

Textbook of
Applied
Microbiology
and Infection Control
Including Safety

As per the Revised Nursing Syllabus

I Clement



JAYPEE

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Chapter

General Characteristics of Microbes

Chapter Outline

- ◆ Structure and classification of microbes
- ◆ Morphological types
- ◆ Size and form of bacteria
- ◆ Motility
- ◆ Colonization
- ◆ Growth and nutrition of microbes
- ◆ Temperature
- ◆ Moisture
- ◆ Blood and body fluids
- ◆ Laboratory methods for identification of microorganisms
- ◆ Types of staining—simple, differential (Gram's, AFB), special-capsular staining (negative), spore, LPCB, KOH mount.
- ◆ Culture and media preparation: Solid and liquid
 - Types of media: Semi-synthetic, synthetic, enriched, enrichment, selective and differential media
 - Pure culture techniques: Tube dilution, pour, spread, streak plate
 - Anaerobic cultivation of bacteria

TERMINOLOGY

- **Asexual reproduction:** Reproduction in which sex cells are not involved; as by binary fission or budding.
- **Acute:** Having rapid onset, severe symptoms and a short course.
- **Antigen:** Foreign substance when gets into the body induces immune response.
- **Antibody:** Endogenous glycoprotein, which reacts with antigen.
- **Chronic:** Of long duration; denoting a disease with slow progression.
- **Disease:** Pathological condition of the body that presents with group of clinical symptoms and signs; and abnormal laboratory findings.
- **DNA:** A nucleic acid consisting of deoxyribose, phosphoric acid and bases. It is present in chromosomes of the nuclei of cells, is the chemical basis of heredity and the carrier of genetic information for living cells.
- **Endogenous:** Produced or originating from within a cell or organism.
- **Endoplasmic reticulum:** Net work of membranous tubules with in a cell and involved in transport of proteins synthesized on the ribosomes; and synthesis of lipids.
- **Electrolyte:** An ionized salt in blood, tissue fluids and cells.
- **Fastidious:** Requiring precise nutritional and environmental conditions for growth and survival.
- **Hematogenous:** Through the blood stream.
- **Histone:** Positively charged protein that is part of chromatin in eukaryotic cells.
- **Iatrogenic:** Any adverse mental or physical condition induced in a patient through the effects of treatment by a physician or surgeon.
- **Incubation period:** The time interval between exposure and development of disease.
- **Lysosome:** Cell organelle that is part of the intracellular digestive system.
- **Microscopic:** Cannot be observed with naked eye.
- **Macroscopic:** Can be observed with naked eye.
- **Microscope:** Optical instrument that greatly magnifies minute objects.
- **Microorganism:** Minute living body not seen with naked eye.
- **Mitochondria:** Oval shaped cell organelles that contain the enzymes for aerobic stages of cell respiration and thus the site of ATP synthesis.
- **Microtubule/Microfilament:** Tubular structures present in an eukaryotic cell and are important for maintaining rigidity; transporting substances in different directions with in a cell.
- **Nuclear membrane:** A membrane enveloping nucleus of a living cell.
- **Nucleolus:** Structure in the nucleus of a cell made of DNA, RNA, and protein. It is the site of synthesis of ribosomal RNA (rRNA).
- **Postulate:** A supposition or view, usually self-evident that is assumed without proof.

- **Primary stain:** The dye applied first in differential staining procedures.
- **Counter stain:** The dye which stains the microorganism or part of it after decolorization of the primary stain.
- **Mordant:** It is a substance which facilitates the reaction of the primary stain with the material to be stained. It combines with the stain and then facilitate the reaction. Basic mordant reacts with acidic stain and acidic mordant reacts with basic stain.
- **Decolorizer:** It is a chemical added in differential staining procedure to selectively remove the stain from the materials that are not intended to be stained.
- **Pathogen:** Organism that causes disease.
- **Virulence:** Degree of pathogenicity in causing disease which depends on toxin production and invasiveness.
- **Invasiveness:** The ability to penetrate in to the tissues, overcome the host defense, multiply and disseminate widely.
- **Toxicity:** The capacity to damage the tissues.
- **Opportunistic:** Normally harmless organism causing disease during lowered host resistance.
- **Infection:** The result of breakdown in the host-parasite relationship and follows when the balance is tipped in favor of the parasite.

INTRODUCTION

Microorganisms are minute, unicellular organisms that are not visible to the naked eye. The reason they are called microorganisms is that you can only see them when you use a microscope. These organisms make up around sixty percent of the earth's living matter. Microorganisms are organisms that can be harmful as well as helpful. Some microbes can cause severe infections like spoiling food and other items. At the same time, others play an essential role in maintaining an ecological balance. The biological characteristics of microorganisms can be summarized under the following categories—morphology, nutrition, physiology, reproduction and growth, metabolism, pathogenesis, antigenicity, and genetic properties.

STRUCTURE AND CLASSIFICATION OF MICROBES

All organisms that are very small or microscopic in size, and cannot be seen with the naked eye are referred to as microorganisms. Microorganisms are visible under the microscope. Anton van Leeuwenhoek first observed microorganisms under the microscope. Microorganisms include bacteria, archaea, algae, fungi, protozoa, etc. Although viruses are not considered living organisms, sometimes they are also included in the microorganism's category.

Classifications of Micro-organisms

Microorganisms are divided into seven types—bacteria, archaea, protozoa, algae, fungi, viruses, and multicellular animal parasites (helminthes). Each type has a characteristic cellular composition, morphology, mean of locomotion, and reproduction.

Bacteria

Bacteria are classified in the kingdom Monera. It includes Eubacteria and Archaeobacteria. They are all unicellular, have a prokaryotic cell which is devoid of a membrane-bound nucleus, and other organelles such as endoplasmic reticulum, mitochondria, Golgi bodies, etc. Later, archaeobacteria were placed into a different domain of the three-domain system, i.e., Bacteria, Archaea and Eukarya. Bacteria are the most abundant microorganisms and are present almost everywhere. They are classified as gram-positive and gram-negative, based on the Gram's staining pattern.

