PHARMACEUTICAL MICROBIOLOGY

(Including Practicals)

As Per PCI Regulations
Second Year B. Pharm., Semester - III

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PREFACE

Microbiology is a basic science dealing with the study of micro-organisms. Pharmaceutical microbiology is related to microbial aspects of production and testing of drugs, cosmetics and medical devices. Detailed knowledge on microbiological methods is necessary for production and analysis of different pharmaceuticals and other products. Biotechnology is also an advanced field of microbiology.

This **'PHARMACEUTICAL MICROBIOLOGY (Including Practicals)** book has been written by considering the syllabus prescribed by **PHARMACY COUNCIL OF INDIA (PCI).** The book is designed for second year B. Pharm. students (semester-III). All the chapters in the present book are followed by objective type, short answer, long answer and multiple choice questions as per university examination pattern.

The book is a simple, handy, comprehensive and profusely illustrated digest on pharmaceutical microbiology with special stress on the requirement of undergraduates. The basic principles and techniques of diverse microbiological fields have been presented. The realization of the need and importance for students promoted me to write new book as per syllabus of PCI semester pattern. It is also hoped that it will serve as a useful resource for teachers of Pharmaceutical Microbiology. There is no need to use separate book for practicals because all practicals as per syllabus is given in Appendix-I.

I express my sincere thanks to all my teachers, faculty members and students for their kind help, motivation and valuable guidance in writing this book. I express my deep sense of gratitude to my family for their love, support and motivation in academics and research. I am also thankful to team of Nirali Prakashan for their sustained efforts and keen interest in the publication of this book.

Readers are requested to mention any shortcomings in this edition and write the valuable suggestions for the improvement of this book for the next edition.

Kindly send your suggestions to the author.

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SYLLABUS

Theory

Unit I **10 Hours**

Introduction, History of Microbiology, its Branches, Scope and its Importance. Introduction to Prokaryotes and Eukaryotes. 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, Quantitative Measurement of Bacterial Growth (Total & 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 & Acid Fast Staining) and Biochemical Tests (IMViC). Study of Principle, Procedure, Merits, Demerits and Applications of Physical, Chemical Gaseous, Radiation and Mechanical Method 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 Viruses. Classification and Mode of Action of Disinfectants. Factors Influencing Disinfection, Antiseptics and their Evaluation. For Bacteriostatic and Bactericidal Actions Evaluation of Bactericidal & Bacteriostatic. 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 Standardization of Antibiotics, Vitamins and Amino Acids. Assessment of a New Antibiotic.

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.

Practicals **4 Hrs/week**

1. Introduction and study of different equipments and processing, e.g., B.O.D. incubator, laminar flow, aseptic hood, autoclave, hot air sterilizer, deep freezer, refrigerator, microscopes used in experimental microbiology.
2. Sterilization of glassware, preparation and sterilization of media.
3. Sub-culturing of bacteria and fungus. Nutrient stabs and slants preparations.
4. Staining methods- Simple, Grams staining and acid fast staining (Demonstration with practical).
5. Isolation of pure culture of micro-organisms by multiple streak plate technique and other techniques.
6. Microbiological assay of antibiotics by cup plate method and other methods.
7. Motility determination by Hanging drop method.
8. Sterility testing of pharmaceuticals.
9. Bacteriological analysis of water
10. Biochemical test.

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UNIT I

Chapter ... 1

INTRODUCTION OF MICROBIOLOGY

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *The History of Microbiology*
- *Applied branches of Microbiology*
- *The Scope and Importance of Microbiology*
- *Basic Knowledge of Classification of Microorganisms*

1.1 INTRODUCTION

Microbiology is the study of living organisms of microscopic size which include bacteria, fungi, algae, protozoa and viruses. This term was introduced by a French Chemist **Louis Pasteur**, who demonstrated that fermentation was caused by the growth of bacteria and yeasts. He is known as the Father of Microbiology. These microorganisms may be seen only by magnifying their images with the microscope. Microorganisms are present everywhere on earth, including humans, animals, plants, soil, water, food and the atmosphere. Microorganisms can multiply in these habitats by utilizing different nutrients. These microorganisms may be beneficial or harmful to human life.

Microbiology is one of the largest and complex biological science as it deals with many diverse biological disciplines. The branches that come under microbiology are categorized into "pure" and "applied" sciences.

Branches of pure sciences under microbiology are classified as: bacteriology (study of bacteria), mycology (study of fungi), protozoology (study of protozoa) phycology or algology (study of algae), parasitology (study of parasitism and parasites), microbial taxonomy (study of classification of microorganisms), genetics (study of heredity and variations) and immunology (study of mechanism involved in the development of resistance by body to infectious diseases). Microorganisms are classified as bacteria, rickettsia, actinomycetes, fungi, algae, protozoa and viruses.

Bacteria:

These are unicellular, free-living small microorganisms which are visible under the light microscope. They occur in water, food, soil, air and all natural environments. They grow on artificial laboratory media and multiply by binary fission. Cocci are about 1 μm in diameter

and bacilli are 1 to 8 µm in length and 0.1 to 0.5 µm in width. Many bacterial species can cause diseases in plants, animals and human beings. Some species play an important role in the natural cycling of elements (soil fertility) and some others may be useful in industries for preparation of different compounds.

Rickettsiae:

Rickettsiae was first discovered in 1909 by **Howard Taylor Ricketts**. They are small, Gram-negative bacilli and have properties of both bacteria and viruses. Rickettsiae are transmitted to humans primarily by arthropods such as ticks and lice and are cultivated only in living tissues (like viruses). They are obligate intracellular parasites and multiply by binary fission. Epidemic typhus, scrub typhus, rocky mountain spotted fever, rickettsial pox, Q-fever and trench fever are the examples of diseases caused by rickettsiae.

Actinomycetes:

Actinomycetes are Gram-positive bacteria with a fungal morphology. They are non-motile, non-capsulated and filamentous that breaks into bacillary and coccoid elements forming aerial mycelium and substrate mycelium. They play a major role in the cycling of organic matter in the soil ecosystem. They are a rich source of antibiotics, enzymes, biosurfactants, enzyme inhibitors, immunomodifiers, plant growth stimulants and help in the biodegradation of agricultural and urban wastes. Actinomycetes are mainly found in soil, water, compost and other extreme environments.

Fungi:

Fungi are eukaryotic microorganisms which exist as saprophytes, parasites or commensals. Fungi may be simple oval cells (yeasts) or long tubular septate hyphae (moulds) showing true lateral branching. They are aerobic or facultatively anaerobic. They are cultivated in the laboratory like bacteria and reproduce by asexual and sexual processes. Fungi are important sources of antibiotics, enzymes, acids, foods, bakery and alcohol fermentations. Certain fungi also cause human diseases, such as athlete's foot and thrush.

