Chapter 2.1. General Bacteriology: Morphology and Physiology of Bacteria

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MEDICALLY IMPORTANT BACTERIA

Based on Gram stain, the bacteria can be grouped into gram-positive cocci, gram-negative cocci, and gram-positive bacilli, gram-negative bacilli and miscellaneous bacteria (that do not take up/poorly take up Gram stain) **(Table 2.1.1)**.

MORPHOLOGY OF BACTERIA

Shape of Bacteria

Depending on their shape, bacteria are classified into:

- Cocci (singular coccus, from; kokkos, meaning berry) are oval or spherical cells, and
- Bacilli or rods (singular bacillus, meaning rod-shaped).

Cocci are arranged in groups (clusters), pairs, or chains. Similarly, bacilli can be arranged in chains, pairs, and some bacilli are curved, comma-shaped, or cuneiform-shaped **(Table 2.1.2 and Fig. 2.1.1)**.

Based on Gram staining property, both cocci and bacilli are further classified into **(Table 2.1.2 and Fig. 2.1.1)**:

- Gram-positive cocci
- Gram-negative cocci
- Gram-positive bacilli
- Gram-negative bacilli.

Table 2.1.1. Medically important bacteria.

Gram-positive cocci
• *Staphylococcus*—e.g. *S. aureus* • *Streptococcus*—e.g. #-hemolytic streptococci, and pneumococcus • *Enterococcus*—e.g. *E. faecalis, E. faecium*
Gram-negative cocci
Neisseria—e.g. meningococcus and gonococcus
Gram-positive bacilli
• *Corynebacterium*—e.g. *C. diphtheriae* • *Bacillus*—e.g. *B. anthracis* • *Mycobacterium*—e.g. *M. tuberculosis, M. leprae* • Miscellaneous gram-positive bacilli—*Listeria, Actinomycetes* and *Nocardia*
Gram-negative bacilli
• Enterobacterales—e.g. *Escherichia coli, Klebsiella, Proteus, Shigella, Salmonella, Yersinia* • Non-fermenting gram-negative bacilli—e.g. *Pseudomonas, Acinetobacter, Burkholderia* • *Vibrio*—e.g. *Vibrio cholerae* • Fastidious gram-negative bacilli—*Haemophilus, Bordetella, Brucella* • Miscellaneous gram-negative bacilli—*Campylobacter, Helicobacter, Legionella*, etc.
Anaerobic bacterial infections
• Sporing anaerobes—*Clostridium* • Non-sporing anaerobes—*Bacteroides*
Miscellaneous bacteria
• Spirochetes—*Treponema, Borrelia, Leptospira* • Rickettsiae, Chlamydiae and *Mycoplasma*

Table 2.1.2. Classification of bacteria depending on their morphology and Gram staining property.

Bacteria	***Example***
Gram-positive cocci arranged in	

Bacteria	***Example***
Cluster	*Staphylococcus*
Short chain	*Streptococcus*
Long chain	Viridans streptococci
Pairs, lanceolate shaped	Pneumococcus
Pairs or in short chain	*Enterococcus*
Gram-negative cocci arranged in	
Pairs, lens-shaped	Meningococcus
Pairs, kidney-shaped	Gonococcus
Gram-positive bacilli arranged in	
Chain (bamboo stick appearance)	*Bacillus anthracis*
Chinese letter or cuneiform pattern	*Corynebacterium diphtheriae*
Branched and filamentous form	*Actinomyces, Nocardia*
Gram-negative bacilli arranged in	
Pleomorphic (various shapes—cocci, coccobacilli, bacilli, etc.)	*Haemophilus, Proteus*
Coccobacilli	*Acinetobacter*
Comma shaped	*Vibrio cholerae*
Others	
Spirally coiled, flexible	Spirochetes
Bacteria that lack cell wall	*Mycoplasma*

Miscellaneous group: However, some bacteria are weakly Gram stained and hence need special stains for their demonstration, such as:

- Spirochetes *(Treponema* and *Leptospira)*—thin spirally coiled bacilli
- *Mycoplasma* (cell wall deficient free-living bacteria)
- Rickettsiae and chlamydiae are obligate intracellular bacteria.

Bacterial Cell Anatomy

Bacterial cell anatomy comprises the following structures **(Fig. 2.1.2)**:

- The *outer layer* or the envelope of a bacterial cell consists of—(1) a rigid cell wall, and (2) an underlying plasma membrane
- The *cytoplasm* contains cytoplasmic inclusions (mesosomes, ribosomes, inclusion granules, vacuoles) and a diffuse nucleoid containing a single circular chromosome
- Some bacteria may possess additional *cell wall appendages* such as capsule, flagella and fimbriae.

Fig. 2.1.1. Different morphology of bacteria and Gram staining property.