Based on the shape of the cell, bacteria are classified into four main groups that are as follows (Fig. 2.1):

1. Coccus or cocci (spherical)
2. Bacillus or bacilli (rod-shaped)
3. Spirillum or spirilla (spiral)
4. Vibrium or vibrio (comma-shaped)

Eubacteria (Fig. 2.2)

They are true bacteria. They have a rigid cell wall and may contain flagella. They are autotrophic as well as hetero-

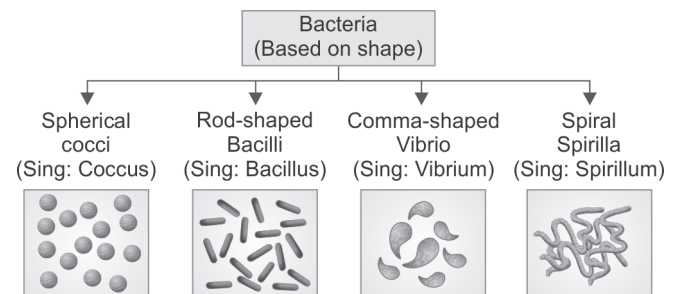


Fig. 2.1: Classification of bacteria based on shape.

1. Bacilli = rod-shaped
2. Cocci = Spherical-shaped
3. Spirilla = spiral and corkscrew-shaped



Fig. 2.2: Types of eubacteria.

trophic. Bacteria reproduce by binary fission and DNA transfer. Some bacteria produce spores under unfavorable conditions. *Mycoplasma* does not contain a cell wall.

- **Photosynthetic autotrophs:** This includes blue-green algae or cyanobacteria. They contain chlorophyll 'a' similar to green plants, and perform photosynthesis. Some cyanobacteria are also capable of fixing atmospheric nitrogen, e.g., *Nostoc* and *Anabaena*.
- **Chemosynthetic autotrophs:** These microorganisms utilize energy derived from the oxidation of inorganic substances such as nitrates, ammonia, sulphur, etc., and produce ATP. These organisms play an important role in nutrient recycling, e.g., purple sulphur bacteria.
- **Heterotrophs:** They are widely distributed and play a key role in the ecosystem as decomposers. They play a significant role in our lives. They are used for the industrial production of antibiotics, organic acids, etc. They also act as nitrogen-fixers, e.g., *Rhizobium* in the root nodules of legumes. Some bacteria are pathogenic to plants and animals causing various diseases, e.g., cholera, tuberculosis, typhoid, botulism, tetanus, citrus canker, fire blight of apple, etc.

Archaeobacteria

They can thrive in extreme environmental conditions. They have different cell wall compositions, which enable them to survive in harsh conditions. The cell membrane of archaea is ether-linked as compared to ester-linked in bacteria. They are further classified into three main groups:

1. **Methanogens:** They are found in marshy areas. They are found in the gut of many ruminating animals and are utilized for the commercial production of methane (biogas).
2. **Halophiles:** They are found in extreme salty areas.
3. **Thermoacidophiles:** They can tolerate extreme temperatures and low pH. They are found in hot springs.

Protozoa

In the Five Kingdom classification, protozoans are classified under the kingdom Protista, which includes

unicellular eukaryotes. Protozoans are heterotrophs. They live as parasites or predators.

On the basis of their mode of locomotion, they are classified into four major groups. They are (Fig. 2.3):

1. **Amoeboid:** They have pseudopodia, which are used for movement and capturing prey, e.g., *Amoeba*, *Entamoeba*, etc.
2. **Flagellated:** These protozoans are flagellated. They are either free-living or parasites, e.g., *Trypanosoma*, the causative organism of sleeping sickness, *Leishmania*, which causes kala-azar, etc.
3. **Ciliated:** They are aquatic and have thousands of cilia present on the body surface, e.g., *Paramecium*. Cilia also help in bringing water laden with food inside the cavity of the gullet.
4. **Sporozoans:** They are non-motile. They produce spores, e.g., *Plasmodium*, the causative organism of malaria, *Toxoplasma*, etc.

Fungi

Fungi are separated into a different kingdom. They are heterotrophic and have a rigid cell wall. They are parasites or saprotrophs. Fungi are microscopic, as well as quite big in size. They are cosmopolitan and grow in warm, humid places. A unicellular fungi—yeast, is used for the industrial production of bread and alcoholic beverages. Penicillium is used for the production of antibiotics.

Some fungi cause diseases in plants and animals, e.g., wheat rust (*Puccinia*), *Candida albicans* causing fungal infection in humans.

Fungi are classified into four main classes based on their morphology and method of spore formation. They are (Fig. 2.4):

1. **Phycomycetes:** They are characterized by the presence of coenocytic mycelium. Spores are produced endogenously in the sporangium, e.g., *Rhizopus*, *Mucor*, etc.
2. **Ascomycetes:** They are commonly known as sac-fungi. The mycelium is branched and septate. The asexual spores are produced exogenously on conidiophores

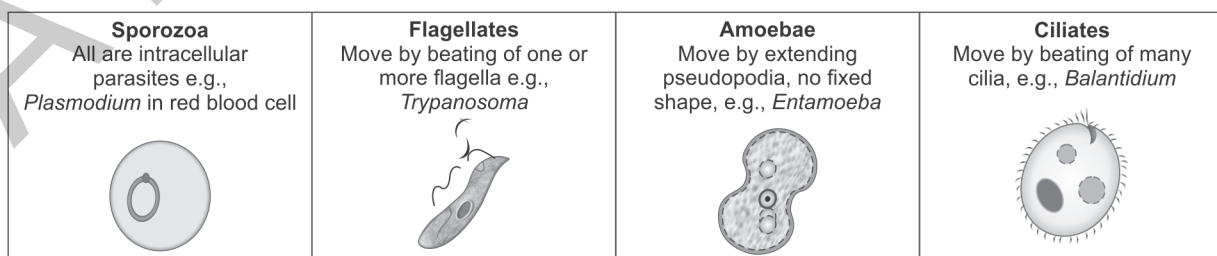


Fig. 2.3: The main divisions of protozoa.

