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Textbook of MICROBIOLOGY for BSc NURSING



2nd Edition

Surinder Kumar

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CHAPTER 2

Microscopy and Morphology of Bacteria

Learning Objectives

After reading and studying this chapter, you should be able to:

- Discuss microscopic methods.
- Explain the principle and describe uses of the following:
  - Darkfield microscopy, phase-contrast microscopy
  - Fluorescent microscopy and electron microscopy
- Differentiate between prokaryotes and eukaryotes.
- Describe anatomy of bacterial cell.
- Describe cell envelope.
- Describe bacterial cell wall.
- Discuss capsule or bacterial capsule.
- Describe bacterial flagellae.
- Describe fimbriae or pili.
- Discuss bacterial spores or endospores.
- Explain L-forms of bacteria.

MICROSCOPY

Microscope is an optical instrument used to magnify (enlarge) minute objects or micro-organisms which cannot be seen by naked eye. In general, microscopy is used in microbiology for two basic purposes: The initial detection of microbes and the preliminary or definitive identification of microbes.

LIGHT MICROSCOPY

Light microscopy refers to the use of any kind of microscope that uses visible light to observe specimens. Here we examine several types of light microscopy. A modern **compound light microscope (LM)** has a series of lenses and uses visible light as its source of illumination (**Fig. 2.1**).

Compound Light Microscopy

Parts of Microscope

An ordinary light microscope consists of different parts, such as the stand, the optical system, the body, the stage, the substage, and the mirror (**Fig. 2.1**).

1. **Stand:** The upright *stand* rests on a heavy *foot* and bears at its upper end an inclined binocular head with two *eye-pieces*, above, and a revolving *nose-piece* bearing several *objectives,* below. The stand rests on a heavy *foot* and the *limb* which bears the optical system.
2. **The optical system:** The stand bears at its upper end an inclined binocular head with two *eye-pieces*, above with (magnification x 5, x 6, or x 10), and a revolving *nose-piece* bearing several *objectives,* below with different magnifying powers. The three objectives most commonly used in microbiology are: (1) a low-power "dry" objective with magnification x 10, (2) a high-power "dry" objective with magnification x 40, and (3) an oil immersion objective with magnification x 100.
3. **The stage:** This is the platform on which material (i.e., the "object") is placed to be examined with a central hole over which the slide with specimen is held by clips. For focusing, the stage is racked upward or downward and the slide can be moved in two directions.

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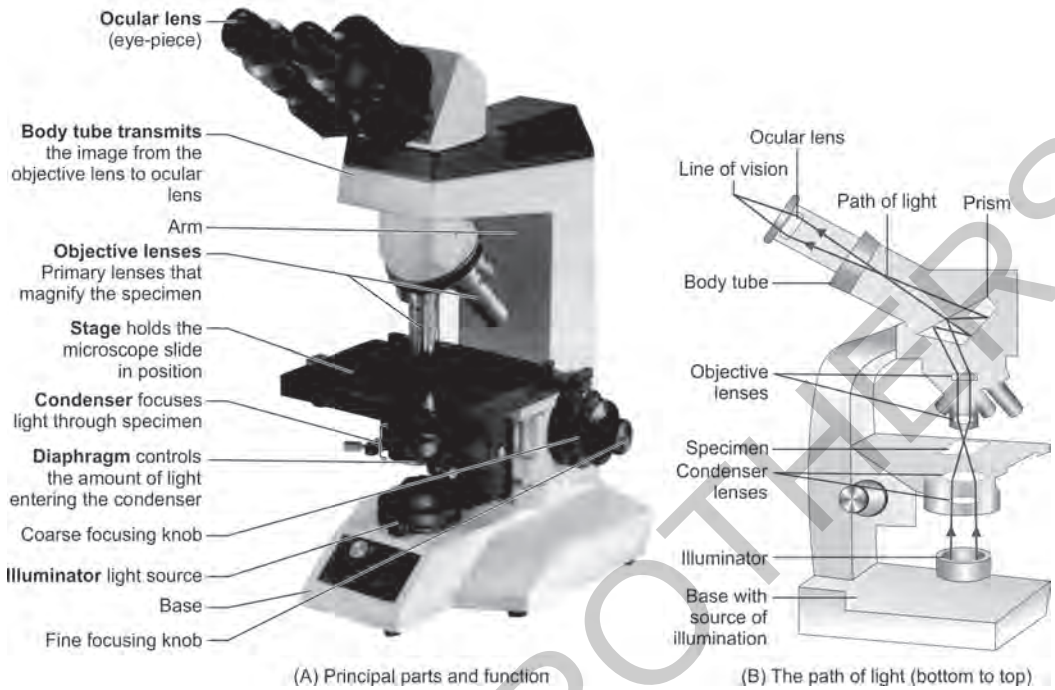

Fig. 2.1: The compound light microscope.