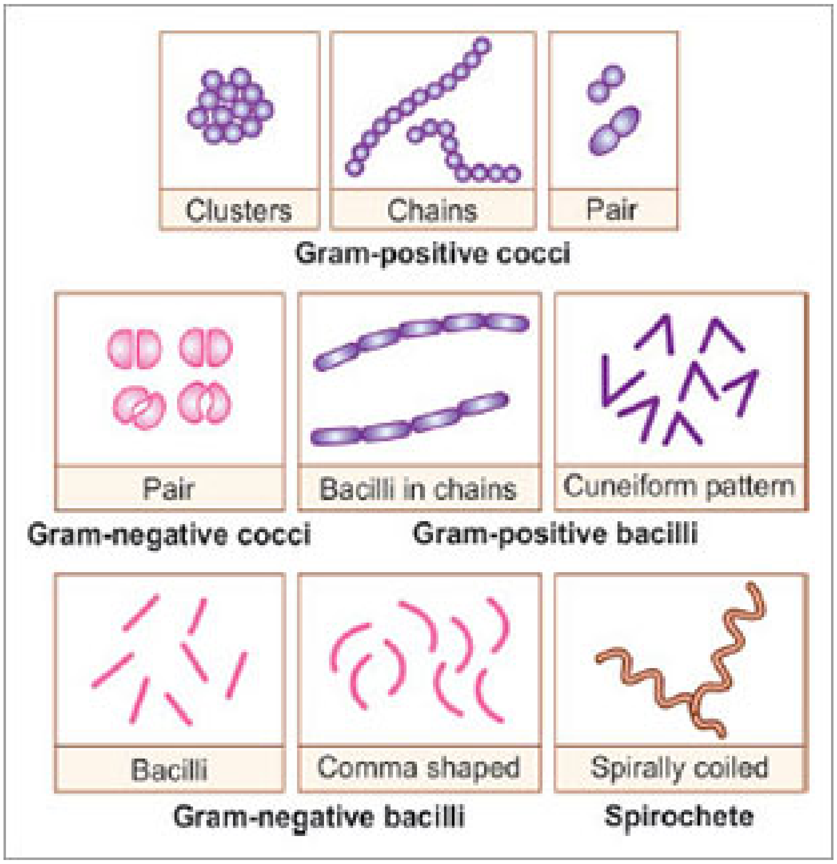
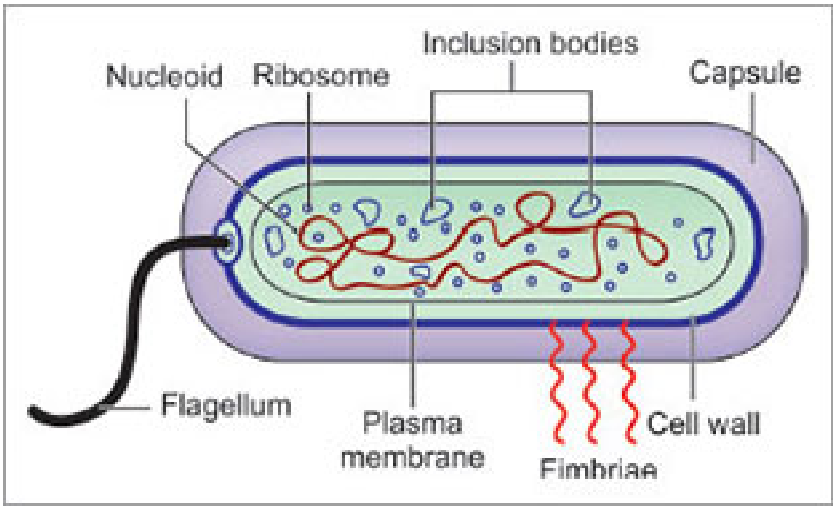


Fig. 2.1.2. Structure of bacterial cell.



Bacterial Cell Wall

The cell wall is a tough and rigid structure, surrounding the bacterium. It is 10–25 nm in thickness.

The cell wall has the following functions:

- It protects the cell against osmotic lysis
- It confers rigidity upon bacteria due to the presence of a peptidoglycan layer in the cell wall
- It is the site of action of several antibiotics
- **Virulence factors:** Bacterial cell wall contains certain virulence factors (e.g. endotoxin), which contribute to their pathogenicity
- **Immunity:** Antibody raised against specific cell wall antigens (e.g. antibody to LPS) may provide immunity against some bacterial infections.

Gram-positive Cell wall

The cell wall of gram-positive bacteria is simpler than that of gram-negative bacteria **(Table 2.1.3)**, made up of the following structure.

- **Peptidoglycan:** It is made up of layers of mucopeptide chains. It is much thicker (50–100 layers thick, 16–80 nm) than the gram-negative cell wall **(Fig. 2.1.3)**
- **Teichoic acid:** Gram-positive cell wall contains a significant amount of teichoic acid; which is absent in gram-negative bacteria. It constitutes major surface antigens of gram-positive bacteria.

Gram-negative Cell wall

The gram-negative cell wall is thinner and more complex than the gram-positive cell wall, comprising of the following components **(Fig. 2.1.4 and Table 2.1.3)**.