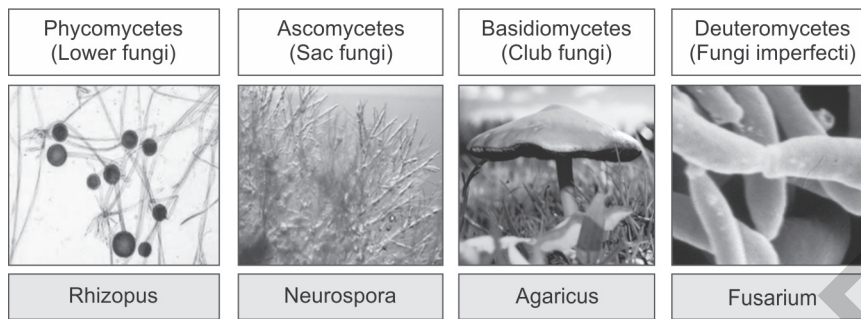


Fig. 2.4: Classification of fungi.

and sexual spores are produced endogenously within asci, e.g., *Penicillium*, *Saccharomyces* (yeast), *Aspergillus*, *Claviceps* and *Neurospora*, etc.

- Basidiomycetes:** Asexual spores are not formed. The basidiospores are exogenously produced, e.g., *Puccinia* (rust), mushrooms, *Ustilago* (smut), etc.
- Deuteromycetes:** Commonly called imperfect fungi due to absence of sexual stage in the life cycle. Most fungi are decomposers and help in nutrient recycling, e.g., *Colletotrichum*, *Alternaria* and *Trichoderma*.

Algae

Unicellular photosynthetic organisms like diatoms, golden algae and dinoflagellates are placed under the kingdom Protista. They are microscopic and are plankton. They are photosynthetic and chief producers in the sea.

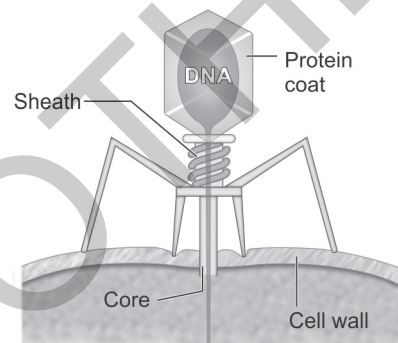
- **Diatoms:** They have a silicious cell wall which fits like a soap box. They are photosynthetic and 'diatomaceous earth' is used for polishing, filtration of oil, etc.
- **Dinoflagellates:** They are marine, and photosynthetic and appear in different colors. They have two unequal flagella, one longitudinal and one transverse. The cell wall has stiff cellulose plates. They are known to cause red tides due to rapid multiplication, e.g., *Gonyaulax* (a red dinoflagellate).

Virus

Viruses are tiny and smaller in its size, ranging between 30–50 nm. Viruses do not contain cells and usually lack a cell wall but are surrounded by a protective protein coating called the capsid. It can be seen as a genetic element and is characterized by the combined evolution of the virus and the host. They contain either RNA or DNA as the genetic material.

Classification of Viruses (Fig. 2.5)

- Viruses can be classified primarily on their phenotypic characteristics, core content, chemical composition,



vs

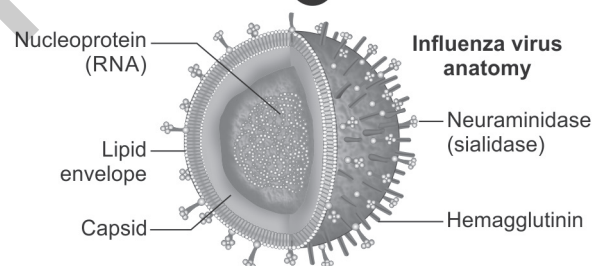


Fig. 2.5: Classification of viruses.

capsid structure, size, shape, modes of replication and other viral genome structures.

- The Baltimore classification is the most commonly used for studying the system of virus classification. This system was developed by an American biologist David Baltimore in the 1970s, for which he was awarded the Nobel Prize.

The below virus information describes the classification of viruses based on their different criteria.

- **Classification based on the presence of nucleic acid:**
 - DNA virus:** The virus, having DNA as its genetic material. There are two different types of DNA virus:
 1. *Single-stranded (ss) DNA virus:* Picornaviruses, Parvovirus, etc.
 2. *Double-stranded (ds) DNA virus:* Adenovirus, Herpes virus, etc.

RNA virus: The virus, having RNA as its genetic material.

There are two different types of RNA virus:

1. *Double-stranded (ds) RNA virus:* Reovirus, etc.
2. *Single-stranded (ss) RNA virus:* It is further classified into two Positive sense RNA (+RNA) and negative sense RNA (-RNA). Polioviruses, hepatitis A, rabies virus, influenza virus are examples of single-stranded RNA virus.

DIFFERENCES BETWEEN PROKARYOTES AND EUKARYOTES

See **Table 2.1**.

BACTERIAL STRUCTURE

It is a gel-like matrix composed of water, enzymes, nutrients, wastes, and gases and contains cell structures such as ribosomes, a chromosome, and plasmids. The cell envelope encases the cytoplasm and all its components. Unlike the eukaryotic (true) cells, bacteria do not have a membrane enclosed nucleus (**Fig. 2.6**).

Salient Features of Bacteria

Salient features of bacteria are the following:

- Unicellular prokaryotic cell
- Present in different shape, size and arrangement

Table 2.1: Differences between prokaryotes and eukaryotes.