Protozoa:

Protozoa are unicellular eukaryotic microorganisms. They are differentiated on the basis of morphological, nutritional and physiological characteristics. They are generally 2 to 200 µm in size and have complex life cycles. Some protozoal species are easily cultivated in laboratory media like bacteria and others are intracellular parasites. All protozoa require large amounts of moisture for growth and activity. They reproduce by asexual and sexual processes. Some species can cause diseases such as malaria and sleeping sickness.

Algae:

Algae are unicellular or multicellular aerobic eukaryotic microorganisms which mainly occur in aquatic environments. They contain chlorophyll and are photosynthetic.

Reproduction occurs by asexual and sexual processes. Some algae can be easily cultured in the laboratory by using artificial media. They are mainly used as food supplements and in pharmaceutical preparations. Agar, used in culture media is prepared from different types of algae.

Viruses:

Viruses are non-cellular small microorganisms (20 to 300 nm) which can only be seen under electron microscope. They are 10 to 100 times smaller than most bacteria. They are obligate intracellular parasites. Viruses comprises an ultramicroscopic fragment of nucleic acid, either DNA or RNA, surrounded by a sheath of protein. They are responsible for many human diseases including AIDs, hepatitis, influenza, polio and chickenpox.

1.2 HISTORY OF MICROBIOLOGY

The existence of the microbial world was unknown until the invention of microscopes because microorganisms are too small to be seen clearly by the naked eye. The principle of telescope and microscope was accidentally discovered in 1609 by **Zacharias Jansen** (Dutch spectacle maker). The first to apply this invention of microscope to medicine was Professor **Athanasius Kircher** (1602 – 1680). He examined the blood of patients suffering from plague and through a microscope saw countless masses of small worms invisible to the naked eye. He attributed these worms like structures to be the causative agents of plague. In 1665, **Robert Hooke** (1635 – 1703) an English scientist used a simple lens that magnified objects. He examined the slices of cork from the bark of an oak tree. He observed that cork was made of tiny boxes that he referred to as 'cells'. The credit for developing a compound microscope with multiple lenses and describing the world of the microbes goes to **Robert Hooke**.

Antony Van Leeuwenhoek (1632 – 1723) was the greatest of all microscopists. He made many observations through the microscope. The Leeuwenhoek's microscope consists of spherical lens was mounted between two small metal plates. The culture or specimen was placed on the point of a blunt pin attached to back plate and was brought into focus by manipulating screw. He was the first to observe and accurately describe the shape of human red blood cells as well as the little agents of disease 'animalcules'. He took the scurf from the root of decayed tooth and mixed it with clean rain water and saw the mobility in animalcules. In 1683, he described and sketched different forms of animalcules such as rods, spheres and spiral shapes which are nothing but the morphological forms of bacteria. This was first recorded observation of bacteria. He discovered major classes of bacteria, protozoa, algae, yeasts, erythrocytes and spermatozoa.

Antony Van Leeuwenhoek, (Dutch merchant) had little formal education but his keen interest in nature made him examine a variety of materials. Glass grinding and preparation of lenses was his hobby and this led him to assemble 250 simple microscopes. He often used

a magnifying glass to study the weaves of the various types of cloth. He observed and measured a large number of minute living organisms, including bacteria and protozoa and communicated this to the Royal Society of London in 1684. In his letter of 1684, he described his experiments on the inhibitory effect of acetic acid on microorganisms. Leeuwenhoek transmitted his discoveries to the Royal Society in the form of a long series of letters written in Dutch. Most of these letters were transmitted and published in English in the Proceedings of Royal Society.

1.2.1 Contributions of Louis Pasteur

The credit for providing foundation to bacteriology as a science goes to **Louis Pasteur** (1822 – 1895), the French organic chemist.

Louis Pasteur was the first scientist who demonstrated that air contains microscopically observable organised structures. He passed a large quantity of air through a tube that contained a plug of guncotton to serve as a filter. The guncotton was then removed and dissolved in a mixture of alcohol-ether and the sediment was examined microscopically. He found that this sediment contained not only organic matter but also a large number of small microorganisms.

He performed a series of experiments to prove that although microorganisms were present in the air they were not spontaneously produced. **Pasteur** took boiled meat and infused it several swan-neck flasks (Fig. 1.1). The flask's opening were freely open to air but curved so that gravity would cause any air borne dust particles to deposit in the lower part of the neck. The flasks were heated to sterilize the broth and then incubated. No growth occurred even though the contents of the flasks were exposed to air. **Pasteur** pointed out that no growth took place because dust and germs had been trapped on the walls of the curved necks but if the necks were broken off so that dust fell directly down into the flask, microbial growth commenced immediately. In 1861, **Pasteur** finally resolved the controversy of spontaneous generation versus biogenesis and proved that microorganisms are not spontaneously generated from inanimate matter.

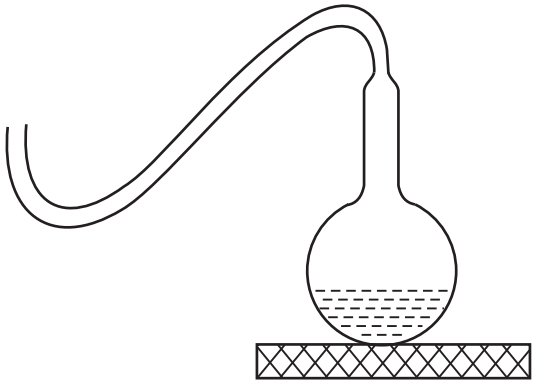


Fig. 1.1: Louis Pasteur's swan neck flask

Pasteur also showed that microorganisms are not evenly distributed in the atmosphere and that their number varies from place to place. For this experiment, he took a large number of sealed flasks containing boiled and cooled infusions and opened a few at a time, for a short period, at various places and then resealed them. Out of the 20 flasks which he opened and resealed on a dusty road, 8 showed spoilage, while out of the 20 that he opened on the top of a mountain, only 5 showed spoilage and out of the 20 that he opened near a glacier, only 1 showed spoilage. After incubation, only those flasks carrying micro-organisms from air showed growth and spoilage. From these experiments, **Pasteur** concluded that air contained microorganisms and their number varied from place to place.