- **Peptidoglycan layer:** It is very thin (2 nm) compared to that of the gram-positive cell wall, made up of 1–2 layers of mucopeptide chain
- **Outer membrane:** This is a phospholipid layer that lies outside the thin peptidoglycan layer. It serves as a protective barrier to the cell. Outer membrane proteins (OMP) or porin proteins help in the transport of smaller molecules and also they are target sites for many antibiotics
- **Lipopolysaccharide (LPS):** This layer is unique to gram-negative bacteria, which is absent in gram-positives. It consists of three parts as:

  1. *Lipid A or the endotoxin*: It is an important virulence factor for gram-negative bacteria
  2. *Core polysaccharide*: It projects from lipid A region
  3. *O side chain*: O antigens are used for serotyping for bacteria and they also induce antibody formation.

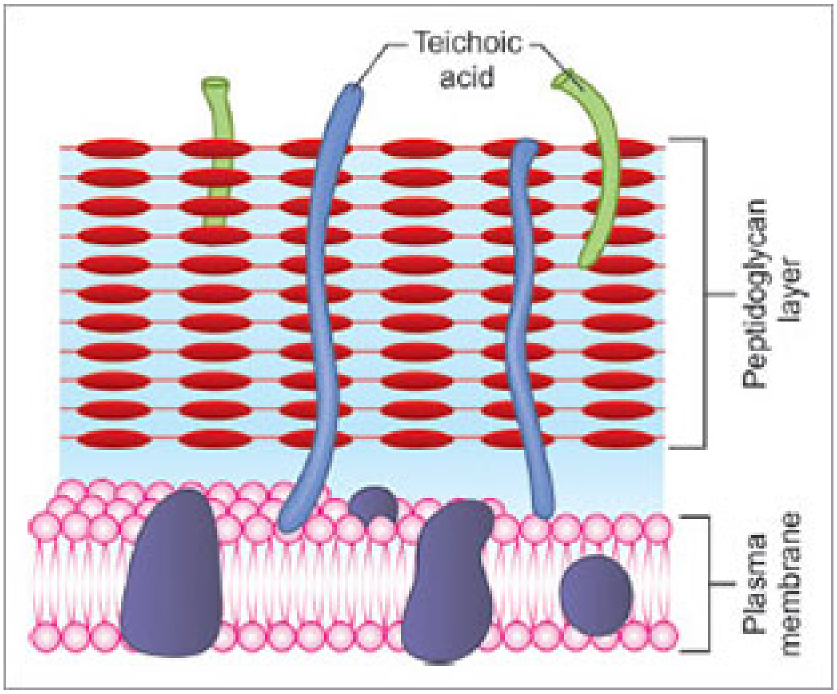
- **Periplasmic space:** It is the space between the inner cell membrane and outer membrane. It encompasses the peptidoglycan layer.

Table 2.1.3. Differences between gram-positive and gram- negative cell wall.

Characters	***Gram-positive cell wall***	***Gram-negative cell wall***
Peptidoglycan layer	Thicker (16–80 nm)	Thinner (2 nm)
Lipid content	Nil or scanty (2–5%)	Present (15–20%)
Lipopolysaccharide	Absent	Present

Characters	*Gram-positive cell wall*	*Gram-negative cell wall*
		(endotoxin)
Teichoic acid	Present	Absent

Fig. 2.1.3. Structure of gram-positive cell wall.



Cell Membrane

The plasma membrane is essential for the survival of the bacteria. It is 5–10 nm thick, and composed of bilayered phospholipid in which several proteins are embedded **(Figs. 2.1.2 to 2.1.4)**. The plasma membrane serves important functions in the bacterial cell such as:

- It is a *semipermeable membrane* that acts as an osmotic barrier; selectively allows particular ions, nutrients into the cell, and also helps in waste excretion

Fig. 2.1.4. Gram-negative cell wall.

