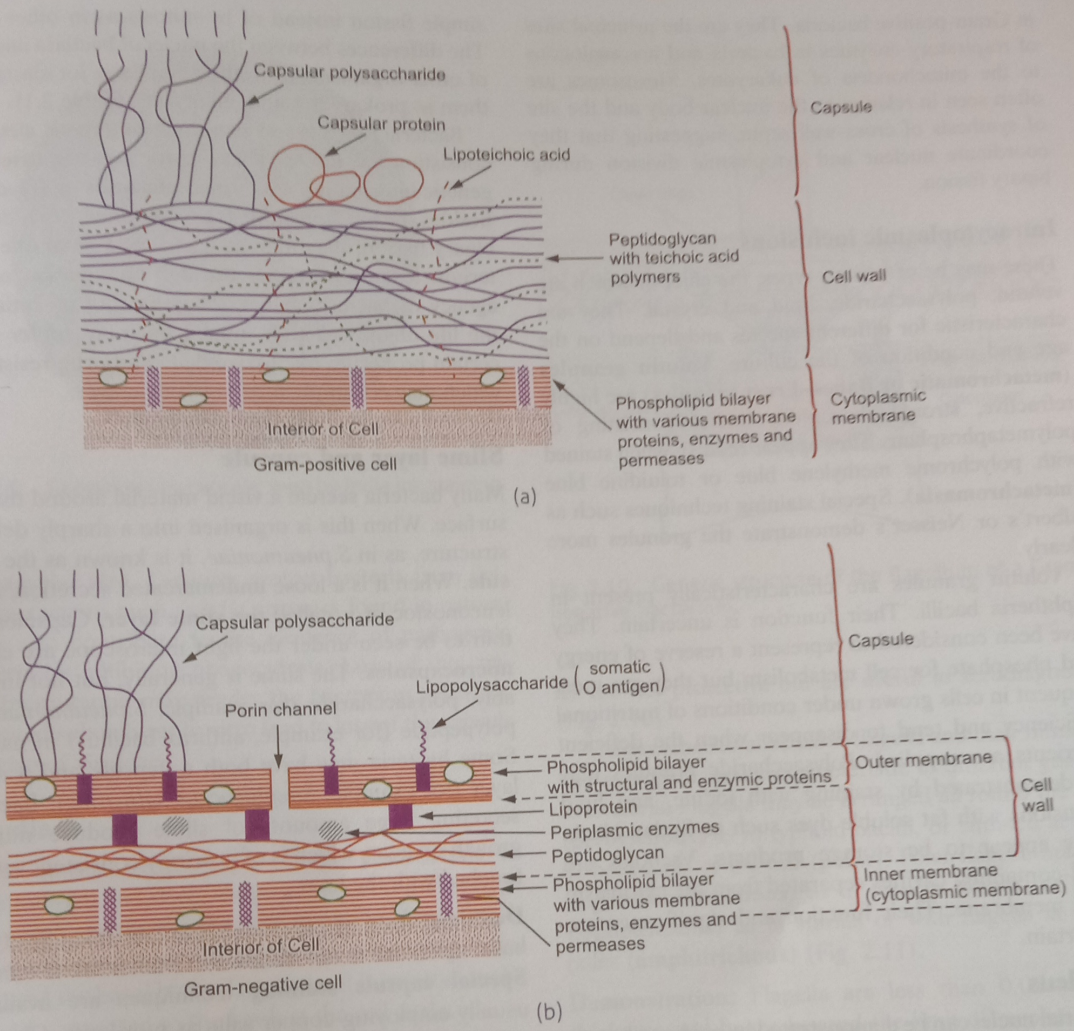



Fig. 2.8 (a) Gram-positive and (b) Gram-negative cell wall

## Cytoplasm

The bacterial cytoplasm is a colloidal system of a variety of organic and inorganic solutes in a viscous watery solution. It differs from eukaryotic cytoplasm in not exhibiting internal mobility (**protoplasmic streaming**) and in the absence of endoplasmic reticulum or mitochondria. The cytoplasm stains uniformly with basic dyes in young cultures but becomes increasingly granular with age. The cytoplasm contains ribosomes, mesosomes, inclusions and vacuoles.

## Ribosomes

These are centres of protein synthesis. They are slightly smaller than the ribosomes of eukaryotic cells (sedimentation constant 70 S) and are seen integrated in linear strands of mRNA to form polysomes.

## Mesosomes (chondroids)

These are vesicular, convoluted or multilaminated structures formed as invaginations of the plasma membrane into the cytoplasm. They are more prominent

in Gram-positive bacteria. They are the principal sites of respiratory enzymes in bacteria and are analogous to the mitochondria of eukaryotes. Mesosomes are often seen 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

These may be of various types, the chief of which are volutin, polysaccharide, lipid and crystal. They are characteristic for different species and depend on the age and condition of the culture. **Volutin granules** (**metachromatic or Babes–Ernst granules**) are highly refractive, strongly basophilic bodies consisting of polymetaphosphate. They appear reddish when stained with polychrome methylene blue or toluidine blue (**metachromasia**). Special staining techniques such as Albert's or Neisser's demonstrate the granules more clearly.