Sl. No.	Character	Prokaryotes	Eukaryotes
1.	Term Origin	Greek for "primitive nucleus"	Greek for "true nucleus"
2.	Definition	Organisms made up of cell(s) that lack a cell nucleus or any membrane-encased organelles	Organisms made up of cells that possess a membrane-bound nucleus as well as membrane-bound organelles
3.	Major groups	Bacteria, Archae, and Bluegreen algae	Algae, fungi, protozoa, plants, animals
4.	Origin	Around 3.5 billion years ago.	Around 2 billion years ago
5.	Size (approximate)	0.5–3.0 μm	>5 μm
6.	Cell type	Usually unicellular (some cyanobacteria may be multicellular)	Usually multicellular
7.	Complexity	Simple	Complex organization.
8.	Nucleus location	Free in the cytoplasm, attached to mesosomes	Contained in membrane bound structure
9.	Nucleus membrane	No nuclear membrane	Classic membrane present
10.	Nucleolus	Absent	Present
11.	Chromosome number	One	More than one
12.	Chromosome shape	Circular	Linear
13.	Genes	Expressed in groups called operons.	Expressed individually
14.	Genome	DNA haploid genome	DNA diploid genome
15.	DNA base ratio (G+C %)	28–73	About 40
16.	DNA wrapping on proteins	Multiple proteins act together to fold and condense prokaryotic DNA. Folded DNA is then organized into a variety of conformations that are supercoiled and wound around tetramers of the HU protein	Eukaryotes wrap their DNA around proteins called histones
17.	Genome nature	Efficient and compact with little repetitive DNA	With large amounts of non-coding repetitive DNA
18.	Membrane-bound organelles	Absent	Present
19.	Ribosomes (sedimentation coefficient)	70S (50S + 30S) smaller	80S (60S + 40S) larger
20.	Ribosome's location	Free in cytoplasm or bound to cell membrane	Attached to rough endoplasmic reticulum
21.	Mitochondria	Absent	Present
22.	Golgi bodies	Absent	Present
23.	Endoplasmic reticulum	Absent	Present

Contd...

Contd..

Sl. No.	Character	Prokaryotes	Eukaryotes
24.	Mesosomes	Present. Performs the function of Golgi bodies and mitochondria and also help in the separation of chromosome during cell division	Absent
25.	Lysosomes	Absent	Present
26.	Peroxisomes	Absent	Present
27.	Chloroplasts	Absent; chlorophyll scattered in the cytoplasm	Present (in plants)
28.	Fimbriae	Prokaryotes may have pili and fimbriae (appendage that can be found on many gram-negative and some gram-positive bacteria)	Absent
29.	Microtubules	Absent or rare	Present
30.	Centrosome	Absent	Present except in flowering plants
31.	Cytoskeleton	May be absent	Present
32.	Glycocalyx	Present	Only in some
33.	Cytoplasmic streaming	Absent	Present
34.	Cytoplasmic membrane	Does not contain sterols (except <i>Mycoplasma</i>)	Contains sterols
35.	Cell wall	Complex structure containing protein, lipids, and peptidoglycans	Present for plant cells and fungi; otherwise absent
36.	Muramic acid	Present	Absent
37.	Movement	Simple flagellum , if present	Complex flagellum , if present
38.	Respiration	Via cytoplasmic membrane	Via mitochondria
39.	Energy production site	Electron transport chain located in the cell membrane	Within membrane bound mitochondria
40.	Metabolic rate	Higher due to larger surface area to volume ratio	Comparatively slow
41.	Reproduction	Asexual (binary fission)	Sexual and asexual/Mitotic division
42.	Generation time	Shorter	Comparatively longer
43.	Genetic recombination	Partial, unidirectional transfer	Meiosis and fusion of gametes
44.	Zygote	Merozygotic (partially diploid)	Diploid
45.	Extrachromosomal DNA	Plasmid	Inside the mitochondria
46.	DNA replication	Occurs in cytoplasm	Occurs in the nucleus
47.	Transcription and translation	Occurs simultaneously	Transcription occurs in nucleus and then translation occurs in cytoplasm

- The cell lack nucleus and membrane-bound cell organelles
- Bacterial DNA is found in the cytoplasm and not packaged to form chromatin as in eukaryotic cell
- Bacteria cell is 10 times smaller than the human cell
- The diameter of a bacteria cell is $\sim 1 \mu\text{m}$ (10^{-6} m)
- The outer covering of a bacteria cell is the cell wall, which is rigid and provides structural integrity
- The bacteria cell wall is made up of peptidoglycan or murein
- Different shapes of bacteria cell are the characteristic feature of a bacteria species
- Bacteria cells may contain external appendages like cilia, flagella, etc.
- Bacteria can be photoautotrophs, chemoautotrophs or parasites.

Structure of Bacterial Cell (Fig. 2.7)

1. Capsule

- Capsule is 0.2 μm thick viscus layer outer layer to the cell wall.
- Capsule is 98% water and 2% polysaccharide or glycoprotein/polypeptide or both.

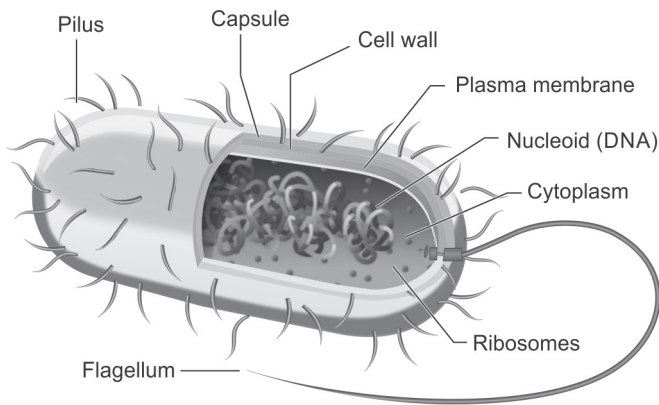


Fig. 2.6: Bacterial structure.

- There are two types of capsule.
 - a. **Macro-capsule:** Thickness of 0.2 μm or more, visible under light microscope.
 - b. **Microcapsule:** Thickness less than 0.2 μm , visible under electron microscope.
 - c. Capsule is very delicate structure. It can be removed by vigorous washing. Capsule is most important virulence factor of bacteria.