Pasteur made an intensive study of beer and wine fermentation. He found that wine spoilage was caused by the growth of undesirable contaminating microorganisms. After some experimentation, he showed that wine did not undergo spoilage if it was held for a few minutes at 50 to 60°C. This gave rise to the new process of preserving wine, fruit juices, milk etc. and was called 'Pasteurisation'. **Pasteur** studied a number of other fermentation processes and showed that not only each type of fermentation was accomplished by a specific type of microorganism but also that each fermentation required specific environmental conditions. He showed that the agent responsible for alcoholic fermentation could flourish in an acid medium while microorganisms for lactic fermentation could grow best only at a neutral pH. During the studies on butyric acid fermentation, **Pasteur** discovered the existence of life in the absence of oxygen. He reasoned that oxygen was inhibitory to the growth of butyric acid bacteria. **Pasteur** also found that many other microorganisms, including yeast could grow either in the presence or in the absence of oxygen. He designated life in the presence of oxygen as 'aerobic', in the absence of oxygen as 'anaerobic' and in presence or absence of oxygen as 'facultative anaerobic'.

Pasteur opened the field of sterilization by stating that boiling rendered the fluids sterile. He soon found that ordinary water had some microorganisms even after boiling. **Pasteur** established the practice of heating fluid material to 120°C under pressure of sterilisation (autoclave). He also introduced the practice of sterilizing glassware by dry heat at 170°C. Pasteur's achievements in sterilization were closely followed by many other researchers. An important investigation was made by **Tyndall**, who observed that actively growing bacteria were easily destroyed by boiling whereas in the inactive phase, they were comparatively resistant. He introduced the method of sterilization by repeated heating with appropriate intervals. This method is known as Tyndallisation.

In 1880, **Pasteur** isolated the bacterium responsible for chicken cholera and grew it in pure culture. But on the day of his public demonstration he failed to repeat his experimental results. The chicken survived after injecting the isolated bacterial culture and this result surprised him. On subsequent experiments he came to the conclusion that on long preservation or subculturing, the virulence power of the pathogenic bacteria was destroyed.

Such microbial culture with decreased virulence was referred to as attenuated culture. Such attenuated culture does not cause infection but induces immunity in host.

Pasteur further introduced a vaccine for anthrax and rabies. He knew that the causative agent of rabies attacked the brain and spinal cord. He took spinal cords from rabbits which died from the disease and suspended these cords in dry sterile air. By this process, the rabies microorganisms in the nervous tissue lost their virulence and the emulsion of the spinal cord was used as a vaccine. It was found to be effective in dogs. Joseph Meister was the first boy cured from rabies by this vaccine.

1.2.2 Robert Koch and Anthrax

The first direct demonstration of the role of bacteria in causing disease was provided by **Robert Koch** (1843 – 1910). He was a German physician, who first isolated *Bacillus anthracis*, the causative agent of anthrax. His method of preparing, fixing and staining film preparation of bacteria using aniline dyes, opened a new area in bacteriological techniques. In 1881, he described his method of preparing cultures on solid media and the isolation of pure strains of bacteria from single colonies was made possible. Till 1881, solid media using potato and gelatin were in practice for isolation of fungi. **Robert Koch** added nutrient agar, a solid transparent medium for isolation of bacteria.

In 1882, he discovered *Mycobacterium tuberculosis* and in 1883 the *Vibrio cholera*. In 1905, Koch was awarded the Nobel Prize in Medicine for this discovery. 'Koch phenomenon' is the immunity to the second infecting dose of the tubercle bacillus. Koch was Director of the Prrussian Institute of Infectious Diseases which was eventually renamed The Robert Koch Institute.

The most notable contribution of **Koch** was the establishment of the causal relationship between a microorganisms and a specific disease by applying a set of criteria referred to as Koch's postulates. According to these postulates, a microbe may be accepted as a causative agent of an infectious disease only if it satisfies the following conditions.

The postulates are:

1. The microorganisms must be present in every case of the disease.
2. The microorganisms must be isolated from the diseased animal and grown in pure culture.
3. The disease must be reproduced when a pure culture of the microorganisms is inoculated into a susceptible host.
4. The same microorganism must be reisolated from the experimentally infected host.

1.2.3 Germ Theory of Disease

Pasteur investigated silkworm disease and proved that the disease was due to a protozoan parasite. Even before **Pasteur** had proved by experiments that bacteria are the

causative agent of some diseases: **Girolamo Fracastoro** in 1546 wrote a treatise in which he said disease was caused by minute "seed" and was spread from person to person. In 1762, **Von Plenciz** not only stated that living agents are the cause of diseases but suspected that different germs were responsible for different diseases. The first man to present real evidence that living organisms were the cause of disease was **Augustino Bassi** (1773-1856). In 1836, he demonstrated that a fungus which caused a disease in silkworm could be transmitted from one silkworm to another silkworm.

Lord Joseph Lister (1827-1912), a famous English surgeon in 1867, introduced antiseptics for the preventation and cure of wound infections. He developed a system of antiseptic surgery designed to prevent microorganisms from entering wounds by the application of phenol on surgical dressings. He also heat sterilized the instruments to be used during surgery. Joseph Lister was the first to introduce aseptic techniques for control of microbes by the use of physical and chemical agents. Because of his important contributions, **Lister** is known as the father of antiseptic surgery.

The confirmed evidence that microorganisms actually cause disease, came from experiments performed by Robert Koch. The proofs which support the Germ theory of disease became 'Koch Postulates'. **Richard Petri**, Koch's assistant designed a special plate to hold solid culture media. This plate has great significance in microbiology and is referred as 'Petri plate'. The period from 1857 to 1914 is considered as the 'golden age of microbiology' because most significant advances made during this period led to the establishment of microbiology as a science. Discovery of the major pathogens following establishment of Germ theory of disease is given in Table 1.1.

1.2.4 Discovery of Chemotherapeutic Agents

Paul Ehrlich (1854 – 1915) in 1904 found that the dye Trypan red was active against trypanosome that causes African sleeping sickness. This dye with antimicrobial activity was referred to as a 'magic bullet'. Subsequently, in 1910, Ehrlich in collaboration with **Sakahiro Hata**, a Japanese physician, introduced the drug Salvarsan (arsenobenzol/ 606 arsenic) as a treatment for syphilis caused by *Treponema pallidum*. Ehrlich continued his experimentation until in 1912 he discovered neosalvarsan. This gave him the title "the father of chemotherapy". He coined the word 'chemotherapy' meaning the treatment of infectious diseases with chemicals that are antagonistic to the pathogen without damaging the host.

