A Text Book Of

PHARMACEUTICAL MICROBIOLOGY

As Per PCI Regulations

SECOND YEAR B. PHARM.
Semester III

Dr. S. B. Bhise
Formerly, Principal,
Govt. College of Pharmacy
KARAD.

Mrs. M. S. Bhise
Ex. Hon. Lecturer,
Government College of Pharmacy
KARAD.

Dr. Lalit Lata Jha
Principal, School of Pharmacy,
Parul University, Gujarat

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Abhyudaya Pragati, 1312, Shivaji Nagar,
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Email : niralipune@pragationline.com

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Preface

Pharmacy Council of India, New Delhi, the apex regulatory body controlling Pharmacy Profession in the country has come out with the regulations viz. Bachelor of Pharmacy (B. Pharm) course regulations 2014 for B. Pharm degree program based on CBSC pattern. Pharmaceutical Microbiology has been included in the course of study for Semester III. This course has been designed to provide basic knowledge required to understand the science of Microbiology with special reference to Pharmacy.

The subject of Microbiology has relevance to pharmaceutical sciences in two areas: Medical Microbiology as related to Pathogens of Diseases and Industrial Microbiology as related to antibiotics. The book has been divided into five chapters.

First chapter deals with basic principles of microbiology including history, scope, importance and structural features of microbes along with microscopy.

Second chapter deals with staining techniques. It also deals with principles and procedures for sterilization. In addition, it discusses equipments used for sterilization and indicators used to test sterilization.

Third chapter deals with fungi and viruses. It includes discussion about classification of and factors related to disinfectants and antiseptics. It highlights on evaluation of bacteriostatic/bacteriocidal agents and sterility testing of various products.

Fourth chapter discusses about aseptic area and microbial bioassays. It outlines environmental cleanliness.

Fifth chapter provides information on spoilage and preservation of pharmaceutical products. It provides basic information on cell-culture.

Being a basic subject of Medical Sciences large number of books are available on this subject that give minute details in voluminous form. We felt it necessary to scan the voluminous information and gather the relevant information that may cater to the needs of students of second year Bachelor of Pharmacy.

Our sincere thanks to Shri. D. K. Furia, Publisher, Prof. S. B. Gokhale and the staff members of Nirali Prakashan including Mr. Malik Shaikh, Mrs. Anjali Muley, Varsha Bodke, Sameer and Roshan Khan for timely publishing the book.

Dr. S. B. Bhise

Mrs. M. S. Bhise

Dr. Lalit Lata Jha

Syllabus

Unit I **10 Hours**

Introduction, history of microbiology, its branches, scope and its importance.

Introduction to Prokaryots and Eukaryots.

Study of ultra-structure and morphological classification of bacteria.

Nutritional requirements, raw materials used for culture media and physical parameters for growth, growth curve, isolation and preservation methods for pure cultures, cultivation of anaerobes, and quantitative measurement of bacterial growth (total and viable count).

Study of different types of phase contrast microscopy, dark field microscopy and electron microscopy.

Unit II **10 Hours**

Identification of bacteria using staining techniques (Simple, Gram's and Acid-fast staining) and biochemical tests (IMViC).

Study of principle, procedure, merits, demerits and applications of physical, chemical and mechanical methods of sterilization.

Evaluation of the efficiency of sterilization methods.

Equipments employed in large scale sterilization.

Sterility indicators.

Unit III **10 Hours**

Study of morphology, classification, reproduction/replication and cultivation of Fungi and Virus.

Classification and mode of action of disinfectants.

Factors influencing disinfection, antiseptics and their evaluation for bacteriostatic and bactericidal actions.

Evaluation of bacteriostatic and bactericidal agents.

Sterility testing of products (solids, liquids, ophthalmic and other sterile products) according to IP, BP and USP.

Unit IV **08 Hours**

Designing of aseptic area, laminar flow equipments; study of different sources of contamination in an aseptic area and methods of prevention, clean area classification.

Principles and methods of different microbiological assay. Methods for standardisation of antibiotics, vitamins and amino acids.

Assessment of a new antibiotic and testing of anti-microbial activity of a new substance.