4. **The substage:** A built-in *lamp* in the foot of the microscope passes a beam of light upward through a *field diaphragm* (iris). The beam is focused on to the specimen by a *substage condenser* which is attached beneath the stage, centered by condenser centration adjustments and moved upward or downward by the turning of a *condenser focusing adjustment*. An *aperture diaphragm* (iris) with a lever for its control is incorporated in the condenser mounting.

Methods of Use of Light Microscope

1. Place the microscope at a convenient position on the bench
2. Check to ensure that the objectives and eye-pieces are free from dust and immersion oil
3. Rack up fully the substage condenser, i.e., until its top surface is within about 1 mm below the upper surface of the stage (undersurface of the slide)
4. Check that the filter carrier in the substage is in its correct position
5. Fully open the substage and lamp irises
6. Switch on the lamp at low intensity and then increase the intensity
7. Place the slide with the object on the stage, so that it is held by the stage clips
8. First view the specimen with a low power dry objective. Use the fine adjustment only to obtain and maintain exact focus
9. Adjust the distance between the eye-pieces so that a single field is seen
10. If continued examination with the **low power objective** is required, diffuse the light uniformly over the field by defocusing the condenser either by racking it down or swinging out its top lens
11. Before proceeding to use a **high-power objective**, check that the iris diaphragms are open, the condenser is focused
12. Before using an **oil-immersion objective**, place a moderately large drop of immersion oil on the middle of the specimen
    i. Rotate the nose-piece until the oil immersion lens is in position

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ii. With the eye at the level of the stage, use the *coarse* focusing adjustment slowly to raise the stage until the oil on the slide makes contact with the objective

13. Apply the eyes to the eye-pieces and, while watching, slowly focus the objective *away* from the slide by lowering the stage with the coarse focusing adjustment
14. Wipe the oil from the objective with a clean tissue after use. Turn down the light intensity control to low and then switch off the light

MORPHOLOGY OF BACTERIA

Microorganisms are generally regarded as living forms that are microscopic in size and relatively simple, usually unicellular, in structure. The bacteria are single-celled organisms that reproduce by simple division, i.e., binary fission.

NAMING AND CLASSIFYING MICROORGANISMS

Microorganisms are a heterogeneous group of several distinct classes of living beings. Whittaker's system recognizes five-kingdoms of living things–**Monera (bacteria), Protista, Fungi, Plantae, and Animalia**. Five kingdoms have been modified further by the development of **three domains, or Superkingdoms** system—the **Bacteria,** the **Archaea** (meaning ancient), and the **Eukarya.**

NOMENCLATURE

The system of nomenclature (naming) for organisms in use today was established in 1735 by Carolus Linnaeus. Scientific names are latinized. Scientific nomenclature assigns each organism two names—the genus (plural: genera) is the first name and is always capitalized; the specific epithet (species name) follows and is not capitalized. The organism is referred to by both the genus and the specific epithet, and both names are underlined or italicized. With the initial of the genus followed by the specific epithet, e.g., *Staphylococcus aureus* (*Staphylococcus-Genus; aureus-Species*).

COMPARISON OF PROKARYOTIC CELLS EUKARYOTIC CELLS

All living organisms on earth are composed of one or the other of two types of cells ***prokaryotic*** cells and ***eukaryotic*** cells (**Table 2.1**).

Table 2.1: Principle differences between prokaryotic and eukaryotic cells.

Characteristic	*Prokaryotic*	*Eukaryotic*
Size (approximate)	0.5–3 µm	>5 µm
Nucleus		
• Nuclear membrane	Absent	Present
• Nucleolus	Absent	Present
• Chromosome	One (circular)	More than one (linear)
• Deoxyribonucleoprotein	Absent	Present
• Division	By binary fission	By mitosis
Cytoplasm		
• Cytoplasmic streaming	Absent	Present
• Mitochondria	Absent	Present
• Golgi apparatus	Absent	Present
• Lysosomes	Absent	Present
• Pinocytosis	Absent	Present
• Endoplasmic reticulum	Absent	Present
Chemical composition		
• Sterol	Absent	Present
• Muramic acid	Present	Absent
Examples	Eubacteria, Archaea All bacteria and blue-green algae	Fungi, slime molds, protozoa, higher plants, and animals including humans

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1. **Prokaryotes—Bacteria and Archaea**: All bacteria and blue-green algae are prokaryotes.
2. **Eukaryotes—Eukarya:** Other algae (excluding blue-green algae), fungi, slime molds, protozoa, higher plants, and animals are eukaryotic **(Table 2.1)**.