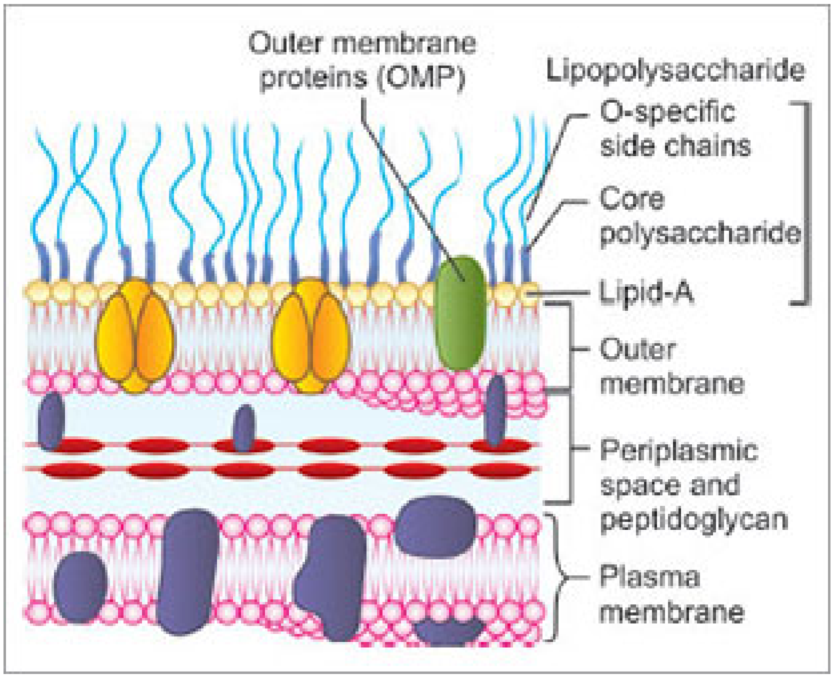
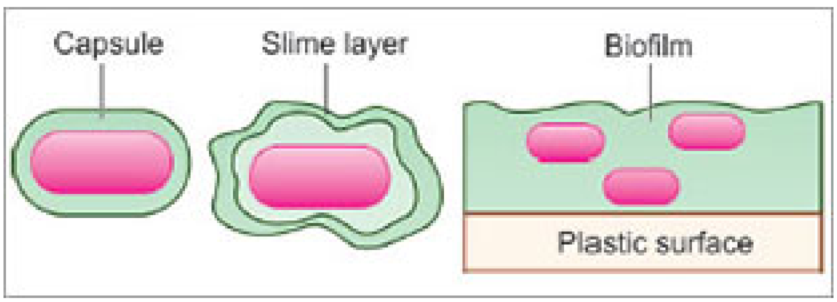


Fig. 2.1.5. Capsule, slime layer and biofilm.



- It acts as the site for a variety of crucial metabolic processes such as respiration, synthesis of lipids and cell wall, etc.

Cytoplasmic Matrix

Bacterial cytoplasm is mainly composed of water and is packed with the following structures.

- **Ribosomes:** They are the sites for protein synthesis; composed of rRNA and ribosomal proteins. Ribosomes are integrated with the mRNA and at this site, the genetic codons of the mRNA are translated into peptide sequences
- **Intracytoplasmic inclusions:** They are the storage sites of nutrients/energy present in some bacteria. They are formed by the bacteria under nutritional deficiency conditions. For examples, include glycogen granules and metachromatic granules
- **Mesosomes:** They are invaginations of the plasma membrane. They possess respiratory enzymes and are involved in bacterial respiration; are analogous to the mitochondria of eukaryotes
- **Nucleoid:** Bacteria do not have a true nucleus, but the genetic material is located in an irregularly shaped region called the nucleoid. There is no nuclear membrane or nucleolus

- Bacteria possess a single haploid chromosome, comprising circular double-stranded DNA
- Bacterial DNA divides by simple binary fission.
- Bacteria also possess extrachromosomal DNA called *plasmids*.

Cell Wall Appendages

Capsule and Slime Layer

Some bacteria possess a layer of amorphous viscid material lying outside the cell wall called *capsule*. When the capsule is in the form of unorganized loose material, it is called a *slime layer (Fig. 2.1.5)*.

Function/Uses

Most of the bacterial capsules are polysaccharide in nature—e.g. pneumococcus, meningococcus, *Haemophilus influenzae*, *Klebsiella pneumoniae*. In *Bacillus anthracis*, the capsule is polypeptide in nature.

The capsule has various functions as follows:

- **Bacterial virulence**: (i) Capsule protects the bacterium from phagocytosis and from the action of host cell lysozymes. (ii) It also helps in biofilm formation. Biofilm is an extracellular polysaccharide layer, which helps in bacterial adhesion to foreign body surfaces and thereby promotes diseases such as prosthetic valve endocarditis and catheter-associated urinary tract infections, etc.
- **Identification:** Capsular antigens can be used for the identification and typing of bacteria
- **Used as vaccine:** Capsular antigens of a few bacteria are used for vaccine preparation; e.g. pneumococcus, meningococcus, and *Haemophilus influenzae* serotype-b.

Demonstration of Capsule

Capsule can be detected by various methods as follows:

- **Negative staining** by India ink and nigrosin stain: Capsule appears as a clear refractile halo around the bacteria against a black background
- **M'Fadyean capsule stain:** It is used for demonstration of the capsule of *Bacillus anthracis* by using polychrome methylene blue stain
- **Quellung reaction** for demonstrating capsule in *Streptococcus pneumoniae* by adding capsular antisera mixed with methylene blue
- **Latex agglutination test:** Capsular antigens can be detected in the sample (e.g. CSF) by latex agglutination test by using specific anticapsular antibodies coated on latex particles. This is available for pneumococcus, *Haemophilus influenzae* and meningococcus.

Flagella

Flagella are thread-like appendages, protruding from the cell wall. They measure 5–20 μm in length and 0.01–0.02 μm in thickness.