Volutin granules are characteristically present in diphtheria bacilli. Their function is uncertain. They have been considered to represent a reserve of energy and phosphate for cell metabolism but they are most frequent in cells grown under conditions of nutritional deficiency and tend to disappear when the deficient nutrients are supplied. Polysaccharide granules may be demonstrated by staining with iodine, and lipid inclusions with fat soluble dyes such as Sudan black. They appear to be storage products. Vacuoles are fluid-containing cavities separated from the cytoplasm by a membrane. Their function and significance are uncertain.

### Nucleus

Bacterial nuclei can be demonstrated by acid or ribonuclease hydrolysis and subsequent staining for nuclear material. They may be seen by electron microscopy. They appear as oval or elongated bodies, generally one per cell. Some cells may possess two or more nuclear bodies due to asynchrony between nuclear and cytoplasmic division.

Bacterial nuclei have no nuclear membrane or nucleolus. The nuclear deoxyribonucleic acid (DNA) is not associated with basic protein. The genome consists of a single molecule of double-stranded DNA arranged in the form of a circle, which may open under certain conditions to form a long chain, about 1 mm in length. The bacterial chromosome is haploid and replicates by simple fission instead of by mitosis as in other cells. The differences between the nuclei of bacteria and that of other organisms form the main basis for classifying them as prokaryotes and eukaryotes (Table 2.1).

Bacteria may possess extranuclear genetic elements consisting of DNA. These cytoplasmic carriers of genetic information are termed **plasmids** or **episomes**. Besides being transmitted to daughter cells during binary fission, they may be transferred from one bacterium to another either through conjugation or the agency of bacteriophages. They are not essential for the life of the cell they inhabit but may confer on it certain properties like toxigenicity and drug resistance which may constitute a survival advantage.

### Slime layer and capsule

Many bacteria secrete a viscid material around the cell surface. When this is organised into a sharply defined structure, as in *S.pneumoniae*, it is known as the **capsule**. When it is a loose undemarcated secretion, as in leuconostoc, it is called the **slime layer**. Capsules too thin to be seen under the light microscope are called **microcapsules**. The slime is generally, but not invariably, polysaccharide (for example, *S.pneumoniae*) or polypeptide (for example, anthrax bacillus) in nature. Some bacteria may have both a capsule and a slime layer (for example, *Streptococcus salivarius*). Bacteria secreting large amounts of slime produce mucoid growth on agar, which is of a stringy consistency when touched with the loop.

**Demonstration of capsule**: Slime has little affinity for basic dyes and is not visible in Gram-stained smears. **Special capsule staining** techniques are available, usually employing copper salts as mordants. Capsules may be readily demonstrated by **negative staining in wet films with India ink,** when they are seen as clear halos around the bacteria, against a black background (Fig. 2.9).

Capsular material is antigenic and may be demonstrated by **serological methods**. When a suspension of a capsulated bacterium is mixed with its specific anticapsular serum and examined under the microscope, the capsule becomes very prominent and appears 'swollen' due to an increase in its refractivity. This **capsule swelling or Quellung reaction**, described by Neufeld (1902), was widely employed for the typing of *S.pneumoniae* in the pre-sulphonamide days when lobar pneumonia used to be treated with specific

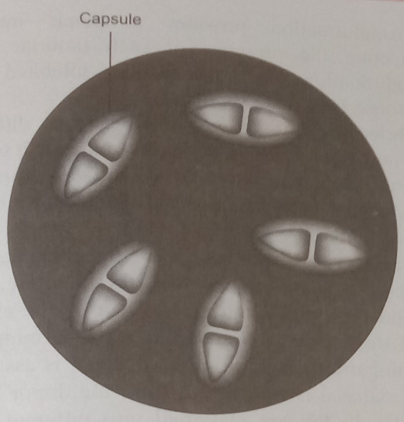



Fig. 2.9 *S.pneumoniae* capsule seen by India ink staining

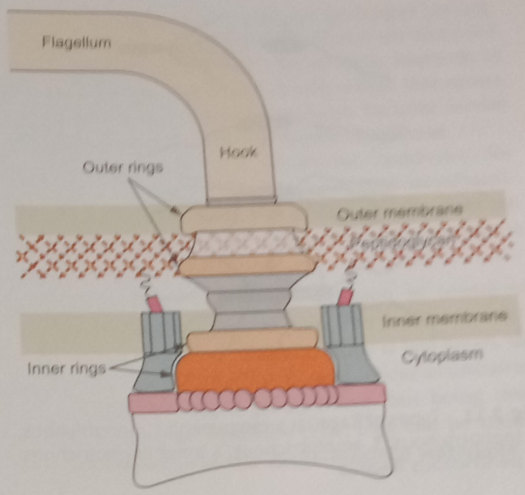



Fig. 2.10 General structure of the flagellum of a Gram-negative bacterium.

anticapsular sera. Capsules protect bacteria from deleterious agents such as lytic enzymes found in nature. They also contribute to the virulence of pathogenic bacteria by inhibiting phagocytosis. Loss of the capsule by mutation may render the bacterium avirulent. Repeated subcultures in vitro lead to loss of the capsule and also of virulence.

## Flagella

Motile bacteria, except spirochetes, possess one or more unbranched, long, sinuous filaments called flagella, which are the organs of locomotion. Each flagellum consists of three distinct parts, the filament, the hook and the basal body. The filament is external to the cell and connected to the hook at the cell surface.