Size of Bacteria

The unit of measurement in bacteriology is the micron or micrometer:

- 1 micron (μ) or micrometer (μm) = a millionth part of a meter or a thousandth of a millimeter.
- 1 millimicron (mμ) or nanometer (nm) = one thousandth of a micron or one millionth of a millimeter.
- 1 Angstrom unit (A) = one tenth of a nanometer

The diameter of the smallest body that can be resolved and seen clearly with naked eye is 200 μm. Bacteria of medical importance generally measure 0.2–1.5 μm in diameter and about 3–5 μm in length. To see bacteria a light microscope must be used. Following types of microscopes are used for examination of bacteria.

Study of Bacteria

Stained Preparations

Because most microorganisms appear almost colorless when viewed through a standard light microscope, we often must prepare them for observation. Staining simply means coloring the microorganisms with a dye that emphasizes certain structures.

Routine methods for staining of bacteria involve drying and fixing smears, procedures that kill them. Fixing simultaneously kills the microorganisms and attaches them to the slide. It also preserves various parts of microbes in their natural state with only minimal distortion. Various staining techniques are commonly used in bacteriology **(See Chapter 6)**.

MORPHOLOGY OF BACTERIA

Shape of Bacteria

Depending on their shape, bacteria are classified into several varieties **(Fig. 2.2)**:

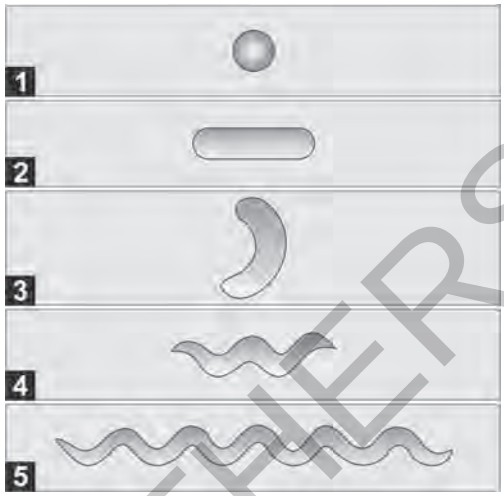

Fig. 2.2: Shape of bacteria: 1. coccus; 2. bacillus; 3. vibrio, 4. spirillum, 5. spirochete.

1. **Cocci:** Cocci (from kokkos meaning berry) are spherical, or nearly spherical
2. **Bacilli:** Bacilli (from baculus meaning rod) are relatively straight, rod shaped (cylindrical) cells. In some of the bacilli the length of the cells may be equal to width. Such bacillary forms are known as coccobacilli and have to be carefully differentiated from cocci
3. **Vibrios:** Vibrios are curved or comma-shaped rods
4. **Spirilla:** Spirilla are rigid spiral or helical forms
5. **Spirochetes:** Spirochetes (from speira meaning coil and chaite meaning hair) are flexuous spiral forms
6. **Mycoplasma:** Mycoplasma are cell wall deficient bacteria and hence do not possess a stable morphology

Arrangement of Bacterial Cells

Pathogenic bacterial species appear as sphere (cocci), rods (bacilli), and spirals. Bacteria sometimes show characteristic of cellular arrangement or grouping **(Figs. 2.3A and B)**.

Anatomy of the Bacterial Cell

The principal structures of the bacterial cell are shown in **Figure 2.4**.