Ehrlich tested new dyes and aniline pigments as histological stains and showed that all dyes can be classified as acidic, basic or neutral. He developed differential staining methods for red and white blood cells laying the foundation of hematology. In 1882, Ehrlich developed a staining procedure to demonstrate the acid fast property of tubercle bacilli which is known as Ziehl- Neelson staining technique. Ehrlich propounded new theory in the field of immunology, explaining the production of antibodies that he quantified in various sera and formulated the standardization of antitoxin into units related to a fixed and invariable standard. In 1908, Paul Ehrlich received the Nobel Prize in Medicine for his studies on immunity.

Table 1.1: Discovery of causative agents of microbial diseases

Disease	Causative agent	Discoverer	Year
Leprosy	*Mycobacterium leprae*	Hansen	1874
Anthrax	*Bacillus anthracis*	Robert Koch	1876
Actinomycosis	*Actinomyces bovis*	Bollinger	1877
Gonorrhoea	*Neisseria gonorrhoeae*	Albert Neisser	1879
Typhoid fever	*Salmonella typhi*	Eberth	1880
Malaria	*Plasmodium* spp.	Laveran	1880
Pyogenic infections	*Staphylococcus aureus*	Alexander Ogston	1881
Tuberculosis	*Mycobacterium tuberculosis*	Robert Koch	1882
Cholera	*Vibrio cholerae*	Robert Koch	1883
Diphtheria	*Corynebacterium diphtheriae*	Theodor Klebs	1883
Tetanus	*Clostridium tetani*	Arthur Nicolaier	1885
Pneumonia	*Streptococcus pneumoniae*	Fraenkel	1886
Meningitis	*Neisseria meningitids*	Weichselbaum	1887
Gastroenteritis	*Salmonella enteritidis*	August Gaertner	1888
Gas gangarene	*Clostridium perfringens*	William Welch	1892
Plague	*Yersinia pestis*	Alexander Yersin	1894
Botulism (food poisoning)	*Clostridium botulinum*	Van Ermengem	1896
Dysentery	*Shigella dysenteriae*	Kiyoshi Shiga	1898
Bird flu	Avian influenza virus	WHO	1900
Syphilis	*Treponema pallidum*	Schaudin	1905
Whooping cough	*Bordetella pertussis*	Bordet and Gengou	1906
Rocky mountain spotted fever	*Rickettsia rickettsii*	Ricketts	1909
Leptospirosis	*Leptospira interrogans*	Inada and Ido	1914
Creutzfeldt - Jakob disease	CJD prion	Creutzfeldt	1920
Q-fever	*Coxiella burnetii*	Derric	1935
Monkey pox	Monkey pox virus	WHO	1958
Hepatitis A	Hepatitis A virus	Purcell et al.	1972
AIDS	Human immunodeficiency virus	Lac Montagnier	1983
Gastritis	*Helicobacter pylori*	Warren and Marshall	1983
Leukemia	Human T-cell virus	Robert Gallo et al	1984
Respiratory disease (SARS)	SARS Coronavirus	Carlo Urbani	2003

Gerhardt Domagk (1895 – 1964) found that prontosil, an azo dye derived from para-aminobenzene sulphonamide was active against specific bacteria. The first success in treating streptococcal infections with sulphonamide was reported in 1935. In 1929, **Sir Alexander Fleming** (1881 – 1955) accidentally discovered a substance produced by *Penicillium notatum*. He extracted from the fungus a compound which he called penicillin that could destroy several pathogenic bacteria. Fleming's work was practically unnoticed until 1940 when **Sir Howard W. Florey** and **E.B. Chain** at Oxford University, developed methods for industrial productions of penicillin in England. The commercial production of penicillin in the USA began in 1941 during the Second World War. *Penicillium notatum* has been replaced with *Penicillium chrysogenum* for the commercial production of penicillin. **S. A. Waksman** (1888 – 1973) at the Rutgers University, USA discovered another antibiotic, streptomycin, produced by two strains of actinomycetes *Streptomyces griseus* in 1944. Major contribution in the search for microorganisms producing antibiotics came from Waksman, who showed that actinomycetes are prolific producers of a variety of antibiotics.

1.3 APPLIED BRANCHES OF MICROBIOLOGY

The prominent applied branches of microbiology are classified as follows:

(i) Medical Microbiology: It deals with the study of causative agents of infectious diseases in human beings. It deals with etiology, pathogenesis, laboratory diagnosis, treatment, epidemiology and control of infection. Medical microbiology has close links with other disciplines such as pathology, clinical medicine, pharmacology cum therapeutics and preventive medicine.

(ii) Pharmaceutical Microbiology: It deals with the study of microorganisms which are responsible for the production of antibiotics, enzymes, vaccines, vitamins and other pharmaceutical substances. It is also the study of microorganisms which can contaminate pharmaceutical products, raw materials, manufacturing areas and foods. It includes study of microorganisms which are used in the treatment of industrial waste material, ayurvedic plant growth promotion, steroid biotransformation and biological indicators. It also includes methods of sterilisation and disinfection, microbiological testing of pharmaceuticals, sterile product preparation and diagnosis of diseases and treatment.

(iii) Industrial Microbiology: It is the study of industrially useful microorganisms in the production of alcoholic beverages, vitamins, amino acids, enzymes, antibiotics and other drugs. It also includes fermentation techniques used for the production of different compounds. Breweries have greatly benefited by understanding the role of specific microbes in fermentation.

(iv) Microbial Biotechnology: It is the scientific manipulation of living organisms, especially at the molecular and genetic level to produce useful products such as antibiotics, enzymes and amino acids.

(v) Food Microbiology: It deals with the interaction of microorganisms and food in relation to food bioprocessing, food spoilage, food borne diseases, their prevention and includes preparation and preservation of food products. Food microbiology also includes the study of normal flora of foods, their significance and the techniques used for protection of food from microbial contamination.

(vi) Soil Microbiology: It is the study of microbial flora and the interactions among soil microorganisms. It also includes the biogeochemical role of soil microorganisms.

(vii) Agricultural Microbiology: It is the study of relationships of microorganisms and crops with an emphasis on the control of plant diseases and improvement of yields. Many microbial species under group of fungi, bacteria and viruses cause a number of plant diseases. Nitrogen fixing microbes act as biofertilizers that help to plant for growth and metabolism.

(viii) Aquatic Microbiology: Aquatic microbiology is the study of microorganisms and their activities in fresh and marine waters including lakes, rivers, bays, estuaries and seas. It also includes water purification, microbiological examination and biological degradation of wastes.

(ix) Air Microbiology: It deals with the role of aerospora in contamination and spoilage of food and dissemination of plant and animal diseases through air. It also includes microbial population in air.