**Structure:** The hook–basal body portion is embedded in the cell envelope. The hook and basal body are antigenically different. Mechanical detachment of the filament does not impair the viability of the cell. The flagella are 3–20 µm long and are of uniform diameter (0.01–0.013 µm) and terminate in a square tip. The wavelength and thickness of the filament are characteristic of each species but some bacteria exhibit biplicity, that is, they have flagella of two different wavelengths. Flagella are made up of a protein (**flagellin**) similar to keratin or myosin. Though flagella of different genera of bacteria have the same chemical composition, they are antigenically different. Flagellar antigens induce specific antibodies in high titres. Flagellar antibodies are not protective but are useful in serodiagnosis (Fig. 2.10).

The presence or absence of flagella and their number and arrangement are characteristic of different genera of bacteria. Flagella may be arranged all round the cell (**peritrichous**) as in typhoid bacilli, or situated at one or both ends of the cell (**polar**). Polar flagella may be single (**monotrichous**) as in cholera vibrios, in tufts (lophotrichous) as in spirilla or with flagella at both poles (**amphitrichous**) (Fig. 2.11).

**Demonstration:** Flagella are less than 0.02 µm in thickness and hence beyond the limit of resolution of the light microscope. They may, in some instances, be seen under **dark ground illumination**. They can be visualised by **special staining techniques** in which their thickness is increased by mordanting, or by **electron microscopy**.

**Motility:** Due to the difficulty of demonstrating flagella directly, their presence is usually inferred from the motility of bacteria. Motility can be observed by noting the spreading type of growth on a semisolid agar medium. Under the microscope, active motility has to be differentiated from the passive movements of the cells, either due to air currents or due to Brownian movement. Bacterial motility may range from the slow

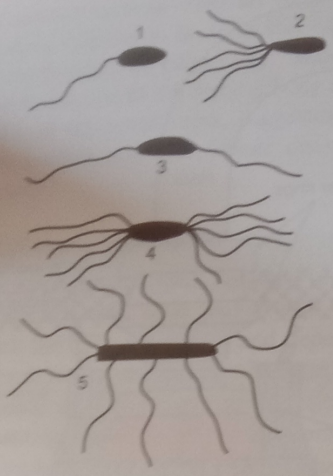



Fig. 2.11 Types of flagellar arrangement: 1. monotrichous; 2. lophotrichous; 3. amphitrichous; 4. amphilophotrichous; 5. peritrichous flagella

'stately' motion of peritrichate bacteria (for example, *Bacillus*) to the darting movement of polar flagellated vibrios. The cholera vibrio may move as quickly as 200 µm per second.

## Fimbriae

Some Gram-negative bacilli carry very fine, hair-like surface appendages called fimbriae or pili. They are shorter and thinner than flagella (about 0.5 µm long and less than 10 nm thick) and project from the cell surface as straight filaments. At least eight morphological types of pili are known, classifiable as either **common** or **sex pili** on the basis of their function. Pili comprise self-aggregating monomers of pilin. They originate in the cell membrane. Fimbriae can be seen only under the electron microscope. They are unrelated to motility and are found on motile as well as non-motile cells. They are best developed in freshly isolated strains and in liquid cultures. They tend to disappear following subculture on solid media.

Fimbriae function as organs of adhesion, helping the cells to adhere firmly to particles of various kinds. This property may serve to anchor the bacteria in nutritionally favourable microenvironments. Fimbriated bacteria form surface pellicles in liquid media. Many fimbriated cells (for example, *Escherichia, Klebsiella*) agglutinate red blood cells of guinea pigs, fowl, horses and pigs strongly, human and sheep cells weakly, and ox cells scarcely.

Hemagglutination provides a simple method of detecting the presence of such fimbriae. The hemagglutination is specifically inhibited by D-mannose (mannose sensitive).

Fimbriae are antigenic. As members of different genera may possess the same fimbrial antigen, it is necessary to ensure that the bacterial antigens employed for serological tests and preparation of antisera are devoid of fimbriae.

**A special type of fimbria are the sex pili.** These are longer and fewer in number than other fimbriae. They are found on 'male' bacteria and help in the attachment of those cells to 'female' bacteria, forming hollow conjugation tubes through which, it is assumed, genetic material is transferred from the donor to the recipient cell. Pili are classified into different types (for example, F, I) based on susceptibility to specific bacteriophages.

## Spore

Some bacteria, particularly members of the genera *Bacillus* and *Clostridium* have the ability to form highly resistant resting stages called spores. Each bacterium forms one spore, which on germination forms a single vegetative cell. Sporulation in bacteria, therefore, is not a method of reproduction. As bacterial spores are formed inside the parent cell, they are called endospores.

While the exact stimulus for sporulation is not known, it occurs after a period of vegetative growth and is presumed to be related to the depletion of exogenous nutrients. Sporulation is initiated by the appearance of a clear area, usually near one end of the cell, which gradually becomes more opaque to form the '**forespore**'. The fully developed spore has at its core the nuclear body, surrounded by the spore wall, a delicate membrane from which the cell wall of the future vegetative bacterium will develop. Outside this is the thick spore cortex, which in turn is enclosed by a multilayered tough spore coat. Some spores have an additional outer covering called **exosporium,** which may have distinctive ridges and grooves (Fig. 2.12). New antigens appear on sporulation.