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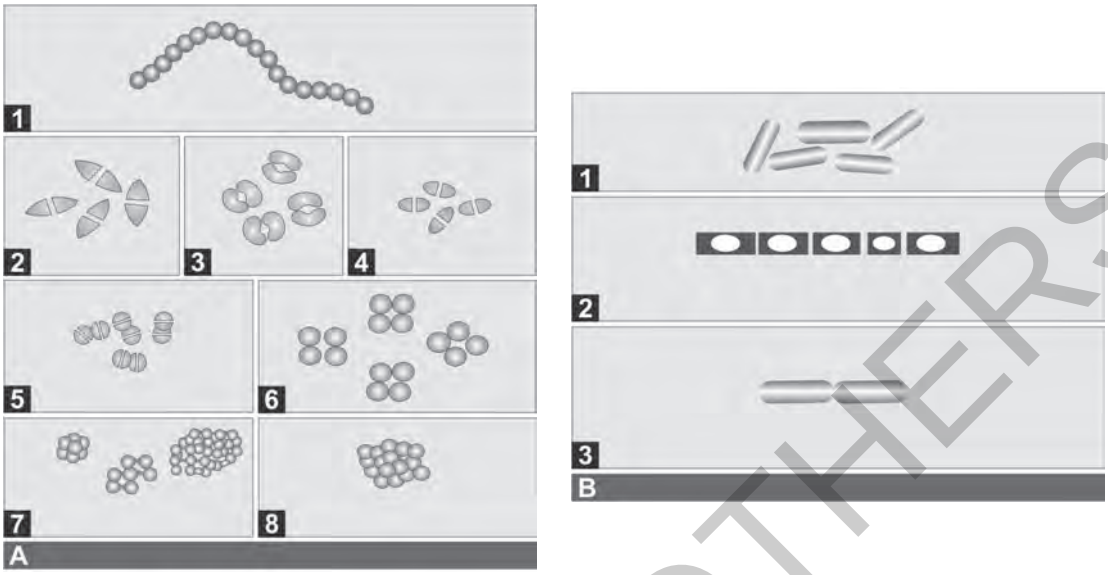

Figs. 2.3A and B: Arrangement of bacteria. (A) Cocci: 1. streptococci, 2. pneumococci, 3. gonococci, 4. meningococci, 5. *Neisseria catarrhalis*, 6. Gaffkya tetragena, 7. sarcina, 8. staphylococci. (B) Bacilli: 1. bacilli in cluster, 2. bacilli in chains (*Bacillus anthracis*) 3. diplobacilli (*K. pneumoniae*).

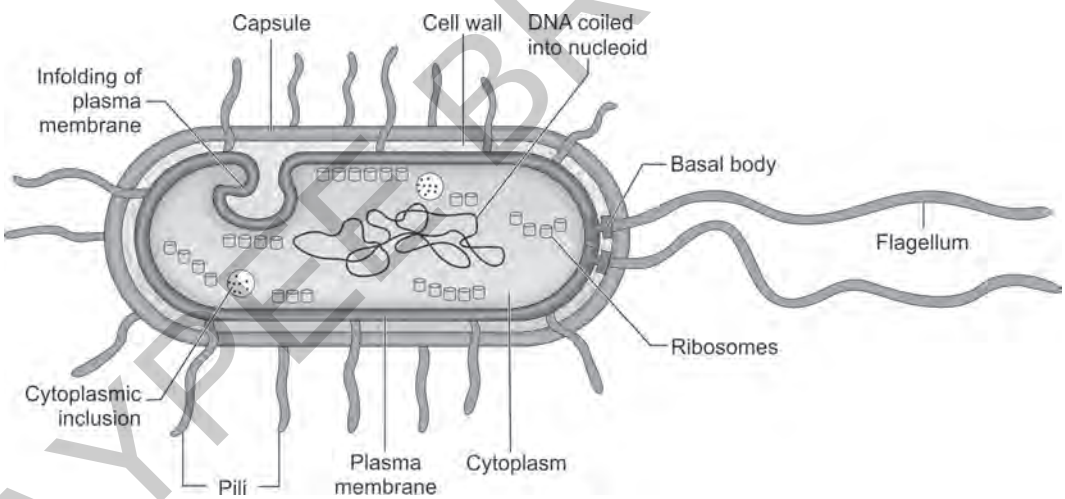

Fig. 2.4: Anatomy of a bacterial cell.

Bacterial Cell Components

It can be divided into:

A. Cell envelope and its appendages.
   a. **The outer layer or cell envelope** consists of two components:
      1. **Cell wall**
      2. **Cytoplasmic or plasma membrane**—beneath cell wall
   b. Cellular appendages—capsule, fimbriae, and flagella

B. **Cell interior:** Those structures and substances that are bounded by the cytoplasmic membrane compose the cell interior and include **cytoplasm, cytoplasmic inclusions, (mesosomes, ribosomes, inclusion granules, vacuoles) and a single circular**

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chromosome of deoxyribonucleic acid (DNA).

Cell Envelope and Its Appendages

The Outer Layer or Cell Envelope

1. Cell wall: The cell wall is the layer that lies just outside the plasma membrane. It is thick, strong, and relatively rigid.

Gram-positive bacterial cell wall: In gram-positive bacteria, the cell wall consists mainly of peptidoglycan and teichoic acids **(Fig. 2.5)**.