Function:

1. It helps in attachments as well as it prevents the cell from desiccation and drying.
2. Capsule resists phagocytosis by WBCs.

II. Flagella

- It is 15–20 nm hair like helical structure emerges from cell wall.

- Flagella are not straight but is helical. It is composed of flagellin protein (globular protein) and known as H antigen.
- Flagella have three parts. Basal body, hook and filament.

Function: It helps motility of the bacteria.

III. Pili or Fimbriae

- Pili are hollow filamentous and non-helical structure.
- They are numerous and shorter than flagella.
- Pili are the characteristic feature of gram -ve bacteria.
- Pili are composed of pilin protein.
- Bacteria containing pili: *Shigella*, *Proteus*, *Neisseria gonorrhoeae*, *E. coli*.

Function:

- **Attachment:** Pili help the bacteria to attach the host cell surface. Most of the human pathogens of respiratory tract, urinary tract are attached with the help of pili.
- Pili (fimbriae) possess antigenic property.
- **Specialized function:** Some pili are modified for specialized function, e.g., sex pilus (F-pili) helps in transfer of DNA from donor to recipient cell during conjugation.
- F-pili also act as receptor for bacteriophage.

IV. Sheath

- Some bacteria forming chain or trichome are enclosed by a hollow tube like structure known as sheath.
- Aquatic bacteria mostly form sheath.
- Some sheathed bacteria are; *Sphaerotilus*, *leptothrix*, *clonothrix*, etc.

Glycocalyx: A coating or layer of molecules external to the cell wall. It serves protective, adhesive, and receptor functions

Bacterial chromosome or nucleoid: The site where the large DNA molecule is condensed into a packet. DNA is the code that directs all genetics and heredity of the cell

Filus: An elongate, hollow appendage used in transfers of DNA to other cells and in cell adhesion

Mesosome: An extension of the cell membrane that folds into the cytoplasm and increases surface area

Flagellum: Specialized appendage attached to the cell by a basal body that holds a long rotating filament. The movement pushes the cell forward and provides motility

Fimbriae: Fine, hairlike bristles from the cell surface that helps in adhesion to other cells and surfaces

Inclusion/Granule: Stored nutrients such as fat, phosphate, or glycogen deposited in dense crystals or particles that can be tapped into when needed

Cell wall: A semirigid casing that provides structural support and shape for the cell

Cell membrane: A thin sheet of lipid and protein that surrounds the cytoplasm and controls the flow of materials into and out of the cell pool

Ribosomes: Tiny particles composed of protein and RNA that are the sites of protein synthesis

Fig. 2.7: Bacterial cell structure.

Function:

- Mechanical support.
- Sometime sheath is impregnated with ferric or manganese hydroxide which provide strength to sheath.

V. Prosthecae

- Prosthecae is semi-rigid extension of cell wall and cell membrane.
- One bacteria may contain one or many prosthecae.
- Some prosthecae develop bud at the tip and hence helps in reproduction.
- Some prosthecate bacteria are: *Caulibacter*, *Stella*, *Prosthecobacter*, and *Hyphomicrobium*.

Function:

- Prosthecae increase surface area for nutrition absorption. It is usually formed in bacteria living in very dilute environment where concentration of nutrition is low.
- Helps in adhesion.
- Asexual reproduction by budding.

VI. Stalk

- It is non-living ribbon like tubular structure.
- It is formed by excretory product of bacteria.
- Some stalked bacteria are: *Gallionella*, *Planctomyces*.

Function: Helps in attachment to solid surface.

VII. Cell Wall

- It is an important structure of bacteria.
- It gives shape to the organism.
- On the basis of cell wall composition, bacteria are classified into two major group, i.e., gram-positive and gram-negative.

Gram-positive cell wall: Cell wall composition of gram-positive bacteria.

- Peptidoglycan
- Lipid
- Teichoic acid

Gram-negative cell wall: Cell wall composition of gram-negative bacteria

- Peptidoglycan
- Outer membrane:
 - Lipid
 - Protein
 - Lipopolysaccharide (LPS)

Peptidoglycan:

- It consists of glycan backbone formed by repeated unit of NAG (n-acetyl glucosamine) and NAM (N-acetyl muramic acid) and the glycan backbone is cross linked by peptide bond.

- Peptidoglycan layer is present in cell wall of both grams positive as well as gram-negative bacteria. However, gram-positive have thick layer of peptidoglycan.

Teichoic acid:

- Teichoic acid is water soluble polymer of glycerol or ribitol phosphate present in gram-positive bacteria.
- It constitutes about 50% of dry weight of cell wall.
- It is the major surface antigen of gram-positive bacteria

Outer membrane:

- It is an additional layer present in gram-negative bacteria.
- It is composed of lipid bilayer, protein and lipopolysaccharide (LPS).

LPS:

- LPS is composed of lipid-A and polysaccharide.
- **Lipid-A:** It is phosphorylated glucosamine disaccharide. It is antigenic.
- **Polysaccharide:** It consists of core-polysaccharide and O-polysaccharide.

VIII. Cell Membrane

- Cell membrane is the inner layer that lies inside the cell wall and encloses the cytoplasm.
- It is also known as cytoplasmic membrane or plasma membrane.
- It is about 80 nm thick.
- Cell membrane of bacteria is composed of phospholipid and proteins.

Function: It is selectively permeable as it allows passing selective substances such as sugar, amino acids across it.

XI. Nucleus

- Nucleus is the most important part of the cell.
- It controls and directs all the cellular activities and stores hereditary information of cell.
- Bacterial nucleus is known as nucleoid; it lacks nuclear membrane, nucleoplasm and nucleolus.
- Bacterial DNA is naked (lacked histone protein).

Function:

- It contains and stores hereditary information of the cell.
- It controls all cell activities.

X. Ribosome

- Bacterial ribosome is of 70s type.
- Ribosomes are rounded granules found freely floating in the cytoplasm.
- Ribosomes are known as universal cell organelle because it is found in both bacterial cell and eukaryotic cell.