General aspects- environmental cleanliness.

Unit V **07 Hours**

Types of spoilage, factors affecting the microbial spoilage of pharmaceutical products, sources and types of microbial contaminants, assessment of microbial contamination and spoilage.

Preservation of pharmaceutical products using antimicrobial agents, evaluation of microbial stability of formulations.

Growth of animal cells in culture, general procedure for cell culture; primary, established and transformed cell cultures.

Application of cell cultures in pharmaceutical industry and research.

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Chapter ... 1

BASICS OF MICROBIOLOGY

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to

- Understand history, branches and scope of microbiology
- Understand background about prokaryotes and eukaryotes
- Understand ultrastructure and classification of bacteria
- Understand nutrition and patterns of growth
- Understand isolation and preservation methods of pure culture
- Understand different types of microscopies

1.1 INTRODUCTION

The word microbiology originates from three Greek words viz. micros, bios and logia. Micros means small; bios means life and logia means study. Thus microbiology is the study of microorganisms, which are unicellular (single cell), multi-cellular (cell colony), or acellular (lacking cells). Microbiology encompasses numerous sub-disciplines like virology, parasitology, mycology and bacteriology. Virology is the study of viruses which are sub-microscopic, parasitic particles of genetic material contained in a protein coat and virus-like agents. Parasitology is the study of parasites, their hosts and the relationship between them. Mycology is the branch of microbiology related to fungi. Bacteriology deals with morphology, ecology, genetics and biochemistry of bacteria.

1.1.1 History of Microbiology

English scientist, Robert Hooke (1635-1703) made first observations about fungi among the specimens of cells which he viewed in mid 1600s. Thereafter a Dutch merchant, Anton van Leeuwenhoek (1632-1723) made careful observations about microorganisms, which he called animalcules. He pioneered the use of simple single-lensed microscope of his own design. Until his death in 1723, he revealed microscopic world to provide accurate descriptions of protozoa, fungi and bacteria. Athanasius Kircher (1602-1680) was the first to observe microorganisms. Later, scientists debated the theory of spontaneous generation, which stated that microorganisms arise from lifeless matter such as beef broth. The theory

was disputed by Francesco Redi (1626-1697) and further by Lazzaro Spallanzani (1729-1799), showing that boiled broth does not give rise to microscopic forms of life.

Louis Pasteur (1822-1895) in late 1800s performed numerous experiments to discover why wine and dairy products became sour and he found that bacteria were responsible for it. Pasteur disproved spontaneous generation of life. His work proved that microorganisms were in the air and can cause disease. Pasteur postulated germ theory of disease, which states that microorganisms are the causes of infectious disease.

German scientist, Robert Koch (1843-1910) provided proof to Pasteur's postulates by cultivating anthrax bacilli and few other types of microorganisms. He then injected pure cultures of anthrax bacilli into mice and showed that the bacilli caused anthrax. He then proposed Koch's postulates which provided a set of principles where by other microorganisms could be related to different diseases.

In late 1800s and first decade of 1900s, the germ theory of diseases was further consolidated. This period is called as Golden age of microbiology during which many agents of different infectious diseases were identified. Many of the etiologic agents of microbial disease were discovered leading to the ability to halt epidemics by interrupting spread of microorganisms.

Inspite of these advances in microbiology, it was rarely possible to render life saving therapy to an infected patient. World's first antibiotic substance benzyl penicillin (Penicillin G) from the mould, *Penicillium notatum* was discovered by Alexander Fleming (1881-1955) in 1928. After World War II, antibiotics were introduced to medicine. The incidence of pneumonia, tuberculosis, meningitis, syphilis and many other diseases declined with the use of antibiotics.

Martinus Beijerinck (1851-1931) is considered founder of virology and environmental microbiology. He is credited with conceptual discovery of tobacco mosaic virus, enrichment culture, nitrogen- fixing/sulphate reducing bacteria. His discovery identified azotobacter and rhizobium bacteria.

However, work with viruses could not be effectively performed till development of electron microscope in 1940s. In that decade, knowledge of viruses developed rapidly. In 1950s and 1960s, vaccines were developed for viral diseases like polio, measles, mumps and rubella.