- **Peptidoglycan**: It is thicker and stronger than those of gram-negative bacteria.
- **Teichoic acid:** In addition, the cell walls of gram-positive bacteria contain ***teichoic acids.***

Gram-negative cell wall: The gram-negative cell wall is structurally quite different from that of gram-positive cells **(Fig. 2.6)**. It consists of **peptidoglycan and lipoprotein, outer membrane, and lipopolysaccharide.**

1. **Peptidoglycan layer**: It is bonded to lipoproteins covalently in the outer membrane and plasma membrane and is in the **periplasmic,** a gel-like fluid between the outer membrane and plasma membrane.

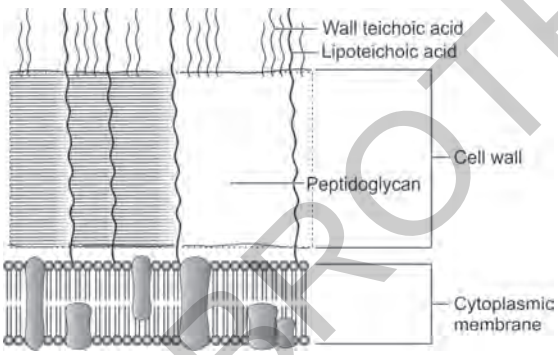

Fig. 2.5: Gram-positive cell wall.

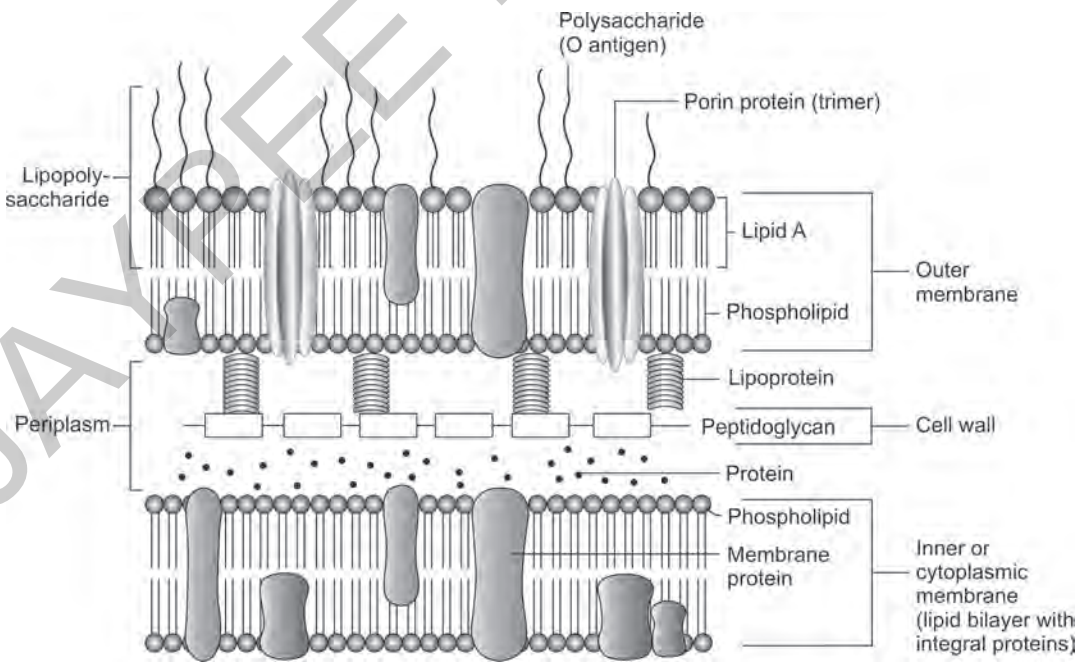

Fig. 2.6: Gram-negative cell wall.

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2. **Lipopoprotein:** Lipopoprotein, or murein lipoproteins attach to the peptidoglycan, and to the outer membrane.
3. **Outer membrane:** External to the peptidoglycan and attached to it by lipoprotein is the outer membrane. It is bilayered structure. Its inner leaflet is composed of phospholipid and outer leaflet by lipopolysaccharide (LPS).
4. **Lipopolysaccharide (LPS):** Lipopolysaccharide (LPS) consists of three components:
   i. **Lipid A** is the lipid portion of LPS and is embedded in the top layer of the outer membrane. Lipid A functions as an **endotoxin**. All the toxicity of the endotoxin is due to lipid A, i.e., pyrogenicity, lethal effect, tissue necrosis, etc.
   ii. **The core polysaccharide** is attached to lipid A.
   iii. **O polysaccharide**: It is known as **O antigen**. Differences between cell wall of gram-positive and gram-negative bacteria are shown in **Table 2.2**.