- Chemically the ribosomes are made up of nucleic acids (particularly RNA and proteins).

Function: It helps in protein synthesis.

XI. Mesosome

Mesosome is a spherical or round sac like structure found commonly in gram-positive bacteria.

Function: It is the site for respiration in bacterial cell

XII. Cytoplasm

- It is colorless, viscous fluid present inside cell membrane.
- All the cell organelles and inclusions are found floating in cytoplasmic fluid.
- It contains proteins, lipid, minerals, nucleic acids, glycogen, water etc.

Function:

- It helps to distribute water, oxygen as other substances throughout the cell.
- Literally, the entire cellular content including nucleus and other cell organelle are floating in cytoplasm.

XIII. Spores (Endospore)

- Spore is metabolically dormant structure produced during unfavorable condition by the process called sporulation.
- Sporulation occurs during late log phase or early stationary phase.
- Under favorable condition spores germinate to give vegetative cell.

CLASSIFICATIONS OF BACTERIA

I. Size of Bacteria

The size of bacteria can be averaged to 2 μm with a diameter of 0.5 μm . Bacteria are known to display a wide variety of size and shapes. They are about one-tenth the size of a eukaryotic cell. Different shapes of bacteria are used to categorize bacteria. Different shapes of a bacterial cell are:

- **Spherical:** Cocci
- **Rod-shaped:** Bacilli
- Spiral bacteria
- **Comma shaped:** Vibrio

Spherical: Cocci

Cocci can be single or multiple in a group of 2, 4, 8, etc. Cocci bacteria can be round, oval or elongated or bean-shaped.

- The cell wall of coccus shaped bacteria may be gram-positive with thick peptidoglycan layer, or gram-negative with the thin peptidoglycan cell wall.

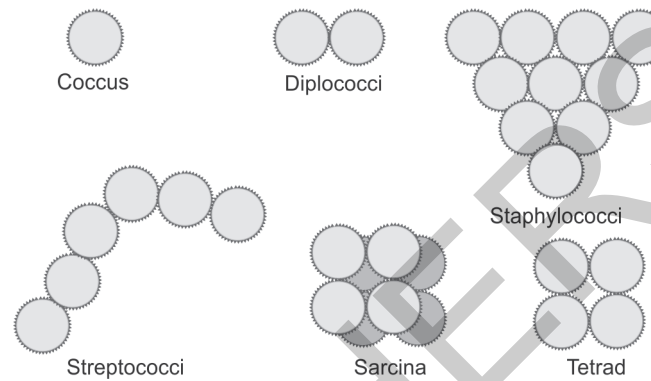


Fig. 2.8: Arrangements of cocci.

- The cells do not separate after cell division. The plane of cell division changes the shape of the bacteria.

According to the arrangement of cells, cocci bacteria are subdivided into various subtypes (**Fig. 2.8**):

- **Monococcus:** Bacteria exist as a single spherical cell.
- **Diplococcus:** Cells are arranged in pairs after cell division. Examples of diplococcus bacteria are: Gram-negative bacteria—*Neisseria* spp, *Moraxella catarrhalis*; Gram-positive bacteria—*Streptococcus pneumoniae*, *Enterococcus* spp, etc.
- **Streptococcus:** The cocci are joined in a plane and arranged in a chain pattern. These are non-motile, aerobic and gram-positive bacteria that cause many diseases. Examples of streptococci are *Streptococcus mutans*, *Streptococcus pyogenes*, *Streptococcus bovis*, *Streptococcus agalactiae*, etc.
- **Tetrads:** Tetrads are arranged in a group of 4 cells. The cell division occurs in two different planes. Examples of tetrads cocci bacteria are *Micrococcus* spp, *Pediococcus*, etc.
- **Staphylococcus:** Cells are arranged in an irregular cluster, which looks like grapes. This is due to the division in three planes. Examples of *Staphylococcus* are *Staphylococcus aureus*, *Staphylococcus*, *Haemolyticus*, etc.
- **Sarcinae:** Sarcinae bacteria are anaerobic gram-positive bacteria. They occur as a group of 8 cells. It is found in the family Clostridiaceae. It is found in the large intestine and skin. Examples of sarcinae are: *Clostridium maximum*, *Sarcina aurantiaca*, *Sarcina ventriculi*, *Clostridium maximum*, *Micrococcus luteus*, etc.

Rod-shaped Bacilli

These are rod-shaped bacteria. They also occur singly or as multiple cells attached after the cell division.

- Bacilliform bacteria are found in many different taxonomic groups of bacteria.
- Bacilliform bacteria can be gram-positive, e.g., *Actinomyces*, *Clostridium*, *Bacillus* or gram-negative bacteria, e.g., *Escherichia*, *Klebsiella*, *Salmonella*, *Streptobacillus*, etc.
- Bacteria of genus *Bacillus* are gram-positive, rod-shaped bacteria:
 - They are obligate aerobe or facultative anaerobic bacteria
 - They can form endospores and live for years as dormant and resistant to heat, radiation and disinfectants
 - They can survive extreme heat and temp as high as 420°C
 - They are the most abundant bacteria and found everywhere
 - Mostly non-parasitic, free-living species
 - Two parasitic and pathogenic bacillus species are *Bacillus anthracis* causing anthrax and *Bacillus cereus*, which causes food poisoning.

Based on the arrangement of rod shaped cells, bacilli are classified into various types (Fig. 2.9):

- **Bacillus:** Single unattached cell, that looks like a rod. Examples are *Bacillus cereus*, *Salmonella enterica*, etc.
- **Diplobacilli:** Two rods are attached to each other and found in pairs after cell division. Examples are *Moraxella bovis*, *Klebsiella rhinoscleromatis*, etc.
- **Streptobacilli:** Due to cell division in one plane, bacilli are arranged in a chain. Genus *Streptobacillus* contains gram-negative, aerobic or facultative anaerobic bacteria. Examples are *Streptobacillus moniliformis*, *Streptobacillus felis*, etc.
- **Coccobacilli:** These are short compared to other bacilli and oval in shape, they appear like a coccus. Examples are *Chlamydia trachomatis*, *Haemophilus influenzae*, *Gardnerella vaginalis*, etc.