- **Arrangement:** There are various patterns of arrangement of flagella with respect to the bacterial surface **(Figs. 2.1.6A to D)**:
    - Peritrichous (flagella distributed over the entire cell surface), e.g. *Escherichia coli*

- Monotrichous (single polar flagellum), e.g. *Vibrio cholerae*
- Lophotrichous (multiple polar flagella)
- Amphitrichous (single flagellum at both the ends).

- **Ultrastructure of flagella:** Flagellum is composed of three parts—(i) filament, (ii) basal body, and (iii) hook
- **Bacterial motility:** Flagella confer motility to the bacteria (organ of locomotion). Bacteria can produce characteristic type of motility which helps in their identification **(Table 2.1.4)**
- **Detection of flagella:** Flagella can be demonstrated by:
  - Direct demonstration by electron microscopy
  - Indirect means by demonstrating the motility by hanging drop method, dark ground or phase contrast microscopy.

Figs. 2.1.6A to D. Types of bacterial flagellar arrangement: *A.* Peritrichous; *B.* Monotrichous; *C.* Lophotrichous; *D.* Amphitrichous.

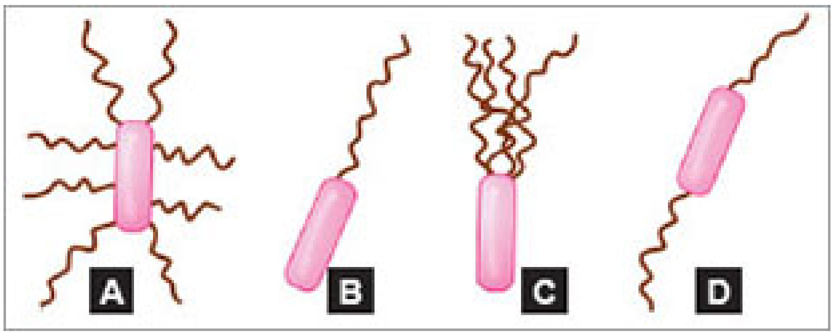


Table 2.1.4. Types of motility shown by different bacteria.

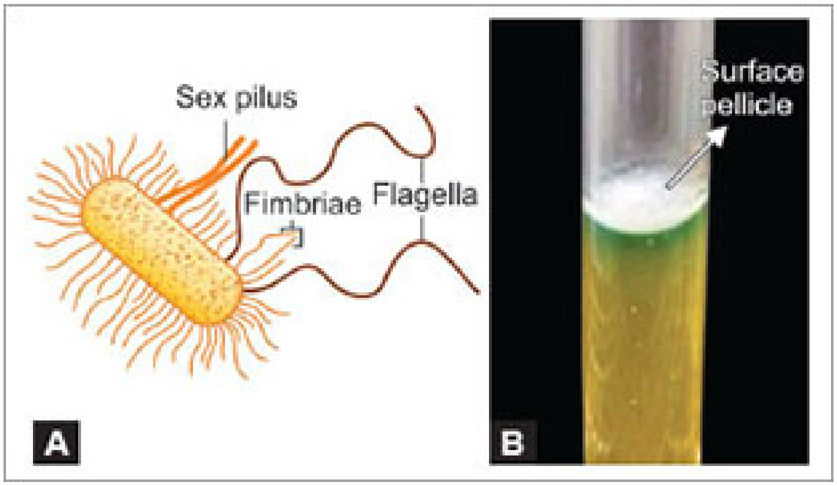
Types of motility	***Bacteria***
Tumbling motility	*Listeria*
Gliding motility	*Mycoplasma*
Stately motility	*Clostridium*
Darting motility	*Vibrio cholerae*
Swarming on agar plate	*Proteus*
Corkscrew motility	Spirochetes

Fimbriae or Pili

Fimbriae or pili are short, fine, hair-like appendages, smaller to flagella, but numerous in number. According to the functions, pili are of two types **(Fig. 2.1.7A)**.

1. **Common pili or fimbriae:** They help in *bacterial adhesion* to epithelial surfaces helping in colonization. They are present in gram-negative and some gram-positive bacteria
2. **Sex pili:** They help in bacterial conjugation by forming a conjugation tube through which the bacterial gene transfer takes place.

Figs. 2.1.7A and B. *A.* Differentiation between fimbriae, sex pilus and flagella; *B.* Surface pellicle (arrow showing).



Source: Department of Microbiology, JIPMER *(with permission)*.

They are only found in gram-negative bacteria.

Detection of fimbriae: Fimbriae can be detected either directly by electron microscope, or indirectly through the formation of the *surface pellicle*. It is a thin layer formed at the surface of liquid culture **(Fig. 2.1.7B)**.

L Form (Cell Wall Deficient Forms)

They are the cell wall deficient bacteria, discovered by E Klieneberger. She named it as L form after its place of discovery, i.e. Lister Institute, London (1935).