2. *Cytoplasmic (plasma) membrane (**Table 2.2**)*
Structure: It is thin (5–10 nm thick), elastic and can only be seen with electron microscope.
Functions of cytoplasmic membrane:
i. **Semipermeable membrane**
ii. **Housing enzymes and proteins**
iii. **Generation of chemical energy**
iv. **Cell motility**
v. **Mediation of chromosomal segregation during replication**

Cellular Appendages

Capsule or slime layer

Structure: Many bacteria synthesize large amount of extracellular polymer in their natural environments. When the polymer forms a condensed, well-defined layer closely surrounding the cell, it is called the capsule as in the pneumococcus. If the polymer is easily washed off and does not appear to be associated with the cell in any definite fashion, it is referred as a slime layer.

Composition of capsules and slime layers: Capsules and slime layers usually are composed of polysaccharide (e.g., *Pneumococcus*) or of polypeptide in some bacteria (e.g., *Bacillus anthracis* and *Yersinia pestis*).

Capsulated bacteria: *Streptococcus pneumoniae,* several groups of streptococci, *Neisseria meningitidis, Klebsiella, Haemophilus influenzae, Yersinia* and *Bacillus.*

Demonstration of capsule

i. **Gram stain:** Slime has little affinity for basic dyes and is not visible in Gram stained smears.
ii. **Special capsule staining techniques**: Usually, employing copper salts as mordants.
iii. **India ink staining (negative staining):** The capsule appears as a clear halo around the bacterium, against a dark background in the film **(Fig. 2.7)**.
iv. **Electron microscope**
v. **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. It is known as the **capsule-swelling reaction** or **Quellung reaction** (Quellung-*Ger; swelling*), described by Neufeld (1902).

Table 2.2: Comparison of cell walls of gram-positive and gram-negative bacteria.

Characteristic	*Gram-positive*	*Gram-negative*
1. Thickness	Thicker	Thinner
2. Peptidoglycan	Thick layer (16–80 nm)	2 nm (thin layer)
3. Teichoic acid	Present	Absent
4. Variety of amino acids	Few	Several
5. Aromatic and sulfur-containing amino acids	Absent or scant	Present
6. Lipids	Absent or scant	Present
7. Porin proteins	Absent	Present
8. Periplasmic region	Absent	Present

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Fig. 2.7: Pneumococci negatively stained with India ink to show capsule.

Functions of capsule

i. **Virulence factor—by inhibiting phagocytosis**
ii. **Protection of the cell wall**
iii. **Identification and typing of bacteria**

Flagella

Motile bacteria possess one or more unbranched, long, sinuous filaments called **flagella**, which are the organs of locomotion.

Structure: They are 3–20 μm long and are of uniform diameter (0.01–0.013 μm) and terminate in a square tip. Flagella consists of largely or entirely of a protein, **flagellin**.

Parts and Composition

Each flagellum consists of three parts**:**

1. Filament
2. Hook
3. Basal body

Arrangement/Types (Fig. 2.8)

These are four types of flagella arrangement:

1. **Monotrichous**—single polar flagellum, e.g., cholera vibrio
2. **Amphitrichous**—single flagellum at both ends, e.g., *Alcaligenes faecalis.*
3. **Lophotrichous**—tuft of flagella at one or both ends, e.g., spirilla
4. **Peritrichous**—flagella surrounding the cell, e.g., typhoid bacilli

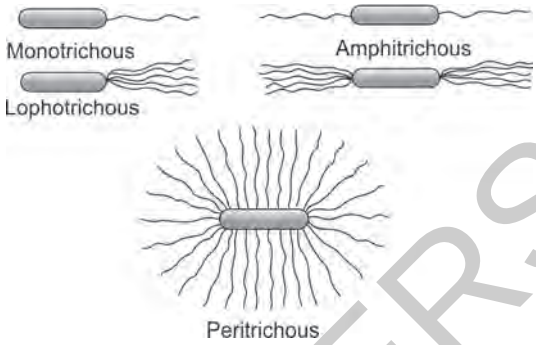

Fig. 2.8: Arrangement of flagella.

Demonstration of Flagella

Flagella are about 0.02 μm in thickness and hence beyond the resolution limit of the light microscope. The following methods are used for its demonstration:

i. **Dark ground illumination**
ii. **Special staining methods**: In which their thickness is increased by mordanting
iii. **Electron microscopy**
iv. **Indirect methods**: Indirect methods by which motility of bacteria can be seen or demonstrated.
   a. Microscopically in fluid suspensions (in a hanging drop or under a coverslip)
   b. **By spread of bacterial growth as a film over agar,** e.g., swarming growth of *Proteus* spp.
   c. **Turbidity spreading through semisolid agar,** e.g., Craigie tube method.