Young spores are seen attached to the parent cell. The shape and position of the spore and its size relative to the parent cell are species characteristics. Spores may be **central** (equatorial), **terminal** or **subterminal**. They may be oval or spherical. They may or may not distend the bacillary body (Fig. 2.13).

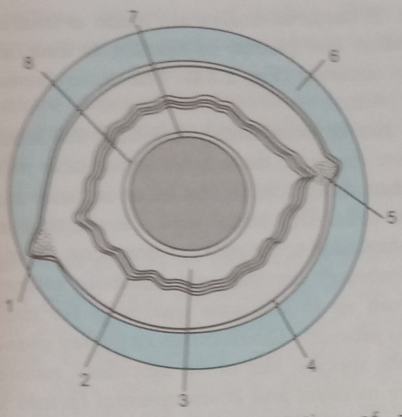



Fig. 2.12 Diagrammatic representation of a bacterial spore: 1. germinal groove; 2. outer cortical layer; 3. cortex; 4. internal spore coat; 5. subcoat material; 6. outer spore coat; 7. cytoplasmic membrane; 8. cell wall primordium.

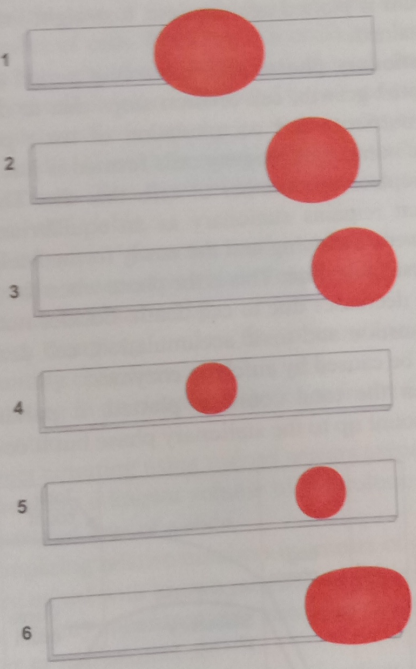



Fig. 2.13 Types of bacterial spores: 1. central, bulging; 2. subterminal, bulging; 3. terminal, spherical; 4. central, not bulging; 5. subterminal, not bulging; 6. terminal, oval

**Resistance:** Bacterial spores constitute some of the most resistant forms of life. They may remain viable for centuries. They are extremely resistant to desiccation and relatively so to chemicals and heat. Though some spores may resist boiling for prolonged periods, spores of all medically important species are destroyed by autoclaving at 120°C for 15 minutes. Methods of sterilisation and disinfection should ensure that spores are also destroyed. Sporulation helps bacteria survive for long periods under unfavourable conditions.

When transferred to conditions conducive for growth, spores germinate. The spore loses its refractility and swells. The spore wall is shed and the germ cell appears by rupturing the spore coat and elongates to form the vegetative bacterium.

**Demonstration:** Spores may be seen in unstained preparations as refractile bodies. The forespore stains intensely, but once the spore envelope is laid down, the spore does not stain readily. Spores appear as unstained areas in Gram-stained preparations, but being more acid fast than the vegetative cells, they can be stained by a **modification of the Ziehl–Neelsen technique**.

## Pleomorphism and involution forms

Some species of bacteria exhibit great variation in the shape and size of individual cells. This is known as pleomorphism. Certain species (for example, plague bacillus, gonococcus) show swollen and aberrant forms in ageing cultures, especially in the presence of high salt concentration. These are known as involution forms. Many of the cells may be non-viable. Pleomorphism and involution forms are often caused by defective cell wall synthesis. Involution forms may also develop due to the activity of autolytic enzymes.

## L forms

Kleineberger-Nobel, studying cultures of *Streptobacillus moniliformis*, observed swollen cells and other aberrant morphological forms and named them L forms, after Lister Institute, London, where the observation was made. L forms are seen in several species of bacteria, developing either spontaneously or in the presence of penicillin or other agents that interfere with cell wall synthesis. L forms may be unstable in that the morphological abnormality is maintained only in the presence of penicillin or other inducing agents, or stable, when the aberrant form becomes the permanent feature of the strain and is retained in serial subcultures. L forms resemble mycoplasma in several ways, including morphology, type of growth on agar and filterability. It is possible that mycoplasmas represent stable L forms of as yet unidentified parent bacteria.

# PHYSIOLOGY OF BACTERIA

## GROWTH AND MULTIPLICATION OF BACTERIA

### Cell division

**Multiplication:** Bacteria divide by binary fission. When a bacterial cell reaches a certain size, it divides to form two daughter cells. Nuclear division precedes cell division and, therefore, in a growing population, many cells carrying two nuclear bodies can be seen. The cell divides by a constrictive or pinching process, or by the ingrowth of a transverse septum across the cell. In some species, the daughter cells may remain partially attached after division.