Modern microbiology has reached many areas like pharmaceutical products, use of quality control methods in food and dairy products, control of disease-causing microorganisms in consumable water and the industrial application of microorganisms. Microorganisms are now used to produce vitamins, amino acids, enzymes and growth supplements. They are useful in manufacture of many foods including fermented dairy products like sour cream, yoghurt and butter milk. They are also used in fermented foods like pickles, breads and alcoholic beverages.

One of the major areas of applied microbiology is biotechnology. In this discipline, genes responsible for production of a protein are identified, separated and transferred to a suitable microorganism. The technique is called as genetic engineering. The suitable microorganism is now cultivated to produce proteins in large quantity. The technique was first industrialised for manufacture of insulin in 1960s.

1.1.2 Branches of Microbiology

The branches of microbiology can be classified into pure and applied sciences.

The branches of pure microbiology are as follows:

- **Bacteriology:** The study of bacteria.
- **Mycology:** The study of fungi.
- **Protozoology:** The study of protozoa.
- **Phycology (Algology):** The study of algae.
- **Parasitology:** The study of parasites.
- **Immunology:** The study of immune system.
- **Virology:** The study of viruses.
- **Nematology:** The study of nematodes

The branches of applied microbiology are as follows:

- **Medical microbiology:** The study of pathogenic microbes.
- **Pharmaceutical microbiology:** The study of microorganisms producing pharmaceutical products.
- **Industrial microbiology:** The study of microbes used for industrial processes.
- **Microbial biotechnology:** The study of microbial manipulation at genetic/molecular level.
- **Food microbiology and dairy microbiology:** The study of microbes causing food spoilage, food borne illness and food products developed by fermentation.

1.1.3 Scope and Importance of Microbiology

The microbiology has influence on genetics, agriculture, food science, ecology and immunology as well as medicine.

- **Genetics:** It mainly involves engineered microbes to make hormones, vaccines, antibiotics and many other useful products for human being.
- **Agriculture:** It involves influence of microbes on agriculture, soil microbiology and prevention of diseases which mainly damage useful crops.
- **Food science:** It involves prevention of spoilage of food and food-borne diseases and uses of microbes to produce cheese, yoghurt, pickles and beer.

- **Immunology:** It involves study of immune system which protects human body from pathogen.
- **Medicine:** It deals with identification of plans and measures to cure diseases of human beings and animals which are infectious to them.
- **Industry:** It involves use of microorganisms to produce antibiotics, steroids, alcohol, vitamins and amino acids.
- **Aero-microbiology:** It helps in studying microorganisms present in atmosphere which is useful in preservation and preparation of food, food-prone diseases, and their ultimate prevention.
- **Beverage microbiology:** It involves study of microorganisms useful in making of beer, wine and a variety of alcoholic beverages like whisky, brandy, rum, gin, vodka.
- **Exo-microbiology:** It helps in exploration of life in the outer space.
- **Geochemical microbiology:** It helps in the study of coal, mineral deposits and gas formation: prospecting the deposits of gas and oil, coal, recovery of minerals from low grade ores.
- **Waste treatment microbiology:** It involves treatment of domestic and industrial effluents or wastes by lowering biological oxygen demand (BOD) and chemical oxygen demand (COD).
- **Pharmaceutical microbiology:** It involves manufacture of antibiotics like penicillins, chloramphenicol, ciprofloxacin, tetracycline, amino glycoside antibiotics, rifampicin, etc. on large scale.

1.1.4 Introduction to Prokaryotes

Life on earth is classified into five kingdoms. The biggest division is between the cells of prokaryote kingdom (the bacteria) and those of other four kingdoms (Animals, Plants, Fungi and Protoctista), which are all eukaryotic cells. Prokaryotic cells are smaller and simpler than eukaryotic cells and do not have a nucleus. Concept of prokaryote and eukaryote can be easily depicted as follows:

Prokaryote means “before carrier bag” i.e. without a nucleus.

Eukaryote means “good carrier bag” i.e. with a nucleus.