Fimbria or Pili

Structure and synthesis: Many gram-negative bacteria have short, fine, hair-like surface appendages which are called **fimbriae or pili**. They are shorter and thinner than flagella (0.1–1.5 μm in length and uniform width between 4 and 8 nm) and emerge from the cell wall. They originate in the **cytoplasmic membrane** and are composed of protein termed **pilins** like flagella.

Demonstration of Fimbriae

1. Electron microscopy
2. Hemagglutination

Functions of Pilli

Two classes can be distinguished on the basis of their function: **Ordinary (common) pili** and **sex pili**.

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Cell Interior

1. **Cytoplasm:** The cytoplasm of the bacterial cell is a viscous watery solution or soft gel, containing a variety of organic or inorganic solutes, and numerous ribosomes and polysomes.
2. **Ribosomes:** The ribosomes are the location for all bacterial protein synthesis.
3. Mesosomes (Chondrioids)
   Structure: These are convoluted or multilaminated membranous bodies formed as invaginations of the plasma membrane into the cytoplasm.
   Functions of mesosomes
   i. Compartmenting of DNA
   ii. Sites of the respiratory enzymes
4. **Intracytoplasmic inclusions:** These bodies are usually for storage. They consist of **volutin (polyphosphate), lipid, glycogen, starch or sulfur.**
5. **Bacterial nucleus:**
   Structure: The genetic material of a bacterial cell is contained in a single, long molecule of double-stranded deoxyribonucleic acid (DNA) which can be extracted in the form of a closed circular thread about 1 mm (1,000 μm) long, about 1,000 times the length of the cell. The bacterial chromosome is haploid and replicates by simple fission. Bacterial nucleus does not possess nuclear membrane.
   Plasmids: Bacteria may possess extranuclear genetic elements in the cytoplasm consisting of DNA termed *plasmids* or *episomes*.
   Function of plasmids: Plasmids are not essential for host growth and reproduction they inhabit, but may **confer on it certain properties**, such as drug resistance, enhanced pathogenicity, which may constitute a survival advantage.
6. **Bacterial spore:**
   A number of gram-positive bacteria, such as those of the genera *Clostridium* and *Bacillus* can form a special resistant dormant structure called an **endospore** or, simply, **spores.** Endospore develop when essential nutrients are depleted. Sporulation in bacteria, therefore, is not a method of reproduction but of preservation **(Fig. 2.9)**.

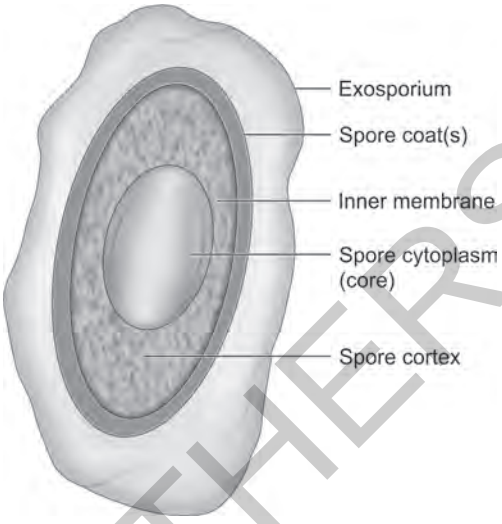

Fig. 2.9: Bacterial spore (cross-section).

Sporulation: Spore formation, sporogenesis or sporulation normally commences when growth ceases due to lack of nutrients, depletion of the nitrogen or carbon source (or both) being the most significant factor.
Stages: It is a complex process and may be divided into several stages.

- **Spore septum**
- F**orespore:** The spore septum becomes a **double-layered membrane** that surrounds the chromosome and cytoplasm. Structure, entirely enclosed within the original cell, is called a **forespore.**
- **Spore coat:** The forespore is subsequently completely encircled by dividing septum as a double layered membrane. The two spore membranes now engage in active synthesis of various layers of the spore. The inner layer becomes the **inner membrane**. Between the two layers there is laid **spore cortex** and outer layer is transformed into **spore coat** which consists of several layers. In some species **from** outer layer also develops **exosporium** which bears ridges and folds **(Fig. 2.9)**.
  Germination: Germination is the process of conversion of a spore into vegetative cells under suitable conditions.

Shape and position of spores

Spores may be central (equatorial), subterminal (close to one end), or terminal **(Fig. 2.10)**.