**Generation time:** The interval of time between two cell divisions, or the time required for a bacterium to give rise to two daughter cells under optimum conditions, is known as the generation time or population doubling time. In coliform bacilli and many other medically important bacteria, the generation time is about 20 minutes. Some bacteria are slow-growing; the generation time in tubercle bacilli is about 20 hours and in lepra bacilli as long as about 20 days. As bacteria reproduce so rapidly and by geometric progression, a single bacterial cell can theoretically give rise to $10^{21}$ progeny in 24 hours, with a mass of approximately 4000 tonnes!

### Growth

In actual practice, when bacteria are grown in a vessel of liquid medium **(batch culture)**, multiplication is arrested after a few cell divisions due to depletion of nutrients or accumulation of toxic products. By the use of special devices for replenishing nutrients and removing bacterial cells **(chemostat or turbidistat)**, it is possible to maintain **continuous culture** of bacteria for industrial or research purposes. When pathogenic bacteria multiply in host tissues, the situation may be intermediate between a batch culture and a continuous culture; the source of nutrients may be inexhaustible but the parasite has to contend with the defence mechanisms of the body. Bacteria growing on solid media form colonies. Each colony represents a clone of cells derived from a single parent cell. In liquid media, growth is diffuse.

### Bacterial growth curve

When a bacterium is seeded into a suitable liquid medium and incubated, its growth follows a definite course. If bacterial counts are made at intervals after inoculation and plotted in relation to time, a growth curve is obtained (**Fig. 2.14**). The curve shows the following phases:

1. **Lag phase:** Immediately following the seeding of a culture medium, there is no appreciable increase in the size number, though there may be an increase in the size of the cells. This initial period is the time required for adaptation to the new environment, during which the necessary enzymes and metabolic intermediates are built up in adequate quantities for multiplication to proceed. The duration of the lag phase varies with the species, size of the inoculum, nature of the culture medium and environmental factors such as temperature.
2. **Log (logarithmic) or exponential phase:** Following the lag phase, the cells start dividing and their numbers increase exponentially or by geometric progression with time. If the logarithm of the viable count is plotted against time, a straight line will be obtained.
3. **Stationary phase:** After a varying period of exponential growth, cell division stops due to depletion of nutrients and accumulation of toxic products. The number of progeny cells formed is just enough to replace the number of cells that die. The viable count remains stationary as an equilibrium exists between the dying and the newly formed cells.
4. **Phase of decline:** This is the phase when the population decreases due to cell death. Besides nutritional exhaustion and toxic accumulation, cell death may also be caused by autolytic enzymes.

When the total count is plotted, it parallels the viable count up to the stationary phase but it continues

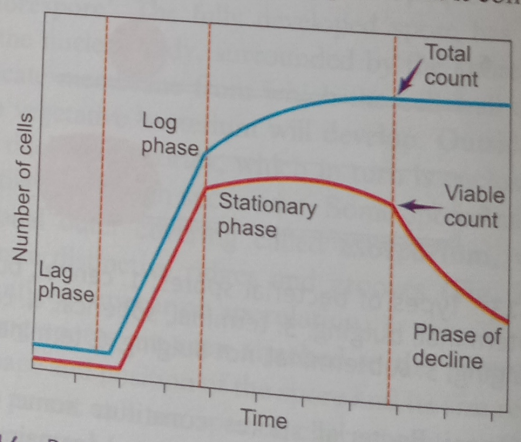



**Fig. 2.14** Bacterial growth curve. The viable count shows the lag, log, stationary and decline phases. In the total count, the phase of decline is not evident.

steadily without any phase of decline. With autolytic bacteria, even the total count shows a phase of decline.

The various stages of the growth curve are associated with **morphological** and **physiological** alterations of the cells.

**Lag phase** maximum cell size is obtained towards the end of the lag phase.

**Log phase**, cells are smaller and stain uniformly.

**Stationary phase**, cells are frequently Gram variable and show irregular staining due to the presence of intracellular storage granules. Sporulation occurs at this stage. Also, many bacteria produce secondary metabolic products such as exotoxins and antibiotics.

**Phase of decline** involution forms are common.

### Bacterial counts

Bacterial growth may be considered at two levels: increase in the size of the individual cell and increase in the number of cells. The former is ordinarily limited and when the critical size is reached, the cell divides, except when cell division is inhibited by substances like penicillin or acriflavine or by growth in magnesium deficient media. Growth in numbers can be studied by bacterial counts. Two types of bacterial counts can be made: total and viable.

- The **total count** gives the total number of cells in the sample, irrespective of whether they are living or not. It can be obtained by:
  - direct counting under the microscope using counting chambers,
  - counting in an electronic device as in the Coulter counter,
  - direct counting using stained smears prepared by spreading a known volume of the culture over a measured area of a slide,
  - comparing relative numbers in smears of the culture mixed with known numbers of other cells,
  - by opacity measurements using an absorptiometer or nephalometer,
  - by separating the cells by centrifugation or filtration and measuring their wet or dry weight, and
  - by chemical assay of cell components such as nitrogen.
- The **viable count** measures the number of living cells, that is, cells capable of multiplication. Viable counts are obtained by **dilution or plating methods**. In the dilution method, the suspension is diluted to a point beyond which unit quantities do not yield growth when inoculated into suitable liquid media (extinction). Several tubes are inoculated with varying dilutions and the viable count calculated statistically from the number of tubes showing growth. This method does not give accurate values but is used widely in water bacteriology for estimation of the '**presumptive coliform count**' in drinking water. In the plating method, appropriate dilutions are inoculated on solid media, either on the surface of plates or as pour plates. The number of colonies that develop after incubation gives an estimate of the viable count. The method commonly employed is that described by **Miles and Misra** (1938) in which serial dilutions are dropped on the surface of dried plates and colony counts obtained.