The word prokaryote comes from two Greek words: pro and karyon. Pro means before and karyon means nut or kernel. A prokaryote is a unicellular organism that lacks a membrane-bound nucleus, mitochondria or any other membrane bound organelle. (See Fig. 1.1)

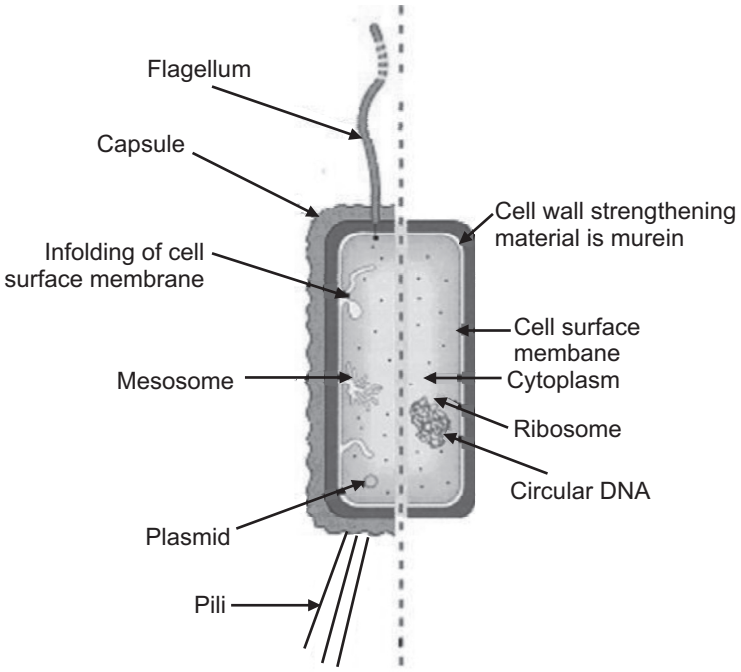


Fig. 1.1: A typical prokaryotic cell

Prokaryotes are divided into two domains : Archaea and Bacteria. Prokaryotes reproduce without fusion of gametes. The first living organisms are thought to have been prokaryotes.

In prokaryotes, all the intracellular water soluble components (proteins, DNA and metabolites) are located together in the cytoplasm enclosed by the cell membrane, rather than in separate cellular compartments. Some prokaryotes like cyanobacteria may form large colonies. Others like myxobacteria, have multicellular stages in their life cycles.

Structure:

Prokaryotes have a prokaryotic cytoskeleton. Flagellin is the building-block of the helically arranged flagellum. Flagellin is one of the most significant cytoskeletal protein providing structural background of chemotaxis, the basic cell physiological response of bacteria.

Most prokaryotes have a size between 1-10 μm, but vary in size from 0.2-750 μm.

Prokaryotic cells have following common structures:

- **Flagellum:** It is a long, whip like protrusion that aids cellular locomotion used by both Gram positive and Gram negative organisms.
- **Cell membrane:** It surrounds the cell's cytoplasm and regulates the flow of substances in and out of the cell.
- **Cell wall (except genera Mycoplasma and Thermoplasma):** It is an outer covering of most cells that protects the bacterial cell and gives it shape.

- **Cytoplasm:** It is a gel like substance composed mainly of water which contains enzymes, salts, cell components and various organic molecules.
- **Ribosome:** It is a cell structure responsible for protein production.
- **Nucleoid:** It is an area of the cytoplasm which contains the prokaryote's single DNA molecule.
- **Glycocalyx:** It is present only in some types of prokaryotes. It is a glycoprotein-polysaccharide covering surrounding the cell membranes.
- **Inclusions:** It contains inclusion bodies like ribosomes and larger masses scattered in the cytoplasmic matrix.

Prokaryotic cells have various shapes; the four basic shapes are as follows:

- **Cocci:** Spherical.
- **Bacilli:** Rod shaped.
- **Spirochaete**: Spiral shaped.
- **Vibrio:** Comma shaped.

Prokaryotic cells have following characteristics:

- They have no organelles, no membrane enclosed nucleus, and no histones. In rare cases, they contain phospholipids and sterols.
- They have 70S ribosomes.
- They have a cell wall composed of peptidoglycan-containing muramic acid.
- They are haploid with a single chromosome.
- They have coupled transcription and translation.