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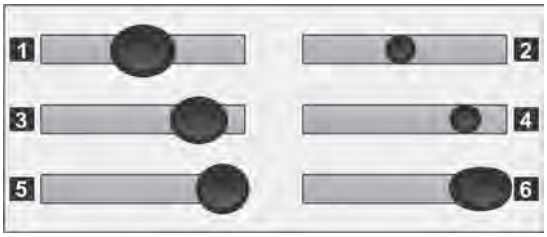

Fig. 2.10: Types of spores. 1. central, bulging, 2. central, not bulging, 3. subterminal, bulging, 4. subterminal, not bulging, 5. terminal, spherical, 6. terminal, oval.

The appearance may be spherical, ovoid or elongated, and being narrower that the cell, or broader and bulging it. The diameter of spore may be same or less than the width of bacteria (Bacillus), or may be wider than the bacillary body producing a distension or bulge in the cell (Clostridium).

Resistance

Bacterial spores constitute some of the most resistant forms of life. Spores of all medically important species are destroyed by autoclaving at 120°C for 15 minutes. Methods of sterilization and disinfection should ensure that spores also are destroyed. Sporulation helps bacteria survive for long periods under unfavorable environments.

Demonstration

i. **Gram stain:** Spores appear as an unstained refractile body within the cell.
ii. **Modified Ziehl–Neelsen (ZN) staining:** Spores are slightly acid-fast and may be stained differentially by a modification of the Ziehl–Neelsen method. Ziehl–Neelsen staining with 0.25–0.5% sulfuric acid (instead of 20% sulfuric acid as used in conventional method) as decoloring agent is used for spore staining.

Uses of spores

1. **Importance in food, industrial, and medical microbiology**
2. **Sterilization control:** For proper sterilization, spores of certain species of bacteria are employed as indicator, e.g., *Bacillus stearothermophilus* which is destroyed at a temperature of 121°C for 10–20 minutes (same temperature and time as used in autoclaving). Prior to its use, these spores may be kept in autoclave. Proper sterilization is indicated by the absence of the spores after autoclaving.
3. **Research**

L-FORMS OF BACTERIA (CELL-WALL-DEFECTIVE ORGANISMS)

Abnormal forms of the bacteria were named **L-forms** after the Lister Institute, London (hence, the "L").

Colonies of L-phase organisms on agar media are small and have a characteristic "**fried egg"** appearance.

L-forms are **nonpathogenic to laboratory animals.**

KEY POINTS

Microscopy: A simple microscope consists of one lens; a compound microscope has multiple lenses.

Anatomy of Bacterial Cell

A. Cell envelope and its appendages
B. Cell interior

Endospores are a dormant stage for survival during adverse environmental conditions.

IMPORTANT QUESTIONS

1. Name various microscopic instruments used in microbiology and describe the working principle of compound microscope.
2. Describe briefly the anatomy of bacterial cell.
3. Draw a labeled diagram of bacterial cell. Write briefly on cell wall of bacteria.
4. Write short notes on:
   a. Bacterial cell wall
   b. Capsule or bacterial capsule
   c. Bacterial flagella
   d. Bacterial spores or endospores

MULTIPLE CHOICE QUESTIONS

1. **Which of the following is not a distinguishing characteristic of prokaryotic cells?**
   a. They usually have a single, circular chromosome
   b. They lack membrane enclosed organelles
   c. They have cell walls containing peptidoglycan
   d. They lack a plasma membrane

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2. Peptidoglycan layer of cell wall is thicker in:
   a. Gram-positive bacteria
   b. Gram-negative bacteria
   c. Fungi
   d. Parasites

3. All the following statements are true for lipopolysaccharide, *except*:
   a. It consists of three components: Lipid A, core polysaccharide and O polysaccharide.
   b. Lipid A functions as an endotoxin
   c. It is an integral part of the cell wall of the gram-positive bacteria
   d. Polysaccharide represents a major surface antigen of the bacterial cell

4. A tuft of flagella present at one or both ends of bacterial cell is known as:
   a. Monotrichous
   b. Amphitrichous
   c. Lophotrichous
   d. Peritrichous

5. Which of the following is not true about fimbriae?
   a. They originate in the cytoplasmic membrane.
   b. They are composed of protein.
   c. They may be used for attachment.
   d. They may be used for motility.

6. Which one of the following bacteria is cell wall deficient?
   a. *Escherichia coli*
   b. *Streptococcus aureus*
   c. *Mycoplasma*
   d. *Treponema pallidum*

7. All of the following are spore forming bacteria, *except*:
   a. *Clostridium botulinum*
   b. *Bacillus anthracis*
   c. *Bacillus subtilis*
   d. *Vibrio cholerae*

ANSWERS

1. d	**2. a**	**3. c**	**4. c**
5. d	**6. c**	**7. d**	

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