## BACTERIAL NUTRITION

The bacterial cell has the same general chemical pattern as the cells of other organisms. The principal constituent of bacterial cells is water, which represents about 80 per cent of the total weight. Proteins, polysaccharides, lipids, nucleic acids, mucopeptides and low molecular weight compounds make up the rest. Bacterial metabolism is closely similar to that of other organisms, exemplifying the 'unity of biochemistry'. There are, however, some differences which are exploited in selective toxicity and chemotherapy.

### Factors that affect growth

For growth and multiplication of bacteria, the minimum nutritional requirements are **water, a source of carbon, a source of nitrogen and some inorganic salts.** Water is the vehicle for the entry of all nutrients into the cell and for the elimination of all waste products. It participates in metabolic reactions and also forms an integral part of the protoplasm.

- Bacteria can be classified nutritionally, based on their **energy requirements** and on their ability to synthesise essential metabolites. Bacteria which derive their energy from sunlight are called **phototrophs** and those that obtain energy from chemical reactions are called **chemotrophs.** Bacteria that can synthesise all their organic compounds are called **autotrophs.** Those that are unable to synthesise their own metabolites and depend on preformed organic compounds are called **heterotrophs**. Autotrophs are able to utilise atmospheric carbon dioxide and nitrogen. They are

capable of independent existence in water and soil and are of no medical importance, though they are of vital concern in agriculture and the maintenance of soil fertility. Heterotrophic bacteria are unable to grow with carbon dioxide as the sole source of carbon. The nutritional requirements of heterotrophs vary widely. Some may require only a single organic substance such as glucose, while others may need a large number of different compounds such as amino acids, nucleotides, lipids, carbohydrates and co-enzymes.

- Bacteria require a supply of **inorganic salts**, particularly the anions phosphate and sulphate, and the cations sodium, potassium, magnesium, iron, manganese and calcium. These are normally present in the natural environment where bacteria live but will have to be supplied in culture media. Some ions such as cobalt may be needed in trace amounts.
- Some bacteria require certain **organic compounds** in minute quantities. These are known as growth factors or bacterial vitamins. **Growth factors** are called **essential** when growth does not occur in their absence, and **accessory** when they enhance growth, without being absolutely necessary for it. In many cases, bacterial vitamins are identical to the vitamins necessary for mammalian nutrition, particularly those belonging to the B group: thiamine, riboflavin, nicotinic acid, pyridoxine, folic acid and vitamin B12.

If a microorganism requiring an essential growth factor is inoculated into a medium containing an excess of all other nutrients, its growth will be proportional to the amount of the limiting substance added. Within a certain range, the concentration of the growth factor will bear a linear relationship to the growth of the organism. This is the principle of microbiological assays, which provide a very sensitive and specific method for the estimation of many amino acids and vitamins, as in the determination of vitamin B12 using *Lactobacillus leichmannii*.

- **Oxygen requirement and metabolism:** Depending on the influence of oxygen on growth and viability, bacteria are divided into aerobes and anaerobes. **Aerobic bacteria** require oxygen for growth. They may be obligate aerobes like the cholera vibrio, which will grow only in the presence of oxygen, or facultative anaerobes which are ordinarily aerobic but can also grow in the absence of oxygen, though less abundantly. Most bacteria of medical importance are facultative anaerobes. **Anaerobic bacteria**, such as clostridia, grow in the absence of oxygen and the **obligate anaerobes** may even die on exposure to oxygen. **Microaerophilic** bacteria are those that grow best in the presence of **low oxygen tension**.

The reason for the apparent toxicity of oxygen for anaerobic bacteria is not well understood. It has been suggested that in the presence of oxygen, hydrogen peroxide and other toxic peroxides accumulate. The enzyme catalase which splits hydrogen peroxide is present in most aerobic bacteria but is absent in anaerobes. Another reason is that obligate anaerobes possess essential enzymes that are active only in the reduced state.

The influence of free oxygen is related to the metabolic character of the bacterium. Aerobic bacteria obtain their energy and intermediates only through oxidation, involving oxygen as the ultimate hydrogen acceptor, while the anaerobes use hydrogen acceptors other than oxygen. Facultative anaerobes may act in both ways. In the case of aerobes, where the ultimate electron acceptor is atmospheric oxygen (aerobic respiration), the carbon and energy source may be completely oxidised to carbon dioxide and water. Energy is provided by the production of energy-rich phosphate bonds and the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). This process is known as **oxidative phosphorylation**. Anaerobic bacteria use as electron acceptors compounds such as nitrates or sulphates instead of oxygen (anaerobic respiration).

A more common process in anaerobic metabolism may be a series of oxidoreductions in which the carbon and energy source acts as both the electron donor and the electron acceptor. This process is known as **fermentation** and leads to the formation of several organic end products such as acids and alcohols, as well as of gas (carbon dioxide and hydrogen). During the process of fermentation, energy-rich phosphate bonds are produced by the introduction of organic phosphate into intermediate metabolites. This process is known as **substrate-level phosphorylation**. The energy-rich phosphate groups so formed are used for conversion of ADP to ATP.