- When bacteria lose cell wall, they become spherical irrespective of their original shape. This may occur spontaneously or after exposure to lysozyme or cell wall-acting antibiotics such as penicillin
- L forms play a role in the persistence of pyelonephritis and other chronic infections
- Some bacteria like *Mycoplasma* lack cell wall permanently and has been suggested that they may represent stable L form.

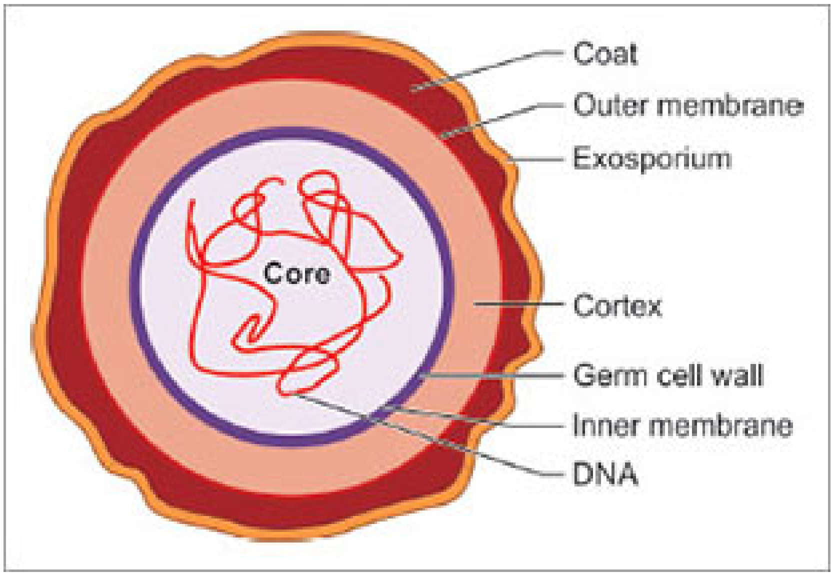
Bacterial Spores

Spores are highly resistant resting stage of the bacteria formed in unfavorable environmental conditions as a result of the depletion of exogenous nutrients.

Structure

The bacterial spore comprises several layers. From the innermost to the outermost, the layers are—core, cortex, coat, and exosporium **(Fig. 2.1.8)**.

Fig. 2.1.8. Structure of bacterial spore.



- The core is the innermost part containing the DNA material and is walled off from the cortex by an inner membrane and the germ cell wall
- Cortex and the coat layers lie external to the core and are separated from each other by an outer membrane
- The outermost layer is called the exosporium.

Sporulation

Sporulation refers to the process of formation of spores from the vegetative stage of bacteria. Sporulation commences when growth ceases due to a lack of nutrients. The mature spore formed is extremely resistant to heat and disinfectant.

Germination

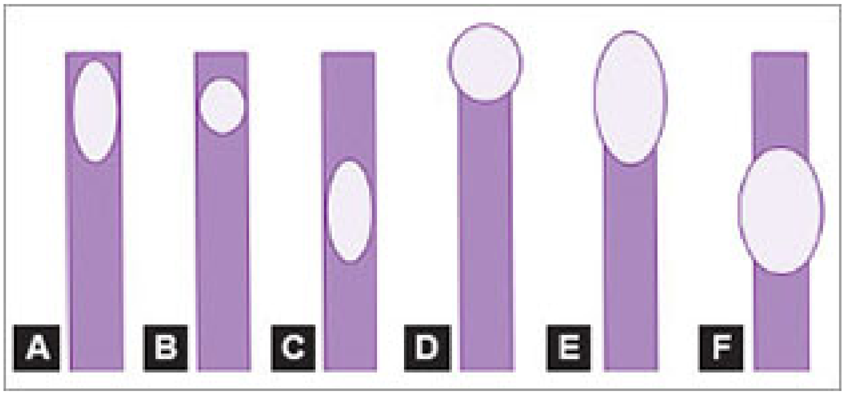
It is the transformation of dormant spores into active vegetative cells when grown in a nutrient-rich medium.

Shape and Position of Spores

For a given species, the position, shape, and relative size of the spore are constant.

- **Position:** Spores may be central, subterminal, or terminal **(Figs. 2.1.9A to F)**
- **Shape:** They may be oval or spherical
- **Width:** The diameter of the spore may be the same or less than the width of bacteria (non-bulging spore—e.g. as in *Bacillus*), or may be wider than the bacillary body producing a bulge in the cell (bulging spore, e.g. as in *Clostridium*).

Figs. 2.1.9A to F. Position and shape of spores: *A*. Non-bulging, oval and terminal; *B*. Non-bulging, round, and subterminal; *C*. Non-bulging, oval and central; *D*. Bulging, round and terminal; *E*. Bulging, oval and terminal; *F*. Bulging, oval, and central.



Sporicidal Agents

Spores are resistant to most of the routinely used disinfectants. Only limited agents called sterilants are capable of killing the spores, e.g. autoclave, ethylene oxide sterilizer, etc. (refer to Chapter 41).