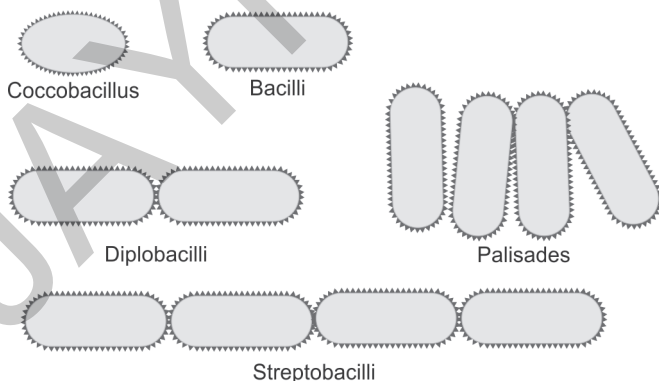


Fig. 2.9: Arrangement of bacilli.

- **Palisades:** The bacilli after cell division bend and therefore arranged in a palisade, fence-like structure. Example: *Corynebacterium diphtheriae*.

Spiral Bacteria

These bacteria are spiral or helical in shape (Fig. 2.10).

Based on the thickness, flexibility and motility of the cell, they are further divided into two types:

1. **Spirillum:** These are gram-negative, rigid bacteria having external flagella. Examples are *Spirillum*, *Campylobacter jejuni*, *Helicobacter pylori*, etc.
2. **Spirochete:** These bacteria are spiral, thin and flexible. They have internal periplasmic flagella. These are pathogenic species that cause various serious diseases. Examples are *Leptospira*, *Treponema pallidum*, etc.

Comma-shaped Vibrio

These are curved and appear like a comma.

- These are mostly gram-negative bacteria
 - They are known to cause various food borne diseases
 - *Vibrio* sp is facultative anaerobes and has 2 chromosomes which replicate independently.
- Examples are *Vibrio cholerae*, *Vibrio parahaemolyticus*, etc.

Other than the main four shapes, the bacterial cell possess various different morphology namely:

1. **Filamentous:** They have a long filamentous structure (Fig. 2.11). Example: *Candidatus savagella*.
2. **Star-shaped:** It resembles a star. Example: *Stella humosa*, *Stella vacuolata*.
3. **Rectangular:** These are box or rectangular shaped. Example: halophilic bacteria such as *Haloarcula vallismortis*.
4. **Pleomorphic:** these have ability to change their shape and size due to various external factors like pressure,

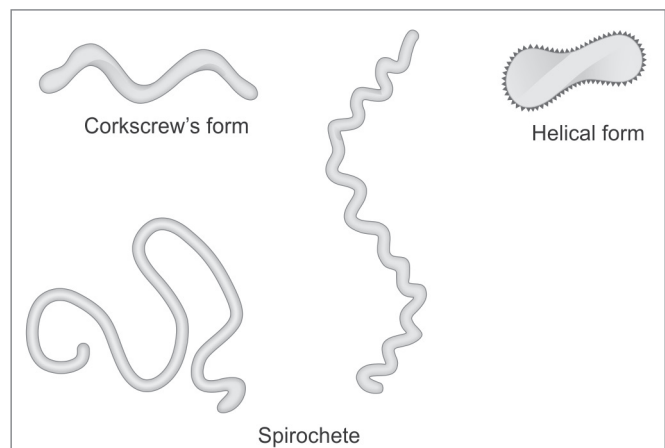


Fig. 2.10: Spiral bacterias.

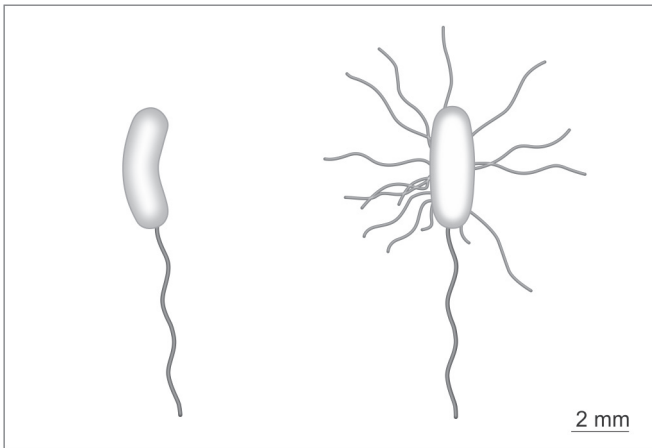


Fig. 2.11: Filamentous structure of bacteria.

environmental stress, etc. Example: *Mycoplasma pneumoniae*, *Mycoplasma genitalium*.

5. **Appendaged:** They are also known as budding bacteria. They can be non-motile or motile with flagella. Example: *Hyphomicrobium*, *Rhodomicrobium*.
6. **Trichome:** Trichomes are a chain of vegetative cell. This is often covered with slimy sheath as in cyanobacteria. Example: *Thiothrix nivea*.
7. **Lobed:** These have a lobed structure and mostly found in hot and volcanic springs. They are flagellated and have an irregular shape. They are acidophiles and thermophiles. Example: *Sulfolobus acidocaldarius*, *Sulfolobus solfataricus*.
8. **Fusiform:** These are spindle-shaped, bulged in the middle and the end is tapered. Example: *Fusobacterium necrophorum*.
9. **Stalked:** A stalk develops at one end of bacteria due to asymmetrical cell division. Example: *Caulobacter crescentus*.
10. **Sheathed:** Bacteria cell is covered with sheath. They are found in water. Example: *Leptothrix*, *Clonothrix*.