(x) Epidemiology: It is concerned with the monitoring, control and spread of diseases in communities.

1.4 SCOPE AND IMPORTANCE

Pharmaceutical microbiology may be defined as that part of microbiology which has special applications in the pharmaceutical field. This is one of the branch of applied microbiology. Applications of microbiology in the pharmaceutical area are described as follows:

(i) Production of antibiotics: Today's 2/3rd of antibiotics are produced from micro-organisms. Pharmaceutical or industrial microbiology concerns itself with the isolation of antibiotic producing microorganisms from natural environments such as soil or water. Many antibiotics are isolated from natural microorganisms by the process of fermentation. e.g. Penicillin from *Penicillium* species, Streptomycin from *Streptomyces griseus*, Tetracycline from *Streptomyces aureofaciens,* Chloramphenicol from *Streptomyces venezuelae* etc.

(ii) **Production of enzymes, vaccines and other pharmaceutical products:** The detection and assay of pharmaceutical and chemical products of microbial activity is a part of pharmaceutical microbiology. Many microbial cells convert raw materials or substrates into valuable organic compounds such as butanol, ethanol, acetone etc. Microbial cells also produce intracellular and extracellular enzymes and these enzymes are important for the success of pharmaceutical fermentation processes. They are associated with the microorganisms ability to attack, degrade and utilize components of the medium and to accumulate fermentation products e.g. amylases, proteases, lipases, invertases, oxidase, catalase, cortisone reductase, phospholipases, cellulases, chitinases etc. Biosurfactants have lot of applications in agriculture, food industries, industrial cleaning, leather, paper and metal industries, textiles, cosmetics and the pharmaceutical industry. Different types of biosurfactants are synthesized by a number of microorganisms e.g. *Acinetobacter* species, *Bacillus* species, *Pseudomonas* species, *Rhodococcus* species etc.

(iii) **Diagnosis of diseases and treatment:** Different tests are used to detect infectious microorganisms e.g. ELISA, Widal test. Antimicrobial susceptibility testing is mainly used for selection of antibiotics for the treatment of microbial infections.

(iv) **Treatment of industrial waste material:** Most industrial processes produce waste waters, salts, organic matter and spent media and these may be toxic. Many microbial species are used for decomposition of such waste materials and organic components e.g. actinomycetes, fungi, protozoa etc. Anaerobic bacteria in anaerobic digestion tanks are active in the decomposition of organic matter by yielding simple organic molecules such as acids, alcohols, glycerol and amines. Cyanide is produced on an industrial scale for use in metal extraction, electroplating and steel industry which is degraded by *Streptomyces lavendulae*.

(v) **Plant growth promotion:** Many microbial cells present in soil, play an important role in soil fertility, herbicidal resistance, insect resistance, change in protein/oil content and enhanced quality of plant products e.g. *Rhizobium* species, *Rhodospirillum* species, *Azotobacter* species, *Agrobacterium rhizogenes*, *Pseudomonas* species, *Acetobacter* species etc. Many nitrogen fixing soil micro-organisms are useful for providing nitrogen for the growth of crops and enzymes released from microorganisms are helpful for plant growth, germination of seeds, flavouring and fruiting of medicinal plants. Soil microbial populations are the key element in recycling plant nutrients and thus they play a major role in the maintenance of soil fertility and soil health. The rhizospheric bacteria may release various plant growth promoting substances as secondary metabolites, which may promote plant growth directly or indirectly. Such beneficial rhizopheric bacteria are often referred to as plant growth promoting rhizobacteria (PGPR). *Pseudomonas* is

one of the extensively studied PGPR, which promotes growth of plants by protecting plant health from soil borne phytopathogens and their toxins. *Azotobacter chroococcum* is a well known free living nitrogen fixing bacterium capable of synthesizing and secreting plant growth promoting substances like thiamine, riboflavin, IAA, gibberellin etc. and is frequently used as a nitrogenous biofertilizer for a number of crops.

(vi) Sterile product preparation: Pharmaceutical microbiology plays a major role in preparation of sterile products. It deals with preparation of sterile rooms, aseptic techniques, detection of microbes by sampling and sterility testing of different sterile preparations. Sterility testing is carried out to support batch releases of sterile pharmaceuticals and medical devices. Settle plates, contact plates and air sampling techniques are used for detection of microorganisms in sterile rooms.

(vii) Sterilization: Different pharmaceutical products are sterilized by killing micro-organisms. This process of killing microbes is called sterilization. Moist heat sterilization, dry heat sterilization, membrane filtration, gaseous sterilization and chemical sterilization are the methods used for killing microorganisms. Methods of sterilization are decided depending on the properties of products and general type of microbes present in the products.

All the sterilization processes may be validated by using biological indicators. A biological indicator (BI) is a preparation of microorganisms usually bacterial spores that are contained in filter papers, porcelain cylinders or test tubes which serves as a challenge to the effectiveness of a given sterilization process or cycle e.g. Moist heat sterilization (autoclave) - *Bacillus stearothermophilus*, Dry heat sterilization (hot air oven) - *Bacillus subtilis*, Radiation sterilization - *Bacillus pumilis*, Membrane filtration - *Pseudomonas diminuta* etc.

(viii) Steroid biotransformation: Steroids are physiologically active compounds of complex structures e.g. cholesterol, ergosterol, testosterone, progesterone etc. Important steroids can be produced by microbiological transformations of naturally occuring steroids. Microbial conversions of steroids are brought about by enzymes secreted by selected strains of microorganisms e.g. *Streptomyces* species, *Rhizopus* species, *Aspergillus* species, *Penicillium* species.

(ix) Identification of microorganisms: Two vital functions of pharmaceutical microbiology and biotechnology are the enumeration and identification of microorganisms found in products and the manufacturing environment. There are many microbial cells present in the environment that are useful for the production of antibiotics, enzymes and other pharmaceutical substances. These micro-organisms are isolated and identified by morphological, biochemical, cultural, microscopic characteristics and genetic studies.

(x) Testing of pharmaceuticals:

(a) Raw material and finished products: Tests for the detection of *Escherichia coli, Salmonella* species, *Pseudomonas aeruginosa and Staphylococcus aureus* in pharmaceutical raw materials and finished products are described in the Indian Pharmacopoeia (IP), United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and British Pharmacopoeia (BP). These organisms are given special attention because they represent particular infection hazards to the patient or their presence is a criterion for the quality of raw materials. This test is mainly applied to raw materials of natural or biological origin such as starch, gum, gelatin, talc etc. The microbiological quality of non-sterile pharmaceutical or cosmetic materials can be controlled by the adoption of one of the two types of standards or both standards. The first is a limit on the total numbers of viable micro-organisms in a given product (TVC, total viable count) and the second is the exclusion of specific pathogens. Water is always tested for total microbial count and presence of *Escherichia coli*.