Prokaryotic organisms include:

- **Typical bacteria :** They have normal peptidoglycan. They may be normal flora or may be pathogenic in human. They do not have a sexual growth cycle.
- **Mycoplasmas :** They are the smallest and simplest bacteria which are self-replicating. They lack a cell wall. They are the only prokaryotes containing sterols.

1.1.5 Introduction to Eukaryotes

Eukaryotic cells are generally larger and more complex than prokaryotic cells. They contain a variety of cellular bodies called as organelles. The organelles function in the activities of the cell and are compartments for localising metabolic function. Microscopic protozoa, unicellular algae and fungi have eukaryotic cells. Structural details of a typical eukaryotic cell is depicted in Fig. 1.2.

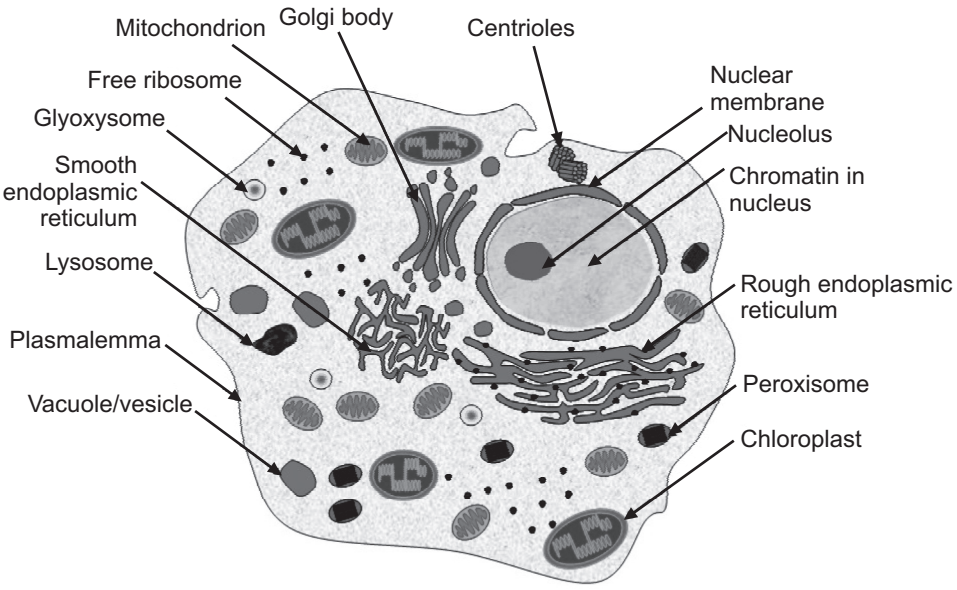


Fig. 1.2: Eukaryotic cell

Structure:

Eukaryotic cells have the following components:

- **Nucleus:** It is composed primarily of protein and DNA, organised into linear units called chromosomes, also known as chromatin. Functional segments are called as genes.
- **Histones:** These are nuclear proteins which provide a supporting framework for DNA in chromosome.
- **Nuclear envelope:** It is an outer membrane for nuclear content. It is a double-membrane structure consisting of two lipid layers with pores in the membrane.
- **Nucleoli:** Every nucleolus is an RNA-rich area where ribosomes are assembled before passing out of the nucleus into the cytoplasm.
- **Cellular organelles:** Within the cytoplasm, there are a number of microscopic bodies called as organelles. They are as follows:
  - **Endoplasmic reticulum (ER):** These are series of membranes which extend throughout the cytoplasm. In some places, ER is studded with sub-microscopic bodies called ribosomes; such ER are termed as rough ER, while others without ribosomes are termed as smooth ER.
  - **Ribosomes:** These are 80S bodies where the amino acids are bound together to form proteins.
  - **Cisternae:** These are spaces within ER membranes.
  - **Golgi body (Golgi apparatus):** These are a series of flattened sacs, usually curled at the edges. The outermost sac bulges away to form drop-like vesicles known as secretory vesicles.