Demonstration of Spores

- **Gram staining:** Spores appear as unstained refractile bodies within the cells
- **Modified Ziehl–Neelsen staining:** Spores are weakly acid-fast and appear red color when ZN staining is performed using 0.25–0.5% sulfuric acid as a decolorizer
- **Schaeffer–Fulton stain:** It is a special staining technique to demonstrate spores.

Applications

Spores of certain bacteria are employed as indicators of proper sterilization. Absence of the spores (inability to grow) after autoclaving or processing in hot air oven indicates proper sterilization.

- Spores of *Geobacillus stearothermophilus* are used as sterilization control for autoclave and plasma sterilizer
- Spores of *Bacillus atrophaeus* are used as sterilization control for hot air oven and ethylene oxide sterilizer.

PHYSIOLOGY OF BACTERIA

Bacterial Growth and Nutrition

Water constitutes about 80% of the total bacterial cell. The minimum nutritional requirements essential for the growth of bacteria include sources of carbon, nitrogen, hydrogen, oxygen, and small amounts of inorganic salts such as sulfur, phosphorus, and sodium, etc.

Some fastidious bacteria require additional growth factors called *bacterial vitamins*, which need to be added to the culture medium for their growth; e.g.—*Haemophilus influenzae* requires niacin.

Bacterial Cell Division

Bacteria divide by *binary fission*. The nuclear division precedes cytoplasmic division and then the two daughter cells get separated. In a few bacteria, the daughter cells may remain partially attached even after cell division; so that the bacterial cells are arranged in pairs or chains (e.g. streptococci) or clusters (e.g. staphylococci).

Rate of Multiplication in Bacteria

Generation time is the time required for a bacterium to give rise to two daughter cells under optimum conditions. The generation time for most of the important pathogenic bacteria (e.g. *Escherichia coli)* is 20 minutes. In *Mycobacterium tuberculosis,* it is about 10–15 hours and for *Mycobacterium leprae*, it is about 12–13 days.

Bacterial Count

The bacterial count may be expressed in terms of the total count and viable count. *The total count* indicates the total number of bacteria (live or dead) in the specimen. *Viable count* measures the number of living (viable) cells in the given specimen.

Bacterial Growth Curve

When a bacterium is inoculated into a suitable liquid culture medium and incubated, its growth follows a definite course. When the bacterial count of such culture is determined at different intervals and plotted in relation to time, a *bacterial growth curve* is obtained comprising of four phases **(Table 2.1.5 and Fig. 2.1.10)**.

1. **Lag phase:** It is the period between inoculation and the beginning of the multiplication of bacteria. After inoculating into a culture medium, bacteria do not start multiplying immediately but take some time to build up enzymes and metabolites

    - Bacteria increase in size due to the accumulation of enzymes and metabolites

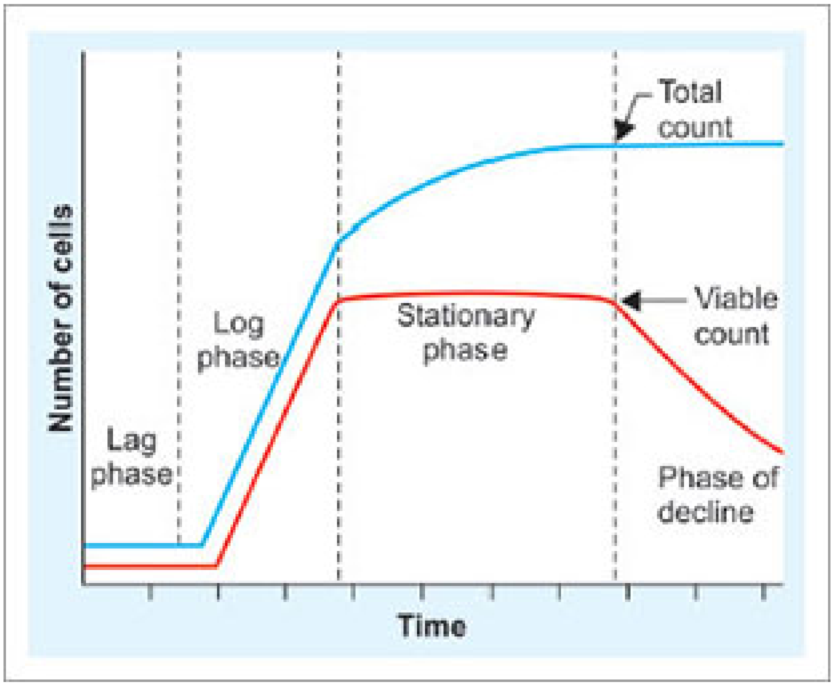
    - Bacteria reach their maximum size at the end of the lag phase.

2. **Log phase:** In this phase bacteria divide exponentially so that the growth curve takes a shape of a straight line.

Table 2.1.5. Various phases of bacterial growth curve.