(b) Microbiological assays of antibiotics: The antibiotic bioassay provides a potency for the over all biological activity of an antibiotic preparation. Activity or potency is quoted in terms of units of a recognised international standard, specifically defined by pharmacopoeias. Agar diffusion (plate assays) and turbidometric assays permit an estimate of antibiotic potency through direct comparison of a test antibiotic with an approved, well-calibrated reference substance. Test microorganisms are used for the test that are non-pathogenic, sensitive to the action of antibiotic and capable of rapid growth.

(c) Evaluation of disinfectants: Effective cleaning and disinfection of manufacturing facilities are crucial to the achievement and maintenance of high quality standards required for medicine and medical devices. Selection of cleaning agents (detergents) and disinfectants will be influenced by the range and composition of the materials, number and types of microorganisms, maximum residual levels acceptable on completion etc. Phenol coefficient test, cup plate method, turbidometric method and Kelsey Sykes test are mainly used for evaluation of different disinfectants.

(d) Antimicrobial preservative efficacy testing: Different types of pharmaceutical products and cosmetics are protected from attack by micro-organisms by using preservatives. This is to protect the user from dangerous infections and to prevent spoilage and deterioration of a product. Preservatives are intended to protect a product from spoilage due to microorganisms introduced by the user and those which unavoidably arise

during the manufacturing process. There is an extensive range of chemical substances available for potential use in preservative systems. The preservatives should be non-toxic, easily available and have broad spectrum activity. Cup-plate method, turbidometric method and total viable counting techniques are used for determination of antimicrobial efficacy of preservatives.

(e) Endotoxin testing: Bacterial endotoxin or pyrogens have a number of physiological effects following intravenous injections such as fever, activation of the cytokine system, endothelial cell damage and intravascular coagulation. The bacterial endotoxins test (BET) is most important in microbiology laboratories involved with quality control of parenterals and medical devices. LAL test, gel-clot method, chromogenic end-point, kinetic turbidometric assay and kinetic chromogenic assay are used for detection of endotoxins.

(f) Tests for support of the sterility assurance system: Sterility testing includes many of the basic principles applicable in running an aseptic area and microbiology laboratory that deal with the testing in support of the sterility assurance system. Sterility testing is mainly performed for parenteral preparations and other sterile pharmaceuticals to detect presence of microorganisms. The interpretation of results from sterility testing can be confused by the risk of microbiological contamination during the test and masking of a genuine non-sterile unit. Sterility tests are mainly performed in laminar air flow cabinets following all aseptic precautions.

Bioburden testing is used to estimate the number of microorganisms in the product prior to sterilization. Pre-sterilization counts carried out in support of specific sterilization processes can involve selective sample treatments. The methods usually used for environmental monitoring in aseptic areas are settle plates, active air sampling, contact plates, swabs for surfaces and garments. These methods provide an approximate guide to the level of microorganisms present and contribute to the knowledge of the true microbiological state of a clean room, aseptic area and laminar air flow cabinet or isolator.

1.5 CLASSIFICATION OF MICROORGANISMS

Taxonomy is the science that deals with the logical arrangement of living things into categories. **Aristotle** (4^{th} Century B.C.) was probably the first to group all organisms and categorised them as either plants or animals. In 1735, **Carolus Linnaeus** (Swedish botanist) named thousands of plants and animals and classified them in the Kingdoms Plantae and Animalia. He also devised the binomial (Genus and species) scheme of nomenclature.

Until the eighteenth century, the classification of living organisms was placed into two Kingdoms, Plant and Animal. The German Zoologist, **E. H. Haeckel** in 1866 proposed a third Kingdom 'Protista', to include those unicellular microorganisms that are typically neither plants nor animals. Protista contains bacteria, algae, fungi and protozoa but viruses are not classified as protista (non-cellular organism). Bacteria are classified as lower protista and algae, fungi and protozoa are classified as higher protista.

Many biologists have accepted the system proposed by **Robert H. Whittaker** in 1969 for the classification of living organisms. He proposed that all living beings can be classified under five kingdoms based on the nutrition and absorption of food materials. The prokaryotic organisms are included under the kingdom monera (lower protista). These organisms do not have the ingestive mode of nutrition. The unicellular eukaryotic microorganisms are placed in kingdom protista (higher protista). In microalgae it is photosynthetic, in protozoa it is ingestive and some other protista it is absorptive. Whittaker explained that some organisms assimilate carbon dioxide by means of photosynthesis while some obtaining through nutrients absorbed from other organisms and some obtaining organic nutrients by way of ingestion. He classified plants, fungi and animals in three separate kingdoms based on the mode of nutrition. In the scheme of Whittaker, microorganisms are accommodated in three of the five kingdoms.

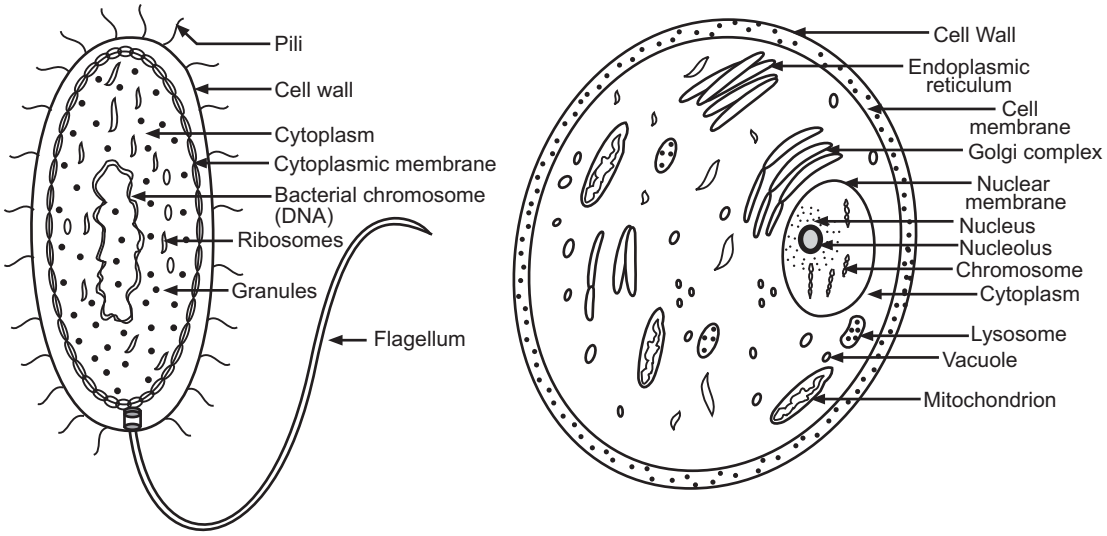
This system places all living things (except viruses) into five kingdoms based on cellular organisation and nutritional patterns; the Monera, Protista, Fungi, Animalia and Plantae. Kingdom Monera includes all unicellular prokaryotes (e.g. bacteria, cyanobacteria) and Kingdom Protista includes unicellular eukaryotic cell (e.g. microalgae, protozoa). The multicellular and multinucleate eukaryotic organisms are found in the Kingdom Fungi, Kingdom Plantae and Kingdom Animalia which mainly utilize nutrients by absorption, photosynthesis and ingestion, respectively. Viruses are acellular microorganisms that have neither cells nor cell membrane. They are composed of few genes which are protected by protein coat. Viruses are act as dead components outside of the host but inside host, they are alive and reproduce by utilizing host cell.