II. Mode of Nutrition

Phototrophs

- Those bacteria which gain energy from light.
- Phototrophs are further divided into two groups on the basis of source of electron:
 1. **Photolithotrophs:** These bacteria gain energy from light and uses reduced inorganic compounds such as H₂S as electron source, e.g., *Chromatium okenii*.
 2. **Photoorganotrophs:** These bacteria gain energy from light and uses organic compounds such as succinate as electron source.

Chemotrophs

- Those bacteria gain energy from chemical compounds.
- They cannot carry out photosynthesis.
- Chemotrophs are further divided into two groups on the basis of source of electron.
 1. **Chemolithotrophs:** They gain energy from oxidation of chemical compound and reduce inorganic compounds such as NH₃ as electron source, e.g., *Nitrosomonas*.
 2. **Chemoorganotrophs:** They gain energy from chemical compounds and uses organic compound such as glucose and amino acids as source of electron, e.g., *Pseudomonas pseudoflava*.

Autotrophs

- Those bacteria which uses carbon dioxide as sole source of carbon to prepare its own food.
- Autotrophs are divided into two types on the basis of energy utilized to assimilate carbon dioxide, i.e., photoautotrophs and chemoautotrophs.
 1. **Photoautotrophs:** They utilized light to assimilate CO₂. They are further divided into two groups on the basis of electron sources, i.e., photolithotropic autotrophs and photoorganotropic autotrophs.
 2. **Chemoautotrophs:** They utilize chemical energy for assimilation of CO₂.

Heterotrophs

- Those bacteria which uses organic compound as carbon source.
- They lack the ability to fix CO₂.
- Most of the human pathogenic bacteria are heterotrophic in nature.
- Some heterotrophs are simple, because they have simple nutritional requirement. However, there are some bacteria that require special nutrients for their growth; known as fastidious heterotrophs.

III. Basis of Temperature Requirement

Bacteria can be classified into the following major types on the basis of their temperatures response as indicated below:

Psychrophiles

- Bacteria that can grow at 0°C or below but the optimum temperature of growth is 15°C or below and maximum temperature is 20°C are called psychrophiles.
- Psychrophiles have polyunsaturated fatty acids in their cell membrane which gives fluid nature to the cell membrane even at lower temperature.

Examples: *Vibrio psychroerythrus*, *Vibrio marinus*, *Polaromonas vaculata*, *Psychroflexus*.

Psychrotrophs (Facultative Psychrophiles)

Those bacteria that can grow even at 0°C but optimum temperature for growth is (20–30)°C.

Mesophiles

- Those bacteria that can grow best between (25–40)°C but optimum temperature for growth is 37°C.
- Most of the human pathogens are mesophilic in nature.
- Examples: *E. coli*, *Salmonella*, *Klebsiella*, *Staphylococcus*.

Thermophiles

- Those bacteria that can best grow above 45°C.
- Thermophiles capable of growing in mesophilic range are called facultative thermophiles.
- True thermophiles are called as Stenothermophiles, they are obligate thermophiles.
- Thermophiles contains saturated fatty acids in their cell membrane so their cell membrane does not become too fluid even at higher temperature.
- Examples: *Streptococcus thermophilus*, *Bacillus stearothermophilus*, *Thermus aquaticus*.

Hyperthermophiles

- Those bacteria that have optimum temperature of growth above 80°C.
- Hyperthermophiles are often isolated from areas, such as deep geothermal heated oil-containing stratifications, shallow coastal and deep-water hot sediments, hydrothermal vents, and 4,000 m below the sea level.
- Examples: *Thermodesulfobacterium*, *Aquifex*, *Pyrolobus fumari*, *Thermotoga*.

IV. Oxygen Requirement

Obligate Aerobes

- Require oxygen to live.
- Example: *Pseudomonas*, common nosocomial pathogen.

Facultative Anaerobes

- Can use oxygen, but can grow in its absence.
- They have complex set of enzymes.
- Examples: *E. coli*, *Staphylococcus*, yeasts, and many intestinal bacteria.

Obligate Anaerobes

- Cannot use oxygen and are harmed by the presence of toxic forms of oxygen.

- Examples: *Clostridium* bacteria that cause tetanus and botulism.

Aerotolerant Anaerobes

- Cannot use oxygen, but tolerate its presence.
- Can break down toxic forms of oxygen.
- Example: *Lactobacillus* carries out fermentation regardless of oxygen presence.

Microaerophiles

- Require oxygen, but at low concentrations.
- Sensitive to toxic forms of oxygen.
- Example: *Campylobacter*.

V. Basis of pH of Growth

Acidophiles

- These bacteria grow best at an acidic pH.
- The cytoplasm of these bacteria is acidic in nature.
- Some acidophiles are thermophilic in nature, such bacteria are called Thermoacidophiles.
- Examples: *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*, *Thermoplasma*, *Sulfolobus*.

Alkaliphiles

- These bacteria grow best at an alkaline pH.
- Example: *Vibrio cholerae* optimum pH of growth is 8.2.

Neutrophiles

- These bacteria grow best at neutral pH (6.5–7.5).
- Most of the bacteria grow at neutral pH.
- Example: *E. coli*.

VI. Basis of Osmotic Pressure Requirement

Halophiles

- Require moderate to large salt concentrations.
- Cell membrane of halophilic bacteria is made up of glycoprotein with high content of negatively charged glutamic acid and aspartic acids. So high concentration of Na⁺ ion concentration is required to shield the –ve charge.
- Ocean water contains 3.5% salt. Most such bacteria are present in the oceans.
- *Archaeobacteria*, *Halobacterium*, *Halococcus*.

Extreme or Obligate Halophiles

- Require very high salt concentrations (20 to 30%).
- Bacteria in dead sea, brine vats.

Facultative Halophiles

Do not require high salt concentrations for growth, but tolerate up to 2% salt or more.

Textbook of Applied Microbiology and Infection Control Including Safety

Salient Features

- A thoroughly revised and updated book as per the revised Indian Nursing Council (INC) syllabus 2021–2022.
- Covers both applied microbiology and infection control including safety, as BSc Nursing syllabus.
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