	Lag	***Log***	***Stationary***	***Decline***
Bacteria divide	No	Yes	Yes	No
Bacterial death	No	No	Yes	Yes
Total count	Flat	Raises	Raises	Flat
Viable count	Flat	Raises	Flat	Falls
Special features	Accumulation of enzymes and metabolites Attains a maximum size	Uniformly stained Metabolically active Small size	Gram variable *Produce:* Granules, spores, exotoxin, bacteriocin	*Produce:* Involution forms

Fig. 2.1.10. Bacterial growth curve.



At this stage, the bacterium is:

- Smaller in size and biochemically active
- Uniformly stained: It is the best time to perform the Gram stain.

3. **Stationary phase:** After the log phase, the bacterial growth ceases almost completely due to exhaustion of nutrients, accumulation of toxic products, and autolytic enzymes

- The number of progeny cells formed is just enough to replace the number of cells that die
- Hence, the number of viable cells remains stationary as there is almost a balance between the dying cells and the newly formed cells. But the total count keeps rising. In this phase:
  - Bacterium becomes gram-variable
  - More storage granules are formed
  - Sporulation occurs in this phase
  - Bacteria produce exotoxins, antibiotics, and bacteriocins.

4. **Decline phase:** Gradually, the bacteria stop dividing completely; while the cell death continues due to exhaustion of nutrients, and accumulation of toxic products. Involution forms are seen in this phase. There is a decline in the viable count but not in total count.

Factors Affecting Growth of Bacteria

Several environmental factors affect the growth of the bacteria.

Oxygen

Based on their oxygen requirements bacteria are classified as:

- **Obligate aerobes:** They can grow only in the presence of oxygen (e.g. *Pseudomonas, Mycobacterium tuberculosis, Bacillus)*
- **Facultative anaerobes:** They are aerobes that can also grow anaerobically (e.g. most of the pathogenic bacteria, e.g. *Escherichia coli, Staphylococcus aureus*, etc.)
- **Microaerophilic bacteria:** They can grow in the presence of low oxygen tension, i.e. 5–10% of oxygen (e.g. *Campylobacter* and *Helicobacter)*
- **Obligate anaerobes:** These bacteria can grow only in absence of oxygen, as oxygen is lethal to them (e.g. *Clostridium tetani)*.

Temperature

Most of the pathogenic bacteria grow optimally at 37°C (i.e. human body temperature). However, the optimal temperature range varies with different bacterial species.

- **Psychrophiles**—grow best at temperatures below 20°C, e.g. *Pseudomonas*
- **Mesophiles**—grow within a temperature range 25°C and 40°C, e.g. most of the pathogenic bacteria
- **Thermophiles**—these bacteria grow at a high-temperature range of 55°C–80°C, e.g. *Geobacillus stearothermophilus.*

Other Important Factors Affecting Growth of Bacteria

- **Carbon dioxide:** Organisms that require higher amounts of carbon dioxide (5–10%) for growth are called *capnophilic bacteria.* Examples include *Brucella abortus, Streptococcus pneumoniae,* etc.
- **pH:** Most pathogenic bacteria grow between pH 7.2–and pH 7.6. Very few bacteria (e.g. lactobacilli) can grow at acidic pH and *Vibrio cholerae* are capable of growing at alkaline pH
- **Light:** Bacteria (except phototrophs) grow well in darkness. Photochromogenic mycobacteria produce pigments only on exposure to light
- **Moisture and desiccation:** Moisture is an essential requirement for the growth of bacteria because 80% of the bacterial cell consists of water. Some organisms like *Treponema pallidum* and *N. gonorrhoeae* die quickly after drying, while *M. tuberculosis* and *S. aureus* may survive drying for several weeks.

EXPECTED QUESTIONS

1. **I. Write an essay on:**

   Describe in detail the structure and function of the cell wall and cell membrane of gram-negative bacilli with the help of a diagram.

2. **II. Write short notes on:**

   1. Bacterial capsule.
   2. Bacterial growth curve.

3. **III. Multiple Choice Questions (MCQs):**

   1. **The cuneiform arrangement is characteristic of:**

a. *Staphylococcus*

b. *Streptococcus*

c. *Corynebacterium diphtheriae*

d. *Bacillus anthracis*

2. **A bacterial capsule can be best demonstrated by:**

a. Gram staining

b. Acid-fast staining

c. Negative staining

d. Albert staining

3. **The bacterial structure involved in motility is:**

a. Ribosome

b. Pili

c. Mesosome

d. Flagella

4. **Which of the following cocci–arrangement is wrong?**

a. Chain-*Streptococcus*

b. Pair-Pneumococcus

c. Chain-Gonococcus

d. Cluster-*Staphylococcus*

5. **Lipopolysaccharide is a component of cell wall of:**

a. Gram-positive bacteria

b. Gram-negative bacteria

c. Virus

d. Fungi

6. **Bacterial structure involved in respiration is:**

a. Ribosome

b. Pili

c. Mesosome

d. Flagella

Answers

1. c	**2.** c	**3.** d	**4.** c	**5.** b	**6.** c