1.5.1 Prokaryotes and Eukaryotes

All living organisms are made up of cells and it is structural and functional unit of life. The cell was first discovered by **Robert Hook** (1665). Organisms that are made up of single cell are known a single-celled or unicellular organisms and from many cells are known as multicellular organisms. All organisms are classified into Prokaryotic (primitive nucleus) and Eukaryotic (true nucleus). These terms are first proposed by **Edward Chatton** (1937). A prokaryotic cells lacks a membrane bound organelles, such as nucleus (Fig. 1.2). The bacteria and cyanobacteria have a prokaryotic cells while fungi, protozoa, algae, plant and animal have eukaryotic cell.

Prokaryotics are simple, small cells while eukaryotic cells are complex, larger size single celled or multicellular. Differences in cellular structure of prokaryotics and eukaryotes include the presence of mitochondria and chloroplasts, the cell wall and the structure of chromosomal DNA (Table 1.2).

The chromatin bodies remain scattered inside the cytoplasm or prokaryotes and asexual division occurs by binary fission. The chromatin bodies are enclosed by a nuclear membrane in a eukaryotic cell and cell division occurs by asexual as well as sexual division. The eukaryotic cells have a variety of membrane-bound components lacking in prokaryotes. These components are called organelles which includes endoplasmic reticulum, golgi bodies, lysosome and mitochondria. Protein synthesis in eukaryotic cells is carried out on ribosomes associated with the endoplasmic reticulum, a network of internal membranes. Golgi apparatus consists of a stack of many flattened, disc-shaped sacs known as cisternae. The main function of endoplasmic reticulum and golgi apparatus is the synthesis of lysosomes, which helps in digestion of intracellular molecules. Lysosome is the membrane bound organelle found only in eukaryotic cells, which contains hydrolytic enzyme. Mitochondria is called as 'power house of cell' and it is responsible for making ATP by redox reactions. Chloroplasts are found in algae and plants which helps in converting energy sunlight into chemical energy through photosynthesis. Both mitochondria and chloroplasts have their own DNA and are capable of synthesizing particular proteins. Cell wall and chloroplast are not found in the animal cell. The eukaryotic cells possess cytoskeleton, which is an interconnected system of fibres, threads and interwoven molecules that render structure to the cell.



(a) Prokaryotic cell **(b) Eucaryotic cell**

Fig. 1.2: Prokaryotic and Eukaryotic cell structures

Both prokaryotes and eukaryotes contain ribosomes which plays major role in protein synthesis. Prokaryotic ribosomes (70S) are smaller than eukaryotic ribosomes (80s). The unit of measurement is the Svedberg (s) unit. In prokaryotes, there are 50S and 30S subunits of

ribosomes, whereas in eukaryotic cells there are 60S and 40S subunits. Prokaryotic ribosomes exist free in the cytoplasm, while eukaryotic ribosomes are usually bound to membranes of endoplasmic reticulum. Several prokaryotic and eukaryotic cells contain organells called flagella and cilia. Flagella are long, and hair like and extend from the cell to permit cell movement. Cilia are shorter but more in number than flagella.

The cell membrane lies at the surface of all cells and is composed primarily of proteins and lipids. Many prokaryotic and eukaryotic cells contain cell wall, a structure outside the cell membrane. Almost all bacteria have a thick, rigid cell walls; whereas eukaryotes, the algae, the fungi and plants have cell walls. Cell wall of fungi contains a polysaccharide called chitin while bacteria possess a different polysaccharide called peptidoglycan.

Prokaryotes contain only a single, circular loop of stable chromosomal DNA; while eukaryote DNA is linear, found on tightly bounded and organized chromosomes. Prokaryotes also differ from eukaryotes in the structure, packing, density and arrangement of their genes on the chromosome. Prokaryotes have compact genomes compared to eukaryotes because prokaryotes genes lack introns and large non-coding regions between each gene.

Table 1.2: Comparison of Prokaryotes and Eukaryotes

Character	Prokaryotes	Eukaryotes
1. Nucleus:		
Nuclear membrane	Absent	Present
Nucleolus	Absent	Present
Chromosome	One (circular)	More than one (linear)
Mitotic division	Absent	Present
Deoxyribonucleoprotein	Absent	Present
2. Cytoplasm:		
Cytoplasmic streaming	Absent	Present
Pinocytosis	Absent	Present
Mitochondria	Absent	Present
Lysosomes	Absent	Present
Golgi apparatus	Absent	Present
Endoplasmic reticulum	Absent	Present
Chloroplasts	Absent	May be present
Membrane - bound (true) vacuoles	Absent	Present
Ribosomes	70S, distributed in the cytoplasm	80S arrayed one membranes as in endoplasmic reticulum, 70S in mitochondria and chloroplasts
Gas vacuoles	Can be present	Absent
Mesosomes	Present	Absent

3.	**Outer cell structures:**		
	Cytoplasmic membranes	Generally sterols absent	Sterols present
	Cell wall	Peptidoglycan present	Peptidoglycan absent
	Locomotor organelles	Simple fibril	Multifibirilled with 'g + z' microtubules.
	Pseudopodia	Absent	Present in some.
4.	**Examples:**	Bacteria, rickettsiae, chlamydiae, actinomycetes.	Fungi, protozoa, algae, plants, animals.