In determining the growth of aerobic and anaerobic bacteria, what is more important than the presence or absence of oxygen is the state of oxidation of the environment. The oxidising or reducing condition of a system is indicated by the net readiness of all the components in that system to take up or part with

electrons. This is known as the **oxidation–reduction (redox) potential** of the system. The redox potential of a medium is best estimated by measuring the electrical potential difference set up between the medium and an unattackable electrode immersed in it. This electrode potential (Eh) is measured in millivolts. The more oxidised the system, the higher the potential. A simpler, though less accurate, method of measuring the redox potential is using oxidation–reduction indicators such as methylene blue, and noting the change in colour.

- **Carbon dioxide**: All bacteria require small amounts of carbon dioxide for growth. This requirement is usually met by the carbon dioxide present in the atmosphere, or produced endogenously by cellular metabolism. Some bacteria like *Brucella abortus* require much higher levels of carbon dioxide (5–10 per cent) for growth, especially on fresh isolation (capnophilic).
- **Temperature**: Bacteria vary in their requirement of temperature for growth. For each species, there is a 'temperature range', and growth does not occur above the maximum or below the minimum of this range. The temperature at which growth occurs best is known as the '**optimum temperature**', which in the case of most **pathogenic bacteria** is 37°C. Bacteria which grow best at temperatures of 25–40°C are called **mesophilic**. All parasites of warm-blooded animals are mesophilic. Within the group of mesophilic bacteria, some like *Pseudomonas aeruginosa* have a wider range (5–45°C), while others like the gonococcus have a restricted range (30–39°C).

**Psychrophilic** bacteria are those that grow best at temperatures below 20°C, some of them even growing at temperatures as low as –7°C. They are soil and water saprophytes and, though not of direct medical importance, may cause spoilage of refrigerated food. Another group of non-pathogenic bacteria, the **thermophiles**, grow best at high temperatures, 55–80°C. They may cause spoilage of underprocessed canned food. Some thermophiles (like *Bacillus stearothermophilus*) form spores that are exceptionally thermoresistant. Extremely thermophilic bacteria have been identified which can grow at temperatures as high as 250°C.

Bacteria also differ in the effect of temperature on viability. Heat is an important method for the destruction of microorganisms (sterilisation), moist heat causing coagulation and denaturation of proteins and dry heat causing oxidation and charring. Moist heat is more lethal than dry heat. The lowest temperature that kills a bacterium under standard conditions in a given time is known as the **thermal death point**. Under moist conditions most vegetative, mesophilic bacteria have a thermal death point between 50 and 65°C and most spores between 100 and 120°C.

At low temperatures some species die rapidly but most survive well. Storage in the refrigerator (3–5°C) or the deep freeze cabinet (–30 to –70°C) preserves cultures. Rapid freezing as with solid carbon dioxide or the use of a stabiliser such as glycerol, minimises the death of cells on freezing.

- **Moisture and drying**: Water is an essential ingredient of bacterial protoplasm and hence drying is lethal to cells. However, the effect of drying varies in different species. Some delicate bacteria like *Treponema pallidum* are highly sensitive, while others like staphylococci withstand drying for months. Spores are particularly resistant to desiccation and may survive in the dry state for several decades. Drying in vacuum in the cold (freeze drying or lyophilisation) is a method for the preservation of bacteria, viruses and many labile biological materials.
- **Hydrogen ion concentration**: Bacteria are sensitive to variations in pH. Each species has a pH range, above or below which it cannot survive, and an optimum pH at which it grows best. The majority of pathogenic bacteria grow best at neutral or slightly alkaline reactions (pH 7.2–7.6). Some acidophilic bacteria such as lactobacilli grow under acidic conditions. Others, such as the cholera vibrio, are very sensitive to acid, but tolerate high degrees of alkalinity. Strong solutions of acid or alkali (5% hydrochloric acid or sodium hydroxide) readily kill most bacteria, though mycobacteria are exceptionally resistant to them.
- **Light**: Bacteria (except the phototrophic species) grow well in the dark. They are sensitive to ultraviolet light and other radiation. Cultures die if exposed to sunlight. Exposure to light may influence pigment production. Photochromogenic mycobacteria form a pigment only on exposure to light and not when incubated in the dark.
- **Osmotic effect**: Bacteria are more tolerant to osmotic variation than most other cells due to the mechanical strength of their cell walls. Sudden exposure to hypertonic solutions may cause osmotic withdrawal of water and shrinkage of protoplasm—plasmolysis.

This occurs more readily in Gram-negative than in Gram-positive bacteria. Sudden transfer from a concentrated solution to distilled water may cause plasmoptysis (excessive osmotic imbibition leading to swelling and rupture of the cell).

- **Mechanical and sonic stress:** Though bacteria have tough cell walls, they may be ruptured by mechanical stress such as grinding or vigorous shaking with glass beads. They may also be disintegrated by exposure to ultrasonic vibration.