1.5.2 Classification of Bacteria

The **Bergey's Manual of Determinative Bacteriology** published by **Williams and Wilkins Co. Baltimore, U.S.A.** (1974), is the international standard for bacterial taxonomy. It deals with the classification of bacteria (19 parts) and contains descriptions of all species known at the time of its publication.

Bergey's manual was revised between 1984 and 1989 and appeared in four volumes with a new name as Bergey's Manual of Systematic Bacteriology. In these four volumes, bacteria have been classified in 33 sections based on morphological and biochemical characteristics. Bacterial groups are divided into four volumes.

(i) Gram-negative bacteria (1984) of general, medical and industrial importance.

(ii) Gram-positive bacteria (1986) other than actinomycetes.

(iii) Gram-negative bacteria (1989) with distinctive properties, cyanobacteria and archaeobacteria and

(iv) Actinomycetes (1989) as Gram-positive filamentous bacteria.

The Bergey's Manual of Systematic Bacteriology places all bacteria in the Kingdom Procaryotae which in turn is divided into four divisions which are as follows:

Kingdom Procaryotae

Division I: Gracilicutes (Thin skin): Prokaryotes with a complex cell wall structure characteristic of Gram-negative bacteria (e.g. non-photosynthetic bacteria).

Division II: Firmicutes (Strong skin): Prokaryotes with a cell wall structure characteristic of Gram-positive bacteria (e.g. rods, cocci, actinomycetes).

Division III: Tenericutes (Soft or tender skin): Prokaryotes that lack a cell wall (e.g. mycoplasma).

Division IV: Mendosicutes (Skin with faults): Prokaryotes with unusual cell walls (e.g. archaeobacteria).

All bacteria are classified as kingdom, division, class, order, family, tribe, genus, section, series, species and individual. All microorganisms have a double name, usually from Latin or Greek stems. The name consists of the genus to which the organism belongs and a species modifier that further describes the genus name. Hence this system is called the binomial system e.g. *Escherichia coli*, *Corynebacterium diphtheriae* etc.

QUESTIONS

(A) Objective type questions:

1. Draw the structure of Prokaryotic cell
2. Define the term with suitable examples:
   - (a) Phycology
   - (b) Taxonomy
   - (c) Aquatic microbiology
   - (d) Industrial microbiology
   - (e) Prokaryotes
   - (f) Aseptic room
   - (g) Endotoxin
   - (h) Preservatives

(B) Short answer questions:

1. Define 'Microbiology'. Explain different applied branches of microbiology.
2. Differentiate between Prokaryotic cell and Eukaryotic cell.
3. Write in detail about contribution of Louis Pasteur in the field of microbiology.
4. Write a note on:
   - (d) Classification of bacteria.
   - (e) Eukaryotic cell.
   - (a) Koch's postulates
   - (b) Leeuwenhoek's microscope.
   - (d) Germ theory of disease.
   - (e) Swan neck flask.

(C) Long answer questions:

1. Write in detail, importance and scope of Pharmaceutical Microbiology.
2. Explain in short about different types of microorganisms.
3. Explain in short developments in Pharmaceutical Microbiology.

(D) Multiple choice questions:

1. _____ is Prokaryotic microorganism.
   - (a) Fungi
   - (b) Rickettsiae
   - (c) Algae
   - (d) Viruses

2. Chloroplasts are mainly present in _____
   (a) Animal cell (b) Plant cell
   (c) Bacterial cell (d) Fungal cell
3. _____ is mainly present in prokaryotic cell.
   (a) Mitochondria (b) Endoplasmic reticulum
   (c) Golgi apparatus (d) Mesosomes
4. Where does energy production occur in prokaryotes?
   (a) Mitochondria (b) Endoplasmic reticulum
   (c) Cytoplasmic membrane (d) Golgi apparatus
5. Antibiotics are produced by a large group of _____
   (a) Bacteria (b) Fungi
   (c) Algae (d) Actinomycetes
6. The concept of sterilization was introduced by _____
   (a) Robert Koch (b) Joseph Lister
   (c) Alexander Fleming (d) Louis Pasteur
7. Petri plate was designed for solid culture media by _____
   (a) Robert Koch (b) Robert Petri
   (c) Richard Petri (d) Alexander Fleming
8. *Mycobacterium tuberculosis* was discovered by _____
   (a) Robert Koch (b) Louis Pasteur
   (c) Albert Neisser (d) Kiyoshi Shiga
9. Leptospirosis is caused by _____
   (a) *Helicobacter pylori* (b) *Coxiella burnetti*
   (c) *Leptospira interrogans* (d) *Rickettsia rickettssii*
10. Syphilis is caused by _____
    (a) *Treponema pallidum* (b) *Bordetella pertussis*
    (c) *Yersinia pestis* (d) *Clostridium tetani*
11. Which of the following diseases was used as the basis for Koch's postulates?
    (a) Tuberculosis (b) Anthrax
    (c) Diphtheria (d) Syphilis
12. Pasteur developed the following vaccines except _____
    (a) Rabies (b) Anthrax
    (c) Chicken cholera (d) B.C.G.

■■■

Chapter ... 2

STRUCTURE AND NUTRITION OF BACETRIA

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *Morphological Classification of Bacteria*
- *Structure of Bacterial Cell with different Parts and Functions*
- *Nutritional requirements to Bacterial Cell*
- *Different Types and Applications of Media*
- *Physical Parameters and their effect on Growth*

2.1 INTRODUCTION

Bacteria are unicellular, free-living, microscopic microorganisms capable of performing all the essential functions of life. They possess both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Bacteria are prokaryotic microorganisms that do not contain chlorophyll. They occur in water, soil, air, food and all natural environments. They can survive extremes of temperature, pH, oxygen tension and atmospheric pressures.

Bacteria are very small microorganisms which are visible under the light microscope. Generally, cocci are about 1 μm in diameter and bacilli are 1 to 8 μm in length and 0.1 to 0.5 μm in width. Bacteria are stained by staining reagents and then visualised under higher powers of magnification (1000 ×) of compound microscope. An electron microscope is used for clear visualisation of internal structures of the bacteria.

Suitable artificial media containing sources of carbon, nitrogen, hydrogen, oxygen, phosphorous and other elements such as sodium, potassium, magnesium and iron in very small amounts have been used for cultivation of micro-organisms in the laboratory. Media are an artificially prepared mixture of various nutrients for the growth and multiplication of microorganisms (singular-medium). A wide variety of culture media are employed by the microbiologist for the isolation, growth, purification, maintenance and identification of microorganisms. A culture media must supply suitable carbon, nitrogen, energy sources and other nutrients.