## BACTERIOCINS

Gratia (1925) observed the production of a highly specific antibiotic substance by one strain of *E.coli* which was active against another strain of the same species. The name colicin was given to such substances produced by *E.coli* and other members of the family Enterobactericeae. With the recognition that colicin-like substances are produced by several other bacteria as well, the generic name **bacteriocin** was proposed for the group of highly specific antibiotic-like substances produced by certain strains of bacteria which are active against other strains of the same or different species. Bacteriocins are given specific names based on the bacterial species of origin, for example **colicins** from *E.coli*, **pyocins** from *Ps.pyocyanea* (*aeruginosa*), **megacins** from *B.megaterium* and **diphthericins** from *C.diphtheriae*.

Bacteriocins are proteins but some may have associated lipopolysaccharides derived from the cell walls of bacteria producing them. Bacteriocins and phages resemble each other in a number of respects. Both adsorb on the surface of susceptible bacterial cells on specific receptor sites, some of which may be the same for phages and bacteriocins. Under the electron microscope, some bacteriocins, especially pyocins, appear like the tail structures of phages. They may be considered products of defective phage genomes, able to code only for parts of phage particles.

The **synthesis** of bacteriocins is determined by the presence in bacteria of colicinogenic factors (**Col factors**). Col factors are episomes and can be transmitted from cell to cell by conjugation or transduction. Certain physical and chemical agents (UV rays, nitrogen mustard) induce colicin production by the cells harbouring Col factors.

A cell producing a bacteriocin is immune to it but may be sensitive to other bacteriocins. Bacteriocins have very specific activity on bacteria, being capable of killing some but not all strains of a species. The specificity is made use of in typing certain species such as *S.sonnei*, *Proteus* sp, *Ps.aeruginosa*. Bacteriocins kill susceptible cells without lysing them.

While phage typing schemes are generally based on the sensitivity of the test strains to the lytic action of phages, **bacteriocin typing schemes** depend on the ability of bacteriocins produced by the test strain to kill standard indicator strains of bacteria. The usual method of bacteriocin typing employs the plate diffusion technique. The test bacterium is inoculated as a broad streak on the centre of a culture medium, the bacterial growth is scraped off and the remaining cells killed by exposure to chloroform vapour. Standard indicator strains of bacteria are then streaked at right angles to the original inoculum. After incubation, the pattern of inhibition of the indicator strains represents the bacteriocin type of the test bacterium.

## RECAP

- The kingdom protista has been divided into two groups: prokaryotes and eukaryotes.
- Bacteria and blue-green algae are prokaryotes, fungi, other algae and protozoa are eukaryotes.
- Bacteria possess a single circular chromosome (eukaryotes have multiple linear chromosomes) and have muramic acid (eukaryotes do not).
- The morphological study of bacteria requires the use of microscopes. The microscopes currently in use are:
  - Optical or light microscope (bright field microscope)
  - Phase contrast microscope

- Fluorescent microscope
- Dark field/ground microscope
- Electron microscope

- Staining techniques used for study of bacteria are:
  - Simple stains (methylene blue, basic fuchsin), where all bacteria are stained the same colour
  - Negative stains
  - Impregnation methods
  - Differential stains, where stains impart different colours to different bacteria or bacterial structures (Gram stain, acid fast stain)
- Depending on their shape, bacteria are classified into cocci (spherical or oval), bacilli (rod-shaped), vibrios (comma-shaped curved rods), spirilla (rigid spiral forms), spirochetes (flexuous spiral forms) and actinomycetes (branching filamentous bacteria).
- A bacterial cell has a rigid cell wall, a phospholipid cytoplasmic) membrane, flagella, fimbriae and pili. Gram-positive cell wall is 90% peptidoglycan and Gram-negative cell wall is composed of lipopolysaccharide which also contains lipid A, a toxic substance that imparts the pathogenic virulence associated with some Gram-negative bacteria.
- Bacteria divide by binary fission. The time interval between two cell divisions is the generation or population doubling time. This may vary from 20 minutes (coliform bacilli) to 20 hours (tubercle bacilli) to 20 days (lepra bacilli).
- The bacterial growth curve consists of a lag phase (no appreciable increase in number), a log phase (an exponential increase in bacterial number), a stationary phase (no increase or decrease in number) and a decline phase (decrease in the bacterial population due to cell death).
- Bacteria vary in their requirements of temperature for growth. Mesophilic bacteria grow best at temperatures of 25–40°C, psychrophilic bacteria at temperatures below 20°C and thermophilic bacteria at high temperatures, 55–80°C.
- Bacteriocins are a group of highly specific antibiotic-like substances produced by certain strains of bacteria active against other strains of the same or different species. Bacteriocins are given specific names based on the bacterial species of origin, for example colicins from *E.coli*, pyocins from *Ps.pyocyanea* (*aeruginosa*), megacins from *B.megaterium* and diphthericins from *C.diphtheriae*.

## SHORT ANSWERS

1. Principles of Gram staining
2. Draw a labelled diagram of the structure of a bacterial cell.
3. L forms of bacteria
4. Bacterial cell wall (Gram-positive, Gram-negative)

## SHORT NOTES

1. Structure of flagella
2. Arrangment of flagella with diagram
3. a) Spores
   b) Arrangement of spores
4. Bacterial growth curve
5. Bacterocins
7. Capsule